

# Herbicide impacts on exotic grasses and a population of the critically endangered herb *Calystegia affinis* (Convolvulaceae) on Lord Howe Island

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**Abstract:** Introduced perennial grasses are capable of altering the habitat of native species, causing reductions in population size and vigour, and potentially affecting life-history processes such as survival, pollination and seedling recruitment. We examined the utility of herbicide treatment on two exotic grasses, *Pennisetum clandestinum* (Kikuyu) and *Stenotaphrum secundatum* (Buffalo grass) to restore the habitat of *Calystegia affinis*, a critically endangered species endemic to Lord Howe and Norfolk Islands. Using two herbicides, Asset (designed to affect only grasses) and Glyphosate (a general herbicide), we compared effectiveness in reducing grass cover on a population of *Calystegia affinis*. We protected *Calystegia* plants from the herbicides by ensuring their leaves were covered by plastic bags during herbicide application. Both herbicides were similarly effective in reducing grass cover after four weeks and had no noticeable adverse affect on *Calystegia* (suggesting the plastic bag protection was effective). After 26 weeks, Glyphosate was more effective in maintaining a reduced grass cover. Plots treated with either herbicide had a greater relative increase in abundance of *Calystegia* stems compared to untreated controls. The Glyphosate treatment resulted in the greatest relative increase in stem abundance, but this was not significantly greater than in the Asset treatment. We consider that spraying with Glyphosate treatment, with follow-up monitoring and spot-spraying, will assist the recovery of the *Calystegia affinis* population. Ultimately, the maintenance of a weed-free zone at the forest edge will provide suitable habitat for additional recruitment of this and other native species.

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## Introduction

Weeds are recognised as a significant threat in many plant communities around the world. In the past few centuries, humans have had a propensity to introduce plants from one country or area to another. Without predators and a lack of competition to keep them in check, introduced plants may flourish and their spread can become a key threat to local biodiversity (Vitousek & Walker 1989; Adair 1995; Mack & D'Antonio 1998; Hoffman *et al.* 2004; Jackson 2005; Williams *et al.* 2005). In Australia, at least 57 nationally-listed endangered species have been identified as being at risk from weeds (Leigh & Briggs 1992). In New South Wales (NSW), over 300 threatened native plant species are considered at risk from exotic plants (Coutts-Smith & Downey 2006). Competition from weeds is therefore a significant factor increasing the risk of extinction faced by threatened species, as well as contributing to their continued decline.

Environmental weeds posing a significant threat in Australia include a range of life forms (Thorpe & Lynch 2000), and in NSW, several exotic grasses have been recognised as a threat to native plant communities (NSW Scientific Committee Determination 2003). Many other grass species, introduced for use in pastures or restoration projects, have escaped from cultivation and become weedy, particularly in coastal and island habitats. One such species is Kikuyu grass (*Pennisetum clandestinum*), a South African native planted in many countries as a pasture grass. Once established, it can spread vigorously and can tolerate a wide range of climatic zones from temperate to subtropical (Holm *et al.* 1977). Another grass species, St. Augustine or Buffalo grass (*Stenotaphrum secundatum*), native to coastal areas of North America, can similarly thrive in temperate and subtropical areas of the world (Gillham 1960; Campos *et al.* 2004, US Forest Service, Pacific Island Ecosystems at Risk (PIER) – accessed November 2007).

Lord Howe Island (lat 31° 35'S; long 159° 05'E) is a remote subtropical island approximately 500 km off the coast of NSW. Of over 100 endemic plant species on the island (Green 1994), eight are currently listed as endangered under the NSW *Threatened Species Conservation Act* (1995) and two as critically endangered on the Federal *Environment Protection and Biodiversity Conservation Act* (1999). Several of these threatened plants are at risk from competition from Kikuyu and Buffalo grass. Kikuyu was introduced to Lord Howe Island as a pasture grass about 1900 (Pickard 1984) while Buffalo was introduced in the 1940s. Both species have thrived in the subtropical maritime climate. Kikuyu and Buffalo grass now cover much of the cleared lowland areas of the Island (Pickard 1984; I. Hutton pers obs.). At the boundary of pastures and the forest edge, these grasses creep into the forest understorey and smother seedlings of native species, preventing recruitment of native forest species. Introduced grasses may also compete with native plants for water and nutrients, hindering the growth, fecundity and establishment of native species (Gillham 1960; Orr *et al.* 2005; Denslow *et al.* 2006; Minchinton *et al.* 2006).

The impact of weeds on a native species will be dependent on the distribution, abundance and life history of the plant. Many threatened species exist only in very low numbers, or in a restricted area, or both. Due to the small size of habitat and range of such threatened species, introduced plant species may have detrimental impacts on their survival. Of particular concern on Lord Howe Island is the Critically Endangered (*EPBC Act* 1999) twiner *Calystegia affinis* Endl. (family Convolvulaceae). *Calystegia affinis* is endemic to Lord Howe and Norfolk Islands (about 900 km northeast). Until recently *Calystegia affinis* was only known on Lord Howe Island from a 1937 collection (J.D. McComish 77A Kew, NSW), and Rodd and Pickard (1983) presumed the plant to be locally extinct. However, a specimen was collected in 1985 at the western end of the area known as Old Settlement. Surveys in 1999 verified the location of the species, but did not find any additional plants at the forest edge around the perimeter of Old Settlement (Hutton & Telford 1999). Subsequent surveys for rare plants (in 2001 and 2002) located another three plants in remote mountain areas at around 500m altitude – one on Mount Lidgbird and two on Mount Gower (Hutton 2001, 2004).

Species from the genus *Calystegia* (*Calystegia* has about 25 species in the tropics and subtropics) have been reported as being clonal, with reproduction often largely by vegetative means (Ushimaru & Kikuzawa 1999; Arafeh & Kadereit 2006) and have extensive, thin, underground rhizomes that produce many leafy stems above ground (Wolf *et al.* 2000). It is likely that *Calystegia affinis* at Old Settlement is also clonal, and the hundreds of stems appearing through the Kikuyu grass may all be one plant, connected by stems and underground rhizomes throughout the grass. Some plants that reproduce clonally can live for decades or centuries (Laberge *et al.* 2000) and the *Calystegia affinis* population

at Old Settlement has been there at least since 1985, and is probably the same plant collected in 1937.

The dense cover of Kikuyu and Buffalo grass at Old Settlement is considered likely to be having an impact on the recruitment and persistence of the *Calystegia affinis* (NSW Scientific Committee 2002) and control of exotic grasses in *Calystegia affinis* habitat is an action in the Lord Howe Island Biodiversity Management Plan (DECC 2007). The aim of this study was to trial herbicide treatment at the Old Settlement site, to assess the suitability of herbicides for broader *Calystegia affinis* habitat restoration and to examine the impact of reduced grass competition on the *Calystegia* population. We compared the effectiveness of two herbicides, Nufarm Asset Herbicide (active ingredient haloxyfop) and Glyphosate on stem dieback of the introduced Kikuyu and Buffalo grass, and examined the impact of these herbicides on the survival of existing stems and the emergence of new stems of *Calystegia affinis*.

## Methods

### *Study site and species*

The study site is a south-east facing lower slope of Dawson's Ridge, above the Old Settlement flats, just inside the Permanent Park Preserve fence line (Fig. 1). The soil is basalt-derived loam containing small scattered basalt rock. The adjacent forest is closed rainforest dominated by *Drypetes deplanchei* and *Cryptocarya triplinervis* (Pickard 1983). At the site *Calystegia affinis* is intermingled with dense growth of Kikuyu and Buffalo grass to 50 cm tall. Where the forest canopy is dense and shades the ground up-slope, both grasses and *Calystegia* are absent, but in some areas where tree cover is less dense, native grasses and *Calystegia* occur. Here occasional *Calystegia* stems twine around small stems of other species up to 60 cm above ground level; otherwise *Calystegia* is strictly decumbent. Remnant native species occurring with *Calystegia affinis* include *Parsonsia howeanum*, *Rapanea platystigma*, *Cryptocarya triplinervis*, *Leucopogon parviflorus*, *Smilax australis*, *Commelina cyanea*, *Lobelia sp.*, *Carex brunnea* and *Dodonaea viscosa*.

### *Initial plant density*

To estimate the *Calystegia affinis* density we used 24, 1m x 1m random quadrats recording (on 31/10/2006) the number of *Calystegia* stems in each quadrat emerging through the grass cover.

### *Herbicide treatments*

It was considered that if any habitat restoration were to take place, the best areas would be adjacent to the native forest, rather than in the middle of the introduced pasture grasses. Eight 5m x 5m quadrats dominated by exotic grasses were selected at the boundary of the forest and pasture (Fig. 1).

All quadrats were permanently marked with hardwood stakes and individually numbered with aluminium tags. GPS readings were recorded for the southwest corner of each quadrat. Within each quadrat the number of *Calystegia affinis* stems was recorded.

Herbicide spray was applied to half the quadrats on 1/11/2006. A low dosage (1:200) Glyphosate-based herbicide was applied to two quadrats, and the grass-specific herbicide Asset (at a dosage rate of 1:200) was applied to two quadrats. A 1:200 Glyphosate treatment is often used in vegetation restoration programs as this dosage kills weeds but does not affect native plant species. No treatment was applied to the remaining four quadrats.

To make identification of individual *Calystegia affinis* stems easier, all individual stems within the quadrat were marked with plastic flagging tape prior to herbicide treatment. To avoid herbicide damage to *Calystegia*, individual plastic sandwich bags were carefully placed over any leaves and stems to minimise the probability of herbicide uptake (Buchanan 1995).

At four weeks and 26 weeks after treatment, the cover of all species in the quadrats was assessed. For grass cover, this included a separate estimate of both living and dead material. All *Calystegia* stems that had died, and any new ones that had grown, were counted.

#### Data Analysis

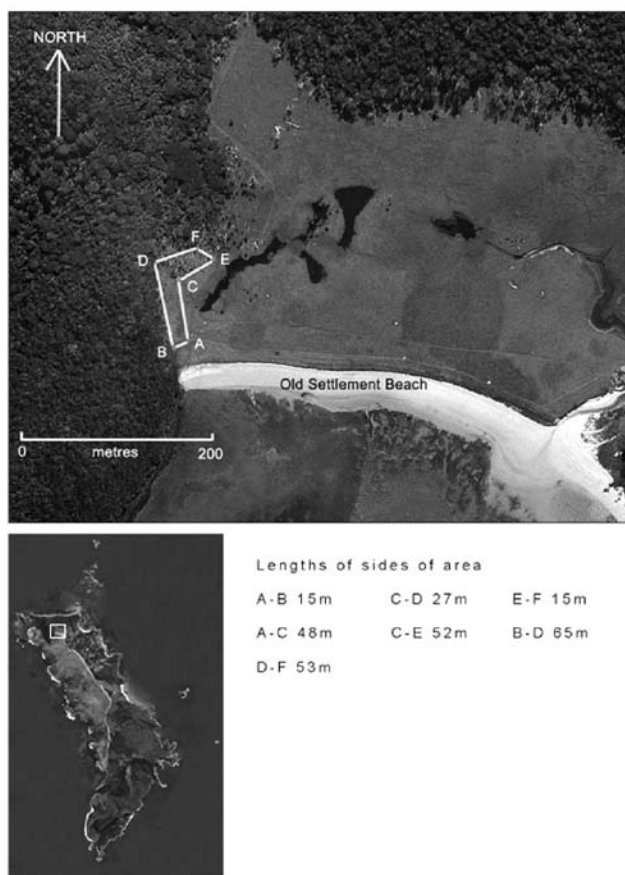
At four week (short-term) and 26 weeks (mid-term), we compared the three treatments (Glyphosate, Asset and no application of herbicide) in terms of the reduction in cover of live exotic grass using a 1 Factor ANOVA. At four weeks only, we examined the proportion of stems of *Calystegia affinis* that had died since the initiation of the study with a 1 Factor ANOVA. At 26 weeks, we also examined proportional change in the number of stems of *Calystegia* using a 1 Factor ANOVA. For all analyses, Cochran's Test was used to test for homogeneity of variances. Where heterogeneous variances were detected, the data were arcsine transformed (Underwood 1981). For the 26 week comparison of proportion of exotic grass killed, the data were heterogeneous even after arcsine transformations and the ANOVAs were performed on the raw data with a conservative approach to rejecting the null hypothesis in order to avoid Type 1 errors (i.e. the null hypothesis was not rejected unless the probability of the F-ratio in the ANOVA was  $<0.01$ ). Where ANOVAs were significant, individual means were compared using Student-Newman-Keuls tests (Zar 1984).

#### Results

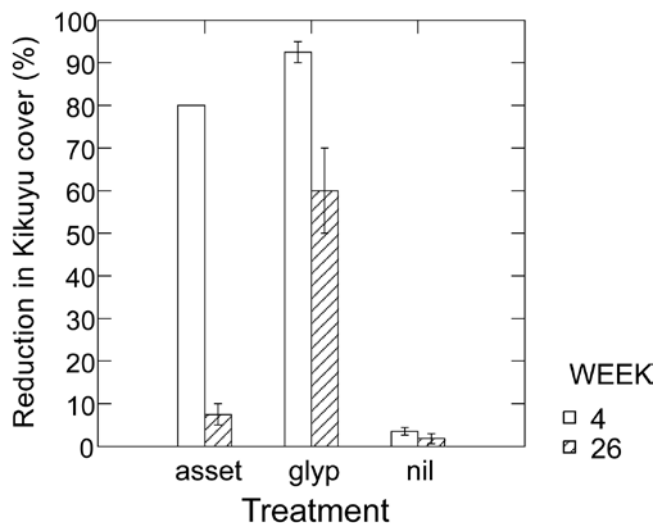
The density of *Calystegia affinis* at 31/10/2006 in the 1m x 1m quadrats ranged from 0 to 5 stems  $m^{-2}$  (mean  $1 \pm 0.3$  stems  $m^{-2}$ ). As the study area covers some 2313  $m^2$ , we estimate that it contained some 2313 ( $\pm 648$ ) *Calystegia affinis* stems. Across the 25  $m^2$  treatment plots, the density of *Calystegia affinis* ranged from 0.2 to 4.4 stems  $m^{-2}$ . The absolute number of stems per 25  $m^2$  plots was also very variable (ranging from 5 to 19 in six of the plots, with two plots with large numbers (98 and 110)).

We identified 15 plant species in the study area– five introduced weeds (*Pennisetum clandestinum*, *Stenotaphrum secundatum*, *Paspalum* sp., *Lilium formosanum*, *Vicia sativa*), and 10 native species – twiners and climbers (*Calystegia affinis*, *Commelina cyanea*, *Parsonia howeanum*, *Smilax australis*), herbs (*Lobelia* sp., *Carex brunnea*), small shrubs (*Dodonaea viscosa*, *Leucopogon parviflorus*) and occasional stunted individuals of the tree species (*Cryptocarya triplinervis* and *Rapanea platystigma*).

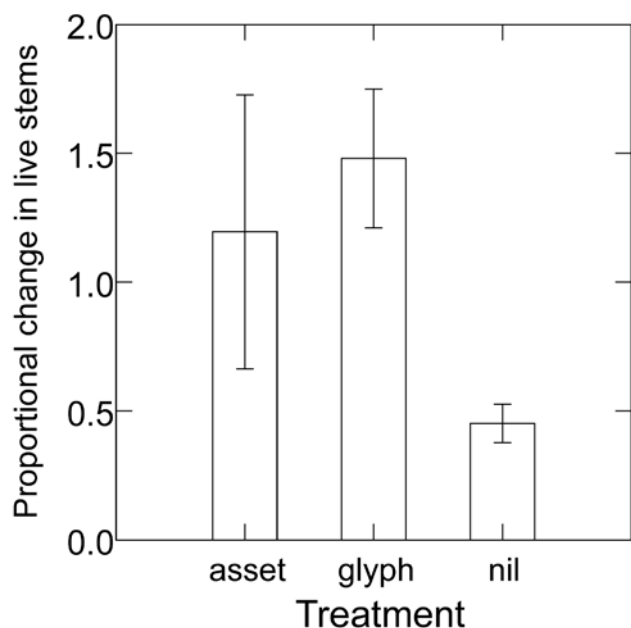
On many leaves of *Calystegia affinis* both within study quadrats and across the whole area, there was evidence of extensive leaf damage, caused by the beetle *Arsipoda parvula*. New leaves appeared to be free of this damage, and older leaves more affected. The impact of this beetle on *Calystegia affinis* is unknown. This beetle is also found in



**Fig. 1.** Location of *Calystegia affinis* study site on Lord Howe Island.



**Fig. 2.** Mean reduction in exotic grass cover ( $\pm 1$  standard error) after herbicide treatment. Four weeks after treatment (open bars); 26 weeks after treatment (filled bars).



**Fig. 3.** Proportional change in the number of living stems of *Calystegia affinis* 26 weeks after application of treatments. A value of 1 means no change. Values of less than 1 represent a decline, values of more than 1 an increase. Bars are means  $\pm 1$  standard error.

Queensland and is known to graze on other species within the Convolvulaceae family.

At four weeks after the application of the herbicide treatments there was a marked reduction ( $P < 0.001$ ) in the cover of live exotic grass in both the Glyphosate and Asset compared to only a very small reduction in the control plots (Figs. 2,4). At 26 weeks, there was still a marked reduction for the Glyphosate treatment ( $P < 0.001$ ), while the Asset and controls were comparable (Figs. 2,4).

The initial impact of spraying on *Calystegia affinis* stems at four weeks was different across the herbicide treatments ( $P = 0.024$ ). There was slightly less stem death in the Asset treatment than the control, while Glyphosate was not different from either. Essentially, the experimental bagging of *Calystegia* leaves minimised any negative effect of the herbicides on living stems. There was some initial production of new stems in the first four weeks after herbicide treatment, but all stems died back over summer between the four and 26 week sampling. At 26 weeks, there was no significant difference across all treatments ( $P = 0.058$ ) in live stems as a proportion of the original number of stems observed. There was however, a trend for a greater proportion of new stems in both herbicide treatments than in the control (Fig. 3).

## Discussion

Targeted herbicide spraying had the desired effect of reducing exotic grass cover while doing minimal damage to *Calystegia affinis* plants (Fig. 4). This contrasts with weed control regeneration attempts in other habitats where non-selective herbicides may significantly impact upon native species (Brown *et al.* 2002; Matarczyk *et al.* 2002; but see Brown 2006 for success with a selective herbicide). The protection of individual *Calystegia affinis* stems using plastic bags while herbicide treatment was carried out was effective, and minimal loss of *Calystegia* was caused by herbicide spraying. Though some *Calystegia* stems present at the start of the trials (marked with ribbon) died, new ones grew within four weeks, in both the treated and the control quadrats.

Both herbicides were effective in reducing the cover of exotic grasses after four weeks. However, over the longer term (26 weeks) there was more regrowth of exotic grass cover in the Asset treatment than in the Glyphosate treatment (Figs. 2,4). Thus, in this situation, the Glyphosate treatment was ultimately more effective in reducing exotic grass cover than the grass-specific Asset herbicide. Longer periods of reduced exotic grass cover should allow more new *Calystegia affinis* stems to be produced and/or fewer stems dying from being overgrown by grass.

In this study, there was only one herbicide treatment spraying, and after 26 weeks considerable introduced grass regrowth had taken place. Further treatment with the Glyphosate herbicide could be beneficial to prevent more grass regrowth,



Asset Treatment: Pre-application Nov 2006



Glyphosate treatment: Pre-application Nov 2006



Asset Treatment: 1 week after treatment



Glyphosate treatment: 1 week after treatment



Asset Treatment: 4 weeks after treatment



Glyphosate treatment: 4 weeks after treatment



Asset Treatment: 26 weeks after treatment



Glyphosate treatment: 26 weeks after treatment

**Fig. 4.** Photographic comparison of one plot each of Asset and Glyphosate treatments from pre-treatment (November 2006) to 1 week, 4 weeks and 26 weeks post-treatment. There was continuous grass cover in the control plots.

provided *Calystegia affinis* plants are protected during any herbicide application. Other alternatives to reduce grass regrowth may be less practical, eg mowing (it would be hard to ensure stems of *Calystegia affinis* were not destroyed), or hand-weeding (too labour intensive for the size of the area to be treated).

All stems of *Calystegia affinis* that were present at the start of the trial (and marked with plastic ribbons) had died after 26 weeks, regardless of herbicide treatment. This may be a feature of the life history of this species. However, the summer from December 2006 to March 2007 was extremely dry for Lord Howe Island. Rainfall figures were December 62 mm (average 106 mm), January 18 mm (average 127 mm), and February 37 mm (average 122 mm) (Bureau of Meteorology LHI). This dry period may have been the cause of the death of these stems. Competition from introduced grasses and the browsing by beetles may have contributed. All of the new leaves that were present after 26 weeks were very healthy in appearance, and bright green, with no insect damage.

Irrespective of herbicide treatment, more *Calystegia affinis* stems were present after 26 weeks than we observed after 0 or 4 weeks; but the quadrats treated with herbicide had a much greater increase in new stems (Fig. 3). This difference was not significant due in part to the small sample size and variation in the data. If the observed trend is real, then treatment of introduced Kikuyu and Buffalo grass with herbicide does benefit the overall vigour of the threatened *Calystegia affinis* as indicated by the increased number of new stems after herbicide treatment compared to the untreated control plots. At any rate, the observed reduction in live exotic grass cover at 26 weeks under the Glyphosate treatment is also likely to enhance growth and survival of *Calystegia* stems.

We noted that in the area around two of the quadrats, there were many small native plants that were stunted and being smothered by Kikuyu and Buffalo grasses. We believe that if these plants are freed from the grasses they will slowly recover normal growth resulting in a semi-shaded area that may provide suitable habitat for *Calystegia affinis*. In previous surveys (Hutton & Telford 1999; Hutton 2001, 2004), and while carrying out this trial, it was observed that *Calystegia* does not grow into the heavily shaded forest canopy edge, but where it is fully exposed, where it faces competition from introduced Kikuyu and Buffalo grass. Consequently, there may be a need to maintain open edges to allow *Calystegia* plants to persist at the site.

As the biomass of exotic grasses is removed, other secondary weeds from adjacent pastures are likely to invade (e.g., Scotch thistle, *Onopordum acanthium*, and Vetch, *Vicia sativa*). Treating the introduced grasses with herbicide and leaving the dead grass mat *in situ* will provide mulch to inhibit secondary weeds for some time.

## Conclusions and recommendations

Following the results of this study we suggest that the area adjacent to this trial be further treated with Glyphosate to improve the habitat for *Calystegia affinis*. Prior to herbicide treatment, all surviving native shrubs, trees, twiners and grasses should be freed up of Kikuyu and Buffalo grasses by gently pulling the grasses off, using secateurs where necessary. Abundance of all plant species in the restoration area should be recorded. The number of *Calystegia* stems should be recorded and each stem marked with coloured ribbon and protected by placing inside plastic bags just prior to herbicide treatment. The area should then be treated with 1:200 Glyphosate spray, being careful to minimise any herbicide overspray reaching native plants. Wherever native plants occur, a protective shield (e.g. cardboard or stiff plastic) should be used to shield the plant while spraying adjacent grass. Twenty-four hours after treatment all plastic bags should be removed from *Calystegia* plants.

At two, four and six months after herbicide treatment the site should be checked for any live exotic grasses and if necessary treated with 1:200 Glyphosate herbicide using a hand-held one litre spray pack. The number and condition of all *Calystegia* stems, and other native plants should be recorded.

The adjacent forest area has a groundcover of native *Carex* species; these also occur in the disturbed site. These species are common groundcovers in equivalent habitats elsewhere on Lord Howe and it may be beneficial to propagate them at the Island nursery, for planting in the restoration site (when the dead kikuyu has broken down), to provide groundcover that will inhibit weed invasion. Plots established during this trial may be used for ongoing monitoring, including population numbers, recruitment and life history observations.

Green (1994) has suggested that Lord Howe and Norfolk Island populations of *Calystegia affinis* may perhaps be separate subspecies, so a DNA comparison of the Lord Howe and Norfolk Island populations to determine if they are distinct species or subspecies would be a valuable addition to our understanding of this rare species (Wolf *et al.* 2000, Arafah & Kadereit 2006). Observations on flowering material indicate the flower corolla of the Lord Howe Island plants are approximately 3.5 cm long compared with 2.5 cm long on Norfolk Island plants (Peter Green pers. comm.).

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