Research reports

Effect of foliar herbicides on the germination and viability of Siam weed (*Chromolaena odorata*) seeds located on plants at the time of application

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Summary

This paper reports a field study undertaken to determine if the foliar application of herbicides fluroxypyr (150 mL 100 L^{-1} a.i.) and metsulfuron-methyl (12 g 100 L^{-1} a.i.) were capable of reducing the germination and viability of *Chromolaena odorata* (L.) R.M.King & H.Rob. (Siam weed) seeds at three different stages of maturity. After foliar application of fluroxypyr germination of mature seeds was reduced by 88% and intermediate and immature seeds were reduced by 100%, compared to the control. Fluroxypyr also reduced the viability of mature, intermediate and immature seeds by 79, 89 and 67% respectively, compared to the control. Metsulfuron-methyl reduced germination of intermediate and immature seeds by 53 and 99% respectively compared to the control. Viability was also reduced by 74 and 96% respectively, compared to the control. Mature seeds were not affected by metsulfuron-methyl as germination and viability increased by 2% and 1% respectively, as compared to the control. These results show that these herbicides are capable of reducing the amount of viable seed entering the seed bank. However depending on the treatment and stage of seed development a percentage of seeds on the plants will remain viable and contribute to the seed bank.

This information is of value to Siam weed eradication teams as plants are most easily located and subsequently treated at the time of flowering. Knowledge of the impact of control methods on seeds at various stages of development will help determine the most suitable chemical control option for a given situation.

Introduction

Siam weed (*Chromolaena odorata* (L.) R.M. King & H.Rob., previously *Eupatorium odoratum* L.) is considered to be one of the world's worst invasive weeds. It is a native of the rainforests of Central and Southern America, where it thrives in early successional stages, but is then suppressed by the growth of other native plants (Binggeli 1997). In non-native locations, Siam weed has the ability to become established in disturbed areas such as clearings, riverbanks, road sides and tracks. Siam weed forms dense thickets which suppress the growth of native vegetation species (Ambika and Jayachandra 1989). Siam weed is also a problem as it out competes pastures and crops, is toxic to stock, causes human health issues and is a fire hazard.

Several small infestations of Siam weed were first identified in Australia in 1994 (Waterhouse 1994). These infestations were located along the Tully River and at Bingil Bay in Far North Queensland (Csurhes and Edwards 1998). By 2006, Siam weed's distribution was concentrated in the Tully/Innisfail and Thuringowa/Townsville regions in north Queensland. Five outer infestations also occurred at Mossman, along the Russell River, and in the Herberton/Mt Garnet area (Galway and Brooks 2006). Predictive modelling suggests that if Siam weed is not controlled it has the potential to spread throughout and devastate large areas of northern Australia and the east coast (Kriticos *et al.* 2005, Galway and Brooks 2006).

Siam weed can spread quickly as individual plants are fast growing (20 mm day^{-1}) and produce large quantities of viable seed; over 87,000 seeds per plant in a single flowering season are reported by Kushwaha *et al.* (1981). In Australia, flowering predominately occurs between May and June, but some plants also flower between September and October.

Due to the restricted nature of infestations in Australia at present, eradication of this invasive weed is considered possible, and several States, Territories and the Commonwealth of Australia are contributing funding towards an eradication program which has been underway since 1994. This funding mainly supports a strategic control team within the Queensland Department of Primary Industries and Fisheries that works in conjunction with other local and state government departments and the community to locate and eradicate Siam weed (Galway and Brooks 2006).

Foliar application of herbicide is the predominant method of control implemented for situations where physical removal is not feasible (Galway and Brooks 2006). Triclopyr/picloram is the registered herbicide for Siam weed (Setter and Campbell 2002) although in some situations such as where Siam weed is closely intertwined with native species, it is not considered suitable for application because of the potential risk to non-target plants (Galway and Brooks 2006). Other herbicides such as metsulfuron-methyl and fluroxypyr which are the focus of the current study have been tested as alternatives and proven effective (J.S. Vitelli unpublished data).

Whilst the ability to kill plants is the primary requirement for herbicides to be used on Siam weed, it would be advantageous if they could also kill seeds located on the plant at the time of spraying. This is particularly pertinent as Siam weed has proven difficult to locate within the dense vegetation of the Wet Tropics bioregion in north Queensland and many infestations are found during the flowering season when the distinct flowers make detection easier.

The literature reports several examples where the application of foliar herbicides has adversely affected the viability of seeds present on plants (Fawcett and Slife 1978, Steadman *et al.* 2006). Previous research has been undertaken on Siam weed to quantify whether triclopyr/picloram could kill seeds at various stages of maturity, but the results indicated that there was no significant effect, irrespective of the stage of maturity (Setter and Campbell 2002). It would be advantageous to discover if alternatives such as fluroxypyr or metsulfuron-methyl had a significant impact on the germination and viability of Siam weed seeds.

Therefore, the objective of this study was to determine the effects of fluroxypyr and metsulfuron-methyl on the germination and viability of Siam weed seeds of different maturity groups located on plants at the time of application.

Materials and methods

Experimental design

A 3 × 3 × 2 factorial experiment was established in October 2006 north-west of Wangan (S 17°33.204', E 145°58.250') in north Queensland. A split-split-plot design was incorporated, with herbicide treatment (untreated control, or applications of either metsulfuron-methyl or fluroxypyr) allocated to main plots, inflorescence maturity (immature, intermediate and mature) allocated to sub plots and timing of measurements (before and after spraying) allocated to sub-sub plots.

The experimental site was 500 × 500 m in size and located in an area considered representative of Siam weed infestations within the Wet Tropics bioregion of north Queensland. A total of 17 free-standing mature plants were selected, tagged and randomly assigned to be sprayed with either metsulfuron-methyl (six plants) or fluroxypyr (six plants), or remain as an untreated control (five plants). This resulted in six replications for each of the herbicide treatments and five for the control.

On each plant immature, intermediate and mature inflorescences were selected and tagged using the classifications previously adopted by Setter and Campbell (2002) which are summarized as follows: Immature inflorescences were characterized by buds that were mostly closed, sepals were green, the visible outer part of the petals white, and the achenes were very light in colour. Intermediate inflorescences had flower buds that were mostly open, sepals were green, the petals were lilac, and achenes were light-mid brown. Mature inflorescences had petals and sepals that were light-mid brown, most petals had fallen off, and achenes were very dark brown or black. For each maturity stage sufficient inflorescences were tagged to allow for the removal of two samples of approximately 500 seeds from each individual plant immediately before and three weeks after spraying which was undertaken on the 27th of October 2006. The two samples were taken before and after treatment to show that changes in viability and germination were caused by the herbicide and not time.

Following collection, two sub-samples of 50 seeds were randomly selected from individual seed samples and placed on moist Whatman™ No. 4 filter papers in 90 mm Petri dishes. These samples were then placed in an incubator set on 12 hour day and night cycles. The day and night temperatures were approximately 32°C and 23°C respectively. Lights were also set for the 12 hour day cycle. All Petri dishes were moistened daily with distilled water.

Germinated seeds were counted, removed and recorded every second day over a period of 28 days, with seeds classified as germinated if they had an emergent radicle of approximately 2 mm or longer. Seeds that failed to germinate were tested for viability using the procedure previously described by Setter and Campbell (2002). Seed viability was measured by the total of the germinated seeds in addition to the number of ungerminated seeds that were considered viable. Both germination and viability were expressed as a percentage of the total number of seeds tested.

Herbicide application

A diaphragm pump was used to spray the foliage of plants with either fluroxypyr (Starane *200) or metsulfuron-methyl (Brush-Off®) until spray dripped off the foliage. The plants were sprayed with a dosage of 9000 mL a.i. ha^{-1}, and a spraying volume of 6000 L ha^{-1} for fluroxypyr and with a dosage of 533 g a.i. ha^{-1}, and a spraying volume of 4444 L ha^{-1} for metsulfuron-methyl. Parafinic oil (Uptake* Spraying Oil) was added to both herbicide treatments at a rate of 330 mL 100 L^{-1} a.i. to increase adhesion of the herbicide. This dosage, application rate and method was recommended in previous research (J.S. Vitelli unpublished data) and was the rate used by eradication teams. Control plants were left untreated.

Statistical analysis

An analysis of variance test was applied to both the germination and viability data. Treatment means were separated by Fisher's Protected LSD test at $P < 0.05$.

Results

Seed germination

For seed germination, a significant ($P < 0.05$) herbicide treatment × timing × maturity stage interaction occurred (Table 1).

Prior to the application of treatments both immature and intermediate seed lots exhibited minimal germination (less than 2%). In contrast germination of mature seeds ranged between 31–53% across all treatments. By the post-treatment recording, germination of untreated immature and intermediate seeds had increased significantly, averaging 37% (Table 1). Germination of mature seeds, on the other hand, had not changed significantly from pre-treatment levels.

Irrespective of the herbicide applied, germination of both immature and intermediate seeds (average of 1.4%) was significantly lower than the untreated controls. In contrast, only fluroxypyr had a detrimental effect on the germination (average of 5%) of mature seeds (Table 1).

Seed viability

For seed viability, a highly significant interaction ($P < 0.01$) occurred among herbicide treatment, timing of measurement and maturity stage (Table 2). Before treatment application, immature and intermediate seeds exhibited minimal viability (<1%) in comparison to mature seed lots, which ranged between 41 to 64%. Three weeks after treatment, the viability of immature and intermediate seeds in untreated controls had increased markedly and was not significantly different to that of the mature seeds, averaging 50% across the three maturity stages (Table 2).

Table 1. Germination (%) of Siam weed seed at three stages of maturity collected immediately before and three weeks after the foliar application of metsulfuron-methyl and fluroxypyr. Values followed with the same letter are not significantly different (P <0.05).

Herbicide treatment	Timing	Maturity stage		
		Immature	Intermediate	Mature
Control	Before	0.0f	0.2f	50.0ab
Metsulfuron-methyl	Before	0.0f	0.0f	31.0e
Fluroxypyr	Before	0.0f	1.6f	53.0a
Control	After	40.6cd	32.5de	43.2bc
Metsulfuron-methyl	After	0.2f	5.5f	44.2abc
Fluroxypyr	After	0.0f	0.0f	5.0f

Table 2. Viability (%) of Siam weed seed at three stages of maturity collected immediately before and three weeks after the foliar application of metsulfuron-methyl and fluroxypyr. Values followed with the same letter are not significantly different (P <0.05).

Herbicide treatment	Timing	Maturity stage		
		Immature	Intermediate	Mature
Control	Before	0.0e	0.2e	62.0a
Metsulfuron-methyl	Before	0.0e	0.0e	41.4b
Fluroxypyr	Before	0.0e	0.16e	63.8a
Control	After	51.2b	43.6b	55.8ab
Metsulfuron-methyl	After	2.3de	11.3cd	56.6ab
Fluroxypyr	After	17.5c	5.0de	11.5cd

The application of both metsulfuron-methyl and fluroxypyr significantly reduced the amount of immature and intermediate viable seeds present three weeks after treatment (<18% across both herbicide treatments) when compared with the untreated controls (Table 2). In contrast, only fluroxypyr significantly reduced the viability (average of 11.5%) of mature seeds.

Discussion

In general, the foliar application of both metsulfuron-methyl and fluroxypyr reduced the germination and viability of immature and intermediate Siam weed seeds located on plants at the time of application. Moreover, fluroxypyr had the added advantage of destroying the mature seeds.

Previous studies have reported that foliar application of herbicides (paraquat, 2,4-D and glyphosate) early in the reproductive stage of Siam weed plants reduce seed production and germination (Mummigatti *et al.* 1995). Similar results were found during a study on the impacts of herbicide treatment on the viability of rigid ryegrass (*Lolium rigidum* L.) seed (Steadman *et al.* 2006).

Mummigatti *et al.* (1995) suggested that Siam weed seeds were most vulnerable to herbicide treatment during the early stages of development. Setter and Campbell (2002) did not record any significant effects on viability following the application of the herbicide triclopyr/picloram (Grazon DS®) to Siam weed seeds ranging in maturity from immature to mature. There was, however, some discussion regarding the immature seeds tested which exhibited minimal germination and viability at both sampling times (pre- and post-treatment), irrespective of whether they were sprayed or untreated. The short duration between sampling times may not have provided sufficient time to determine whether the treated seeds had been deleteriously affected by chemicals or whether they would eventually develop into viable seeds (Setter and Campbell 2002). Although a similar three week time period between sampling events was used in the present study, immature and intermediate seeds did significantly increase in viability when left untreated, allowing the low viability in the herbicide treatments to be attributed to the direct effects of spraying.

Besides the direct effects of spraying on seed viability, Steadman *et al.* (2006) also found that herbicides could affect the development of emerged seedlings. In their study on rigid ryegrass seed, a high percentage of emerging radicles were not as healthy as those in untreated controls. They assumed that many of these specimens would fail to develop. Similarly in the current study, many Siam weed seedlings within herbicide treatments appeared to be smaller and have brown unhealthy roots soon after germinating. Further research for an extended period of time is warranted to quantify whether such seedlings would eventually die.

Overall, the information gained from this study has the potential to benefit the Siam weed eradication program in Australia. It can be concluded that there are herbicides that could be used to treat plants that have flowered and set seeds and subsequently reduce the number of viable seeds entering the seed bank. Nevertheless there may still be a percentage of seeds on plants that will remain viable and lead to replenishment of the seed bank. Where possible, the best scenario is still to control all Siam weed plants before they have the opportunity to reach reproductive maturity.

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