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Glyphosate resistance in junglerice (*Echinochloa colona*) and alternative herbicide options for its effective control

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Research Article

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Abstract

Control of glyphosate-resistant (GR) junglerice is a challenging task in eastern Australia. There is limited information on the efficacy and reliability of alternate herbicides for GR populations of junglerice, especially when targeting large plants and when temperatures are high. A series of experiments were conducted to confirm the level of glyphosate resistance in three populations of junglerice and to evaluate the efficacy of alternate herbicides for the control of GR junglerice populations. The LD_{50} of glyphosate of B17/7, B17/34, and B17/35 populations was found to be 298, 2,260, and 1,715 g ae ha^{-1}, respectively, suggesting that populations B17/34 and B17/35 were highly resistant to glyphosate. Glyphosate efficacy was reduced at high-temperature (35 C day/25 C night) compared with low-temperature conditions (25 C day/15 C night), suggesting that control of susceptible populations may also be reduced if glyphosate is sprayed under hot conditions. Preemergence herbicides dimethenamid-P (1,000 g ai ha^{-1}) and pendimethalin (1,500 g ai ha^{-1}) provided 100% control of GR populations (B17/34 and 17/35). Postemergence herbicides, such as clethodim (60 or 90 g ai ha^{-1}), glufosinate (750 g ai ha^{-1}), haloxyfop (52 or 78 g ai ha^{-1}), and paraquat (400 or 600 g ai ha^{-1}), applied at the four-leaf stage provided 100% control of GR populations. For larger junglerice plants (eight-leaf stage), postemergence applications of paraquat (400 or 600 g ai ha^{-1}) provided greater weed control than clethodim, glufosinate, and haloxyfop. A mixture of either glufosinate or haloxyfop with glyphosate provided poor control of GR junglerice populations compared with application of glufosinate or haloxyfop applied alone. Efficacy of glufosinate and haloxyfop for the control of GR populations decreased when applied in the sequential spray after glyphosate application. This study identified alternative herbicide options for GR junglerice populations that can be used in herbicide rotation programs for sustainable weed management.

Introduction

Junglerice is a problematic weed in important summer crops of eastern Australia: sorghum [*Sorghum bicolor* (L.) Moench], mungbean [*Vigna radiata* (L.) R.Wilczek], corn (*Zea mays* L.), rice (*Oryza sativa* L.), and cotton (*Gossypium hirsutum* L.) (Osten et al. 2007; Pratley et al. 2008; Walker et al. 2010; Shabbir et al. 2019). This weed harms Australian crop production, as it competes with crops for water and soil nutrients (Mahajan et al. 2019; Mutti et al 2019). Junglerice can produce a considerable number of seeds (12,380 to 20,280 seeds per plant), especially when grown under fallow conditions (Squires et al. 2021). Furthermore, its emergence in multiple cohorts is a great challenge for season-long weed control (Wu et al. 2004). It is essential to control junglerice during the summer season to reduce crop competition and weed seed replenishment in the soil, as well as to enhance resource use efficiency (Mahajan et al. 2020). Llewellyn et al. (2016) estimated that junglerice could cost Australian grain growers AU$14.6 million annually when assessed in terms of yield loss and control.

Most of the growers in Australia follow winter cropping–based production systems and give much attention to stored soil moisture from summer season rains for subsequent winter crops (ABARES 2021; Dolling et al. 2006). Therefore, weed-free conditions in the summer season are critical for conserving soil moisture for subsequent winter crops. The no-till production system is quite popular in eastern Australia, where growers rely heavily on glyphosate for pre-seeding and summer-fallow weed control (Llewellyn et al. 2012). However, the evolution of glyphosate-resistant (GR) populations of junglerice in this region has made the control of this weed difficult

(Thornby et al. 2013; Walker et al. 2004). A recent study in eastern Australia revealed that the resistance factor of glyphosate in some populations of junglerice ranged from 6- to 15-fold (Mahajan et al. 2020). A better understanding of the GR behavior of these populations is critical for sustainable weed control. Previous studies reported that glyphosate resistance levels in junglerice could vary with temperature and that glyphosate's efficacy can be reduced at high temperatures (Nguyen et al. 2016; Shrestha et al. 2018). However, only two papers have been published, and more information is required on the response of GR and glyphosate-susceptible (GS) populations of junglerice when sprayed in different temperature conditions.

For sustainable weed control, it is important to evaluate alternative herbicides when cases of herbicide resistance start to appear for specific weeds (Beckie 2006; Peterson et al. 2018). A wide range of preemergence and postemergence herbicides could reduce the selection pressure caused by the overuse of a single, commonly used herbicide by providing flexibility in herbicide rotation programs for sustainable control. Under fallow situations, the right choices of preemergence herbicide may reduce costs by avoiding the use of multiple knockdown applications when multiple cohorts of junglerice appear (Davidson et al. 2019). Preemergence herbicides, such as pendimethalin and atrazine, are popular for junglerice control in Australia (Davidson et al. 2019). However, information on alternate preemergence herbicides, such as dimethenamid-P and prosulfocarb + *S*-metolachlor for junglerice control, is limited in Australia. Also, there are reports that atrazine does not provide effective control of junglerice in some pockets of Australia (Heap 2008).

Alternative postemergence herbicides must be identified for controlling survivors of GR populations. Information is limited on the response of GR junglerice populations of this region to postemergence herbicides, such as clethodim, glufosinate, haloxyfop, and paraquat. The efficacy of postemergence herbicides varies with the growth stage of weeds, and it is important to find the appropriate weed growth stage to achieve maximum herbicide efficacy (Chauhan and Abugho 2012; Singh and Singh 2004).

Stopping seed production of junglerice in one season could reduce the weed pressure in subsequent seasons (Walsh et al. 2013). Allowing survivors of GR populations to set seeds may further increase the spread of resistance. Two-pass application (sequential application, known as double-knock in Australia) of herbicide tactics are designed to reduce such survivors by controlling survivors of one treatment (first knock) with a follow-up treatment (second knock), so that minimum seed is set on these survivors (Preston 2019). Sequential application of herbicide tactics also reduces the reliance on one herbicide and thereby reduces the risk of herbicide resistance. It is important to find the best double-knock or sequential application of herbicide treatment for GR populations of junglerice so that the survivor seed set is reduced.

Herbicide mixture programs that incorporate different modes of action could prove to be an effective part of a sustainable weed program dealing with resistant weed populations, provided herbicide combinations have a synergistic effect so as to be more effective (Werth et al. 2011). It is important to find the best herbicide mixture for sustainable weed control and prolong the usefulness of herbicides.

A series of pot experiments were conducted to answer the following questions:

(i) How does the response of GR junglerice populations differ with various doses of glyphosate?
(ii) How does temperature influence glyphosate efficacy in GR and GS populations?
(iii) What preemergence herbicides options exist for controlling GR populations?
(iv) What postemergence herbicides options exist for effective GR populations control, and what is the appropriate plant stage when the herbicide should be applied?
(v) Which herbicides should be applied in a sequential application or double knock or in herbicide mixtures to effectively control GR junglerice populations?

Materials and Methods

Experiments were conducted at the research facility of the weed science unit at Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Gatton, Australia. All experiments were repeated twice during the spring–summer seasons of 2019 and 2020, and herbicide treatment in each experiment has been provided in Table 1. Seeds of three populations (B17/7, B17/34, and B17/35) of junglerice were used in different studies. Seeds were collected from eastern Australia in March 2017 and the respective GPS coordinates of B17/7, B17/34, and B17/35 populations, were 27.5000°S, 151.6967°E; 28.5830°S, 150.3689°E; 29.9580°S, 152.1517°E.

Seeds of each population were collected from 40–50 plants spread over an area of >1 ha. Seeds from these populations were multiplied in a common environment in December 2018 at the Gatton research farm. Populations were separated using plastic sheets to avoid any outcrossing. Fresh seeds were collected and stored at room temperature (25 ± 2 C) until used for experimental purposes. In each study (except Experiment 2), pots were kept on benches under natural light and temperature conditions (open area). In each study, plants were kept well-watered and fertilized.

General Protocol

Postemergence herbicides were sprayed at the four-leaf stage of junglerice. Preemergence herbicides were sprayed immediately after sowing. Herbicides were sprayed using a research track sprayer equipped with Teejet XR 110015 flat-fan nozzles (BA Pumps and Sprayer, Queensland, Australia) calibrated to an output spray volume of 108 L ha^{-1}. Plants were allowed to grow for 28 d after treatment (DAT) of herbicide application to determine herbicide efficacy. Plants were assumed dead if they did not have at least one new leaf at 28 DAT. Plant biomass was measured at 28 DAT. Plants were harvested from the base of the plants and dried in an oven at 70 C for all experiments conducted.

Experiment 1. Glyphosate Dose–Response Experiment

In this experiment, seeds of each population were sown in pots (11 cm diam and 10 cm height) filled with potting mix (Centenary Landscape, Qld, Australia). Initially, 12 seeds were sown in each pot at 0.5 cm depth and after establishment, five plants per pot were maintained. The experiment was conducted in a factorial randomized-block design with three replications, where the first factor was population (B17/7, B17/34, and B17/35), and the second factor was glyphosate dose [0 (no herbicide), 285, 570, 1,140, 2,280, and 4,560 g ae ha^{-1}]. The recommended dose of glyphosate was 570 g ae ha^{-1} for junglerice control under fallow conditions in Australia. In the second year there were three replications of each herbicide dose. For mortality and biomass reduction percentage, surviving

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Table 1. Outline of herbicide treatments in different experiments.

Experiment 1. Glyphosate dose–response experiment	Experiment 2. Effects of temperature on glyphosate efficacy	Experiment 3. Performance of preemergence herbicides		Experiment 4. Performance of postemergence herbicides		Experiment 5. Performance of sequential and herbicides mixture	
Glyphosate dose [0 (no herbicide), 285, 570, 1,140, 2,280, and 4,560 g ae ha^{-1}]	Glyphosate dose (0, 285, 570, 1,140, 2,280, and 4,560 g ae ha^{-1}).	Herbicide treatments	Dose (g ai ha^{-1})	Herbicide treatments	Dose (g ai ha^{-1})	Herbicide treatments	Dose (g ae/ai ha^{-1})
		Nontreated control	–			Nontreated control	–
		Atrazine	1,800	Nontreated control	–	Glyphosate	1,140
		Atrazine	2,700	Clethodim	60	Glufosinate	750
		Dimethenamid	540	Clethodim	90	Haloxyfop	78
		Dimethenamid	810	Glufosinate	500	Paraquat	600
		Imazethapyr	70	Glufosinate	750	Glyphosate fb Glufosinate	1,140 fb 750
		Imazethapyr	105	Haloxyfop	52	Glyphosate fb Haloxyfop	1,140 fb 78
		Isoxaflutole	75	Haloxyfop	78	Glyphosate fb Paraquat	1,140 fb 600
		Isoxaflutole	113	Paraquat	400	Glyphosate + Glufosinate	1,140 + 750
		Pendimethalin	1,000	Paraquat	600	Glyphosate +Haloxyfop	1,140 + 78
		Pendimethalin	1,500			Glyphosate + Paraquat	1,140 + 600
		Prosulfocarb + S-metolachlor	1,380			LSD	–
		Prosulfocarb + S-metolachlor	2070				
		S-metolachlor	960				
		S-metolachlor	1,440				

plants and shoot biomass data of each pot at 28 d after herbicide application were converted into survival percentage or percent shoot biomass reduction compared with the nontreated control:

(Surviving plants or shoot biomass of nontreated pot – Survived plants or shoot biomass of treated pot)/(Surviving plants or shoot biomass of nontreated pot × 100).

Experiment 2. Effects of Temperature on Glyphosate Efficacy

This experiment was conducted in a factorial randomized-block design with three replications, where the first factor was the temperature regime, the second factor was population (B17/7, B17/34, and B17/35), and the third factor was glyphosate dose (0, 285, 570, 1,140, 2,280, and 4,560 g ae ha^{-1}).

Two automatic temperature-controlled glasshouse bays were used to keep plants in the experiment at the required temperature regime. One glasshouse bay was maintained at a high-temperature regime, day/night temperature of 35/25 C (12 h/12 h), and the second glasshouse bay was maintained at a low-temperature regime, day/night temperature of 25/15 C (12 h/12 h).

Seeds of each population were sown in pots (11 cm diam and 10 cm height) filled with potting mix (Centenary Landscape, Qld, Australia). Initially, 12 seeds were sown in each pot at 0.5 cm depth and after establishment, five plants per pot were maintained. Plants were grown in the appropriate glasshouse bay and sprayed using a research track sprayer as mentioned in the general protocol. Plants were only removed from the glasshouse bay for glyphosate application and then immediately returned to maintain the desired temperature regime. Mortality percentage and biomass reduction of each pot were assessed by following the similar procedure as described in Experiment 1.

Experiment 3. Performance of Preemergence Herbicides

This experiment was conducted with seven preemergence herbicides (pendimethalin, prosulfocarb, isoxaflutole, imazethapyr, atrazine, dimethenamid-P, and *S*-metolachlor) at two doses of each herbicide. The experiment was conducted separately for each population. Therefore, there were a total of 15 treatments, including a nontreated control for each population (B17/7, B17/34, and B17/35) that were tested in a randomized-block design with three replicates. Pots were filled with potting mix, and 12 junglerice seeds were sown in each pot at a depth of 0.5 cm. Preemergence herbicides were sprayed immediately after sowing using a research track sprayer as mentioned in the general protocol. Pots were kept dry until 24 h after spray, and were watered thereafter with a sprinkler system. At 28 DAT, mortality percentage and biomass reduction of each pot were assessed by following the similar procedure as described in Experiment 1.

Experiment 4. Performance of Postemergence Herbicides

This experiment was conducted with four postemergence herbicides (clethodim, glufosinate, haloxyfop, and paraquat) at two doses of each herbicide. The experiment was conducted separately for three populations (B17/7, B17/34, and B17/35) in a factorial randomized-block design with three replicates. The first factor was the leaf stage (four-leaf and eight-leaf), and the second factor was the nine herbicide treatments, including the nontreated control. Plants were grown and sprayed in a similar way as mentioned in Experiment 1.

Experiment 5. Performance of Sequential Application of Herbicides and Herbicides Mixtures

This experiment was conducted separately for the two GR populations (B17/34 and B17/35). Eleven herbicide treatments were tested in a randomized complete-block design with three replications. Treatments were composed of four herbicides (glyphosate, paraquat, glufosinate, and haloxyfop) at different applications combinations. Herbicide mixture treatments were applied at the four-leaf stage of plants only. In double-knock or sequential-application herbicide treatments, the first knock was applied at the plant four-leaf stage, and the second knock was applied 10 d after the first application knock.

Statistical Analyses

In Experiments 1 and 2, mortality and biomass reduction (as a percentage compared to the nontreated control), data were regressed over herbicide treatments using a four-parameter log-logistic model in SigmaPlot 14.0 Notebook (Systat Software, San Jose, CA).

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

where y = mortality percentage or percentage biomass reduction, y_0 = bottom of curve, a = difference of top and bottom of curve, x_{50} = dose required to kill 50% plants or plant growth, b = slope of curve, and x = herbicide dose. The fitness of the selected model was determined using R^2 values (best fit).

In Experiments 3, 4, and 5, data were subjected to the ANOVA using the GENSTAT 16th edition (VSN International, Hemel Hempstead, UK) to test for treatment–by–experimental run interaction. Where the ANOVA found significant treatment effects, means were separated at $P \leq 0.05$ using Fisher's protected LSD test. Data were also validated to meet the assumptions of normality and variance before analyzing.

Results and Discussion

Experiment 1. Glyphosate Dose–Response Experiment

Junglerice populations B17/7, B17/34, and B17/35 survived at glyphosate rates of 285, 4,560, and 2,280 g ha^{-1}, respectively (Table 2, Figure 1). At glyphosate dose 2,280 g ha^{-1}, the survival rates of B17/34 and B17/35 were 100% and 93%, respectively. LD_{50} values of glyphosate for B17/7, B17/34, and B17/35 populations were found to be 298, 2,260, and 1,715 g ha^{-1}, respectively. Similarly, dose for 50% reduction (GR_{50}) values of glyphosate for B17/7, B17/34, and B17/35 populations were 273, 529, and 597 g ha^{-1}, respectively (Table 2, Figure 1). These results suggest that the B17/7 population is GS, and populations B17/34 and B17/35 are GR. These results confirmed a previous study in which the B17/34 and B17/35 junglerice populations were found to be highly resistant to glyphosate (Mahajan et al. 2019). The GR_{50} value of each population was lower than the LD_{50} value, because biomass data were taken 28 DAT. The surviving plants did not have enough time to grow.

The occurrence of GR populations in these regions warrants the necessity of integrated weed management strategies (chemical, cultural, and mechanical tactics) for the management of junglerice. Strategies like stewardship guidelines must be followed to reduce the selection pressure of resistant populations. It is necessary to control these populations at an early stage before they set seeds in fallows as well as cropland situations. In Australia, glyphosate resistance in junglerice was first reported in 2007 (Storrie et al.

Table 2. Parameter estimates for glyphosate dose–response curves of three populations of junglerice in Experiment 1.[a,b]

Population	*a*	*b*	LD_{50}/GR_{50}	y_0	R^2
			Mortality[c]		
			%		
B17/7	100 (1.1)	−31 (0.9)	298 (0.3)	−7.5 (0.1)	0.99
B17/34	92 (0.1)	−33 (0.1)	2,260 (0.1)	−2.7 (0.1)	0.99
B17/35	100 (0.1)	−6.3 (0.1)	1,715 (1.4)	−0 (0.1)	0.99
			Biomass reduction		
			%		
B17/7	100 (3)	−29 (1)	273 (0.4)	−18 (0)	0.99
B17/34	110 (12)	−1 (0.3)	529 (121)	0.3 (4.2)	0.99
B17/35	105 (5.5)	−1.6 (0.2)	597 (52)	0.4 (3.1)	0.99

[a]There were five plants in each pot before spraying.
[b]Abbreviations: *a*, Difference of top and bottom of curve; *b*, slope of curve; LD_{50}, lethal dose (in g ha^{-1}) for 50% mortality; GR_{50}, dose (in g ha^{-1}) for 50% growth reduction; y_0,: bottom of curve.
[c]Values in parentheses indicate ± standard error. The curve is a four-parametric logistic regression model fitted to data.

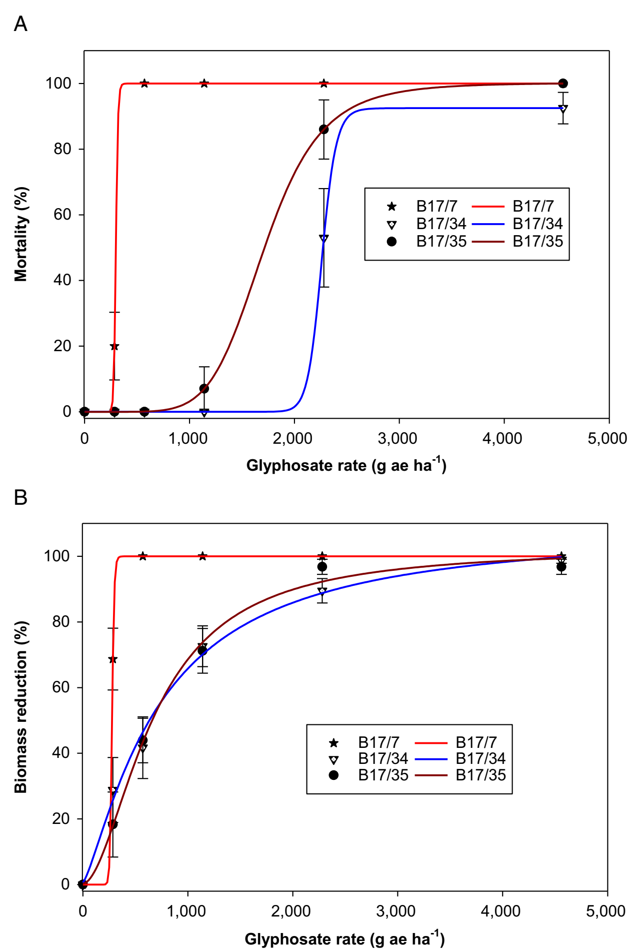

Figure 1. Glyphosate dose–response curve of three populations of junglerice for (A) plant mortality (%), and (B) biomass reduction (%). The curve is a four-parametric logistic regression model fitted to data.

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Table 3. Parameter estimates for glyphosate dose–response curves of three populations of junglerice in Experiment 2.[a]

Population	*a*	*b*	LD_{50}/GR_{50}	y_0	R^2
		Mortality[b] %			
Low-temperature regimes					
B17/7	100 (1.6)	−29 (0.8)	145(0.1)	4.0 (0.1)	0.99
B17/34	106 (13)	−2.1 (0.8)	353 (70)	−3.9 (9.8)	0.97
B17/35	106 (19)	−2 (1.0)	318 (90)	−5.0 (14)	0.95
High-temperature regimes					
B17/7	99 (1.3)	−14 (44)	493 (231)	−0.01 (0.9)	0.99
B17/34	106 (7.0)	−2.1 (0.3)	1,266 (104)	0.99 (2.3)	0.99
B17/35	97 (0.6)	−4 (0.1)	1,323 (9)	0.16 (0.25)	0.99
		Biomass reduction %			
Low-temperature regimes					
B17/7	100 (4)	−27 (0.6)	132 (0.2)	−1.9 (0)	0.99
B17/34	99 (7)	−4 (1.1)	242 (20)	−1.8 (5.9)	0.99
B17/35	100 (0.6)	−2.6 (0.01)	168 (1.3)	0.1 (0.5)	0.99
High-temperature regimes					
B17/7	99 (0.4)	−4 (0.2)	116 (1.5)	−0.1 (0.4)	0.99
B17/34	109 (22)	−1.4 (0.6)	714 (251)	−4.1 (9.4)	0.97
B17/35	95 (17)	−2.1 (0.9)	931 (224)	11 (7)	0.97

[a] LD_{50}, lethal dose (g ha^{-1}) for 50% mortality; GR_{50}, dose (g ha^{-1}) for 50% growth reduction; y_0, bottom of curve; *a*, difference of top and bottom of curve; *b*, slope of curve.
[b] Values in parentheses indicate ± standard error. The curve is a four-parametric logistic regression model fitted to data.

2008). Glyphosate-resistant junglerice populations have also been reported from other parts of the world (Alarcon-Reverte et al. 2013). It was suggested that repeated and intensive use of glyphosate in the no-till production system of eastern Australia has evolved GR populations (Gaines et al. 2012; Storrie et al. 2008).

Experiment 2. Effects of Temperature on Glyphosate Efficacy

At low-temperature regimes, 60% of plants of the B17/7 population survived at a glyphosate rate of 143 g ha^{-1}; however, at the high-temperature regime, 100% of plants of the B17/7 population survived at this rate of glyphosate (Table 3, Figure 2). For the B17/34 population, at low-temperature regimes, plant mortality was 100% at a glyphosate rate of 1,140 g ha^{-1}; however, at the high-temperature regimes, 55% of plants survived glyphosate application at this rate. Similarly, for the B17/35 population, at low-temperature regimes, plant mortality was 100% at a glyphosate rate of 1,140 g ha^{-1}; however, at the high-temperature regimes, 67% of plants survived glyphosate application at this rate.

The LD_{50} values of the B17/7 population at low- and high-temperature regimes were 145 and 493 g ha^{-1}, respectively (Table 3, Figure 2). Similarly, GR_{50} values of the B17/7 population at low- and high-temperature regimes were 131 and 116 g ha^{-1}, respectively (biomass reduction, Table 3, Figure 3). For the B17/34 population, LD_{50} and GR_{50} values of glyphosate increased from 353 to 1,266 g ha^{-1} and 242 to 714 g ha^{-1}, respectively, at high-temperature regimes compared with low-temperature regimes. For the B17/35 populations, LD_{50} and GR_{50} values of glyphosate increased from 318 to 1,323 g ha^{-1} and 167 to 931 g ha^{-1}, respectively, at high-temperature regimes compared with low-temperature regimes. The differing LD_{50} values in Experiments 1 and 2 might be due to different environmental conditions; Experiment 1 was conducted in an open environment, whereas Experiment 2 was conducted in controlled environmental conditions (fixed day/night temperatures; 12 h/12 h).

This study confirmed that glyphosate efficacy for junglerice control increased at low-temperature conditions compared with

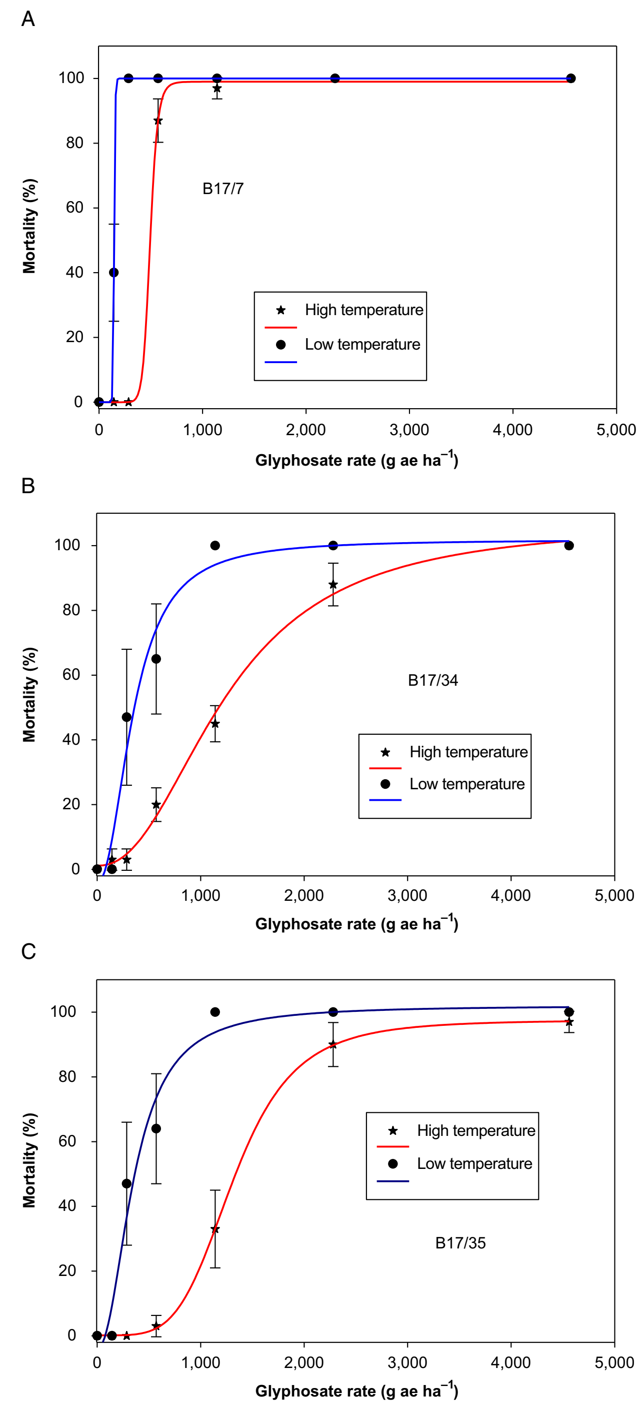

Figure 2. Glyphosate dose–response curve of three populations of junglerice for plant mortality (%) of (A) B17/7, (B) B17/34, and (C) B17/35. The curve is a four-parametric logistic regression model fitted to data.

high-temperature conditions, and the response of junglerice plants to glyphosate varied with populations. It was reported that the poor control of GR weeds at high-temperature conditions might be due to the interaction of temperature and resistance mechanisms (Ganie et al. 2017; Nguyen et al. 2016). A recent study on annual sowthistle (*Sonchus oleraceus* L.) revealed that at low temperatures (19–24 C), GS plants did not survive at a glyphosate rate of 570 g ae ha^{-1}, however, at

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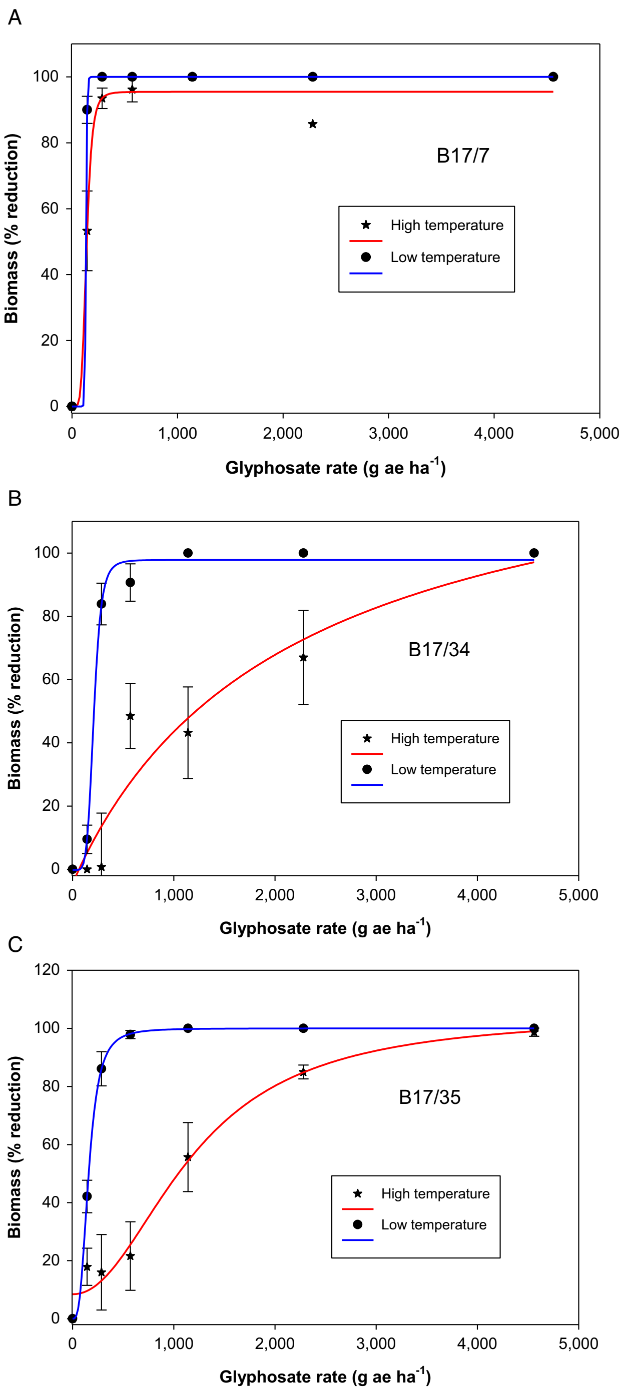

Figure 3. Glyphosate dose–response curve of three populations of junglerice for biomass reduction (%) of (A) B17/7, (B) B17/34, and (C) B17/35. The curve is a four-parametric logistic regression model fitted to data.

the high temperatures (28–30 C), 83% of GS plants survived (Chauhan and Jha 2020). Similarly, 58% of GR plants of annual sowthistle survived with a glyphosate rate of 2,280 g ae ha^{-1} when applied during high-temperature regimes, whereas mortality was 100% when applied during low-temperature regimes (Chauhan and Jha 2020).

Increased efficacy of glyphosate at low-temperature conditions might be due to greater absorption of glyphosate by junglerice plants at low temperatures. Previous studies revealed that the uptake of glyphosate by junglerice plants was 48% to 66% at 20 C but decreased to 21% to 42% at 30 C (Nguyen et al. 2016). In another study, Tanpipat et al. (1997) reported that junglerice seedlings grown at 20 C died earlier with glyphosate application than those grown at 35 C, suggesting faster absorption of glyphosate by plants at low-temperature conditions. These authors suggested that high-temperature conditions increased transpiration in plants that could cause slow absorption and translocation of glyphosate and activity in plants. These studies suggest that the efficacy of glyphosate for junglerice control can be improved if glyphosate was applied during the evening hours when temperature conditions are low for improved absorption (Ou et al. 2018). Application of glyphosate in high-temperature conditions may cause poor control of junglerice, increase infestation of resistant populations, and lead to high weed seed production and subsequent reinfestation. In fallow conditions, it is better to control junglerice in the spring season compared with the summer seasons, as the temperature conditions in spring are lower.

This study suggests that growers need to check temperature conditions before glyphosate application. Increased rates of glyphosate during high-temperature conditions may improve the control of junglerice; however, there is a risk of evolution of highly GR populations with the use of high rates. Over-reliance on glyphosate for the control of junglerice in fallow conditions should be reduced, particularly during hot summers (high-temperature conditions), and farmers could opt for alternative postemergence herbicides.

Table 4. Emergence percentage of junglerice populations (B17/7, B17/34, and B17/35) at 28 d after preemergence herbicides application.[a]

Herbicide treatments	Dose g ai ha^{-1}	Emergence %		
		B17/7	B17/34	B17/35
Nontreated control	–	48.6	36.1	69.2
Atrazine	1,800	41.1	36.1	63.3
Atrazine	2,700	41.1	34.4	56.9
Dimethenamid-P	540	1.7	1.1	0.6
Dimethenamid-P	810	0	0	0
Imazethapyr	70	50.6	26.1	62.2
Imazethapyr	105	44.7	34.7	54.2
Isoxaflutole	75	51.4	37.2	69.4
Isoxaflutole	113	50.3	29.4	68.1
Pendimethalin	1,000	2.8	0	0
Pendimethalin	1,500	0	0	0
Prosulfocarb + *S*-metolachlor	1,380	52.5	29.7	60.3
Prosulfocarb + *S*-metolachlor	2,070	40	17.8	41.9
S-metolachlor	960	16.4	6.9	13.3
S-metolachlor	1,440	6.1	0.6	1.7
LSD ($P \leq 0.05$)	–	13.3	8.7	17.1

[a]Statistical analysis was done separately for each population. In each pot, 15 seeds were sown.

Experiment 3. Performance of Preemergence Herbicides

Dimethenamid-P applied at 810 g ai ha^{-1} provided 100% control of each population (B17/7, B17/34, and B17/35). Pendimethalin and *S*-metolachlor also inhibited germination of each population (Table 4). Each herbicide treatment resulted in lower biomass than the untreated control (Table 5). Atrazine, imazethapyr, and isoxaflutole treatments suppressed junglerice effectively,

Table 5. Aboveground biomass of junglerice populations (B17/7, B17/34, and B17/35) at 28 d after preemergence herbicides application.[a]

Herbicide treatments	Dose g ai ha^{-1}	Aboveground biomass g pot^{-1}		
		Population		
		B17/7	B17/34	B17/35
Nontreated control	–	0.87	0.50	0.64
Atrazine	1,800	0.35	0.25	0.34
Atrazine	2,700	0.32	0.23	0.37
Dimethenamid-P	540	0.001	0.001	0.001
Dimethenamid-P	810	0	0	0
Imazethapyr	70	0.29	0.18	0.39
Imazethapyr	105	0.29	0.10	0.18
Isoxaflutole	75	0.28	0.21	0.23
Isoxaflutole	113	0.24	0.15	0.30
Pendimethalin	1,000	0.08	0	0
Pendimethalin	1,500	0	0	0
Prosulfocarb + *S*-metolachlor	1,380	0.43	0.08	0.23
Prosulfocarb + *S*-metolachlor	2,070	0.20	0.05	0.13
S-metolachlor	960	0.04	0.01	0.014
S-metolachlor	1,440	0.004	0.001	0
LSD ($P \leq 0.05$)	–	0.18	0.08	0.18

[a]Statistical analysis was done separately for each population.

although they did not result in the complete eradication of junglerice populations,.

This study suggests that dimethenamid, pendimethalin, and *S*-metolachlor are the best herbicides for preemergence control of GR populations of junglerice. A previous study suggested that junglerice has multiple cohorts (Wu et al. 2004); therefore, the use of residual herbicides (dimethenamid-P, pendimethalin, and *S*-metolachlor) against junglerice could provide season-long weed control. However, these herbicides must be evaluated under crop situations for their selectivity to different crops. There is also a need to study plant-back issues while using these herbicides. The activities of these three preemergence herbicides will vary under different soil, moisture, and climatic conditions, but again, these are fairly well known for these herbicides. Therefore, validation of these herbicides for the control of GR populations should be investigated under field conditions.

Experiment 4. Performance of Postemergence Herbicides

The tested herbicides (clethodim, glufosinate, and haloxyfop) provided effective control (when assessed in terms of mortality percent and biomass reduction) of the three populations of junglerice when plants were treated at the four-leaf stage (Tables 6 and 7). When plants were treated at the eight-leaf stage, paraquat and glufosinate at 750 g ha^{-1} provided excellent (>97%) control of junglerice populations. Clethodim and haloxyfop provided poor control (~50%) of junglerice when plants were treated at the eight-leaf stage. Glufosinate at 500 g ha^{-1} resulted in lower mortality of B17/7 when applied at the eight-leaf stage compared with the four-leaf stage. The aboveground biomass of clethodim-treated plants was similar to the untreated control when these herbicides were applied at the eight-leaf stage. This study suggests that the efficacy of clethodim was reduced when the plant size was larger. In summary, when the plant size of the junglerice was small (four-leaf stage), clethodim, glufosinate, paraquat, and haloxyfop provided superior weed control. However, if farmers are unable to spray at an early stage of plants, then paraquat could be highly effective for the control of junglerice populations. These studies need field evaluation for further confirmation of results.

Reduced efficacy of herbicides against weeds such as signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster], goosegrass [*Eleusine indica* (L.) Gaertn.], and fall panicum (*Panicum dichotomiflorum* Michx.) was observed when clethodim was applied at a later stage of plants (Burke et al. 2002). Glufosinate provided poor control of goosegrass when applied to taller (15-cm) plants compared with smaller plants (10-cm height) (Eytcheson and Reynolds 2019).

A previous study on tropical weeds revealed that the efficacy of postemergence herbicides against junglerice, Chinese sprangletop [*Leptochloa chinenensis* (L.) Nees], and southern crabgrass [*Digitalia ciliaris* (Retz) Koel] increased when applied at the four-leaf stage (87% to 98%) compared with the eight-leaf stage (53% to 64% control) (Chauhan and Abugho 2012). Likewise, delayed application of trifloxysulfuron against Johnsongrass [*Sorghum halepense* (L.) Pers.] and redroot pigweed (*Amaranthus retroflexus* L.) caused poor efficacy and resulted in the poor control of these weeds, as the plants were larger at the time of spray (Singh and Singh 2004).

Experiment 5. Performance of Sequential and Herbicides Mixture

This study was conducted with GR populations B17/34 and B17/35 (Table 8). As expected, glyphosate at 1,140 g ha^{-1} did not control junglerice effectively and caused only 50% mortality in both populations. Glufosinate at 750 g ha^{-1} effectively controlled both populations, and the mortality in treated plants was >95% (Table 8). Paraquat and haloxyfop caused 100% mortality in both populations. The efficacy of haloxyfop and glufosinate for the mortality of the B17/35 population was reduced when these herbicides were applied in the sequential spray after glyphosate application. Regarding biomass, each herbicide treatment had lower biomass than the untreated control. Among herbicide treatments, plants treated with glyphosate (1,140 g ha^{-1}) had higher biomass than other herbicide treatments.

These results suggest that paraquat, haloxyfop, and glufosinate are quite effective in controlling the GR populations. Therefore, in the fields or under fallow situations, where GR populations have occurred, these herbicides can be successfully used to control these populations. Poor control of GR populations was observed when these herbicides were mixed or applied in sequential spray, suggesting that the sequential application is not useful and that such application strategies may increase the cost of control. Herbicide mixtures or sequential herbicide applications have been found to be very effective for controlling season-long weed control, difficult weeds, and in certain situations, GR weeds (Davidson et al. 2019; Widderick et al. 2013).

In summary, our results demonstrated that junglerice populations B17/34 and B17/35 were highly resistant to glyphosate. GR junglerice populations have been increasing in the no-till production system of eastern Australia in the past few years, especially in fields having a long history of glyphosate use. Therefore, integrated weed management strategies and improved stewardship guidelines are required in those regions to restrict the spread of these populations to other regions of Australia. High-temperature conditions reduced the efficacy of glyphosate. Therefore, for improved control of junglerice populations, it is advisable to spray during the evening hours when temperature conditions are relatively low.

Table 6. Survival percentage of junglerice populations (B17/7, B17/34, and B17/35) at 28 d after postemergence herbicides application (treated at the four- and eight-leaf stage of the plant).[a]

Herbicide treatments	Dose g ai ha–1	Plant survival %					
		B17/7		B17/34		B17/35	
		Four-leaf stage	Eight-leaf stage	Four-leaf stage	Eight-leaf stage	Four-leaf stage	Eight-leaf stage
Nontreated control	–	100	100	96.7	100	100	100
Clethodim	60	0	53.3	0	50	0	50
Clethodim	90	0	53.3	0	50	0	50
Glufosinate	500	3.3	45.6	0	0	0	20
Glufosinate	750	0	13.3	4.2	0	0	0
Haloxyfop	52	0	50	0	50	0	50
Haloxyfop	78	0	50	0	50	0	50
Paraquat	400	0	0	0	3.3	0	3.3
Paraquat	600	0	3.3	0	0	0	0
LSD ($P \leq 0.05$)	–	29.2		27.0		27.8	

[a]LSD values have been provided for the interaction effect of leaf stage and herbicide treatments. Before spray, there were five plants in each pot.

Table 7. Aboveground biomass of junglerice populations (B17/7, B17/34, and B17/35) at 28 d after postemergence herbicides application (treated at the four- and eight-leaf stage of plants).[a]

Herbicide treatments	Dose g ai ha^{-1}	Aboveground biomass g pot^{-1}					
		B17/7		B17/34		B17/35	
		Four-leaf stage	Eight-leaf stage	Four-leaf stage	Eight-leaf stage	Four-leaf stage	Eight-leaf stage
Nontreated control	–	1.0	2.1	1.3	1.4	0.9	1.6
Clethodim	60	0	1.7	0	1.0	0	1.2
Clethodim	90	0	1.3	0	1.3	0	1.3
Glufosinate	500	0.001	0.2	0	0	0	0.001
Glufosinate	750	0	0.001	0.001	0	0	0
Haloxyfop	52	0	1.9	0	0.7	0	1.4
Haloxyfop	78	0	1.6	0	0.8	0	1.0
Paraquat	400	0	0	0	0.001	0	0.001
Paraquat	600	0	0.01	0	0	0	0
LSD ($P \leq 0.05$)	–	0.9		0.5		0.7	

[a]LSD values have been provided for the interaction effect of leaf stage and herbicide treatments. Before spray, there were five plants in each pot.

Table 8. Survival percentage and aboveground biomass of glyphosate-resistant junglerice populations (B17/34 and B17/35) in relation to mixtures and sequential application of herbicides.[a]

Herbicide treatments	Dose g ae/ai ha^{-1}	Plant survival %		Aboveground biomass g pot^{-1}	
		B17/34	B17/35	B17/34	B17/35
Nontreated control	–	100	100	32.9	29.5
Glyphosate	1,140	50.0	50	4.7	5.2
Glufosinate	750	3.3	0	0.2	0
Haloxyfop	78	0	0	0	0
Paraquat	600	0	0	0	0
Glyphosate fb[b] glufosinate	1,140 fb 750	23.3	30	0.3	0.3
Glyphosate fb haloxyfop	1,140 fb 78	33.3	43.3	0.8	1.5
Glyphosate fb paraquat	1,140 fb 600	0	16.7	0	0.1
Glyphosate + glufosinate	1,140 + 750	0	0	0	0
Glyphosate +haloxyfop	1,140 + 78	0	0	0	0
Glyphosate + paraquat	1,140 + 600	0	0	0	0
LSD ($P \leq 0.05$)	–	26.6	26.0	2.3	2.7

[a]Evaluation was done 28 d after treatment. Statistical analysis was done separately for each population.
[b]Abbreviation: fb, followed by.

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Alternative options must be identified to control GR junglerice populations. This research identified preemergence herbicides, such as dimethenamid-P, pendimethalin, and *S*-metolachlor, that can be used successfully for managing GR populations. The use of preemergence herbicides could provide season-long weed control in crops as well as in fallow situations. Postemergence herbicides, such as clethodim, paraquat, haloxyfop, and glufosinate, could be used successfully in managing GR populations if applied at the right time (four-leaf stage). Larger plants of GR junglerice, especially in fallow situations, can be effectively controlled with paraquat. Alone, application of glufosinate (750 g ai ha^{-1}), haloxyfop (78 g ai ha^{-1}), and paraquat (600 g ai ha^{-1}) provided >98% control of GR populations; therefore, there is no advantage in using these herbicides as a mixture with glyphosate. The sequential application of glufosinate and haloxyfop, when followed by glyphosate application, resulted in worse efficacy of glufosinate and haloxyfop than the sole application of glufosinate and haloxyfop, indicating that the stage of plants played a crucial role for high efficacy of glufosinate and haloxyfop. In sequential spray, glufosinate and haloxyfop were applied at a late stage of growth; this study provided evidence that when these herbicides were applied at a late stage or to larger plants, the efficacy was reduced. Therefore, for the control of GR populations of junglerice, glufosinate and haloxyfop must be applied at the four-leaf stage of the plant.

Overall, our findings identified alternative preemergence (dimethenamid-P, pendimethalin, and *S*-metolachlor) and postemergence (glufosinate, haloxyfop, and paraquat) for control of GR-junglerice populations that can be used in herbicide rotation programs for delaying the problem of herbicide resistance. Judicious use of these herbicides in combination with agronomic practices (tillage, sowing time, row spacing) could reduce the spread of these populations by providing effective control.

As mentioned above, junglerice plants are prolific, and seeds can be easily dispersed through winds and other means; therefore, strategies to reduce the survival from preemergence and postemergence herbicides are critical. No preemergence herbicide could provide complete prevention of weeds from emergence when weeds have multiple flushes/cohorts. Moreover, the efficacy of preemergence herbicides is highly influenced by soil texture, moisture, and climatic conditions. Growers wait for the time of peak emergence of multiple cohorts to avoid repeated sprays and to save costs on herbicides and fuels, and by that time, early cohorts become large in size and have passed the optimum spray stage. Therefore, further studies are needed to evaluate herbicide mixtures or sequential applications of preemergence herbicides (dimethenamid-P, or pendimethalin, or *S*-metolachlor) with postemergence herbicides, such as paraquat, for the minimum survivors of GR populations. Late applications of effective postemergence herbicides, such as paraquat, in the sequential or double-knock tactic, and herbicides mixtures with effective preemergence herbicides (dimethenamid-P, or pendimethalin, or *S*-metolachlor), may prove a tool to provide control or prevent seed production of GR junglerice.

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