

## Trial of herbicide control methods for sisal *Agave sisalana* in the arid island environment of Aldabra Atoll, Seychelles

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### SUMMARY

Sisal *Agave sisalana* is an invasive alien plant species of concern at the UNESCO World Heritage Site of Aldabra Atoll in the Seychelles. Physical control efforts since the 1970s to remove sisal from Aldabra have only been partially successful because the roots cannot be completely removed, resulting in continuous control efforts. We conducted a seven month herbicide trial, using different herbicide concentrations with two application methods, to determine the most effective and feasible control method for sisal. We also checked effects on surrounding native plants. The highest treatment mortality was from 50% herbicide concentration applied directly to the cut growth tip, which resulted in 80% sisal mortality after four months. Fewer treated plants died at lower herbicide concentrations and more small plants died than large plants. No sisal plant died that was foliar sprayed, only cut, or in the control group. There were no visible negative effects of any treatment on the surrounding native flora. The results indicate that chemical control of sisal is effective at high herbicide concentration applied directly to the cut growth tip. A full-scale eradication of sisal from Aldabra has been started based on the trial results.

### BACKGROUND

Invasive alien species are one of the top four drivers of biodiversity loss (Sanderson & Moulton 1998, Mooney *et al.* 2005). Invasive alien plants are a particular threat to native island plant communities and their control is widely used in habitat restoration (Caujapé-Castells *et al.* 2010). The main control techniques are chemical and physical, although bio-control methods are also used for major pests (Flory & Clay 2009). Early detection and response is the most cost- and labour-effective, and also the most successful way to address invasive alien plants problems (Clout & Williams 2009). In insular ecosystems there can be additional constraints, such as high sensitivity of endemic habitats, the necessity to reduce disturbance wherever possible and difficulty of site access.

An invasive alien plant that has spread to non-native areas around the world is sisal *Agave sisalana* (Agavaceae) (ISSG 2014). Sisal is a large succulent perennial plant which is native to Mexico and has been introduced into tropical and sub-tropical areas as an ornamental and cultivated plant, notably in Brazil, Tanzania, Madagascar and Kenya for fibre production (Nobel 1988, Jacobson 2005). Sisal produces monospecific stands which exclude native flora, contribute to habitat homogenisation, and reduce biodiversity and food sources for native wildlife (Badano & Pugnaire 2004). The species is a sexually sterile clone, probably of hybrid origin, and produces no seed. Reproduction occurs via bulbils (young plantlets that root where they fall, following development on a 5–6 m tall inflorescence), and via sprouting of new plants from elongated underground stolons (Nobel 1994, Weber 2003, Gentry 2004).

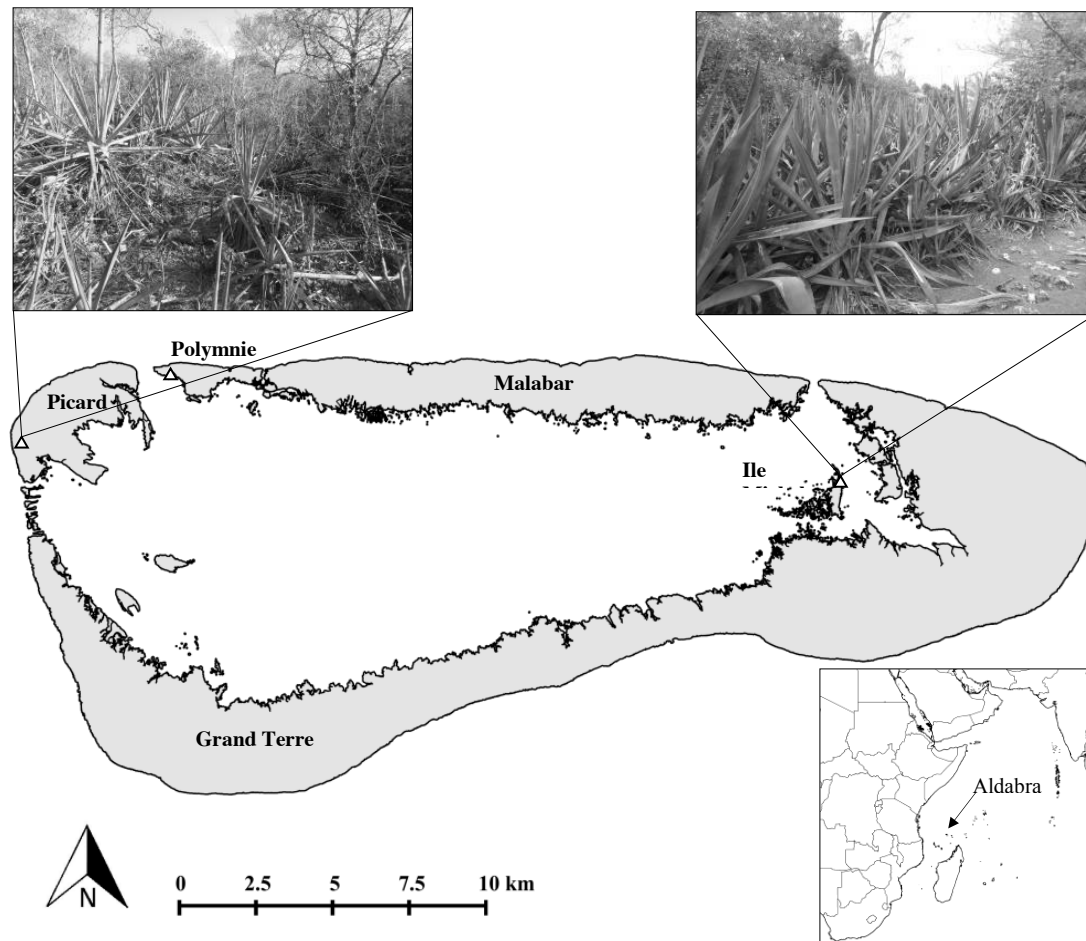
Sisal has long been considered an alien plant of concern at the UNESCO World Heritage Site of Aldabra Atoll (9°24'S, 46°20'E; 34 × 14.5 km; Figure 1) in the Seychelles; a large raised coral atoll consisting of a rim of four main islands (Figure 1) with a total land area of approximately 152.5 km<sup>2</sup>.

Aldabra has been strictly protected since 1976, was inscribed on the UNESCO World Heritage list in 1982 and has been managed since 1979 by the Seychelles Islands Foundation. Aldabra's mean annual rainfall is approximately 975 mm, which is unevenly distributed throughout the year, with most rain falling during the wet season from January to April with the remainder of the year being dry or very dry (Seychelles Islands Foundation, unpublished data).

Sisal was identified on Aldabra as a target species for control as early as 1971 (Stoddart 1971) and between 1972 and 1976 large patches (800 m<sup>2</sup> and larger) were manually removed from several locations. Clearing of patches near the settlement area on Picard alone accumulated at least 2100 man hours in 1974 (Seychelles Islands Foundation, unpublished data). In the mid-1970s, coordinated control efforts on Aldabra to remove sisal patches started by uprooting plants, followed by removal and/or burning. This type of control was successful in residential and frequently visited areas (e.g. the area around Picard settlement; Figure 1). The hard porous limestone substrate of Aldabra, however, enables plants to root deeply in the rock, making it difficult to remove them completely. This resulted in re-sprouting of sisal around the atoll after initial removal and thus in continuous control efforts. These efforts, over many years, led to Aldabra's current limited distribution of sisal at only three locations; Picard, Ile Michel and Anse Polymnie (Figure 1, Table 1).

The difficulties of physical control led us to investigate the potential for chemical control, which has been successfully used in many eradication programs elsewhere (Soria *et al.* 2002, Wotherspoon & Wotherspoon 2002). Herbicide trials on *A. sisalana* and *A. americana* (century plant) in Australia showed several herbicide-carrier-application combinations to have positive results (>90% above-ground plant mortality; Foley & Bolton 1990, Bickerton 2006). On Aldabra, herbicide should reach the difficult to remove roots of the sisal plants, preventing re-sprouting and ultimately leading to potential eradication of this invasive plant from the atoll. An important consideration for chemical control on Aldabra, however, is that

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**Figure 1.** Location of Aldabra Atoll in the Indian Ocean (inset bottom right), present locations of sisal patches on Picard, Polymnie and Ile Michel (white triangles) and inset pictures showing sisal patches on Picard and Ile Michel.

herbicide carriers are often environmental pollutants (e.g. diesel), which are particularly undesirable in sensitive ecological areas. Herbicide has consequently not been considered previously for large-scale use on Aldabra.

Our aim was to conduct a small-scale herbicide trial on individual plants to determine the most effective and feasible control method for sisal with minimal risk to Aldabra's environment. We used different herbicide concentrations with two common application techniques: cut-stump and foliar spray methods. Based on the outcome of the experimental trials we determine the best control method and the total effort required for a full-scale eradication of sisal from Aldabra.

## ACTION

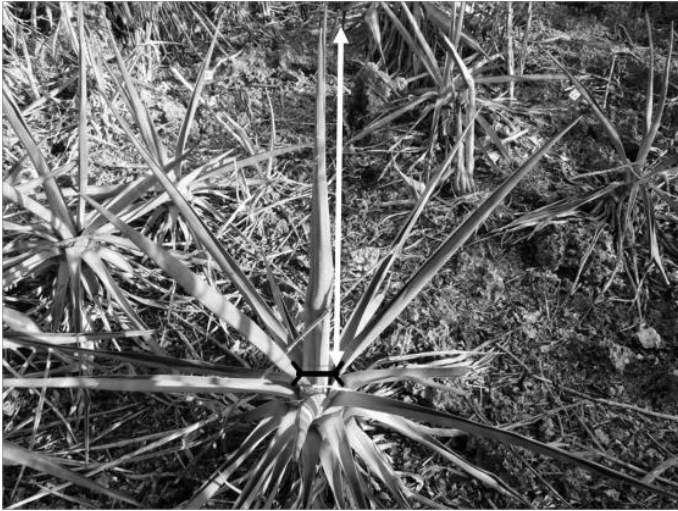
The plants in the Picard sisal patch were selected for the trial as it was the easiest to access and monitor regularly. The patch consisted of approximately 200 plants loosely clustered in an area of approximately 400 m<sup>2</sup> interspersed and

surrounded by native scrub 2-3 m high. Each of the plants was marked with a numbered tag and allocated to one of three height classes as an indicator of plant age before treatment: plants 0.2–0.5 m in height (small, 69 plants); plants 0.5–1 m in height (medium, 78 plants); and plants >1 m in height (large, 27 plants). Young plants (<0.5 m in height) that were directly situated below or in close contact with 'mother' plants were excluded from the trial to prevent possible interference through roots. To control for size differences, all target plants were randomly divided equally among the three height classes and across treatments.

The herbicide used was Tordon 101™ (240 g/l 2,4-Dichlorophenoxyacetic acid and 65 g/l picloram), which is a commercially available herbicide combination. This herbicide was selected because it is readily degraded when exposed to sunlight on the surface of plant foliage and soils (Tu *et al.* 2001). Tordon 101™ (hereafter referred to as 'herbicide') is most effective when applied during the growth season, and periods of stress or dormancy should be avoided (Tu *et al.* 2001, Dow AgroSciences 2010). Observations of sisal on

**Table 1.** Current known sisal patches on Aldabra with their characteristics and locations (see Figure 1).

Location	Number of plants	Approximate area (m)	Description
Picard	200	15 × 30	Patch interspersed and surrounded by native scrub.
Anse Polymnie	30	2 × 2	Small clump of medium sized plants.
Ile Michel	2,000–3,000	50 × 30	Dense patch, produces several flowering plants yearly.



**Figure 2.** Sisal plant showing central growth stem (white vertical line) and location of cut (black horizontal line) for herbicide application.

Aldabra showed that leaves tended to be folded inward during dry periods, limiting access for herbicide application to the leaf surface. Our trial was therefore carried out after the first rains at the end of the dry season (June–October), with treatment conducted during 17–19 November 2013 in dry conditions.

Herbicide solution was coloured with red food dye to mark treatment and applied using a 1.5 L hand-held plant sprayer. Two application methods were used: spray application, which consisted of spraying the top and bottom surfaces of all leaves, including the central growth stem; and neat-to-cut application, in which the central growth stem was cut as low as possible (Figure 2) and herbicide solution was applied immediately after cutting (within 20 s). The manufacturer's recommended herbicide concentrations were 50% for neat-to-cut methods and 1% for direct leaf-stem applications (Dow AgroSciences 2013). We used water for the herbicide dilution. To determine the lowest effective herbicide concentration we tested eight treatments: (1) Removal of growth tip only without herbicide (n=22); (2) High concentration (50%) herbicide applied neat to cut (n=20); (3) Medium concentration (20%) herbicide applied neat to cut (n=20); (4) Low concentration (5%) herbicide applied neat to cut (n=21); (5) High concentration (2%) herbicide sprayed on whole plant (n=21); (6) Medium concentration (1%) herbicide sprayed on whole plant (n=20); (7) Low concentration (0.5%) herbicide sprayed on whole plant (n=21); and (8) No treatment (control, n=21). The treatment of cutting without herbicide application was included to determine whether only cutting has a significant effect on plant mortality.

Following treatment, sisal plants were monitored monthly for seven months. The health of each plant was visually assessed and recorded as either 'healthy': plant had at the most a few minor blemishes; 'unhealthy': plant alive but with necrotic tissue; or 'dead': no living (green) tissue could be seen (following Bickerton 2006). Native plants surrounding treated sisal plants were also carefully examined every month to check for signs of herbicide effects such as leaf-dropping, necrotic tissue or plant death.

Due to the low frequency of mortality events in most treatments and time intervals, we conducted three different analyses. We used pairwise binomial tests to compare proportions of mortality at high, medium and low herbicide concentration with that of the control group (Crawley 2007).

We used a generalised linear model with a quasi-binomial error distribution to account for over-dispersion to test for differences in mortality (binary response) six months after treatment with plant size and herbicide concentration (both factors with three levels). Both explanatory variables were fitted as main effects. Lastly, temporal differences in neat-to-cut treatment on mortality at three and six months were tested with a mixed effect model and a binomial error distribution, fitted with the *glmer* function of the *lme4* package (Bates *et al.* 2014). The two-level factors month and herbicide concentrations were included in the model as fixed effects and plant ID was fitted as a random effect to account for repeated sampling. Analyses were conducted in R 3.1.1 (R Core Team 2014).

## CONSEQUENCES

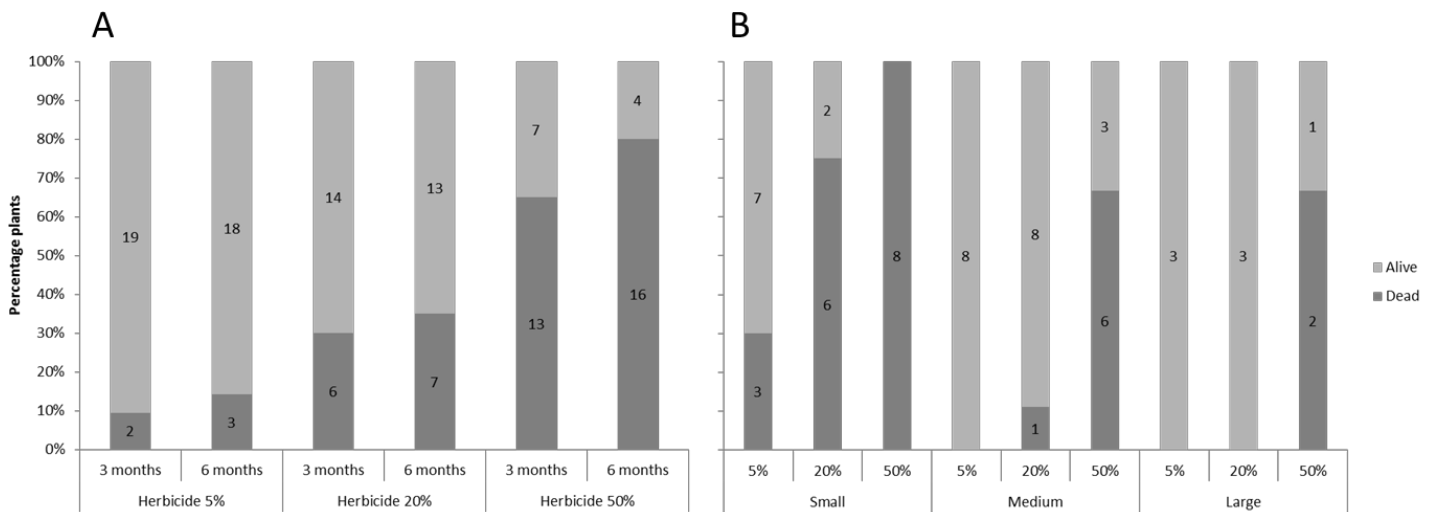
The most effective treatment was neat-to-cut with herbicide concentrations of  $\geq 20\%$  (pairwise binomial tests; high concentration: 80% died after six months compared to 0% in the control group;  $\chi^2 = 24.3$ ,  $p < 0.0001$ ; medium: 35% died after six months;  $\chi^2 = 7.00$ ,  $p = 0.0082$ ; Figure 3). Fewer plants died at low and medium herbicide concentrations of neat-to-cut treatments than at high concentration six months after treatment, and smaller plants were more affected than larger plants. More small plants died after neat-to-cut treatments than larger plants (small plants: 65%; medium plants: 27%; large plants: 22%; Figure 3, Table 2). Only small plants were killed by all three herbicide concentrations in neat-to-cut treatments, while only 50% herbicide concentration killed medium and large plants (Figure 3B). All plants that were dead by the end of the experiment had died after 5–6 months, and final levels of mortality could only be determined six months after treatment, irrespective of herbicide concentrations (Figure 3, Table 3). Most neat-to-cut treated plants showed initial signs of stress and those that recovered did so after four months and were recorded as healthy after seven months. Final success rate of a treatment could only be confirmed up to seven months after application.

No plant in the control or cut only treatments died. Cutting the growth tip weakened 25% of sisal plants within six weeks after treatment, but these plants recovered swiftly and after three months almost no negative effects were detectable.

Spray treatments were highly ineffective: 10% of sprayed plants showed temporary and weak negative effects (leaf discolouration and necrotic tissue on leaf edges). At low herbicide concentration these effects lasted for 6–8 weeks, and at high concentration two plants were unhealthy up to four months after treatment. All plants in the spray treatments (n = 62) recovered and no sprayed plant died.

**Table 2.** Results of the generalised linear model testing main effects of plant size and herbicide concentration on sisal mortality after neat-to-cut treatment. Reference levels of plant size was 'small plants' and of herbicide concentration was 'high concentration'.

Variable	Coeff.	S.D.	t	p
Intercept	4.57	1.19	3.83	<b>0.0003</b>
Large plants	-4.13	1.40	-2.95	<b>0.005</b>
Medium plants	-3.75	1.12	-3.36	<b>0.001</b>
Low concentration	-5.47	1.30	-4.20	<b>&lt; 0.0001</b>
Medium concentration	-3.35	1.06	-3.16	<b>0.003</b>



**Figure 3.** Percentage mortality of sisal plants treated with 5%, 20% or 50% concentration of herbicide neat to the cut central growth stem: (A) after three and six months; and (B) different sisal plant size groups (small, medium and large) after six months.

No non-target effects were recorded throughout the experiment and all native plants surrounding the treated sisal were unaffected by the herbicide (independent of application technique). The effort required for treatment of approximately 50 sisal plants in a low density sisal patch, with herbicide applied directly to the cut growth tip, was about one hour with a recommended team size of two people (i.e. two person hours). The amount of herbicide needed for the neat-to-cut application is dependent on plant size. It is approximately 5–15 ml per plant for plants <1 m, but plants >1 m required up to 40 ml per plant as the cut surface is larger than that of smaller plants.

## DISCUSSION

Our results show that targeted herbicide application rather than only physical removal can be used successfully to control or eradicate sisal in sensitive habitats. Neither removal of the growth tip alone nor spraying plants without cutting the growth tip was effective when using low concentrations of herbicide. The most effective control method for sisal in the arid environment of Aldabra was the direct application of high herbicide concentration (50%) to the cut growth tip. High mortality was also seen in smaller plants at lower herbicide concentrations, suggesting that the greater biomass of larger plants after growth tip removal reduces the efficacy of the herbicide. Mortality of sisal plants with this treatment was similar to previous studies (Foley & Bolton 1990, Bickerton 2006) although the use of different herbicide combinations and concentrations rules out a direct comparison of these results.

Tunison and Zimmer (1992) found foliar spray with 5% Garlon® 4 (active ingredient; triclopyr) effective but noted that it was only partially effective on small plants.

Even the application of the highest concentration of herbicide caused no visible harmful effects to native vegetation on Aldabra. We also show that it is not essential to use carriers (surfactants) in herbicide application on sisal as up to 80% mortality was achieved with a herbicide–water mix. Follow-up treatment, however, is necessary to achieve 100% mortality, even using a high herbicide concentration.

Following the results of the trial, a full-scale eradication of sisal from Aldabra was launched using the most effective method of applying herbicide directly to the cut growth tip of each plant. The effort and herbicide requirements of the eradication were calculated from the trial data, allowing the operation to be budgeted and planned efficiently. Environmental and economic considerations led to a final herbicide concentration between medium and high trial concentrations for the eradication. Given the size differences in mortality in the trial, a larger stump surface (than only the growth tip) was created by cutting off the top half of the plant, i.e. removing the rosette of upper leaves using a pruning saw or shears, and applying herbicide to the resulting cut surface. Monitoring of the eradication progress so far has indicated that sisal mortality following the modified methods is equal to or higher than the trial mortality.

Our experimental trial and further honing of the most effective technique for eradication provides evidence for an effective method of controlling invasive sisal in an arid and sensitive island ecosystem.

**Table 3.** Results of the generalised mixed-effect model testing time and herbicide concentration effects on sisal mortality at neat-to-cut treatment. Reference levels of month was ‘three months’ and of herbicide concentration was ‘high concentration’.

Variables	Coefficient	S.D.	z	p
Intercept	12.38	3.20	3.86	< 0.0001
6 months	15.06	4.81	3.13	0.001
Low concentration	-40.52	9.73	-4.16	< 0.0001
Medium concentration	-37.84	8.82	-4.29	< 0.0001
6 months × low concentration	-0.58	6.73	-0.09	0.93
6 months × medium concentration	-2.10	5.58	-0.38	0.71

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