final report

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Distribution, invasiveness, biology and control of rubber bush (Calotropis procera) in northern Australia

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Abstract

We investigated the weed risk posed by rubber bush (*Calotropis procera*) on rangelands in northern Australia. Rubber bush establishes most prolifically in disturbed environments where there is reduced plant competition. Mature plants have remarkable survivability and seed profusely. Mating (solid pollinia transfer) relies on specific insect (Hymenoptera) pollinators during warm months (October-February). Fruit production is aided by self-compatibility, peaking in medium-density (250-500 individuals/ha) stands. Low but similar fruit production per plant occurs at low stand densities, but in large dense stands pollinator limitation causes low fruit set per plant. Each fruit contains ~430 wind-dispersed seeds. Some seeds (~7.5%) are blown >1 km making range expansion of 1 km every 2-3 years possible. Seeds have high germinability (85-100%), no dormancy, and germination success is temperature and moisture dependent. Seeds exposed to high soil surface temperatures rarely germinate, while buried seeds germinate without fail. Overall, rubber bush exerts considerable propagule pressure resulting in significant invasive potential. Distribution modelling shows that currently it has not saturated its potential range. The milkweed bug *Oncopeltus fasciatus* affects the fruit, and natural dieback events also reduce populations in some areas. Several herbicides can be used effectively to control rubber bush using a range of techniques. Mechanical methods require that the root system is severed 10-20 cm below ground, and that follow-up control is undertaken to treat new plants favoured by the disturbance.

Executive Summary

Several prioritisation processes have listed rubber bush (*Calotropis procera*) as an important economic and environmental weed in northern Australia's rangelands. However, there has been conjecture regarding whether rubber bush spread is a symptom of poor land condition, and whether dense infestations can occur on well-managed lands. We addressed these questions, as well as how best to control it, in this MLA funded collaborative research project (July 2010-May 2015).

Under normal field conditions, post-germination and sapling mortality of rubber bush is generally high and if there are high plant densities, reproductive maturity is delayed. Once mature, plants have remarkable survivability and are capable of profuse seed production. The mating system requires specific insect pollinators (Hymenoptera) and is plant density-dependent. Plants tend to flower between 4 to 11 months and are capable of producing pods after 12 months.

The complex flower morphology forces the withdrawal of solid pollinia by the pollinator, limiting pollination to larger wasp and bee species. In some areas (e.g. the Northern Territory), many or all of these insects may be absent during the colder months, which means that most fruit set occurs in hotter months, even though rubber bush flowers year-round. Fruit production per plant (fecundity) peaks at intermediate population densities (250-500 plants/ha). Generally, low pollinator pressure limits rubber bush fruit production at high population densities and, to a lesser extent, at low population densities too, because there is low pollen transfer efficiency between widely scattered plants.

Rubber bush seeds are wind dispersed. Some seeds (~7.5%) are blown >1 km so range expansion of 1 km every 2-3 years is possible. With the exception of one insect species (*Oncopeltus fasciatus*) that is a pre-dispersal seed predator, there are no post-dispersal seed predators. The seeds are highly germinable resulting in a short lived seed bank (<2 years) if favourable temperature and rainfall conditions occur. Therefore, land managers can effectively control rubber bush patches in a 2-3 year timeframe by targeting mature individuals first, prioritising the eradication of small isolated populations and mature singletons wherever they occur, and following up with seedling control. Where control of all mature plants cannot be achieved in one year, thinning plants in the first year so they are below intermediate densities as much as possible, will exploit the density-dependent reproduction of the plant, thus reducing pod production and replenishment of soil seed banks.

Rubber bush is not overly competitive in intact rangelands and establishes mostly in disturbed environments where plant competition is reduced. In exclosure experiments, seeds germinated mainly on disturbed ground after being buried. Treatments that removed grass cover and turned over the soil surface displayed the best germination success (10-20%). When rubber bush and barley Mitchell grass (*Astrebla pectinata*) were grown together in competition trials, barley Mitchell grass suppressed the shoot and root mass of rubber bush, while rubber bush reduced the shoot mass of the grass species. Thus, grazing should be managed to maintain a healthy pasture to supress the establishment and growth of rubber bush. Otherwise, dense stands capable of reducing the herbage yield of grasses are more likely to establish.

Although rubber bush has a foothold throughout northern Australia, distribution modelling shows that currently it has not saturated its potential range, with large areas still under threat. Mean temperature, wind speed, beef density, distance to roads, mean rainfall and, to a lesser extent, vegetation type, were the most important factors influencing the distribution of rubber bush. Under climate change, the future potential distribution of rubber bush will extend into the southern and eastern parts of the Northern Territory, border regions of Western Australia, and western Queensland. Contraction of suitable conditions in the north-western region of the Northern Territory is likely. Overall, the future climate scenario predicts there will be more suitable areas across northern Australia for rubber bush. This highlights the need to manage this species as an invasive weed.

Several new herbicide options for rubber bush control have been identified. Foliar spraying using two chemicals (2,4-D amine and imazapyr/glyphosate) gave satisfactory mortality, but efficacy was best on smaller plants (≤1.5 m in height). A range of options for treating low densities or small patches were also found, including methods that can be incorporated into routine activities (such as checking bores). They include: the use of triclopyr/picloram (Access™) applied in a diesel mixture using basal bark techniques; cut stumping using neat glyphosate or a picloram based gel on small-medium sized plants; and ground applications of the residual herbicides hexazinone and tebuthiuron. For treatment of large dense infestations, aerial applications of tebuthiuron were found to be highly effective, but must comply with vegetation management laws of respective states and territories.

In terms of mechanical control, high mortality is achievable provided plant stems are severed 10–20 cm below ground. If not, a large proportion of stems reshoot vigorously from the root collar. Large-scale seedling recruitment should be expected after any mechanical treatment, necessitating the need for follow-up control over at least two years to avoid re-infestation of sites. Natural large-scale dieback was also observed at several sites in Queensland and more recently in the Northern Territory. While there is a historical precedent for diebacks of rubber bush associated with long runs of drier than usual years in the Victoria River District, in this project a leaf spot disease (*Passalora calotropidis*) has been implicated. Further research is recommended to ascertain the exact cause or synergy of causes (e.g. low rainfall may promote leaf spot), which resulted in a 96% decline since December 2012 in a population in the Gulf of Carpentaria Region.

Overall, rubber bush has the potential to be a medium risk invasive species in northern Australia. Like most weed species, the life history of rubber bush is adapted to efficient seed dispersal and early germination of seed, but it cannot easily invade intact pastures and habitat. Consequently, rubber bush favours disturbed sites and its invasive potential is, in large, part due to land management practices that cause disturbance to intact habitat (roadsides, stock yards, around bores). Seed and pollination biology and wind dispersal of seed combine to ensure that reproduction is density-dependent. This density dependence should be exploited when controlling rubber bush, by destroying mature plants wherever they occur, by repeated thinning of established stands to low density stands. These can be managed more easily and removed within a two year period. The removal of isolated singletons is essential to slow rates of invasion across the landscape. Control measures should be monitored over at least a 2-3 year period. Rubber bush has not spread to the full extent of its potential current range, and climate change scenarios suggest that its range will expand in the future. Thus, controlling rubber bush now is encouraged to avoid future spread and loss of pastoral productivity.

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1 Background

Rubber bush (*Calotropis procera*), a native of tropical and subtropical Africa and Asia (Grace 2006), is spreading across northern Australia (Grace 2006). However, its current distribution is only a small proportion of its potential distribution, which could include most of the rangelands of northern Australia (Grace 2006).

Thick infestations of rubber bush (Plate 1) reduce pastoral production and native biodiversity (Grace 2006). The plant is toxic, although there are few reports of domestic animals dying from it (Grace 2006). During dry periods, cattle will browse rubber bush plants and can keep them in check. Whether rubber bush is a highly competitive plant capable of replacing pastures in good condition, or a weed of disturbed or degraded areas (Bastin et al*.* 2003, Grace 2006) is the subject of the research presented in this report.

A national literature review (Grace 2006) and the outcomes of a MLA sponsored rubber bush workshop (Tennant Creek 2007), established that there was a paucity of information available on rubber bush in Australia, and identified key research needed to fully understand its invasiveness and how best to control it.

In 2010, MLA approved and funded a collaborative research project on rubber bush, involving the Department of Agriculture and Fisheries, Charles Darwin University and the Northern Territory Department of Land Resource Management with input from a range of stakeholders. The priority was to better understand the invasiveness, biology/ecology and distribution of this invasive weed and how to control it in a range of situations.

Plate 1. A thick rubber bush infestation in the Barkly Tablelands region of the Northern Territory

2 Projective Objectives

1. Describe key aspects of rubber bush ecology relevant to management.
2. Quantify the distribution and rate of spread of rubber bush at several locations.
3. Quantify the invasiveness of rubber bush under different disturbance regimes and land types using a series of competition and exclusion studies.
4. Develop improved control options including:
    - 4.1 Complete testing of products that can be used as part of day-to-day activities to control isolated rubber bush plants.
    - 4.2 Trial and compare current and new herbicide products for control of rubber bush.
    - 4.3 Conduct a seasonality trial to quantify efficacy of herbicides under different seasonal conditions and growth stages.
    - 4.4 Monitor the effectiveness of aerial applications of tebuthiuron for broad-scale control of rubber bush in suitable habitats.
5. Monitor areas being affected by a dieback phenomenon to quantify whether rubber bush plants recover or eventually die, which will have significant implications on the population dynamics of infestations.
6. Identify the most practical and cost-effective control options for rubber bush.
7. Develop recommendations for managing rubber bush in different environments and with different levels of rubber bush intrusion.

3 Ecology and Invasiveness

Invasiveness is the ability of a plant to establish, reproduce and disperse within an ecosystem and has been associated with reductions in native plant richness (Grice 2006, Benke et al.et al. 2011). Biological invasions can cause irreversible losses of biodiversity (Oliveira et al. 2009). Thus, it is important to evaluate the invasiveness of rubber bush for at least three reasons. Firstly, the transition of a plant from naturalised to invasive status must be understood to manage it effectively – often the transition is the culmination of many concurrent synergistic processes that may escape notice. Secondly, this knowledge is required to support decision making at a policy level (i.e. plant categorisation) and, thirdly, to address knowledge gaps that may exist regarding the species in northern Australia.

Researching the invasiveness of a weed has the obvious potential benefit of predicting its invasive potential prior to introduction or after introduction (Grime 1979, Hodgson et al.et al. 1999, Pheloung et al. 1999, Morales & Aizen 2002, Ricciardi & Cohen 2007, Richardson & Rejmánek 2011). In their discussion of concepts and definitions, Richardson et al.et al. (2000) advised of the importance of having clearly defined, widely accepted terminology to describe the status of alien plants, so as not to confound the objective formulation of priorities for management and, in our case, research. While agreeing with this view, we adopt the following definitions proposed by Richardson et al. (2000) and others:

a) **Invasive** plants are naturalised plants that reproduce offspring, often in large numbers, at considerable distances from parent plants.
b) **Weeds** are plants that grow at sites where they are not wanted and that usually have detectable economic or environmental effects.

The methods of estimating invasiveness are based on four principles, namely, that invasiveness is due to a) introduction history; b) species traits; c) ecological processes; and

d) evolutionary processes. In terms of introduction history, the question is whether invasion is caused primarily by propagule pressure; for species traits, the question is whether invasiveness is enhanced by fitness or dispersal traits; for ecological processes, the question is whether realised niches facilitate invasiveness. Finally, in terms of evolutionary processes, it is whether the plant has evolved and increased its competitive ability in the introduced range (Van Kleunen et al. 2010). We adopt the working hypothesis in this report that the invasiveness of rubber bush arises due to a combination or combinations of the above.

It is argued that rapid evolution challenges the prediction of invasiveness (Whitney & Gabler 2008), and rapid evolution could be a factor in the recent spread of rubber bush. However, given its distribution worldwide, length of residence and the qualities it shows in very diverse environments, including Brazil and Hawaii, it would have had to evolve extremely quickly in several different directions within fairly short periods to develop novel mutualisms, yet aspects of its phenotype are remarkably consistent. In particular, the rubber bush present in Australia shows very little flower polymorphism (Forster 1998), compared to flowers in the native range that are sometimes white, for example, suggesting that the local populations may have developed from a few initial introductions. Finally, strong selection in alien species within a new range is thought to destroy all but the pre-adapted individuals (Mack et al. 2000). The process of assessing the invasiveness of rubber bush entails identifying and quantifying functional traits that enable the plant to be a “winner” where others are “losers” (Hodgson et al. 1999). As rubber bush’s invasiveness has become evident in Australia within a relatively short period (~25-50 years), measurement of its invasiveness needs to take into account its introduction history, species traits and ecological processes. For this reason, our study largely excluded evolutionary trends.

3.1 Reproduction

3.1.1 Methodology

3.1.1.1 Flower morphology

The gross and fine structures of the rubber bush flower that contribute to successful fruiting were investigated. A series of laboratory and field experiments were undertaken to evaluate the determinants of pollination success, such as flower anthesis, pollinia viability and sucrose production.

3.1.1.2 Mating system and pollination ecology

Pollinators and their flower visitation behaviours were monitored at Tennant Creek (19°42'56.28" S 134°17'36.05"E), Helen Springs (18°25'16.64"S 133°53'35.49"E), Sandover (21°48'16.70"S 135°6'38.21"E), Muckaty (18°38'4.76"S 133°49'44.11"E) and Katherine. (14°28'8.03"S 132°20'27.42"E) over three years. The pollinator status of an insect was verified by examining whether individuals of the species carried pollinia or not. To determine visitation behaviour, video clips of equal length (10 minutes) were recorded on a branch of each of five focal plants at each site during peak insect visitation activity. The effect of flowering plant density on pollinator visitation rates, and consequently the reproductive performance of individual plants, was assessed by recording fruit set on focal plants at known population densities over the same period.

3.1.1.3 Breeding system

Four rubber bush populations were examined in breeding system experiments, namely Katherine (KA), Helen Springs Roadside (HSR), Helen Springs Hillside (HSH) and Tennant Creek (TC). A sample of 165 plants (330 umbels) distributed as follows: 85 at KA, 40 at HS (R), 20 at HS (H), and 20 at TC, were examined.

One umbel per plant was bagged to test for autogamy. For each umbel, all open flowers were removed and buds bagged in netting to exclude pollinators. On the same plant, all flowers on one umbel were hand-pollinated with pollen from that plant, all the flowers on another umbel were hand-pollinated with pollen from a different population 2-5 km away, and then bagged. Hand-pollination was achieved using a pin to gently open the stigmatic slit and insert a single pollinium into the stigmatic chamber. A third umbel was bagged after removing open flowers without hand-pollination. After two weeks, the abscission of petals and formation of small bulbous swellings at the base of the flower was taken as evidence of the onset of fruiting. The level of self-compatibility was determined by calculating the self-compatibility index (i.e. the ratio of fruit formed in selfed to crossed flowers). A plant was considered self-incompatible if the ratio was <0.2 (Etcheverry et al. 2008).

To determine if seedling growth differed depending on whether they originated from selfed or crossed fruit, seeds (n = 116) from selfed and crossed fruit from one plant at Helen Springs were germinated in a greenhouse. Emerging seedlings were allowed to grow for three months and their height, leaf length and width were then measured. This experiment was repeated for selfed and crossed seeds from KA and HS (R), to assess if there are population level differences in the performance of selfed and crossed seedlings.

3.1.1.4 Flowering and fruiting phenology

The flowering and fruiting phenology of plants may contribute to their invasive character, particularly if their phenology helps to ensure reproductive success under diverse climatic conditions. In addition, it has been observed that climate change modifies the phenology of both plants and animals (Visser & Both 2005, Cleland et al. 2007), and could consequently influence existing mutualisms. The flowering and fruiting phenologies of rubber bush were monitored over a period of two years across nine sites located in Queensland and the Northern Territory (Table 1), to determine the length of the fruiting season relative to the flowering season. At the Queensland sites, we recorded the sizes of plants, checked for the presence/absence of flowers and recorded the number of fruit on focal plants every month, while at the Northern Territory sites, we recorded the stem numbers and sizes, plant height, crown width, number of umbels and fruit on focal plants monthly.

Table 1. Names and coordinates of the phenology observation sites.

Site name	Coordinates
Big Bend	19°51'15.4"S 146°08'33.5"E
Bluff Downs	19°40'29.4"S 145°32'04.1"E
Helen Springs Paddock	18°24'50.0"S 133°53'40.6"E
Helen Springs Hillside	18°27'39.3"S 133°55'09.7"E
Muckaty Bore	18°38'01.2"S 133°50'37.4"E
Muckaty Paddock	19°05'40.3"S 145°16'07.4"E
Christmas Creek Trainline	19°05'40.3"S 145°16'07.4"E
Nardoo	18°21'33.6"S 139°31'00.4"E
Tennant Creek	19°42'50.4"S 134°17'26.0"E

3.1.2 Results

3.1.2.1 Flower morphology

Rubber bush has a complex flower with pollen contained in sacs called pollinia, whose successful transfer requires insect vectors (Plate 2a-b., Plate 3). The stigmatic disc is ~5.5 mm wide and 5.32 mm deep. The nectar is held in cuculli and the depth of the nectaries determines the type of insects that can access the nectar.

There was some variation (significantly different at two sites – Table 2) in the measured morphology of the flower. Very few flowers (<1%) on plants in recently established stands possessed square rather than pentagonal stigmatic discs – perhaps an indicator of increased negative allelic frequencies due to high levels of inbreeding.

Table 2. Comparison of flower sizes of rubber bush across sites.

Site	Width of stigmatic disc (mm) mean ± s.e.	Height of the staminal column (mm) mean ± s.e.
SO	5.58 ± 0.05a[1]	5.10 ± 0.06a
MU	5.58 ± 0.04a	5.42 ± 0.06b
HS	5.60 ± 0.04b	5.33 ± 0.04a
TC	5.07 ± 0.04b	5.42 ± 0.03a

[1] Means are presented with their standard errors and within columns different letters denote significant differences at $P < 0.05$.

The ovaries are linked to the five corners of the pentagonal stigmatic disc, such that insertion of a pollinium into any of the five chambers between the anther flaps can result in the formation of a fruit. There are two pollinia connected at the corpusculum (making up the pollinarium) and a translator arm, all of which are involved in the transfer of pollen by insects (Plate 4a-d).

a

b

Plate 2. Top (a) and side (b) views of a rubber bush flower showing the landing surfaces and pentagonal stigmatic disc and aspects of the flower that were measured.

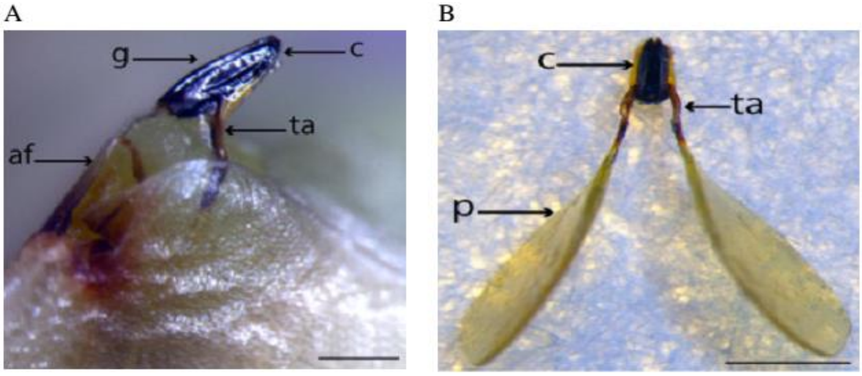


Plate 3. Microscopic structure of a rubber bush flower: (a) position of the pollinarium, with intact corpusculum and translator arm of an (b) extracted pollinarium. Abbreviations: g - groove, c – corpusculum, ta – translator arm, af – anther flaps, p – pollinium (Greenfield 2013).

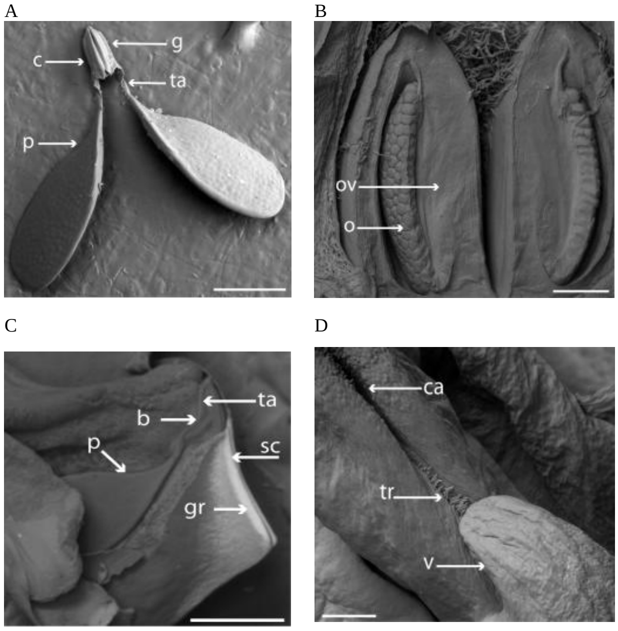


Plate 4. Scanning electron micrograph of the fine structure of the rubber bush flower. Abbreviations: c-corpusculum, ca – cavity, g – groove, ov - ovary, o – ovules, p – pollinium, ta – translator arm, sc – stigmatic chamber, tr – trichomes, v- vesicle. Scale bar = 500 µm (Greenfield 2013).

3.1.2.2 Mating system and pollination ecology

The size of the stigmatic disc and the arrangement of the nectaries relative to the opening of each stigmatic chamber on the flower are adaptations that attract generalist Hymenopteran pollinators, such as carpenter bees (*Xylocopa aruana*), honey bees (*Apis mellifera*) and wasps, to land on the flower and vector pollen.

There was a gradual species shift in the Hymenopteran pollinator assemblage along a north to south gradient of the sample sites, with carpenter bees and honey bees forming most of the pollinator community in Katherine, and wasps being dominant at the southern end of the gradient. Moving towards the central regions of Australia, the pollinator assemblage predominantly comprised various families of larger wasps (Table 3). Two species of wasps, namely *Delta latreillei* and *Sphex fumipennis,* were particularly common pollinators across sites, other than Katherine, while other wasp species were only found at specific sites.

The wasps were most active during the hottest times of the day (1030h-1530h). Pollinator visitation patterns (Table 3) indicated a high proportion of within plant pollen transfer (geitonogamy) in most populations, and insects probed 4-5 open flowers on an umbel before moving to another umbel on the same plant.

All insect vectors (Table 3) were absent during the colder months of the year (April to August) and thus there were few or no fruit on rubber bush plants during these months. Insect visitors started appearing on flowers at the beginning of September, and the first wave of mature fruit set seeds towards the end of October (i.e. after about eight weeks).

Table 3. Species of bees and wasps, their size (length), visitation patterns and the site(s) where they were found. Visitation patterns* FF – flower to flower on the same umbel; UU – umbel to umbel on same plant; PP – plant to plant.

Family	Sub-family	Name	Pattern*	Locations where found	Length (cm)
Vespidae	Eumeninae	*Delta latreillei*	FF	TC, HS, SO	3
		Epiodynerus nigrocintus	FF	HS	1.5
		Abispa ephippium	UU	TC	2.5
	Polistinae	*Polistes signata*	UU/FF	HS, TC	1.3
Apidae	Apinae	*Apis mellifera*	FF	KA, TC	1
Anthophoridae	Xylocopinae	*Xylocopa aruana*	UU	KA	2.2
Megachilidae	Megachilinae	*Hackerapis* sp.	UU	HS	0.9
	Lithurginae	*Lithurgus* sp.	UU	HS	1.5
Sphecidae	Sceliphrinae	*Sceliphron laetum*	UU/FF	TC, MU	3
	Sphecinae	*Sphex fumipennis*	PP	MU, TC, HS, SO	2.4
		Sphex sericius	FF	TC	2
		Prionyx sp.	FF/UU	TC, SO	2.3
		Sphex sp.	FF	TC	2.2
Crabronidae	Bembicinae	*Sphecius pectoralis*	FF	MU	2.7
Pompilidae	Pepsinae	*Hemipepsis* sp.	UU	HS, MU	2.5
	Pompilinae	*Heterodontonyx australis*	UU	HS	1.6
	Pompilinae	*Turneromyia* sp.	FF	TC, SO	1.5
Scoliidae	Scoliinae	*Trisciloa ferruginea*	FF	TC, SO	2
		Radumeris radula	FF	TC	4.2

The probability of successful pollinium transfer was low (~0.04%), but the effect of one successful transfer, in terms of viable seeds per fruit, is significant due to the large number of pollen grains contained in one pollinium (~450-500) that accords with the number of seeds per fruit.

3.1.2.3 Breeding system

Overall, 30.7% of all hand pollinations across sites were successful, of which 57.1% were in crossed and 42.9% in selfed flowers. Fruit abortion was common in manipulated and un-manipulated plants, particularly during the peak fruiting season. The self-compatibility index for HS Roadside was 0.29, for TC 1.18, for HS Hillside 0.01, for KA 1.29, while only crossed flowers formed fruit at the MU site. We conclude that rubber bush is a facultative out-crosser, capable of both selfing and crossing.

Seedlings from crossed seed did not differ significantly from the selfed seeds in a number of leaves, but grew taller and had a significantly larger overall leaf surface area than those from selfed seed ($F_{1,\,114} = 5.2$, $P = 0.02$). The mean crossed seedling height was 9.37 ± 0.24 cm and selfed 7.27 ± 0.34 cm (mean ± SE; applies to all other data presented in a similar manner, unless stated otherwise), while mean number of leaves was 5.51 ± 0.12 per seedling. In a separate but similar experiment involving selfed and crossed seeds from different populations and plants (i.e. HS and KA), crossbred HS seedlings had significantly heavier root and shoot mass and grew taller than the selfed seedlings, however, no difference was observed in KA seedlings in all measured parameters (Fig. 1).

3.1.2.4 Flowering and fruiting phenology

The flowering and fruiting trends observed at the four sites in Queensland were different from those in the Northern Territory, in that Queensland sites tended to fruit all year round, while the Northern Territory plants ceased fruiting in winter altogether, although they were in flower. An increase in the number of flowers in 2014 did not result in a concurrent increase in the number of fruit compared to the previous years at the Queensland sites (Fig. 2), the probability of fruiting remained stable, which may be indicative of stagnating pollinator numbers. At all sites in the Northern Territory, the mean number of pods peaked in January and the fruiting season was shorter in 2012 compared to 2013 (Fig. 3.) Year to year fruiting differences were not related to the level of flowering as plants were in flower throughout the year (Fig. 4), but there were few or no fruit during the colder winter months due to insects overwintering (hibernating).

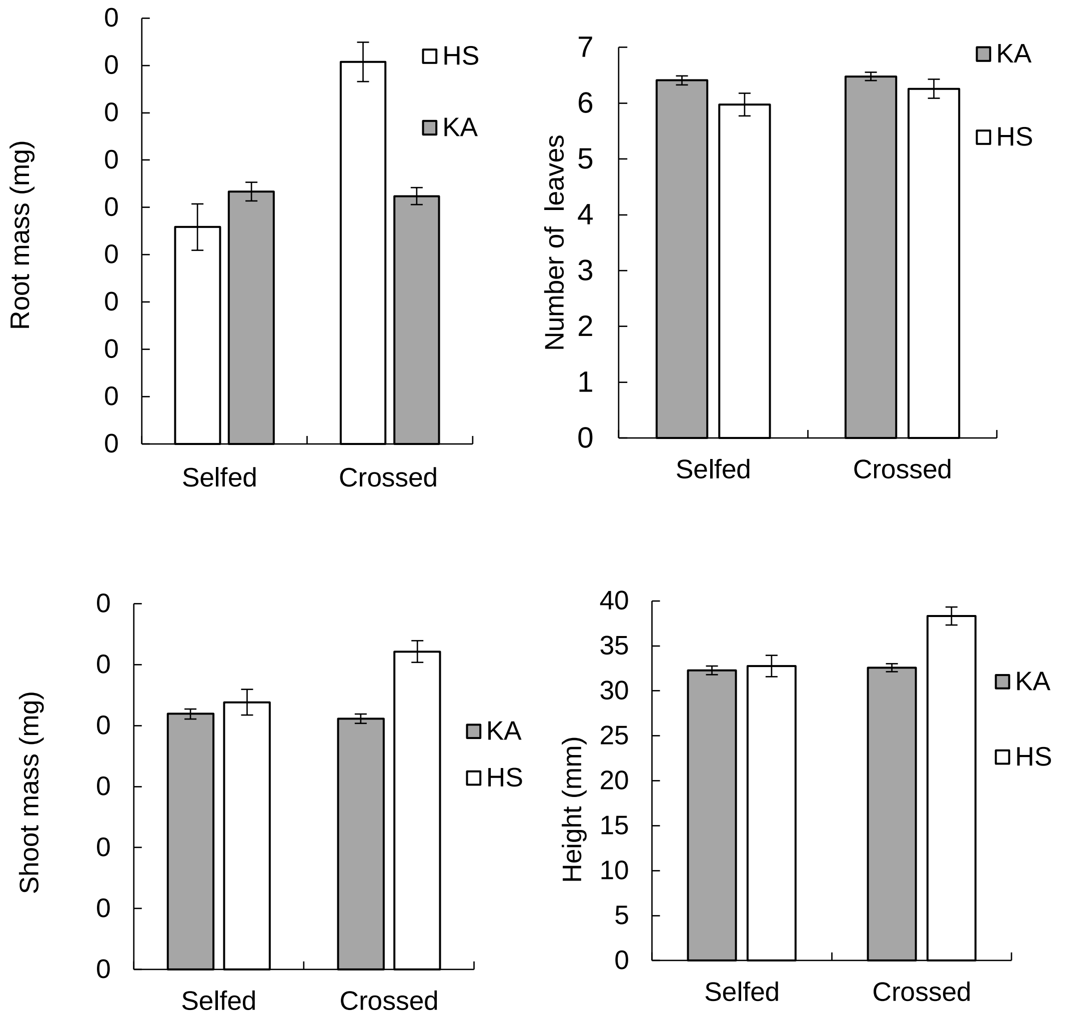


Fig. 1. Mean shoot mass, root mass, number of leaves and height after three months, of crossed and selfed seedlings grown from seed in a greenhouse experiment. The vertical bar above each column represents the standard error.

Across seasons and years, a total of 1240 phenology observations were made in the Northern Territory and Queensland locations. The Queensland data were analysed to characterise how the number of flowers and fruits varied across months and years (Fig. 2). The overall mean number of pods/plant across sites and months at the Queensland sites was 2.04 ± 0.33 (n = 673). Some sites had significantly higher mean fruit occurrences/month than others (i.e. Big Bend 11.16 ± 3.96 compared with Christmas Creek 0.93 ± 0.24). Both month and site had a significant influence on the number of fruit (i.e. not all months had the same number of fruits: month $F_{17, 568} = 9.53$, $P < 0.05$; site $F_{3, 568} = 15$, $P < 0.05$). It is worth noting that even a few umbels can produce fruit provided there are pollinators. The Northern Territory sites had an overall mean of 65.55 ± 3.29 umbels/plant/month and 5.08 ± 0.58 pods/plant/month (n = 567) (Fig. 3). Rubber bush plants flowered throughout the year (Fig. 4), with minor variation in the mean number of umbels/plant/month among sites; however, fruit set occurred mainly from September to February in the Northern Territory, whilst low numbers of fruit were found all year round in Queensland. It is worth noting that fruiting started earlier in Katherine (August), probably due to the predominant pollinator species in Katherine being different from other regions (carpenter and honey bees versus wasps elsewhere).

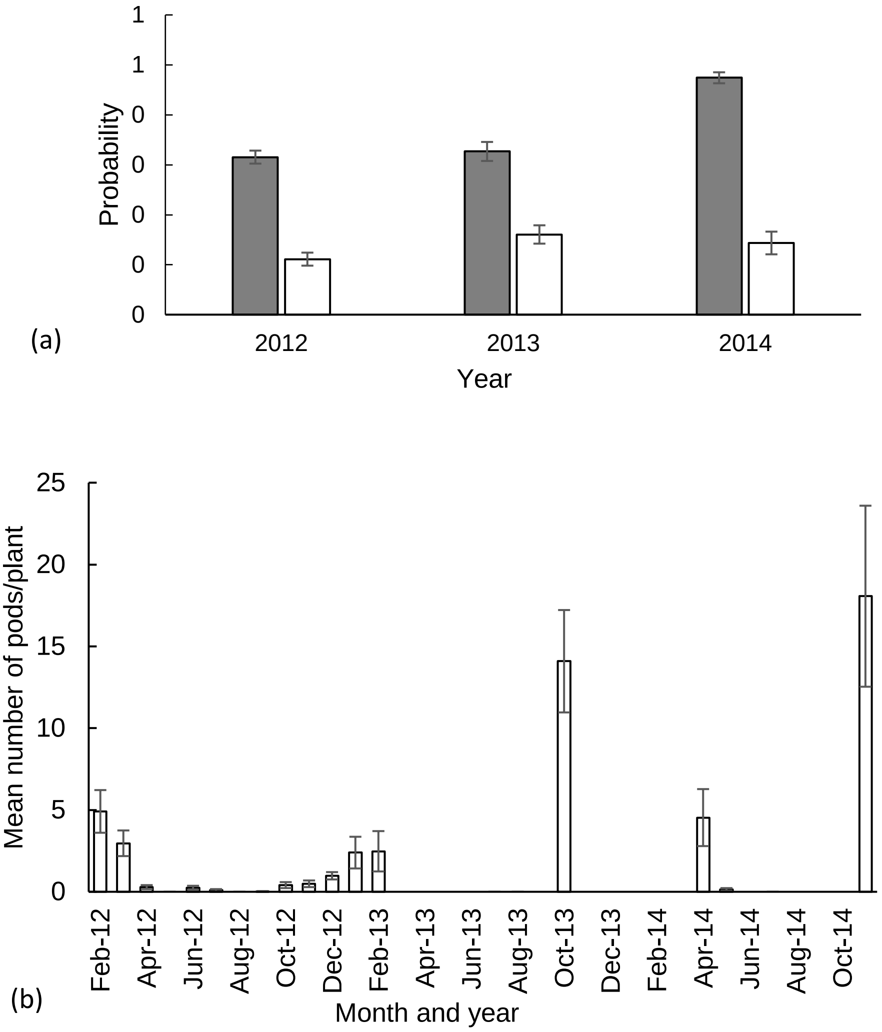


Fig. 2. The probability of flowering (shaded) and fruiting (unshaded) (a) across years and (b) mean number of pods during specific months at the Queensland sites. The vertical bar above each column represents the standard error.

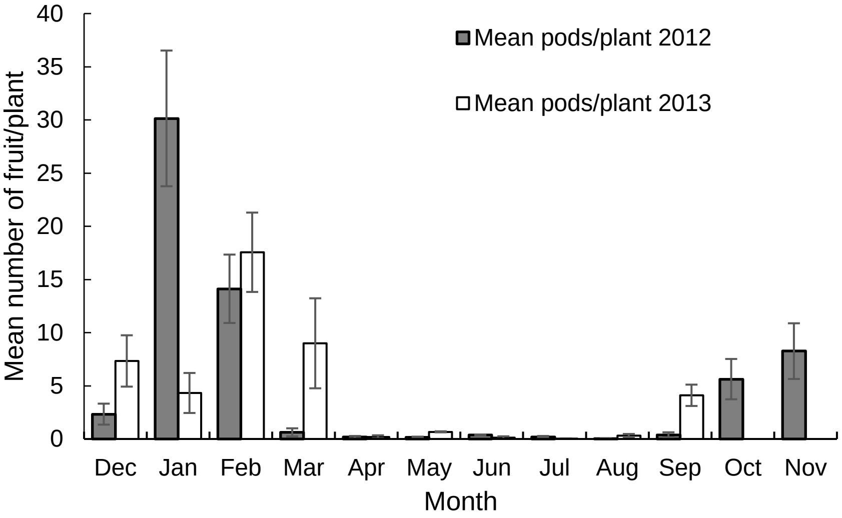


Fig. 3. Mean number of pods/plant/month in the Northern Territory populations. The vertical bar above each column represents the standard error.

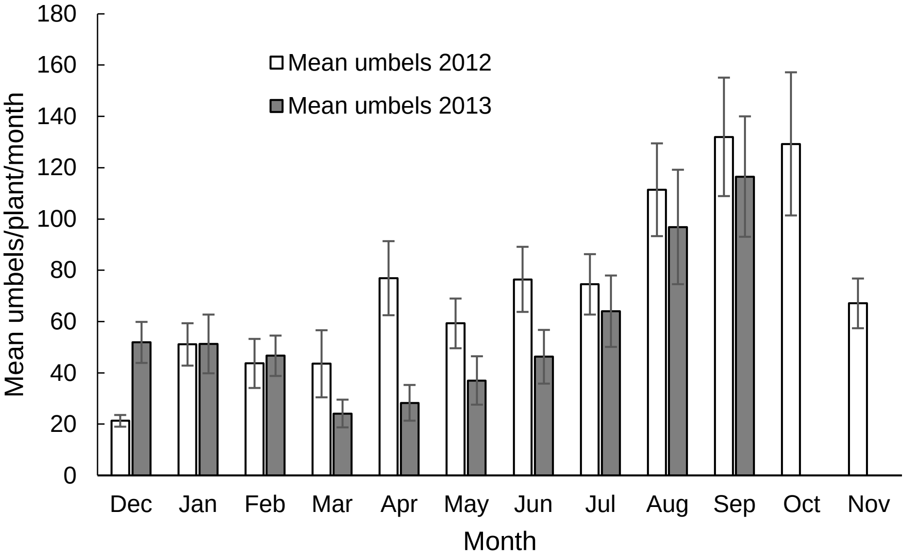


Fig. 4. Mean number of umbels/plant/month in the Northern Territory populations. The vertical bar above each column represents the standard error.

3.2 Seed longevity

3.2.1 Methodology

The potential longevity of rubber bush seeds was quantified in a seed burial trial undertaken at the Tropical Weeds Research Centre in Charters Towers between 2009 and 2012. Seeds were exposed to different soil types (clay and river loam), levels of pasture cover (present and absent) and burial depths (0, 2.5, 10 and 20 cm). Two fresh seed lots were tested at separate times to determine if exposure to different environmental conditions influenced seed longevity. An initial collection of ripe follicles of rubber bush was undertaken in December 2008 from two locations: 43 km south-east (20°13’S, 146°38’E) and 15 km north-west of Charters Towers (19°59'S, 146°30 E). This was before commencement of the rubber bush project but is included to allow comparison with a second collection of seeds that was made possible due to funding from the project. The second seed lot was collected in October 2011 from an infestation of rubber bush located in the Gulf of Carpentaria Region (18°13'S, 140°38'E). Full details on the methodology and results are provided in a scientific paper published by The Rangeland Journal (see Appendix 1).

3.2.2 Results

Annual rainfall, recorded at the experimental site between 2009 and 2012, was consistently greater than the long-term mean for Charters Towers (658 mm), averaging 1105, 1323, 1037, 832 mm per annum, respectively. For the first five months of 2013 before the second seed lot finished being tested in May 2013, 452 mm of rainfall was recorded (Fig. 5). Despite high annual rainfall, the first and second seed lots were exposed to different seasonal patterns of rainfall, particularly in the first 12 months, which was a critical period in the longevity of soil seed banks of rubber bush. After burial in March 2009, the first seed lot received 118 mm of rainfall for the remainder of the autumn period. This was followed by a very dry winter and spring period where only 21 mm of rainfall were recorded, before the onset of a wet summer where 493 mm fell. In contrast, the second seed lot buried in January 2012 received 218 mm of rainfall in February, followed by 319 mm during autumn. Even the winter period received high rainfall with 189 mm being recorded. However, the following spring was dry (32 mm) and summer rainfall was below average, with 294 mm recorded (Fig. 5).

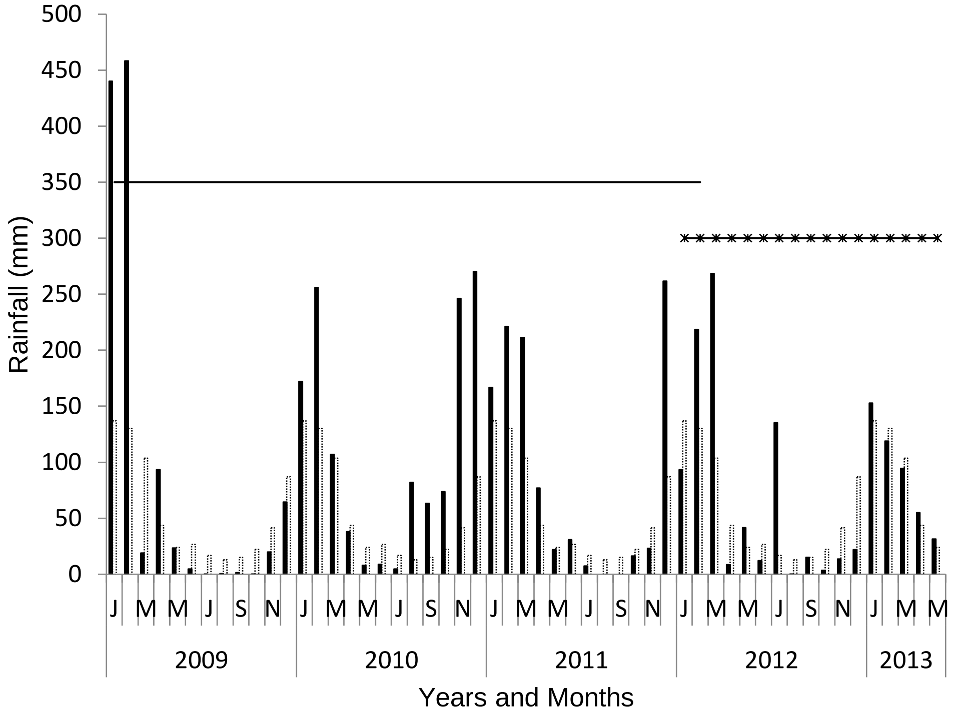


Fig. 5. Monthly rainfall (■ mm) at the research site between March 2009 and May 2013, and the average monthly rainfall (□ mm) for Charters Towers associated with burial duration for seed lot 1(_____) and seed lot 2 (xxxx).

In the first seed lot, initial seed viability and germinability was high, averaging 85.0 ± 2.4% (per cent of total seed number) and 100% (per cent of viable seeds), respectively. Following burial, significant burial depth × burial duration ($P < 0.001$) and soil type × burial duration ($P < 0.01$) interactions were recorded for seed viability. In contrast, the level of pasture cover did not have a significant influence ($P > 0.05$) on seed viability over time. Viability declined most rapidly in the first three months, particularly in seed lots that were buried. After six months, <1% of buried seeds remained viable, compared with 28% of surface-located seeds (Fig. 6). This rapid decline in viability coincided with high germination of seeds in the field in the first three months after burial. On average, 92% of seeds germinated within three months if buried, significantly more ($P < 0.05$) than surface-located seeds which averaged only 38% (Fig. 7). No viable seeds were retrieved from buried seed lots after 18 months, whilst surface-located seed lots had no viable seed after 24 months.

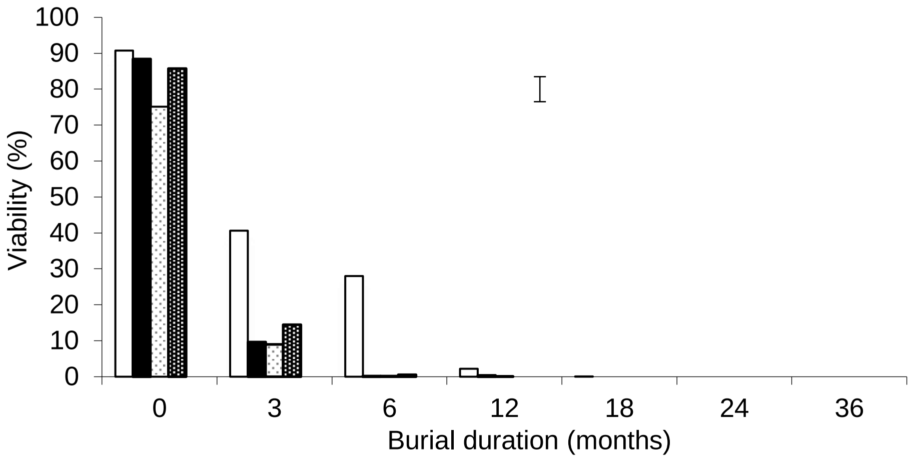


Fig. 6. Viability (%) at the time of testing of the first seed lot of rubber bush as affected by burial depth (□ 0 cm, ■ 2.5 cm, ░10 cm and ▓ 20 cm) and burial duration. The vertical bar indicates the least significant difference at *P*=0.05.

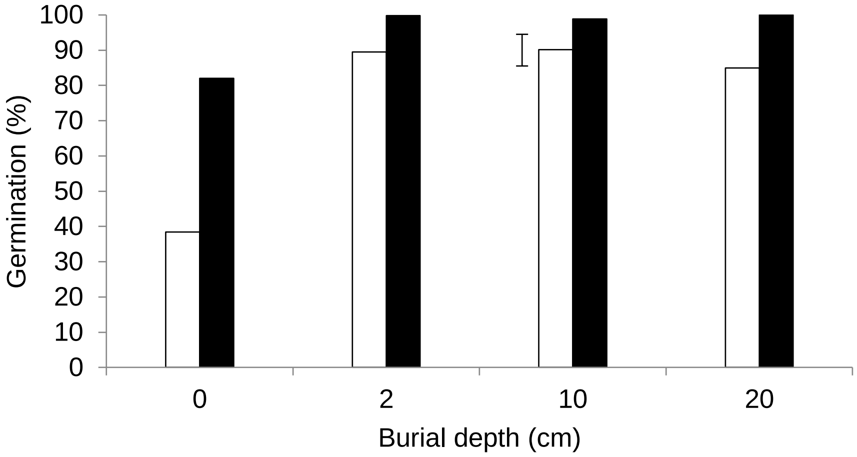


Fig. 7. The proportion (%) of the first (□) and second (■) seeds lot of rubber bush that germinated in the field at different depths, three months after burial. The vertical bar indicates the least significant difference at *P*=0.05.

With regards soil type, a more rapid rate of decline in viability occurred in the clay soil compared with the river loam soil (Fig. 8). No viable seeds were retrieved from the clay soil after 18 months, whilst nil viability was recorded in the river loam soil at the 24-month retrieval.

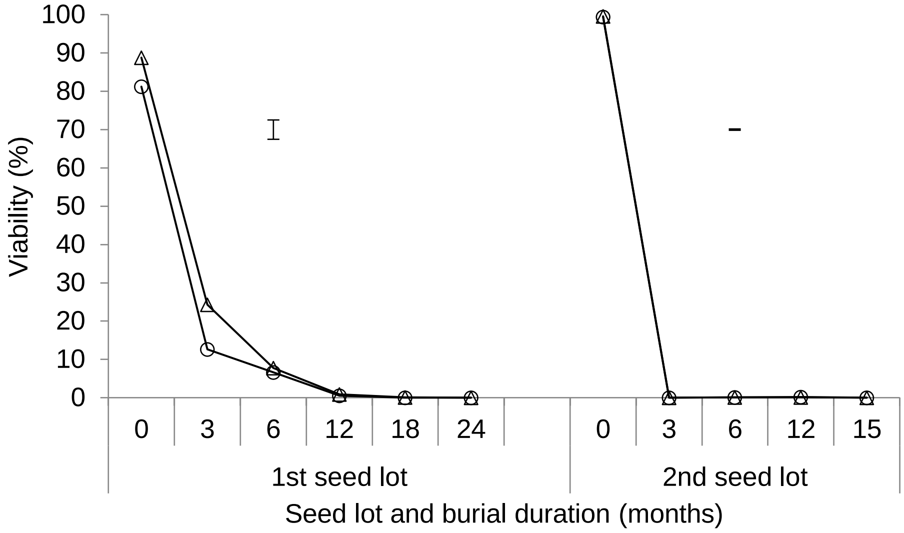


Fig. 8. Viability of the first and second seed lots of rubber bush as affected by soil type (○ clay and ∆ river loam) and burial duration. Vertical bars indicate the least significant difference at *P*=0.05.

In the second seed lot, initial seed viability and germinability was extremely high, averaging 99.5 ± 0.1% (per cent of total seed number) and 100% (per cent of viable seeds), respectively. Following burial, viability was significantly affected by burial duration ($P < 0.05$), but not by soil type, pasture cover or burial depth ($P = 0.93$). The rate of decline in viability was faster than that of the first seed lot tested.

After 3 months, no viable seed was recorded across all burial depths, soil types and pasture cover treatments. However, subsequent 6 and 12 month assessments recorded 0.1% viability. No viable seeds were retrieved from any seed lots 15, 18 or 24 months after burial. As for the first seed lot, the rapid decline in viability was associated with a high percentage of seeds germinating in the field, particularly those buried below ground. Surface-located seeds averaged 82% germination after three months compared with 99% for those buried between 2 and 20 cm (Fig. 7).

3.3 Seed biology and fruit production

3.3.1 Methodology

The reproductive capacity and the relationship between size and reproductive performance of rubber bush plants were investigated at five sites (HS Hillside, HS Roadside, MU Paddock, MU Bore and TC) over a period of two years. Additionally, the mean number of seeds per fruit was determined at six populations (Cowan Downs (CD), Bluff Downs (BD), Helen Springs (HS), Muckaty (MU) Tennant Creek (TC) and Katherine (KA)) across Queensland and the Northern Territory.

Life history traits, including seed germination traits, are important indicators of plant invasiveness, because they influence individual establishment and survival. Plasticity of trait response constrains range expansion into new environments. Accordingly, we examined the effects of environmental temperature on both individual seed production (mean numbers of seeds per fruit) and germination performance (germinability G; mean germination time MGT) of rubber bush across an environmental (temperature and rainfall) gradient in northern Australia. The germination response is closely related to the ability of an invasive species to establish and spread at a locality. Seeds were collected from the seven locations mentioned above across the Northern Territory and Queensland (Fig. 9) and their characteristics (i.e. number of seeds per pod, mass), ability to germinate at different depths (0, 3 and 6 cm) and at different temperatures (20, 25, 30, 35, 40 and 45 °C) were tested together with the germination response to water stress. The temperature levels used simulated soil temperature levels observed in the field.

A total of 103 plants were grown in a greenhouse to assess growth at different plant densities densities (i.e. 2, 3, 4, 6, 8 and 12 seedlings/pot, 3 replicates) and age at reproductive maturity. At the age of nine months, several plants had outgrown the greenhouse, therefore a subsample of 31 plants (2, 3, 4, 6, 7 and 9) were selected and followed to the end of the experiment. The minimum age at first flowering was recorded. It was taken that once plants flowered they could form fruit, however, competition for pollinators meant smaller plants need to grow taller (~2 years) to attract pollinators.

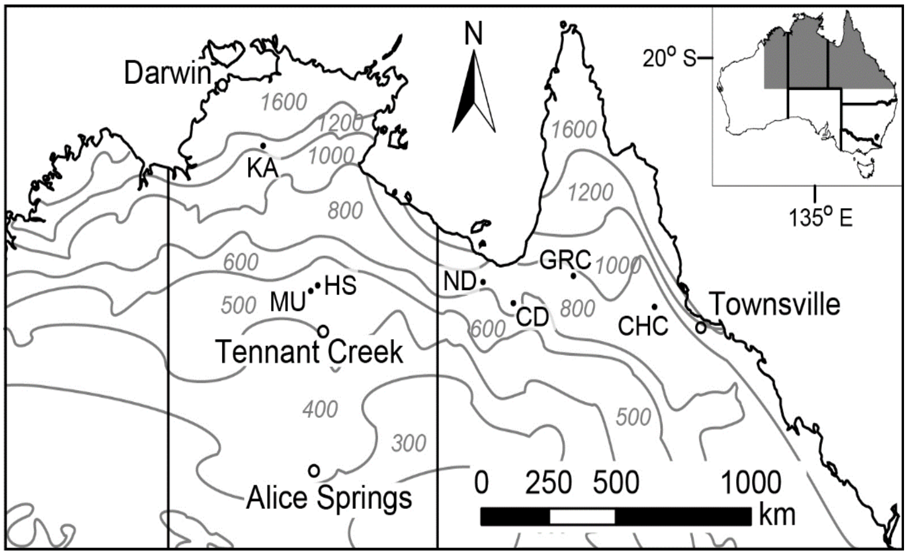


Fig. 9. Locations of Rubber Bush populations from which seed samples were collected (Grey contours are isohyets; Geoscience 2010).

3.3.2 Results

The density of plants differed significantly between MU and MUB and the remainder of the sites, and density at a site influenced the number of fruit set on individual plants (Fig. 10). The number of flowers on a plant was only significantly different at TC (Table 4). Among the studied populations, the density of rubber bush plants was lowest at TC site, while the mean number of fruit set was higher at the site by a wide margin compared to the rest of the sites (i.e. HSH, HSR, MU, MUB).

Table 4. Reproductive parameters of rubber bush estimated at five populations in the Northern Territory (HSH-Helen Springs Hillside, HSR-Helen Springs Roadside, MU-Muckaty, MUB – Muckaty bore, TC – Tennant Creek).

Variable	Site				
	HSH	HSR	MU	MUB	TC
Mean density (plants/ha)*	510±25.5a	520±39.7a	690±49.5b	840±51.3b	393.4±20.8a
Mean pods/plant	4.1	7.0	5.7	0.9	68.7
Mean seed output/plant	1776	3032	2469	390	29761
Mean plant height (m)	1.83±0.1	2.7±0.1	2.42±0.1	2.96± 0.1	3.02±0.2
Adjusted umbels/plant*	58.19a	43.19a	56.55a	36.8a	121.68b
Flowers/Umbel*	11.30±0.47bc	11.5±0.91bc	10.0±0.47b	6.40±0.22a	12.7± 0.96c
Ratio of flowers : fruit	160.38	70.96	99.21	261.69	22.49

*Values within the row with different letters are significantly different ($P < 0.05$).

There was no Allee effect (i.e. no monotonic decline in mean individual fitness at lower densities or population size) and fruit production per plant (fecundity) was lower above and below intermediate densities (250-500 plants/ha) of flowering plants (Fig. 10). Low pollinator pressure at low plant densities and pollinator satiation (i.e. greater than normal pollen limitation) at high plant densities may account for these fruit production trends. In support of this proposition, per plant pollinator visitation rates were low at low and high plant densities, and greatest at intermediate densities, while pollen supplementation experiments showed that rubber bush is pollinator limited even at intermediate densities.

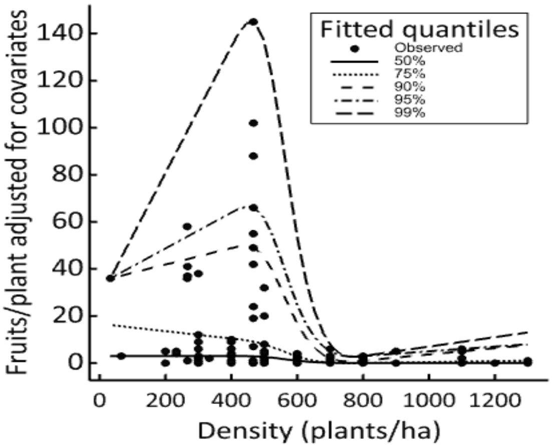


Fig. 10. The relationship between reproductive performance and plant density; fruit production peaks at intermediate plant density.

Of the six sites monitored to quantify the number of seeds produced per fruit, the Katherine population produced the most seeds per fruit on average, with Cowan Downs (CD) producing the least seeds per fruit (Fig. 11), probably due to greater resource availability, thereby allowing for greater numbers of ovules in a flower. However, seed numbers per pod did not vary significantly ($F_{5, 53} = 1.48$, $P = 0.21$) among the populations (433.2 ± 19.0) but seed mass did (6.90 ± 0.28 mg), with no negative correlation between the measures ($F_{6,119} = 18.74$, $P < 0.05$).

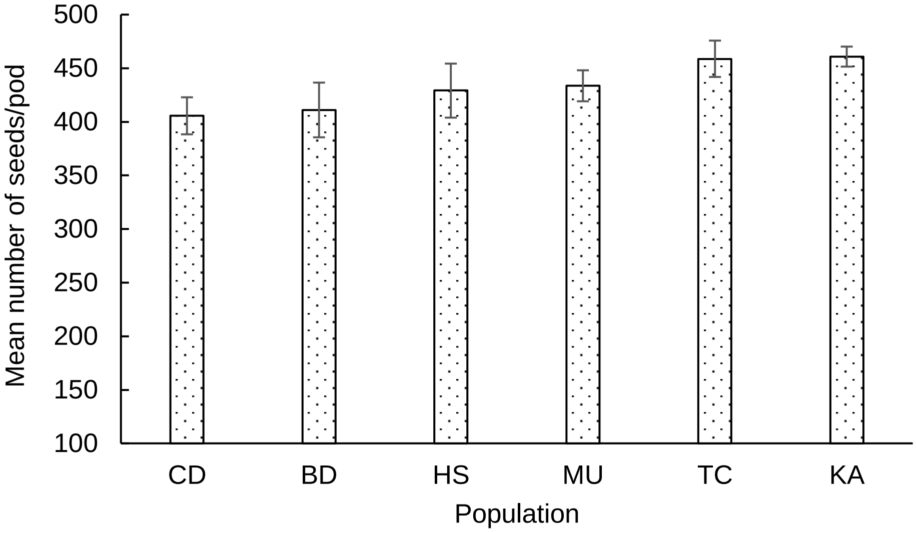


Fig. 11. The number of seeds in a fruit at various populations in Queensland and the Northern Territory (Mean ± s.e. = 433.19 ± 7.92). The vertical bar above each column represents the standard error.

Testing of seeds from a range of sites, revealed that the optimum germination temperature for rubber bush was 30 °C (mean time to germination 2.6 to 3.3 days – Table 5; average percentage germination of 80.9 to 100%), with temperatures ≥40 °C inducing seed quiescence (cf. dormancy) (Fig. 12-14). Planting depth influenced seedling emergence with almost no germination by seeds on the soil surface but 88.5% germination at 3 cm depth. Although seeds had high germinability at 30 °C, large numbers of seeds that germinated too early or too late in the hot wet season dried and became unviable. Nevertheless, a germination rate of 1% of all seeds could translate into a large number of plants due to the abundant seeds (high propagule pressure) produced in one season.

Phenotypic plasticity in both germination and mean germination time was lower at the ends of the environmental gradient, with faster mean germination times at the invasion fronts ensuring rapid transition from reliance on endosperm resources to photosynthesis, thereby increasing the probability of seedling survival.

Table 5. Various germination indices (mean ± SE) and parameters for rubber bush seeds from seven populations, germinated at 30 °C in controlled temperature incubation cabinets. G=Germinability; MGT=Mean Germination Time (days); MGR=Mean Germination Rate; CV=coefficient of variation of MGT; Mass (mg) = mass of a seed in milligrams (HS-Helen Springs Hillside, CHC-Christmas Creek, GRC- Gilbert River Crossing, CD-Cowan Downs, ND – Nardoo, MU-Muckaty, KA-Katherine).

Index	CHC	GRC	CD	ND	HS	MU	KA
G (%)	85.9 ± 8.8	80.9 ± 12.7	100	99.3 ± 0.7	97.9 ± 3.2	82.2 ± 9.7	97.5 ± 2.9
MGT (d)	2.6 ± 0.14	3.2 ± 0.3	2.9 ± 0.2	2.8 ± 0.2	2.8 ± 0.04	3.3 ± 0.1	3.06 ± 0.4
MGR (d^{-1})	0.38± 0.02	0.31 ± 0.04	0.34 ± 0.02	0.35 ± 0.03	0.36 ± 0.01	0.3 ± 0.01	0.33 ± 0.04
CV(MGT)	28 ± 3	31 ± 9	22 ± 6	12 ± 4	22 ± 4	27 ± 7	28 ± 3
Mass (mg)	5.2 ± 0.1	8.3 ± 0.2	8.1 ± 0.2	7.00 ± 0.1	7.8 ± 0.5	5.7 ± 0.3	6.1 ± 0.3
Viability	99	98	99	100	98	100	98

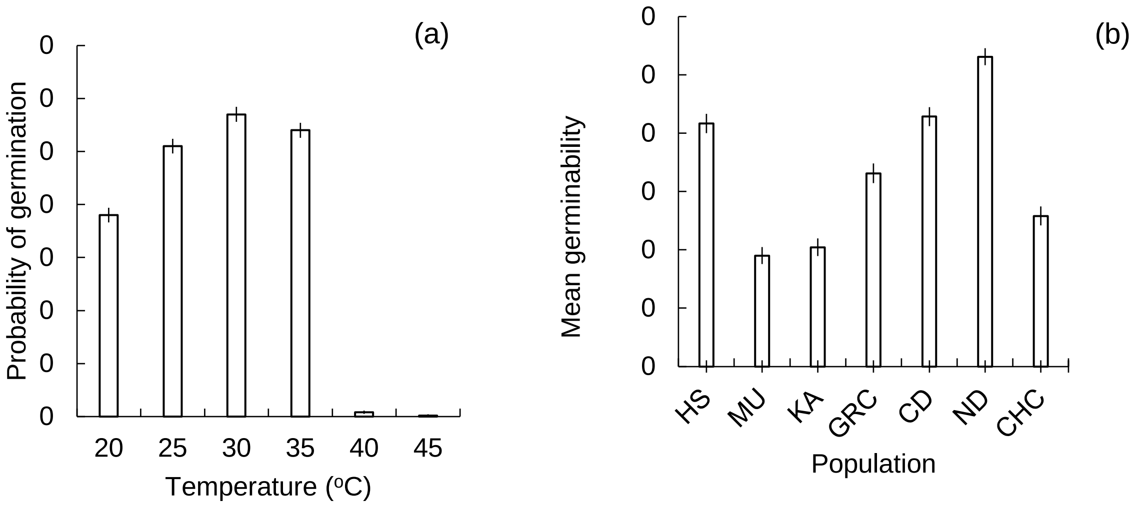


Fig. 12. (a) Probability of seed germination for a given temperature (R^2= -0.35, $F_{5,\ 6299}$ =437.95, P<0.001), and (b) Mean germinability of seeds from different populations across all temperature levels. The vertical bar above each column represents the standard error.

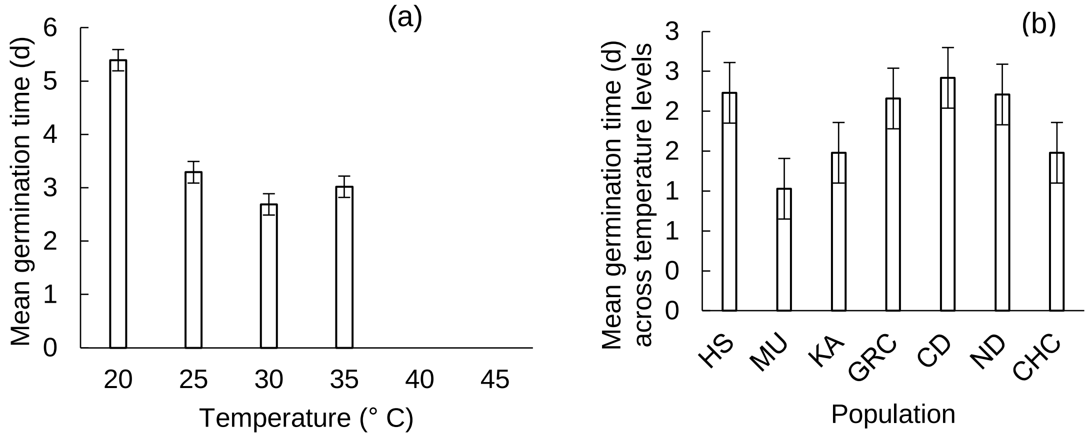


Fig. 13. (a) Mean germination time for all seed samples at various temperatures, and (b) Mean germination time of seeds from different populations (sites arranged from highest to lowest summer temperature). The vertical bar above each column represents the standard error.

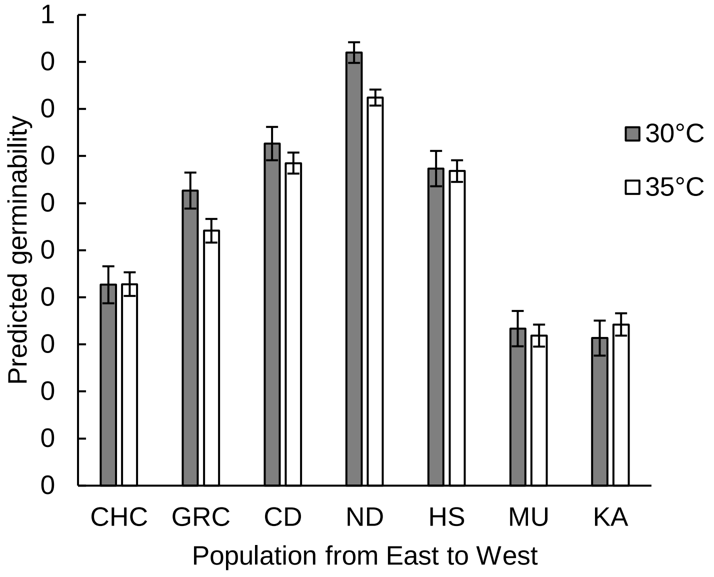


Fig. 14. Germination performance at 30 and 35 °C of rubber bush seeds from different populations. The vertical bar above each column represents the standard error.

The effect of water stress (Water potential-MPa) on seed germinability depended on the temperature. Optimum germination temperature was modified by an interaction between water potential and the germination temperature, for instance higher germination percentage was attained at 20 °C across water potentials (Fig. 15) and the effect was significant ($F_{2,372}$ = 275.5, $P < 0.05$).

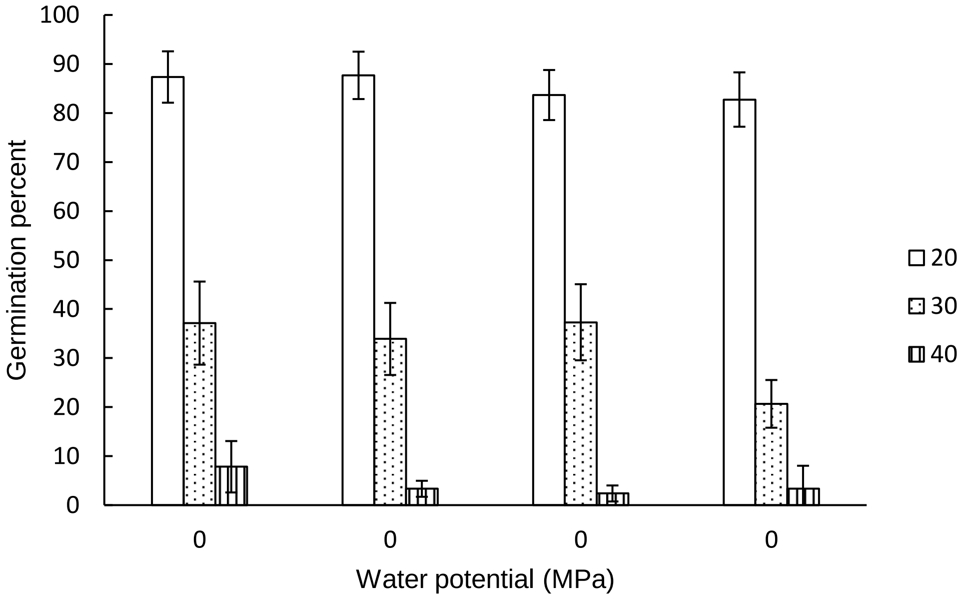


Fig. 15. Germination temperatures of rubber bush seeds at various water potentials. The vertical bar above each column represents the standard error.

In the age to reproductive maturity study, plants grown at low density (2 plants/pot) had few resource constraints, displayed rapid growth and flowered at 11 months of age. At higher seedling densities (≥ 6 plants/pot), plant growth was suppressed over time, with individuals growing slowly if at all. Thus plant density at establishment and at maturity had significant negative effects on plant size (stem diameter; $F_{5, 30} = 9.94$, $P < 0.001$ and height $F_{5, 30} = 5.82$, $P = 0.001$). Small plant size results in a competitive disadvantage for pollinators that delays reproduction even if plants produce flowers.

3.4 Seed Dispersal

3.4.1 Methodology

3.4.1.1 Dispersal modes

A search of the literature and anecdotal evidence was used to identify the modes of dispersal that have been reported for rubber bush.

3.4.1.2 Dispersal kernels

Seed dispersal was investigated at three sites in the Barkly Region that differed in topography. Two sites had flat terrain while one was hilly. Tests were performed during windy, gentle breeze and calm periods of the day, to obtain different wind speeds. Each time, freshly harvested seeds (with wings or pappi) were released from various heights and their dispersal distance measured. Before each simulation, the ground at the experimental site was inspected to ensure there were no residual seeds. The direction of dispersal on release was marked. The distances travelled by seeds were sampled by distance categories or bins, and seeds at different distances were counted in quadrats.

3.4.2 Results

3.4.2.1 Dispersal modes

The search of the literature in combination with anecdotal evidence indicated that rubber bush is mainly wind dispersed, although other mechanisms, such as water and human mediated dispersal (e.g. contaminated vehicles and machinery), play a role. Rubber bush is currently spreading down several water courses and the dispersal of seeds on vehicles/machinery or by human beings occurs, but probably plays a minor role compared to wind. We also observed limited instances (Plate 5) where rubber bush seedlings were emerging from cattle dung, but do not believe that the fruits are generally sought after by livestock as a food source. It is most likely that they are inadvertently consumed at some locations during periods when cattle actively eat the leaves and smaller stems of rubber bush.

Plate 5. Rubber bush seedlings emerging from cattle dung.

Rubber bush seeds possess a coma (silky tuft) that confers buoyancy, both in air and water. On particularly hot days, the seeds are lifted by thermal currents, and once they attain a certain height, may be transported over relatively long distances (>1 km). In the Barkly Tablelands, there are few impediments to the movement of airborne seeds as most of the vegetation is short, or predominantly grassland. Secondly, there are few topographical barriers, such as mountains, and wind dispersal efficiency is maximised. In the Gulf of Carpentaria, it is reported that many seeds float on water, thus some rubber bush populations occur on the shores of islands, interfering with turtle nesting (Bill Jackson, personal communication).

3.4.2.2 Dispersal kernels

The dispersal distance of rubber bush seeds was bimodal and depended on the degree of uplift attained by the seeds at the point of release. The seeds attained higher uplift in gentle wind during hot weather when thermal air currents were strongest. Seeds can be blown long distances after attaining sufficient uplift. Most seeds, however, landed between 10 and 40 metres from the parent plant, with approximately 7.5% of the seeds blown out of sight. Some of the key factors that determined how high a seed was carried included the temperature of the ground surface, which creates thermal currents, seed terminal velocity, wind speed and the height from which the seed was released (Fig. 16). The milkweed bug (*Oncopeltus fasciatus*) was common on branches and below the canopies of rubber bush plants (Enock Menge, personal observation). It laid eggs in fruit and fed on seeds that fell under the canopy of parent plants, thus seeds that dispersed farther escaped predation.

Field observations of young populations that were adjacent to older ones at the three sites showed that groups of seedlings are likely to establish at distances of 1000-1500 m from parent populations after long-distance dispersal. The near dispersal kernel has a greater proportion of seeds falling between 5-20 meters from the parent plant and is responsible for localised population saturation.

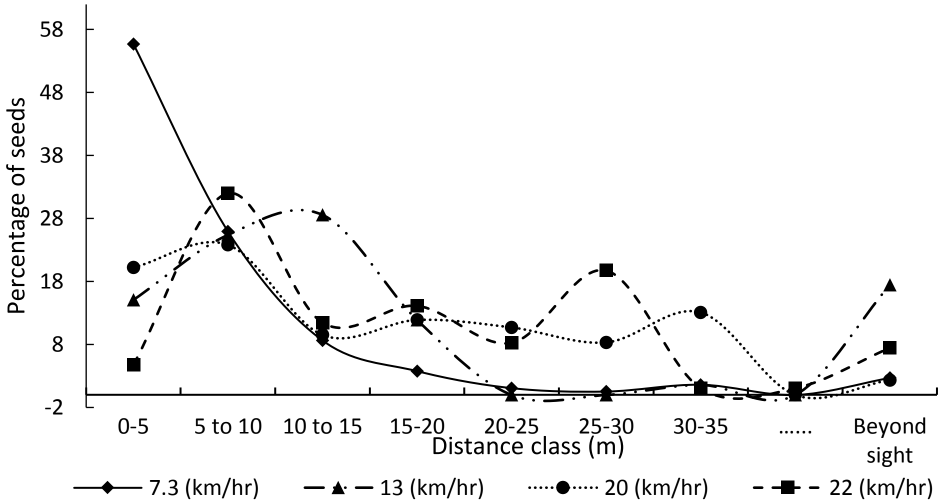


Fig. 16. Dispersal kernel of rubber bush seeds at various wind speeds.

3.5 Competition and invasiveness

3.5.1 Methodology

3.5.1.1 Exclosure experiment

The competitive ability of rubber bush was assessed by sowing seeds on plots subjected to various levels of disturbance within fenced exclosures at two sites with different soil and environmental conditions. The soil at Helen Springs was mainly deep and black cracking clay soil, while at Katherine, the soil was red and sandy. Helen Springs receives about 500 mm annual rainfall while Katherine receives ~1200 mm annual rainfall.

At Helen Springs, there were 12 exclosures while at Katherine, there were 10 exclosures, each with 10 treatment subplots per exclosure. Each of the subplots was treated by one of the following: CL – the grass was clipped to simulate light grazing, CLHO – the grass was clipped and the subplot hoed, CLSD – clipped and then seeds broadcast on the ground, CLHOSD – clipped, hoed and seeds broadcast on the ground, HO – hoed removing all grass, HOSD – hoed and seeds raked into the soil to simulate high levels of disturbance, GZ – plot outside the exclosure, grazed, GZSD – outside the exclosure, grazed but seeds broadcast on the ground, UN – undisturbed, UNSD – undisturbed and seeds broadcast on the ground. Germination performance was monitored for a month. The experiment was repeated twice at each site, i.e. November 2011 and 2012 at Helen Springs; and March and November 2014 at Katherine.

3.5.1.2 Competition experiment

Barley Mitchell grass seeds (*Astrebla pectinata)* were collected from several plants from Helen Springs and grown in pots in a greenhouse together with and separately from rubber bush seeds collected from the same site. Plants were grown in pots filled with cleaned river sand and were irrigated twice daily for five minutes. Growth performance indicators, such as height, stem diameter, leaf width and length, were recorded. The grass and rubber bush seedlings were harvested after three months and the shoot and root dry matter of both species determined. Plant material was dried for 72 hours in an oven at 60 °C and weighed. The performance of each species in mixture was compared with their performance in monoculture.

3.5.2 Results

3.5.2.1 Exclosure experiment

At both field sites (i.e. Katherine and Helen Springs), the experimental evidence that seeds need to be covered by soil to germinate was confirmed. Earlier laboratory experiments demonstrated that temperature was an important determinant of germination performance and that soil cover likely helped to modify the temperature environment thus enhancing germination. At both HS and KA, no seed germination was recorded in undisturbed subplots where seeds were sown on the soil surface (Fig. 17).

The treatments (subplots) represented increasing levels of disturbance to the soil surface and, accordingly, increasingly disturbed conditions for rubber bush seed germination. The treatments that removed grass cover, as well as turned over the soil surface, displayed the best germination success at both Helen Springs and Katherine (10-20%; $F_{9, 119} = 12.7$, $P < 0.001$; and $F_{9, 89} = 3.9$, $P < 0.05$ respectively). A Mantel Test to compare germination performances at HS and KA showed they were comparable ($r = 0.56$).

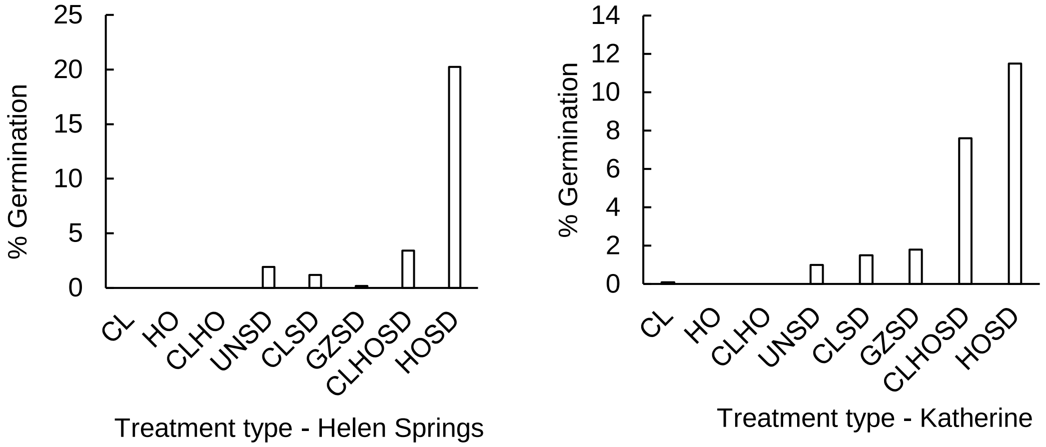


Fig. 17. Germination of seeds in exclosure plots at different levels of disturbance. CL – clipped, HO – hoed, CLHO – clipped and hoed, UNSD – undisturbed and seeded, CLSD – clipped and seeded, GZSD – grazed and seeded, CLHOSD – clipped hoed and seeded, and HOSD – hoed and seeded.

3.5.2.2 Competition experiment

The shoot mass of barley Mitchell grass was significantly reduced ($F_{1, 106} = 15.42$, $P < 0.05$) when grown together with rubber bush (0.06 ± 0.01 g) compared to the monoculture (0.1 ± 0.01 g). In contrast, the mean root mass of barley Mitchell grass was not significantly different between the mixed (0.03 ± 0.004 g) and monoculture grass treatments (0.04 ± 0.01).

The root biomass of rubber bush plants was significantly reduced by the presence of grass seedlings in the same medium ($F_{1, 132} = 37.51$, $P < 0.05$). Mean root mass of rubber bush in mixed culture was 0.05 ± 0.01 g compared to 0.13 ± 0.01g when grown as a monoculture.

Similarly, the shoot biomass of rubber bush plants was significantly reduced ($F_{1, 132} = 41.84$, $P < 0.05$), with mean shoot mass in monoculture averaging 0.31 ± 0.03 g, compared to only 0.1 ± 0.01 g in mixed culture.

Whereas rubber bush affects the development of the shoot in the grass species, the grass species is a stronger competitor as it reduces both the shoot and root biomass of rubber bush. The reduction in the shoot mass of barley Mitchell grass suggests that pasture production is likely to be reduced within rubber bush infestations.

3.6 Distribution and rate of spread

3.6.1 Methodology

Several sources were used to compile a comprehensive list of rubber bush occurrences throughout Australia. Locations were sourced from the online database Australia's Living Atlas (ALA 2014) as well as various Herbaria. In addition, rubber bush locations from surveys undertaken by the Northern Territory Department of Land Resource Management (Weeds Branch), together with records from on-ground surveys throughout the Northern Territory carried out by the authors during this study, were included. In total, 5976 rubber bush occurrence records were collated for mainland Australia. Most records were confined to northern Australia and data and analyses were accordingly restricted to this region.

The MaxEnt tool (MaxEnt version 3.3.3k) available at http://www.cs.princeton.edu/~schapire/maxent/ was used to model the current and potential distribution of rubber bush. MaxEnt is a species distribution modelling software package that uses presence-only data to estimate species' potential distributions by finding the maximum entropy distribution (Elith et al. 2006, Philips et al. 2006, Dudík 2007). The environmental variables that characterise the environment at the species detection localities constrain the output distribution, and these constraints are expressed as simple functions of the environmental variables called features. A suite of environmental variables relating to the topography, fire history, soil properties, climate and land use of the Northern Territory, were identified for inclusion in the modelling to determine the major factors that were contributing to the spread of rubber bush (Appendix 2).

As strong co-linearity can influence the results of MaxEnt models, a Principal Components Analysis (PCA) was run on all continuous variables, using the GenStat Statistical Package (VSN International 2012), prior to model building. PCA was conducted using a correlation matrix (i.e. data were standardised to account for differences in unit measurements between variables) on all of the data layers, except the categorical variables. If variables were strongly correlated with one another, then the variable with the largest combined PC1 and PC2 scores was selected for inclusion in MaxEnt modelling, while all other correlated variables were discarded.

Jack-knife tests and response curves were used to determine the importance of each environmental variable and to illustrate the effect of selected variables on the probability of rubber bush occurrence (Appendix 3). Jack-knife tests provide an alternative estimate of variable importance where each variable is excluded in turn, and a model created with the remaining variables, then a model is created using each variable in isolation. Response curves plot the specific environmental variables on the x-axis and the predicted probability of suitable conditions, as defined by the logistic output, on the y-axis. Upwards trends for variables indicate a positive relationship and downward movements represent a negative relationship; the magnitude of these movements indicates the strength of the relationship. The MaxEnt model produced 10 species-distribution probability maps based on ten-fold cross-validation. The 10 probability maps were then averaged to obtain a habitat suitability map for rubber bush.

MaxEnt also offers a ‘projection’ option which allowed us to model the potential distribution of rubber bush under predicted changed climate conditions. The model was first trained on the environmental variables corresponding to current climate conditions, and then projected using the same future climate scenario variables. We sourced future climate spatial data from the Tropical Data Hub (https://eresearch.jcu.edu.au/tdh). To reduce uncertainty in single global circulation model (GCM) predictions and offset errors in individual GCMs, we used a multi-model ensemble average from the 18 GCMs provided for the RCP 4.5 emission scenario (Gleckler et al. 2008, Pierce et al. 2009). We projected future potential distribution using mean daily temperature (BioClim1) and mean annual rainfall (BioClim12), as they were the only environmental variables available that corresponded to those used in the current climate models.

A weed risk assessment (WRA) was also performed using the information generated from the current research project and the published literature as inputs into the WRA tool developed by the Commonwealth Department of Agriculture, Forestry and Fisheries (Department of Agriculture 2015).

3.6.2 Results

Currently, rubber bush is mainly confined to the Northern Territory, northern Queensland and north eastern border regions of Western Australia (Fig. 18). A closer inspection of the presence data (based on when locations were recorded) over the last four decades, shows that this species has expanded its range since the 1940s and still continues to spread. This is aided mainly by a mixed mating system, a pollination system that is generalized at the level of Order Hymenoptera - widely distributed effective pollinators in northern Australia, and efficient wind dispersal of seed. Range expansion was assessed as part of evaluating the weed risk posed by rubber bush. The main environmental predictors of current distribution were distance to roads (25.3%), average rainfall (21%), mean temperature (21%) wind (14.6%), beef density (13.6%) and vegetation type (4.6%). The models showed that the current potential distributional range of rubber bush has not been saturated.

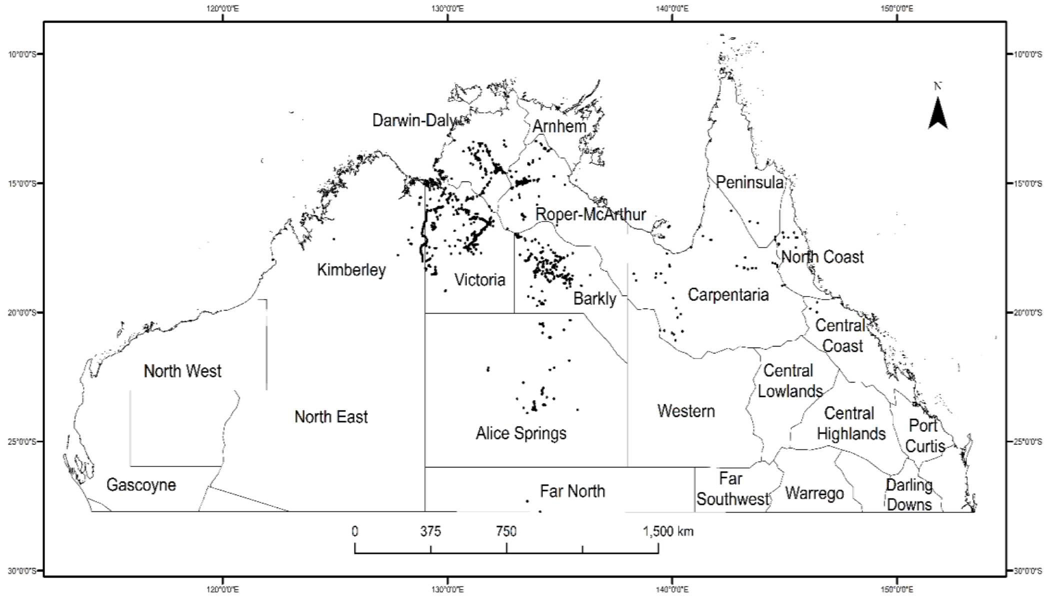


Fig. 18. Current distribution of rubber bush across northern Australia (1940-2012) based on the sources outlined previously.

The projected distribution based on the future climate scenario extended further eastward within the Northern Territory's Roper-McArthur, Barkly and Alice Springs regions (Fig. 19a-c), spreading into Queensland's Carpentaria and Western regions. There was also some projected spread into Cape York Peninsula. In Western Australia the main concentration of projected spread was in the north-west and north-east regions with some spread occurring into the northern Kimberley region of Western Australia. A slight contraction of suitable conditions in the Northern Territory's Darwin-Daly region and in the arid centre around Alice Springs was also projected. Overall, the future climate scenario predicts there will be more suitable conditions throughout the Northern Territory for rubber bush (Fig. 20). Thus, rubber bush has not saturated its current potential distribution, and climate change projections indicate that it will expand its current range in all surrounding states.

Model performance was good (mean AUC±SD = 0.897 ± 0.021). Change in temperature, based on the projected variables, provides more novel climatic conditions suitable for rubber bush expansion than rainfall. Similar to the current potential distribution model, distance to roads, average rainfall, mean temperature, wind, beef density and vegetation type were included in the projection model. The projection model was expanded to include the whole of northern Australia from northern Queensland to Western Australia. The model predictions consistently showed a southward expansion of the suitable range of rubber bush under projected future climate scenarios. In the Northern Territory, there is reduction in the current non-suitable range under future climatic conditions (25.7%), while the increase in the suitable range is considerable (between 22.2-715.8%; Table 6).

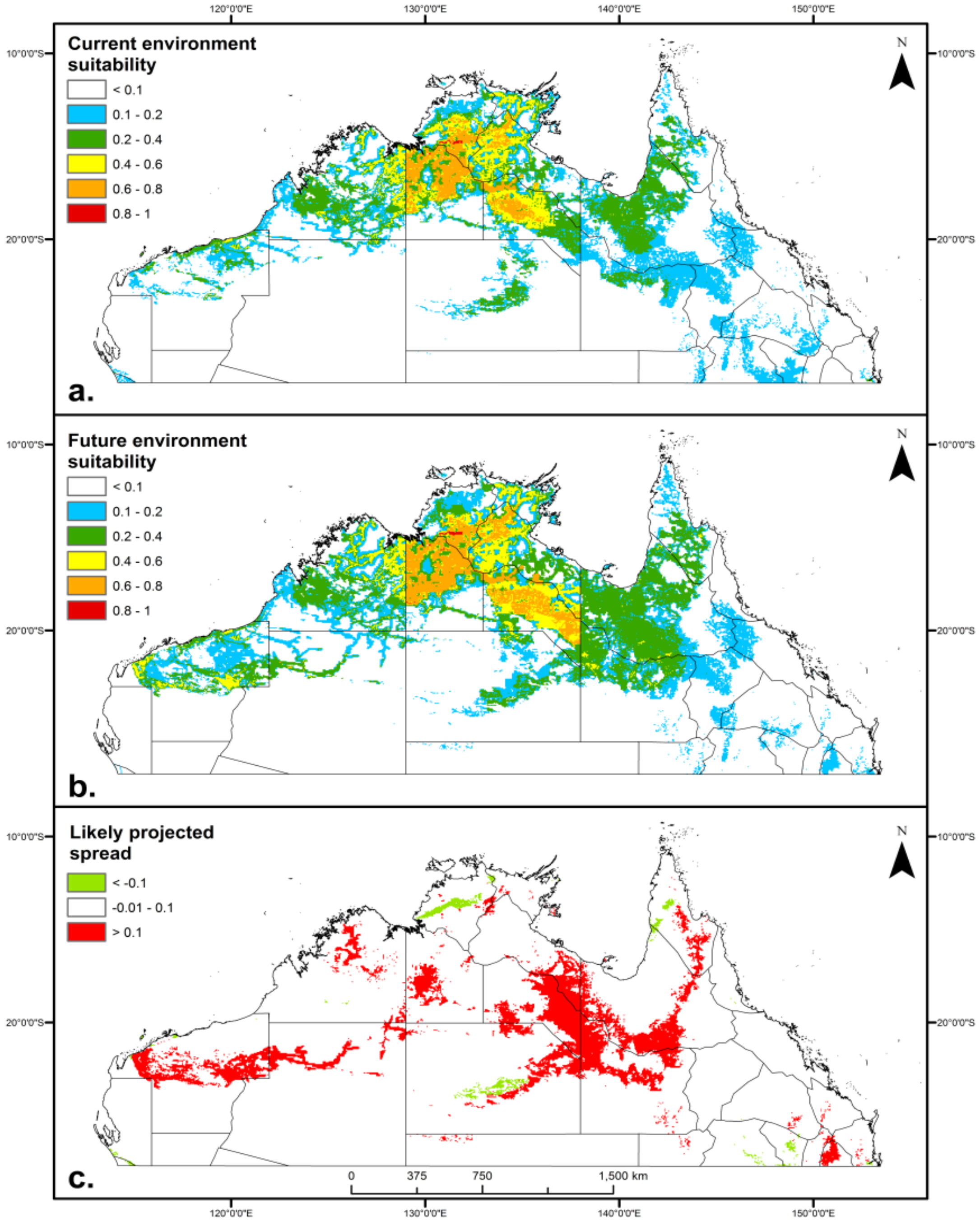


Fig. 19. Current and potential future distribution of rubber bush across northern Australia: (a) Current environment suitability, (b) future environment suitability (under climate change) and (c) areas most at risk of future spread. For figures a and b, areas classified as less than 0.1 (clear) are unsuitable, with suitability increasing thereafter up to the most favourable areas with ratings of 0.6-1 (orange and red). For figure c, the greatest spread is anticipated in areas classified as >0.1 (red).

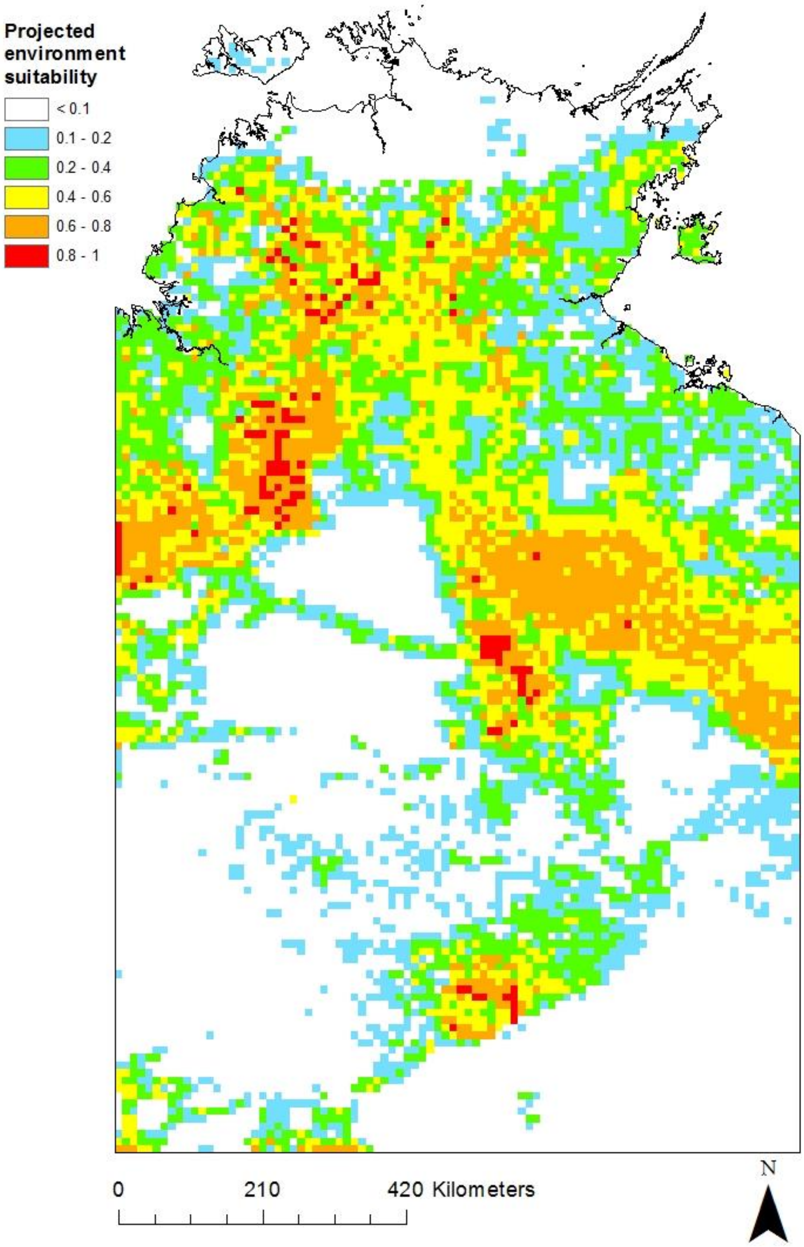


Fig. 20. Potential future distribution of rubber bush in the Northern Territory. Red, orange and yellow colours show high probability of suitable conditions for rubber bush, while green and blue colours represent low predicted probability of suitable conditions. White areas indicate areas not suitable for rubber bush.

Table 6. Northern Territory potential distribution range for rubber bush in the current- and future-climate scenarios, as predicted by MaxEnt. Probability of presence varies from non-suitable areas (<0.1) to suitable areas (>0.1). Areas suitable for rubber bush are expected to increase in a future-climate scenario.

Probability of presence	Current-climate area (km$^{2)}$	Future-climate area (km^2)	% change
< 0.1	702800	522200	-25.7
0.1 - 0.2	130400	159400	22.2
0.2 - 0.4	158300	175000	10.5
0.4 - 0.6	102600	163600	59.5
0.6 - 0.8	59800	119800	100.3
0.8 – 1	1900	15500	715.8

In completing a weed risk assessment (WRA) on rubber bush using the tool developed by the Department of Agriculture, Forestry and Fisheries, it was possible to answer 37 questions partitioned into 8 on biogeography, 10 on undesirable attributes and 19 on its biology and ecology (Appendix 4). This resulted in a final score of 28 (Appendix 5), which is equivalent to a 'reject' if rubber bush was under consideration for introduction into Australia (Department of Agriculture 2015), where the tolerance level is a score of 6. In terms of the relevance of the score to the current situation, it implies that rubber bush is a moderate to high risk invasive plant in diverse parts of the world including Australia, Brazil and Hawaii (Grace 2006, Rangel & Nascimento 2011a, Leal et al. 2013) and is likely to become a major weed risk under future climate scenarios.

3.7 Discussion

Several aspects of the ecology, invasiveness and distribution of rubber bush were investigated in the course of the research presented here. Rubber bush is invasive mainly because a single fruit produces seeds in profusion (~430 seeds per fruit; i.e. immense propagule pressure) that are wind dispersed, which is a reliable generalised dispersal system common to many invasive plant species (Sen et al. 1968, Sharma & Amritphale 2008). Several species of native wasps, carpenter bees and honey bees are effective pollinators of rubber bush (Willmer 1988, Leys 2000), and given the wide distribution and moderate abundance of these Hymenopterans across north Australia, rubber bush is likely to have effective pollinators at most localities in tropical Australia.

Soil surface temperature was found to have a profound effect on the germinability of seeds, such that seeds that were not buried did not germinate. Thus, seeds must be covered or buried in the soil to germinate. The distribution and current spread of rubber bush in north Australia is unsurprisingly associated with disturbance by pastoral practices. There is clearly a need to avoid cattle trampling seeds into the soil and affording them the much needed soil cover for germination, particularly in hotter environments. Overall, rubber bush is a poor competitor of native species at the seed and early seedling stage, and does not easily invade intact rangelands. It has remarkable survivability when mature due to prolific resprouting (Csurhes 2009, Biosecurity-Queensland 2013). Thus, ripping and burning are not suitable or effective control methods of rubber bush. Resprouting allows rubber bush to persist through the latter disturbances and regenerate from the soil seed bank when conditions are suitable.

3.7.1 Reproduction

The synorganisation of the flower, while apparently complex and unusual in an invasive species, provides reproductive assurance through self-compatibility and the transfer of hundreds of pollen grains in solid pollinia (agglutinated pollen grains in a single sac) in one pollination event. The successful transfer of a single pollinium results in several hundred seeds produced per fruit and, under normal circumstances, the propagule pressure that confers considerable invasive potential (Eisikowitch 1986b, Forster 1998, Fabricante et al. 2013).

The mutualism between generalised Hymenopteran pollinators and this invasive plant species is a good example of a functional form of biotic interaction that influences invasion success. We investigated the pollination ecology of rubber bush with the latter premise in mind. In some instances, invasive plants are unable to spread in a new environment due to reproductive failure brought about by a lack of suitable pollinators (failure of pollination mutualism). This is usually taken into account when evaluating the weed risk posed by a plant species. The rubber bush flower has a complex morphology and a pollination system requiring the extraction of solid pollinia (pollen sacs) by the pollinator which, in turn, limits pollination to larger wasps and a few larger bee species (Ali & Ali 1989). Nevertheless, several native Hymenopterans with wide distribution in northern Australia are capable of pollinating it. Some of the wasp species are native to dry and hot areas, and their life histories require that they hibernate over winter (Evans 1966, Leys 2000, Tryjanowski et al. 2010). Thus although rubber bush flowers year-round, fruit set generally only occurs during the hotter and wetter months of summer in the Northern Territory and exposes rubber bush to pollinator limitation, whereas it fruits throughout the year in Queensland (Moodley et al. 2015).

Shifts in climate and/or range expansion by both rubber bush and pollinator species could extend the fruiting season and accelerate invasion by rubber bush, bearing in mind that it only takes one successful pollination event, even a self-pollination event, for the production of hundreds of seeds in a single fruit. Interestingly, the mean number of seeds per fruit (~430 seeds) for Australian populations is almost twice the number per fruit observed in populations in India (native range) (230 ± 19 [$\bar{x}$ ± SE]) (Sharma & Amritphale 2008). The latter may arise because the Australian environment is more resource rich and thus more pollen grains are produced in the pollinium, as well as more ovules in the fruits. Whatever the explanation, more seeds per fruits exerts twice the propagule pressure on the Australian environment, and highlights the greater invasive potential of rubber bush in Australia than elsewhere where it is also considered a weed.

Wind dispersal poses one important challenge for invasive plants – that is, the lack of mates when plants establish as singletons (Lipow & Wyatt 2000, Petanidou et al. 2012, Chrobock et al. 2013). When plants are not self-compatible, the single individual is unable to reproduce until a second plant establishes and this slows the process of invasion. Invasive species however, are known to have a facultative breeding system where they can reproduce through self-fertilisation or cross-pollination (Petanidou et al. 2012). Rubber bush uses self-compatibility to achieve reproductive assurance that confers significant invasive potential and is also able to cross breed.

The flowering and fruiting phenology of plants may contribute to their invasive characteristics, particularly if their phenology helps to ensure reproductive success under diverse climatic conditions. In addition, climate change modifies the phenology of both plants and animals (Visser & Both 2005, Cleland et al. 2007), and could consequently influence existing mutualisms in the future. In Australia, rubber bush has a characteristically long flowering season. This is thought to be a response to low pollinator pressure (Alonso 2004, Chrobock et al. 2013), which ultimately results in stand density-dependent rates of fruiting and seed set. In its native range, rubber bush flowers for a short period of the year (Willmer 1988, Sharma & Amritphale 2008). The long flowering season in Australia and other invaded regions maximises reproductive opportunity (and invasive potential) where Hymenopteran pollinators are generally at low densities and even absent during the winter months (Evans 1966). Under future climate scenarios, temperatures are predicted to increase and the wet season is likely to be shorter and the dry season longer. Thus, it is likely that the fruiting season for rubber bush will be longer. Management from year to year will need to factor in the observed changes in the lengths of seasons. Rubber bush stands in the northern regions of the range of rubber bush where pollinators do not ‘over-winter’ (or hibernate) should be a high priority for control.

3.7.2 Seed dispersal and biology

Rubber bush seeds possess a coma (silky tuft) that is an adaptation for wind dispersal, and our observations show that wind dispersal is responsible for localised population saturation. Major long distance dispersal events, such as from continent to continent or from region to region, were almost certainly mediated by humans. However, we could not ascertain if humans have continued to disperse rubber bush seeds, and the genetic analyses needed to determine whether rubber bush has expanded its range from several or a few points of introduction (Cain et al. 2000) is a future research priority (see Section 5.3). Human supported seed dispersal could release the invasion process and rubber bush populations from low genetic diversity caused by the limitations of a self-compatible breeding system (Ward 2006).

At the local level, wind dispersal is an effective and reliable dispersal agent of rubber bush seeds. The overall local dispersal kernel across all sites and conditions was bimodal where most of the seeds were dispersed to a near distance and a small percentage (~7.5%) of the seeds was blown beyond sight, potentially contributing to long-distance dispersal. Seed uplifting probability sets an upper bound on the probability of long-distance colonisation (Nathan et al. 2002). Thermal currents are common in the Barkly region particularly when there is strong sunshine and gentle wind. Although the proportion of seeds that were uplifted was less than 0.1 in this study, this low incidence is still important because a viable population can start with a single plant. Careful management of grazing is critical, particularly for paddocks that are downwind from any large infestations, because with 7.5% of seeds dispersing over relatively long distances, these plants escape the challenges associated with high-density populations, such as competition for resources and pollinators, consequently having high establishment success and thus need to be eradicated.

The near dispersal kernel had a greater proportion of seeds falling between 5-20 m from the parent plants, thus over time, plants that are close together are likely to have higher than expected genetic similarities, and consequently inbreeding. This is indirectly supported by evidence of lower phenotypic plasticity of germination traits in populations at potential invasion fronts in both the west and east of rubber bushes range in north Australia. Nevertheless, small founder populations in wind dispersed species have been reported to attain rapid genetic and demographic homogenization thus overcoming negative effects of inbreeding (Machon et al. 2003).

Rubber bush plants produce thousands of wind-dispersed seeds during the fruiting season. Wind dispersal causes seed to be deposited at random in different microsite conditions. Thus, unsurprisingly, rubber bush seeds are adapted to cope with these varied conditions, for example, by having quiescent (Yang et al. 2012) seeds. Ordinarily, non-dormant seeds cannot complete germination when one or more of the primary requirements (temperature, moisture, light, oxygen) is sub-optimal, such as if the temperature is not in a critical range (Dalling et al. 2011). In dormant seed, the seed coat prevents imbibition and the seed has to undergo certain treatment(s) to break dormancy, such as scarification, chilling or several drying and cooling cycles. In contrast, quiescent seeds germinate immediately conditions are suitable and do not require strictly defined dormancy-breaking events to germinate. Rubber bush seeds are mostly quiescent and were observed to produce a gelatinous water retaining substance around the emerging radical when germination was interrupted, which offers some protection in the short-term to fluctuating environmental conditions (Huang et al. 2008, Yang et al. 2012). Although seed lots used in our study exhibited high germinability, the literature does report a few instances of low-level dormancy in rubber bush. Between 2% and 35% of rubber bush seeds collected from an Indian population (Amritphale et al. 1984) and ≤ 6% from a Brazilian population, were reported to be dormant (Labouriau & Valadares 1976). Low-level dormancy may prolong the longevity of some rubber bush seed banks beyond those reported in the current study.

Surprisingly, at low water potential, rubber bush seeds mostly germinated at temperatures below 30 °C, perhaps an indication that water stress lowers the optimum germination temperature in rubber bush, as in some other wind dispersed species, such as Egyptian broomrape (*Orobanche aegyptiaca*) (Kebreab et al. 1999, Kebreab & Murdoch 2000) that are adapted to dry environments. Imbibed seeds deteriorate rapidly if conditions are not conducive to germination (e.g. exposed on the soil surface or an unsuitable substrate), and have much reduced viability and longevity (of the order of a few months). Combined with profuse seed production and wind dispersal, seed quiescence thus ensures rubber bush maximises opportunities within its regeneration niche.

The high germination rates of rubber bush seed under hot tropical temperatures in our study was consistent with those reported in a Brazilian study (Labouriau & Valadares 1976), where germination was highest between 23 and 33 °C. It is plausible that longevity could be extended in areas that receive periods of extreme temperatures (too hot or cold) that may prevent germination. For example, Labouriau & Valadares (1976) recorded little germination once temperatures reached ≥34 °C, but germination of these same seeds once temperatures fell to 30 °C. Besides these potential inhibitory effects on germination, surface seeds exposed to very high temperatures in summer may become unviable.

The ability of rubber bush to germinate in soils at depth suggests that light is not a necessary condition for germination and is consistent with findings of Leal et al. (2013) who reported high germination under a range of light intensities ranging from 0 to 100%.

In summary, the high germinability of rubber bush seed results in it having a relatively short lived seed bank (<2 years) if favourable temperature and rainfall conditions prevail (Bebawi et al. 2015). It is therefore dependent on regular replenishment from reproductive adult plants for substantial seedling emergence. Consequently, large scale recruitment is most likely within the first 12 months of control programs, and land managers could potentially achieve effective control of rubber bush patches in a 2-3 year timeframe, if they are able to kill all original plants and undertake follow-up control frequently enough to prevent any new plants from reaching reproductive maturity. This is based on the assumption that replenishment of the seed bank is not occurring from external sources (e.g. wind and water dispersal). With regards to the frequency that follow up control would need to be undertaken, plants in the age to reproductive maturity study took at least 11 months to flower at low plant densities. In a pot trial in Queensland, plants took an average of six months to flower (range of 4-8 months) and 13.5 months (range of 13-14 months) to produce pods (Bebawi et al. 2015). Therefore, follow up control activities undertaken ~12 monthly should avoid replenishment of soil seed banks, although six monthly intervals would be preferable, to provide two opportunities to find and control any new plants before they reach reproductive maturity.

3.7.3 Competitiveness and invasiveness

As a general rule, invasive species that have generalised breeding systems and seed biology, tend to overlap with native species in habitat and resource use, and are thus not good competitors and cannot easily invade intact native habitat (Lockwood et al. 2007). Support for competitive resistance of intact native habitats to invasion by weed species comes from grasslands (Petryna et al. 2002) and shrublands (Keeley et al. 2003). Rubber bush is no exception to this rule and displayed no to low rates of establishment in intact native habitat, but establishment rates of 10-20% of seeds in disturbed native habitat, particularly where superficial soils had been turned over by disturbance (see Vincke et al. 2010), for example by cattle or on roadside berms. Once established, rubber bush plants persist even in arid environments, mainly due to superior drought resistance of mature plants (Boutraa 2010, Boutraa & Akhkha 2010, Tezara et al. 2011) and the ability to re-sprout (Grace 2006).

Our competition experiment demonstrated that rubber bush suppressed the shoot mass of the native barley Mitchell grass. However, the grass species was a more effective competitor, reducing both the root and shoot mass of rubber bush. Interactions between an invading species and a native one may take many forms including allelopathy (Cheam 1984a,b) and exploitation competition (i.e. differential efficiency of resource acquisition) (Vance 1984). Any reduction in grass production is a major concern to graziers as pastures are the main feed resource for cattle. Thus, rubber bush is an undesirable component of rangelands because, where established in paddocks, it does reduce the amount of pasture produced by native grass species. In contrast, Cheam (1984a,b) showed that the invasive buffel grass (*Cenchrus ciliaris*) had a competitive interaction with rubber bush, such that rubber bush was excluded by the grass. Overall, rubber bush is not overly competitive and does not easily invade intact rangeland, accounting for why it is most abundant in disturbed rangelands.

Understanding the relationship between disturbance and establishment success is essential because disturbance is an important component of any ecosystem that shapes community species composition (Hobbs & Huenneke 1992). Disturbance may facilitate plant species invasions (Hierro et al. 2006). Our exclosure experiments clearly demonstrated under natural conditions in the field that seeds deposited on the soil surface were unlikely to germinate, but germinated at rates of 10-20% where the soil treatments simulated trampling and turnover by cattle. Thus, in the wild, although rubber bush produces numerous efficiently dispersed seeds, populations do not always grow as fast as expected. The need to manage propagule pressure (i.e. prevent successful seedling establishment) was clearly indicated in the exclosure experiments.

From observations in the field, rubber bush populations are often associated with areas receiving high grazing pressure by cattle (e.g. close to watering points) and facilitated by animal/mechanical disturbance, as predicted by Hierro et al. (2006). Cattle bury rubber bush seeds ensuring high germination. Surprisingly, the role of soil type on the germination performance of rubber bush seeds in the exclosures was negligible, as there was no difference between black cracking soils (Barkly Tablelands) and red sandy soils (Katherine).

3.7.4 Distribution and rate of spread

Two important ecological questions are central to determining the invasiveness of rubber bush: (1) does the current distribution of rubber bush cover all of its environmentally determined potential distribution; and (2) will the range of the species expand or contract with climate change? To answer these questions we used species distribution models (SDM's). The ability to predict future species distributions is important and has become common due to the availability of powerful software packages that are able to match the occurrences of a species with environmental variables (Philips *et al*. 2006, Thuiller *et al*. 2009). These features are then used as constraints for predicting future distribution. Maximum Entropy Modelling (MaxEnt) tool is one such software package (Philips & Dudik 2007) and has been used with high reliability in several cases (i.e. Kumar & Stohlgren 2009) and was adopted for our study. Among the predictor variables of current distribution of rubber bush, the most important were mean distance to roads, mean rainfall and mean temperature. The other environmental factors were wind (speed and direction) and beef density. Vegetation type had only a slight influence (4.6%) on current rubber bush distribution, which is consistent with our current understanding of the species' ecology. Mean distance to roads and beef density are factors associated with rangeland management practices that may predispose an area to infestation. For example, during the dry season the maintenance of roads on properties and animal trampling around watering points buries seeds, thus improving the likelihood of seed germination at these disturbed sites. Currently rubber bush occupies only a part of its potential range, and furthermore, its occurrence is closely associated with road infrastructure, bores, mustering yards and disused mine sites. Thus, its range expansion is strongly associated with range management practices/human activity. Some minor changes in infrastructure (such as use of water tanks in place of earthen dams or 'turkey nests') may help mitigate this mode of spread. A review of grazing management practices may be warranted in some cases depending on the invasive risk profile of a property.

Projecting rubber bush distribution under future climate conditions, we used mean rainfall and mean temperature, which we have demonstrated have significant effects on seed germination. Temperature may have other effects besides directly acting as a cue for various physiological processes. It affects pollinator-flower mutualisms in that temperature determines availability of pollinators (Eisikowitch 1986b, Willmer 2011). Because rubber bush is wind dispersed, the dispersal distances will be influenced by topography and changes to wind dynamics under future climate (Nathan & Muller-Landau 2000, Nathan et al. 2011). Based on temperature and rainfall projections of future climate scenarios, rubber bush may expand its range by between 22.2-715.8% across the Northern Territory and the neighbouring states. There are low-density populations of rubber bush in the Kimberley region of Western Australia, and the species tolerates salty soils to establish on beach sand in the Gulf of Carpentaria where it interferes with turtle breeding. Rubber bush has, however, not occupied all its suitable range mainly because of scarcity of suitable microsites (i.e. disturbed sites) and dispersal limitations. Projected climate changes are likely to lengthen the fruiting season of existing populations and increase the invasive capacity. Properties that neighbour large infestations are at risk, thus a regional approach to the management of rubber bush is advisable. Overall, the future climate scenario predicts there will be more suitable conditions for rubber bush throughout the Northern Territory and neighbouring States. This prediction is similar to the predicted spread of other woody weeds such as prickly acacia (*Vachellia nilotica*) (Kriticos et al. 2003) and parkinsonia (*Parkinsonia aculeata*) (Van Klinken et al. 2009).

3.7.5 Controlling rubber bush

While the ultimate aim of any control program would be the total eradication of rubber bush this is not always possible at a site for logistical reasons, cost, and because regeneration from soil seed banks or by resprouting will inevitably demand several follow-up treatments. The dispersal mechanisms of rubber bush also means that reinvasion from neighbouring infestations is also a possibility. Nevertheless, our research findings provide some best practice guidelines for managing rubber bush:

1. Timing control activities to coincide with winter (cooler months or dry season in the Northern Territory) is not only practical and advisable, but also likely to achieve greater success because pollinators are not abundant and thus, the few plants that escape treatment will produce relatively fewer fruit in the following summer season.
2. With fruit production peaking at intermediate stand densities (isolated = 1-100 plants in an area at densities of 1-5 plants/ha; low density = <250 plants/ha; medium density = 250-500 plants/ha; high density >500 plants/ha), management of this weed should take advantage of low pollinator pressure at low stand densities to aid in controlling naturalised stands:
    a. Because ‘low’ density populations are pollen limited and produce fewer fruits per plant, control programs should aim at initially reducing stand densities to no reproductive trees standing and then undertake follow-up to remove seedlings that may appear afterwards.
    b. High density stands are pollinator limited and produce as many fruits per plant as low density stands. Controlling these often large populations may best be achieved in stages (for logistical or cost reasons), provided that stand densities are reduced to low levels in one year, and there is follow-up treatment as soon as possible to remove survivors and recruits, preferably within 6 months.

   c. On no account should control methods leave stands in medium density condition, because at these densities reproduction is optimised and maximum numbers of fruits per plant are produced. One is essentially farming rubber bush and maximising fruit production under these conditions.
   d. Where triage is required at a locality containing various densities, landholders should consider targeting medium density stands first, then high density stands, followed by low density and isolated stands.
   e. Control (removal of individuals) activities should aim at killing all rubber bush plants at a locality within two consecutive years. A follow-up third year is advisable to remove regeneration by seeding and from the soil seed bank.
   f. Lastly, isolated trees must be removed wherever encountered because a self-compatible breeding system allows single individuals to exert considerable propagule pressure, and establish a stand with in a few years if pollinators are present.
3. Avoid putting cattle into paddocks or disturbing established rubber bush stands during the fruiting and seeding period (September to February). This will minimise burial of seed through trampling by cattle, and in turn, germination and seedling establishment will be much reduced.
4. Avoidance of grazing on such paddocks during fruiting may also help minimise germination and establishment of new plants. Intense grazing pressure on a paddock that removes ground cover will lead to the establishment of rubber bush if seeds are present in the soil. Pastoralists can reduce establishment rates by avoiding putting cattle into paddocks that have fruiting rubber bush plants in them.
5. Where livestock feed on rubber bush without negative effects (e.g. parts of the Barkly and Katherine regions and in the Gulf of Carpentaria) (Plate 6), allowing them to browse on the plants just before the onset of summer, and the peak flowering period, may also disrupt reproduction by rubber bush. However, this management practice should not be seen as a replacement for direct control methods that remove plants; only as an aid to thinning stands.

Plate 6. A rubber bush stand after severe browsing by cattle in the Barkly region.

6. At this time, no native herbivores are known that regulate rubber bush stand density. A seed eating insect (*Oncopeltus fasciatus*) was found on fruits or below the canopy at several of the sites in the Northern Territory and further research is required to investigate its bio-control potential. Natural large-scale dieback of stands was also observed at several sites in Queensland (See section 4.10.7) and more recently, in the Barkly Tablelands Region of the Northern Territory. While there is a historical precedent for diebacks of dense rubber bush stands, associated with long runs of drier than usual years in the 1980s in the Victoria River District (Foran et al. 1985, Bastin et al. 2003), in the diebacks observed in this project a leaf spot disease has been implicated. Further research is recommended to ascertain the exact cause or synergy of causes (e.g. low rainfall may promote leaf spot), which resulted in a 96% decline since December 2012 in a population in the Gulf of Carpentaria Region.
7. A recommended practice is to ensure that all motor vehicles used in the transportation of stock from infested to uninfested areas are cleaned of contamination, and hay from infested areas should not be taken to uninfested areas. Anecdotal evidence suggests that seeds also float on water, thus giving rise to populations on islands off Queensland's Gulf of Carpentaria. Control of rubber bush along water courses is thus a priority.
8. Mean temperature is predicted to increase under future climate conditions, with a shorter wet season and a warmer and longer dry season. Thus, the fruiting season for rubber bush is likely to be longer and aid further range expansion into regions adjacent to its current range. Management from year-to-year will need to factor in the observed changes in the lengths of fruiting seasons and interventions must be timed to occur before fruit set, which in turn, may be determined by the timing of abundance of Hymenopteran pollinators. Monitoring the latter may provide insights on when to initiate removal of rubber bush in seasonally cooler parts of its range. In terms of the influence of phenology on management strategies, the same strategy cannot be followed in Queensland and the Northern Territory.
9. Rubber bush management zones could be established from Western Australia through the Northern Territory to Queensland based on population sizes. On the basis of available data, rubber bush populations in areas south of the 20th Parallel are sparse and could be targeted for eradication. Populations found in areas between the 20th and the 14th parallels are mostly extensive and dense. These populations require a combination of maintenance management and grazing management to minimize establishment of new plants. Reduction in populations in the severely invaded areas should become evident on infested properties within three to four years (i.e. 3-4 wet seasons). Locations to the east of the Tablelands Highway and west of Alice Springs, have generally proven less susceptible to rubber bush invasion over the last several decades. These locations should be monitored continuously because if they get invaded, it will be a sign of the failure of certain environmental filters that have limited the extensive colonisation of the areas.

3.8 Weed risk assessment of rubber bush

Rubber bush has the reproductive capacity, efficient seed dispersal mechanisms, adaptation of germination responses to unpredictable environmental conditions and plant longevity as a result of resprouting, all of which make it currently a low to moderately invasive plant species in the Australian environment, with the potential to become a high risk weed in the future. A weed risk assessment using the DAF Weed Risk Assessment system and incorporating the findings of this project produced a score of 28. On the basis of tolerance limits proposed under the DAF system, 7 is the basis for ‘rejecting’ a species under evaluation (Department of Agriculture 2015) (See Appendix 4). Additionally, the plant is already invasive in Brazil where it was introduced around the same time as in Australia (Grace 2006, Leal et al. 2013) and in Hawaii (PIER 2011). Certain regional populations, such as those on the Barkly Tableland and the Victoria River district, have had long residence and act as source populations for future spread.

A regional approach to their management is warranted to mitigate risks to adjoining areas where rubber bush has yet to establish. Like most weed species, the life history of rubber bush is adapted to seed dispersal and early germination of seed, and it cannot easily invade intact pastures and habitat. Consequently, rubber bush favours disturbed sites and its invasive potential is, in large, part due to land management practices that cause disturbance to intact habitat (roadsides, stock yards, around bores). Seed and pollination biology related to the mode of seed dispersal cause reproduction to be density-dependent. This density dependence should be exploited in controlling rubber bush by destroying mature plants wherever they occur, by repeated thinning of established stands to low density stands and the removal of isolated plants. Control measures should be applied over at least a 2-3 year period. Rubber bush has not spread to the full extent of its potential current range and climate change scenarios suggest that its range will expand in the future. Thus, controlling rubber bush now is encouraged to avoid future spread and loss of pastoral productivity.

4 Control

4.1 Site Details

Chemical and mechanical control of rubber bush was investigated on cattle properties or sites adjoining neighbouring properties. There were six trial sites in total including four in far north Queensland and two in the Northern Territory (Fig. 21). The sites in the Gulf of Carpentaria in Queensland (Sites 1, 2 and 3) were all located in pulled gidyea (*Acacia cambagei*) country with the soils primarily shrinking and expanding deep, red, brown or grey cracking clays. At site 4, scattered rubber bush was growing within the ballast of the old railway line that had connected Greenvale to Townsville. It was the only woody vegetation present but would have originally been eucalypt woodland and the soil under the ballast material was a dark brown to yellow brown sandy loam. Sites 5 and 6 were both located in the Mitchell grass Bioregion of the Northern Territory, where the soils are characteristically uniform fine-textured soils (vertisols) and typically grey or brown cracking-clays (Fisher et al. 2002).

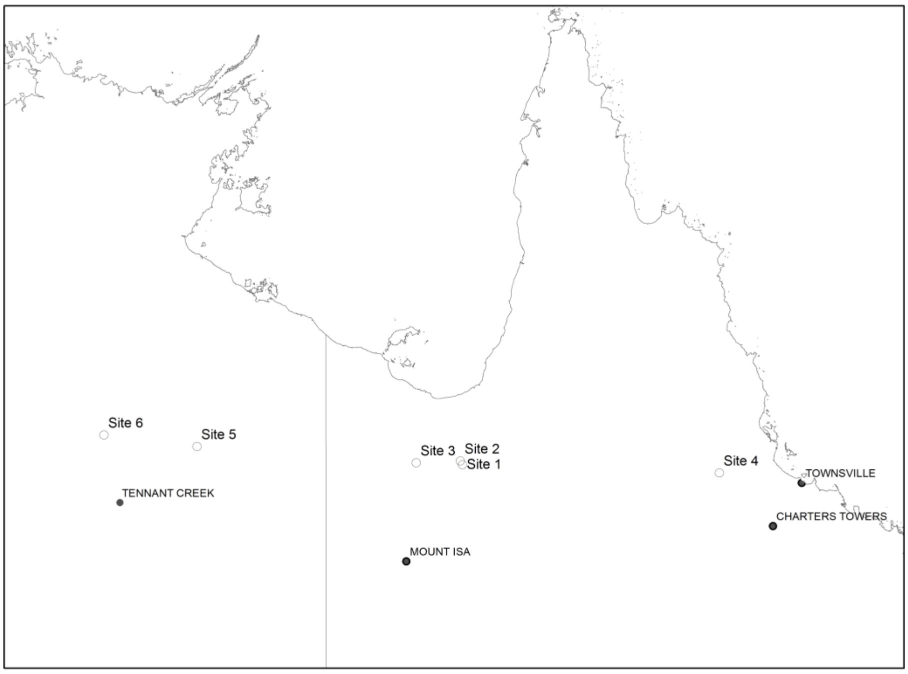


Fig. 21. The location of field sites where control research was undertaken on rubber bush during the project.

Annual rainfall received at the sites whilst trials were being undertaken is shown in Table 7 and was based on SILO data drill output (Queensland Government 2015). Long term mean average rainfall for each site is also presented and was obtained from records of the closest official Bureau of Meteorology location that measured rainfall. The information was accessed using Climate Data Online (Bureau of Meteorology 2015).

Table 7. Annual rainfall (mm) received at control sites whilst research was being undertaken and the long term average for these sites based on SILO data drill output.

Year	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
2011	907	936	970	740		
2012	724	726	706	666		
2013	432	439	285	484		
2014	513	534	462		690	940
2015*	184	179	163		466	327
Long term mean	708	708	612	649	400	454

*up until 28 March 2015

4.2 Control treatments trialled

In total, 11 separate trials were undertaken to test the efficacy of numerous herbicides applied using a range of techniques. Three trials focussed on finding additional foliar options to the two chemicals (metsulfuron-methyl and imazapyr) that are currently registered in a minor use permit issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA; PER 12497). Initially, a preliminary screening experiment was undertaken, followed by a rate refinement trial, which incorporated a seasonality component. A third study focussed on trying to explain the poor efficacy of metsulfuron-methyl when compared with previous research findings reported in Vitelli et al. (2008). Cut stump and frilling applications were tested in an initial trial incorporating two herbicides, which was followed by a specific experiment to refine rates for glyphosate using the cut stump technique. Testing of residual herbicides for control of rubber bush was undertaken, with an initial screening of hexazinone and tebuthiuron based products demonstrating their potential. Three further studies (two on tebuthiuron and one on hexazinone) were then undertaken to refine rates. Besides different rates, the tebuthiuron trials incorporated placement position and seasonality into the experimental design. Efficacy of aerial applications of tebuthiuron was also monitored following commercial applications on the property where site 4 was located.

With most of the application techniques mentioned above used in several trials, a full description is provided below to avoid duplication in subsequent sections (Table 8). Thereafter, reference is only made to the application technique tested in respective trials.

Table 8. Application techniques implemented during the control research, the equipment used and how herbicides were applied.

Application technique	Description
Foliar – backpack applicator	A 15 L back pack sprayer (Swissmex®) with an adjustable solid cone nozzle was used, with the foliage of each plant sprayed to the point of run-off.
Foliar – spray rig	An 800 L capacity dual reel Quik Spray® unit was used. It was operated at 400 kpa pressure to spray the whole plant (foliage and stems) to the point of run-off.
Basal bark - traditional	An 8 L handheld pneumatic sprayer (Swissmex®) with a 0.6 m wand and an adjustable full cone nozzle was used to spray the entire circumference of the lower 30-40 cm of each plant stem. Spraying continued until the mixture had soaked in and started running down the stem.
Basal bark - thinline	An 8 L handheld pneumatic sprayer (Swissmex®) with a 0.6 m wand and an adjustable full cone nozzle was used to spray the entire circumference of the lower 5 cm of each plant stem. Spraying continued until the mixture had soaked in and started running down the stem.
Cut stump	Plants were usually cut off as close to the ground level as possible (unless otherwise specified) using a brush cutter with a metal blade attachment. Once cut, the herbicide mixture was immediately (within ~5 seconds) applied to the cut surface. For glyphosate and triclopyr/picloram (Access™) applications, an 8 L handheld pneumatic sprayer (Swissmex®) with a 0.6 m wand and an adjustable full cone nozzle was used, with the cut area sprayed until the point of runoff. Picloram gel (Vigilant™) applications involved applying a 3-5 mm thick layer of the gel over the cut surface.

Application technique	Description
Frilling	A series of blazes were inserted into the stems of rubber bush ~20-30 cm above ground at ~10 cm intervals using a tomahawk. Chemical was then inserted into the cuts at predetermined rates.
Hand application of granular tebuthiuron (Graslan™) and hexazinone (several products)	A portable set of scales was used to accurately obtain the quantity required based on the canopy area of individual rubber bush plants. The granules were then applied by hand to either the base of plants and/or evenly distributed under the canopy, depending on the experimental design and methodology of respective experiments.
Spotgun® application of liquid hexazinone (Velpar® L)	A Spotgun® was used to apply individual applications of either 2 or 4 mL of herbicide mixture per metre of plant height. Spot applications were made at the base of rubber bush plants and if several were applied they were spread out evenly around the base of the plant.
Aerial application of tebuthiuron (Graslan™)	All aerial applications of tebuthiuron (Graslan™) were coordinated by Dow AgroSciences with pellets distributed using a fixed wing aircraft. The aircraft operated on a swathe width of 22 m and application was carried out using a half overlap pattern.

Besides the herbicide research, the potential of mechanical control was also evaluated through an initial trial to identify the cutting depth needed to kill plants, followed by a stick raking demonstration. Monitoring of a dieback phenomenon was also undertaken to quantify whether it would have a long term impact on rubber bush populations and, if so, to consider implications for implementation of control activities.

The methodology, results and discussion associated with the findings of the various trials is covered in the following sections.

4.3 Statistical analyses

For all trials where percentage mortality was based on experimental units comprising several plants (usually 10-20), analysis of variance using Genstat (Version 16) was undertaken to determine if significant treatment differences occurred at $P < 0.05$. If there were, Fishers Protected l.s.d. test was used to determine which treatment means differed from each other.

For some data sets, arcsine transformations were undertaken, particularly if there was a concentration of data close to zero and 100%. However, before doing so, the value of 0% was substituted by (1/4n) and the value of 100% by (100 -1/4n), as described by Steel & Torrie (1981).

For trials where the experimental units were individual plants, analysis of individual tree survival data was undertaken using a Generalised Linear Model on binomial proportions. This was applicable to the cutting depth trial and the experiment that investigated the influence of season, placement and application rate on efficacy of ground applications of tebuthiuron.

Because of the pseudo-replication that had to be applied to monitor the efficacy of aerial applications of tebuthiuron, testing for significant treatment differences using analysis of variance has not been undertaken. Instead, for each treatment applied, average data and associated standard errors ($\bar{x}$ ± s.e.) are presented for respective monitoring periods. Changes over time are then discussed on a treatment by treatment basis.

4.4 Foliar and basal bark herbicides

4.4.1 Methodology

4.4.1.1 Preliminary foliar screening trial

A split plot experiment was established in April 2011 at site 1, with herbicide treatments allocated to main plots and size class allocated to sub plots. Main plots averaged ~10×10 m in size and were separated by a two metre buffer. Each treatment was replicated three times.

In total, 22 treatments (Table 9) were selected for spraying onto 10 small (≤1.5 m) and 10 medium sized plants (>1.5 to 3 m). Either one or two rates of commercially available chemicals containing the active ingredients 2,4-D amine, 2,4-D amine + metsulfuron-methyl, 2,4-D/picloram, aminopyralid/fluroxypyr, fluroxypyr, glyphosate, metsulfuron-methyl, triclopyr, triclopyr/picloram, and triclopyr/picloram/aminopyralid were selected for spraying using a backpack applicator (see Table 8). Four new products not commercially available at the time were also included. Three of these [aminopyralid/metsulfuron-methyl (Stinger™), 2,4-D/picloram/aminopyralid (FallowBoss™Tordon™), triclopyr/picloram/aminopyralid (Tordon™ Regrowth Master)] are now commercially available, but one is not, and after discussion with the manufacturer, it will not be progressed at this stage. As such, for commercial in confidence reasons the efficacy data will not be presented in this report. For comparative purposes, metsulfuron-methyl, the currently recommended foliar herbicide in the Minor Use Permit (PER12497) for control of rubber bush in pastures, was included. An untreated control plus the most frequently used herbicide application of basal barking (see Table 8) using triclopyr/picloram (Access™) was also included for comparison. When a chemical's label indicated a particular wetting agent was needed, it was included at the recommended rate (Table 9).

Table 9. Herbicides and rates applied during the screening trial on rubber bush, which included 20 foliar treatments compared against a basal bark treatment and a control (untreated).

Herbicide (Active ingredient)	Trade name	Rates applied	
		g a.i./100L	g or mL product/100L
2,4-D amine (625 g/L)*	Amine 625	500	800
2,4-D amine (625 g/L)*	Amine 625	625	1000
2,4-D (300 g/L)/picloram (75 g/L)	Tordon™ 75-D	195/49	650
2,4-D (300 g/L)/picloram (75 g/L)/aminopyralid (7.5 g/L)	FallowBoss™ Tordon™	180/45/5	600
2,4-D amine (625 g/L) + metsulfuron-methyl (600 g/kg)*	Amine 625 +Brush-Off®	250+12	400+20
2,4-D amine (625 g/L) + metsulfuron-methyl (600 g/kg)*	Amine 625 + Brush-Off®	500+18	800+30
Aminopyralid (10 g/L)/ fluroxypyr (140 g/L)	Hotshot™	7/98	700
Aminopyralid (375 g/L)/metsulfuron-methyl (300 g/L)*	Stinger™	15/12	40
Aminopyralid (375 g/L)/metsulfuron-methyl (300 g/L)*	Stinger™	23/18	60
Fluroxypyr (333 g/L)*	Starane™ Advanced	300	900
Glyphosate (470 g/L)	Roundup® Dual Salt Technology®	470	1000
Glyphosate (540 g/L) †	Roundup PowerMAX®	470	870
Metsulfuron-methyl (600 g/kg)*	Brush-Off®	12	20
Metsulfuron-methyl (600 g/kg)*	Brush-Off®	18	30
Triclopyr (600 g/L)	Garlon™ 600	360	600
Triclopyr (200 g/L)/picloram (100 g/L) ‡	Tordon™ Double Strength	100/50	500
Triclopyr (200 g/L)/picloram (100 g/L)/aminopyralid (25 g/L) ‡	Tordon™ Regrowth Master	120/60/15	600
Triclopyr (300 g/L)/picloram (100 g/L) /aminopyralid (8 g/L)*	Grazon™ extra	225/75/6	750
Triclopyr (240 g/L)/picloram (120 g/L) (in diesel)	Access™	400/200	1667
Control (untreated)	n/a		

*BS1000, †Pulse® or ‡Uptake™ were added as wetting agents at 100, 200 or 500 mL of product/100 L, respectively.

Prior to application of treatments, all small and medium sized plants to be treated were tagged and their height, basal diameter and number of stems were recorded. On average, small plants had a height of 0.67 ± 0.01 m, a basal diameter of 1.74 ± 0.09 cm and 1.24 ± 0.02 stems. In contrast, medium sized plants had an average height of 1.87 ± 0.01 m, a basal diameter of 8.61 ± 0.08 cm and 3.40 ± 0.05 stems.

All plants were very healthy at the time of spraying which was undertaken between 18-21 April 2011. Following implementation of treatments, mortality was assessed at 14 (June 2012) and 24 months (April 2013) after treatment (MAT).

4.4.1.2 Rate refinement and seasonality foliar trial and comparison of two basal bark techniques

The experiment was established in April 2012 at site 2 using a completely randomised design, incorporating 30 treatments each replicated three times. Fifteen of these were initially applied in autumn to primarily refine rates, with four of these also applied in winter, spring and summer to quantify if there were any seasonal influences. Experimental units comprised discrete clumps of 15 medium sized (1.5 to <3 m high) rubber bush plants.

A total of 12 foliar herbicide treatments were selected (Table 10); the three most effective herbicides from the screening trial (i.e. 2,4-D amine, glyphosate, and triclopyr/picloram/aminopyralid); a slightly higher rate of 2,4-D/picloram/aminopyralid, the same two rates of metsulfuron-methyl as used in the first trial, a mixture of 2,4-D amine + metsulfuron-methyl, and two rates of imazapyr/glyphosate.

The metsulfuron-methyl treatments were repeated because of the poor results achieved in the first trial, which were inconsistent with the findings from earlier herbicide trials undertaken on rubber bush (Vitelli et al. 2008). Imazapyr/glyphosate was also included at two rates. Imazapyr alone had proven effective previously (Vitelli et al. 2008) and with glyphosate demonstrating high efficacy in the first trial, this herbicide warranted inclusion. An untreated control plus basal barking using triclopyr/picloram (Access™) was added for comparison. A new thinline basal bark technique for application of triclopyr/picloram (Access™) was also opportunistically included to collect efficacy data, and to compare it against the traditional basal bark technique. These treatments were implemented in autumn between 30 April and 2 May 2012.

The seasonality component involved spraying the lower rates of the foliar herbicides 2,4-D amine (500 g a.i./100 L), glyphosate (470 g a.i./100 L), metsulfuron methyl (12 g a.i./100 L) and triclopyr/picloram/aminopyralid (150/50/4 g a.i./100 L) and the traditional basal bark application of triclopyr/picloram in winter (19-20 July 2012), spring (31 October to 2 November 2012) and summer (6-8 February 2013).

Table 10. Herbicides and rates applied during the rate refinement and seasonality trial, which included 12 foliar treatments, compared against two basal bark treatments and a control (untreated).

Herbicide (Active ingredient)	Trade name	Rates applied[‡]	
		g a.i./100L	g or mL product/100L
2,4-D amine (625 g/L)	Amine 625	500	800
2,4-D amine (625 g/L)	Amine 625	625	1000
2,4-D (300 g/L)/picloram (75 g/L)/aminopyralid (7.5 g/L)	FallowBoss™ Tordon™	195/49/5	650
2,4-D amine (625 g/L) + metsulfuron-methyl (600 g/kg)	Amine 625 + Associate®	500+12	800+20
Glyphosate (570 g/L)	Roundup® attack™	470	825
Glyphosate (570 g/L)	Roundup® attack™	570	1000
Imazapyr (150g/L)/glyphosate (150 g/L)	Arsenal® Xpress	97.5/97.5	650
Imazapyr (150g/L)/glyphosate (150 g/L)	Arsenal® Xpress	150/150	1000
Metsulfuron-methyl (600 g/kg)	Associate®	12	20
Metsulfuron-methyl (600 g/kg)	Associate®	18	30
Triclopyr (300 g/L)/picloram (100 g/L) /aminopyralid (8 g/L)	Grazon™ extra	150/50/4	500
Triclopyr (300 g/L)/picloram (100 g/L) /aminopyralid (8 g/L)	Grazon™ extra	225/75/6	750
Triclopyr (240 g/L)/picloram (120 g/L) (in diesel)*	Access™	400/200	1667
Triclopyr (240 g/L)/picloram (120 g/L) (in diesel) [†]	Access™	2400/1200	10000
Control	n/a		

*****Traditional basal bark technique applied to bottom 30 cm of stem.

†Thinline basal bark technique applied to bottom 5 cm of stem.

‡For all foliar treatments, the surfactant BS1000 was added at a rate of 100 mL/100 L.

Prior to allocation of treatments, the selected rubber bush plants were tagged and their height and basal diameter recorded. On average, plants were 2.10 ± 0.01 m high and had a basal diameter of 11 ± 0.1 cm. On the day before spraying was due to occur for respective seasonal times, leaf coverage (proportion of maximum leaf cover expressed as a percentage) and the presence of flowering or podding on plants was recorded.

Following implementation of treatments, the level of leaf brownout was recorded after 6-7 weeks and mortality was assessed at 12 and 24 MAT.

Foliar spraying and basal bark spraying was undertaken using the same equipment and application methods used in the screening trial (See Table 8). The only exception was the thinline basal bark technique where only the lower 5 cm of the stem was sprayed, compared with 30-40 cm for the traditional technique.

Temperature and humidity conditions during spraying at the different seasonal times were measured using a pen-type digital thermometer/hygrometer (Table 11). A Kestrel 1000® handheld wind meter was used to record wind speeds.

Table 11. Average temperature (ºC), humidity (%) and wind speed (km/hr) at the different seasonal times that foliar spraying was undertaken.

Environmental conditions	Seasonal conditions			
	Autumn	Winter	Spring	Summer
Temperature (ºC)	34	25	35	35
Humidity (%)	43	30	45	45
Wind speed (km/hr)	0.5	1.7	0.7	0.9

4.4.1.3 Effect of water type and temperature on efficacy of foliar applications of metsulfuron-methyl

The poor efficacy obtained using metsulfuron-methyl in the earlier two trials (see results section) contrasted greatly with published information from previous research (Vitelli et al. 2008) which reported high mortality when applied at 12 g a.i./100 L. Water quality and ambient temperatures at the time of spraying are two possible factors that could have contributed to this variability. To test this assumption, a field experiment was initiated in October 2011 at site 1, incorporating a 2×2 factorial component. Factor A comprised two types of water (town and bore) and Factor B two temperature conditions at the time of spraying (moderate and hot). An untreated control was also included for comparison.

Treatments were laid out using a completely randomised design, replicated three times. Experimental units comprised 15 medium sized plants located within plots that were ~10 m × 10 m in size. Prior to allocation of treatments all selected plants were tagged and their height, basal diameter and number of stems were recorded. On average, plants were 2.36 ± 0.05 m high, with a basal diameter of 11 ± 0.7 cm and 3 ± 0.2 stems.

Foliar spraying and monitoring of climatic conditions (temperature and wind speed) were undertaken in a similar manner to the previous trials. Town water for the trial came from the Charters Towers water supply and bore water from a bore located within 5 km of the trial site. To achieve different temperature conditions under which spraying was undertaken, moderate treatment applications were conducted between 7–10 am, and hot treatment applications between 1-4 pm. This resulted in a 10 ºC difference between the moderate and hot treatments, which averaged 27 ºC and 37 ºC, respectively.

Following implementation of treatments, mortality was assessed at 8 (20 June 2012), 12 (30 October 2012) and 24 MAT (2 November 2013).

4.4.2 Results

4.4.2.1 Preliminary foliar screening trial

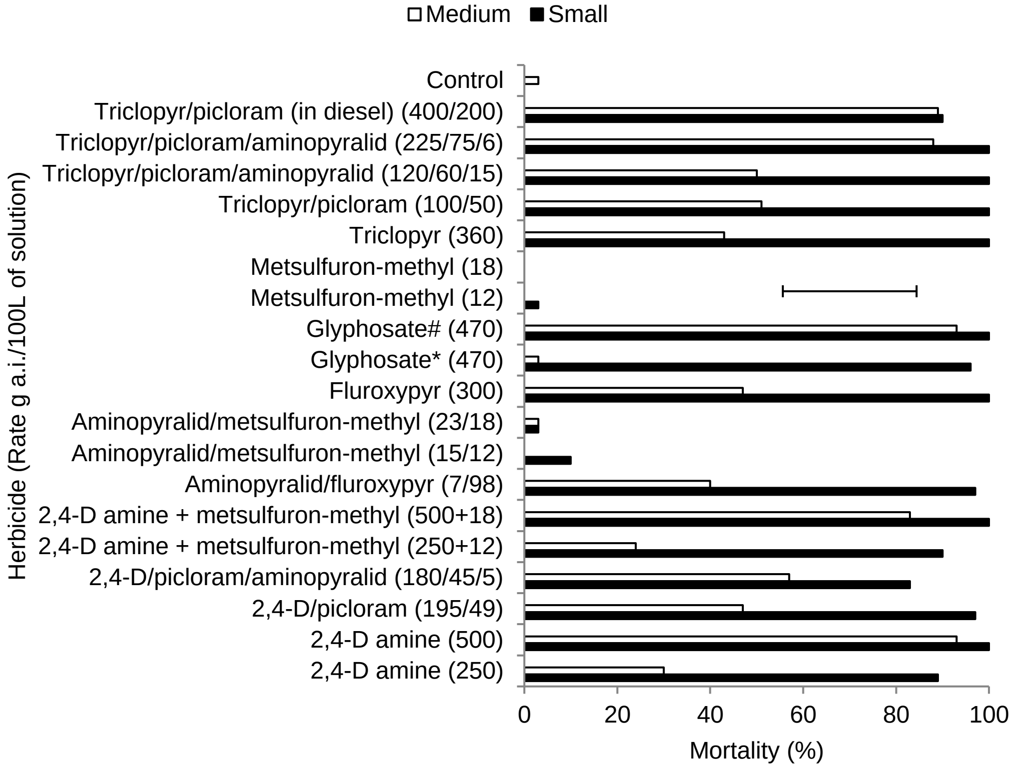
Significant differences (P < 0.01) in rubber bush mortality were recorded at 14 and 24 MAT between the 22 herbicide treatments as well as the two size classes. There was also a significant (P < 0.01) herbicide × size class interaction (Fig. 22). For some treatments, the level of mortality increased between the 14 and 24 MAT assessments, but during this time plants became affected by a form of dieback which appears to have increased mortality further, rather than the herbicides themselves. For example, in the control treatment (untreated) mortality was <4% 14 MAT but increased to an average of 20% 24 MAT. As such, emphasis is placed on the 14 MAT assessments to distinguish differences associated with efficacy of the herbicide treatments.

Over all size classes, three foliar herbicides gave >90% mortality 14 MAT. They were the highest rate of 2,4-D amine (Amine 625), the 540 g a.i./L formulation of glyphosate (Roundup PowerMax®), and triclopyr/picloram/aminopyralid (Grazon™ extra). In comparison, basal barking using triclopyr/picloram (Access™) in a diesel carrier averaged 89%, which was not significantly different to the aforementioned herbicides. Despite being applied at the same rate of active ingredient, the two glyphosate treatments differed significantly (P > 0.05) from each other. The one containing potassium salt only (Roundup PowerMax®) demonstrated higher efficacy than the one containing both potassium and mono-ammonium salts (Roundup® Dual Salt Technology®).

In terms of size classes, small plants were much more susceptible to foliar spraying, with average mortality of 73% recorded 14 MAT, compared with 42% mortality of medium sized plants.

The significant herbicide × size class interaction was associated with several of the herbicide treatments, which demonstrated low efficacy on medium sized plants but high efficacy on small plants. This was most pronounced for the lower rates of 2,4-D amine (Amine 625) and 2,4-D amine + metsulfuron methyl (Amine 625 + Brush-Off®), and the single rates of 2,4-D/picloram (Tordon™ 75-D), aminopyralid/fluroxypyr (Hotshot™), the 470 g a.i./L formulation of glyphosate (Roundup® Dual Salt Technology®), fluroxypyr (Starane™ advanced), triclopyr (Garlon™ 600), triclopyr/picloram Tordon™ Double Strength, and Triclopyr/picloram/aminopyralid (Tordon™ Regrowth Master).

(a)



(b)

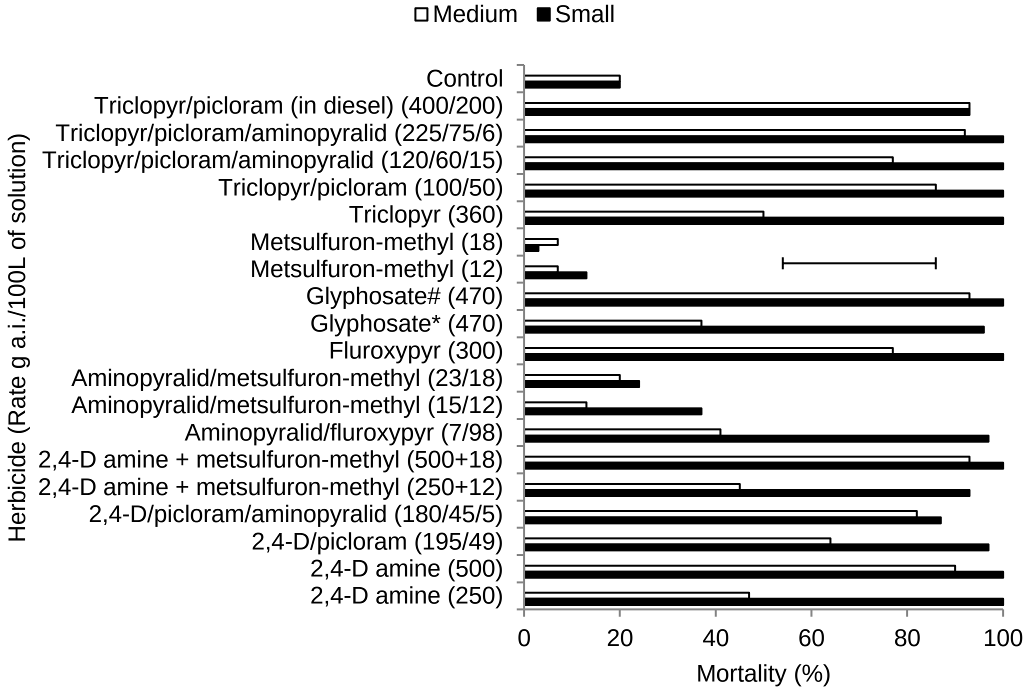


Fig. 22. Effects of herbicides on mortality of small and medium sized rubber bush plants (a) 14 and (b) 24 MAT during the screening trial. Horizontal bars indicate the l.s.d. at P = 0.05. (* 470 g a.i./L formulation of glyphosate; $^{\#}$540 g a.i./L formulation)

4.4.2.2 Rate refinement and seasonality trial

Significant differences (P < 0.05) in initial leaf brownout and mortality (12 and 24 MAT) occurred between herbicide treatments applied to refine rates for foliar control of rubber bush (i.e. those applied between 30 April and 2 May 2012). All herbicide treatments caused greater than 90% brownout of the original leaf material present at the time of spraying, with only the two rates of metsulfuron-methyl (94-95%) and the lower rate of imazypyr/glyphosate (93%) not causing 100% brownout. However, this high brownout did not translate to high mortality in all treatments.

Of the foliar herbicides, imazapyr/glyphosate achieved ≥98% mortality 24 MAT at both rates applied and the highest rate of 2,4-D amine recorded 91% mortality (Fig. 23). 2,4-D/picloram/aminopyralid achieved a moderate level of control (78%) after 24 months with the remainder recording ≤60% mortality. Metsulfuron-methyl was the worst performing herbicide, with mortality ≤23% for both rates applied. Both basal bark applications gave 100% control.

All control plants remained alive at the end of the study but they had been severely affected by dieback (as reported in the screening trial); in most instances their original stems had been killed and they had reshot from the base. Plants remaining alive after herbicide treatment were also affected by the dieback and, for most treatments, there was an increase in mortality between the 12 and 24 MAT assessments.

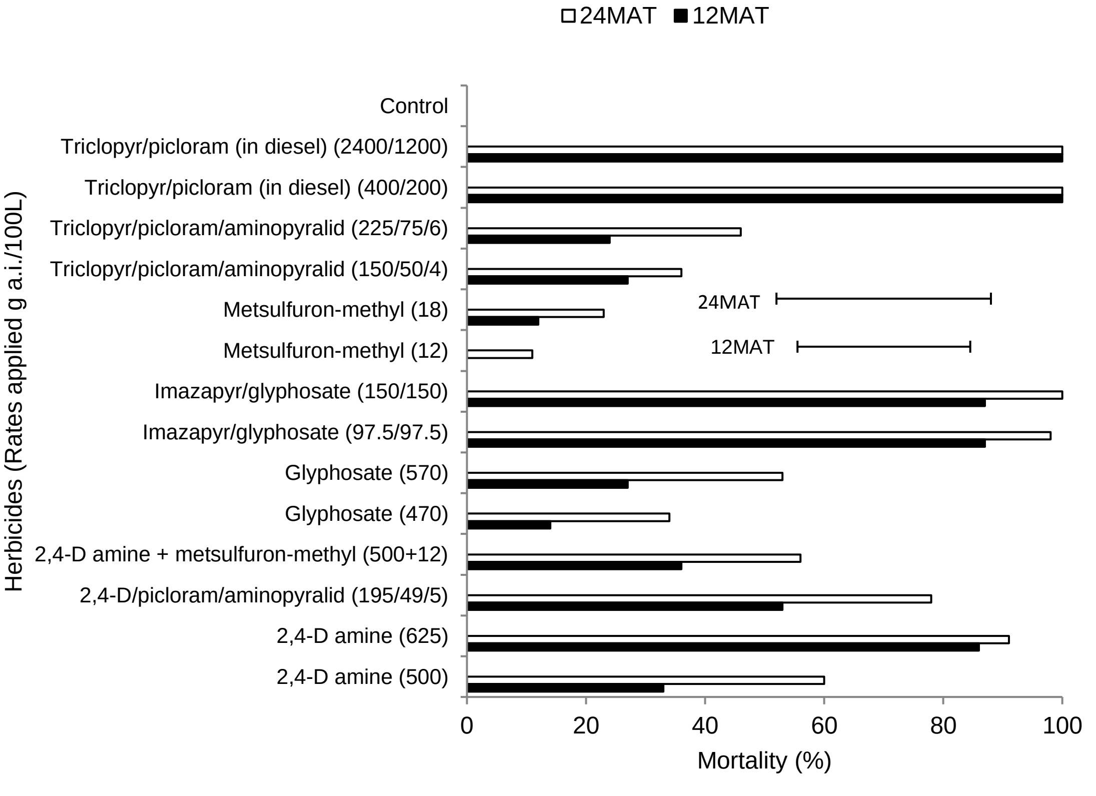


Fig. 23. Effect of herbicides on mortality of rubber bush plants 12 and 24 MAT during the second trial. The horizontal bar indicates the l.s.d. at P = 0.05.

With regards the seasonality component, plants sprayed during the winter period had a greater proportion of leaf material than those in autumn, spring and summer, which ranged between 53-60%. Almost all plants (≥98%) had flowers present during winter and spring applications, compared with 71 and 85% during the autumn and summer periods, respectively. Only a small percentage of plants had pods on them (3-8%) when sprayed, across the four seasonal times (Table 12).

Table 12. The proportion of leaf on plants and the percentage of plants with flowers and pods at the different seasonal times when foliar and basal bark spraying was undertaken.

Plant measurements	Seasonal conditions			
	Autumn	Winter	Spring	Summer
Leaf on plants (%)	60	73	57	53
Flowering plants (%)	71	98	99	85
Plants with pods (%)	8	3	5	4

A significant season × herbicide interaction ($P < 0.001$) for the level of initial brownout and subsequent plant mortality occurred at both 12 and 24 MAT. Within treatment variability increased markedly between the 12 and 24 MAT treatments, which coincided with increased prevalence of dieback (Fig. 24).

The majority of treatments caused major brownout (>90%) of leaves present on plants at the time of spraying, except summer applications of glyphosate (40%) and winter applications of metsulfuron-methyl (30%). Mortality however, differed markedly. The basal bark application of triclopyr/picloram consistently gave 98-100% mortality, while metsulfuron-methyl gave high mortality (98% 24 MAT) in summer, but low mortality (≤11%) for the other seasonal times. Highest mortality of 2,4-D amine (57-76%) was achieved following summer, autumn and winter applications. Mortality of glyphosate and triclopyr/picloram/aminopyralid treatments varied the least with seasons 12 MAT, but was consistently low (<50% 24 MAT).

Treatment costs (exclusive of the wetting agent) for the most effective foliar herbicides (2,4-D amine, imazapyr/glyphosate) were calculated and compared against those currently registered in PER12497 (metsulfuron-methyl and imazapyr). Calculations were based on April 2015 prices and the average amount applied to plants during the trial (i.e. 1030 mL/plant). 2,4-D amine (800-1000 mL of Amine 625/100 L of mixture) and imazapyr/glyphosate (650-1000 mL of Arsenal® Xpress/100 L of mixture) cost $0.05-$0.06 and $0.50-$0.78/plant, respectively. In comparison, metsulfuron methyl (20 g of Brush-Off®/100 L of mixture) and imazapyr (500 mL of Unimaz™/100 L of Mixture) cost $0.04 and $0.40/plant, respectively. For the basal bark treatments, the traditional and thinline techniques cost $1.02 and $1.76/plant, respectively. This was based on the price of Access™ in April 2015, $1.34 for diesel, and applications of 390 and 195 mL of mixture/plant for the traditional and thinline techniques, respectively.

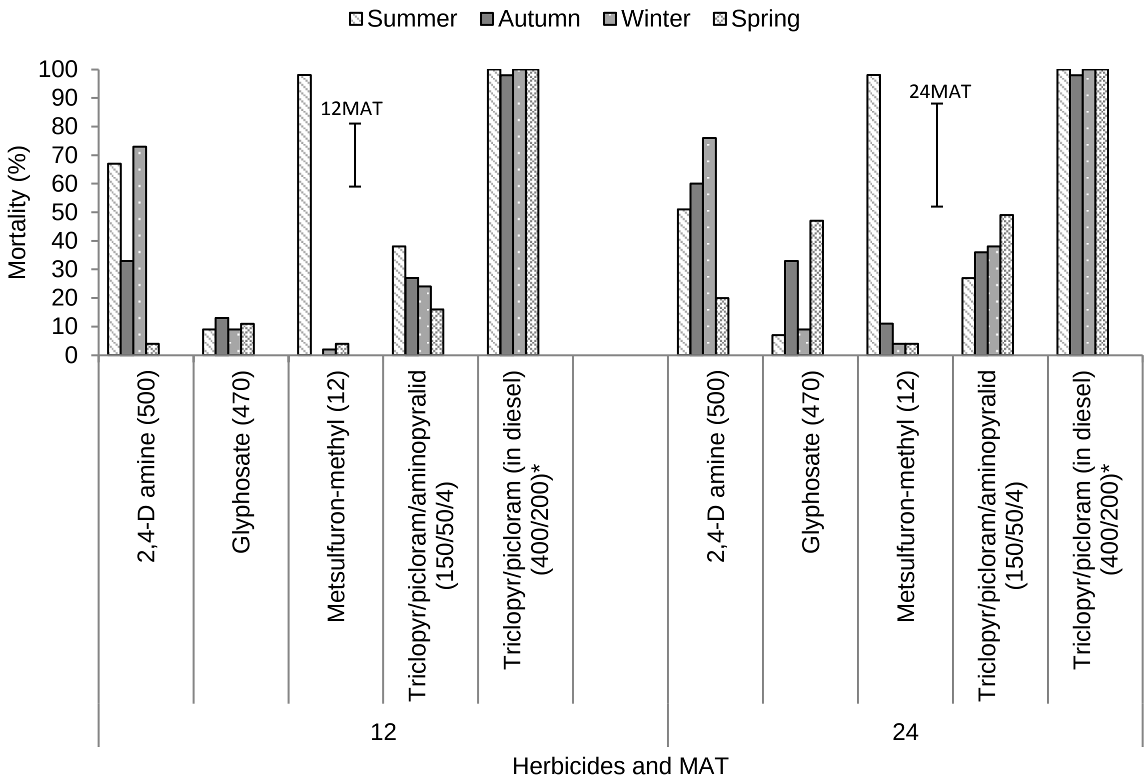


Fig. 24. Effect of four foliar and one basal bark* herbicide on rubber bush mortality 12 and 24 MAT, following spraying in summer, autumn, winter or spring. Vertical bars indicate the l.s.d. (at P = 0.05) for respective monitoring times.

4.4.2.3 *Effect of water type and temperature on efficacy of foliar applications of metsulfuron-methyl*

As for the previous two trials, initial efficacy (8-12 MAT) of foliar applications of metsulfuron-methyl was generally poor (<50%) across all treatments. While, there were still some significant treatment differences 8 MAT, this was not the case thereafter.

At 8 MAT a significant difference ($P < 0.05$) in mortality occurred between temperature treatments but not water type treatments ($P > 0.05$), neither was there a significant interaction between the two factors ($P > 0.05$). Mortality was highest when spraying was undertaken under moderate temperature conditions compared with hot temperatures, averaging 44% and 26% respectively. Overall, all herbicide treatments recorded significantly higher mortality ($P < 0.05$) than the untreated control. All untreated control plants remained alive 8 MAT, whilst on average, 35% of herbicide treated plants were dead.

At 12 and 24 MAT, no significant differences were detected between water types or temperature treatments and there was no significant interaction ($P > 0.05$). There was still, however, a significant difference between all herbicide treatments and the untreated control. Across all treatments (including the control) mortality increased over time, which seemed to coincide with the appearance of dieback on plants. At 12 and 24 MAT, control plots averaged 4 and 36%, respectively. In contrast, herbicide treated plants averaged 48 and 83% mortality after 12 and 24 months, respectively.

4.5 Cut stump and frill applications

4.5.1 Methodology

4.5.1.1 Initial cut stump and frilling trial

Triclopyr/picloram (Access™) is included in a minor use permit (PER12497) for the control of rubber bush using the cut stump and basal bark techniques, however it has to be mixed in diesel. To test other practical options that landholders could easily transport around as part of day to day activities, an efficacy trial using cut stump and frilling techniques was undertaken using the readily available herbicide glyphosate and a picloram gel that comes in an easily transportable tube.

A 3×3 factorial experiment was implemented in June 2011 at site 1 using a randomised complete block experimental design. Factor A comprised three herbicide treatments [untreated control, glyphosate (Roundup PowerMax® which contained 540 g a.i./L), picloram gel (Vigilant™ which contained 43 g a.i./L)]. Factor B was three application techniques (cut stump at ground level or at 20 cm, or frilling). All treatment combinations were replicated three times and experimental units consisted of clusters of 15 medium sized rubber bush plants.

Prior to allocation of treatments all selected plants were tagged, and their height, basal diameter and number of stems was recorded. On average, they were 2.14 ± 0.02 m high, had a basal area of 70.16 ± 1.56 cm^2 and 4.1 ± 0.06 stems.

Cut stump and frilling treatments were applied on 21/22 June 2011 using the methodology described in Table 8. Glyphosate was applied neat onto the cut surface for the cut stump treatment and at a rate of ~2 mL of product per cut for the frilling treatment. Picloram gel applications involved applying a 3-5 mm thick layer of the gel over the cut surface for cut stump treatments and ~2 mL of product per cut for the frilling treatment.

Mortality assessments were undertaken at 12 (24/25 June 2012) and 25 MAT (26/27 July 2013).

4.5.1.2 Rate response of glyphosate using the cut stump technique

Based on the findings of the initial cut stump and frill trial, a randomised complete block experiment was implemented at site 1 in July 2012 to identify the minimal application rate of glyphosate that would still cause high mortality of rubber bush. To achieve this, the efficacy of two different concentrations of glyphosate applied to cut stumps at 2-3 rates was tested. The standard 360 g a.i./L formulation (Roundup®) was included at 120, 180 and 360 g a.i/L of spray solution. A stronger 570 g a.i./L formulation (Roundup® Attack™) was also included at 190 and 570 g a.i./L of spray solution. For treatments that did not involve neat applications of herbicide, water was added to make the required concentrations. A control treatment was also included for comparison, which involved cutting plants but not applying any herbicide.

In total there were six treatments, each replicated three times, with experimental units comprising clusters of 15 medium sized plants. Prior to allocation of treatments, all selected plants were tagged, and their height, basal diameter and number of stems were recorded, along with whether they were flowering and/or podding. On average, plants were 2.07 ± 0.01 m high, had a basal diameter of 10 ± 0.01 cm and 2.75 ± 0.1 stems. They were all flowering but only 6% had pods on them at the time.

Treatments were applied on 23-24 July 2012 using the cut stump methodology outlined in Table 8. Mortality assessments were undertaken at 12 (23 July 2013) and 24 MAT (23 July 2014).

4.5.2 Results

4.5.2.1 Initial cut stump and frilling trial

At both 12 and 24 MAT, a significant ($P < 0.05$) herbicide treatment × application technique interaction influenced mortality of rubber bush plants (Fig. 25). Differences were most pronounced at 12 MAT with dieback becoming very prevalent thereafter and confounding the results.

At 12 MAT minimal mortality (<10%) occurred if plants were cut stumped (at either the base or 20 cm) or frilled but not treated with any herbicide. If neat glyphosate (540 g a.i./L) was applied following frilling or cut stumping at the base, high mortality (>95%) was achieved. This was not the case for plants cut stumped at 20 cm above ground, where mortality averaged only 9%. The application of picloram paste following frilling or cut stumping resulted in 87–98% mortality, with all three application techniques not significantly different from each other ($P > 0.05$).

At 24 MAT, mortality in the nil herbicide treatments had increased to 71-78% across the three application techniques. The glyphosate and picloram gel herbicide treatments exhibited high mortality following cut stumping and frilling, although cut stumping at 20 cm using glyphosate still produced poorer results (80% mortality) than the other treatment combinations (>95% mortality).

The cost of herbicide for cut stumping was similar for both application heights (i.e. ground level or 20 cm) but varied markedly between the glyphosate and picloram gel treatments, averaging $0.15 and $3.50/plant, respectively. This was based on April 2015 prices and an average dose of 17 and 21 mL/plant for the glyphosate and picloram gel treatments, respectively. Similarly for frilling, glyphosate was cheaper than the picloram gel, averaging $0.16 and $2.00/plant, respectively. This was based on April 2015 prices and an average dose of 18 and 21 mL/plant for the glyphosate and picloram gel treatments, respectively.

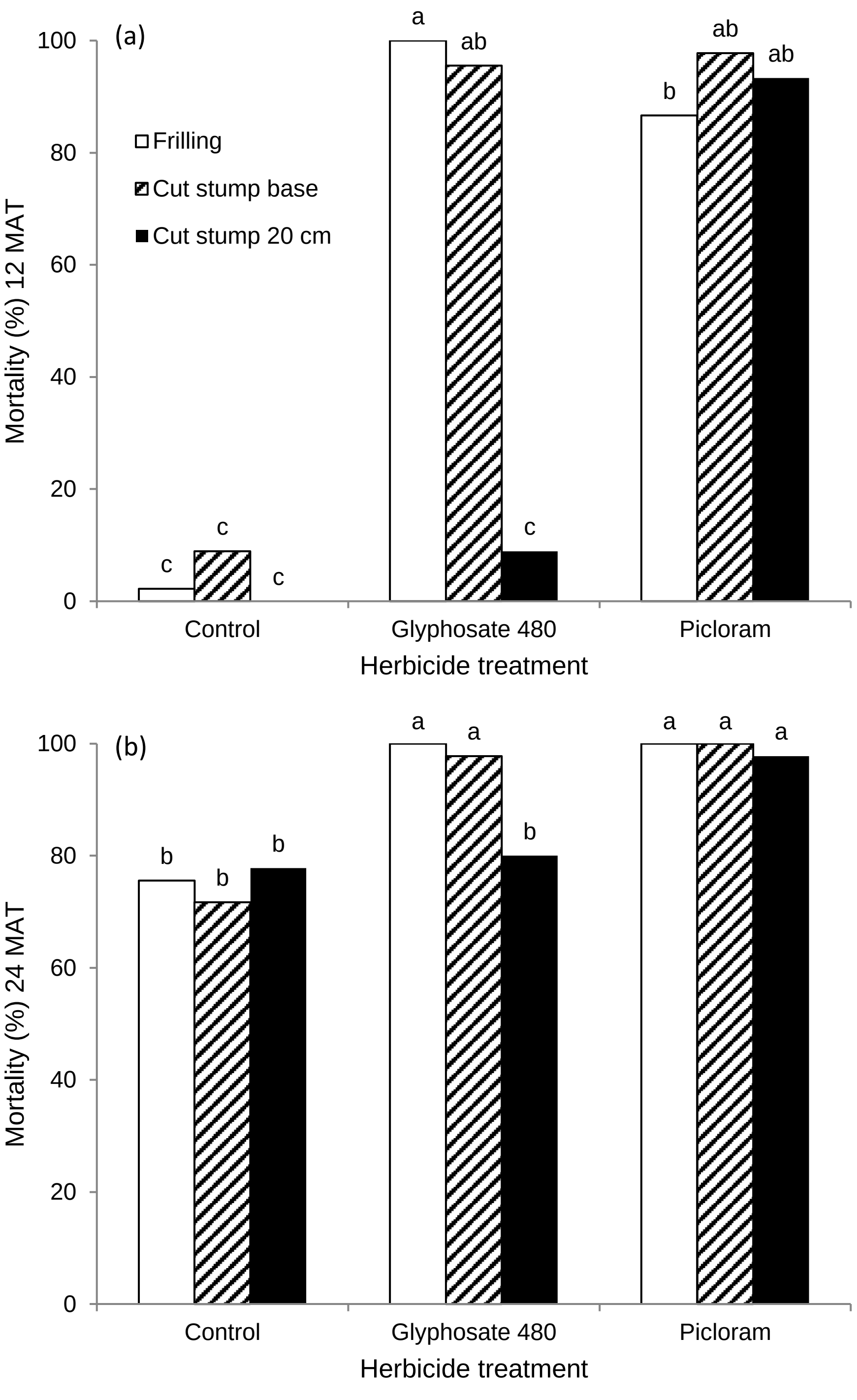


Fig. 25. The efficacy of nil (control), glyphosate (540 g a.i./L) and picloram gel (43 g a.i./L) herbicide applications on mortality of rubber bush plants (a) 12 and (b) 24 MAT, using the cut stump (base and 20 cm aboveground) and frilling techniques. Within graphs, columns followed by the same letter are not significantly different ($P < 0.05$).

4.5.2.2 Rate response of glyphosate using the cut stump technique

At 12 MAT plant mortality was ≥73% for all treatments that received a glyphosate application after plants were cut stumped and was significantly higher ($P < 0.05$) than the control treatment which averaged 2% mortality (Table 13). Mortality tended to increase with increasing concentration of glyphosate in the mixture applied to treat plants and at 360 g a.i./L of mixture and higher mortality was ≥98% (Fig. 26).

At 24 MAT mortality had continued to increase in all treatments, including the control, and appeared to coincide with the increased presence of dieback more so than the effect of the treatments.

The cost of herbicide to treat individual plants ranged from $0.06 to $0.26, generally increasing as the concentration of glyphosate in the mixture applied to the cut stumps increased. This was based on April 2015 prices for the glyphosate products used and applications of between 9-32 mL of glyphosate product/plant, depending on the rate and concentration of particular products. The quantity of mixture applied ranged between 28-32 mL/plant across the five herbicide treatments.

Table 13. The mortality of rubber bush 12 and 24 MAT following cut stump application of two glyphosate formulations at 2-3 rates. Within columns, mortality values followed by the same letter are not significantly different ($P < 0.05$) from each other.

Herbicide (active ingredient)	Trade name	Rates (g a.i./L Spray solution	Cost/plant ($)	Mortality (%)	
				12 MAT	24 MAT
Glyphosate (360 g/L)	Roundup®	120	0.06	73c	87b
Glyphosate (360 g/L)	Roundup®	180	0.10	76bc	89ab
Glyphosate (360 g/L)	Roundup®	360	0.21	98ab	100a
Glyphosate (570 g/L)	Roundup® Attack™	190	0.08	82abc	91ab
Glyphosate (570 g/L)	Roundup® Attack™	570	0.26	100a	100a
Control				2d	29c

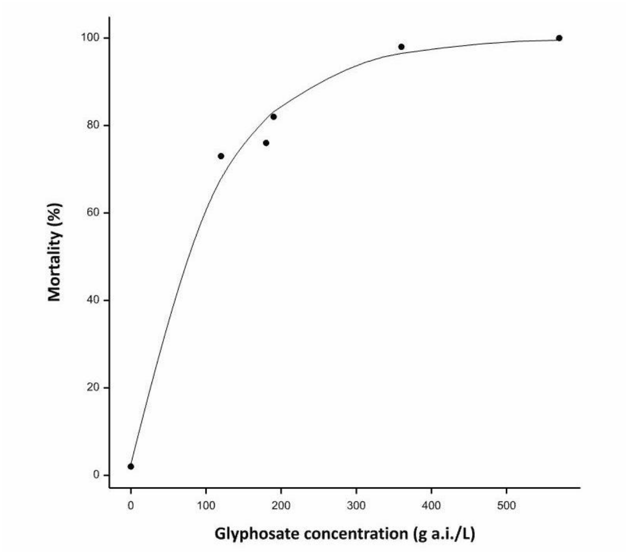


Fig. 26. The effect of increasing concentrations of glyphosate on rubber bush mortality 12 MAT, following application to cut stumps. A regression equation (R^2= 0.98) has been fitted; $y = 100-97.4888*(0.99081^x)$, where y is plant mortality and x is glyphosate concentration.

4.6 Ground and aerially applied residual herbicides

4.6.1 Methodology

4.6.1.1 Screening trial

A randomised complete block experiment comprising five treatments and three replications was established in October 2011 at site 1 to test the efficacy of ground applications of residual herbicides containing hexazinone and tebuthiuron. Three products containing hexazinone and one containing tebuthiuron (Table 16) were selected for application using the highest recommended rate on respective labels. An untreated control was also included for comparison.

Table 16 Herbicides and rates applied in the screening trial of ground applications of residual herbicides.

Herbicide (Active ingredient)	Trade name	Rates applied	
		g a.i.	g or mL product
Hexazinone (250 g/L)	Velpar® L	1 g/m*	4 ml/m*
Hexazinone (200 g/kg)	Velmac® G	1 g/m$^{2\ \dagger}$	5 g/m^{2†}
Hexazinone (100 g/kg)/ Bromacil (50 g/kg)/ Diuron (50 g/kg)	Dymac® G	0.5/0.25/0.25 g/m^{2†}	5 g/m^{2†}
Tebuthiuron (200 g/kg)	Graslan™	0.4 g/m^{2†}	2 g/m^{2†}
Control	Control		

* per metre of plant height; †per m^2 of canopy

Treatments were applied to clusters of 10 plants spaced at least four metres apart from each other and from neighbouring clusters. Prior to application of treatments, plants to be treated were tagged and their height, basal diameter, number of stems, and average canopy size were recorded. On average, they were 2.3 ± 0.03 m high, with a basal diameter of 10.6 ± 0.23 cm, 3.3 ± 0.09 stems, and an average canopy width of 2.22 ± 0.05 m.

Treatments were applied (see Table 8 for details) on 6 November 2011 at the selected rates (Table 9) with the chemical placed around the base of plants as close as possible. Given the root structure of rubber bush, which mostly comprises a large tap root and a few lateral roots, this was considered the best approach to maximise uptake of the herbicide.

Mortality assessments were undertaken at 8 (21 July 2011), 12 (30 October 2012), 18 (25 May 2013) and 24 MAT (30 October 2013).

4.6.1.2 Influence of season, placement and application rate on efficacy of ground applications of tebuthiuron

A completely randomised factorial experiment was established in February 2011 at site 4 to test the efficacy of ground applications of tebuthiuron (Graslan™) applied at two seasonal times (summer and spring), two placement positions (at the base or evenly under the canopy) and four rates (0, 0.2, 0.4 and 0.8 g a.i. per m^2 of canopy cover). Experimental units comprised individual rubber bush plants, with each treatment replicated 15 times.

Prior to application of treatments, plants to be treated were tagged and their height, basal diameter, and average canopy size were recorded. On average, they were 1.91 ± 0.06 m high, with a basal diameter of 6.39 ± 0.31 cm and a canopy width of 1.68 ± 0.07 m.

Summer treatments were implemented on 15 February 2011 and spring treatments on 20 September 2011. The selected rates were applied either to the base of plants or evenly distributed under the canopy of plants as outlined in Table 8.

Mortality assessments at 24 MAT were undertaken on 15 February 2013 and 24 September 2013 for summer and spring applications, respectively.

4.6.1.3 Refinement of application rates and placement of tebuthiuron

A completely randomised factorial experiment was established in October 2011 at site 3 to test the efficacy of ground applications of tebuthiuron (Graslan™) at two placement positions (at the base or evenly under the canopy) and four rates (0, 0.4 0.6 and 0.8 g a.i. per m^2 of canopy cover). Experimental units comprised 10 individual rubber bush plants that were spaced over 3 m apart, with each treatment replicated three times.

Prior to application of treatments, plants to be treated were tagged and their height, basal diameter, number of stems and average canopy size were recorded. On average, they were 2.40 ± 0.02 m high, had a basal diameter of 11.74 ± 0.22 cm, 3.6 ± 0.02 stems, and a canopy width of 2.54 ± 0.04 m. They were all flowering but only 3% had pods on them at the time.

Treatments were implemented on 7 November 2011 by applying the selected rates of tebuthiuron either to the base of plants or evenly distributing it under the canopy of plants as outlined in Table 8.

Mortality assessments were undertaken at 12 (6 November 2012) and 24 MAT (1 November 2013).

4.6.1.4 Efficacy of aerial applications of tebuthiuron (Graslan™)

Opportunistic monitoring of aerial applications of tebuthiuron (Graslan™) were undertaken in the vicinity of site 3 after the landholder used Dow AgroSciences to undertake control of gidyea regrowth that contained rubber bush, along with some patches of other invasive weeds such as parkinsonia.

In November 2011, 295 ha were treated, mostly at a rate of 2.5 kg a.i./ha, although a few strips were flown by the aircraft at 3 and 3.5 kg a.i./ha to test the response to higher rates. In November 2012, an additional 465 ha were treated at 2.5 kg a.i./ha and ~25 ha were treated at 2 kg a.i./ha to test if a lower rate would control the gidyea regrowth and rubber bush.

Prior to treatment, three transects 2 m wide by 40 m long were established in each area designated to receive a specific rate of tebuthiuron. (Note that three transects were shortened to 25–30 m due to higher densities of plants.) For both 2011 and 2012 applications, three 40 m transects were also established in neighbouring untreated areas that were similar in vegetation structure to the treated areas. All plants within transects had their height and basal diameter measured, and whether they were flowering and/or podding at the time of treatment, were recorded.

The density of rubber bush in the first treatment area averaged 3800 ± 300, 8000 ± 900, 4800 ± 2000 and 6000 ± 500 plants/ha for the untreated control and 2.5, 3 and 3.5 kg a.i./ha treatments, respectively. In the second area, the control and 2.0 and 2.5 kg a.i./ha treatments averaged 15 600 ± 3 900, 38 400 ± 13 000 and 19 300 ± 3600 plants/ha, respectively.

Despite differences in the density of plants between treatments, the size structure (in terms of height) was fairly similar for both areas treated (i.e. in 2011 and 2012).The demography of the transects suggested that they were both in the expansion stage with plants less than 1 m in height the dominant size class (Fig. 27).

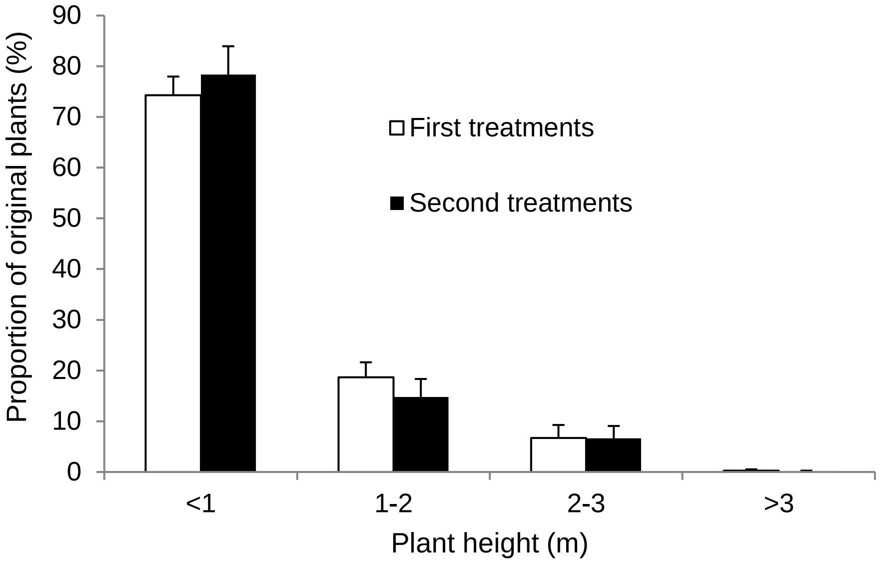


Fig. 27. The size class structure (height) of rubber bush plants in the first and second areas treated with aerial applications of tebuthiuron. The bar above each vertical column represents the standard error of the mean.

Quarterly assessments of transects were undertaken for three years following the 2011 treatments, and six monthly assessments were undertaken for two years following the 2012 treatments. Each time, plant mortality and the appearance of any new seedlings within transects was recorded. Unfortunately, two of the untreated control transects associated with the 2011 treatments had to be discarded due to them being accidentally treated with tebuthiuron.

4.6.1.5 Rate response of liquid hexazinone

Promising preliminary results from the screening trial of several residual herbicides led to the establishment of a completely randomised experiment in September 2012 at site 2 to refine rates for liquid hexazinone (i.e. Velpar® L). There were six treatments, each replicated three times. Experimental units comprised clusters of 15 plants spaced at least four metres apart from each other and from neighbouring clusters.

The treatments comprised five rates of hexazinone (0, 0.25, 0.5, 0.75 and 1 g a.i./m of plant height) with water added to respective rates, if required, to enable a 4 mL dose of mixture to be applied per metre of plant height. A neat application of 0.5 g a.i per metre of plant height was also included and equated to an application of 2 mL of product per metre of plant height. It was included to test whether the volume of liquid was important for effective application of liquid hexazinone.

Prior to application of treatments, plants to be treated were tagged and their height, basal diameter, and whether they had flowers and/or pods were recorded. On average, they were 2.32 ± 0.02 m high and had a basal diameter of 11.43 ± 0.19 cm. All plants were flowering but only 2% had pods.

Treatments were implemented (see application details in Table 8) on 3 November 2012 by applying doses of respective treatment mixtures to the base of plants.

Mortality assessments at 24 MAT were undertaken on the 9 November 2014.

4.6.2 Results

4.6.2.1 Screening trial

A significant difference (P < 0.05) occurred between herbicide treatments at 8, 12, 18 and 24 MAT. The granular hexazinone was the fastest acting herbicide and hexazinone/bromacil/diuron the slowest, with the liquid hexazinone and tebuthiuron in between (Fig. 28). Nevertheless, by 24 MAT all herbicide treatments exhibited high mortality (97-100%) and were significantly different to the untreated control, which averaged 30%. This mortality in control treatments appears to be associated with a dieback phenomenon which started affecting plants between 8 and 12 MAT. Despite differences in the rate of mortality over time between herbicide treatments, their effect on rubber bush plants was similar. Initially, chlorosis of the leaves occurred which was followed by stem death and then some reshooting at the base. This regrowth then became chlorotic and the plants eventually died.

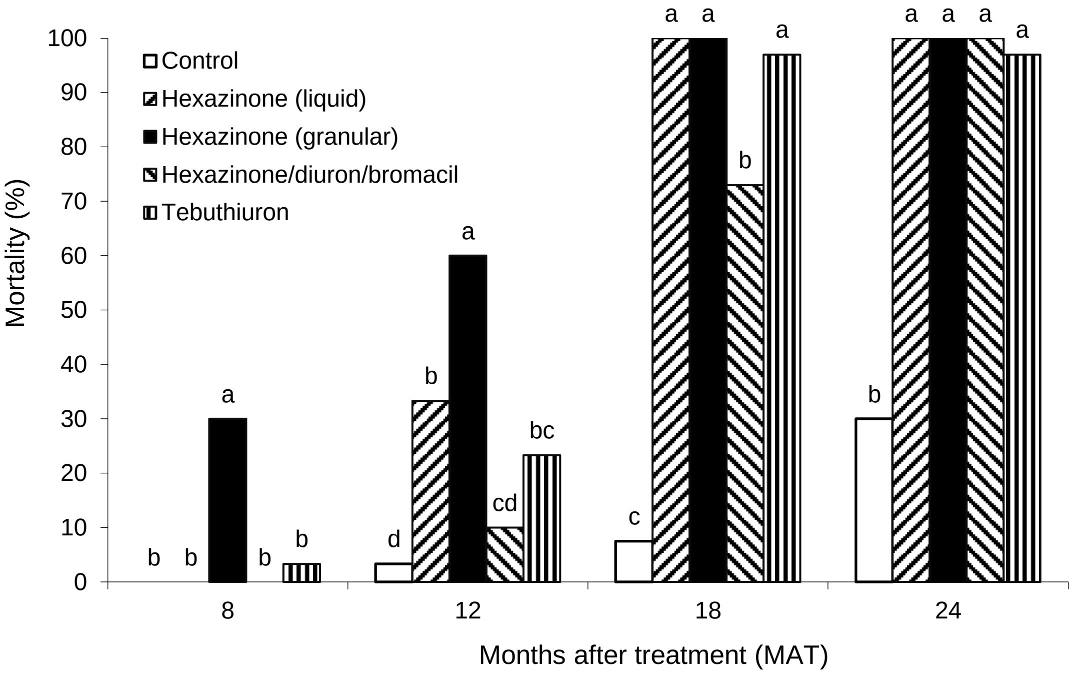


Fig. 28. Efficacy (% mortality) of four ground applied residual herbicides on rubber bush compared against an untreated control, 8, 12, 18 and 24 MAT. For each assessment time, bars with the same letters are not significantly different from each other (P < 0.05).

4.6.2.2 Influence of season, placement and application rate on efficacy of ground applications of tebuthiuron

February applications of tebuthiuron resulted in low mortality (19.7 ± 1.3%) of rubber bush 24 MAT, irrespective of the rate applied or the placement position. Spring applications resulted in significantly higher mortality (P < 0.05) particularly as the application rate increased (Fig. 29). Placement position (i.e. at the base or evenly distributed under the canopy) did not have a significant influence ($P > 0.05$) but there was a trend towards higher mortality at the lowest rate of tebuthiuron if placed at the base of plants.

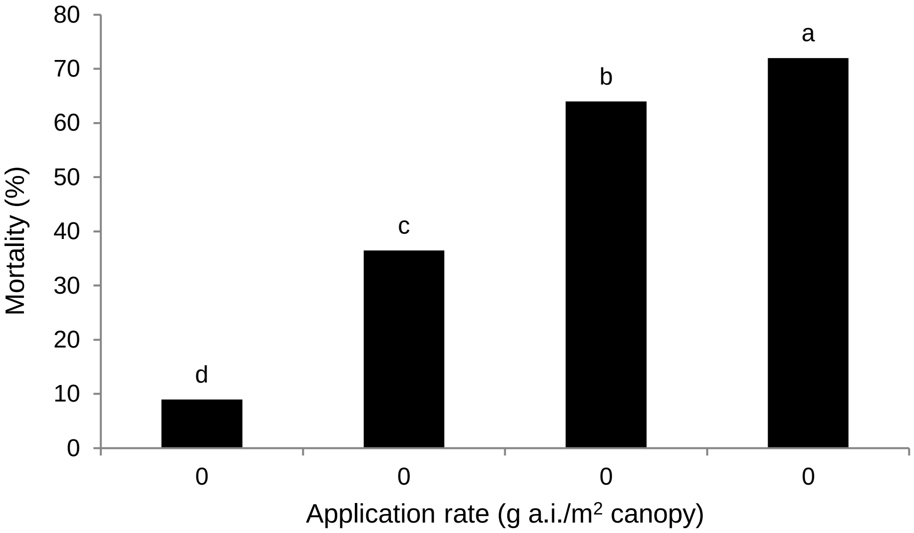


Fig. 29. The effect of tebuthiuron rates on mortality of rubber bush 24 MAT at site 4 following spring application. Bars with the same letters are not significantly different from each other ($P < 0.05$).

4.6.2.3 Refinement of application rates and placement of tebuthiuron

There was no significant difference ($P > 0.05$) in mortality between the rates of herbicide applied or the placement position of the herbicide and no significant rate × placement interaction ($P > 0.05$). All herbicide treatments were, however, significantly different to the untreated control ($P < 0.05$). At 12 and 24 MAT, the average mortality of control plants was 7% and 50%, respectively. In contrast, the herbicide treatments average mortality was 81% 12 MAT with this increasing to 100% 24 MAT (Fig. 30). Between 12 and 24 MAT some control plants started showing evidence that they may have come in contact with tebuthiuron. Initially, chlorosis of the leaves occurred which was followed by stem death and then some reshooting at the base. This regrowth then became chlorotic and the plants eventually died.

The cost of herbicide to treat individual plants with the lowest effective rate of 2 g/m^2 was \$0.18, which was based on April 2015 prices for Graslan™ and an average application of 11 g/plant.

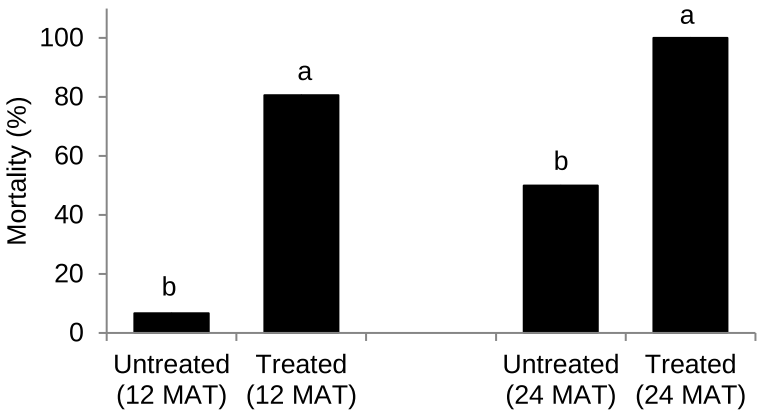


Fig. 30. Differences in mortality between untreated and tebuthiuron treatments (across all rates and placement positions) at site 3, 12 and 24 MAT. For each assessment time, bars with the same letters are not significantly different from each other ($P < 0.05$).

4.6.2.4 Efficacy of aerial applications of tebuthiuron (Graslan™)

In the first area treated aerially with tebuthiuron in November 2011, high mortality of rubber bush was recorded 24 MAT, even at the lowest rate of 2.5 kg a.i./ha (Fig. 31). In contrast, rubber bush in the neighbouring untreated area rapidly increased in density during the first 12 months of monitoring, before natural attrition of young seedlings occurred. Nevertheless, there was still the equivalent of 14 375 and 11 750 plants/ha after 24 and 36 months, respectively. The first seedling in treated transects was recorded 30 MAT and by 36 MAT there were on average 31.25 ± 31.25 seedlings/ha, across all tebuthiuron treatments.

At the time, the application cost to apply tebuthiuron aerially at the designated rates ranged between ~$185-$220/ha, but treatment cost will vary depending on the distance from an aerial spray contractor's base, proximity of an airfield to the site to be treated, and any bulk discounts that may be available from suppliers (Graham Fossett, personal communication).

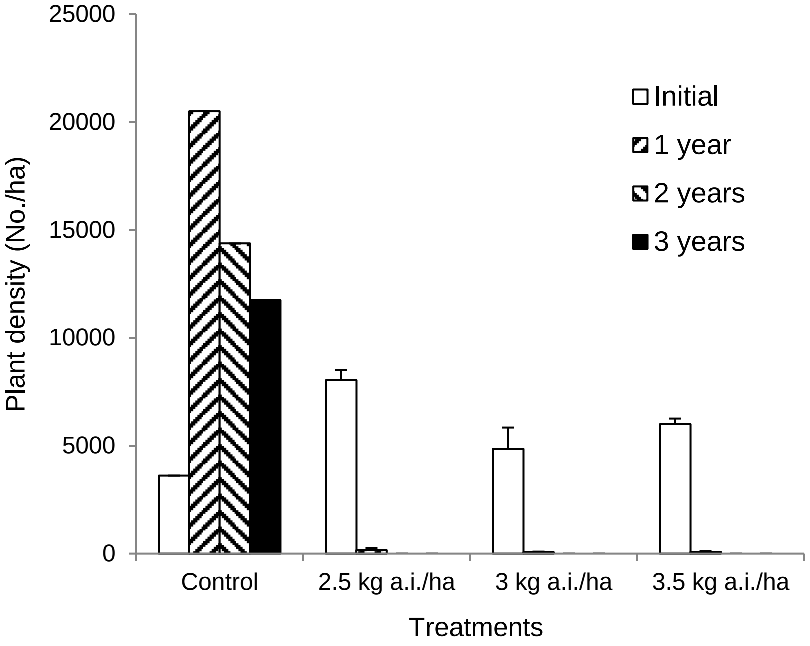


Fig. 31. Average plant density over time for transects treated with 2.5, 3 and 3.5 kg a.i. of tebuthiuron/ha in 2011 and an untreated control. The bar above each vertical column represents the standard error of the mean (No error bars are presented for the control treatment, because only one transect was monitored after two control transects were accidentally treated with tebuthiuron).

In the second area treated aerially with tebuthiuron (in November 2012), mortality was again high for transects that received 2.5 kg a.i./ha, although a small proportion (3.5%) of plants remained alive 24 MAT. In contrast, the 2 kg a.i./ha treatment failed to kill 23% of the original plants (Plate 7). The untreated control experienced a slight decrease in density over time (Fig. 32) despite 444 new seedlings/ha being recorded over the two years. New seedlings were recorded within 6 months in the 2 and 2.5 kg a.i./ha treatments and averaged 267 ± 267 and 125 ± 72/ha for the two years after treatment, respectively.

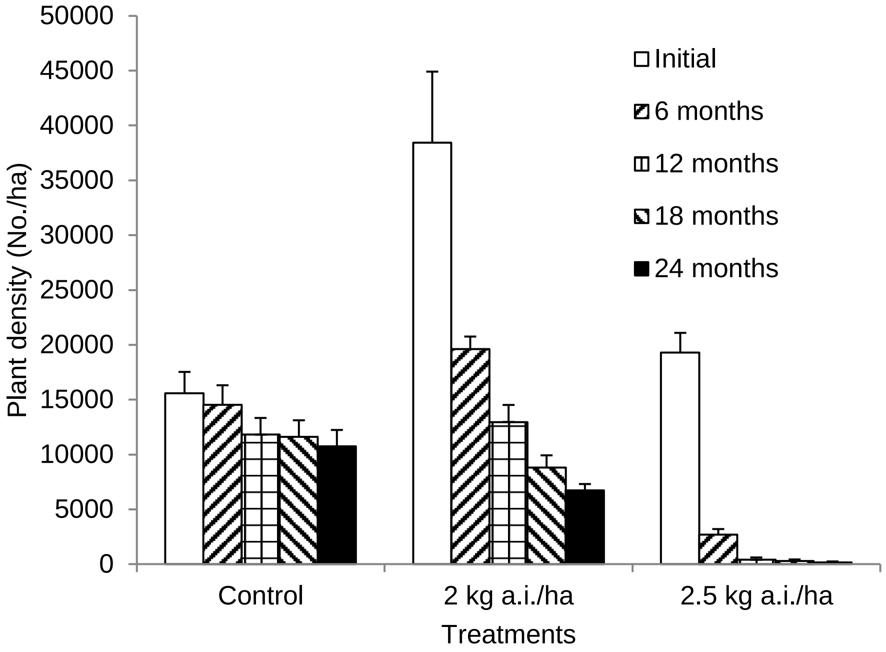


Fig. 32. Average plant density over time for transects treated with 2.0 and 2.5 kg a.i. of tebuthiuron/ha in 2012 and an untreated control. The bar above each vertical column represents the standard error of the mean.

Plate 7. Rubber bush transects prior to aerial applications (November 2011) in the first treated area of 2 (top left) and 2.5 kg a.i./ha (bottom left) and 24 MAT (November 2013) for the 2 (top right) and 2.5 kg a.i./ha (bottom right) treatments.

4.6.2.5 Rate response of liquid hexazinone

Two years after implementation of treatments, a highly significant difference in plant mortality was recorded ($P < 0.001$). All hexazinone treatments exhibited greater than 90% mortality and were not significantly different from each other, irrespective of the rate applied (Fig. 33). They were, however, all significantly different to the untreated control which averaged 29% mortality 24 MAT.

The amount of liquid applied in individual doses did not make a significant difference ($P > 0.05$) with efficacy at a rate of 0.5 g a.i./metre of plant height similar irrespective of whether a neat 2 mL or a diluted 4 mL (equal parts herbicide and water) dose was applied.

The cost of herbicide to treat individual plants with 2 and 4 mL of hexazinone/m of plant height was $0.13 and $0.28, respectively. This was based on April 2015 prices for Velpar® L and an average application of 11 g/plant.

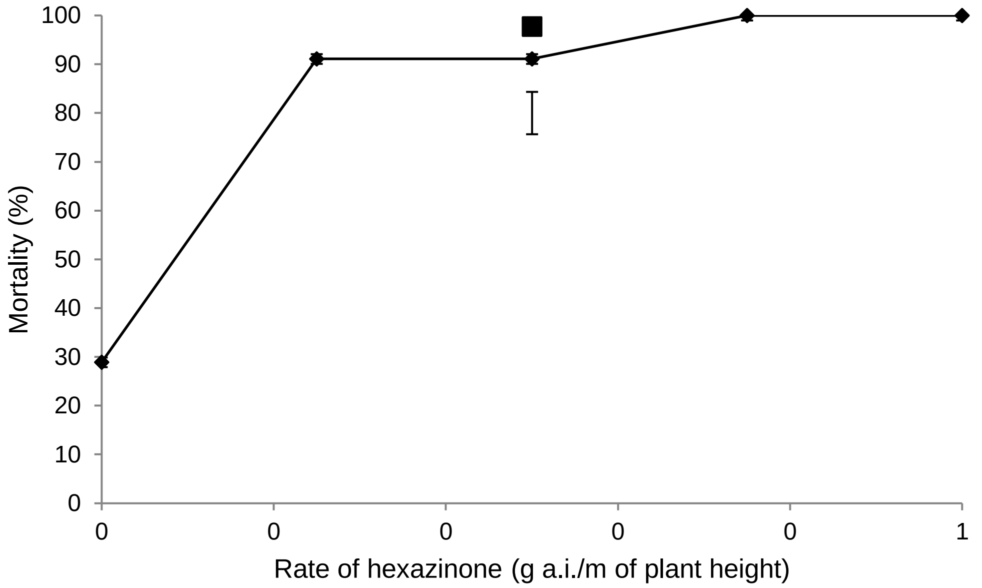


Fig. 33. The mortality (♦) of liquid hexazinone applied in 4 mL doses at 0, 0.25, 0.5, 0.75 and 1 g a.i./m of plant height. The efficacy of a neat application of 0.5 g a.i/m of plant height applied using 2 mL doses is also included (■) for comparison, along with a least significant bar to highlight differences between treatments at $P = 0.05$.

4.7 Testing of the most promising herbicide treatments in the Northern Territory

4.7.1 Methodology

To test whether the most promising ground applied herbicide treatments identified from the Queensland research would perform consistently for rubber bush elsewhere, a trial was established in the Northern Territory. This trial was also intended as a demonstration for landholders interested in management of rubber bush. Northern Territory staff from the Department of Land Resource Management also coordinated the implementation of two demonstrations in the Barkly Tablelands on the use of aerial applications of tebuthiuron to control rubber bush, based on the findings of the Queensland work. However, the efficacy of the demonstrations was not fully apparent at the time of completion of the rubber bush project and consequently is not incorporated into this report.

With regards to efficacy of ground applications, in May 2014 a randomised complete block experiment comprising 12 treatments (Table 17), each replicated three times, was established in the Barkly Tablelands region. Two replicates were located at site 5, whilst the third was at site 6. Each block had plots sufficient in size (an average of 361 m^2 per plot) to contain 20 medium to large sized rubber bush plants.

The foliar treatments were applied in May 2014 using a Quik Spray® unit (see Table 8 for application details). Both traditional and thinline basal bark treatments and cut stump applications were also applied in May 2014 (see Table 8). Ground applications of the residual herbicides tebuthiuron and hexazinone were manually applied towards the end of October 2014.

Rubber bush plants were evaluated for live growth six months after application (November 2014) for the foliar, basal bark and cut stump treatments. However, due to the onset of the wet season, only two replicates could be completed. Further assessments of these treatments, as well as the ground applied residual herbicides, will continue after cessation of the project.

Table 17. Herbicide treatments implemented in the northern Territory based on the findings from the Queensland trials.

Control method	Herbicide (Active ingredient)	Trade name	Application rate (g a.i.)
Basal bark (Traditional)	Triclopyr/picloram	Access™	40/20 g a.i./10 L mixture
Basal bark (Thinline)	Triclopyr/picloram	Access™	240/120 g a.i./10 L mixture
Cut stump	Triclopyr/picloram	Access™	40/20 g a.i./10 L mixture
Cut stump	Glyphosate	Squareup 360™	360 g a.i./1 L (neat)
Cut stump	Picloram	Vigilant™	43 g a.i./kg (neat)
Foliar	2,4-D amine	Amine 625	625 g a.i./100 L mixture
Foliar	Metsulfuron-methyl	Brush-off®	12 g a.i/100 L mixture
Foliar	Metsulfuron-methyl + Triclopyr/picloram	Brush-Off® + Picloram + Triclopyr 400	12 g a.i/100 L mixture + 150/50 g a.i./100 L mixture
Soil applied	Tebuthiuron	Graslan™	0.3 g a.i./m^2 of canopy cover
Soil applied	Tebuthiuron	Graslan™	0.4 g a.i./m^2 of canopy cover
Soil applied	Hexazinone	Velpar® L	1 g a.i/m of height (neat)
Control			

4.7.2 Results

After six months, the two basal bark treatments using triclopyr/picloram and the foliar application of 2,4-D amine were the most promising with >90% of rubber bush plants showing no signs of live growth (Table 18). Effectiveness of cut stump applications of triclopyr/picloram was lower but not significantly different from above-mentioned treatments, averaging 77%. However, there was variation between replicates, with one replicate recording 95% and the other only 60% mortality.

Cut stumping using neat glyphosate produced moderate results, with 60% of plants showing no signs of live growth after six months. This is better than plants treated with picloram gel, which averaged only 15%.

Foliar applications of metsulfuron-methyl alone and in a mixture with triclopyr/picloram performed poorly, with >70% of plants still displaying live growth.

Table 18. The percentage of plants exhibiting no live growth (above ground) six months after application of treatments (mean across two replicates). Figures followed by the same letters are not significantly different from each other ($P < 0.05$). Missing values are for treatments not yet evaluated.

Control method	Herbicide (Active ingredient)	Application rate (grams active ingredient)	No live growth (%)
Basal bark (Traditional)	Triclopyr/picloram	40/20 g a.i./10 L mixture	95a
Basal bark (Thinline)	Triclopyr/picloram	240/120 g a.i./10 L mixture	97a
Cut stump	Triclopyr/picloram	40/20 g a.i./10 L mixture	77ab
Cut stump	Glyphosate	360 g a.i./1 L (neat)	60bc
Cut stump	Picloram	43 g a.i./kg (neat)	15cd
Foliar	2,4-D amine	625 g a.i./100 L mixture	92a
Foliar	Metsulfuron-methyl	12 g a.i/100 L mixture	12cd
Foliar	Metsulfuron-methyl + Triclopyr/picloram	12 g a.i/100 L mixture + 150/50 g a.i./100 L mixture	27cd
Soil applied	Tebuthiuron	0.3 g a.i./m^2 of canopy cover	-
Soil applied	Tebuthiuron	0.4 g a.i./m^2 of canopy cover	-
Soil applied	Hexazinone	1 g a.i/m of height (neat)	-
Control			0d

4.8 Mechanical control

4.8.1 Methodology

4.8.1.1 Effect of cutting depth

A completely randomised experiment comprising four treatments was initiated on 7 October 2011 at site 1. The aim of this experiment was to determine if mortality of rubber bush plants would occur if the main tap root was severed beneath the soil surface, similar to what is achieved using cutter bar and bladeplough style equipment. Treatments involved an untreated control (not cut) and three cutting depths (0, 10 and 20 cm below ground).

Experimental units were individual rubber bush plants spaced over 2 m apart, with each treatment replicated 15 times. Prior to application of treatments, plants to be treated were tagged and their height and basal diameter were recorded. On average, they were 1.72 ± 0.03 m high and had an average basal diameter of 7.63 ± 0.16 cm.

A mattock was used to sever the roots for the below-ground cuts (i.e. 10 and 20 cm) and a forestry-type pruning saw was used to cut stems level with the ground surface for the 0 cm treatment. Once treatments were implemented, the exact depth that cuts were made was measured, and averaged 11.2 ± 0.55 cm and 19 ± 0.36 cm for the 10 and 20 cm below ground treatments, respectively.

Post-treatment mortality assessments were undertaken 4 (February 2012), 8 (26 June 2012), 12 (30 October 2012), 16 (11 February 2013), 21 (27 July 2013) and 24 MAT (27 October 2013). If plants were alive, the number of stems, their height and whether there were any flowers or pods were recorded.

4.8.1.2 Stick raking demonstration

In February 2012, the manager of the property used a front-end loader with a stick rake attachment to clear ~5 ha of dense rubber bush near site 1. To estimate the efficacy of this technique, a 25 m × 25 m area was demarcated within the 5 ha area, two weeks prior to treatment application. The location (from a fixed point), height and basal diameter of all plants were then recorded in the 25 m x 25 m area. Post treatment assessments were undertaken 5 (21 July 2012) and 13 MAT (March 2013) to quantify the level of mortality of original plants and the amount of seedling regrowth. Observations of flowering and podding were also made during post treatment assessments.

4.8.2 Results

4.8.2.1 Effect of cutting depth

Cutting height had a significant ($P < 0.05$) effect on the mortality of rubber bush plants. All plants cut at ground level reshot after treatment, whilst all plants cut at 10 or 20 cm below ground were killed (Fig. 34). Some mortality of control and 0 cm plants occurred from 12 MAT (October 2012) onwards and coincided with the appearance of dieback during this time.

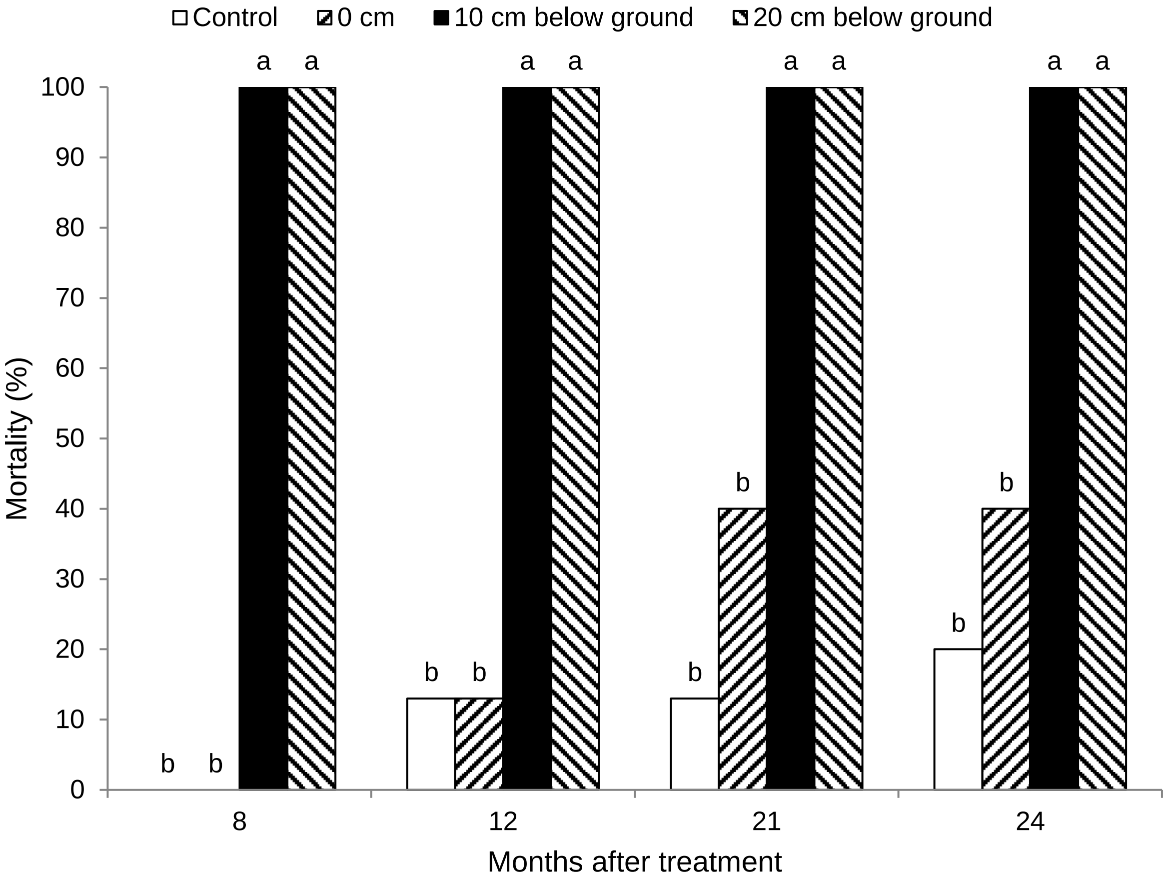


Fig. 34. The effect of cutting depth on mortality of rubber bush plants 8, 12, 21 and 24 MAT. Within each assessment time, bars with the same letters are not significantly different from each other (P < 0.05).

Cutting plants off close to ground level caused them to vigorously reshoot, with the number of stems peaking at 7.4 ± 0.54 (4 MAT), more than twice the number of stems compared to control plants at the same time (Fig. 35). The height of cut plants also increased rapidly, and by 12 MAT they were only 26 cm shorter than untreated control plants (Fig. 35). At 8 MAT all cut plants were flowering again and by 12 MAT a small percentage (15%) were podding. The impacts of the dieback resulted in a rapid reduction in plant height in both the control and 0 cm treatment from 12 MAT onwards. While plants responded to dieback by reshooting from the base (mainly between 12 and 16 MAT), many of these stems eventually died. By 24 MAT, the average number of live stems per plant was less than two for both control and 0 cm treatments (Fig. 35).

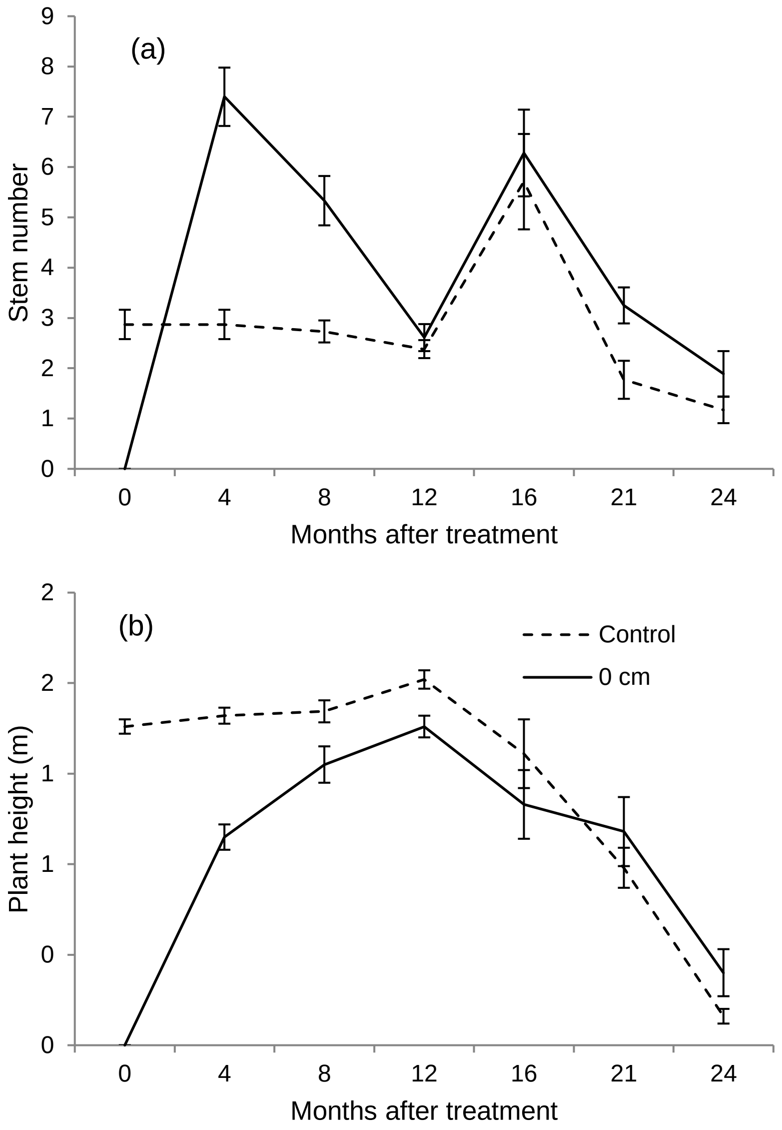


Fig. 35. Changes in (a) stem number and (b) plant height for live control plants and those cut off at ground level (0 cm treatment). Vertical bars represent the standard error.

4.8.2.2 Stick raking demonstration

Thirteen months after stick raking was undertaken, 36% of the original plants remained alive, with all having reshot from the base after being cut-off close to ground level. There were also 784 new seedlings/ha, which was more than the original population density of 496 plants/ha. When combined with surviving plants, the total rubber bush population 13 MAT was 976 plants/ha, an increase of 97% compared to the original density. Surviving original plants averaged 1.13 m in height with 50% flowering, but only 8% with pods on them. New seedlings ranged between 30-75 cm in height.

4.9 Dieback monitoring

4.9.1 Methodology

To understand the population impacts of a dieback phenomenon that started affecting rubber bush plants in control trials undertaken at the Gulf of Carpentaria field sites, six transects were established in a dieback affected area (~2 ha) of site 1 in December 2012. Transects were two metres wide and long enough (9 to 35 m) to contain ~70 plants. When the transects were established, the majority of plants (57%) had an average basal diameter of 2-5 cm (Fig. 36). Small plants (<2 cm basal diameter) comprised 26% of the population and large plants (>10 cm basal diameter) only 3%.

Dieback assessments were undertaken using a 1-5 rating scale (Table 19), where 1 was no affect and 5 was death of plants. Assessments were undertaken 7 (27 July 2013), 11 (3 November 2013), 17 (5 May 2014) and 23 months (5 November 2014) after establishment of the transects. Each time, dieback assessments of each plant were made and the number of new seedlings and their location were recorded. Whether plants were flowering or podding was also noted.

Table 19. Rating scores used to quantify the affect that the dieback was having on rubber bush plants within transects at site 1.

Rating score	Description
1	No affect
2	Branch tip death, leaves dropping off
3	Branch death, reshooting from main stem
4	Branch and stem death, may be reshooting from base
5	Dead

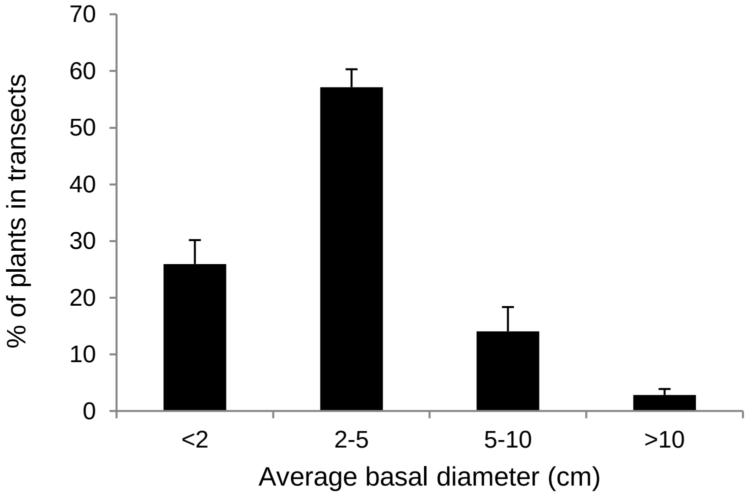


Fig. 36. The initial size class structure (average basal diameter) of the dieback affected area at site 1. The bar above each vertical column represents the standard error of the mean.

4.9.2 Results

Over the two years of monitoring, 96% of the original 28 250 ± 4950 plants/ha died and only 50 seedlings/ha were recruited, mostly in the first seven months. Consequently, there was only 1250 ± 1100 plants/ha present in December 2014 (Fig. 37).

The level of damage imposed by the dieback on plants increased over time (Fig. 38), with many plants initially rated 3 or 4 progressing to a rating of 5 (i.e. dead) two years later (Plate 8). At the time of initial assessments, six percent of plants had flowers, however no flowering was recorded in subsequent monitoring. No podding was recorded throughout the entire monitoring period, including at the time of the initial assessment.

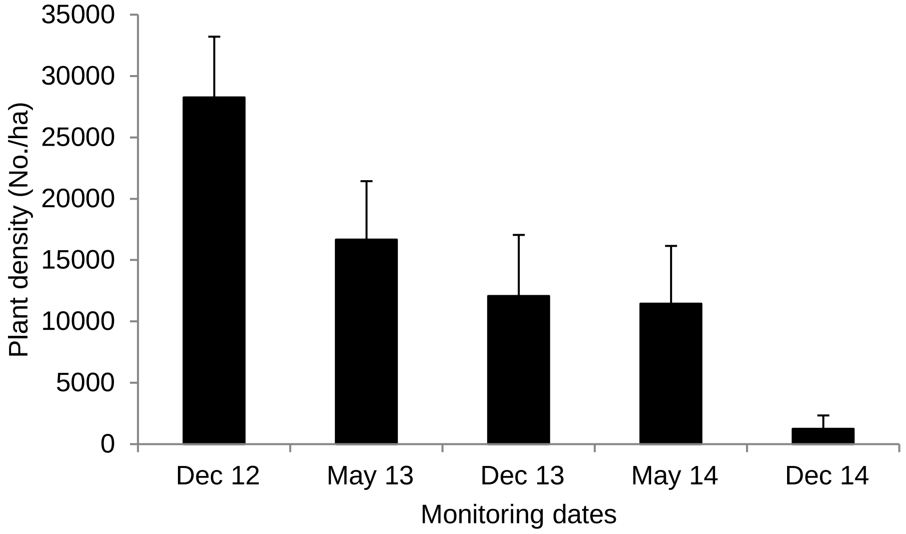


Fig. 37. Changes in rubber bush density (plants/ha) over time in dieback affected transects. The bar above each vertical column represents the standard error of the mean.

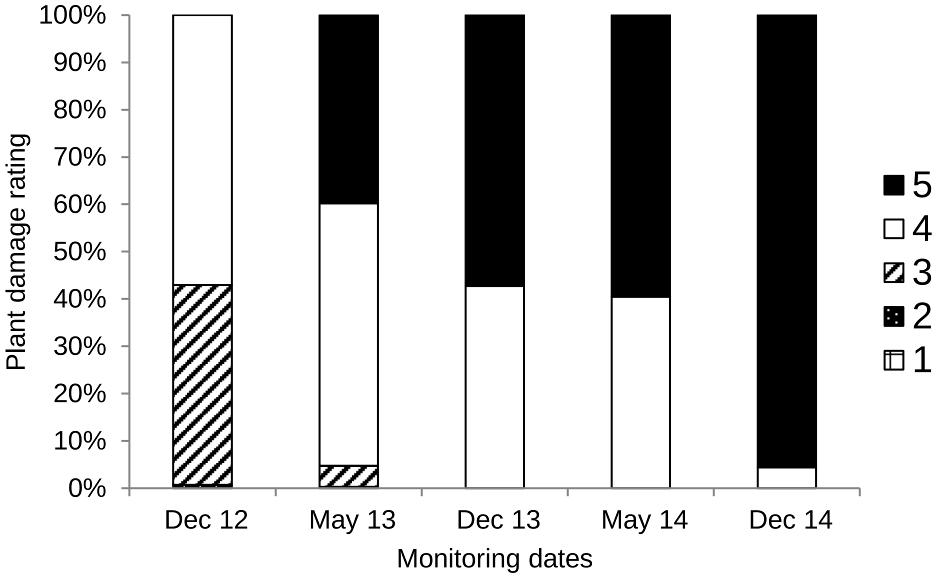


Fig. 38. Damage assessment ratings (see Table 19) for each monitoring period, expressed as a percentage of live plants.

Plate 8. Rubber bush plants within a dieback transect at the start (December 2012) (left) of monitoring and two years later (November 2014) (right) at site 1.

4.10 Discussion

The control research in this project expanded on previous studies (Vitelli et al. 2008), by confirming some of the results obtained in this earlier research, as well as identifying several additional options capable of giving effective control of rubber bush. Though it was a difficult plant to achieve consistently high efficacy using some techniques (particularly foliar applications), others such as basal barking and ground and aerial applications of residual herbicides, consistently caused high mortality. The pilot mechanical trial identified the level of damage needed to kill rubber bush, but results from a stick raking demonstration highlighted a capacity to rapidly replace killed plants through new seedlings. During the course of the control research, a dieback phenomenon started deleteriously affecting rubber bush plants, and appeared to have an impact on the efficacy of some of the applied treatments (particularly foliar spraying). Regular monitoring of transects at an affected site indicated that it could have a major impact on populations. Detailed discussion on the results of the various techniques tested and the impacts of the dieback are provided below.

4.10.1 Foliar herbicides

In the trials undertaken, high mortality was achieved with foliar spraying using some herbicides, but there were inconsistences between trials, between the seasonal times that applications were made, and between previously reported trials (Vitelli et al. 2008). The size of plants also appears to have been an influence on herbicide efficacy; overall, mortality tended to be higher for smaller plants.

Imazapyr/glyphosate, 2,4-D amine and metsulfuron-methyl were the only herbicides to give high mortality of the larger rubber bush plants in Trial 2, for at least one of the application times. Vitelli et al. (2008) had previously reported excellent mortality of rubber bush using imazapyr at rates of 125-250 g a.i./100 L. In the current study, the lower rate of imazapyr/glyphosate contained only 97.5 g a.i. of imazapyr/100 L of solution, but there was an equal proportion of glyphosate that could have been contributing to mortality of rubber bush. Nevertheless, restrictions associated with imazapyr means that chemicals containing this active ingredient can only be applied in situations such as roadsides and right of ways, not in pastures. Metsulfuron-methyl is currently the most cost effective option for treating a range of broadleaf weeds in pastures, and has previously been reported to give high mortality of rubber bush when applied at a rate of 12 g a.i./100 L (Vitelli et al. 2008). In the current trials, high mortality was achieved only once at an application rate of 12 g a.i./100 L, but for the rest of the times it was sprayed, efficacy was poor, even at an application rate of 18 g a.i./100 L. Anecdotally, several landholders have reported similar variation in mortality after using this herbicide, bringing into question whether it should be recommended as a reliable option for treating rubber bush. In contrast, 2,4-D amine consistently gave good control of rubber bush during autumn applications, but a higher rate (625 g a.i./100 L) was needed to provide high mortality of the larger plants in trial 2. However, even at the higher rate, 2,4-D amine would be a more cost effective alternative than most of the other options, except metsulfuron-methyl.

In conclusion, the results from the trials suggest that efficacy from foliar spraying can be reliably high if applied to small to medium plants up to 1.5 m high. More variation occurs when treating larger plants, so alternate control techniques, such as basal barking, should be considered. 2,4-D amine and imazapyr based products could give good efficacy in non-pasture situations, whilst in pastures 2,4-D amine would be most applicable. Despite the variability of metsulfuron-methyl, the fact that high mortality was achieved in summer during the seasonality trial, in combination with its excellent efficacy in previous studies (Vitelli et al. 2008), suggests that landholders should test it in their situations along with options such as 2,4-D amine. These two chemicals are compatible and could be included in a mixture at little extra expense given the low cost of metsulfuron-methyl. While this combination did not perform any better in the current studies than 2,4-D amine on its own, it may do so in some situations and would also control a broader range of other weeds that may be growing in association with rubber bush. To maximise uptake of herbicide by rubber bush, it is recommended that landholders not only spray the foliage of rubber bush, but also the stems.

The presence of dieback was a confounding factor during the foliar trials, and appeared to be at least partly responsible for increasing the variability of the results within treatments as well as increasing mortality over time. While some plant death in untreated controls was most likely associated with dieback, herbicide treated plants may possibly have been more susceptible to the dieback following treatment. This is ironic as, in hindsight, some of the plants could have been affected by the dieback at the time treatments were applied which may have contributed to the poorer than expected results. Previous research on the woody weed rubber vine (*Cryptostegia grandiflora*) has shown that efficacy of foliar applications of herbicide is reduced by increasing presence of the leaf rust *Maravalia cryptostegiae* (Vitelli & Madigan 1999). This could possibly be similar for rubber bush and land managers should therefore only spray plants when they are in healthy condition and with maximum leaf coverage that is ideally not affected by any leaf diseases.

4.10.2 Basal bark applications

The inclusion of basal bark treatments in the foliar trials for comparative purposes provided the opportunity to confirm the efficacy of triclopyr/picloram using both the traditional method and the newer thinline technique. Vitelli et al. (2008) had previously reported up to 87% mortality using the traditional basal bark technique. In the current study, mortality exceeded 95% mortality across both techniques and irrespective of the season of application. Anecdotally however, some landholders have had mixed results basal barking rubber bush. This could be associated with the different bark structure of rubber bush compared with several other woody weeds that they may have been used to, such as prickly acacia. Rubber bush has a corky bark that soaks up more chemical before run-off, which is recommended for basal barking. The spray mixture does not spread as easily as it does for some other plants, so it is critical that the applicator directly sprays all the way around the plant.

Basal barking for control of rubber bush using triclopyr/picloram has been included in a minor use permit (PER12497) for several years, but the manufacturer of this product is now progressing full label registration for both the traditional and thinline techniques.

4.10.3 Cut stumping and frilling

Cut stumping using triclopyr/picloram (Access™) mixed in diesel has been previously identified as an effective technique for controlling isolated plants and low density infestations of rubber bush (Vitelli et al. 2008). The current research has found that good control can also be obtained through neat applications of glyphosate or pasting the cut surface with a picloram based gel, particularly when plants are cut off close to ground level. Both these options have the added advantages of requiring no mixing with diesel and being easy to transport. Furthermore, equipment to cut plants off does not need to be sophisticated for small plants; pruning saws and a range of garden clippers are more than adequate and easy to carry around. As plants become larger, more heavy duty equipment, such as brush cutters and chainsaws, will be required. For these plants, triclopyr/picloram (Access™) may be the best herbicide to apply after the plants are cut off close to ground level. The plants used in the trials were of a medium size and efficacy on larger plants cannot be guaranteed. This may be a possible reason for the poor results obtained in the Northern Territory trial (see below). Alternatively, dieback may have made the plants more susceptible to the imposed treatments and potentially contributed to the high mortality that was obtained. Nevertheless, these techniques warrant being considered for inclusion in a revision of the current minor use permit (PER12497) for control of small to medium sized plants, but in the interim some additional plants of varying sizes will be treated to support the previous findings.

Frilling also proved effective using both glyphosate and picloram gel herbicides but, given the multi-stemmed nature of rubber bush, it would be a time consuming exercise for treating anything but isolated or very low density infestations. Nevertheless, not much equipment needs to be transported and frilling will also be considered for inclusion in the minor use permit.

4.10.4 Ground and aerially applied residual herbicides

Both ground and aerial applications of residual herbicides proved highly effective at controlling rubber bush plants. Distribution of chemical over the whole infested area, such as in aerial application, also minimises seedling regrowth. Where individual plants were treated, large scale seedling recruitment was observed at times in between the treated areas.

Across all of the trials undertaken, the lowest rate of tebuthiuron that was still capable of causing high mortality of rubber bush was 2.5 kg a.i./ha *(*i.e. 0.25 g a.i./m^2). A rate of 2.0 kg a.i./ha was ineffective when applied either to individual plants based on canopy size or when evenly distributed through aerial applications. This is similar to previous research by Vitelli et al. (2008) who reported ≤20% mortality following application of tebuthiuron at rates of 1.5-2.0 kg a.i./ha.

Efficacy of tebuthiuron was best if applied prior to the onset of the wet season and not during it, which is consistent with the recommendations on the labels of respective products for treatment of woody vegetation (including weeds) generally. There also did not appear to be any difference in efficacy between placing it directly at the base of plants or spreading it evenly under the canopy of plants when treating individual plants or using a broad-scale application based on a unit area.

Variation in the efficacy of tebuthiuron between sites (particularly between site 4 and those in the Gulf of Carpentaria [sites 1-3]) when applied at comparable rates may have been associated with soil type differences. The clay soils in the Gulf of Carpentaria appeared to be conducive to high mortality, although feedback from chemical company representatives following commercial applications on two neighbouring properties in that region suggested that, in some instances, differences in clay content had an influence on mortality (Graham Fossett, personal communication). On the property next door to where site 3 was located, a rate of 3.0 kg a.i./ha of tebuthiuron was required on a fine textured (medium clay) soil to achieve comparable control to that recorded at site 3 using 2.0 kg a.i/ha (Graham Fossett, personal communication). Reeves (unpublished data) also reported poor efficacy of tebuthiuron when applied at rates of 0.4-1.0 g a.i./m^2 in northern Western Australia on a sandier soil type.

Based on the findings, a revision of the current minor use permit (PER12497) will be submitted to include both ground and aerial applications of tebuthiuron. However, given the potential variability of tebuthiuron, before commencing broad-scale control of rubber bush, it may be advantageous for land managers to firstly determine its efficacy on a smaller scale and at a few rates for their particular soil types. This will help identify whether it will be an effective option for them. If native vegetation is also present amongst the rubber bush, any existing vegetation management laws would need to be considered for respective states and territories before applying tebuthiuron. If after considering all these factors, aerial application of tebuthiuron is considered the most cost effective option, the use of reputable aerial operators with suitable application equipment and mapping technology will ensure accurate product placement.

Liquid hexazinone proved highly effective in killing rubber bush at rates ranging from 0.25 to 1 g a.i./m of plant height. However, like tebuthiuron, the poor efficacy reported following its use previously (Reeves, unpublished data) could be associated with site specific differences, such as soil types. Testing on a small scale should therefore be undertaken before conducting broad-scale control. In the interim, liquid hexazinone will be included for consideration by the APVMA in a revision of the current minor use permit (PER12497), most likely at a rate of 0.5-1 g a.i./m, with the lower rate for small plants (≤1.5 m) and the higher rate for larger ones. If approved, land managers will also have to consider any existing vegetation management laws for respective states and territories before applying hexazinone.

4.10.5 Testing of the most promising herbicide treatments in the Northern Territory

Basal barking with triclopyr/picloram mixed with diesel using both the traditional and newer thinline technique demonstrated high mortality in the Northern Territory trial, consistent with the Queensland trials and the findings of Vitelli et al. (2008). Similarly, 2,4-D amine was also the best of the foliar treatments which is similar to the findings from Queensland, although some variation occurred between trials in Queensland.

Discrepancies between the Queensland and Northern Territory trials occurred in the efficacy of cut stump applications. In Queensland, the application of neat glyphosate and a picloram gel caused high mortality, but results were poor in the Northern Territory. Triclopyr/picloram (Access™) also gave mixed results in the Northern Territory when applied as a cut stump treatment, which contrasts with earlier work by Vitelli et al. (2008), who reported high mortality.

While no efficacy data was yet available on the soil applied residual herbicides in the Northern Territory at completion of the project, personal observations after 11 months suggested that they were performing in a similar manner to the Queensland trials at the same time, and should end up providing high mortality. The aerial demonstrations of tebuthiuron also looked promising and consistent with the Queensland findings at a comparable stage, with small plants observed to be dead and any large plants remaining alive displaying severe necrosis.

Overall, the results reaffirm previous discussions that there could be treatment variation for several of the treatments identified for rubber bush, and that small areas should be treated first to confirm whether good efficacy can be achieved using a particular technique/product before implementing large scale control activities. This will avoid unnecessary expenditure and minimise the amount of follow up control that may be needed.

4.10.6 Mechanical control

The cutting depth trial quantified the potential to obtain high mortality of rubber bush using equipment, such as cutter bars and blade ploughs, capable of severing the roots system of plants. Those that cut the plant off at ground level will cause minimal mortality. The plants will reshoot vigorously and end up with many more stems than were there initially. This will make follow up control even more difficult. The stick raking demonstration resulted in moderate mortality. Surviving plants were mostly those that had been cut/broken off at ground level. This demonstration also showed the propensity of rubber bush to re-establish through seedling recruitment, which for many weeds is often promoted under the disturbed conditions created by mechanical techniques (McKenzie et al. 2004; Bebawi et al. 2011). Such promotion of seedling regrowth can be advantageous if follow up control is undertaken before these plants reach reproductive maturity. This is particularly pertinent for rubber bush given its short seed longevity with most seedling regrowth likely to occur within the first 24 months after implementation of treatments. If this seedling regrowth is controlled, under most situations landholders should expect minimal seedling recruitment thereafter, unless previous conditions have been dry and not conducive to germination, or if further seed input is occurring from external sources (i.e. through wind or water dispersal from neighbouring infestations). If follow up control is not undertaken, initial mechanical control can exacerbate the problem and facilitate rapid expansion of rubber bush in infested areas.

4.10.7 Dieback monitoring

Over the two years that monitoring was undertaken, high mortality of rubber bush plants occurred from a dieback phenomenon that was first observed at the site in 2012. The symptoms were consistent with those of a leaf spot disease (*Passalora calotropidis*) that was reported by Wilkinson et al. (2005) after the senior author collected it in 2002 from rubber bush plants growing on sand dunes near the township of Karumba, north Queensland. At the time, this was the first recording of this disease in Australia, but since then it has been observed at several locations in Queensland and the Northern Territory. Wilkinson et al. (2005) reported that in the early stages, the disease expresses itself as a dark lesion on either surface of the leaf with a halo of chlorotic yellow tissue. As the lesion increases in size, the chlorotic zone spreads, bounded by the leaf veins. On the lower leaf surface, the dark centres grow in size and eventually become covered in mycelium, bearing spores in poorly defined concentric rings. As the disease progresses, the entire leaf becomes yellow, followed by abscission. In the absence of supporting leaves, the branch tips die back, extending to the whole branch as the disease progresses (Wilkinson et al. 2005). During the current project, all of these symptoms were observed. Plants appeared capable of reshooting vigorously if the initial branches were killed, but this new growth often became infected at some stage and, in most instances, the plants eventually died.

Passalora calotropidis was included in a list of fungal pathogens of rubber bush and was mentioned as a potential biological control agent (Baretto et al. 1999) for countries where it had invaded. Baretto et al. (1999) also suggested that the fact that it was already present in several countries throughout Central and South America indicated that it may have been spread or introduced at the same time rubber bush was introduced. If this was the case for Australia, it has been here earlier than first reported by Wilkinson et al. (2005), as rubber bush has been in the country for a long time, with reports of it being naturalised at two locations in northern Queensland in 1935 (Grace 2006). This is plausible, because Australia was not included in the large mycological study undertaken on rubber bush by Baretto et al. (1999).

While *P. calotropidis* is considered the most likely cause of the dieback being observed on rubber bush, several other pathogens have been isolated from stem sections of dieback infected plants (Isahak 2013). Some of these, when sprayed onto seedlings of rubber bush, demonstrated an ability to cause similar symptoms to those observed in the field, resulting in high mortality rates. Further research is needed to quantify exactly what causal agent is responsible for the widespread dieback being observed. This work could also lead into possible opportunities for development of a mycoherbicide for rubber bush.

Irrespective of the causal agent, the presence of a damaging dieback that is affecting the population dynamics of rubber bush needs to be factored into management strategies. Monitoring of affected areas needs to continue to determine whether it will persist and provide long term impacts on populations. If dieback does persist, it could be an alternate explanation for the large reduction in rubber bush that was observed at a vegetation monitoring site in the Victoria River district of the Northern Territory (Bastin et al. 2003). At the time, the decline in rubber bush was attributed to a combination of competition from perennial grasses and a series of years that received below average rainfall during the wet season. This could be the reason, but it is also feasible that the dieback had reached this location and started having a deleterious effect on the rubber bush plants. If the dieback was reducing the vigour of rubber bush plants, this may also have provided a more competitive advantage to the pasture grasses present.

Whilst the unintentional introduction of *P. calotropidis* may provide a level of biocontrol on rubber bush in Australia, several promising insects have also been identified overseas and warrant investigation (Dhileepan 2014). In particular, there are three pre-dispersal seed predators, the Aak weevil (*Paramecops farinosus*), the Aak fruit fly (*Dacus persicus*) in the Indian subcontinent and the Sodom apple fruit fly (*Dacus longistylus*) in the Middle East (Dhileepan 2014). In Australia there are no native Calotropis species and only two native Australian genera – Cynanchum and Tylophora (with 23 species) in the same tribe Asclepiadeae as *Calotropis* spp. This makes rubber bush an ideal target for classical biological control in Australia (Dhileepan 2014).

4.11 Conclusion

The control research has emphasised the importance of choosing the correct technique depending on the size and density of rubber bush and the situation it is growing in. Ensuring the selected technique is applied correctly is also important to avoid variable results. Smaller plants are much easier to kill than large mature ones, and it is critical that infestations are treated as soon as possible after discovery, thereby reducing the opportunity for populations to expand and spread. This will minimise control costs, which is important as there are no cheap options to effectively treat large dense infestations and, in most instances, it would be difficult to recoup the investment through increased productivity of the land. Several options that could be applied to control isolated rubber bush plants or small patches found during day to day activities (such as when checking bores) have been identified in this project and do not require a lot of equipment (e.g. such as hand application of tebuthiuron). For landholders trying to control large dense infestations, aerial application of tebuthiuron has proven a highly effective option, but there may be variability across soil types and legislative restrictions in timbered areas that landholders will have to consider. These options, along with any of the other effective herbicide treatments identified in this project, will be included in a revised minor use permit for control of rubber bush. For some, respective chemical companies will be approached to see if they would be willing to progress label registration. Already, label registration is occurring for basal bark control of rubber bush using triclopyr/picloram (Access™) mixed with diesel. The influence of a dieback phenomenon during the project has been an unexpected factor that has not only confounded some of the research findings, but also provided some promise that a level of population control may be imposed on rubber bush infestations in the absence of traditional control work. However, more work is needed to determine the long term implications of this dieback and commencement of a complimentary biocontrol program to source and introduce some of the promising insects that have been identified by other researchers (Dhileepan 2014) would be advantageous.

5 Recommendations

The ecological, invasiveness, spread/distribution and control research reported in previous sections has identified key findings that land managers should take into consideration when developing management strategies for control of rubber bush. These include:

- Most of the seed produced by rubber bush is dispersed locally, but a small percentage (~7.5%) can be blown considerable distances (>1 km) by wind, allowing it to potentially extend its range fairly rapidly (1 km every 2-3 years). Consequently, land managers need to be on the look-out for this weed over their whole property and not just in the immediate vicinity of infestations, or along watercourses, which is another vector for spread.
- Plants established through long-distance dispersal (i.e. outliers) escape from the challenges associated with established high-density populations, such as competition for resources and pollinators, and could therefore potentially grow faster and reach reproductive maturity more quickly. Furthermore, self-compatibility and solid pollinia (with hundreds of pollen grains) confer reproductive assurance so that one successful pollination event, even self-pollination, means that a single mature plant can establish a stand of rubber bush within 2-3 years. Maintenance of healthy pastures will slow the growth and development of rubber bush and increase mortality of young plants, but control of small outbreaks in new areas (i.e. not previously known to have rubber bush) or outliers arising from core infestations, should be a priority to prevent establishment or slow the spread of rubber bush, respectively.
- Where rubber bush is scattered over the landscape, control should be undertaken before the population reaches densities (~250-550 plants/ha) that are conducive to maximum fecundity, which will facilitate exponential increases in plant density under favourable environmental conditions.
- Given the variability in reproduction that can occur across locations, largely due to the presence or absence of pollinators, land managers should monitor populations prior to implementation of control activities, particularly their reproduction (e.g. how many pods they are producing). This will give them an appreciation of the level of seedling recruitment that they can expect, which may influence the control options they choose to use. For example, if there have been lots of seeds produced and the soil seed bank is high, the use of residual herbicides might be applicable to minimise seedling establishment.
- Rubber bush tends to have a short-lived seed bank (<2 years) under average or above average rainfall conditions, due to its seed characteristics (high germinability and minimal dormancy). It is therefore dependent on regular replenishment from reproductive adult plants for substantial seedling emergence/regrowth to occur. Consequently, large scale recruitment is most likely to occur within the first 12 months of control programs, and land managers could potentially achieve effective control of rubber bush patches in a 2-3 year timeframe if they are able to kill all original plants, and undertake follow-up control frequently enough to prevent any new plants from reaching reproductive maturity. This is based on the assumption that replenishment of the seed bank is not occurring from external sources (e.g. wind and water dispersal). The frequency that follow up control would need to be undertaken would be ~12 monthly to avoid replenishment of soil seed banks, although six monthly intervals would be preferable to provide two opportunities to find and control any new plants before they

reach reproductive maturity. If broad-scale control of residual herbicides has been implemented, the timing of follow up control may possibly be extended until the residual activity ceases (could be up to 2-3 years).

- Where livestock feed on rubber bush without negative effects, allowing them to browse on the plants before the onset of summer when they are most reproductive, may be a useful strategy for disrupting reproduction of rubber bush.
- Seedling emergence and establishment will be enhanced through disturbances such as overgrazing, the trampling effect of livestock during wet periods, and implementation of mechanical techniques. Pastoralists could reduce establishment rates by avoiding putting cattle into paddocks that have fruiting rubber bush plants in them to avoid trampling effects.
- Rubber bush does not appear to be an overly competitive plant, so setting stocking rates that maintain pastures in healthy condition will reduce seedling emergence and survival, and slow the growth and development of plants. However, if thick stands of rubber bush have established, they will reduce pasture production and will need to be controlled to increase the productivity of the land. In some instances, re-sowing with suitable pasture species may be required.
- Individual plants or small patches of rubber bush could be effectively treated using a number of herbicide techniques, some of which require minimal equipment easily carried by most forms of transport used on properties (e.g. all-terrain vehicles, utilities etc.). These include hand applications of tebuthiuron (e.g. Graslan™), backpack spraying of liquid hexazinone (e.g. Velpar® L), basal barking (either traditional or thinline) using triclopyr/picloram (Access™), and cut stumping with glyphosate based products, a picloram gel (Vigilant™ II), or triclopyr/picloram (Access™). For cut stumping, a wide range of equipment (from hand saws and garden clippers up to brush cutters with metal blade attachments) could potentially be used, depending on the size and density of rubber bush. Correct application of all techniques is critical, as rubber bush will reshoot otherwise.
- Application of some foliar herbicides (e.g. 2,4-D amine, imazapyr) could also be effective for scattered to medium density infestations, with efficacy most likely to be best on smaller plants (<1.5 m) and those that are in very healthy condition with maximum leaf coverage at the time. Because efficacy appears to be best when the whole plant is thoroughly sprayed, there appears to be minimal scope for effectively using aerially-applied foliar herbicides for control of rubber bush.
- For broad-scale control of dense rubber bush, aerial application of tebuthiuron is the most cost effective herbicide treatment that can provide high mortality of original plants and residual control of seedling growth. This technique will be restricted to areas permissible under vegetation management legislation of respective states and territories, and some variability in efficacy may occur between soil types.
- Control of rubber bush using mechanical treatments could be effective using equipment (e.g. blade ploughs, cutter bars) that cuts plants off below ground (e.g. 10–20 cm). If plants are only cut off at ground level, such as by bulldozers or graders, a large proportion may reshoot vigorously. Large scale seedling regrowth should be expected after mechanical control, unless plants have not produced many pods in the previous years. Stimulating the emergence of seedlings can be beneficial by helping deplete the seed bank more rapidly. However, land managers need to be able to control this seedling growth before it reaches reproductive maturity and starts replenishing the seed

bank, otherwise the problem can be exacerbated. Therefore, follow up control should be planned at the time of implementation of mechanical techniques.

- The long term implications of a dieback phenomenon on rubber bush populations in northern Australia are not fully apparent at this stage, but dieback can reduce reproduction and plant densities. Land managers planning on controlling infestations that may be displaying signs of dieback need to consider the best time to initiate activities. If it is becoming widely established and starting to adversely affect the growth and reproduction of rubber bush, immediate action may not be necessary, except perhaps to kill any healthy plants. It may be more appropriate to allow natural attrition to occur with follow up control of surviving plants undertaken later on. Conversely, implementing control when the plant is less vigorous from the dieback may increase the effectiveness of control programs particularly if the seed bank is lower than normal. However, the immediate efficacy of some techniques (such as foliar spraying) would be reduced by the dieback.

On a broader scale, distribution modelling shows that, although rubber bush has a foothold throughout northern Australia, it has not saturated its potential range with large areas still under threat. Furthermore, under climate change, the future potential distribution of rubber bush may extend into new areas, including the southern and eastern parts of the Northern Territory, border regions of Western Australia, and western Queensland. Land managers in prone areas need to be made aware of rubber bush by responsible authorities/agencies, and encouraged to control plants in the early stages of invasion if found, to prevent it impacting on livestock enterprises. Strategically, rubber bush management zones could be established from Western Australia through the Northern Territory to Queensland based on population sizes as recommended in section 3.7.5.

5.1 Identification of the most practical and cost-effective control options for rubber bush

Based on the findings from the rubber bush project, previous research studies (Reeves, unpublished data; Vitelli et al. 2008), and anecdotal evidence provided by individuals/organisations that have actively controlled rubber bush, an attempt has been made to identify the most practical and cost-effective control options for rubber bush (Table 20). An economic evaluation of control techniques was not an official objective of the rubber bush project, but in identifying the most appropriate techniques, comparative costings developed by Vitelli et al. (2008) were used in the decision making process. It is also important to note that some of the specific herbicides that will be recommended for respective techniques are not currently registered for control of rubber bush. Approval will be sought from the APVMA for their inclusion in a revision of PER12497.

Table 20. Evaluation of the suitability of potential control options for rubber bush at different densities.

Technique	Appropriate densities*	Suitability†	Comments
Mechanical - (grubbing plants out)	I,L	✓	A range of machines could be used including front-end loaders, graders and bulldozers. They will be ineffective if the plant is only cut-off at ground level and not completely removed.
Mechanical - (severing the root system off below ground)	L,M,D	✓ ✓	A range of equipment could be used including front (i.e. Ellrott plough®) and back mounted blade ploughs and cutter bar devices attached to various sized machines, ranging from tractors to large bull dozers. For broad-scale control using machinery, large scale seedling recruitment may occur.
Foliar spraying	I,L,M	✓ ✓	Generally best on smaller plants (<1.5 m) that are in healthy condition at the time of application and not affected by dieback.
Cut stump	I,L	✓ ✓	A range of equipment could be used from pruning saws to brush cutters with metal blades. Plants should be cut-off close to ground level and the cut stump sprayed within 5 seconds. Neat glyphosate‡ and a picloram gel (Vigilant™ II‡) (smaller plants only) and triclopyr/picloram (Access™) at a rate of 1 L/ 60 L of diesel would be options to apply to the cut stumps.
Basal bark	I,L,M	✓ ✓ ✓	Can be applied all year round to treat all size classes using triclopyr/picloram (Access™). Both the traditional and thinline‡ technique can be effective, but it is critical that the stem is sprayed to the point of runoff all the way around and to the required height: 30-40 cm for the traditional rate of 1 L/60 L of diesel and 5 cm for the thinline technique using 1 L/10 L of diesel.
Hand application of tebuthiuron (e.g. Graslan™)‡	I,L,M	✓ ✓ ✓	Rates ranging from 0.3–0.4 g a.i./m^2 of canopy should provide good control of original plants, although there could be some soil type differences. If soil seed banks are high at the time of application significant seedling regrowth outside of the herbicide impact zone (i.e. between treated plants) may occur.

Technique	Appropriate densities*	Suitability†	Comments
Spot gun application of liquid hexazinone (e.g. Velpar L) ‡	I,L,M	✓✓✓	Rates ranging from 0.5–1 g a.i./m of plant height (higher rate for larger plants) should provide good control of original plants, although there could be some soil type differences. If soil seed banks are high at the time of application significant seedling regrowth between treated plants may occur.
Aerial application of tebuthiuron‡	H	✓✓✓	Rates ranging between 2.5-3 kg a.i./ha should provide good control. Most appropriate for infestations in habitats with low tree cover and away from watercourses. Not only controls the original plants but also provides residual control to minimise seedling regrowth. Need to consider vegetation management legislation and variability across soil types.
Fire	L,M,H	✓	Based on anecdotal evidence only and individual one off fires. Whether a regular fire regime would impose a level of control is unknown, but warrants investigation.

*Appropriate densities: I – Isolated (1-100 plants in an area at densities of 1-5 plants/ha), L – low <250 plants/ha), M – medium (250-500 plants/ha), H – high (>500 plants/ha)

†Suitability of control options: ✓ - low ✓✓ - moderate ✓✓✓ - very good

‡Not currently registered, but approval will be sought from the APVMA for their inclusion in a revision of PER12497. The thinline basal bark technique for rubber bush will be registered in the future by Dow AgroSciences, the manufacturer of Access™.

5.2 Managing rubber bush in different environments and with different levels of rubber bush intrusion

Taking into consideration the suite of techniques that could be used to control rubber bush, recommendations are provided for managing it in different environments and with different levels of rubber bush intrusions (Table 21).

Table 21. Recommendations for managing rubber bush in different environments.

Situation	Density*	Recommendation
Mitchell grass downs country or areas that have been cleared previously (e.g. gidyea country)	I,L,M	Individual plants or small patches could be controlled in numerous ways using herbicides, including cut stumping, basal barking, spot gun application of liquid hexazinone, hand application of tebuthiuron or foliar spraying. For low to medium densities, basal barking, spot gun application of liquid hexazinone or hand application of tebuthiuron would be the recommended techniques. For the latter two options, some variation in efficacy between soil types may occur, so testing on a small scale first would be advisable. Foliar applications could also be used but would be best on smaller plants. Machinery capable of cutting the plants off below ground could give good results, but the localised disturbance may promote seedling recruitment.
	H	Aerial application of tebuthiuron would be the most cost effective technique to control not only the original infestation but also to provide residual control to minimise seedling regrowth. Rates may need to vary depending on soil type. Mechanical control may be a preferred option if landholders want to control rubber bush as well as regrowth of native vegetation (e.g. gidyea) that had been previously cleared. The use of blade ploughs or cutter bar devices that cut plants off below ground should give good control but seedling regrowth may be significant and require follow up control. If follow up control of light to medium density regrowth is required, basal barking, spot gun application of liquid hexazinone or hand application of tebuthiuron would be appropriate techniques. For dense seedling regrowth, some natural attrition may occur but foliar spraying whilst the seedlings are still small should be an effective option. Before commencing either aerial herbicide applications or mechanical control, vegetation management regulations of respective states/territories will need to be considered. Re-establishing a competitive pasture should also be a priority and may require re-seeding.

Situation	Density*	Recommendation
Timbered areas including along riparian corridors.	I,L,M,H	For all densities, control options are largely restricted to individual herbicide techniques, particularly basal barking. Cut stumping would be suitable for small outbreaks and foliar spraying could be used for up to medium level densities, particularly where populations contain a large proportion of smaller plants. Ground application of residual herbicides will be restricted in this environment due to potential off target damage to native tree species and in some cases proximity to water courses.
Agricultural non-crop areas, commercial and industrial areas, fence lines and rights-of-way.	I,L,M	In these environments, densities tend to range from new outbreaks of individual plants, to small patches, to medium density situations. Repeated cut stump, basal bark or foliar spraying using imazapyr based herbicides would be most effective for ongoing control of rubber bush in these situations. Consideration could be given to the use of liquid hexazinone or hand application of tebuthiuron if there is limited risk to non-target plants.

*Appropriate densities: I – Isolated (1-100 plants in an area at densities of 1-5 plants/ha), L – low <250 plants/ha), M – medium (250-500 plants/ha), H – high (>500 plants/ha)

5.3 Recommendations for future research and dissemination of key findings to stakeholders

Whilst the research undertaken within this project has greatly increased our knowledge of the ecology, invasiveness, spread/distribution and control of rubber bush, it has also identified some additional research that could be pursued to advance management of rubber bush in Australia. This includes:

- Genetic research to determine the genetic variability within Australian populations and to quantify the inter-relatedness of populations across northern Australia – whether rubber bush has established from multiple introductions or not, will assist with planning effective regional management strategies. This information would also be beneficial if a biological control program was to be initiated, particularly if the country of origin can be determined.
- Implementation of biological control research to build on opportunistic sampling undertaken whilst researchers have been looking for agents for other priority weeds (e.g. bellyache bush and prickly acacia). Whilst a dieback phenomenon is currently affecting infestations at several locations and may provide a level of biocontrol on rubber bush in Australia, several promising insects have also been identified overseas and warrant investigation. In particular, there are three pre-dispersal seed predators, the Aak weevil (*Paramecops farinosus*), the Aak fruit fly (*Dacus persicus*) in the Indian subcontinent and the Sodom apple fruit fly (*Dacus longistylus*) in the Middle East (Dhileepan 2014). In Australia there are no native Calotropis species and only two native Australian genera –

Cynanchum and Tylophora (with 23 species) in the same tribe Asclepiadeae as *Calotropis* spp. This makes rubber bush an ideal target for classical biological control in Australia (Dhileepan 2014).

- Further research to quantify the impacts of rubber bush on productivity of livestock enterprises and on biodiversity. The competition research undertaken in this project through a pot trial demonstrated that rubber bush can affect pasture yields. Field research to quantify density thresholds where pasture production is adversely affected, along with supporting economic analysis to show if there are any financial impacts at an enterprise level, would be desirable.
- On-going monitoring of the impacts of the dieback phenomenon on rubber bush to confirm the causal agent, and determine long term implications from a management perspective.
- Testing of some of the most effective control options on different soil types. A lot of the control research in this project was undertaken on clay soils. Anecdotal evidence and the findings from previous research suggest that efficacy may possibly be different on other soil types, particularly red soils. Residual herbicides are most likely to be influenced by soil type and it would be advantageous if they were tested on other soil types, particularly red soils.
- More demonstration style activities to test some of the best control strategies identified during the project on a larger scale. This will further extend the findings of the research to stakeholders, whilst allowing collection of some economic data. Completion of a cost benefit-analysis would also help guide decision making, but some additional field data on the impact of rubber bush on pasture production may need to be collected beforehand.
- Investigations into livestock-rubber bush interactions to ascertain whether there are any toxicity risks, what the triggers are that cause animals to eat it readily at times in some situations, but not others, and whether utilisation by livestock can be incorporated more into management strategies for control of rubber bush.
- Quantifying whether fire regimes involving a series of fires could impose population level control of rubber bush in suitable environments.

To disseminate the key recommendation from the project and maximise adoption, particularly by the grazing industry, we have commenced several initiatives and recommend several others that could be undertaken with further funding support. They include:

- Running a series of field days in the major regions being impacted by rubber bush. A well-attended event was held in the Gulf of Carpentaria in Queensland (Nardoo Station) during July 2013. It would be beneficial to also hold similar events in locations where rubber bush is only in the early stages of invasion, but predicted to expand if not controlled through early intervention.
- A wide range of community group, natural resource management and government staff involved in weed management have either been directly/indirectly involved in the rubber bush project, and are now well placed to provide the latest recommendations for management of rubber bush, either on an individual basis or at relevant events (e.g. field days).
- Weed fact sheets on priority weeds are produced by state/territory agencies responsible for overseeing pest management in these jurisdictions. Those on rubber bush are either currently being updated or will be in the near future to incorporate key findings/recommendations from the project. In particular, a wider range of control options

will be included and more detail on the ecology of rubber bush will be provided. These fact sheets are often the primary source of information for those trying to manage problematic weeds and are one of the first documents to show up during internet searches.

- To compliment these fact sheets, a technical note is also currently being compiled by the Northern Territory Government, which will focus more specifically on the outcomes of the rubber bush project and the implications for management of rubber bush.
- A priority will be to liaise with respective chemical companies to obtain label registrations where possible for the most effective herbicide options identified during the project. Already, steps are underway to include rubber bush on the Access™ herbicide label. A revision of a current minor use permit (PER12497) which is due to expire on the 31 December 2015 will also be submitted and include additional recommendations from the projects findings (such as ground and aerial application of tebuthiuron). Having registered herbicide options means that those responsible for giving advice (such as suppliers of rural products) will not only be able to provide legal recommendations, but they will also be able to more easily find recommendations for rubber bush through mechanisms such as fact sheets, chemical company brochures/websites and pesticide search engines (e.g. Infopest www.infopest.com.au/, and the APVMA website www.apvma.gov.au/).
- As mentioned in the research recommendations section, the establishment of some demonstration/adaptive management sites in strategic areas would be advantageous if additional funding could be procured. This will not only further extend the findings of the project and allow testing of management options on a larger scale, but also hopefully prove a catalyst for affected landholders to work together to combat this weed. Given its dispersal mechanisms (e.g. wind and water) an individual approach will not be as effective as a collective response involving neighbouring properties.
- The undertaking of a social science survey would also be beneficial to find out first hand, any barriers that may prevent adoption of management strategies for rubber bush and how these could be overcome.
- Finally, opportunities for the use of social media and other technologies to extend key messages from the project should be explored. For example, the control research highlighted the importance of correct application of several of the techniques used. Production of YouTube videos that explain the application of individual techniques would be invaluable for those not experienced with them and/or control of rubber bush.

6 Success in achieving objectives

Performance is reviewed against each of the seven objectives.

1. Describe key aspects of rubber bush ecology relevant to management.

This objective was achieved. The project has investigated in detail several aspects of the ecology of rubber bush including its reproductive biology, flowering and fruiting phenology, age to reproductive maturity, seed bank persistence and dispersal mechanisms.

2. Quantify the distribution and rate of spread of rubber bush at several locations.

This objective was achieved. The spread of rubber bush has been investigated at two levels: (1) seed dispersal kernels were investigated at a local scale; and (2) the current and potential distribution of rubber bush has been modelled at a landscape scale. For the former, the ability to disperse seeds was investigated at three sites in the Barkly Region of the Northern Territory that differed in topography. For the latter, two important ecological questions central to determining the invasiveness of this species had to be answered, namely: (1) does the current distribution of rubber bush cover all its potential distribution range or not? and (2) will the range of the species expand or contract with climate change? These questions were answered using species distribution models (SDMs) that covered northern Australia.

3. Quantify the invasiveness of rubber bush under different disturbance regimes and land types using a series of competition and exclusion studies.

This objective was achieved. Field experiments incorporating different levels of competition and disturbance were conducted twice near Katherine on a red soil site, and twice on the Barkly Tableland (Helen Springs Station) on a black soil site.

4. Develop improved control options including:
    - 4.1. complete testing of products that can be used as part of day-to-day activities to control isolated rubber bush plants
    - 4.2. trial and compare current and new herbicide products for control of rubber bush
    - 4.3. conduct a seasonality trial to quantify efficacy of herbicides under different seasonal conditions and growth stages
    - 4.4. monitor the effectiveness of aerial applications of tebuthiuron for broad-scale control of rubber bush in suitable habitats.

All control objectives were achieved. A range of products and techniques were tested for control of isolated plants including cut stump applications using glyphosate or a picloram gel, both traditional and thinline basal bark techniques using triclopyr/picloram mixed with diesel, and ground applications of soil applied residual herbicides. Two extensive foliar herbicide trials were completed to compare current and new herbicide products, some of which had not been put on the market at the time of testing. A seasonality trial incorporating summer, autumn, winter and spring applications of four foliar and one basal bark applied herbicide was undertaken. We also investigated why one of the foliar herbicides (metsulfuron-methyl) identified as being highly effective in previous research performed poorly in the current project. To do this, efficacy under different water types and temperatures at the time of application was tested. The effectiveness of aerial applications of tebuthiuron was monitored in two trials. The first involved three rates (12.5, 15 and 17 kg of product/ha) and based on preliminary findings resulted in further applications (10 and 12.5 kg of product/ha) to see if even lower rates would be effective and potentially reduce application costs.

A pilot mechanical trial was also implemented to ascertain the level of damage necessary to cause high mortality of rubber bush. This was supported by a demonstration where rubber bush was controlled using a stick rake mounted on the front of a large front end loader, with the level of mortality and seedling regrowth recorded.

5. Monitor areas being affected by a dieback phenomenon to quantify whether rubber bush plants recover or eventually die, which will have significant implications on the population dynamics of infestations.

This objective was achieved. Six transects each containing at least 70 rubber bush plants were established in the Gulf of Carpentaria region of Queensland and monitored at six monthly intervals for two years. At commencement of the monitoring, the site was heavily infested with dieback and all recorded plants displayed some level of damage.

6. Identify the most practical and cost-effective control options for rubber bush.

This objective was achieved and is incorporated into the recommendations section of the final report. The most practical and cost-effective control options were identified after careful consideration of the key findings from the control research undertaken in the current project, the findings from previous herbicide research undertaken in northern Queensland, and the lessons learnt from several landholders who had been undertaking control activities on their properties for several years.

7. Develop recommendations for managing rubber bush in different environments and with different levels of rubber bush intrusion.

This objective was achieved and was developed after consideration of all available information at the time, including the outcomes from the current project, previous research findings from both in Australia and overseas, and the personal experiences of landholders, government weed officers and other stakeholders (e.g. Natural Resource Management bodies and community groups) involved in rubber bush management.

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8 Publications from the research

Bebawi FF, Campbell SD, Mayer RJ (2015) Seed bank longevity and age to reproductive maturity of *Calotropis procera* (Aiton) W.T. Aiton in the dry tropics of northern Queensland. *The Rangeland Journal* **37**, 239-247.

Campbell S, Roden L, Crowley C (2013) Calotrope (*Calotropis procera*): A weed on the move in northern Queensland. In ‘Proceedings of the 12th Queensland Weed Symposium’. (eds. M O’Brien, J Vitelli, D Thornby) pp. 11-14 (The Weed Society of Queensland: Brisbane, Australia)

Campbell S, Heard T, Galea V, van Klinken R (2013) Where do we stand with weeds from a research perspective. In 'Proceedings of the Northern Beef Research Update Conference'. pp. 43-48 (North Australia Beef Research Council: Gympie, Australia)

Humphrys M, Campbell S, Steel D (2015) Expanding the range of control options for *Calotropis procera* (rubber bush) in the Barkly Tablelands. In 'Proceedings of the 18th Australian Rangelands Conference' (Australian Rangelands Society: Alice Springs; Northern Territory)

8.1 Articles submitted or under preparation

Menge EO, Bellairs SM, Lawes MJ. Germination patterns of *Calotropis procera* (Aiton) W.T. Aiton R. Br. (Apocynaceae) seeds from different populations in Australia. *Weed Research* (*In revision*).

Menge EO, McConchie CA, Brown G, Lawes MJ. Pollinator guild and mating system of *Calotropis procera* (Ait.) W.T. Aiton (Asclepiadaceae) in an invaded range. *Conference* (*Submitted*).

Menge EO, McConchie CA, Lawes MJ. Density dependence in *Calotropis procera* (Ait.) R.Br. (Apocynaceae) - an invasive milkweed. *Austral Ecology* (*In revision*).

Menge EO, Wilson A, Oliveira SLJ, Lawes MJ. Evaluating the potential distributional range of *Calotropis procera* (Ait.) R.Br. (Apocynaceae), using MaxEnt models. *Environmental Monitoring and Assessment* (*In preparation*).

Menge EO, Bellairs S, McConchie CA, Lawes MJ. The invasiveness of *Calotropis procera* – An updated Weed Risk Assessment. *Plant Protection Quarterly (In preparation)*.

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10 Appendices

10.1 Appendix 1: Seed longevity paper on rubber bush published in the Rangeland Journal

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Seed bank longevity and age to reproductive maturity of *Calotropis procera* (Aiton) W.T. Aiton in the dry tropics of northern Queensland

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Abstract. Understanding the reproductive biology of *Calotropis procera* (Aiton) W.T. Aiton, an invasive weed of northern Australia, is critical for development of effective management strategies. Two experiments are reported on. In Experiment 1 seed longevity of *C. procera* seeds, exposed to different soil type (clay and river loam), pasture cover (present and absent) and burial depth (0, 2.5, 10 and 20 cm) treatments were examined. In Experiment 2 time to reach reproductive maturity was studied. The latter experiment included its sister species, *C. gigantea* (L.) W.T. Aiton, for comparison and two separate seed lots were tested in 2009 and 2012 to determine if exposure to different environmental conditions would influence persistence. Both seed lots demonstrated a rapid decline in viability over the first 3 months and declined to zero between 15 and 24 months after burial. In Experiment 1, longevity appeared to be most influenced by rainfall patterns and associated soil moisture, burial depth and soil type, but not the level of pasture cover. Experiment 2 showed that both *C. procera* and *C. gigantea* plants could flower once they had reached an average height of 85 cm. However, they differed significantly in terms of basal diameter at first flowering with *C. gigantea* significantly smaller (31 mm) than *C. procera* (45 mm). On average, *C. gigantea* flowered earlier (125 days vs 190 days) and set seed earlier (359 days vs 412 days) than *C. procera*. These results suggest that, under similar conditions to those that prevailed in the present studies, land managers could potentially achieve effective control of patches of *C. procera* in 2 years if they are able to kill all original plants and treat seedling regrowth frequently enough to prevent it reaching reproductive maturity. This suggested control strategy is based on the proviso that replenishment of the seed bank is not occurring from external sources (e.g. wind and water dispersal).

Additional keywords: calotrope, giant rubber bush, reproductive maturity, rubber bush, seed persistence, seed viability.

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Introduction

Calotropis procera (Aiton) W.T. Aiton is a native species of tropical Africa, Arabia and Asia (Everist 1974; Forster 1992). It has become naturalised in South Africa, Australia, South-western USA, Mexico, Pacific and Caribbean Islands, Venezuela, Brazil and Paraguay (Holm *et al*. 1979; Parsons and Cuthbertson 2001; Weber 2003). In Australia, it is most commonly known as calotrope or rubber bush, and is believed to have been introduced as a garden plant or from seed in packing of Afghan cameleers' equipment (Hall 1967; Smith 2011). It has become naturalised in several areas in north Queensland, the Northern Territory and the Kimberley region of Western Australia (Gardner and Bennetts 1956; Chippendale and Murray 1963; Everist 1974; Forster 1992; Department of Agriculture, Fisheries and Forestry 2013).

Most reproduction of *C. procera* is from seeds which have a silky pappus which not only facilitates wind-dispersal over several hundred metres (Francis 2002; Staples and Herbst 2005) but also flotation in water thus promoting dispersal along water bodies, including irrigation and drainage channels (Brandao 1995).

Three species of *Calotropis* have been reported in the literature – *C. procera*, *C. gigantea* (giant rubber bush) and *C. acia* Buch-Ham (Ali 1980; Rahman and Wilcock 1991). In India, three varieties of *C. procera* (Rajarkah, Suklarkah and Sveta mandarah) have been identified in the region of Dhanvantari Nigantu (Sharma *et al*. 2011). Although both *C. procera* and *C. gigantea* are commonly found in Australia (Forster 1992; Parsons and Cuthbertson 2001; Smith 2011; Discover Nature at JCU 2013), there have been no reports of different varieties being present.

In Australia, *C. procera* is considered an invasive weed that threatens the sustainability of pasture production, particularly in the dry tropics of north Queensland (Kleinschmidt and Johnson 1977; Forster 1992; Martin 1996; Vitelli *et al*. 2008; Campbell *et al*. 2013), in the Northern Territory (Miller 2003) and in Western Australia in the Kimberley region (Forster

1992; Smith 2011). It has the ability to form dense infestations, which are difficult and costly to control (Grace 2006; Vitelli *et al*. 2008). Adoption of pasture management practices that promote perennial grasses (Crothers and Newbound 1998; Milson 2000; Parsons and Cuthbertson 2001), in conjunction with chemical control is a recommended strategy for management of *C. procera* invading the tropical savannahs of northern Australia (Vitelli *et al*. 2008). Currently available information on the ecology and biology of *C. procera* does not allow an assessment of how frequently control activities would need to be undertaken and for what duration to achieve effective control.

Parsons and Cuthbertson (2001) tentatively suggested that *C. procera* flowers once plants are 2 years old. Long *et al*. (2008) predicted that seeds would persist for between 1 and 3 years, after *C. procera* was subjected to a laboratory-based, controlledaging test along with 12 other emerging and common weeds of Queensland. To test these assumptions/predictions, two experiments were undertaken. Experiment 1 determined the effect of soil type, pasture cover and burial depth on seed longevity of fresh seeds of *C. procera* and was repeated to test the influence of different environmental conditions, particularly soil moisture. Experiment 2 determined the age to reproductive maturity (flowering and seed production) of both *C. procera* and its sister species, *C. gigantea* (L.) W.T. Aiton]. *Calotropis gigantea* was included for comparison with *C. procera*, to gain an insight into the relative invasiveness of these two species, which appear capable of growing in similar environments in the dry tropics of northern Australia (Dunlop 1987; Forster 1992; Parsons and Cuthbertson 2001; Smith 2011; Discover Nature at JCU 2013).

Materials and methods

Experiment 1: seed longevity

Site description

The experimental site (38 m x 36 m) was located in the grounds of the Tropical Weeds Research Centre, Charters Towers, north Queensland (20º09'S, 146º26'E; elevation 318 m). It was large enough for the longevity of up to 12 different seed lots of various weed species to be tested at any one time under the same treatment conditions.

The site was fenced to exclude livestock, rabbits and kangaroos. It had been previously cleared of woody vegetation and had a ground cover that comprised buffel grass [*Cenchrus ciliaris* L.], Indian couch [*Bothrichloa pertusa* (L.) A.Camus]; dark wiregrass (*Aristida calycina* R.Br.), purpletop chloris (*Chloris inflata* Link), Red Natal grass [*Melinis repens* (Willd.) Zizka], feathertop rhodes grass (*Chloris virgata* Sw.), sabi grass [*Urochloa mosambicensis* (Hack.) Dandy], budda pea (*Aeschynomene indica* L.) and siratro [*Macroptilium atropurpureum* (DC.) Urb.].

Long-term mean annual rainfall for Charters Towers is 658mm with 76% of this occurring during the summer months (December–February) (BOM 2012). The mean maximum daily temperature ranges in summer between 32.6ºC and 34.8ºC and in winter (June–August) between 24.8ºC and 26.6ºC. Specific details on rainfall and ambient temperature at the field site during the study were measured using an on-site automatic weather station (Campbell Scientific, Logan, UT, USA).

Seed collection

An initial collection of ripe follicles of *C. procera* was undertaken in December 2008 from two locations: 43 km south-east (20º13'S, 146º38'E) and 15 km north-west of Charters Towers (19º59'S, 146º30'E). Follicles were placed in jackets of aluminium mosquito gauze ($1m^2$) to dry for 2 weeks in a dry glasshouse before seeds were extracted and pooled. Six-hundred and forty subsamples of 50 seeds were then randomly selected and placed in bags of shade cloth (4 cm x 4 cm x 0.5 cm; 1.1mm x 2.4 mm mesh size) to simplify seed retrieval while maximising soil/seed contact.

A second seed collection of ripe follicles was undertaken in October 2011 from an infestation of *C. procera* located in the Gulf of Carpentaria Region (18º13'S, 140º38'E), 820 km north-west of Charters Towers. The same procedure as that used for the initial seed collection was followed for selection and containment of seed lots.

Experimental design

A factorial combination of 2 soil types x 2 pasture levels x 9 retrieval times x 4 seed burial depths was implemented in four blocks in a multiple split-plot design as described in the following paragraphs.

The main plot treatments were established in March 2008 by digging eight trenches (1.0 m wide x 0.5 m deep x 36 m long) 2 m apart. The soil/landform into which the trenches were dug was heavy clay loam. The trenches were then grouped into four blocks, each comprising two neighbouring trenches. In each block, one trench was randomly filled with river loam and the other with clay soil that had been collected in the vicinity of Charters Towers. These soils arecommonsoil types in the vicinity of Charters Towers on which *C. procera* can occur.

To establish the subplots, half of each trench (i.e. 18 m) was randomly allocated to be kept bare (i.e. pasture excluded) through physical removal of all vegetation whereas the other half was allowed to revegetate from the local seed bank and encroachment from the 2-m buffer strips. After 6 months (September 2008), the revegetated portions (i.e. pasture present) had a dense cover of pasture that was ~40–50 cm high.

The pasture present and pasture excluded portions of each trench were divided into 12 1-m-long by 1-m-wide sections with a 50-cm buffer in between, to enable longevity testing of up to 12 seed lots of various weed species at any one time. For both the first and second seed lots of *C. procera*, subplots were implemented by first randomly selecting one of the 1-m-long sections in both the pasture present and pasture excluded portions of each trench. Within this 1-m-long x 1-m-wide section of trench, nine 12–15-cm-diameter cylindrical holes were dug to a depth of 30 cm using a manually operated auger. They were positioned so that there were three rows each containing three holes, equal distances apart. Each hole was randomly allocated one of nine retrieval dates. These holes were then filled with a cylindrical PVC pipe (11 cm in diameter x 30 cm in height) containing seed bags that had been buried at the designated burialthe depths using the same soil within respective trenches. Seed lots randomly selected to be tested at time zero were not buried, but directly subjected to germination and viability testing.

The PVC pipes were perforated at the base and also had four holes drilled on the sides to allow for drainage. Blotting paper was placed at the base and in the holes to prevent soil loss but allow free drainage. Pipes were systematically filled on site using ~6.5 kg of soil, with a bag of seeds placed at depths of 0, 2.5, 10 and 20 cm. The top of the PVC pipe was then covered with rabbit mesh wire to prevent loss of bags containing seeds placed on the soil surface (0 cm in depth). Sensors connected to two separate data loggers (DT85 model – Data Electronics Pty Ltd, Brisbane, Qld, Australia) were inserted at each burial depth in dummy PVC pipes that were buried at the head of each soil trench to monitor soil temperature (Type K steel encased thermocouples) and soil moisture (SM200) on an hourly basis.

The first seed lot of *C. procera* was buried in March 2009 with retrievals designated to occur 3, 6, 12, 18, 24, 36, 48, 60 and 72 months after burial or until no viable seeds were recorded for two consecutive retrievals. The second seed lot was buried in January 2012, with retrievals designated to occur 3, 6, 12, 15, 18, 24, 36, 48 and 60 months after burial. On each retrieval date, one PVC pipe from each replicate of the 16 soil type x pasture cover plots was randomly retrieved. The bags containing buried seeds were then removed from the PVC pipes and washed gently to remove attached soil particles.

Germination and viability testing

To determine ‘germinability’, remaining intact seeds were removed from the bags and placed in Petri dishes (9 cm diameter) containing two layers of ‘Whatman No. 1’ filter paper moistened with 10 mL of distilled water. These dishes were then placed into a growth cabinet set at 30ºC/20ºC day/night with alternating 12 h of light and dark. Germinable seeds (identified by radicle emergence) were counted and removed daily for 14 days. Seeds that did not germinate were checked for dormancy using the tetrazolium method (Moore 1985). Seed viability (germinable + dormant) was expressed as a per cent of total seeds initially buried.

At the 3-month retrieval, the number of seeds that germinated in the packets was also recorded by counting emerged seedlings. However, this was not possible in later retrievals due to disintegration of emerged seedlings.

Experiment 2: days to flowering and seed production, plant height and basal diameter

The experiment was conducted at the Tropical Weeds Research Centre between September 2006 and December 2008. A completely randomised design with six replications was used to grow single plants of *C. procera* and *C. gigantea* in plastic pots (40 cm in diameter x 40 cm in depth) filled with garden soil potting mix. Pots were regularly watered to field capacity. Seeds of both *C. procera* and *C. gigantea* were collected in August 2006 and came from pods near the Gregory River (Fig. 1) in the Gulf of Carpentaria (18º57’S, 139º27’E). Three seeds of *C. procera* and *C. gigantea* were initially sown and seedlings were subsequently thinned 3 weeks after emergence to one plant per pot. Number of seeds per pod of *C. procera* and *C. gigantea* averaged 486 ± 10.1 (n = 14) and 127 ± 12.2 (n = 14), respectively.

Plant height (cm) and basal diameter (mm) at flowering and seed production (when first swollen follicle was observed) were recorded for each plant, along with days to flowering and seed production.

Data analyses

GENSTAT was used for all statistical analyses (GENSTAT 8.1, VSN International, Hemel Hempstead, Hertfordshire, UK) and Fisher’s least significant differences test was used to determine differences between treatments whenever analysis showed treatment effects to be statistically significant ($P < 0.05$). All statistical analysis concerning seed germination and viability was undertaken on arcsine-transformed data, which was later backtransformed for display.

Fig. 1. Distinct follicle size and shape and inflorescences colour and form of *C. procera* (back) and *C. gigantean* (front).

In Experiment 1, viability data was analysed using a multiple split-plot ANOVA as dictated by the experimental design: 4 blocks x 2 soil types split for 2 pasture levels split for 9 retrieval times split for 4 burial depths. All other data was only analysed at a single time (i.e. germination at 3 months) or was an average over time (i.e. soil moisture content and temperature), and was thus subjected to an analysis of 4 blocks x 2 soil types split for 2 pasture levels split for 4 burial depths.

For Experiment 2, all measurements undertaken on *C. procera* and *C. gigantea* were subjected to one-way ANOVA using a completely randomised design.

Results

Experiment 1 – seed longevity

Rainfall and soil moisture

Annual rainfall, recorded at the site between 2009 and 2012, was consistently greater than the long-term mean for Charters Towers (658 mm), averaging 1105, 1323, 1037, 832 mm per annum, respectively. For the first 5 months of 2013 before the second seed lot finished being tested in May 2013, 452 mm of rainfall was recorded (Fig. 2).

Despite high annual rainfall, the first and second seed lots were exposed to different seasonal patterns of rainfall, particularly in the first 12 months, which was a critical period in the longevity of soil seed banks of *C. procera*. After burial in March 2009, the first seed lot received 118 mm of rainfall for the remainder of the autumn period. This was followed by a very dry winter and spring period where only 21mm of rainfall were recorded, before the onset of a wet summer where 493 mm fell. In contrast, the second seed lot buried in January 2012 received 218 mm of rainfall in February followed by 319 mm during autumn. Even the winter period received high rainfall with 189mm being recorded. However, the following spring was dry (32 mm) and summer rainfall was below average, with 294 mm recorded (Fig. 2).

The prevailing rainfall resulted in no significant difference ($P > 0.05$) in average daily proportion of soil moisture content conditions between the first and second seed lots (average of 0.1%), when calculated for the full duration of burial. However, during the first 3 months when rainfall patterns differed markedly and major reductions in seed viability occurred, average proportion of daily soil moisture content was significantly higher ($P < 0.01$) for the second seed lot than the first one, averaging 0.14 and 0.08, respectively.

Soil type, pasture cover and soil depth did not have a significant effect ($P > 0.05$) on average daily soil moisture content for the first and second seed lots, when calculated for the full duration of burial. If calculated for the first 3 months of burial when major changes in viability occurred, there was no significant difference ($P > 0.05$) during testing of the second seed lot, but a significant soil type x pasture cover interaction ($P < 0.05$) occurred during testing of the first seed lot. The clay soil had consistently higher soil moisture content than the river loam and the pasture-excluded plots had higher soil moisture contents than those where pasture was present, with differences greatest in the clay soil treatment (Fig. 3).

Temperature conditions

The second seed lot was exposed to slightly higher (26.8ºC) average daily soil temperatures than the first seed lot (26.3ºC) when averaged across all soil types, levels of pasture cover and burial depths ($P < 0.05$). During burial of both seed lots, soil type did not have a significant effect ($P > 0.05$) on average daily soil temperatures, but both the level of pasture cover and burial depth did ($P < 0.05$). Temperatures were higher in the absence of pasture cover (Fig. 4) and there was a positive correlation between average daily soil temperature and burial depth ($r = 0.98$) (Fig. 4).

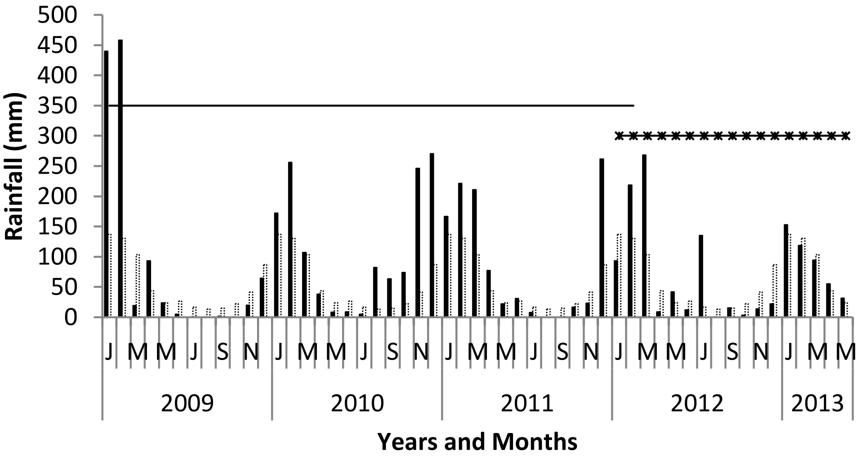

Fig. 2. Monthly rainfall (■ mm) at the research site between March 2009 and May 2013, and the average monthly rainfall (□ mm) for Charters Towers associated with burial duration for seed lot 1(_____) and seed lot 2 (**xxxx**).

Seed viability

First seed lot. Initial seed viability and germinability was high, averaging 85.0 ± 2.4% (per cent of total seed number) and 100% (per cent of viable seeds), respectively. Following burial, significant burial depth x burial duration ($P < 0.001$) and soil type x burial duration ($P < 0.01$) interactions were recorded for seed viability. In contrast, the level of pasture cover did not have a significant influence ($P > 0.05$) on seed viability over time.

Viability declined most rapidly in the first 3 months, particularly in seed lots that were buried. After 6 months, <1% of buried seeds remained viable, compared with 28% of surfacelocated seeds (Fig. 5). This rapid decline in viability coincided with high germination of seeds in the field in the first 3 months after burial. On average, 92% of seeds germinated within 3 months if buried, significantly more ($P < 0.05$) than surfacelocated seeds, which averaged only 38% (Fig. 6). No viable seeds were retrieved from buried seed lots after 18 months, whereas surface-located seed lots had no viable seed after 24 months.

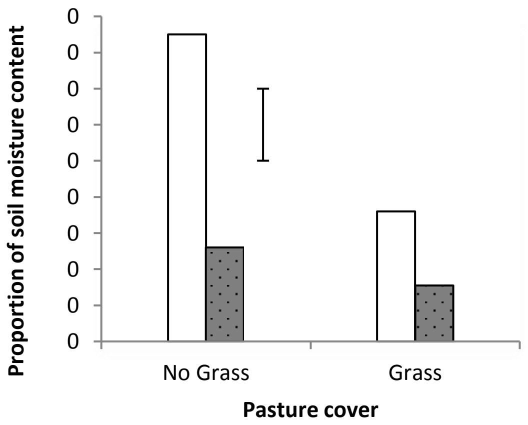

Fig. 3. Average proportion of daily soil moisture content recorded during the first 3 months of burial of the first seed lot, as affected by soil type (clay □ and river loam ■) and level of pasture cover. Vertical bar indicates the least significant difference at $P = 0.05$.

With regards soil type, a more rapid rate of decline in viability occurred in the clay soil compared with the river loam soil (Fig. 7). No viable seeds were retrieved from the clay soil after 18 months, whereas nil viability was recorded in the river loam soil at the 24-month retrieval.

Second seed lot. Initial seed viability and germinability was extremely high, averaging 99.5 ± 0.1% (per cent of total seed number) and 100% (per cent of viable seeds), respectively. Following burial, viability was significantly affected by burial duration ($P < 0.05$), but not by soil type, pasture cover or burial depth ($P = 0.93$). The rate of decline in viability was faster than that of the first seed lot tested.

After 3 months, no viable seed was recorded across all burial depths, soil types and pasture cover treatments. However, subsequent 6- and 12-month assessments recorded 0.1% viability. No viable seeds were retrieved from any seed lots 15, 18 or 24 months after burial. As for the first seed lot, the rapid decline in viability was associated with a high percentage of seeds germinating in the field, particularly those buried below ground. Surface-located seeds averaged 82% germination after 3 months compared with 99% for those buried between 2.5 and 20 cm (Fig. 6).

Experiment 2 – days to flowering and seed production,plant height and basal diameter

Plant height and basal diameter at flowering

Plant height at flowering did not differ significantly between *C. procera* and *C. gigantea* ($P > 0.05$), averaging 85 ± 2 cm. In contrast, *C. procera* had a significantly larger ($P < 0.05$) basal diameter at flowering than *C. gigantea*, averaging 45mm and 31 mm, respectively.

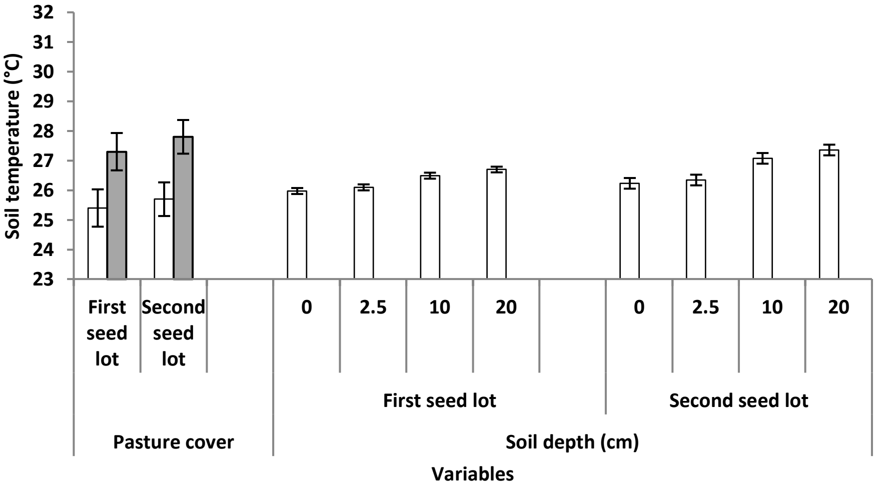

Fig. 4. Average daily soil temperature (ºC) recorded during burial of the first and second seed lots,as affected by pasture cover (□ present and ■ absent) and soil depth. Vertical bars indicate the leastsignificant difference at $P = 0.05$.

Days to flowering and seed production,

Significant differences ($P < 0.05$) in days to flowering occurred between *C. procera* and *C. gigantea* (Fig. 8). Plants of *C. gigantea* took between 116 and 146 days to flower (average, 125 days) after germination, whereas *C. procera* took between 125 and 250 days (average, 190 days). Similarly, days to seed production differed significantly ($P < 0.05$), with *C. gigantea* producing seed between 352 and 365 days after germination (average, 359 days), compared with 399–425 days for *C. procera* (average, 412 days).

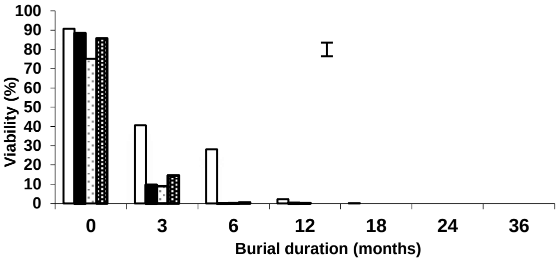

Fig. 5. Viability (%) at the time of testing of the first seed lot of *C. procera* as affected by burial depth (□ 0 cm, ■ 2.5 cm, ░ 10 cm and ▓ 20 cm) and burial duration. Vertical bars indicate the least significant difference at $P = 0.05$.

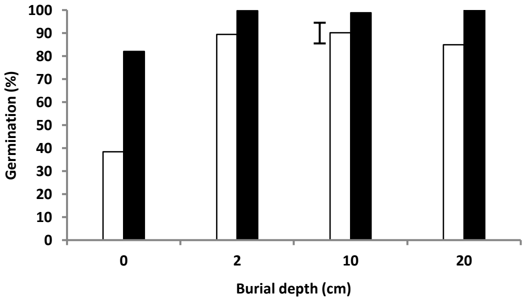

Fig. 6. The proportion (%) of the first (□) and second (■) seeds lot of *C. procera* that germinated in the field at different depths, 3 months after burial. Vertical bar indicates the least significant difference at $P = 0.05$.

Discussion

Under the prevailing environmental conditions experienced during the study, seed longevity of *C. procera* was relatively short (15–24 months) and young plants demonstrated the potential to flower and produce seeds within 6 and 14 months, respectively.

Seed longevity

Based on a dichotomous key, developed by Thompson *et al.* (1997) and which classifies seed longevity into three categories [transient (viable ≤1 year), short-term persistent (viable 1–5 year) and long-term persistent (viable ≥5 year)], our results suggest that *C. procera* has a short-term persistent seed bank depending on prevailing conditions. This is consistent with the earlier prediction of Long *et al.* (2008), who suggested that seeds would persist for between 1 and 3 years.

Germination and viability testing revealed that the seeds of *C. procera* had high viability and germinability, and should, therefore, readily germinate under favourable field conditions. Francis (2002) reported a similar finding with 89% germination recorded 64 days after sowing seeds in a potting mix. Similarly, Leal *et al.* (2013) recorded greater than 95% germination in fresh seeds after 35 days from two populations in north-eastern Brazil.

Calotropis procera seeds readily germinated after rainfall with an associated rapid decline in the proportion of viable seeds remaining over time. This was most evident in the second seed lot tested where <1% viability was recorded after 3 months. These seeds received much more rainfall in the first 3 months after burial than the first seed lot, and consequently would have had more favourable conditions for germination to occur. This differential response between the two seed lots highlights the influence of prevailing environmental conditions on seed longevity, as reported previously (Chambers and MacMahon 1994; Baskin and Baskin 2001). Given that both seed lots were tested during above-average rainfall conditions, it is feasible that seed banks of *C. procera* could persist for longer during droughts due to

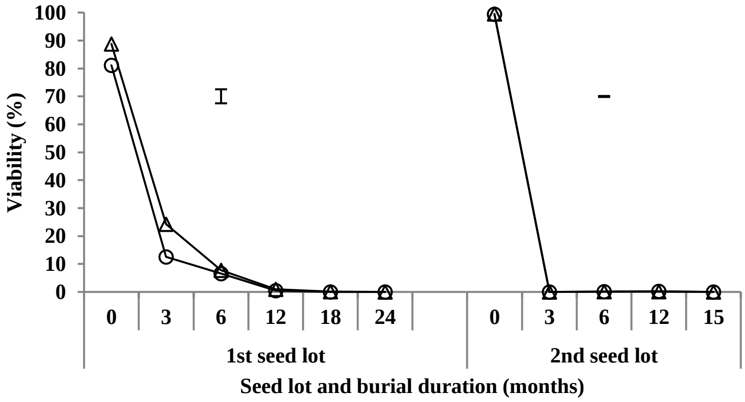

Fig. 7. Viability of the first and second seed lots of C. procera as affected by soil type (○ clay an Δ river loam) and burial duration. Vertical bars indicate the least significant difference at P = 0.05.

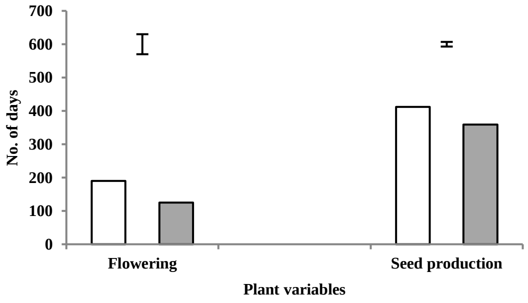

Fig. 8. Average days from the emergence of the first seedling to flowering and seed production of *C. procera* (□) and *C. gigantea* (■). Vertical bars indicate the least significant difference at *P* = 0.05.

fewer opportunities to germinate and this warrants investigation. Studies on *Lantana camara* L. (lantana) and *Jatropha gossypiifolia* L. (bellyache bush) have highlighted major differences in the persistence of soil seed banks due to soil moisture availability. Vivian-Smith and Panetta (2009) predicted that the longevity of soil seed banks of *L. camara* could be reduced by 8 years under high soil moisture content conditions when compared with sites that received natural seasonal rainfall. Similarly, a rainfall-exclusion experiment found that the longevity of seed banks of *J. gossypiifolia* could be extended from ~3 to 4 years under natural rainfall conditions to greater than 10 years in the absence of rainfall (Bebawi *et al*. 2012).

The more rapid decline in viability of the first seed lot when buried in clay soils compared with loams is also most likely due to differences in soil moisture content. The clay soil had higher average daily soil moisture content than the river loam during the first 3 months (autumn) of the experiment, before going into a dry winter. This would have provided more favourable conditions for germination of *C. procera* seed that was in a highly germinable state at the time. A similar occurrence was reported for *J. gossypiifolia*, whose seed bank was depleted quicker due to greater seedling emergence under the higher soil moisture conditions in a clay soil compared with a red duplex soil (Bebawi *et al.* 2012). A more rapid decline in viability of seeds, located below ground compared with those on the surface, has also been reported for other weeds (Panetta 2001; Bebawi *et al*. 2012) and often attributed to greater soil moisture availability, although there are several factors that could have an influence such as seedsoil contact, such as aeration, light availability, temperature and dormancy (Bekker *et al.* 1998; Benvenuti *et al.* 2001; Harrison *et al*. 2007; Vivian-Smith and Panetta 2009). In our study, differences in average daily soil moisture between surfacelocated seeds and those buried below ground were not detected during testing of either seed lots, but this may have been associated with placement of the sensors, which had to be partially buried to keep them in place.

The high germination of *C. procera* under the prevailing temperatures of the present study was consistent with those reported in a Brazilian study (Labouriau and Valadares 1976), where germination was highest between 23ºC and 33ºC. It is plausible that longevity could be extended in areas that receive periods of extreme temperatures (too hot or cold) that may prevent germination. For example, Labouriau and Valadares (1976) recorded little germination once temperatures reached 34ºC or above. Besides these potential inhibitory effects, surface seeds exposed to very high temperatures in summer could possibly lose viability. Ooi *et al.* (2009) indicated that soil temperatures, which increase as a result of global warming, may approach thresholds for seed death in ecosystems where high temperatures are already apparent. They found that, among other plant species, the initial viability of the physically dormant seeds of *Tephrosia sphaerospora* F. Muell. (Fabaceae) declined rapidly from almost 100% to 58% after 70 days exposure to high predicted soil temperatures (70/25ºC).

The ability of *C. procera* to germinate in the field at depth suggests that light is not necessary and is consistent with findings from Leal *et al.* (2013) who reported high germination under a range of light intensities ranging from 0% to 100%. However, the general location of seeds of *C. procera* in the soil profile has not been clarified in this study, nor has the ability of seedlings to emerge from depth. It is important to note that, although the seed lots used in this study exhibited high germinability, the literature does report limited instances of low-level dormancy in *C. procera*. Between 2% and 35% of *C. procera* seeds collected from an Indian population (Amritphale *et al*. 1984) and up to 6% from a Brazil population were reported to be dormant (Labouriau and Valadares 1976). The presence of dormancy may prolong the longevity of seed banks of *C. procera* beyond those reported in the present study.

Flowering and seed production

The average time taken for *C. procera* to produce seeds in the present study was slightly less (412 days) than the 2 years suggested by Parsons and Cuthbertson (2001). Plants did, however, have access to abundant soil moisture and differential responses could occur under drier field conditions or due to different levels of competition from desirable native and/or pasture species. The large lag time observed between flowering and seed production also warrants further consideration to determine whether this is a normal occurrence or not. It was much longer for *C. procera* than for *C. gigantea* (190 days vs 125 days) and was the main reason for the large difference between these two species in the time taken for plants to reach the seed production stage. Both species rely on insect pollination for reproduction (Wanntorp 1974; Ramakrishna and Arekal 1979; Morse 1981; Eisikowitch 1986; Ali and Ali 1989; Grace 2006) and perhaps differences in availability of pollinators could influence when flowers are fertilised and pod production occurs. Additionally, differences in pod size and number of seeds produced per pod may also explain differences between these two species in time taken to reach reproductive maturity, as more time will be required by *C. procera* compared with *C. gigantea* to produce the higher energy requirements (given similar photosynthetic area). It is also likely that the prolific production of seeds by *C. procera* (485.7 ± 10.1) compared with *C. gigantea* (126.5 ± 12.2) may be contributing to the pre-dominance of the former in the Australian rangelands.

Management implications

The short longevity of seeds of *C. procera* highlights the vulnerability of this species in terms of its soil seed bank, which makes it highly amenable for control and possible eradication from an area. It is notable that the apparently successful eradication program targeting a very large incursion of kochia [*Bassia scoparia* (L.) A.J.Scott] in Western Australia has involved a species whose seeds are short-lived (Dodd and Randall 2002).

Based on the findings of the present study, land managers controlling *C. procera* can expect a high density of seedling regrowth in the first 12 months under average or above-average rainfall conditions. If this seedling regrowth is treated along with any original plants that may have been missed or not controlled effectively, little germination should occur as the resident soil seed bank should be very low thereafter. In terms of the frequency of control activities, annual surveillance and treatment of plants should be sufficient in most years to prevent new plants from producing seeds and replenishing soil seed banks. However, as both *Calotropis* species investigated in this study produced seed as early as September (spring), it would be advisable to commence control operations at the onset of spring.

Given its dispersal mainly by wind and water, there is a risk that seed dispersal from neighbouring areas or external sources could occur resulting in ongoing recruitment. This will be particularly pertinent for land managers with large infestations that cannot all be controlled at the same time.

Acknowledgements

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10.2 Appendix 2: Environmental layers used for modelling, the timeframe they cover and their source.

Variable		*Time frame*	*Source*	
Topography	Elevation	1996	U.S. Geological Survey	https://lta.cr.usgs.gov/get_data/
	NVIS major vegetation groups*†‡	2012	Australian Dept. Environment	http://www.environment.gov.au/metadataexplorer/
	Minimum annual bare ground cover	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
	Maximum annual bare ground cover	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
Fire history	Years since last burnt	2000-2013	North Australian Fire Information	http://nafi3-dev.firenorth.org.au/nafi3/
	Fire Frequency	2000-2013	North Australian Fire Information	http://nafi3-dev.firenorth.org.au/nafi3/
Soil properties	Soil fertility	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
	Soil pH (0-30cm)	2011	ASRIS	ttp://www.asris.csiro.au/themes/NationalGrids.html#NationalGrids_Downloads
	Clay content percentage (0-30cm)	2011	ASRIS	ttp://www.asris.csiro.au/themes/NationalGrids.html#NationalGrids_Downloads
	Soil bulk density (0-30cm)	2011	ASRIS	ttp://www.asris.csiro.au/themes/NationalGrids.html#NationalGrids_Downloads
	Plant available water capacity	2011	ASRIS	ttp://www.asris.csiro.au/themes/NationalGrids.html#NationalGrids_Downloads
Climate	Potential evapotranspiration	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Actual evapotranspiration	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Water availability index	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average daily temperature annual*	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average minimum daily temperature annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average maximum daily temperature annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average rainfall annual*	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average rain days >25mm annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average rain days >10mm annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average rainfall during wet season (October to April) annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average rainfall during dry season (April to November) annual	1961-1990	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml

Variable		*Time frame*	*Source*	
	Average relative humidity at 9am annual	1976-2005	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average relative humidity at 3pm annual	1976-2005	Bureau of Meteorology	http://www.bom.gov.au/climate/averages/maps.shtml
	Average wind speed (km/hr)* †	1975-2013	CSIRO	https://data.csiro.au/dap/home?execution=e2s1
Land use	Australia land use*†‡	2012	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_luausg9abll20140506_11a.xml
	Beef density*†	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
	Number of bores (NT)	2014	NT Dept. Land and Resource Management	http://daff.gov.au/abares/aclump/pages/landuse/datadownload.aspx
	(QLD)	2014	QLD Dept. Natural Resource Management	https://www.dnrm.qld.gov.au/mapping-data/queensland-globe
	(WA)	2014	WA Dept. of Water	
	Distance to minor roads (km)*	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
	Minor roads*	2011	Australian Dept. Agriculture	http://data.daff.gov.au/anrdl/metadata_files/pb_mcas11g9ablm03111a01.xml
Future climate	Annual mean temperature (BIO_1) †	2005	WorldClim	http://wallaceinitiative.org/climate_2012/tdhtools/Search/DataDownload.php
	Annual precipitation (BIO_{12}) †	2005	WorldClim	http://wallaceinitiative.org/climate_2012/tdhtools/Search/DataDownload.php

*Variables used in MaxEnt model building for potential *C. procera* distribution.
†Variables used in MaxEnt model building for future *C. procera* distribution.
‡Categorical variables (not used in PCA).

10.3 Appendix 3: Tests and response curves associated with distribution modelling.

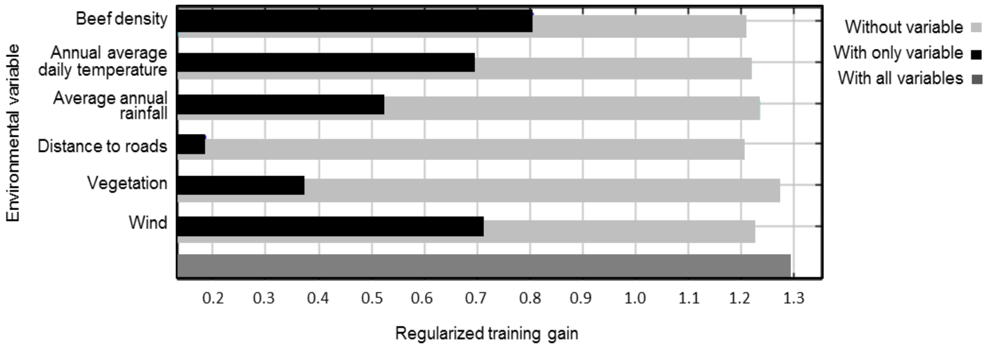

Fig. 1. Jackknife test for evaluating the relative importance of environmental variables for *Calotropis procera* in northern Australia.

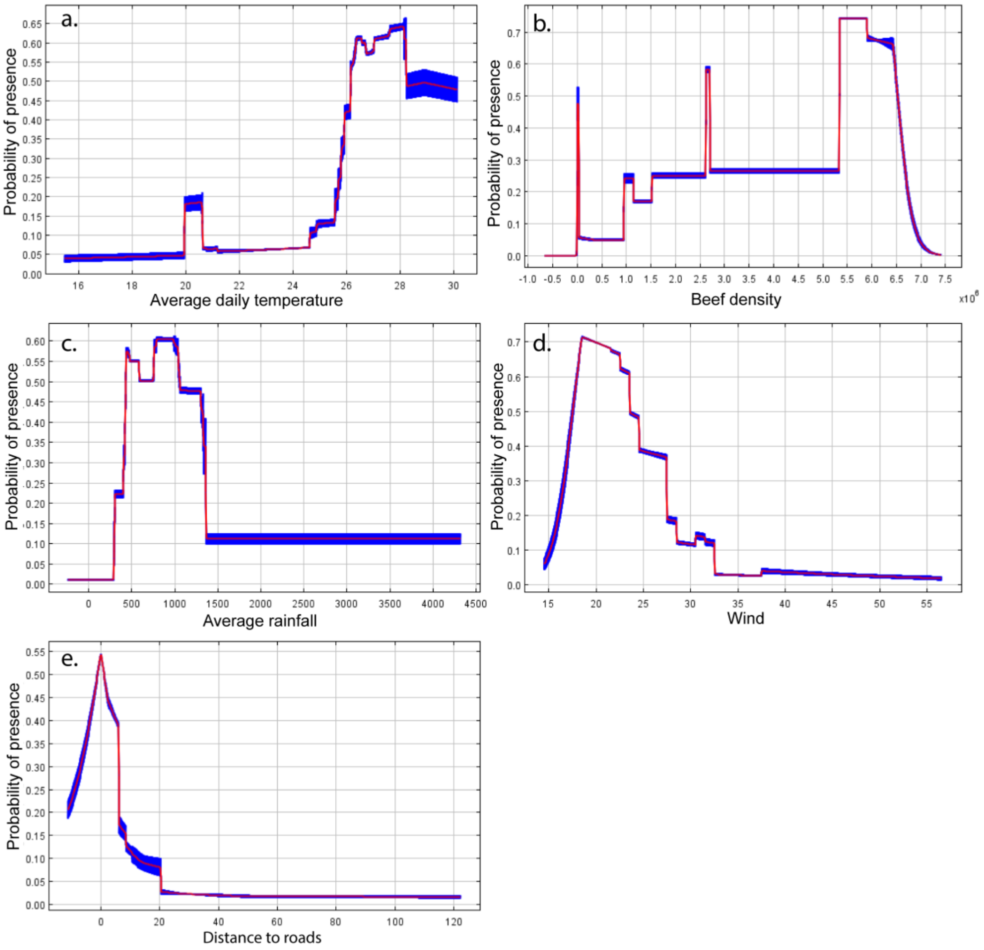

Fig. 2. Response curves for (a) average daily temperature, (b) beef density, (c) average rainfall (d) wind and (e) distance to roads. Response curves illustrate how logistic prediction changes as each variable is varied while keeping all other environmental variables at their average. Lines show the mean ± 1 SE of the response taken from ten models replicated through cross-validation.

10.4 Appendix 4: Supporting data for responses to the WRA questions.

A.	Biogeography			E. Menge 2015	Supporting data
1	Domestication/ cultivation	1.01	Is the species highly domesticated? If answer is 'no' go to 2.01	N	
		1.02	Is species naturalised where grown?		
		1.03	Does the species have weedy races?		
2	Climate and Distribution	2.01	Species suited to Australian climates (0-low; 1-intermediate; 2-high)	2	(Menge, *pers. observ.*; CSIRO 2013)
		2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)	2	(Council of Heads of Australasian Herbaria 1999-2013, CSIRO 2013)
		2.03	Broad climate suitability (environmental versatility)	Y	(Ali & Ali 1989, Forster 1992, Smith 2002, Rangel & Nascimento 2011b, CSIRO 2013)
		2.04	Native or naturalised in regions with extended dry periods	Y	(Bastin et al. 2003a, Grace 2006, Vitelli et al. 2008)
		2.05	Does the species have a history of repeated introductions outside its natural range?	Y	(Parsons & Cuthbertson 2001, Rangel & Nascimento 2011b)
3	Weed Elsewhere: (interacts with 2.01 to give a weighted score)	3.01	Naturalised beyond native range	Y	(Brandao 1995, Parsons & Cuthbertson 2001, Francis 2004, Fabricante et al. 2013)
		3.02	Garden/amenity/disturbance weed	Y	(Menge, pers. observ.,Csurhes 2009)
		3.03	Weed of agriculture/horticulture/forestry	Y	(Grace 2006, Rangel & Nascimento 2011b, Sobrinho et al. 2013)
		3.04	Environmental weed	Y	(Fisher et al. 2002)
		3.05	Congeneric weed	Y	(Grice & Martin 2005)
B.	Biology/Ecology				
4	Undesirable traits	4.01	Produces spines, thorns or burrs	N	
		4.02	Allelopathic	N	(Cheam 1984a,b)
		4.03	Parasitic		
		4.04	Unpalatable to grazing animals	N	(Radunz et al. 1983, Grace 2006)
		4.05	Toxic to animals	N	(Radunz et al. 1983)
		4.06	Host for recognised pests and pathogens		
		4.07	Causes allergies or is otherwise toxic to humans	Y	(Francis 2004, Nelson et al. 2007, Lottermoser 2011)
		4.08	Creates a fire hazard in natural ecosystems	N	(Vitelli et al. 2008)
		4.09	Is a shade tolerant plant at some stage of its life cycle		
		4.1	Grows on infertile soils	Y	(Eisikowitch 1986a, Grace 2006, Csurhes 2009, Fabricante et al. 2013)
		4.11	Climbing or smothering growth habit	N	(Forster 1992)
		4.12	Forms dense thickets	Y	(Parsons & Cuthbertson 2001, Grace 2006)
5	Plant Type	5.01	Aquatic	N	(Eisikowitch 1986b)
		5.02	Grass	N	(Forster 1992)

		5.03	Nitrogen fixing woody plant	N	(Forster 1992)
		5.04	Geophyte	Y	(Eisikowitch 1986a, Tezara et al. 2011)
6	Reproduction	6.01	Evidence of substantial reproductive failure in native habitat	Y	(Madhu et al. 2013)
		6.02	Produces viable seed	Y	(Menge, *pers. observ.,* Sen et al. 1968, Labouriau & Valadares 1976, Ali & Ali 1989)
		6.03	Hybridises naturally		
		6.04	Self-fertilisation	Y	Menge, *pers. observ*.
		6.05	Requires specialist pollinators	Y	(Menge, pers. observ., Eisikowitch 1986a, Ali & Ali 1989)
		6.06	Reproduction by vegetative propagation	Y	(Vitelli et al. 2008)
		6.07	Minimum generative time (years)	1.5	(Menge, *pers. observ.*, Bebawi et al. 2015)
7	Dispersal mechanisms	7.01	Propagules likely to be dispersed unintentionally	Y	(Parsons & Cuthbertson 2001)
		7.02	Propagules dispersed intentionally by people		
		7.03	Propagules likely to disperse as a produce contaminant	Y	(Parsons & Cuthbertson 2001)
		7.04	Propagules adapted to wind dispersal	Y	(Forster 1992, Grace 2006, Bebawi et al. 2015)
		7.05	Propagules buoyant	Y	(Csurhes 2009)
		7.06	Propagules bird dispersed		
		7.07	Propagules dispersed by other animals (externally)		
		7.08	Propagules dispersed by other animals (internally)		
8	Persistence attributes	8.01	Prolific seed production	Y	Menge, *pers. observ.*
		8.02	Evidence that a persistent propagule bank is formed (>1 yr)	N	(Bebawi et al. 2015)
		8.03	Well controlled by herbicides	Y	(Vitelli et al. 2008, Campbell et al. 2013)
		8.04	Tolerates or benefits from mutilation, cultivation or fire	Y	(Vitelli et al. 2008)
		8.05	Effective natural enemies present in Australia	N	(Moeller et al. 2012, Madhu et al. 2013, Dhileepan 2014)

10.5 Appendix 5: Updated WRA for rubber bush in the Australian environment using the DAF system.

Weed Risk Assessment report 29-Apr-15

Botanical name: *Calotropis procera*
Common name: Rubber bush
Name of Assessor: E. Menge

Outcome: 0
Score:
Agricultural 20
Environmental 22
Total 28

	A.	**History/Biogeography**		
A	**1**	*Domestication/ cultivation*	1.01 Is the species highly domesticated? If answer is 'no' go to 2.01	N
C			1.02 Is species naturalised where grown?	
C			1.03 Does the species have weedy races?	
	2	*Climate and Distribution*	2.01 Species suited to Australian climates (0-low; 1-intermediate; 2-high)	2
			2.02 Quality of climate match data (0-low; 1-intermediate; 2-high)	2
C			2.03 Broad climate suitability (environmental versatility)	Y
C			2.04 Native or naturalised in regions with extended dry periods	Y
			2.05 Does the species have a history of repeated introductions outside its natural range?	Y
C	**3**	*Weed Elsewhere*	3.01 Naturalised beyond native range	Y
E			3.02 Garden/amenity/disturbance weed	Y
A			3.03 Weed of agriculture/horticulture/forestry	Y
E			3.04 Environmental weed	Y
			3.05 Congeneric weed	Y
	B.	**Biology/Ecology**		
A	**4**	*Undesirable traits*	4.01 Produces spines, thorns or burrs	N
C			4.02 Allelopathic	N
C			4.03 Parasitic	N
A			4.04 Unpalatable to grazing animals	N
C			4.05 Toxic to animals	N
C			4.06 Host for recognised pests and pathogens	
C			4.07 Causes allergies or is otherwise toxic to humans	Y
E			4.08 Creates a fire hazard in natural ecosystems	N
E			4.09 Is a shade tolerant plant at some stage of its life cycle	
E			4.10 Grows on infertile soils	Y
E			4.11 Climbing or smothering growth habit	N
E			4.12 Forms dense thickets	Y
E	**5**	*Plant type*	5.01 Aquatic	N
C			5.02 Grass	N
E			5.03 Nitrogen fixing woody plant	N
C			5.04 Geophyte	Y
C	**6**	*Reproduction*	6.01 Evidence of substantial reproductive failure in native habitat	Y
C			6.02 Produces viable seed	Y
C			6.03 Hybridises naturally	
C			6.04 Self-fertilisation	Y
C			6.05 Requires specialist pollinators	Y
C			6.06 Reproduction by vegetative propagation	Y
C			6.07 Minimum generative time (years)	1.5
A	**7**	*Dispersal mechanisms*	7.01 Propagules likely to be dispersed unintentionally	Y
C			7.02 Propagules dispersed intentionally by people	
A			7.03 Propagules likely to disperse as a produce contaminant	Y
C			7.04 Propagules adapted to wind dispersal	Y
E			7.05 Propagules buoyant	Y
E			7.06 Propagules bird dispersed	
C			7.07 Propagules dispersed by other animals (externally)	
C			7.08 Propagules dispersed by other animals (internally)	
C	**8**	*Persistence attributes*	8.01 Prolific seed production	Y
A			8.02 Evidence that a persistent propagule bank is formed (>1 yr)	N
A			8.03 Well controlled by herbicides	Y
A			8.04 Tolerates or benefits from mutilation, cultivation or fire	Y
E			8.05 Effective natural enemies present in Australia	N
		Statistical summary of scoring	Score partition: Biogeography	16
			Undesirable attributes	2
			Biology/ecology	10
			Questions answered: Biogeography	8
			Undesirable attributes	10
			Biology/ecology	19

A = agricultural, E = environmental, C = combined