Optimal intervals differ for double knock application of paraquat after glyphosate or haloxyfop for improved control of *Echinochloa colona, Chloris virgata* and *Chloris truncata*

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**Abstract**

The grasses *Echinochloa colona* (L.) Link, *Chloris virgata* Sw. and *C. truncata* R. Br. are major problems in summer follow of the sub-tropical grain region of Australia. Traditionally, these weeds were treated with glyphosate alone, but *E. colona* and *C. truncata* populations have evolved glyphosate resistance, and the weed flora is also being dominated by the glyphosate-tolerant species *C. virgata*. For improved control of these populations, sequential application of glyphosate, or recently haloxyfop, followed by paraquat is being used. The optimal interval between sequential applications of these herbicides needs to be defined for these summer-growing grasses. Pot experiments were conducted using glyphosate or haloxyfop followed by paraquat at intervals from 1 to 21 days. In addition, populations of *E. colona* and *C. truncata* with resistance to glyphosate were compared. The optimal interval between sequential applications differed for the three grasses and the herbicide used for the first application. For the glyphosate-paraquat sequential treatments, the optimal intervals were 1–14 days for GS and GR *E. colona*, 7 days for *C. virgata*, and 7–14 days and 14 days for GS and GR *C. truncata* populations. For the haloxyfop-paraquat treatments, the optimal intervals were 1–21 days for *E. colona*, 1–4 days for *C. virgata* and 1–7 days for *C. truncata*. This treatment achieved 100% control irrespective of resistance status. Thus, sequential application, particularly haloxyfop followed by paraquat is a highly effective tactic for control of these three weeds.

**Keywords:** Glyphosate, Paraquat, Haloxyfop, Glyphosate-resistance, Double knock

1. Introduction

*Echinochloa colona* (L.) Link, *Chloris virgata* Sw. and *C. truncata* R. Br. are common grass weed species infesting summer crops and follow of the sub-tropical cropping region of eastern Australia (Osten et al., 2007; Rew et al., 2005; Walker et al., 2005; Werth et al., 2010). All three species are subject to glyphosate resistance with the world’s first case of glyphosate-resistant (GR) *E. colona* documented in the Australian sub-tropical cropping region. Since then, 101 populations have been confirmed GR in this region (Preston, 2017) and in the tropical Ord River region of Western Australia (Gaines et al., 2012). Four populations of *C. truncata* have been confirmed as GR in the southern part of the sub-tropical cropping region in New South Wales (Preston, 2017) and in 2017 the first two populations of *C. virgata* were confirmed as GR in the northern part of the sub-tropical cropping region (Preston, 2017).

The double knock technique of herbicide application is the sequential application of two knockdown herbicides from different mode-of-action groups (Werth et al., 2010) to the same cohort of weeds in follow. The technique is designed to control any survivors of the first herbicide, to prevent or delay herbicide resistance (Werth et al., 2010). A model produced by Neve et al. (2003a) predicted the double knock of glyphosate followed by paraquat was the most effective strategy for preserving glyphosate susceptibility in no-tillage systems (Neve et al., 2003b). Using the double knock technique, *Lolium rigidum* Gaud. was predicted to develop glyphosate resistance in 17 out of 1000 simulations over 30 years. Paraquat resistance was never predicted (Neve et al., 2003b).

The efficacy of the double knock technique can differ between weed species, herbicides used and the interval between the two herbicides. A study by Borger and Hashem (2007) demonstrated an optimal interval of 2–10 days between applications of glyphosate and...
parasaitic weed control for control of *L. rigidum* in Western Australia. In the subtropical cropping region of eastern Australia, the double knock technique of glyphosate mixed with 2,4-D followed by paraquat, with an optimal interval of 5–10 days, was highly effective for the control of winter-growing GR *Coryza bonariensis* (Worlh et al., 2010; Walker et al., 2012). The double-knock tactic is also widely used in the subtropical cropping region for controlling GR *E. colona* (Widderick et al., 2013).

Since the development of grass weed populations with glyphosate resistance in the subtropical cropping region of Australia, the grain production industry have sought effective alternatives to glyphosate for grass control in fallows (Walker et al., 2004). As an alternative to the traditional glyphosate-paraquat double knock technique, a grass selective herbicide, acetyl coA carboxylase inhibitor (e.g. haloxyfop) is followed by paraquat. This new tactic of haloxyfop-paraquat was shown to be more effective on *C. virgata* than the glyphosate-paraquat tactic (Walker, 2013), and may be an alternative for controlling GR grasses and other problematic summer grass weed species.

However, there is little research into the optimum time between the sequential herbicide applications for summer grass control. This paper reports research into the impact of different intervals between the sequential applications of glyphosate or haloxyfop followed by paraquat on glyphosate-susceptible (GS) and GR populations of *E. colona* and *C. truncata*, and on *C. virgata*, with the objective of defining the optimal interval between sequential applications for these summer grasses.

2. Materials and methods

2.1. Site and seed source

Four pot experiments were conducted in a shade house, designed to mimic a natural environment by not limiting light, under ambient conditions in Toowoomba (27.534°S; 151.993°E). These are referred to as Experiment 1 (which commenced in October 2011), Experiment 2 (February 2012), Experiment 3 (October 2012) and Experiment 4 (February 2013). Pots of 20 cm diameter were filled with a potting mix and topped with 1 cm of seed raising mix (Searles’ Premium Potting Mix and Searles’ Seed Raising Mixture; JC & AT Searle, Kilcoy, Queensland). Seeds were sown on the soil surface using two or three of the following weed populations: GS *E. colona* in Experiments 1, 2 and 4; GR *E. colona* in Experiments 2 and 4; *C. virgata* in Experiments 1 and 3; and GR *C. truncata* in Experiment 3 (Table 1). Glyphosate-susceptible seeds were collected from fields where glyphosate was still effective (*E. colona* from near Dalby, Queensland, *C. truncata* near Tamworth, New South Wales and *C. virgata* near Wellcamp, Queensland), and GR seeds were collected from fields with populations confirmed as having glyphosate resistance (*E. colona* near Bellata, New South Wales, and *C. truncata* near Tamworth, New South Wales) (Preston, 2017). Seedlings were thinned to six per pot, which were watered daily to ensure optimum growth.

2.2. Herbicide application and treatments

The two initial herbicide treatments were glyphosate at 180 g ai ha⁻¹ (Glyphosate CT, 450 g/L, Nufarm Australia Ltd.) or haloxyfop at 20 g ai ha⁻¹ (Verdict™520, Dow AgroSciences Australia Ltd.) with Uptake™ Spraying Oil (Dow AgroSciences Australia Ltd.) at 0.5%. These treatments were applied in a custom-built, single nozzle (TT11015) cabinet sprayer delivering herbicide in 84 L/ha water at 29 kPa, at a speed of 3.3 km/h, at four weeks after the grasses had emerged. At this stage, most plants were at the late-tillering stage, except for *C. truncata* and *E. colona* plants, which had started to flower in Experiments 3 and 4. Subsequently, the weeds either had no sequential herbicide application (interval = Nil) or were sprayed with paraquat at 300 g ai ha⁻¹ (Gramoxone®250, Syngenta Australia Pty. Ltd.) in 110 L/ha water at intervals of 1, 2, 4, 7, 10, 14 or 21 days after the first herbicide treatment.

2.3. Design, measurements and analysis

The treatments of the four experiments (2 or 3 weed populations x 2 initial herbicide treatments x 8 intervals between first and sequential herbicide application) were combined factorially. All experiments followed a split plot design, with pots of each weed population arranged together as a main plot and initial herbicide treatment x interval combinations randomly assigned to pots forming the sub-plots, with three replicate blocks.

At two weeks after the 21-day paraquat application, the green tillers were counted in each pot and the green biomass of survivors in each pot was collected, oven-dried at 80 °C for 48 h and then weighed.

Prior to data analysis, all the treatments with zero biomass or tillers for the majority or all replications were excluded because they violated the assumptions of homogeneity and normality required for the analysis. While not included in the analyses, these treatment averages have been included in data Tables 1 and 2. The remaining data were transformed using the log (x + 0.1) transformation for biomass data and the sqrt (x) transformation for tiller numbers prior to analysis.

Initially, all remaining data was analysed, comparing the impact of the sequential paraquat treatment at different intervals within each of the five weed populations x initial herbicide treatment x experiment, using a common residual variance. This analysis showed a highly significant three-way interaction between weed population x experiment x interval.

The analyses were extended to look at full factorial combinations. Firstly for glyphosate, five groups consisting of two *C. virgata* and three *C. truncata* populations, by all interval treatments were analysed. Secondly for haloxyfop, the same five population groups within experiments by the intervals of 0, 7, 10, 14 and 21 days were analysed. *E. colona* was not included in these analyses due to the large number of zeros. These analyses also showed significant three-way interaction between weed population x experiment x interval for both biomass and tiller number. Subsequently, intervals were statistically compared for each combination of herbicide, population and experiment.

Predictions for the treatments receiving the sequential paraquat application were compared using Fisher’s Protected LSD test. The one-tailed LSD were calculated in order to determine whether the predicted means were significantly greater than zero biomass or tiller number. Linear mixed models were fitted to the data using the Restricted Maximum Likelihood (REML) procedure in GenStat 17th Edition.

3. Results

The embedded factorial analyses showed the three way interactions (weed populations x experiments x interval) were significant for biomass response to glyphosate (P < 0.001) and haloxyfop (P = 0.02) treatments, and for tiller number response to glyphosate (P < 0.001). However, the three way interaction and weed population x interval interaction for tiller number response to haloxyfop treatments were not significant, although the weed population x experiment interaction was significantly different (P = 0.002) and the main effect of interval was significantly different (P = 0.04). Thus, the biomass and tiller responses to interval of the sequential paraquat application are presented for each weed population and experiment (Tables 1 and 2).

3.1. *Echinochloa colona*

The responses of the glyphosate-resistant population of *E. colona* were very similar in both experiments (Tables 1 and 2). Without the paraquat application (interval = Nil), the glyphosate alone application resulted in 2.09 and 5.15 g biomass/pot and 17.1 and 49.3 tillers/pot. In contrast, a minimal amount of biomass and number of tillers survived the haloxyfop alone application in both Experiments 2 and 4.
Weed biomass (g/pot) for *Echinochloa colona*, *Chloris virgata*, and *Chloris truncata* in four experiments (Exp) following initial treatment with either glyphosate or haloxyfop followed by paraquat at different intervals. The ‘Nil’ interval treatment had no sequential application of paraquat. *Echinochloa colona* and *Chloris truncata* populations were either glyphosate-resistant (GR) or glyphosate-susceptible (GS). Assessment was at 14 days after the last second knock.

<table>
<thead>
<tr>
<th>Interval</th>
<th><em>E. colona</em> (GR)</th>
<th><em>E. colona</em> (GS)</th>
<th><em>C. virgata</em></th>
<th><em>C. truncata</em> (GR)</th>
<th><em>C. truncata</em> (GS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial treatment with glyphosate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>2.09</td>
<td>5.15</td>
<td>a</td>
<td>0.40</td>
<td>2.09</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
<td>0.01–0</td>
<td>0</td>
<td>0.1–c</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.01–0</td>
<td>0.1–c</td>
<td>0.13</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0.03–0</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.03</td>
<td>0</td>
<td>0.17</td>
<td>bc</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0.02–0</td>
<td>0.40</td>
<td>bc</td>
</tr>
<tr>
<td>21</td>
<td>0.25</td>
<td>–</td>
<td>0.04–0</td>
<td>0.58</td>
<td>a</td>
</tr>
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</table>

The zero averages were not included in the analysis; – indicates means were mainly based on zeros and were excluded from analysis; # indicates the averages that were not significantly different from zero. Back-transformed means are presented and letters within each experiment and first knock herbicide denote significant differences.

### Table 2

Plant tillers (number/pot) for *Echinochloa colona*, *Chloris virgata*, and *Chloris truncata* in four experiments (Exp) following initial treatment with either glyphosate or haloxyfop followed by paraquat at different intervals. The ‘Nil’ interval treatment had no sequential application of paraquat. *Echinochloa colona* and *Chloris truncata* populations were either glyphosate-resistant (GR) or glyphosate-susceptible (GS). Assessment was at 14 days after the last second knock.

<table>
<thead>
<tr>
<th>Interval</th>
<th><em>E. colona</em> (GR)</th>
<th><em>E. colona</em> (GS)</th>
<th><em>C. virgata</em></th>
<th><em>C. truncata</em> (GR)</th>
<th><em>C. truncata</em> (GS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial treatment with glyphosate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>0.10</td>
<td>0.03</td>
<td>0</td>
<td>0.26</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.1–0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.1–0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0.2–0</td>
<td>1.8</td>
<td>d</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>5.4</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>11.6</td>
</tr>
<tr>
<td>21</td>
<td>2.3</td>
<td>3.3</td>
<td>b</td>
<td>0.6–0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The zero averages were not included in the analysis; – indicates means were mainly based on zeros and were excluded from analysis; # indicates the averages that were not significantly different from zero. Back-transformed means are presented and letters within each experiment and first knock herbicide denote significant differences.

(Table 1 and 2). Irrespective of the first herbicide treatment and interval, all sequential applications of paraquat killed 67–100% of plants, except in the glyphosate-paraquat treatment with a 21 day interval in Experiment 4 (53% killed).

The responses of the GS population of *E. colona* were similar to the GR population for the haloxyfop-paraquat treatments, but not for the glyphosate-paraquat treatments, across the three experiments. In Experiments 1 and 2, the glyphosate-alone treatment resulted in 0.40 and 2.09 g biomass/pot (Table 1) and 28.4 and 36.3 tillers/pot (Table 2), whereas the sequential applications of paraquat with intervals of 1–14 days in both experiments killed 90–100% of plants, as well as for an interval of 21 days in Experiment 1. In contrast, in Experiment 4, the glyphosate-alone treatment resulted in 11.07 g biomass/pot and 86.7 tillers/pot, and the glyphosate-paraquat treatment killed 80–100% of plants with intervals of 1–4 days. With longer intervals, the glyphosate-paraquat treatment was increasingly less effective with increasing interval between the sequential applications. Haloxyfop alone, except in Experiment 2, and all haloxyfop-paraquat treatments, except...
for an interval of 21 days in Experiment 2, resulted in complete death.

### 3.2. Chloris virgata

The responses of *C. virgata* were very similar in both experiments (Tables 1 and 2). The glyphosate-alone treatment resulted in 6.76 and 16.63 g biomass/pot and 61.7 and 51.8 tillers/pot, whereas the haloxyfop-alone treatment produced only 1.63 and 4.03 g biomass/pot and 8.4 and 18.7 tillers/pot in Experiments 1 and 3 respectively. The majority of the glyphosate-paraquat treatments were ineffective with no significant reduction in biomass and number of tillers, except for biomass reduction for the interval of 7 days in both Experiments and for the interval of 14 days in Experiment 3, with 97, 67 and 75% biomass reductions respectively. The haloxyfop-paraquat treatment resulted in complete death for intervals of 1–4 days, followed by a trend of decreasing effectiveness with increasing time interval.

### 3.3. Chloris truncata

Following treatment with glyphosate alone, the glyphosate-resistant population of *C. truncata* produced 10.38 g biomass/pot and 63.6 tillers/pot (Tables 1 and 2). All glyphosate-paraquat treatments resulted in significant 71–89% biomass reduction and 54–72% reduction in number of tillers with no impact of interval. The treatment with haloxyfop alone resulted in 2.07 g biomass/pot and 19.0 tillers/pot. The haloxyfop-paraquat treatments killed 95–100% of plants for intervals of 1–4 days, followed by a trend of gradual loss in effectiveness with increasing time interval.

The responses of the glyphosate-susceptible population of *C. truncata* to the glyphosate-paraquat treatments differed in the two experiments. In Experiment 1, all glyphosate-paraquat treatments were effective in reducing biomass and tiller number compared to glyphosate alone, which produced 2.33 g biomass/pot and 52.9 tillers/pot. In contrast, in Experiment 3, the glyphosate alone treatment resulted in 0.29 g biomass/pot and 9.3 tillers/pot, and only the glyphosate-paraquat treatments with 7 day or 7–10 day intervals significantly reduced biomass and tiller number respectively. In both experiments, the haloxyfop-paraquat treatments at 1 and 2 day intervals resulted in complete death, with a significant reduction in biomass for the majority of the other intervals, except 14 days in Experiment 3 and 21 days in Experiment 1. There was the same trend for tiller numbers in Experiment 1, but tiller numbers in Experiment 3 were not reduced for intervals 7–21 days compared to the haloxyfop alone treatment.

### 4. Discussion

The optimal interval between the sequential applications differed for the three grasses and with the herbicide used for the first application. The optimal intervals for the glyphosate-paraquat sequential treatments were 1–14 days for GS and GR *E. colona*, 7 days for *C. virgata*, and 7–14 days and 14 days for GS and GR *C. truncata* populations respectively. For the haloxyfop-paraquat treatments, the optimal intervals were 1–21 days for *E. colona*, 1–4 days for *C. virgata* and 1–7 days for *C. truncata*, which achieved 100% control irrespective of glyphosate resistance status.

Overall, haloxyfop alone and the haloxyfop-paraquat treatments were more effective than glyphosate alone and the glyphosate-paraquat treatments for the three grasses. Haloxyfop has traditionally been used for selective, in-crop control of grass weeds (Yu et al., 2007). Due to their demonstrated efficacy in crops, the acetyl coA carboxylase inhibitors have been investigated for controlling glyphosate-resistant grasses and *C. virgata* in fallows (Walker, 2013) as an alternative to glyphosate. However, the acetyl coA carboxylase inhibitor herbicides are at high risk for resistance development. The initial gene frequency for resistance toward these herbicides in *L. rigidum* was estimated at 1 plant in 1 million by Diggle and Neve (2001) compared with 1 plant in 100 million for glyphosate. Preston et al. (1999) predicted resistance would evolve after repeated use of acetyl coA carboxylase inhibitor herbicides in 6–8 years compared with > 12 years for glyphosate.

Although haloxyfop has been found to be very effective on the summer grasses in fallows, in Australia it is now recommended only to be used as part of a haloxyfop-paraquat sequential treatment (double knock) to minimise the risk for populations developing resistance to the acetyl coA carboxylase inhibitors (Australian Pesticides and Veterinary Medicines Authority, 2017). At present, numerous populations of *Avena fatua* in Australia have been confirmed with resistance to herbicides that are acetyl coA carboxylase inhibitors. As well, several Australian populations of *Lolium rigidum* have evolved multiple resistance to glyphosate and acetyl coA carboxylase inhibitors (Heap, 2017). Whilst there are no recorded populations of *E. colona*, *C. virgata* and *C. truncata* with multiple resistance to glyphosate and acetyl coA carboxylase inhibitors, there are populations of *E. colona* overseas that have developed multiple resistance to glyphosate and several other herbicide mode of action groups (Heap, 2017).

The efficacy of the glyphosate followed by paraquat double knock was greatly affected by weed species in this study. Glyphosate followed by paraquat was effective for *E. colona* control, ineffective for *C. virgata* and variable for *C. truncata*. These results are consistent with previous studies on *E. colona* that showed the sequential treatment of glyphosate followed by paraquat has been used to successfully control GS and GR *E. colona* (Widderick et al., 2013), but that it was an unreliable tactic for *C. virgata* (Walker, 2013). The reduced efficacy of the glyphosate-paraquat double knock is likely due to the poor control provided by glyphosate as the first applied herbicide (Tables 1 and 2). This poor control placed more reliance on the paraquat to kill the plants. Paraquat is a bipyrpyridyl herbicide that relies on contact and photosynthesis for effective control (Hawkes, 2014). Full plant coverage by herbicide sprays is more difficult to achieve on larger plants. Hence, in our study, the coverage and contact by the paraquat may not have been sufficient to cause plant death, following poor efficacy with the glyphosate. A similar result was obtained by Werth et al. (2010) who found the double knock on *C. bonariensis* was less effective when coverage by paraquat was impeded.

Timeliness of the second herbicide application will be a key factor determining the efficacy of the double knock technique. In general, there was greater flexibility in timing of the second application for controlling *E. colona* than for the two *Chloris* species. For the *Chloris* species, the optimal interval between the first and second herbicide applications was less for haloxyfop treatments than for glyphosate treatments.

As expected, glyphosate alone was ineffective on GR *E. colona* and GR *C. truncata*. In addition, glyphosate alone was also not effective on *C. virgata*, and was variable on GS *E. colona* and GS *C. truncata*. The poor control of *C. virgata* with glyphosate alone is consistent with other field research on this weed in the sub-tropical grain region as reviewed by Walker (2013). This poor control may also explain why the glyphosate-paraquat double knock technique was mostly ineffective on *C. virgata*. Similarly, a potential reason for the ineffective control of GS *E. colona* in Experiment 4, may be due the large plant size at the time of spraying, as Widderick et al. (2013) found previously that glyphosate alone provided good to excellent control on early-tillering glyphosate-susceptible *E. colona*, but variable control on larger plants.

When applied correctly, the double-knock technique provides a useful tactic to delay the evolution of glyphosate resistance (Neve et al., 2003b) and other herbicide resistances (Storrie, 2014). By stopping seed set on surviving weeds, the double knock technique can prevent the proliferation of the weed and its genes; including genes that endow resistance.

### 5. Conclusion

The double knock technique can be an effective tactic for the control
of *C. virgata*, and GR and GS *E. colona* and *C. truncata*. However, efficacy will depend upon the resistance status of the weed and the first herbicide treatment. To achieve maximum efficacy, the second herbicide needs to be applied in a timely manner, especially for the *Chloris* species. In addition, the double knock will be most reliable when applied to smaller weeds. The more effective double knock was with the halox-yfop-parquat application. Field evaluation of the reported double knock techniques should take place to assess efficacy in a field environment. If applying this technique, land managers need to be mindful of the associated herbicide resistance risks and make every effort to stop 100% seed set on survivors.

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**Conflicts of interest**

None.

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**References**


