Evaluation of Di-Bak® Herbicide Capsule System for Control of Chinee Apple (*Ziziphus mauritiana*) in North Queensland

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Summary Chinee apple (*Ziziphus mauritiana* Lam.) is a significant weed in the drier tropics of northern Queensland, Western Australia, and the Northern Territory. Throughout these regions its densely formed thickets influence the structure, function, and composition of rangeland ecosystems thereby outcompeting the native pasture species. The subsequent loss of pasture cover affects the quality of services (i.e., agronomic productivity, livestock carrying capacity, mustering, ecosystem services) obtainable from this diverse, natural resource. In Australia, the management of *Z. mauritiana* is limited to the application of synthetic herbicides and mechanical clearing operations. Whilst their efficacy is undisputed, there are concerns regarding the suitability of synthetic herbicides in ecologically sensitive or low-value habitats. This greater appreciation for environmental stewardship has promoted significant developments in the field of woody weed management.

This study investigates the effectiveness of a novel stem-implantation system for controlling woody weed species in grassland and rangeland environments. A pair of replicated trials were established among a naturally occurring population of *Z. mauritiana* at Alligator Creek, North Queensland (rural locality south of Townsville). The trials differed in dosage level by adjusting the application spacing (10 cm and 15 cm) of four encapsulated synthetic herbicides. An untreated control and benchmark treatment (drill-and-fill application of Tordon® RegrowthMaster) were also assessed for performance comparison. A significant effect ($p < 0.05$) on plant vigor was discerned for both dosage levels within eight months of trial establishment. The highest incidence of mortality was observed among the individuals treated with aminopyralid + metsulfuron-methyl (37.5 mg/capsule and 30 mg/capsule), metsulfuron-methyl (330 mg/capsule) and picloram (1000 mg/capsule), achieving a similar response to the drill-and-fill application of Tordon® RegrowthMaster (200 g/L triclopyr, 100 g/L picloram and 25 g/L aminopyralid). We predict total mortality (~100%) with these preeminent treatments by the next assessment period (15 months), as well as equally effective control under a reduced dosage (by increasing spacing from 10 cm to 15 cm).

Keywords Chinee Apple, *Ziziphus mauritiana*, Woody Weed, Integrated Weed Management, Chemical Control, Stem Implantation

INTRODUCTION

Ziziphus mauritiana Lam. (Chinee Apple, Indian Jujube, Ber) is a deciduous thorny tree or shrub native to south Asia and eastern Africa (Grice 1996, Grice *et al*. 2000, Bebawi *et al*. 2016, Liang *et al.* 2019). In Australia, it was introduced to early mining settlements (e.g., Charters Towers, Ravenswood, Mingela, Hughenden) during the late nineteenth century (1863) for its ornamental and horticultural value (Grice 1996, Grice *et al.* 2000, Bebawi *et al.* 2016). Its current distribution is densest in the northern parts of Queensland (Townsville-Charters Tower region), Western Australia (Northern Kimberley, Pilbara, Dampierland) and the Northern Territory (near Katherine) (Grice 1996, Bebawi *et al.* 2016). Throughout these regions, its densely formed thickets can alter the structure and ecological integrity of rangeland ecosystems by outcompeting native pasture species (Grice 2004, Bebawi *et al*. 2016). This affects the quality of services (agronomic productivity, livestock carrying capacity, water accessibility, mustering) obtainable from this diverse, natural resource (Bebawi *et al.* 2016, Dhileepan 2017, Ani *et al*. 2018).

The management of *Z. mauritiana* is limited to the application of synthetic compounds or mechanical clearing operations (Bebawi *et al.* 2016, Dhileepan 2017). The manual removal of higher density (>150 plants/ha) or isolated infestations can be achieved through stick-raking, blade ploughing or bulldozing of individual trees (terrain and soil type permitting). However, these attempts are often deemed inefficient and cost prohibitive. The basal or cut-stump application of synthetic auxin herbicides (triclopyr, fluroxypyr or picloram carried in diesel) is the most effective approach for the aggressive eradication of lower density (<50 plants/ha) populations. Whilst their efficacy is undisputed, there are concerns regarding the suitability of synthetic herbicides applied with diesel in ecologically sensitive (i.e., riparian zones, woodlands) or low-value habitats (O'Brien *et al.* 2022). Their excessive or imprecise application may result in non-target damage through herbicidal and diesel drift, runoff or leaching into adjacent habitats (O'Brien *et al.* 2022). This greater appreciation for environmental stewardship has promoted significant developments in the field of woody weed management by reducing dosage levels or improving application methods (O'Brien *et al.* 2022).

This study investigates the effectiveness of BioHerbicides Australia's (BHA Pty Ltd) proprietary stem-implantation system and Di-Bak® range of synthetic herbicides for controlling *Z. mauritiana* in rangeland environments. This novel technology was initially developed for the encapsulated delivery of an endophytic bioherbicide in parkinsonia (*Parkinsonia aculeata* L.) (Galea 2021a, b). It has since been expanded to the application of other endophytic organisms, as well as synthetic compounds (herbicides, fungicides, and insecticides) available in dry formulations (Goulter *et al.* 2018, Galea 2021a, Limbongan *et al.* 2021). Unlike its industry counterparts, this device provides a targeted, readily calibrated herbicide application thereby minimising environmental and operator exposure (Goulter *et al.* 2018, Galea 2021a, Limbongan *et al.* 2021, O'Brien *et al.* 2022).

MATERIALS AND METHODS

Experimental Design A pair of replicated trials were established (23 to 25 February, 2021) among a naturally occurring population of *Z. mauritiana* at a cattle property in Alligator Creek, North Queensland (rural locality near the south of Townsville) (19°24'10"S 146°56'24"E). These parallel trials involved the mapping, measurement, and treatment of individual plants with four encapsulated synthetic herbicides sourced from BioHerbicides Australia's (BHA Pty Ltd) Di-Bak® range of registered and developmental products (Table 1). An untreated control and benchmark treatment (drill-and-fill application of Tordon® RegrowthMaster) were also assessed for performance comparison.

The first trial investigated a commercially recommended dosage of one capsule for up to (<) every 10 cm in stem circumference (Goulter et al. 2018, Galea 2021b) by following a randomised complete block design (RCBD) with four blocks. Within each block, the six treatments were randomly assigned to a group of fifteen plants (uniform age and growth). All plants were appropriately tagged, and GPS waypoints recorded for ease of re-location.

Table 1. Active constituents (g/kg) and dosage (mg/capsule) of registered and developmental encapsulated synthetic herbicides sourced from BioHerbicides Australia (BHA Pty Ltd).

	Treatment	Active Constituents (g/kg)	Dose (mg/ capsule)
1	Glyphosate	700	261
2	Aminopyralid +	375	37.5
	Metsulfuron-Methyl	300	30
3	Metsulfuron-Methyl	600	330
4	Picloram	100	1000

The population dynamics of this trial site provided an opportunity to assess the error tolerance (i.e., resilience to human error) of the stem-implantation system by lowering the dosage level (one capsule per 15 cm in stem circumference). This second trial adopted a similar experimental design with three blocks. The same untreated control and benchmark treatment (drill-and-fill application of Tordon® RegrowthMaster) was evaluated given its proximity to the first trial.

Treatment Application The synthetic capsules were delivered via the InJecta® handheld device developed by BioHerbicides Australia (BHA Pty Ltd). The mechanics of this unit are described in-depth by Limbongan *et al.* 2021.

The drill-and-fill application of Tordon® RegrowthMaster (200 g/L triclopyr, 100 g/L picloram and 25 g/L aminopyralid) was achieved with a calibrated drenching syringe (NJ Phillips 5 mL Metal Tree Injector). A series of holes (10 cm spacing) were drilled (10 mm drill diameter) into the sapwood (15 mm to 20 mm depth) of the woody stem at a slight downward angle (45°). The label recommended dose (1 mL at 1:4 dilution with water) of herbicide solution was injected into the drilled holes.

Trial Assessment These trials were assessed at establishment (23 February 2021), three months (24 May 2021) and eight months (26 October 2021) by recording the overall vigour (1 = healthy, 2 = slightly distressed, 3 = moderately distressed, 4 = severely distressed, 5 = dead) of each plant. The 'stress score' was discerned by removing the outermost layer of the bark with a rasp, or with severely affected plants, bark removal with a claw hammer to reveal the colour of the tissue beneath (O'Brien *et al.* 2022). A plant was deemed 'dead' if the stem tissue was dry, brown, or bleached. Whilst, there was underlying moisture and colour (often dull, pale green) observed in the stem tissue of moderately or severely distressed plants. Additionally, an auditory assessment of the internal hydration (i.e., vascular fluids) was conducted by hammering the primary stem to confirm mortality (O'Brien *et al.* 2022).

Data Analysis The treatment effects on 'stress score' were analysed in RStudio® (RStudio Inc, Boston, Massachusetts, United States). A one-way analysis of variance (ANOVA) was performed by taking the mean (μ) of each replication. All pairwise comparisons among treatment means (μ) were estimated with the emmeans (estimated marginal means, also known as least-squares means) package. The compact letter displays (CLD) were corrected with Tukey's Honest Significant Difference post-hoc test.

RESULTS AND DISCUSSION

A significant effect (p <0.05) on plant vigour was discerned for both dosage levels within eight months (i.e., 35 weeks) of trial establishment (Table 2). The highest incidence of mortality was observed among the individuals treated with aminopyralid and metsulfuron-methyl (37.5 mg/capsule and 30 mg/capsule), metsulfuron-methyl (330 mg/capsule) and picloram (1000 mg/capsule), achieving a similar deterioration in plant health to the 'drill-and-fill' application of Tordon® RegrowthMaster (200 g/L triclopyr, 100 g/L picloram and 25 g/L aminopyralid) (Table 2, Table 3). This is evidenced by their rapidly increasing 'stress score' values whereby most plants (>85%) were deemed 'dead' (stress score of five) at thirty-five weeks (Table 3). However, there was no significant difference (p >0.05) individually between these four treatments (Table 2) or under different dosage levels (i.e., treatment-trial interaction). This suggests that the recommended dosage has an error tolerance of at least (≤) 5 cm per capsule for the respective synthetic compounds. We predict total mortality (100%) with these preeminent treatments by the next assessment period (15 months).

The performance of glyphosate (261 mg/capsule) is underwhelming relative to the other synthetic treatments. The reduction in plant vigour was mostly 'moderate' (stress score of three) for both dosage levels (Table 2). The untreated plants (i.e., control) were also slightly distressed at thirty-five weeks (Table 2). However, a degree of seasonal defoliation is expected given the deciduous nature of this species due to moisture stress.

Table 2. The *p*-value, mean (μ) stress score (SS) and standard error (SE) for trial one (10 cm spacing) and two (15 cm spacing) at each assessment period (week 0, 13. 35). The superscript letters (i.e., compact letter displays) denote all pairwise comparisons among treatment means (μ).

	Week					
	0		13		35	
	Trial 1					
p-Value	0.451		9.24×10^{-10}		2.45×10^{-09}	
	SS	SE	SS	SE	SS	SE
Control	1.0^{a}	0	1.0^{c}	0	2.68^{c}	0.073
Tordon® Regrowth Master	1.0^{a}	0	3.92^{a}	0.043	4.80^{a}	0.062
Glyphosate	1.0^{a}	0	2.52^{b}	0.113	3.37^{b}	0.099
Aminopyralid + Metsulfuron-Methyl	1.0^{a}	0	3.67^{a}	0.061	4.98^{a}	0.017
Metsulfuron-Methyl	1.0^{a}	0	3.63^{a}	0.063	5.0^{a}	0
Picloram	1.0^{a}	0	3.88^{a}	0.041	4.97^{a}	0.023
	Trial 2					
p-Value	0.465		2.80×10^{-10}		1.36×10^{-08}	
Control	1.0^{a}	0	1.0^{c}	0	2.62^{c}	0.092
Tordon® Regrowth Master	1.0^{a}	0	3.89^{a}	0.047	4.89^{a}	0.047
Glyphosate	1.0^{a}	0	2.22^{b}	0.077	3.38^{b}	0.143
Aminopyralid + Metsulfuron-Methyl	1.0^{a}	0	3.93^{a}	0.038	5.0^{a}	0
Metsulfuron-Methyl	1.0^{a}	0	3.98^{a}	0.022	5.0^{a}	0
Picloram	1.0^{a}	0	3.73^{a}	0.067	4.91^{a}	0.043

Table 3. The percentage (%) mortality among each treatment for trial one and two at the most recent assessment period (Week 35).

Treatment	Percentage (%) Mortality	
	Trial 1	Trial 2
Control	0.0	0.0
Tordon® Regrowth Master	83.33	88.89
Glyphosate	6.67	13.33
Aminopyralid + Metsulfuron-Methyl	98.33	100
Metsulfuron Methyl	100	100
Picloram	96.67	91.11

This novel technology has been proven successful for the management of other woody weed species: prickly acacia (*Vachellia nilotica*), leucaena (*Leucaena leucocephala*), calotrope (*Calotropis procera*), camphor laurel (*Cinnamomum camphora*), (Goulter *et al*. 2018), mimosa bush (*Vachellia farnesiana*) (Limbongan *et al*. 2021), Chinese elm (*Celtis sinensis*) (O'Brien *et al*. 2022, Galea 2021a). This study has demonstrated that the encapsulated delivery of synthetic compounds is also highly effective against *Z. mauritiana* in grassland and rangeland environments. Unlike its industry counterparts, a minimum recommended lethal dose is delivered directly into the vascular system of the target species whereby the active agent is fully captured internally (Goulter *et al*. 2018, Galea 2021a). This targeted, readily calibrated herbicide application has the potential to (i) lower active ingredient concentrations (~20% to 30%), (ii) minimise the likelihood of environmental exposure to plant protection compounds and (iii) improve operator safety (Galea 2021a). This technology is a possible replacement for foliar or stem spraying, stem-injection or canopy application (Galea 2021a)

Although not presented in this paper, these trials were also replicated in Mulgrave, Northern Queensland during the dry season (late-May 2021). This research is still underway, and the findings will be reported upon trial conclusion.

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REFERENCES

Ani, O., Onu, O., Okoro, G. and Uguru, M. (2018). Overview of Biological Methods of Weed Control. In 'Biological Approaches for Controlling Weeds', eds R. Radhakrishnan, pp. 5-16. (InTechOpen, London, UK).

Bebawi, F.F., Campbell, S.D. and Mayer, R.J. (2016). Seed Bank Persistence and Germination of Chinee Apple (*Ziziphus mauritiana* Lam.). *The Rangeland Journal* 38, 17-25 (DOI 10.1071/RJ15104).

Dhileepan, K. (2017). Biological Control of *Ziziphus mauritiana* (Rhamnaceae): Feasibility, Prospective Agents and Research Gaps. *Annals of Applied Biology* 170, 287-300. (DOI 10.1111/aab.12338).

Grice, A.C. (1996). Seed Production, Dispersal and Germination in *Cryptostegia grandiflora* and *Ziziphus mauritiana*, Two Invasive Shrubs in Tropical Woodlands of Northern Australia. *Australian Journal of Ecology* 22, 49-55. (DOI 10.1111/j.1442-9993.1996.tb00615.x).

Grice, A.C., Radford, I.J. and Abbott, B.N. (2000). Regional and Landscape-Scale Patterns of Shrub Invasion in Tropical Savannas. *Biological Invasions* 2, 187-205.

Grice, A.C. (2004). Weeds and the Monitoring of Biodiversity in Australian Rangelands. *Austral Ecology* 29, 51-58. (DOI 10.1111/j.1442-9993.2004.01364.x).

Galea, V.J. (2021). Stem Implanted Capsules – A Target, Efficient and Sustainable Method for Delivery of Synthetic and Biological Agents to Plantation Crops. Proceedings of the 2nd International Plantation Conference (Universiti Teknologi MARA, Selangor, Malaysia).

Galea, V.J. (2021). Use of Stem Implanted Bioherbicide Capsules to Manage an Infestation of *Parkinsonia aculeata* in Northern Australia. *Plants* 10 (DOI 10.3390/plants10091909).

Goulter, K.C., Galea, V.J. and Riikonen, P. (2018). Encapsulated Dry Herbicides: A Novel Approach for Control of Trees. Proceedings of the 21st Australasian Weeds Conference, eds S. Johnson, L. Weston, H. Wu and B. Auld, pp. 247-250. (The Weed Society of New South Wales Inc., Sydney).

Limbongan, A.A., Campbell, S.D. and Galea, V.J. (2021). Novel Encapsulated Herbicide Delivery Mechanism: Its Efficacy in Mimosa Bush (*Vachellia farnesiana*) Control. *Plants* 10 (DOI 0.3390/plants10112505).

O'Brien, C.J., Mellor, V. and Galea, V.J. (2022). Controlling Woody Weed Chinese Elm (*Celtis sinensis* Pers.) with Stem-Implanted Herbicide Capsules. *Plants* 11 (DOI 10.3390/plants11030444).