Arum Lily Herbicide Experiment

HMAS Stirling, Garden Island

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Summary

Arum Lily Herbicide Experiment

This report details an experiment to determine the effectiveness of two herbicides, Glean® (chlorsulfuron) and Glean® mixed with 2,4-D amine, in controlling Arum Lily (*Zantedeschia aethiopica*) infestation on Garden Island, Western Australia. The experiment was set up as a series of 45, 4x4 m quadrats in 1997, in which eight different spray treatments were applied with one unsprayed control throughout the four year experiment. The treatments were compared using one, two, three and alternate repeat applications of each herbicide throughout the period on five replicates of the nine treatments. Above ground plant numbers were counted annually throughout the experimental period.

In 2001, the HLA-Ecoscape team conducted the final year assessment of the experiment. This involved excavating three randomly assigned, 1x1 m subsamples in each quadrat and conducting above ground counts, below ground counts and dry weight measurements of Arum Lily plant material within the subsamples. A count of total above ground counts was also undertaken in the larger 4x4 m quadrat prior to excavation of the subplots. The data were analysed to test the following hypotheses:

- H_o: There is no significant difference in the number of plants between treatments in the final year;
- H_o: There is no significant difference in the number of daughter plants between treatments in the final year;
- H_o: There is no significant difference in total dry weight between treatments in the final year;
- H_o: There is no significant difference in the number of rotted tubers with active side rhizomes between treatments in the final year;
- H_o: There is no significant difference in the number of plants between years or treatments; and
- H_o: There is no significant difference in the number of plants in each height class between years and treatments.

It was found that the method of estimating above ground plant numbers grossly underestimated the actual number of individual plants, as determined through excavation.

It was found that Glean® mixed with 2,4-D amine generally had a greater effect on reducing plant numbers and biomass than Glean® alone, provided that more than one annual application was made. There was no significant difference between the three treatments with the greatest impact which were:

- Glean® + 2,4-D amine applied in the first two years only;
- Glean® + 2,4-D amine applied for three consecutive years; and
- Glean® + 2,4-D amine applied on two alternate years.

The effectiveness of the above treatments differed significantly to the control (no spray) treatment. Glean® alone was effective in killing the above ground parts of the plant but did not have a significant effect on reducing biomass, indicating survival of the rhizome.

It is therefore recommended that future herbicide control of Arum Lily on Garden Island incorporate the Glean® + 2,4-D amine mixture either in two consecutive years with a break of two years, or in every alternate year. The use of Glean® alone is not recommended as it merely produces dormancy in the rhizome, which then resprouts.

1.0 Introduction

Arum Lily Herbicide Experiment

1.1 Background

Arum Lily (*Zantedeschia aethiopica*) has been identified as the highest weed threat to the ecological integrity of Garden Island, Western Australia (Wykes, 1997). Its management has consumed the highest proportion of time and budget allocated to weed control/environmental management on the island (LeProvost, Dames and Moore, 2000). The problems posed to the environment by the Arum Lily infestation have been identified by Wykes (1997) as:

- Their ability to displace native plant species and their invasiveness into areas that have not been disturbed by human activities;
- Their ability to change the physical structure of the vegetation and wildlife habitat.;
- Toxicity and a resulting reduction of food sources to native animals; and
- Impact on aesthetic values. They are conspicuous and are obviously not a component of native vegetation.

The control of Arum Lily, to date, has been concentrated on herbicide spraying programs, as manual removal was found to have a high impact on the native vegetation (Wykes, pers. comm.) and biological control investigations funded by the Department of Defence (DoD) found no likely candidates (Wykes, pers. comm.). Fire management has been considered but would impact on the fire-intolerant *Callitris* and *Melaleuca* communities of Garden Island. Fire would also be difficult since to be effective it must be of an intensity to heat the underlying soil and rhizome (Plummer, 1997). However fire followed by herbicide control of reshooting arum lily, followed by direct seeding with *Callitris* and *Melaleuca* may be a potential approach for the densely infested *Acacia* scrub community (Wykes, Pers. comm.)

Different spraying strategies have been employed to maximise spraying effectiveness. From 1989 until 1996, chlorsulfuron alone was applied to the areas of highest infestation at a concentration of 2 g/100 L water with 250 mL of wetting agent and a 'red-eye' marker. In 1996, 2,4-D amine was added to the solution and concentration of Glean® increased on advice from John Moore, Department of Agriculture, Albany (Wykes, pers. comm.). The spraying strategy was also changed by taking a systematic approach, commencing treatment in the less severely infested northern areas of the island (Wykes, 1997). In the areas accessible to vehicles, the herbicides have been applied with a hose at a concentration of 5 g chlorsulfuron, 1 L 2,4-D amine (Lo-Odour®) and 250 mL non-ionic surfactant. The majority of applications, however, necessitated hand spraying at a higher concentration of 10 g chlorsulfuron, 2 L 2,4-D amine and 250 mL non-ionic surfactant (Wykes, 1997).

This experiment attempts to ascertain the efficacy of combining 2,4-D Amine with Glean®, and the application regime that is most successful at controlling Arum Lily. Nine different treatments were tested between the years 1997 and 2001 in an area of dense infestation near Beagle Road adjacent to HMAS Stirling Naval Base. Forty-five 4x4 m plots with 5 replications of each treatment were tested each year throughout the experiment, and were then partially excavated in the final year to determine the impact of the treatments on biomass and actual plant numbers based on below ground observations.

1.2 Aims

The aims of the experiment were:

- To determine the degree of success that can be achieved with successive years of spraying using the current treatment;
- To compare this with past treatment;
- To determine the rapidity of return to pre-treatment conditions when spraying ceases; and
- To determine the difference in infestation when treated and not treated.

The current treatment consists of the application of a solution of Glean® (chlorsulfuron) and 2,4-D Amine Lo-Odour® (2,4-dichlorophenoxyacetate acid as dimethylamine salt). The past treatment consisted of spraying with Glean® alone (surfactant wetting agents were added to both solutions). The herbicides were applied by hand spraying, replicating, as much as possible, the behaviour of spraying contractors.

To determine the effectiveness of the two solutions and the rapidity of return to pre-treatment conditions, nine different treatments were established consisting of four different spraying regimes of each solution. There was one control treatment that was not sprayed, to determine the difference in infestation when treated and not treated.

1.3 Biology of Arum Lily

Arum Lily originates from South Africa where it is pre-adapted to the Mediterranean climate found in south west Western Australia (Plummer, 1997). It is a large robust perennial with large, broad shiny leaves, and large, white funnel-shaped inflorescences with a yellow-orange spike inside, bearing numerous tiny male and female flowers (Moore, 1997). The roots consist of white tuber-like rhizomes giving rise to white, fleshy lateral roots (Moore, 1997). The rhizome freely produces offsets as a form of asexual reproduction. A 2 cm rhizome of the dwarf variety of *Z. aethiopica* can produce 25-30 offsets in cold climate conditions and more in warmer climates (Welsh *et. al.* in Plummer, 1997). All of the offsets are capable of independent growth, even when the 'mother' rhizome is killed. Considering the larger size of mature rhizomes found on Garden Island, the potential for vegetative reproduction and the subsequent difficulties of eradication are high.

In addition to the ability to reproduce vegetatively, Arum Lily can reproduce sexually from its flowers. Each flower can produce fruit containing numerous seed that are then dispersed, mainly through the gut of frugivorous birds. They can also be consumed by foxes and stock that subsequently pass and disperse the seed (Moore, 1997). It is also dispersed by human vectors, as it is valued as an ornamental garden plant. This is the most likely explanation for its initial presence and subsequent naturalisation on Garden Island (Moore, 1997).

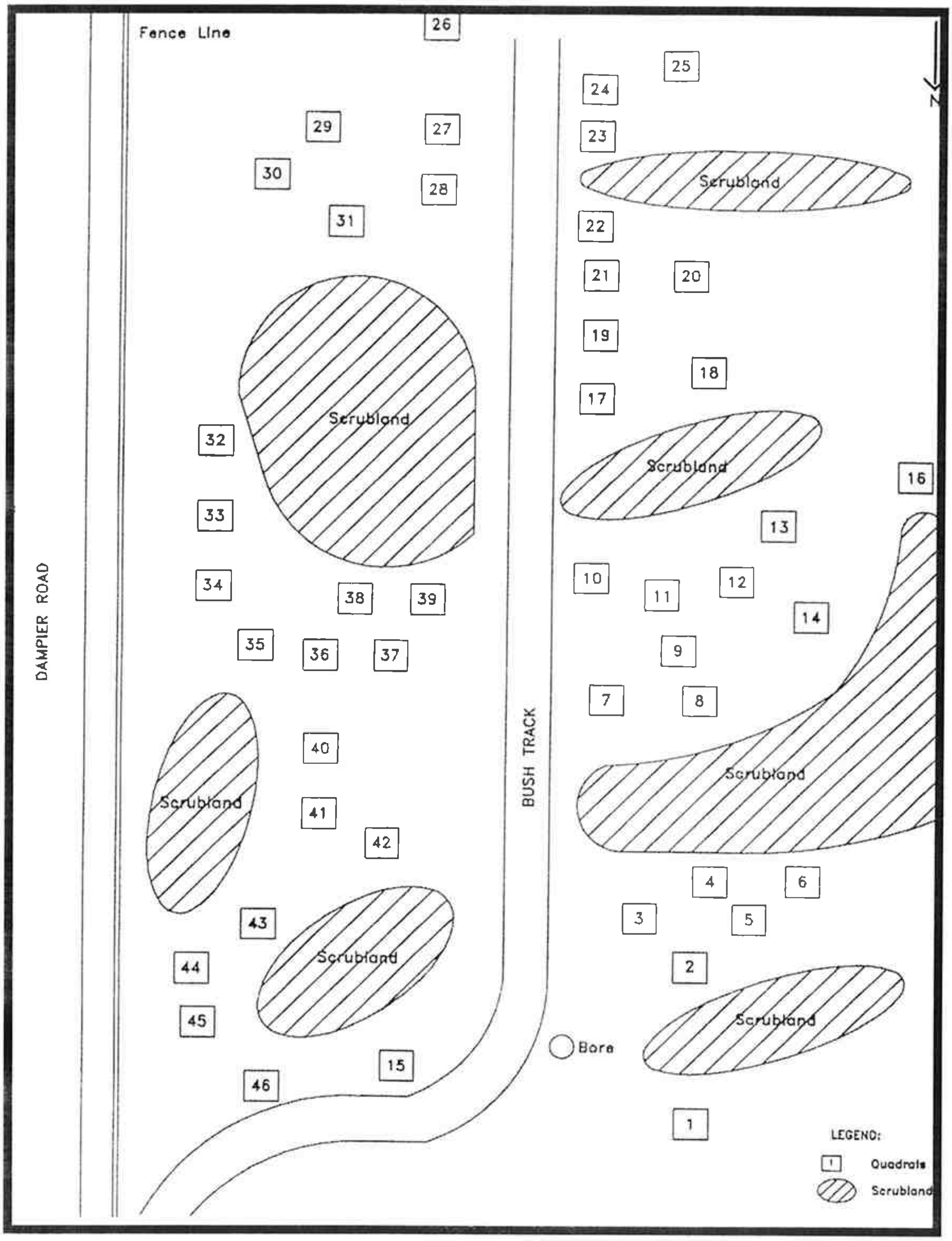
1.4 Report Structure

The report is divided into the following sections:

- Introduction – Outlines the background of the Arum Lily problem, the aims of the experiment and the biology of Arum Lily;
- Methods – Details the methods used in the experiment;
- Analysis – Describes the analytical procedures used to examine the raw data and test for differences between treatments;
- Results – Shows the results of the analysis including relevant tables and graphs;
- Discussion – Highlights the most successful treatments, comments on experimental design and avenues of future study and provides conclusions and recommendations; and

Figure 1. Map Showing location of study site at HMAS Stirling, Garden Island

Figure 2. Map showing quadrat locations (Courtesy PPK Environment and Infrastructure Pty. Ltd).



2.0 Method

Arum Lily Herbicide Experiment – Year 4

2.1 Experimental Area

The experimental area is located on the central axis of Garden Island, adjacent to HMAS Stirling Naval Base and about 200m west of Beagle Road (Wykes *et al.*, 2000). It was judged suitable as it had:

- Not been sprayed since 1994 and so no residual effects of spraying could affect the experiment;
- Moderate Arum Lily infestation rather than scattered or continuous infestation, to decrease variability;
- Relatively uniform topography to decrease variability - on the western edge of the flat, 'upper bench' (B1) land formation; and
- Relatively uniform habitat - generally *Acanthocarpus preisii* scrub dominated by scattered *Acacia rostellifera.*

2.2 Quadrat Design

Forty-five 4x4 m quadrats were established throughout the study site. The quadrat positions were placed in dense *Acanthocarpus preisii* scrub where there was little or no overhanging from trees. Areas of dense infestation of Arum were also avoided, as individual plants/clumps could not be distinguished. The quadrat size of 4x4 m was found suitable for obtaining samples of 30 or more plants (Wykes *et al.*, 2000).

The experimental area was divided into 5 blocks of 9 quadrats. The nine treatments were assigned within each block using random number tables (Appendix One). Each quadrat was marked by a star picket, generally in the north-east corner of the quadrat, while the other corners were marked by small, wooden stakes. A photograph was taken of each quadrat before the experiment commenced, with a similar photograph taken at the end of the experimental period.

2.3 Spray Treatments

The spray compositions used throughout the experiment were:

Glean®:	10 g chlorsulfuron and 250 mL non-ionic surfactant 'wetting agent' in 100 L water.
Glean® + 2,4-D Amine:	10 g chlorsulfuron, 2 L 2,4 dichlorophenoxyacetic acid as dimethylamine salt (Lo-Odour®) with 250 mL non-ionic surfactant 'wetting agent' in 100 L water.

The spray was applied as a short, focussed squirt on a single leaf of each plant from which fluid flowed down its petiole to the base of the plant. Plants in a 0.5 m buffer zone around the treated quadrats were also sprayed to minimise the fall of seeds into the quadrats. The quantity of herbicide applied varied with the amount of infestation present. Less Glean®+2,4-D amine tended to be used than Glean® alone due to the higher volatility of 2,4-D Amine (Wykes *et al.*, 2000).

The quadrats were sprayed according to the regime previously determined (refer to Table 1). In some cases this involved annual spraying from 1997 to 1999. No spraying was conducted after 1999, to allow any dormant underground survivors of the spray treatments to emerge. The dates of the treatments and measurements are included in table 2.

Table 1: Herbicide treatment regimes

Treatment	1997	1998	1999	2000
1	Glean®	No Spray	No Spray	No Spray
2	Glean®	Glean®	No Spray	No Spray
3	Glean®	Glean®	Glean®	No Spray
4	Glean®+2,4D	No Spray	No Spray	No Spray
5	Glean®+2,4D	Glean®+2,4D	No Spray	No Spray
6	Glean®+2,4D	Glean®+2,4D	Glean®+2,4D	No Spray
7	Glean®	No Spray	Glean®	No Spray
8	Glean®+2,4D	No Spray	Glean®+2,4D	No Spray
9	No Spray	No Spray	No Spray	No Spray

Table 2: Dates of Treatments and Measurements

Year	Date Measured	Date Sprayed	Arum Development Stage
0	30/9/1997 – 3/10/1997	6/10/1997 – 7/10/1997	Fully flowering; much seed development; unripe fruits.
1	15/9/1998	18/9/1998	Fully flowering; Some seed development.
2	15/10/1999	21/10/1999	Fully flowering; much seed development; unripe fruits.
3	13/10/2000	No Treatment	N.R.
4	8/11/2001	No Treatment	Flowering finished fruit developed, some ripe.

2.4 Above Ground Counts

Annual counts were made of the number of plants in each quadrat. Plant counts were separated into three height classes; 0-30 cm; 31-60 cm and; >60 cm. As it was difficult to distinguish between a clump of individual plants and a clump of shoots from the same below ground rhizome, a clump of plants were regarded as an individual plant. Stakes had been used to mark plants counted in previous years so as to separate these from the current years new growth. This was not done in the final year as the condition of the stakes was such that they were no longer useful indicators of previous growth.

2.5 Below Ground Counts

At the end of the experimental period (2001), three 1x1 m subsamples were randomly taken from each quadrat using a random number generator[1] (see Appendix Three). They were then excavated to determine the actual number of plants in each subsample, based on the number of shoots with a distinct separate rhizome. The plants were sorted into the three height classes listed above and then counted. Many clumps of plants turned out to be a mass of offsets, each distinctly separate from each other. For this reason these 'daughter plants' were counted as separate plants.

2.6 Total Dry Weight

At the end of the experimental period, all of the Arum Lily plant material in each of the 1x1 m subsamples was removed and oven dried to obtain total dry weight of Arum Lily in each subsample. The plant material was cut to a size suitable for drying. The extreme amount of moisture held in the stems of the Arum Lily meant that an extended period of drying had to be used. The samples were oven dried in paper bags at a temperature of 65°C for 4 days, and measured to 2 decimal places on calibrated electronic balances.

[1] Microsoft Excel

3.0 Analysis

Arum Lily Herbicide Experiment – Year 4

3.1 Hypotheses Tested

The data collected was analysed to test the following null hypotheses:

1. H_o: There is no significant difference in the number of plants between treatments in the final year;

2. H_o: There is no significant difference in the number of daughter plants between treatments in the final year;

3. H_o: There is no significant difference in total dry weight between treatments in the final year;

4. H_o: There is no significant difference in the number of rotted rhizomes with active side rhizomes between treatments in the final year;

5. H_o: There is no significant difference in the number of plants between years and treatments; and

6. H_o: There is no significant difference in the number of plants in each height class between years and treatments.

The hypotheses were tested using analyses according to the type and quality of the data. For this reason the analysis of each hypothesis will be dealt with separately.

3.2 H_o: 1 – Number of Plants in Final Year

The final year counts were based on below ground observations made from each of the 1x1 m subsamples. We were unable to use a parametric nested plot design for analysis, as homogeneity of variance could not be achieved through data transformation. Therefore, the number of plants in the three subsampled cells from each quadrat were averaged to maintain randomness between the samples. The five subsequent means for each treatment were then analysed to determine if a statistically significant difference was detectable between the numbers of plants for each of the nine spraying treatments. Seedlings were excluded from the analysis to replicate the behaviour of spraying contractors (Wykes *et al.*, 2000).

The data displayed heterogenous variances[2] (α = 0.007) and therefore could not be significance tested using a parametric one-way analysis of variance (Sheskin, 1997). Threfore, a non-parametric comparison using the Kruskal-Wallis one-way analysis of variance of ranks was used. Descriptive statistics were also obtained and the mean and standard error of each treatment was graphed, A Student Newman Keuls (SKM) post hoc-test was used to group homogenous subsets.

3.3 H_o: 2 – Number of Daughter Plants in Final Year

The number of daughter plants were not assessed in the final year as it was found that most of these were in fact separate plants (offsets) and so were counted as such. For the purposes of herbicide control, it is important to distinguish between separate plants, as spraying just the 'mother' plant will not kill the offsets and it may in fact exacerbate the problem (Plummer, 1997).

3.4 H_o: 3 – Total Dry Weight in Final Year

The final year dry weight of the three subsampled cells from each quadrat was averaged to maintain randomness. The five subsequent means for each treatment were then analysed to determine if a statistically significant difference was detectable in the dry weight of Arum Lily between each of the nine spraying treatments.

The data displayed heterogenous variances (α < 0.001) and therefore could not be significance tested using a one-way analysis of variance (Sheskin, 1997). Logarithmic transformation of the data using Natural Log [x+1]. (Ln[x+1]) achieved a significance level of 0.047 for equal dispersion, which was deemed acceptable enough to perform a parametric single factor one-way analysis of variance. The resulting mean differences for each treatment were tested for significance using Tukey's Honestly Significant Difference (HSD) test, from which three groups of significant variables were obtained at the 0.05 significance level.

3.5 H_o: 4 – Number of Rotted Rhizomes in Final Year

A test of homogeneity of variance showed unequal dispersion between treatments (α = 0.02) so the data was transformed using the Ln[x+1] transformation, which resulted in an acceptable equal variance (α = 0.364). A single factor one-way analysis of variance was then performed on the transformed data and the differences in variables were tested using Tukey's HSD test.

[2] SPSS – Statistical Package for the Social Sciences

3.6 H_o: 5 – Number of Plants Between Years and Treatments

Levene's test of homogeneity of variance showed unequal dispersion between treatments ($\alpha = 0.006$). Transformation of data did not result in equal variances, so the non-parametric Kruskal-Wallis 2-way analysis of variance of ranks was used. This allowed a test for significant differences between treatments, years and the interaction between treatments and years. A graph was produced depicting the means and standard errors of the above ground counts for each year and treatment to allow a visual comparison to be made. Seedlings were again excluded from the analysis.

3.7 H_o: 6 – Number of Plants in Each Height Class Between Years and Treatments

A Levene test of homogeneity of variances showed unequal dispersion among the data groups. Transformation did not yield equal variances so non-parametric Kruskal-Wallis 2-way analysis of variance of ranks were used to determine differences within each height class. Differences between the mean ranks of year and treatment were tested. Descriptive statistics of the mean and standard error of treatments among years was determined and presented graphically. Seedlings were again excluded from the analysis.

3.8 Comparison of Above and Below Ground Plant Counts

A linear regression analysis was also performed to determine the level of correlation between above ground counts and below ground counts of each height class.

4.0 Results

Arum Lily Herbicide Experiment – Year 4

4.1 H_o: 1 – Number of Plants in Final Year

The number of plants in the final year based on below ground observations was assessed using a one-way Kruskal-Wallis analysis of variance of ranks. This resulted in a calculated value of 27.599 (df=8). As the chi-square critical value at the 0.01 significance level is 20.090 (df=8), we can reject the null hypothesis and conclude there is a significant difference in number of plants between at least two treatments in the final year (Sheskin, 1997).

The dispersion around the mean is shown in Figure 3 by the median, interquartile range and outliers for each treatment.

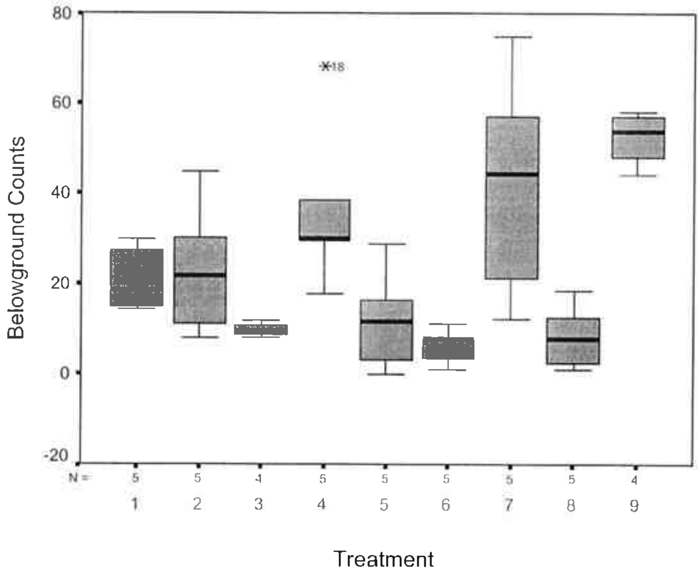


Figure 3: Median, interquartile range and outliers of below ground counts for each treatment.

It can be seen that treatments 3, 6, 8 and 9 show the smallest degree of variability between samples. These treatments are:

- T3 – 3 years of Glean® application (G-G-G-N);
- T6 – 3 years of Glean® + 2,4-D Amine (GA-GA-GA-N);
- T8 – Alternate years of Glean® + 2,4-D Amine (GA-N-GA-N); and
- T9 – Control (N-N-N-N).

Treatment 7 (G-N-G-N) showed the highest variability between samples and also had the highest median value of all sprayed treatments.

The mean and standard error of below ground counts for each treatment (Figure 4), indicates a separation of treatments into three groups. The treatments that appear to have reduced plant numbers the most are 3, 5, 6 and 8. These treatments were:

- T3: Glean® for 3 years (G-G-G-N);
- T5: Glean® + 2,4-D amine for 2 years (GA-GA-N-N);
- T6: Glean® + 2,4-D amine for 3 years (GA-GA-GA-N); and
- T8: Glean® + 2,4-D amine alternate years (GA-N-GA-N).

Treatments 1 and 2 appear to have had an intermediate effect. They consisted of:

- Glean® in the first year only (G-N-N-N); and
- Glean® for 2 consecutive years (G-G-N-N) respectively.

Treatments 4, 7 and 9 had the least effect. These treatments were:

- T4: Glean® + 2,4D amine for 1 year (GA-N-N-N);
- T7: Glean® alternate years (G-N-G-N); and
- T9: Control (N-N-N-N).

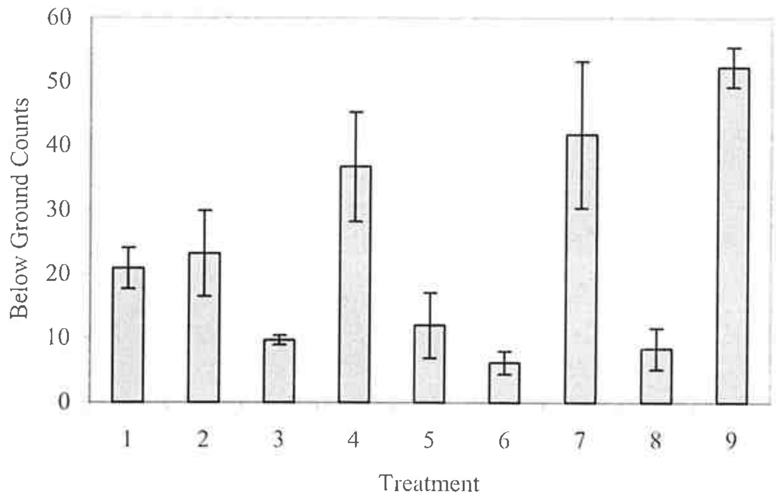


Figure 4: Below ground counts among herbicide treatments (mean ± standard error).

The post-hoc SKM test resulted in three homogenous subsets, similar to those observed in figure 4 but with an overlap between groups. This is shown in table 3.

	Subset and means		
Treatment	1	2	3
6	6.1333		
8	8.3333		
3	9.6250		
5	11.9333		
1	20.8667	20.8667	
2	23.1333	23.1333	
4		36.7000	36.7000
7		41.8000	41.8000
9			52.4167
Sig.	0.389	0.095	0.183

Table 3: Belowground count means for groups in homogenous subsets

4.2 H_o: 3 – Total Dry Weight in Final Year

Differences in total dry weight in the final year were tested using a single factor one-way analysis of variance with data transformed to its logarithmic equivalent. The means and standard errors of each treatment are shown in Figure 5. The treatments that appear to have had the greatest effect are 5 (GA-GA-N-N), 6 (GA-GA-GA-N) and 8 (GA-N-GA-N). Treatments 1 (G-N-N-N), 2 (G-G-N-N) and 3 (G-G-G-N) appear to have had an intermediate effect, and treatments 4 (GA-N-N-N), 7 (G-N-G-N) and 9 (N-N-N-N) had the least effect on Arum Lily biomass.

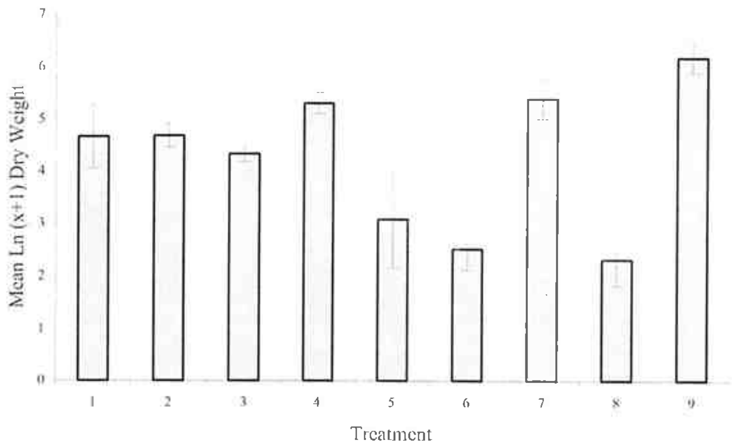


Figure 5: Ln(x+1) dry weights for each treatment (mean ± standard error).

The results of significance testing using Tukey's HSD test are shown in table 4.

Table 4: Significance comparisons between different treatments (mean ln(x+1) dry weight).

Ho: No significant difference in biomass between treatments in the final year, α < 0.05 shaded.

Treatment	1	2	3	4	5	6	7	8	9
1		1.000	1.000	0.986	0.314	0.055	0.970	0.026	0.368
2	1.000		1.000	0.988	0.299	0.051*	0.975	0.024	0.384
3	1.000	1.000		0.860	0.616	0.163	0.798	0.086	0.150
4	0.986	0.988	0.860		0.041	0.004	1.000	0.002	0.917
5	0.314	0.299	0.616	0.041		0.994	0.030	0.960	0.001
6	0.055	0.051*	0.163	0.004	0.994		0.003	1.000	0.000
7	0.970	0.975	0.798	1.000	0.030	0.003		0.001	0.951
8	0.026	0.024	0.086	0.002	0.960	1.000	0.001		0.000
9	0.368	0.384	0.150	0.917	0.001	0.000	0.951	0.000	

The Tukey HSD test gave rise to three homogenous subsets (α = 0.05), displaying significant similarity to each other shown in Table 5. Although there is a high degree of overlap between the groups, it is still possible to determine the treatments that had the greatest effect on Arum Lily biomass. These are treatments 8 (GA-N-GA-N), 6 (GA-GA-GA-N) and 5 (GA-GA-N-N). The group of treatments with the least effect contained treatments 3 (G-G-G-N), 1 (G-N-N-N), 2 (G-G-N-N), 4 (GA-N-N-N), 7 (G-N-G-N) and 9 (N-N-N-N).

Table 5: Homogenous subsets of treatments.

Treatment	Group 1	Group 2	Group 3
8	2.319		
6	2.517	2.517	
5	3.084	3.084	
3	4.340	4.340	4.340
1		4.670	4.670
2		4.690	4.690
4			5.314
7			5.400
9			6.189
Sig	*0.086*	*0.051*	*0.150*

4.3 H_0: 4 – Number of Rotted Rhizomes in Final Year.

The data was transformed using the Ln (x+1), which gave homogenous variances (α = 0.159). The resulting means were tested using Tukey's HSD test and were found to have no significant differences between treatments. A graph of the untransformed means and standard errors of treatments is shown in Figure 6. Although it appears to suggest that treatment 7 (G-N-G-N) has a higher number of rotted rhizomes than the other treatments, the high variability of the samples means that this cannot be proven statistically.

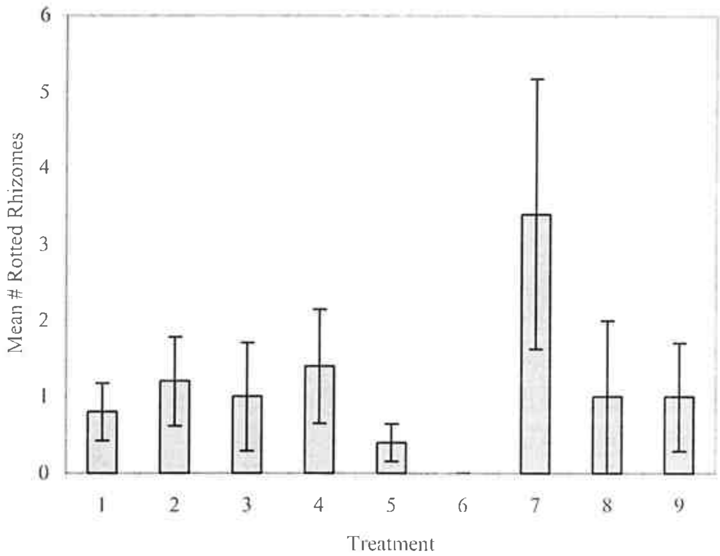


Figure 6: Number of rotted rhizomes in each treatment (mean ± standard error).

4.4 H$_o$: 5 – Number of Plants between Years and Treatments

The results of the non-parametric Kruskal-Wallis 2-way analysis of variance revealed a difference between treatment means and years. There is also an interaction between years and treatments. Descriptive statistics of the mean and standard error of above ground counts between years and treatments are graphed in Figure 7, to show where these differences lie.

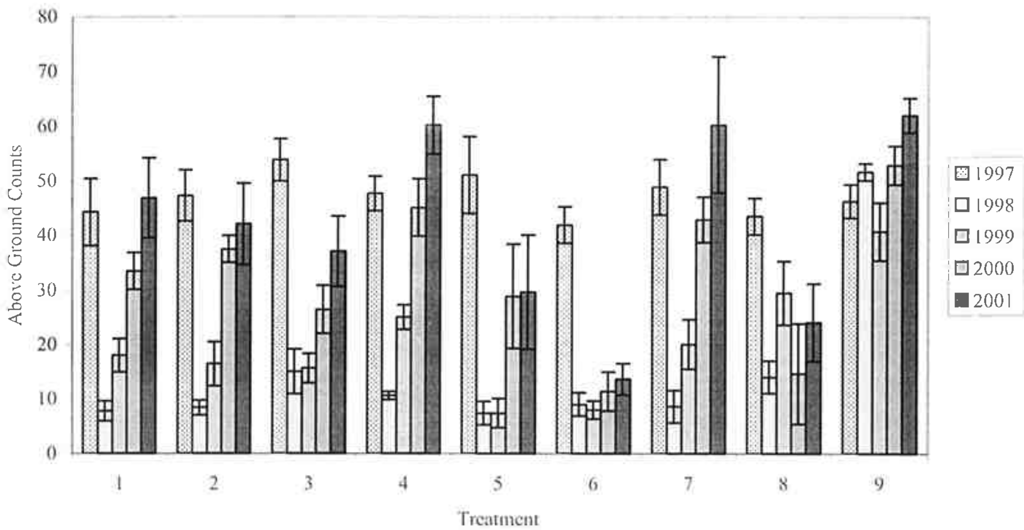


Figure 7: Above ground counts for treatments and years (mean ± standard error)

From this graph it is possible to visually determine trends in plant numbers between treatments during the course of the experiment. All treatments (except the control) caused a rapid fall in the first year with different patterns of recovery, as follows:

- T1. (G-N-N-N): Gradual return to pre-treatment numbers;
- T2. (G-G-N-N): Gradual return to pre-treatment numbers;
- T3. (G-G-G-N): Two year delay with gradual return to less than pre-treatment numbers;
- T4. (GA-N-N-N): Gradual return to greater than pre-treatment numbers;
- T5. (GA-GA-N-N): Two year delay before increase to intermediate levels;
- T6. (GA-GA-GA-N): Little or no recovery;
- T7. (G-N-G-N): Gradual return to greater than pre-treatment numbers;
- T8. (GA-N-GA-N): Intermediate recovery; and
- T9. (N-N-N-N): Control, gradual increase in numbers.

The overall trends in Figure 7 appear to suggest that Arum Lily is able to rapidly recover from Glean® applications while repeated applications of Glean® + 2,4-D amine successfully reduces plant numbers. Treatments 4 and 7 both showed a recovery to higher levels than the initial numbers, suggesting that these treatments seemed to have no effect on reducing Arum Lily numbers over the experimental period.

The increase in control numbers over time suggests that the infestation in this area has not reached its climax. This has implications towards interpreting trends among years, as the control does not remain constant. Returns to pre-treatment conditions in the sprayed treatments, therefore, may not signify a complete null effect if they are still less than the control numbers. All treatments except for 4 (GA-N-N-N) and 7 (G-N-G-N) showed smaller final year numbers than the control.

4.5 H_o: 6 – Number of Plants in Each Height Class Between Years and Treatments

4.5.1 Overall Results

The results of the non-parametric Kruskal-Wallis 2-way analysis of variance performed separately on each height class showed the following response to the null hypotheses:

Height Class 0-30 cm	Ho: No difference between treatments	accept
	Ho: No difference between years	reject
	Ho: No interaction between years and treatments	reject
Height Class 30-60 cm	Ho: No difference between treatments	reject
	Ho: No difference between years	reject
	Ho: No interaction between years and treatments	reject
Height Class >60 cm	Ho: No difference between treatments	reject
	Ho: No difference between years	reject
	Ho: No interaction between years and treatments	reject

There was a significant difference between years in all height classes and a significant difference between treatments in height classes 30-60 cm and >60 cm. There was no significant difference in numbers between treatments in the 0-30 cm height class, although some trends between treatments could be seen in the graph which, although not statistically significant, may help uncover some effects.

4.5.2 0-30 cm Height Class

A graph of mean number of Arum Lily plants in the 0-30 cm height class between treatments and years is shown in Figure 8. All treatments except for 5, 6, 8 and 9 showed a gradual increase in Arum Lily numbers in the 0-30 cm height class after the initial spray treatment. Treatment 5 (GA-GA-N-N) showed low numbers for two years after the initial herbicide application, treatment 6 (GA-GA-GA-N), showed relatively low numbers throughout, treatment 8 (GA-N-GA-N) showed intermediate levels throughout and treatment 9 (control) suggested a slight decrease in numbers over the years.

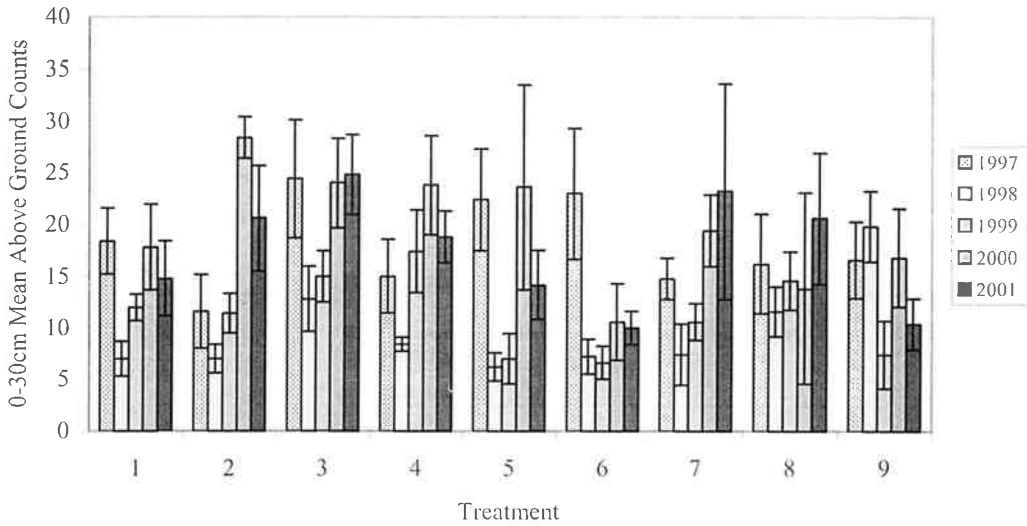


Figure 8: 0-30 cm height class above ground counts for years and treatments (mean ± standard error).

4.5.3 30-60 cm Height Class

A graph of mean number of Arum Lily plants in the 30-60 cm height class is shown in Figure 9. Treatments 1 (G-N-N-N), 4 (GA-N-N-N) and 7 (G-N-G-N) all show greater numbers in the final year than initially. Treatments 3 (G-G-G-N) and 5 (GA-GA-N-N) show an increase to lower than initial levels and treatments 6 (GA-GA-GA-N) and 8 (GA-N-GA-N) show little increase from levels directly after the first herbicide application. Although treatment 8 showed some recovery in the second year, the following two years after the second application of herbicide displayed little reappearance of plants in this height class.

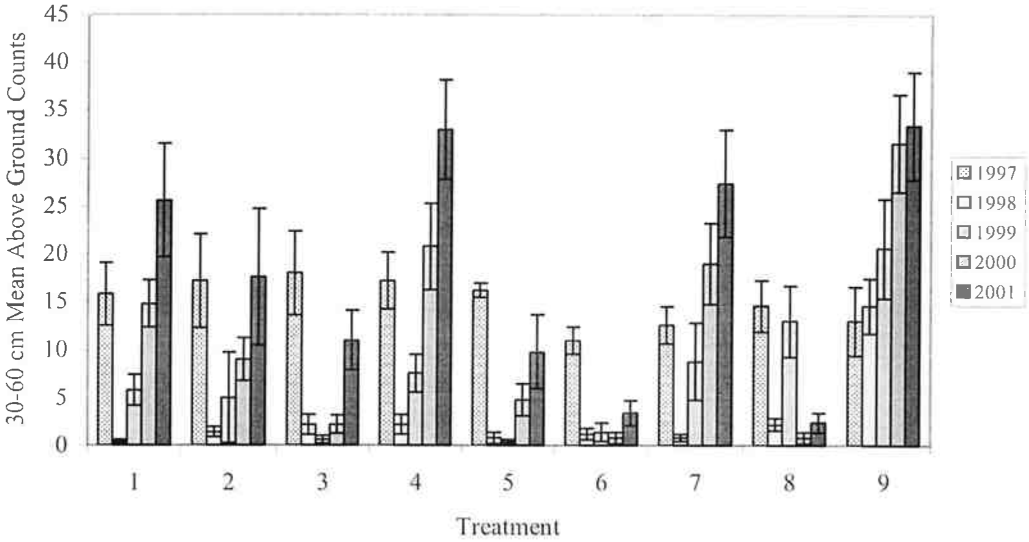


Figure 9: 30-60 cm height class above ground counts for years and treatments (mean ± standard error).

4.5.4 >60 cm Height Class

The mean number of plants in the >60 cm height class are shown in Figure 10. All treatments showed a decrease in numbers of this height class in the final year compared to the initial year except the control. Numbers of plants fell dramatically in the first year after the initial herbicide application in all treatments, with only treatments 1 (G-N-N-N), 2 (G-G-N-N), 4 (GA-N-N-N), 5 (GA-GA-N-N) and 7 (G-N-G-N) showing signs of recovery in this height class. The initial numbers of plants in this height class are quite variable among the treatments making comparisons difficult.

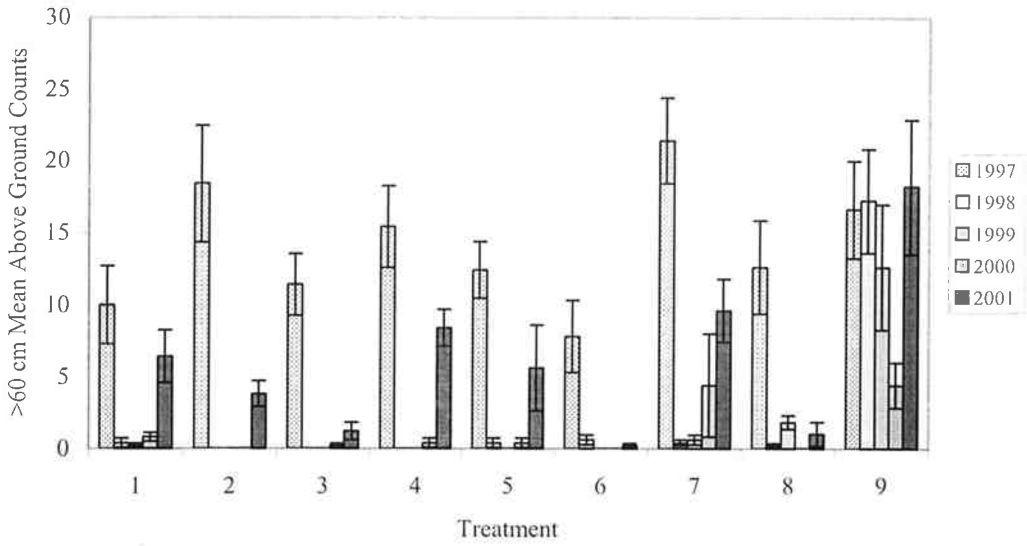


Figure 10: >60 cm height class above ground counts for years and treatments (mean ± standard error).

4.6 Comparison of Above and Below Ground Plant Counts

A linear regression analysis on all of the data collected on above and below ground plant counts gave R^2=0.53, indicating a weak relationship between above ground and below ground counts (Rowntree, 1981).

The data was also separated into total counts for each height class and linear regressions performed on each of the three groups. The regressions gave the following results:

- 0-30 cm height class – R^2=0.01 indicating no correlation;
- 30-60 cm height class - R^2=0.42 indicating a weak correlation; and
- >60 cm height class - R^2=0.05 indicating no correlation (Rowntree, 1981).

5.0 Discussion

Arum Lily Herbicide Experiment – Year 4

5.1 Treatments

5.1.1 Treatment 1 (G-N-N-N)

This treatment involved spraying with Glean® in the first year only. It exhibited an intermediate degree of plant reduction in numbers compared with other treatments. However, there was no statistically significant difference between treatment 1 and the control (α = 0.368). When comparing initial and final year numbers, it can be seen that after an initial reduction, numbers of plants had essentially returned to pre-treatment levels by the final year. This was also shown by a pattern of steady increase in above ground numbers of the 0-30 cm and 30-60 cm height classes over the years. From these results it appears that a single application of Glean® has only a small effect on Arum Lily numbers and biomass in the final year.

5.1.2 Treatment 2 (G-G-N-N)

This treatment was sprayed with Glean® for the first two years only and had no herbicide application for the following two years. The final year below ground counts for this treatment showed a similar mean to treatment 1 but was more variable. Similarly, the biomass in the final year was not significantly different to treatment 1 (α = 1.000). The above ground counts between years also showed a similar recovery pattern to treatment 1 indicating that the additional application of Glean® in the second year did not produce any difference in Arum Lily recovery over the following two years.

5.1.3 Treatment 3 (G-G-G-N)

This treatment was sprayed with Glean® over each successive year of the experiment followed by one year of no herbicide application. There was a low variability in final year numbers, but the mean biomass was not significantly different to treatments 1 and 2, or the control (treatment 9). There did appear to be a reduction in number of plants in the final year compared to treatments 1, 2 and 9, and there also appeared to be a delayed recovery in above ground counts, the majority of which appeared to come from the 0-30 cm height class, suggesting a large number of resprouts. The poor correlation shown between observed and actual numbers in this height class however, means that this observation must be treated with caution. The recovery of above ground parts under continued spraying may also indicate some resistance to the herbicide developing over time. Of the treatments using Glean® only, treatment 3 gave the best result in terms of a reduction in plant numbers, although the biomass in this treatment was essentially the same as treatments 1 and 2. This may be due to Glean® having little effect on underground rhizomes, which become dormant (Moore and Hoskins, 1997) and then resprout once annual herbicide applications cease.

5.1.4 Treatment 4 (GA-N-N-N)

This treatment was sprayed with Glean®+2,4-D amine in the first year only. There were a high number of below ground counts in the final year and a high biomass, that was not found to be statistically different from the control (α = 0.917). The above ground counts over the years indicate a recovery to higher than initial numbers with increases displayed in all height classes. Compared to other treatments, this treatment showed a poor response in terms of numbers and biomass in the final year, as well as a rapid recovery rate to higher than pre-treatment conditions. This treatment displayed a higher number of plants than treatment 1, although it had a significantly similar biomass (α = 0.986). This may be because a single application of Glean® + 2,4-D amine serves merely to kill the mother plant allowing the offsets to increase.

5.1.5 Treatment 5 (GA-GA-N-N)

This treatment was sprayed with Glean®+2,4-D amine for the first two years of the experiment. Although there was a fairly high variability among samples in this treatment, final year below ground numbers and biomass were low. This treatment showed a significant difference in biomass compared with treatment 4 (α = 0.041), 7 (α = 0.030) and also compared with the control (α = 0.001). It was also significantly similar to treatment 6 (α = 0.994) and 8 (α = 0.960). Comparisons between years revealed a delayed, moderate recovery after the third year with no further increases in the final year. The majority of this recovery appeared to come from the 0-30 cm height class after the third year, and from the 30-60 cm height class after the fourth year. This, coupled with the low biomass result in the final year, seems to indicate that the recovery is primarily due to new seedlings spreading from surrounding areas rather than survivorship of existing rhizomes. If this treatment was used in an area of low infestation, lower recovery levels might be expected.

5.1.6 Treatment 6 (GA-GA-GA-N)

This treatment was sprayed with Glean®+2,4-D amine for the entire three years of the spraying period. It had a low variability between below ground numbers of plants in the final year and also had the lowest mean number of plants between treatments. It had a low mean biomass, that was significantly different to treatments 2 (α = 0.051), 4 (α = 0.004), 7 (α = 0.003) and 9 (α < 0.001). It was significantly similar to treatments 5 (α = 0.994) and 8 (α = 1.000). This was the only treatment to show no rotted rhizomes in any of the quadrats suggesting that no resprouting from dead rhizomes had occurred. It showed little signs of recovery throughout the experimental period with all surviving plants appearing to come from the 0-30 cm height class. This would suggest that like treatment 5, any recovery is probably due to recruitment from surrounding infestations.

5.1.7 Treatment 7 (G-N-G-N)

This treatment was sprayed with Glean® on alternate years and had the highest variability in numbers between quadrats of any of the treatments. It had the highest mean number of plants of any treatment apart from the control. The biomass of treatment 7 was significantly higher than treatment 5 (Ho: 0.030), 6 (α = 0.003) and 8 (α = 0.001) and was significantly similar to the control treatment (α = 0.951). This treatment also showed the highest number of rotted rhizomes in the final year which, coupled with the high biomass and numbers, suggests that this treatment regime failed to completely kill the underground rhizome. There was also a rapid recovery from the initial reduction in above ground numbers, which was not affected by the additional application of Glean® in the third year. All height classes appeared to increase over time and there was no further reduction in numbers following the second application. This could indicate a resistance to Glean® occurring after the recovery from the first application.

5.1.8 Treatment 8 (GA-N-GA-N)

This treatment was sprayed with Glean®+2,4-D amine on alternate years over the course of the experiment. It showed a low number of below ground counts in the final year as well as a low biomass. In this respect it was significantly similar to treatments 5 (α = 0.960) and 6 (α = 1.000). The number of above ground plants showed a moderate recovery in the second year after each herbicide application but this recovery seemed to mainly come from the 0-30 cm height class with some from the 30-60 cm height class. It appears likely that this is due to recruitment from surrounding infestation.

5.2 Effect of Single Spray Treatments.

The effect of a single application of herbicide was demonstrated by the results of treatment 1 (G-N-N-N) and treatment 4 (GA-N-N-N). Treatment 1 showed a return to pre-treatment numbers but had lower numbers and less biomass than the control (treatment 9), while treatment 4 showed higher numbers than treatment 1, and a very similar biomass to the control. This appears counter-intuitive given the effectiveness shown by other treatments using the Glean + 2,4-D Amine combination. Plummer (1997) states that killing only the mother rhizome may lead to a multiplication of the problem as the offsets move into the area vacated by the main rhizome. This effect appears to be demonstrated in treatment 4 where a single application of Glean + 2,4-D Amine appears to have killed the main rhizome allowing surviving offsets to grow into its space. In contrast a single application of Glean has not killed the main rhizome, instead it has merely killed the aboveground parts causing that seasons growth to be suppressed giving lower numbers in the final year. This has important implications for the management of Arum lily and demonstrates that repeat applications of herbicide are necessary if long term eradication is desired.

5.3 Herbicide Effectiveness

From the analysis there is a good indication that mixing 2,4-D amine with chlorsulfuron (Glean®) generally is much more effective at controlling Arum Lily than using chlorsulfuron alone, which does not appear to kill the rhizome even under repeated applications. The exception to this is when there is only a single application of the chlorsulfuron/2,4-D amine mixture, which is less effective in reducing Arum Lily numbers than a single application of chlorsulfuron. It can thus be concluded that there needs to be a double 'hit' of the mixture to kill both the main rhizomes and then the surviving offsets and dormant rhizomes. Given the high potential for vegetative reproduction in Arum Lily, this is indeed an important consideration. The double application also does not need to be for two consecutive years (treatment 5); in fact, spraying the mixture on alternate years (treatment 8) appears to produce a marginally better result. The interesting outcome is that an additional application ie three years of consecutive spraying (treatment 6) does not produce a much lower number of plants or a lower biomass in the final year. This is important when considering the cost of weed control. These results confirm early experiments by Moore and Hoskins (1997) on application of Glean® and 2,4-D amine on Arum Lily which were the basis of the current herbicide regime on Garden Island (Wykes, 1997).

5.4 Above Ground Plant Estimates

The results of the study show that above ground estimates of plant numbers is not providing an accurate, nor consistent, estimate of actual Arum Lily plant numbers, as shown by excavation. The high degree of variability in the data also does not allow a standard conversion rate to be used to obtain a more accurate estimate of plant numbers. Above ground plant number estimates tend to grossly underestimate the actual plant numbers.

5.5 Experimental Design

The experiment has provided very useful information on the effectiveness of different herbicides and application rates, as well as the usefulness of undertaking above ground plant counts. However, the differing treatments between years (spray and no-spray) created a high degree of variation in the data between years and treatments, which meant that normality and homogenous variances could not be achieved and non-parametric methods of analysis often had to be used. The results of the study however, have provided a clear indication of which treatments were most and least successful.

5.6 Conclusions and Recommendations

5.6.1 Spray Regimes

From the analysis it appears that there are three treatments that succeeded in reducing both final year numbers and biomass of Arum Lily to the highest extent. These are:

- treatment 5, involving spraying a mixture of chlorsulfuron and 2,4-D amine for the first two years of the four year period;
- treatment 6 involving 3 consecutive applications of the mixture; and
- treatment 8 involving two applications of the mixture in alternate years.

From this, it is recommended that a weed control strategy be based on two applications of a mixture of chlorsulfuron and 2,4-D amine in alternate years. In areas of heavy infestation, follow-up application is required so continuation of the alternate year approach is recommended ie GA-N-GA-N-GA-etc..

Of treatments 5, 6 and 8, treatment 8 has:

- a lower cost than continual application; and
- lower variability than a two consecutive years of application with a 2-year lapse in treatment.

Monitoring of Arum Lily cover/abundance throughout the island should allow longer gaps between spraying once numbers have declined.

5.6.2 Spraying Techniques

Excavation of Arum Lily revealed that most of the 'daughter plants' in a clump were in fact offsets separate from the 'mother' plant. This is an important consideration when hand spraying. In this experiment, the herbicide was applied as a short, focussed squirt on a single leaf of each plant. However, the majority of stems arising from the ground were from separate rhizomes, so it is important that a leaf from each stem receive an application of herbicide.

5.6.3 Future Study

In addition to regular monitoring of Arum Lily infestation throughout Garden Island, it would be beneficial to continue this experiment over a longer time frame comparing different variations of treatment 5 and 8. In particular longer gaps between spray treatments can be examined for their effectiveness in controlling arum infestations once the initial infestation has been reduced. For this aim the existing treatments can be extended in the following manner.

- Treatment 5: (GA-GA-N-N) ➔ N-GA-N-N-
- Treatment 6: (GA-GA-GA-N) ➔ N-GA-N-N
- Treatment 8: (GA-N-GA-N) ➔ N-GA-N-N
- Treatment 9: (N-N-N-N) ➔ N-N-N-N

In this way the spraying regime with the highest effectiveness and the lowest cost can be determined, as well as the effects of a two-year gap between spray treatments once the initial infestation has been reduced. This can be achieved at a lower cost than the 1997-2001 experiment as only four treatments will need to be assessed and a single additional application of Glean + 2,4-D Amine in 2002.

It also may be worthwhile examining different methods of applying herbicide and the time of year of application, given the results of recent studies conducted by Agriculture WA in the Ludlow Tuart Forest. Their study indicated that Glean® on its own was effective in reducing Arum Lily abundance if applied as a wipe rather than a spray, in the middle of the annual growth cycle (during flowering with some early fruit formation. We recommend that caution be exercised when applying the results of other studies to Garden Island, as the ecology of the island may be different to the usual Arum Lily habitats seen on the mainland.

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