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Evaluation of Florpyrauxifen-benzyl for the control of *Cyperus aromaticus* (Navua sedge)

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Abstract: Background: *Cyperus aromaticus* (Navua sedge) is a creeping perennial sedge common to tropical environments, currently threatening many agroecosystems and ecosystems in Pacific Island countries and northern Queensland in Australia.

Objective: A glasshouse study was conducted to evaluate the efficacy of florpyrauxifen-benzyl on *C. aromaticus* plants with and without established rhizomes.

Methods: The plants with established rhizomes were treated at three application times being mowed, pre-flowering and flowering growth stages and plants without established rhizomes were treated at seedling, pre-flowering and flowering growth stages. At each application time, plants were treated with four rates of florpyrauxifen-benzyl: 0, 15, 30 and 60 g a.i. ha^{-1} and control.

Results: There was no mortality in the plants with established rhizomes. Reduction in the number of tillers was observed at four weeks after treatment (WAT) in plants treated with 30 and 60 g a.i. ha^{-1} of herbicide, however, there was new growth from the rhizomes and the number of tillers increased at 8 WAT. Conversely, florpyrauxifen-benzyl provided above 95% control in plants without established rhizomes.

Conclusions: These results indicate florpyrauxifen-benzyl can help manage a new *C. aromaticus* infestation prior to the establishment of rhizomes. However, it has little to no impact on *C. aromaticus* plants with established rhizomes, and other management options should be employed to control them.

Keywords: chemical control; *Kyllinga polyphylla;* weed management; rhizome; creeping perennial

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1. Introduction

Cyperus aromaticus (Ridley) Mattf. & Kukenth (Navua sedge) is an invasive perennial C_4 sedge of tropical environments which forms dense stands with a creeping rhizome. A native of tropical Africa, it has become problematic in many southwest pacific islands, including Fiji, Tahiti, Samoa, Sri Lanka, Malaysia, the Solomon Islands and tropical north Queensland in Australia (Black, 1984; Parsons, Cuthbertson, 1992; Vitelli et al., 2010). This aggressive weed is a major cause of concern in pastures affecting dairy and livestock industries and also in crops such as rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.) and banana (*Musa acuminata* Colla) (Kraehmer et al., 2016; Black, 1984; Shi et al., 2021). It causes environmental problems and is seen growing along roadsides and creeks, in ditches, drains and lawns. It is generally avoided by grazing cattle and provides minimal nutritional value, allowing it to spread more rapidly in pastures. This is a major weed in Fiji where it has reduced the carrying capacity of pastures by upto 40%, thus reducing milk production (Karan, 1975; Kerr et al., 1995). *C. aromaticus* grows most advantageously in places that do not have a distinct dry season and receive more than 2,500 mm of annual rainfall (Vogler et al., 2015). However, it is noted to be well established and growing in the Atherton Tablelands region in north Queensland which receives around 1,400 mm of annual rainfall. In areas of lower rainfall, it can be found growing in low lying, wetter areas or drains (Parsons, Cuthbertson, 1992).

Cyperus aromaticus is an extremely aggressive persistent weed which competes with pastures and crops for light, water, nutrients and space and has the ability to quickly smother pasture lands (Vitelli et al., 2010). The plant reproduces both by seed and vegetatively (Black, 1984; Vitelli et al., 2010), making it a very successful colonizer. Vegetatively, it spreads through the extension of the rhizome system and when viable rhizome fragments are dispersed during cultivation. It also spreads via seeds with each seed head producing approximately 250 seeds. Consequently, a dense stand of this weed (200 plants m^{-2}) may produce 44,400 to 56,700 viable seeds m^{-2} (Vitelli et al., 2010). It is a fast-growing plant, in which the seedlings develop quickly and form flowers 7-9 weeks post-emergence (A. Chadha, unpublished data). A new shoot or tiller is produced from the rhizome at the same time as flowering, which grows similarly to the seedling and produces a flower head and the rhizome again extends by producing another shoot. This process goes on as the plants flower, thus creating an interconnected colony from the underground stem system.

Mechanical control options like crushing, slashing and rotary hoeing are unfeasible and time consuming for large infestations and not successful in managing *C. aromaticus* (Vitelli et al., 2010). The effect of 17 herbicides belonging to different groups was studied by Vitelli et al. (2010) in a herbicide screening trial but only six of the herbicides namely, halosulfuron, glyphosate, hexazinone, imazapic, imazapyr and MSMA were found effective. However, there are environmental concerns such as persistence in soil, off-site movement and lack of selectivity from the use of those herbicides at the rates required to provide above 90% control of *C. aromaticus*. Currently, only one herbicide, halosulfuron-methyl, an ALS inhibitor is registered for control of *C. aromaticus* in pastures in Australia. Although, halosulfuron-methyl was found most effective at controlling *C. aromaticus* as a selective herbicide, its effect on the rhizome is not known and regular applications of the chemical are required (Vogler et al., 2015). To compound this problem, halosulfuron-methyl has a long grazing withholding period of 10 weeks and another application of the herbicide can only be done after 10 weeks of the initial application. Also, relying on, and using one herbicide continuously will increase the chance of herbicide resistance (Heap, 2014; Powles, Gaines, 2016). Against this backdrop, it is essential to test other herbicides which provide an alternate site of action to evaluate their efficacy for *C. aromaticus*.

Florpyrauxifen-benzyl [benzyl-4-amino-3-chloro-6-(4-chloro2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate] is a synthetic auxin which belongs to the arylpicolinate family of group 4 herbicides (Epp et al., 2016). This herbicide exhibits unique herbicidal features for a synthetic auxin, by providing control of broadleaf, grass and sedge species at low use rates (Miller, Norsworthy, 2018b). Synthetic auxins when used as a herbicide, modify protein synthesis, cell division and plant growth and these effects may continue for a long time in plants (Grossmann, 2010). Florpyrauxifen-benzyl when applied to *Cyperus esculentus* L. (yellow nutsedge), *Cyperus iria* L. (rice flatsedge) and *Cyperus difformis* L. (smallflower umbrella sedge) at the rate of 30 g a.i. ha^{-1}, provided 93%, 94% and 95% control, respectively (Miller, Norsworthy, 2018a).

The purpose of this research was to evaluate the potential of florpyrauxifen-benzyl to control *C. aromaticus*. As *C. aromaticus* is capable of spreading both via seeds and its underground rhizome system, management of this species requires target control of both the above ground and underground systems. Hence, the objective of this study are to:

(i) Test florpyrauxifen-benzyl as a potential chemical option for control of *C. aromaticus* plants with established rhizomes.

(ii) Evaluate the efficacy of florpyrauxifen-benzyl on *C. aromaticus* plants grown from seeds, without established rhizomes.

2. Materials and methods

2.1 Rhizome and seed collection

Rhizomes of *C. aromaticus* were collected from two locations in tropical North Queensland in December 2019 (17°47'27"S, 145°57'18"E and 17°23'47.39"S, 145°38'2"E). The above-ground parts were removed, rhizomes washed to remove the soil and wrapped in paper towel to keep them moist until they were potted in the glasshouse three days later. Mature seeds of *C. aromaticus* were collected in July 2019 from Mt Cutcheon Road (17°42'55''S, 146°2'42''E), South Johnstone, Queensland from a roadside area which had a monoculture of *C. aromaticus*. Seeds were stored in dark glass bottles at 19 °C in the seed ecology laboratory, of Federation University, Mount Helen, Victoria, prior to the start of the experiment.

2.2 Experiment set up

Pot trials using rhizomes (Experiment 1) were conducted between December 2019 and May 2020, while trials using seeds (Experiment 2) were conducted between April 2021 and October 2021. Both experiments were carried out in the glasshouse at Federation University. The glasshouse was maintained at day temperatures between 32 °C and 27 °C and a night temperature between 23 °C and 18 °C, and a relative humidity above 80%. Photoperiod ranged between 9-13 h in the glasshouse. The plants were watered twice daily for ten minutes each time using the automatic watering system in the glasshouse to eliminate any water stress.

2.3 Experiment 1: Plants with established rhizomes

The experimental design was a completely randomised two-factor factorial design with five replications measured over five time periods. The first factor was the application timing, based on three growth stages, mowed (the plants were cut at pot rim level to simulate mowing), pre-flowering and flowering stage. The second factor used was four rates of florpyrauxifen-benzyl application, 0x (control), 0.5x (15 g a.i. ha^{-1}), 1x (30 g a.i. ha^{-1}), and 2x (60 g a.i. ha^{-1}). The rates were based on the study conducted on other *Cyperus* species including Yellow nutsedge, Rice flatsedge and Smallflower umbrella sedge (Miller, Norsworthy, 2018a). Each combination of application timing and herbicide rate was replicated five times. Plastic pots measuring 19 cm in diameter and 18 cm in height were filled with potting mix (Van Schaik's Bio Gro Pty Ltd, Mount Gambier, South Australia) composed of 59% composted bark, 32% nursery blend and 9% Coco peat. Four rhizomes, consisting of one small rhizome (2-3 cm length), two medium rhizomes (3-5 cm length) and one large rhizome (5-8 cm length) were planted into each pot to maintain comparison results of rhizome sizes in each pot.

The number of live reproductive tillers were recorded for each plant every fortnight starting from the day of treatment until 8 weeks after treatment (WAT). Each plant was also given a visual score for herbicide damage every fortnight until 8 WAT using the linear rating scale to assess weed control (Frans, 1986), whereby 0 was no visual damage and 100 was complete death of the plant.

2.4 Experiment 2: Plants without established rhizomes

The experimental design was similar to Experiment 1. Ten seeds of *C. aromaticus* were sown at a depth of 0.5 cm and were thinned down to four plants per pot, once the seedlings were established. Three application times were at seedling (four weeks after sowing; mean of 21 ± 0.5 leaves per pot), pre-flowering stage (eight week after sowing; mean of 24 ± 4 tillers per pot) and flowering growth stages (12 weeks after sowing; mean of 27 ± 3 tillers per pot). For each of the application times, plants were sprayed with four fractional herbicide applications to represent a range of concentrations, 0x (control), 0.5x (15 g a.i. ha^{-1}), 1x (30 g a.i. ha^{-1}), and 2x (60 g a.i. ha^{-1}).

The number of live reproductive tillers were recorded at 8 WAT. Visual scoring of herbicide damage was also done at 8 WAT using the linear rating scale to assess weed control (Frans, 1986), whereby 0 was no visual damage and 100 was complete death of the plant.

2.5 Herbicide spraying

The adjuvant Hasten™ (704 g/L ethyl and methyl esters of canola oil fatty acids with 196 g L^{-1} non-ionic surfactants) was added to all florpyrauxifen-benzyl spray treatments at a 2.5% v/v concentration of the spray volume (Miller, Norsworthy, 2018a). A trolley sprayer was used to deliver 150 L ha^{-1} spray solution at a spray pressure of 200 kPa. Minidrift air-inclusion nozzles with a spray angle of 110° and 50 cm distance between the nozzles were used in the boom, maintaining a height of 50 cm above the foliage. Controls were maintained without any herbicide treatment.

2.6 Statistical analyses

Both experiments were repeated twice with a gap of two weeks to investigate any possible differences between experimental results or whether the data could be pooled. It was found that there was no significant difference in the two trials for all the factors tested in both the experiments, hence the data from both trials were combined.

Experiment 1: Linear mixed models were conducted using SPSS to investigate the main effects of application timing, herbicide rate and observation times and their 2- and 3-way interactions. Separate models were used for the number of tillers and visual score for each application time. Trial, application timing and the herbicide rate were considered fixed effects. As several rhizomes were grown in the same pot, it was used as a random effect. Time with an AR1 covariance structure was also treated as a random effect to account for the same plants being measured on several occasions. The significance of the main effects were analysed using Tukey's post-hoc analysis and significant interactions from the mixed models were analysed by investigating the simple main effects with Bonferroni adjustments. All assumptions were checked by investigating the normality and spread of the residuals. The same analyses of main effects and interactions were followed for both experiments 1 and 2.

Experiment 2: Linear mixed models were conducted using SPSS to investigate the main effects of application timing and herbicide rate and their 2- way interactions. Separate models were used for the number of tillers and visual score. Application timing and the herbicide rate were considered fixed effects and pot and plant within pot were considered to be random effect. After investigating various covariance structures for the random effect of pot, it was redundant and removed from the models.

3. Results

Experiment 1: Plants with established rhizomes

No mortality was observed in *C. aromaticus* plants with established rhizomes treated with florpyrauxifen-benzyl in any of the treatments. A strong three-way interaction was observed between application time of treatment with herbicide rate and observation time for both the number of tillers/plant ($p<0.001$) and visual score/plant ($p<0.001$) (Table 1). The three-way interaction was further explored by examining the two-ways interactions for each application timing. Visually this is depicted in Figures 1 and 2. Overall, compared to the control plants, the highest reduction in the number of tillers and the most visual damage caused to the plant was observed in plants treated with 60 g a.i. ha^{-1} of herbicide (Figures 1 and 2).

At mowed application time, plants treated with 30 and 60 g a.i. ha^{-1} of herbicide had no increase in the number of tillers/plant until 4 WAT (Figure 1a). However, there was an increase in the number of tillers/plant thereafter (Figure 1a). Minimal visual damage was observed in the plants treated with 15 and 30 g a.i. ha^{-1} of herbicide. However, the plants treated with 60 g a.i. ha^{-1} of herbicide had significantly higher visual damage compared to plants treated with 15 and 30 g a.i. ha^{-1} of herbicide at all the observation times ($p<0.05$) (Figure 2a).

At pe-flowering application time, plants treated with 30 and 60 g a.i. ha^{-1} of herbicide had a decline in the number of tillers/plant at 2 and 4 WAT (Figure 1b). However, new tillers started emerging from the rhizome post 4 WAT and an increase in the number of tillers was observed at 6 and 8 WAT (Figure 1b). The growth of these new tillers also reduced the score for visual damage at 6 and 8 WAT

Table 1 - Summary of ANOVA for all main effects and their interaction from the mixed models for the number of tillers/plant and visual score/plant for experiment 1 (plants with established rhizomes)

	Number of tillers/plant				Visual score/plant			
	df1	df2	F	p-value	df1	df2	F	p-value
Application time	2	467.0	6.01	0.003	2	299.6	32.83	<0.001
Herbicide rate	3	467.0	87.31	<0.001	3	299.6	409.57	<0.001
Observation time	4	1872	969.1	0.000	4	322.0	534.28	<0.001
Application time * herbicide rate	6	467.0	1.780	0.101	6	299.6	32.89	<0.001
Application time* observation time	8	1872	24.87	<0.001	8	322.0	40.11	<0.001
Herbicide rate* observation time	12	1872	95.48	<0.001	12	322.0	187.55	<0.001
Application time* herbicide rate* observation time	24	1872	8.797	<0.001	24	322.0	27.70	<0.001

Note: df1, df2, F and p refer to the numerator degrees of freedom, denominator degrees of freedom, test statistic and p-value respectively for each treatment or interaction effect from the linear mixed model

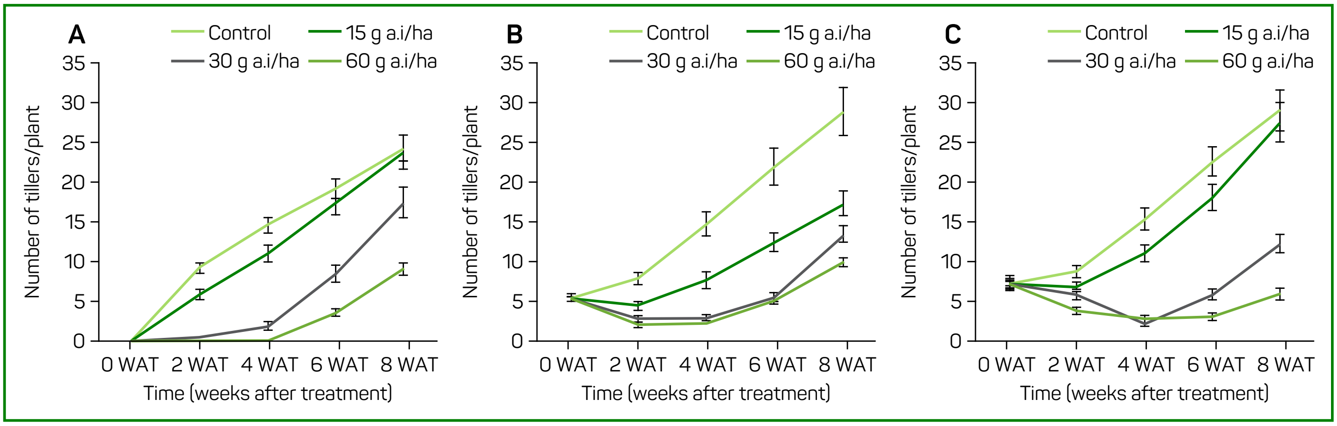

Figure 1 - Means of number of tillers/plant in *Cyperus aromaticus* plants with established rhizomes at 0, 2, 4, 6, and 8 weeks after treatment with various rates of florpyrauxifen-benzyl at three application times; (a) mowed, (b) pre-flowering and (c) flowering. Error bars represent the standard error

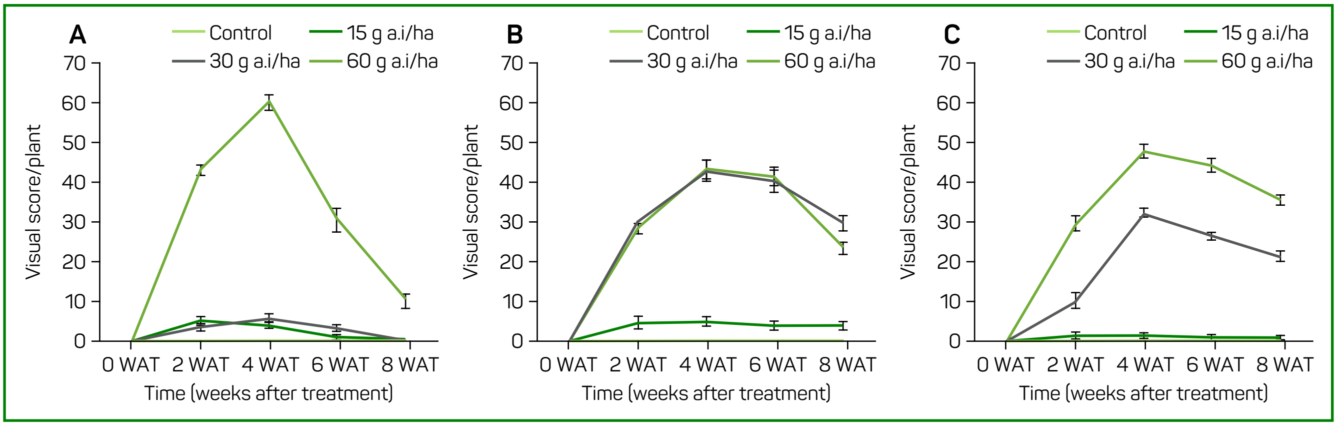

Figure 2 - Means of visual score/plant in *Cyperus aromaticus* plants with established rhizomes at 0, 2, 4, 6, and 8 weeks after treatment with various rates of florpyrauxifen-benzyl at three application times; (a) mowed, (b) pre-flowering and (c) flowering. Error bars represent the standard error

(Figure 2b). The visual damage score was similar in plants treated with 30 and 60 g a.i. ha^{-1} of herbicide which was significantly different to that of control and plants treated with 15 g a.i. ha^{-1} of herbicide (Figure 2b).

Plants treated at the flowering growth stage followed a similar pattern of reduction in tillers after treatment and then the addition of new tillers from the rhizome after 4 WAT. However, a more pronounced effect of herbicide rate was observed in the plants treated at flowering stage. At 8 WAT, plants treated with 60 g a.i. ha^{-1} of herbicide, had the lowest number of tillers/plant (Figure 1c) and highest score for visual damage (Figure 2c). Plants treated with

15 g a.i. ha^{-1} of herbicide and control had similar number of tillers/plant and visual score at 8 WAT which was significantly different to plants treated with 30 and 60 g a.i. ha^{-1} of herbicide (Figure 1c and 2c).

Experiment 2: Plants without established rhizomes

A significant interaction ($p < 0.001$) between the application time of herbicide and the rate of herbicide used in the treatments was observed in both the parameters recorded: number of tillers/plant measured at 8 WAT and visual score at 8 WAT (Table 2). The control had maximum number of tillers in the flowering stage, followed by seedling stage and pre-flowering stage, respectively. However, all three herbicide application rates had significantly few tillers regardless of the application time. All the plants treated with 15, 30 and 60 g a.i. ha^{-1} of florpyrauxifen-benzyl at the seedling stage died and did not have any live tillers. When the plants were treated at the flowering stage, there was no difference in the number of tillers/plant in the plants treated with 30 and 60 g a.i. ha^{-1} of herbicide but they were significantly lower than both the control and plants treated with 15 g a.i. ha^{-1} of herbicide (Table 2). Comparing the effect of application time on the number of tillers/plant when treated with 15 g a.i. ha^{-1} of herbicide, no difference was observed in the plants treated at seedling and pre-flowering growth stages, but they were significantly higher in the plants treated at flowering stage.

In each of the application times, the visual score for herbicide damage was similar in the herbicide rates used of 30 and 60 g a.i. ha^{-1} and were significantly higher than the plants treated with 15 g a.i. ha^{-1} of herbicide and the control (Table 2). The plants treated with 30 and 60 g a.i. ha^{-1} at the seedling and pre-flowering growth stages had significantly higher visual score for herbicide damage compared with plants treated at the flowering stage (Table 2).

4. Discussion

Overall, florpyrauxifen-benzyl at 30 and 60 g a.i. ha^{-1} provided high levels of control (96 to 98%) when applied to seedling or pre-flowering *C. aromaticus* plants which didn't have established rhizomes (Table 2). A similar response of florpyrauxifen-benzyl was observed in other sedges in greenhouse trials (Miller, Norsworthy, 2018a). At 30 g a.i. ha^{-1}, florpyrauxifen-benzyl controlled *C. esculentus*, *C. iria* and *C. difformis* by 93, 94 and 95%, respectively when applied to three to four leaf seedling plants (Miller, Norsworthy, 2018a). Similar to the response of grass weeds to auxin herbicides, when *C. aromaticus* plants were exposed to 30 and 60 g a.i. ha^{-1} of florpyrauxifen-benzyl, swelling was observed near the base, had reduced growth, the leaves turned yellow and then necrotic (Miller, Norsworthy, 2018a).

Florpyrauxifen-benzyl was not found effective at controlling *C. aromaticus* plants with established rhizomes. A reduction in the number of tillers was observed until 4 WAT, however the growth of new tillers from the established rhizomes proved that it is not effective at reducing rhizome viability. Creeping perennial plants like *C. aromaticus* derive an important competitive advantage from their underground storage and proliferation organs, which in this case is the rhizomes (Ringselle et al., 2021). Rhizomes owe their persistence to the large number of dormant buds supported by the stored nutrients (Kolberg et al., 2018, Dalbato et al., 2014). Most of the herbicides, like florpyrauxifen-benzyl only target the above ground biomass and are unable to sufficiently translocate to the dormant buds in the rhizome which can then produce new tillers and continue the life cycle (Gannon et al., 2012). Rhizomes can give rise to new shoots after herbicide application if the herbicide has no residual activity and only systemic herbicides are capable of translocating to the

Table 2 - Impact of herbicide treatment and application timing on number of tillers/plant and visual score of *Cyperus aromaticus* plants without established rhizomes when treated with florpyrauxifen-benzyl

Measure	Herbicide rate	Application time		
		Seedling	Pre-flowering	Flowering
Number of tillers/plant at 8 WAT	Control	8.5 ± 0.33 (a)(3)	6.7 ± 0.41 (a)(2)	12.4 ± 0.59 (a)(1)
	15 g a.i. ha^{-1}	0 ± 0 (b)(2)	0.4 ± 0.08 (b)(2)	4.1 ± 0.31 (b)(1)
	30 g a.i. ha^{-1}	0 ± 0 (b)(1)	0.1 ± 0.04 (b)(1)	0.2 ± 0.12 (c)(1)
	60 g a.i. ha^{-1}	0 ± 0 (b)(1)	0 ± 0 (b)(1)	0.2 ± 0.10 (c)(1)
	p-values	Application time < 0.001; herbicide rate < 0.001; Application time *herbicide rate < 0.001		
Visual score at 8 WAT	Control	0 ± 0 (a)(1)	0 ± 0 (a)(1)	0 ± 0 (a)(1)
	15 g a.i. ha^{-1}	93 ± 2.7 (b)(1)	75 ± 5.1 (b)(2)	29 ± 3.7 (b)(3)
	30 g a.i. ha^{-1}	98 ± 2.3 (c)(1)	96 ± 2.4 (c)(1)	76 ± 2.4 (c)(2)
	60 g a.i. ha^{-1}	100 ± 0 (c)(1)	99 ± 0.5 (c)(1)	85 ± 1.1 (c)(2)
	p-values	Application time < 0.001; herbicide rate < 0.001; Application time *herbicide rate < 0.001		

Note: Values within columns, followed by the same letter (first bracket), are not significantly different at $p \leq 0.05$. Values within rows, followed by the same number (second bracket), are not significantly different at $p \leq 0.05$.

rhizomes (Nelson, Renner, 2002). Using a chemical that translocates completely to the rhizome is potentially an effective herbicide strategy, but even if a few inactive buds escape, the rhizome will maintain the capacity to sprout. A single application is unlikely to kill all the rhizomes in many species, because even a high dose may not allow enough translocation to the rhizomes (Elmore et al., 2019). As opposed to controlling above-ground shoots and seed production, managing the bud bank is far more challenging as it is difficult to achieve translocation of herbicide throughout the extensive underground rhizome system (Zimdahl, 2018).

Although it is understood that florpyrauxifen-benzyl has been developed for use in rice, it was a good candidate to evaluate its activity on *C. aromaticus*, as it is shown to provide high levels of control in other sedge species and grass weeds (Teló et al., 2019). As florpyrauxifen-benzyl is highly effective in controlling seedling and pre-flowering *C. aromaticus* plants without established rhizomes, it can be further tested in field for use in rice fields where *C. aromaticus* is a problem (Kraehmer et al., 2016). Also, florpyrauxifen-benzyl will provide another mode of action to control new infestations of *C. aromaticus* which have grown from seeds and do not have established rhizomes as engaging alternate mode of actions in a weed management program can support weed resistance management (Norsworthy et al., 2012).

Author's contributions

AC, SK, KD: conceptualised and designed the study. AC: data collection. SK, KD, CT, and KD: writing and editing the manuscript.

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