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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 fallows 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.

1. Introduction

Echinochloa colona (L.) Link, *Chloris virgata* Sw. and *C. truncata* R. Br. are common grass weed species infesting summer crops and fallows across 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 2015 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 fallow. 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

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paraquat + diquat 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 *Conyza bonariensis* (Werth 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; GS *C. truncata* 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 × interval interaction for tiller number response to haloxyfop treatments were not significant, although the weed population × 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