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Looking beyond Glyphosate for Site-Specific Fallow Weed Control in Australian Grain Production

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Abstract: Summer annual weed species in northern Australian summer fallows are frequently present at low densities and, increasingly, are glyphosate-resistant, creating the need for alternative herbicides for site-specific weed control. Alternative non-selective herbicide treatments are effective on problematic summer fallow weeds; however, many are yet to be evaluated as site-specific (spot spraying) treatments. This study aimed to identify herbicides that could be used in place of glyphosate to control larger/mature *Chloris virgata* and *Sonchus oleraceus* plants. The response of these weed species to 12 herbicide treatments was evaluated in pot experiments conducted over summer/autumn 2022. Despite herbicide treatments not being consistently effective across both species, there were instances where control was achieved by some herbicide treatments. *S. oleraceus* was controlled (i.e., ≤10% plant survival) by glufosinate-ammonium, paraquat and also with protoporphyrinogen-oxidase (PPO)-inhibiting herbicides saflufenacil, tiafenacil and trifludimoxazin. However, these results were not consistent in repeated studies or for *C. virgata*. Glyphosate was the only herbicide that controlled *C. virgata*. A glyphosate replacement as a spot-spraying treatment was not identified, and until further studies are more successful, alternative approaches are needed to preserve the ongoing effectiveness of this herbicide.

Keywords: feathertop Rhodes grass; sowthistle; spot spraying; site-specific weed control

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1. Introduction

In dryland winter cropping regions across Australia, summer fallow weed control is required to optimise the yield potential of subsequent grain crops [1]. The primary agronomic benefits of good weed control during the summer fallow period (i.e., the period between harvest of one winter crop and planting of the next) are reducing losses of soil moisture and available nutrients [2]. Maintaining weed-free summer fallows has been shown to increase the performance of subsequent winter crops [3,4]. The adoption of conservation cropping based on reduced tillage and crop residue retention by Australian grain growers [5] has resulted in a reliance on herbicides for summer fallow weed control [6]. More specifically, glyphosate, a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [7], has been relied on since its introduction in the 1980s for weed control in Australia's summer rainfall dominant northern grains region [8]. In the ensuing decades, the repeated application of glyphosate has provided an intense selection pressure that has resulted in the widespread evolution of resistant genotypes [9]. Very many cases of glyphosate-resistant weeds have now been reported in the northern grains region [10–12]. A recent herbicide resistance survey in the northern region found that, of the sampled populations, 14% of sowthistle (*Sonchus oleraceus*) and 68% of feathertop Rhodes grass (*Chloris virgata*) were resistant to glyphosate [13]. Cases of glyphosate resistance in *C. virgata* [14] have also been reported in the southern grains region. As a result, there is an increasing and ongoing need for alternative options to control problematic summer fallow weeds.

Until recently, summer fallow spraying frequently involved whole-field herbicide applications for the control of often low weed densities (i.e., less than 1.0 plant m^{-2}). The control of low densities of the commonly occurring weeds, *C. virgata* and *S. oleraceus*, is essential as these species have the potential to establish large persistent seedbanks from small populations [15,16]. The ability to site-specifically apply herbicides to weed plant targets significantly reduces herbicide use with substantial cost savings [17]. These savings can allow for the cost-effective application of frequently more expensive alternative herbicide treatments to reduce the reliance on glyphosate [18]. With summer fallow site-specific spraying frequently delayed due to uncertainties around additional cohort emergence and unfavourable spraying conditions, the size of targeted weeds is often larger than advised in label recommendations. Thus, higher application rates of herbicide treatments are required to control large and frequently glyphosate-resistant weeds [19,20].

Site-specific weed control (SSWC) treatments for fallow weed control are applied using a boom-spray fitted with sensor-based detection systems (e.g., WEED-IT® (Rometron, Steenderen, The Netherlands) and WeedSeeker® (Trimble, Sunnyvale, CA, USA)). These sensors detect living (green) plant material via the near-infrared to red reflectance ratio relative to the non-living background (soil and plant residues) [21]. Sensor-based spot-spraying systems have been in use by Australian growers since they were first evaluated in the 1990s [22].

Whilst not conducted in the context of SSWC, but driven by escalating glyphosate resistance, recent Australian research has focused on identifying alternative herbicide treatments for summer weeds [10,11,19]. A pot-based investigation by Desai et al. [11] demonstrated complete weed control of four to five-leaf *C. virgata* using either 600 g a.i. ha^{-1} of paraquat or 750 g a.i. ha^{-1} of glufosinate-ammonium. Similarly, Chauhan et al. [19] controlled (i.e., $\leq$10% plant survival) eight to ten-leaf glyphosate-resistant C. virgata after applying greater than or equal to 750 g a.i. ha^{-1} of glufosinate-ammonium. The effectiveness of both paraquat (600 g a.i. ha^{-1}) and glufosinate-ammonium (750 g a.i. ha^{-1}) does, however, decrease for larger *C. virgata* plants [19]. This indicates that higher site-specific relevant application rates are required for the control of larger *C. virgata* plants. Regardless, in most instances, when glufosinate-ammonium and paraquat were increased to 1140 g a.i. ha^{-1} [11] or 740 g a.i. ha^{-1} [19], respectively, they were found to be comparable to glyphosate in controlling four to ten-leaf *C. virgata*. Control of six-leaf *S. oleraceus* using paraquat (400 g a.i. ha^{-1}) and glufosinate-ammonium (500 g a.i. ha^{-1}) has also been demonstrated for a glyphosate resistant biotype that was not controlled by 741 g a.i. ha^{-1} of glyphosate [10]. In the same study, the protoporphyrinogen oxidase (PPO) inhibitor saflufenacil controlled four-leaf *S. oleraceus* ($\geq$15.9 g a.i. ha^{-1} application rates), but this rate did not control six-leaf plants [10]. Once again, highlighting the need to apply higher site-specific relevant rates. These studies indicate that there are herbicide treatments that can be used in place of glyphosate as site-specific treatments. The objective of this study was to evaluate herbicide treatments for their potential to be used instead of glyphosate to control *Chloris virgata* and *Sonchus oleraceus* populations when applied as site-specific treatments to older and difficult-to-control plants.

2. Materials and Methods

2.1. Weed Seed Source

To screen site-specific relevant herbicide treatments for their efficacy on *C. virgata* and *S. oleraceus*, a series of pot experiments were conducted in the outdoor growth facility on the Charles Sturt University (CSU) campus, Wagga Wagga (35.03° S, 147.20° E). Weed seed samples used in the pot experiments were sourced from field locations in the Wagga Wagga area (Table 1). Prior to use, seed germinability was assessed in a germination test, where 20–40 seeds were placed on Petri dishes filled with 0.6% (*w/v*) amyl media agar (n = 4) that were then stored at a constant 20 °C. Germinated seeds were counted and removed daily until there was no further germination seven days after the last germinated seed was recorded, and then seed germinability (percent germination) was calculated (Table 1).

Table 1. Species name, collection location, date of collection and germinability for seed samples of *C. virgata* and *S. oleraceus* that were subsequently used in pot experiments. Planting, spraying and harvest dates for pot experiments examining the efficacy of alternative herbicide treatments on these two summer annual weed species conducted at Charles Sturt University, Wagga Wagga, NSW in 2022.

Species	Collection Location	Collection Date	Seed Germinability(%)	Experiment	Designation	Planting	Spraying	Harvest
Chloris virgata	Muttuma, NSW	April 2020	76	1 2	*C. virgata*-R1 *C. virgata*-R2	7 January 2 February	3 February 7 March	4 March 4 April
Sonchus oleraceus	Weethalle, NSW	November 2020	67	1 2	*S. oleraceus*-R1 *S. oleraceus*-R2	7 January 2 February	25 February 1 April	25 March 29 April

2.2. Experimental Design and Weed Propagation

Pot experiments were conducted from January to May 2022, with experiments repeated for each species (Table 1). Over this period, average minimum and maximum temperatures were 14 °C and 27 °C, respectively, and average daily solar exposure was 18 MJ m^{-2} (Figure 1). Prior to planting, 20 cm diameter pots (185 mm deep) were filled with garden loam (140 mm) over approximately 30 mm of gravel. A 12-well planting template was used to create evenly spaced 8 mm diameter holes to a consistent depth of 5 mm for weed seed planting. The weed seeds were covered with a 10 mm layer of coarse sand. Sixty pots were planted for each weed species. After planting, pots were placed in a hoop-house (70% UV block shade cloth) where they were regularly watered to maintain the pots near field capacity and were consistently fertilised with Thrive® (25% N, 5% P and 8.8% K *w*/*w*).

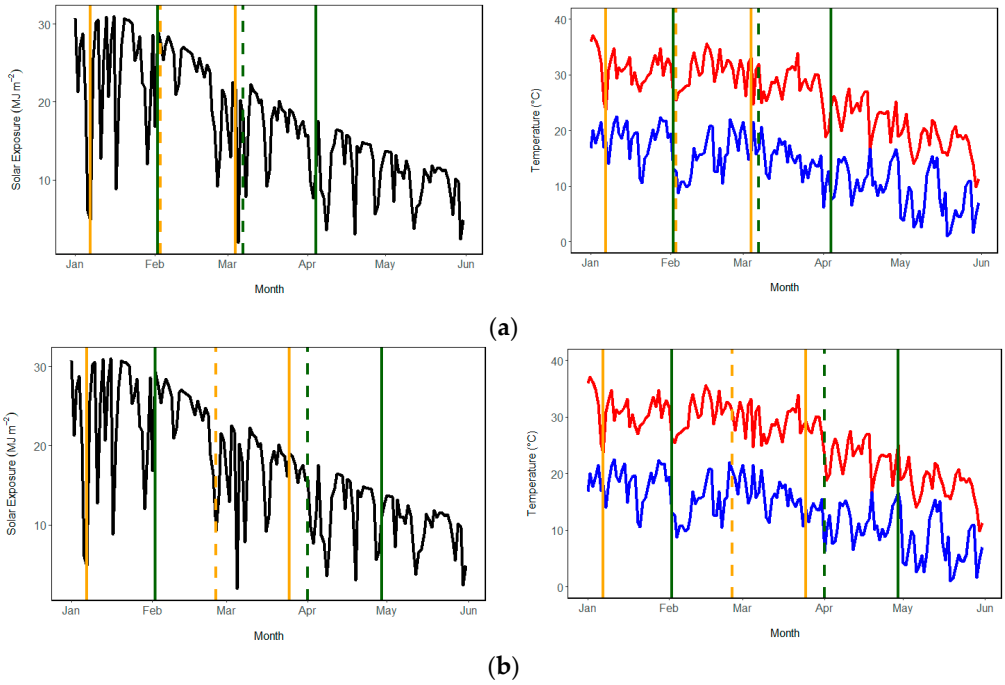

Figure 1. Daily solar radiation and temperature data (from Wagga Wagga Airport) over the duration of the pot experiments for (**a**) *Chloris virgata* and (**b**) *S. oleraceus*. Left-hand side panels represent daily solar exposure (MJ m^{-2}), and right-hand side panels represent daily minimum (blue) and maximum (red) temperatures [23,24]. Solid orange lines denote the start (planting) and finish (harvest) of Experiment 1, and solid green lines denote the start and finish of Experiment 2. Dashed lines denote the day of spray application for Experiment 1 (orange) and Experiment 2 (green).

For each weed species experiment, once weed seedlings were established (1 to 5 true leaves), pots were moved into the grow-out area where seedling density was thinned to 12 plants per pot. The grow-out area was covered by a retractable shade-cloth roof which

remained over the seedlings for 3–6 days; after this period the plants received full sunlight for the remainder of the experiment.

2.3. Application of Herbicide Treatments

C. virgata plants were sprayed once stem elongation had commenced (Z-31) [25], and all *S. oleraceus* plants were sprayed at the commencement of flowering (BBCH-55) [26]. Environmental conditions on the day of spray application varied between experiments (Table 2).

Table 2. Relative humidity at 3 pm (%), solar exposure (MJ m^{-2}) and minimum and maximum temperatures (°C) at Wagga Wagga Airport [23,24] on the day of spray application for each weed species and experiment.

Weed Species and Experiment	Relative Humidity (%)	Solar Exposure (MJ m^{-2})	Temperature (°C)	
			Min	Max
C. virgata-R1	32	28	13	25
C. virgata-R2	37	18	17	32
S. oleraceus-R1	49	9	21	31
S. oleraceus-R2	28	16	6	23

Herbicides and rates selected for testing were largely chosen based on the current label and potential future registrations for use on at least one of the weed species being screened (Table 3). Herbicide treatments were applied in a spray cabinet using a single even-fan nozzle (Teejet® DG95015EVS; Newton, Vic., Australia) at a water delivery rate of 98 L ha^{-1} (210 kPa, 5.9 km h^{-1}). It should be noted that an application error for two treatments in *C. virgata*-R1 resulted in saflufenacil + trifludimoxazin being applied at 18 and 9 g a.i. ha^{-1} (Table 3).

Table 3. Herbicides, application rates and adjuvants used for each treatment applied.

Herbicides	Application Rates (g a.i. ha^{-1})	Adjuvants
Glyphosate	1645	100 mL/100 L (Activator® (Nufarm, Vic., Australia))
Glufosinate-ammonium	750	-
2,4-D	1050	-
Paraquat	1250	-
Amitrole + Ammonium thiocyanate	1250 + 1100	100 mL/100 L (Activator® (Nufarm, Vic., Australia))
Saflufenacil	18 [a]	1 L/100 L (Hasten® (Vicchem, Vic., Australia))
Tiafenacil	18 [a]	1 L/100 L (Banjo® (Nufarm, Vic., Australia))
Saflufenacil + Trifludimoxazin	12 [a] + 6 [a]	1 L/100 L (Hasten® (Vicchem, Vic., Australia))
Paraquat + Amitrole + Ammonium thiocyanate	500 + 1250 + 1100	100 mL/100 L (Activator® (Nufarm, Vic., Australia))
Paraquat + Saflufenacil	500 + 18	1 L/100 L (Hasten® (Vicchem, Vic., Australia))
Paraquat + Tiafenacil	500 + 18	1 L/100 L (Banjo® (Nufarm, Vic., Australia))
Paraquat + Saflufenacil + Trifludimoxazin	500 + 12 [a] + 6 [a]	1 L/100 L (Hasten® (Vicchem, Vic., Australia))

[a] Saflufenical and Trifludimoxazin were applied at 18 and 9 g a.i. ha^{-1} in *C. virgata*-R1.

To avoid exposing plants to rain post-herbicide application, *C. virgata*-R2 and both experiments of *S. oleraceus* were placed undercover in a hoop-house for at least 6 h before being moved back to the grow-out area. With no threat of rain, treated pots in the *C. virgata*-R1 experiment were immediately returned to the grow-out area.

2.4. Data Collection and Analysis

Visual plant injury was assessed weekly up until 28 days after herbicide application. Plant injury was defined as the proportion of desiccated/necrotic tissue across all plants in a pot relative to the untreated control and was recorded as a percentage (i.e., 0% = no visible injury to 100% = no living plants). Plant survival in each pot was assessed 28 days after treatment where plants with chlorotic growing points were identified as

controlled, while growing points that were unaffected or with new growth were identified as survived. Surviving plants were then harvested by cutting off at ground level. The harvested plants were placed into paper bags, one for each pot, which were then dried in a dehydrator for approximately 72 h at 70 °C. Dried samples were weighed and dry biomass per surviving plant was calculated for each pot. These methods were repeated across all weed species and experiments.

In the grow-out area, pots were arranged in a randomised complete block design created in RStudio, Version 4.1.2 [27] using the "design.rcbd" function in the agricolae package, Version 1.3.5 [28]. This design accounted for 13 herbicide treatments (including a control) in four blocks. A blocking design was used to account for any spatial variation such as shading. Statistical analysis was conducted in RStudio [27], using ggplot, Version 3.3.5 [29] and SigmaPlot (SigmaPlot 14.0; Systat Software Inc., San Jose, CA, USA) for data presentation. Paired t-tests were performed on the plant biomass data between experiments for both *C. virgata* and *S. oleraceus* to determine whether the datasets could be combined. To conduct the same analysis on the proportional datasets, separate generalised linear mixed models with logit link-functions considering either visual plant injury or plant survival as a function of the experiment were built [30]. In all generalised linear mixed models, experimental blocks were considered random effects. Applications containing saflufenacil + trifludimoxazin were removed for these analyses in the case of *C. virgata* due to an inconsistency in application rates between experiments (as noted in Table 3). For *C. virgata*, significant differences between experiments were observed in the plant biomass, injury and survival data ($p < 0.05$). For *S. oleraceus*, the plant injury and survival data exhibited significant differences between experiments ($p < 0.05$), whilst plant biomass data were not significantly different between experiments ($p > 0.05$). Since differences between experiments existed in a majority of instances, all experiments for *C. virgata* and *S. oleraceus* were analysed separately.

One-way analyses of variance (ANOVA) with blocking terms were constructed to analyse pot biomass data. Plant survival data underwent an arcsine square-root transformation before being analysed using generalised least square (GLS) regression through the nlme package [31], summarised as:

$$\text{arcsine}(\text{Survival}^{(1/2)}) = \mu + (\text{Herbicide Treatment effect}) + (\text{Block effect}) + \varepsilon \quad (1)$$

Notably, model assumptions were not met for plant survival in *C. virgata*-R1 and so no analysis took place in this instance. Visual plant injury data also underwent an arcsine square-root transformation prior to being analysed using a GLS regression which incorporated weighted variance structures and compound symmetry correlation structures, using the nlme package, Version 3.1.153 [31]. These structures ensured that the model residuals maintained heterogeneity and were independent (which was necessary with the repeated measures design). The model built to analyse the plant injury data can be summarised as:

$$\begin{aligned}\text{arcsine}(\text{Injury}^{(1/2)}) = \mu + (\text{Herbicide Treatment effect}) + (\text{Time effect}) + \\ (\text{Herbicide Treatment} \times \text{Time effect}) + (\text{Block effect}) + \varepsilon\end{aligned} \quad (2)$$

Separate models were built for both weed species and experiments. All GLS regression models underwent an analysis of deviance to consider the significance of each model effect. Post hoc analyses were conducted using either the HSD.test function (for ANOVA) from the agricolae package [28] or the cld function (for GLS) from the multcomp package, Version 1.4.19 [32], both of which adjust for family-wise error using the Tukey method.

3. Results

3.1. Plant Survival

None of the herbicides evaluated, including glyphosate, consistently controlled (i.e., ≤10% plant survival) both summer weed species (*C. virgata* and *S. oleraceus*) across

both experimental runs (Table 4). In general, herbicide treatments failed to control the large established plants (i.e., late tillering for grass weeds and flowering for broadleaf weeds) at growth stages typical of when site-specific spot-spray treatments are applied. Overall, effective control (<10% survival) was only achieved in eight instances, seven of which were in *S. oleraceus*-R1 (glufosinate-ammonium, paraquat, saflufenical, saflufenical + trifludimoxazin and paraquat + PPO inhibitor mixtures), and also glyphosate in *C. virgata*-R2 (Table 4). Differences in herbicide efficacy between experiments for *C. virgata* and *S. oleraceus* were likely due in part to contrasting humidity and cloud cover conditions between respective herbicide application timings that likely lead to variations in herbicide uptake and translocation [33–35]. Clearly, though, the evaluated herbicides and application rates would be not suited for routine use as spot-spraying treatments on older/larger weeds.

Table 4. Plant survival at 28 days after application of post-emergence herbicide treatments applied to *Chloris virgata* at stem elongation and *Sonchus oleraceus* at flowering. Presented means are back transformed. Significant differences ($p < 0.05$) between treatments within the same column are indicated by differing letters. Model root mean square errors are also presented for each experiment.

Treatment	*C. virgata*		*S. oleraceus*	
	R1 [a]	R2	R1	R2
	Plant Survival (%)			
Control	100	100 a	100 a	100 a
Glyphosate	75	3 b	75 ab	15 c
Glufosinate-ammonium	100	25 b	5 def	47 bc
2,4-D	100	100 a	44 bcde	99 ab
Paraquat	100	96 a	2 ef	99 ab
Amitrole + Ammonium thiocyanate	100	100 a	68 abc	93 ab
Saflufenacil	100	100 a	0 f	51 abc
Tiafenacil	100	100 a	57 bcd	100 a
Saflufenacil + Trifludimoxazin	100	100 a	4 def	70 abc
Paraquat + Amitrole + Ammonium thiocyanate	100	99 a	13 cdef	77 abc
Paraquat + Saflufenacil	100	99 a	0 f	52 abc
Paraquat + Tiafenacil	100	92 a	1 ef	93 ab
Paraquat + Saflufenacil + Trifludimoxazin	100	99 a	0 f	89 abc
Root Mean Square Error	-	8	16	19

[a] Model assumptions were not met in *C. virgata*-R1, and so no analysis was conducted.

3.2. *Chloris virgata* Growth

Glyphosate was the most effective herbicide in reducing growth and causing damage to *C. virgata* plants. In both *C. virgata*-R1 and *C. virgata*-R2, glyphosate resulted in the largest biomass reductions, 91% and 98%, and the highest plant injuries of 78% and 99%, respectively (Table 5). Glufosinate-ammonium was the next most effective herbicide; however, its efficacy was inconsistent with greater levels of growth suppression and plant damage during the generally warmer, more humid conditions on the day of spray application in *C. virgata*-R2 (Table 2). In *C. virgata*-R2, the only other herbicides to produce substantial reductions (72 to 81%) in plant biomass were paraquat alone or in combination with PPO inhibitors (i.e., saflufenacil, tiafenacil and saflufenacil + trifludimoxazin) (Table 5). However, as the plant injury ratings indicate, *C. virgata* plants did recover from these treatments (Figure 2).

Table 5. Biomass of surviving *Chloris virgata* plants and visual plant injury at 28 days after the application (DAA) of post-emergence herbicide treatments to plants at stem elongation in two experiments conducted over the summer/autumn. Presented means for visual plant injury are back transformed. Significant differences ($p < 0.05$) between treatments within the same column are indicated by differing letters. Model root mean square errors are also presented for each experiment.

Treatment	Biomass(g Plant^{-1})		Plant Injury at 28 DAA (%)	
	R1	R2	R1	R2
Untreated control	4.25 ab	4.30 ab	0 a	0 a
Glyphosate	0.38 g	0.07 g	78 c	99 f
Glufosinate-ammonium	0.98 fg	0.21 fg	24 b	92 f
2,4-D	4.58 a	4.80 a	6 ab	0 a
Paraquat	3.37 bcd	0.99 efg	14 b	40 d
Amitrole + Ammonium thiocyanate	3.11 cd	2.49 cd	25 b	71 e
Saflufenacil	4.37 ab	3.84 ab	10 b	12 b
Tiafenacil	4.32 ab	4.32 bc	11 b	10 b
Saflufenacil + Trifludimoxazin	1.85 ef	2.55 cd	17 b	16 bc
Paraquat + Amitrole + Ammonium thiocyanate	3.67 abc	1.60 de	9 b	29 bc
Paraquat + Saflufenacil	3.38 bcd	1.21 ef	8 b	37 cd
Paraquat + Tiafenacil	3.70 bcd	1.04 efg	12 b	44 d
Paraquat + Saflufenacil + Trifludimoxazin	2.56 de	0.81 efg	15 b	40 d
Root Mean Square Error	0.36	0.34	10	8

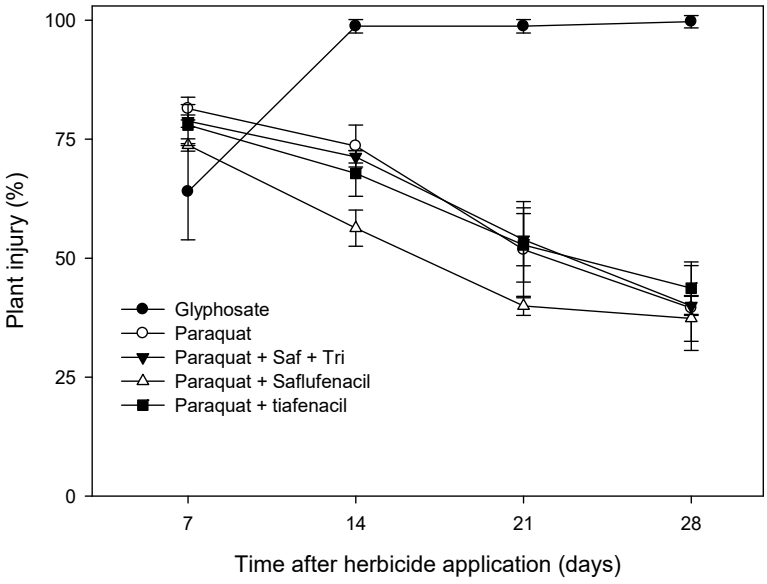

Figure 2. Visual plant injury assessments conducted every 7 days for 28 days after applications of paraquat, glyphosate and mixtures of paraquat and protoporphyrinogen oxidase inhibitors on stem-elongating *Chloris virgata* plants grown in pots in the outdoor growth facility at Charles Sturt University, Wagga Wagga, NSW in 2022. To highlight the differences in speed of activity and to identify recovery from specific herbicide treatment effects, the data presented are from the second (*C. virgata*-R2) of the repeated studies evaluating herbicides for potential use as spot-spray fallow weed control treatments. Presented means are back transformed. Error bars represent the standard error around the mean of three or four replicates. 'Saf + tri' refers to 'Saflufenacil + Trifludimoxazin'.

3.3. Sonchus oleraceus Growth

Herbicide treatment effects on *S. oleraceus* plant growth and resulting plant injury levels were inconsistent between *S. oleraceus*-R1 and *S. oleraceus*-R2, indicating a strong environmental influence on efficacy. Paraquat and PPO inhibitor treatments (except tiafenacil) and their combinations were markedly more damaging to plant growth in the first experimental run than in the second, where the maximum biomass reduction from an alternative herbicide was only 76% (Table 6). In contrast, glyphosate was more effective in

the second experiment than in the first. Saflufenacil and paraquat were more rapid-acting than glyphosate on *S. oleraceus* plants in *S. oleraceus*-R1, reaching maximum visual plant injury at 14 days after application (Figure 3). The addition of paraquat to PPO inhibitors generally led to greater suppression of plant growth, particularly for tiafenacil (from 38% biomass reduction to 82%) and saflufenacil + trifludimoxazin (from 86% biomass reduction to 100%) in *S. oleraceus*-R1.

Table 6. Biomass of surviving *Sonchus oleraceus* plants and visual plant injury at 28 days after application (DAA) of post-emergence herbicide treatments to plants at flowering in two experiments conducted over the summer/autumn. Presented means for visual plant injury are back transformed. Within a column, treatments with the same letters are not significantly different ($p > 0.05$). Model root mean square errors are also presented for each experiment.

Treatment	Biomass(g plant^{-1})		Plant Injury at 28 DAA (%)	
	R1	R2	R1	R2
Control	2.22 a	1.84 a	0 a	0 a
Glyphosate	0.77 ab	0.19 d	68 bcd	97 d
Glufosinate-ammonium	0.82 ab	0.52 cd	82 bcd	54 bc
2,4-D	1.53 ab	1.25 ab	74 bcd	49 bc
Paraquat	0.41 b	1.36 ab	94 cd	13 ab
Amitrole + Ammonium thiocyanate	0.82 ab	1.09 bc	55 bc	56 bc
Saflufenacil	0 b	0.54 cd	100 d	55 bc
Tiafenacil	1.60 ab	1.09 bc	22 ab	16 ab
Saflufenacil + Trifludimoxazin	0.32 b	0.76 bcd	93 cd	34 bc
Paraquat + Amitrole + Ammonium thiocyanate	0.29 b	0.72 bcd	93 cd	72 cd
Paraquat + Saflufenacil	0 b	0.45 cd	100 d	46 bc
Paraquat + Tiafenacil	0.39 b	1.09 bc	95 cd	24 abc
Paraquat + Saflufenacil + Trifludimoxazin	0 b	0.84 bcd	100 d	21 abc
Root Mean Square Error	0.56	0.23	14	12

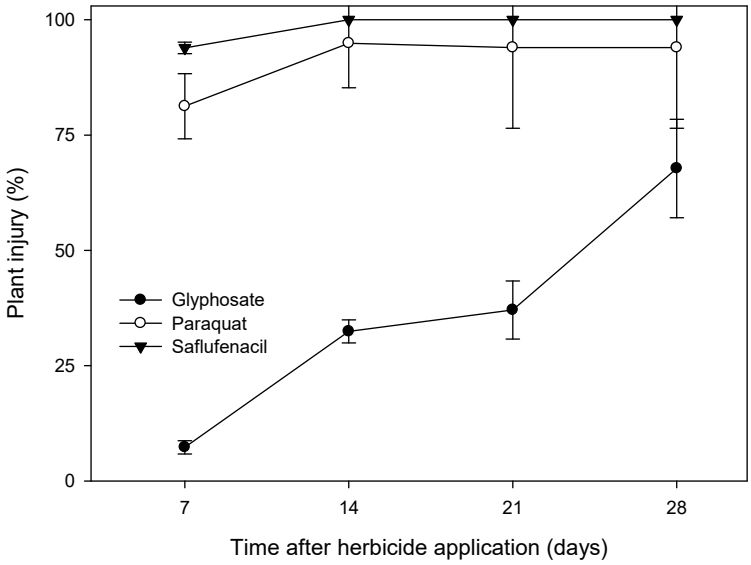

Figure 3. Visual plant injury assessments conducted every 7 days for 28 days after applications of saflufenacil, paraquat and glyphosate on flowering *Sonchus oleraceus* plants grown in pots in the outdoor growth facility at Charles Sturt University, Wagga Wagga, NSW in 2022. To highlight the differences in speed of activity and to identify recovery from specific herbicide treatment effects, the data presented are from the first (*S. oleraceus*-R1) of the repeated studies evaluating herbicides for potential use as spot-spray fallow weed control treatments. Presented means are back transformed. Error bars represent the standard error around the mean of four replicates.

4. Discussion

The reduced herbicide efficacy on generally larger and older weed plants resulted in generally poor and inconsistent weed control by all evaluated treatments; consequently, a suitable alternative to glyphosate was not identified. All herbicides failed to consistently control generally larger and older *C. virgata* and *S. oleraceus* plants. This lack of efficacy was more evident when environmental conditions were less conducive for herbicide uptake and activity. For both paraquat and PPO inhibitors, the lower light conditions experienced in *S. oleraceus*-R1 (Table 2) potentially decreased the immediate destruction of leaf and vascular tissue, improving herbicide mobility [34,35] and, ultimately, efficacy (Table 4). Conversely, the higher light conditions in *S. oleraceus*-R2 likely limited the efficacy of both paraquat and PPO inhibitor herbicides. This is less likely for glufosinate-ammonium, which has been shown to be more effective when exposed to light after spray application [36] and is not limited by its own fast activity [37]. Instead, lower relative humidity on the day of spray application for *S. oleraceus*-R2 potentially limited the translocation and efficacy of glufosinate-ammonium [33] relative to *S. oleraceus*-R1. The substantial size of *C. virgata* [19] at the time of spray application is potentially responsible for failed control of these species (Table 4) by treatments that were effective on plants in *S. oleraceus*-R1. These fast-acting 'contact' herbicides [36,38,39] were potentially 'diluted' amongst the large *C. virgata* plants [40]. As a result, these large plants were able to withstand and outgrow applications of contact herbicides (Figure 2). It should also be noted that there are likely several metabolic [41] and physiochemical [42] differences between the weed species used in the present study that may have also contributed to the inconsistent response of alternate herbicides. More broadly, whilst reasons can be speculated for many of the inconsistencies in herbicide efficacy observed in the present study, these reasons do not excuse inconsistent weed control. The lack of consistency shown by alternate treatments in the present study highlights their inadequacy for completely replacing glyphosate in site-specific summer fallow weed control.

Glyphosate was generally more efficient than any of the individual herbicides assessed in these studies, with the implication that this herbicide will continue to be relied on for SSWC despite a proliferation of glyphosate resistance throughout northern Australian cropping regions [13]. In this study, glyphosate was the only treatment to control *C. virgata* (Table 4) and has previously been at least comparable to a variety of alternate treatments on a susceptible *C. virgata* biotype [11]. This also somewhat contradicts research by Desai et al. [11], who found that paraquat and glufosinate-ammonium were more effective than glyphosate on smaller *C. virgata* plants, highlighting the need for higher application rates of paraquat and glufosinate-ammonium than those used in the present study when controlling larger *C. virgata* plants. Despite not providing control, glyphosate was as or more effective than all alternate herbicide treatments in *S. oleraceus*-R2 (Table 4), similar to the results of Chauhan and Jha [10] on susceptible *S. oleraceus* seedlings. It should, however, be acknowledged that the same level of effectiveness was not consistent between experiments for *C. virgata* and *S. oleraceus*, where seasonal temperature differences (Figure 1) likely influenced the efficacy of glyphosate [10,43]. The results from this present study suggest that glyphosate will, concerningly, remain the primary option for site-specific fallow weed control.

The ever-present threat of glyphosate resistance [13] dictates the need for further research aimed at identifying alternatives. This should include investigating the use of increased application rates, above those used in this investigation (Table 3), to control large summer fallow weeds. Previous research indicates that contact herbicides, such as glufosinate-ammonium and saflufenacil, are rate responsive in controlling *C. virgata* [19] and *S. oleraceus* [10], respectively, suggesting that efficacy would improve at elevated rates. Further research should also include the evaluation of a broader range of alternate non-selective herbicide options, such as glufosinate + PPO inhibitors [44] and paraquat + isoxaflutole [19], as well as sequential applications of alternative herbicides [45], in the context of SSWC.

5. Conclusions

The outcomes of the research presented here highlight the challenge of identifying alternative herbicide options that can be used in place of glyphosate for site-specific fallow weed control. No specific replacement for glyphosate was identified, and indeed, no alternative herbicide treatments for specific summer weed species were found. It is evident that a direct replacement of glyphosate is highly unlikely. Therefore, there is a need for a concerted industry-wide effort towards identifying alternatives, with consideration of physical as well as chemical options for summer fallow weed control. Without effective alternative weed control options, the occurrence and frequency of glyphosate resistance will continue to grow such that this herbicide will soon no longer be an option for weed control in Australian cropping systems.

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