Use of profile analysis of repeated measures in a herbicide trial on blue morning glory (*Ipomoea indica* (Burm.) Merrill)

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Summary

When several dependent variables, evaluated on the same scale, are measured against one or more independent variables using the multivariate approach, progressive differences in response to treatments can be determined. Each subject or a randomly selected group of subjects within each treatment is assessed repeatedly over time. Because the same scale of measurement is used, uniform profile response curves can be produced. This method of analysis has not been used commonly in weed science, but has value in providing a more complete picture of a weed's response to herbicide treatments. As an example a field trial was undertaken to examine the effects of six herbicides, some at several rates of application, when applied as overall sprays to blue morning glory (*Ipomoea indica* (Burm.) Merrill). Results from six assessments, spanning seven weeks, were subjected to profile analysis. While all herbicides damaged the weed, substantial regrowth occurred within the assessment period for all treatments except triclopyr and 2,4-D amine. The most cost effective treatment was 2,4-D amine at 400 g 100 L^{-1} with the addition of Synetrol oil at 0.2%. Another introduced climber, glycine (*Neonotonia wightii* (Arn.) Lackey), was not affected by 2,4-D amine at the applied rates and could replace blue morning glory as a smotherer of native species if 2,4-D amine were the only herbicide employed. This highlights the need for weed management strategies to take into account the full complement of weeds at managed sites.

Introduction

It is not uncommon for the expense and time put into a field trial to be completely wasted because the trial output was based on a single, final assessment and the trial was destroyed by fire or interference from a competitive land utilizer e.g. the site provided badly needed feed for stock. The duration of a woody weed control trial can be as long as two years. If progressive assessments can be recorded prior to an interfering event, then collected data can give informative indications of treatment response. This repeated measure procedure may provide complete response data for some treatments and a predictive model for treatments where the effect was continuing. Such data can also provide cross validation for future trials.

Where analysis of the results from a herbicide trial is based only upon ratings made at a final assessment, commonly utilizing one way analysis of variance, valuable information may be lost. In particular, the rates at which weeds display damage from applied herbicides may vary considerably and may be an important determinant of acceptability to the end user.

Both of these considerations can be accounted for by the use of profile analysis of repeated measures (Tabachnick and Fidell 1989). The primary question addressed by profile analysis is whether different groups have parallel profiles and is assessed by a test of parallelism. Secondly, whether or not groups produce parallel profiles is of interest to determine if any group, on average, scores higher on a collected set of measures than other groups. The third question addressed by profile analysis concerns whether the dependent variable changes significantly over the period of assessment. This is known as profile 'flatness', and is generally relevant only if profiles are parallel (Tabachnick and Fidell 1989).

We are unaware of previous use of profile analysis in weed science. In this paper we explore the use of this statistical method with the results from a herbicide trial on blue morning glory (*Ipomoea indica* (Burm.) Merrill).

The species

Blue morning glory is a perennial vine that probably originated in tropical America but at the time of European settlement had spread to the Old World Tropics (Johnson 1995). It was collected by Banks and Solander at the Endeavour River, Queensland in 1770, but most of the naturalized populations in Queensland have originated from garden escapes (Johnson 1995). Blue morning glory now occurs widely in the Atherton Tableland and throughout the high rainfall coastal areas in south-eastern Queensland and northern New South Wales (Kleinschmidt and Johnson 1979, Floyd 1989). It is locally abundant in areas where rainforest has been cleared and often invades remnant stands of native vegetation. As with other weedy vines, blue morning glory commonly suppresses and kills native shrubs and trees and can pose a major problem to revegetation programs.

Table 1. Herbicide treatments applied to morning glory.

Treatment	Trade name	Herbicide	Product strength (a.i. g L^{-1} or kg^{-1})	Product [A] dilution a.i.	Adjuvant	Rate adjuvant	Application rate (kg a.i. ha^{-1})
1	Control	–	–	–	–	–	–
2	DP600®	Dichlorprop	600	150 g 100L^{-1}	–	–	5.00
3	DP600	Dichlorprop	600	300 g 100L^{-1}	–	–	10.0
4	DP600	Dichlorprop	600	600 g 100L^{-1}	–	–	20.0
5	Garlon 600®	Triclopyr ester	600	102 g 100L^{-1}	–	–	3.40
6	Starane®	Fluroxypyr	200	100 g 100L^{-1}	–	–	3.33
7	Roundup®	Glyphosate	360	360 g 100L^{-1}	–	–	12.0
8	Brush-off®	Metsulfuron methyl	600	3 g 100L^{-1}	Non-ionic[B] surfactant	1:500	0.100
9	Brush-off	Metsulfuron methyl	600	6 g 100L^{-1}	Non-ionic surfactant	1:500	0.200
10	Brush-off	Metsulfuron methyl	600	12 g 100L^{-1}	Non-ionic surfactant	1:500	0.400
11	Amicide 500®	2,4-D amine	500	100 g 100L^{-1}	Rapeseed oil[C]	1:500	3.33
12	Amicide 500	2,4-D amine	500	200 g 100L^{-1}	Rapeseed oil	1:500	6.67
13	Amicide 500	2,4-D amine	500	400 g 100L^{-1}	Rapeseed oil	1:500	13.3

[A] The carrier was water in all cases. [B] Nufarm surfactant. [C] Synetrol oil. ® Registered trade mark.
Volume per treatment over 3 plots = 50 L.

Blue morning glory is perceived by the general community as a major nuisance weed in the Montville-Palmwoods and Maleny areas of south-eastern Queensland. Over the past few years there has been increasing pressure from the community to have this weed declared under the Rural Lands Protection Act. Methods for controlling blue morning glory include cutting plants close to the ground, treating cut stems and scraped roots with glyphosate (1:1 in water) and then spraying the regrowth, also with glyphosate (1:50 in water) (Floyd 1989). In order to explore further options for controlling this weed, a trial was undertaken to test the effectiveness of commonly available herbicides when employed as overall sprays. Pilot trials in the previous summer had indicated that 2,4-D amine and triclopyr showed high activity against blue morning glory.

Materials and methods

Site

The trial was conducted on an abandoned pasture site 3 km east of Montville on the Montville Palmwoods Road, approximately 130 km north of Brisbane. Here blue morning glory was a dominant ground cover, associated with molasses grass (*Melinus minutiflora* Beauv.), glycine (*Neonotonia wightii* (Arn.) Lackey), silverleaf desmodium (*Desmodium uncinatum* (Jacq.) DC.), Japanese sunflower (*Tithonia diversifolia* (Hemsley) A. Gray) and lantana (*Lantana camara* L.). The pasture was bounded by subtropical wet sclerophyll forest, with dominant canopy species *Eucalyptus acmenoides* Schauer and *E. microcorys* F. Muell. in association with araucarian vine forest sub-canopy species.

Experimental design and assessment technique

The experiment was set up as a combination randomized block design (Tabachnick and Fidell 1989, Neter *et al.* 1990) with repeated measures through time. The main factor of interest was herbicide treatment (12 treatments plus a control) (Table 1) applied singly to thirteen 7 × 7 m plots per block. There were three blocks, ranging from easily accessible (Block 1) to accessible with difficulty, owing to a steep slope with rock outcropping (Block 3).

The spray equipment consisted of a Hardi split tub 400 L tank coupled to a Hardi 600 diaphragm pump driven by a Honda GX 120 4 HP motor. The spray hose was fitted with a Hardi 'Black magic' adjustable gun type 60 (max. 60 bar 870 p.s.i.) and a nozzle with the size Hardi 1553-35 insertion disk specification.

Approximately 50 L was applied per treatment over the three replicated plots, i.e. approximately 3400 L ha^{-1}. A variable number of concentrations were used for products when previous observations had indicated good potential for control of this weed. Treatments were applied over periods spanning 15–17 and 21–23 February 1995. The spray unit was run at approximately 1 bar pumping pressure, depending on wind direction and speed and the potential for temperature-dependent droplet evaporation on the day (Anon. 1969).

Assessments were made in four 1 m^2 quadrats per plot, using a rating scale from 1 (dead) to 7 (healthy) (Figure 1). Three of these quadrats were chosen at random at each assessment, and one quadrat was randomly selected at the first assessment

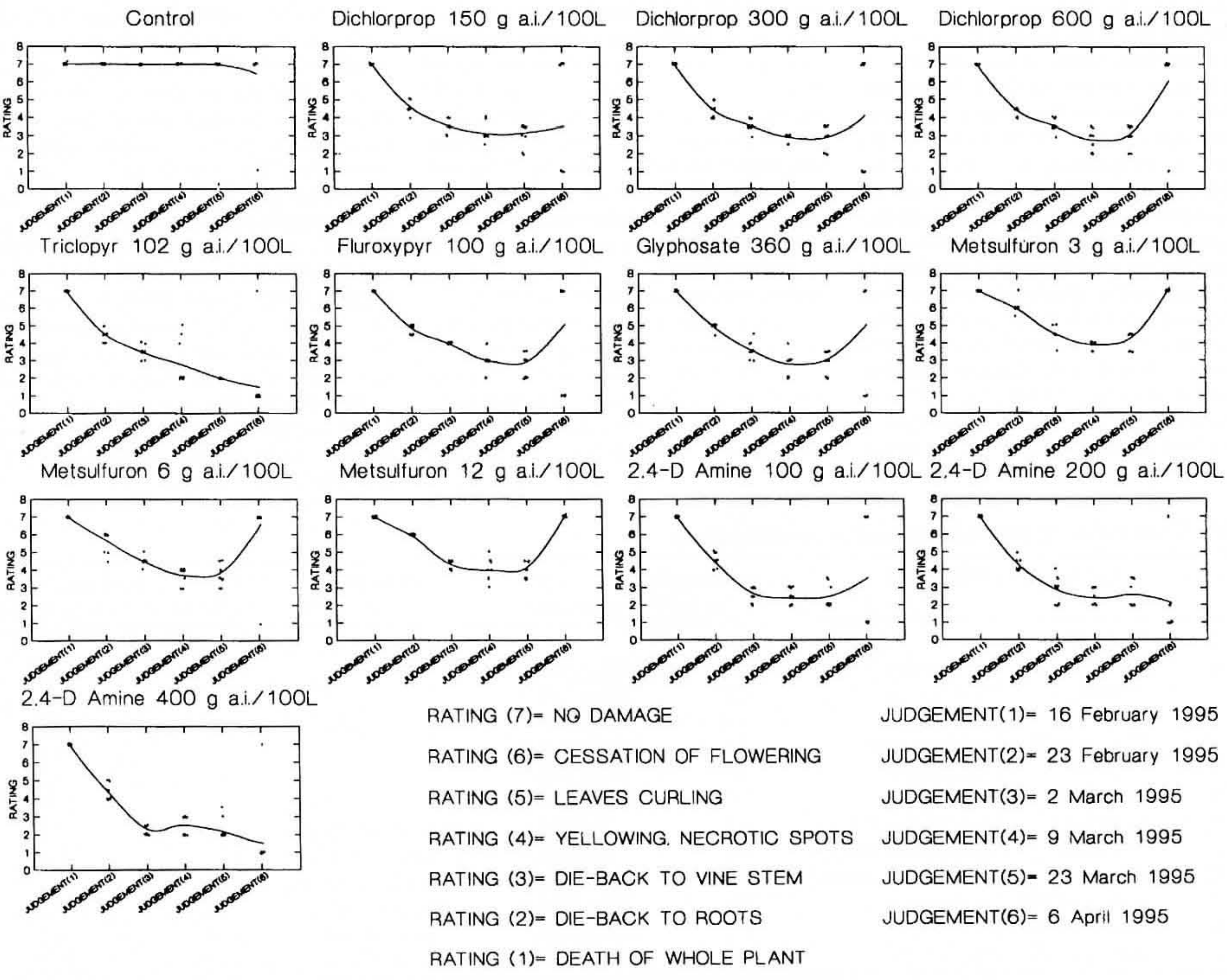

Figure 1. Time course of damage to blue morning glory following application of 12 herbicide treatments.

Table 2. Contrasts generated by using Fisher's least-significant-difference test on means summed across assessments.

Treatment	Treatment	Low mean score	High mean score	F ratio	Probability
13	5	3.31	3.52	1.64	0.2
13	11	3.31	3.75	7.00	0.009
13	12	3.31	3.55	2.10	0.14
12	11	3.55	3.75	1.42	0.23
5	11	3.52	3.75	1.86	0.17
5	6	3.52	4.42	30.3	<0.001
5	7	3.52	4.37	27.1	<0.001
7	6	4.37	4.42	0.09	0.765
2	4	4.14	4.45	3.68	0.05
3	4	4.12	4.45	4.19	0.04

Table 3. Summary of control and costs of effective herbicide treatments.

Herbicide	Concentration spray solution (g 100 L^{-1})	Spray volume (L ha^{-1})	Mean control rating	Cost 100 L^{-1} ($)
2,4-D amine + Rapeseed oil[A]	100	3333	3	2.68
2,4-D amine + Rapeseed oil	200	3333	1.33	4.19
2,4-D amine + Rapeseed oil	400	3333	1	7.20
Triclopyr[B]	102	3333	1	11.47

[A] Synetrol oil added at the rate of 0.2% v/v.
[B] Garlon 600.

and thereafter fixed for the purpose of assessment and obtaining sequential photographic records. Assessments were made on 16, 23 February, 2, 9, 23 March and 6 April 1995.

Analysis

The null hypotheses to be tested were: Ho_1 (no difference between the control treatment and the 12 herbicide treatments) and Ho_2 (no difference between the 12 herbicide treatments with regard to impact on blue morning glory). The significance of differences of herbicide treatments from the control treatment was determined by post analysis hypothesis contrasts with a Bonferroni adjustment for conducting multiple comparisons on pooled treatment responses (S-Means) (Kirby 1993). This process was validated by using Dunnett's test (Wilkinson 1994).

The six assessments (judgements) were treated as dependent variables corresponding to the independent variables 'Block' and 'Treatment'. The computer program Systat 6 for DOS (Wilkinson 1994) was used for preliminary data screening. Potential outliers were isolated using Mahalonabis distances, scrutinized and cross-validated using Cook's distances (Tabachnick and Fidell 1989). In all instances the highlighted cases could be explained as acceptable treatment variation given the high variance in some treatment groups, e.g. dichlorprop treatments 2–4. The need for adjusting some individual data points was negated given the relatively large size of the data set; the influence of these isolated data points was not expected to distort the overall outcome of the experiment.

Profile analysis of repeated measures (Tabachnick and Fidell 1989) was chosen as a method for further analysis, largely because of substantial differences in variance between individual assessment times within treatments. Repeated measures multivariate analysis of variance could not be used because of this commonly encountered violation of homogeneity of covariance. Profile analysis overcomes this restriction by transforming the multiple measurement of the dependent factor (judgements) into separate dependent variables and using a specific multivariate statistical test. The Systat GLM module from the DOS Version 6 (Wilkinson 1994) was used for the profile analysis of repeated measures (Kirby 1993).

Results

Initial scrutiny of the data was undertaken by fitting relationships to the full data set of treatment responses (Figure 1), using distance weighted least squares to smooth the data distributions by treatment. From these relationships it appeared that all treatments were different from the control, with treatments 5, 12 and 13 demonstrating the best long term suppression of the blue morning glory biomass.

S-means were derived from pooling block effects and summing across assessments, and were used by conducting multiple comparisons of the treatment segments. All herbicide treatments were significantly different from the control plots when using the Bonferroni adjusted probabilities on mean comparisons. (However, Dunnett's test showed that treatments containing metsulfuron methyl were not significantly different from the control.) Thus Ho_1 was rejected.

Pooled assessments means compared between the various treatments provided a global perspective of treatment variation in this trial. The trend was indicative of diversity in treatment levels ($F(12, 117) = 75.85, P<0.01$).

The test for parallelism on the block grouping factor was not significant. However, the test for parallelism on the treatment grouping factor was highly significant ($F(60, 585) = 8.63, P<0.01$). An analysis of Wilks' Lambda indicated a >99% probability that the variance, as combined for this test, was accounted for by the difference in shape of the profiles of the 13 treatment groups (Figure 1). Thus, variation in herbicide treatment response was indicated at each assessment time.

The flatness test indicated significant deviation from zero ($F(5, 585) = 326, P<0.01$). The measure of the strength of association between the dependent and independent variable was high, indicated by the Hotelling-Lawley trace of 556 ($P<0.01$). Wilks' Lambda was very small at 0.0018 ($P<0.001$), indicating low error variance. Thus, on overall performance, herbicide treatments produced reductions in weed biomass. The extraction of the control and metsulfuron treatments from the analysis would have reduced the flatness tendency. The judgement effect on Pillai's criterion ≈ eta (η^2) for the judgement × treatment interaction $F(5, 113) = 0.99$ ($P<0.01$) indicated that 99% of the variance in this combination of segments was accounted for by non-flatness of the profiles collapsed over groups. Although closer association to zero flatness can indicate slower acting herbicide activity, this was not indicated in the analysis at assessment 6, where plants treated with metsulfuron methyl were in a recovery state.

The polynomial test indicated that the data across the repeated measures tended to follow a quadratic trend ($F(1, 2) = 495.06, P<0.01$). However, this linear analysis was conducted on the assumption that the herbicide treatment effects could be expected to follow a largely linear trend if monitoring had been conducted on a daily basis. The linear trend was only marginally different from the quadratic ($F(1, 2) = 388.46, P<0.01$).

Other comparisons of interest are presented in Table 2. Treatments 5, 12 and 13 were significantly different from all other treatments, leading to the rejection of Ho_2. Rates of plant degeneration were significantly different for the effective herbicide treatments. At 1 week after application, both the 2,4-D amine and triclopyr ester herbicides showed similar effects ($F(1, 117) = 0.0, P=1$) (Figure 1). By the third assessment the 2,4-D amine treatments had caused more damage than the triclopyr treatment, with 2,4-D amine at 400 g 100 L^{-1} inducing the most deterioration of plant tissue ($F(1,117) = 181.52$, $P<0.01$). However, by assessments four and five, whilst plants in the 2,4-D amine treatments tended to stabilize, triclopyr treated plants continued to deteriorate at a steady rate ($F(1,117) = 1.83$, $P<0.17$). The triclopyr sprayed plot deteriorated at this faster and steadier rate until the final assessment, when both herbicides gave similar results ($F(1,117) = 0.0$, $P=1$). Although the biomass reduction was particularly high, these effective treatments did not actually reach a score of 1 in profile (Figure 1). This was probably due to experimental error in application technique, owing to the multi-layering of the morning glory vines.

Discussion

The statistical approach utilized in this work appears not to have been employed widely in weed control research. The main advantage of studying herbicide efficacy profiles is that at individual assessment times, comparisons may be made of how a herbicide treatment is performing in relation to the control, other treatments or known performance of a documented standard.

Herbicide manufacturers know that the speed of initial knock down is particularly important in determining end user acceptability. The profile statistical output takes into consideration accumulated carry-over effects as more assessments are brought into the analysis. This is particularly relevant where residual chemicals can have diverse initial effects on plant degeneration, e.g. tebuthiuron may have low initial impact while bromacil will tend to have high initial impact (Trevor Armstrong personal communication). Repeat measure analysis and profile analysis both provide a highly sensitive error term derived from the treatment*block interaction. If block matching is non-significant, the error term is adjusted by subtraction of the block effect. This gives consistency in determining the significance of treatment effects (Tabachnick and Fidell 1989). Trials involving sequential biomass reduction or an extended activity time when using such compounds as metsulfuron methyl or residual herbicides (where activity is affected by soil moisture), also suit the repeat measure/profile design in that peaks and troughs are fully represented in the analysis output.

In the present trial the statistical response outcomes were different when comparing repeated measures analysis, and a one way analysis of variance with judgement (6) as the dependent variable and treatment as the independent variable. For example, profile analysis determined that there was a significant difference between treatments 13 and 11, but a one way ANOVA determined that the treatments were similar. Overall, the profile analysis approach is much more sensitive to response differences, compared to an analysis of singly collected data analysed by a one way analysis of variance.

Triclopyr (Treatment 5) and 2,4-D amine (Treatments 11–13) gave the best control of blue morning glory in this trial (Figure 1, Table 2). However, the most cost effective treatment was 2,4-D amine at 400 g 100 L^{-1} with the addition of Synetrol oil at 0.2%. Although there was no significant difference between the degree of control achieved by the 2,4-D treatments and by triclopyr (Table 2), the former would be preferred both on the basis of cost (Table 3) and relative lack of volatility when compared with the triclopyr ester formulation (Anon. 1983). In addition, the regeneration of grasses was prolific on plots treated with 2,4-D (Figure 2), which presumably could suppress reinvasion by blue morning glory seedlings. Continued observations of this trial indicated that such suppression may last for at least 18 months.

The failure of glyphosate to control blue morning glory adequately when applied as a 0.36% a.i. solution (Figure 1) suggests that it would not be wise to apply solutions of less than 0.72% a.i. (Floyd 1989) to regrowth and that application of a 0.54% a.i. solution (Rawling 1989, cited in Swarbrick and Skarratt 1994) could possibly yield variable results. Both of these recommended concentrations, at approximately \$24.00 and \$18.00 per 100 L respectively, are considerably more expensive than the highest rate of 2,4-D amine tested in this trial (Table 3).

If the aim of blue morning glory control is to assist in revegetation of formerly

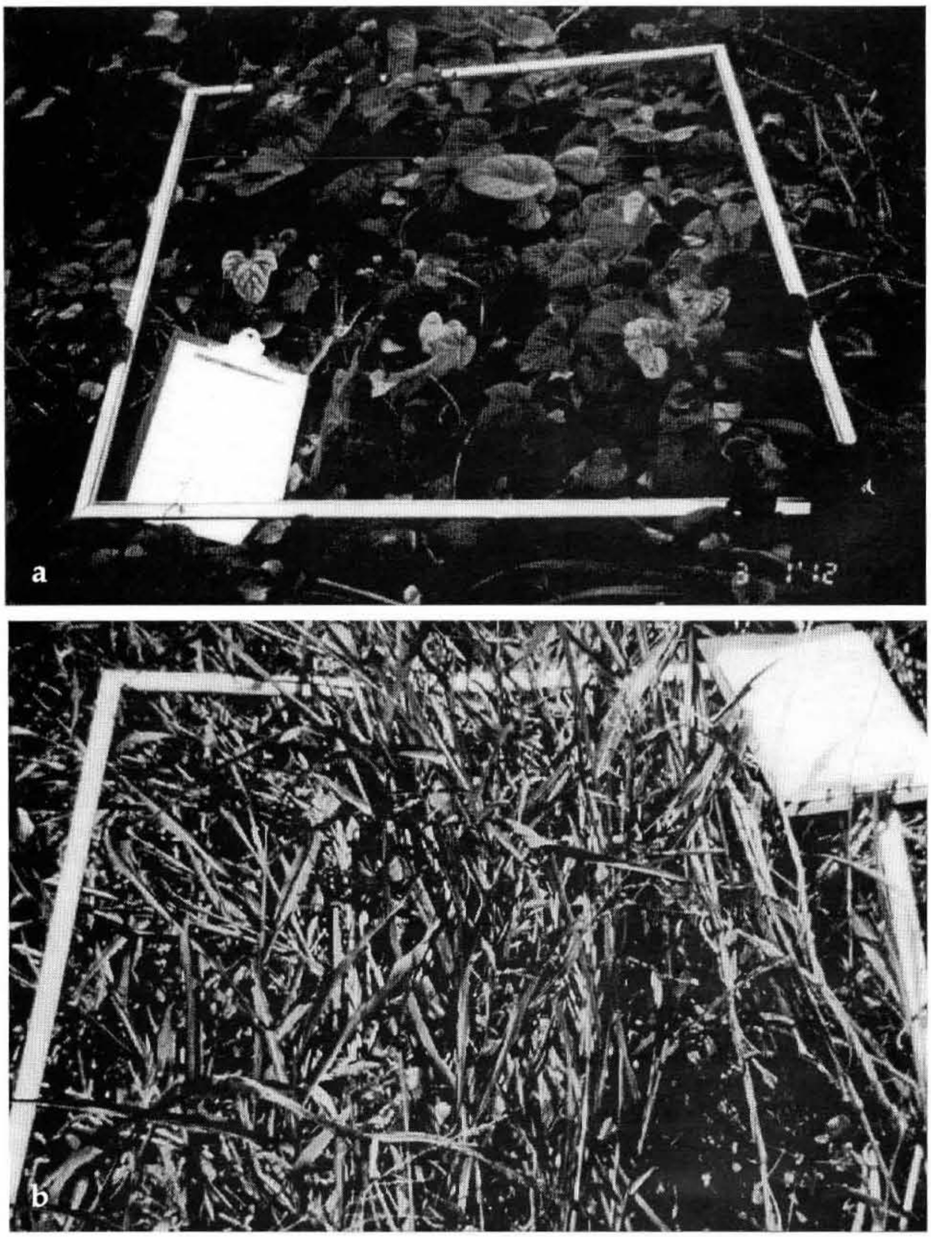

Figure 2. Fixed quadrat at a) 1 week and b) 7 weeks after application of 2,4-amine 500 g L^{-1} at 1:125 with the addition of Synetrol oil at 1:500.

cleared areas using native species, careful consideration must be given to the responses of other weed species to the treatment. Observations made in the present trial indicated that the introduced climber glycine was relatively unaffected by 2,4-D at any of the rates used. This suggests that by controlling one climbing weed species, another climber, just as capable of smothering young native plants, could be favoured. Preliminary work with dicamba has shown this herbicide to be effective against blue morning glory, while controlling both glycine and silverleaf desmodium (E.C. Sparkes, unpublished data). Further research is required to determine the best strategies to reduce the combined impact of introduced climbers upon regenerating native trees and shrubs.

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References

Anon. (1969). The Agricultural Chemicals Distribution Control Act 1966–1968. *In* 'Commercial Operators Manual', p. 33-4. (Department of Primary Industries, Brisbane).

Anon. (1983). Amicide 500 Technical Bulletin Number 4. (Nufarm Limited, Lytton, Queensland).

Floyd, A.G. (1989). The vine weeds of coastal rainforest. Proceedings of the 5th Biennial Noxious Plants Conference, Volume 1, pp. 109-15. (New South Wales Department of Agriculture and Fisheries, Sydney).

Johnson, R.W. (1995). The aliens have landed: An account of the development of the naturalized flora of Queensland. *Proceedings of the Royal Society of Queensland* 105, 5-17.

Kirby, K.N. (1993). 'Advanced data analysis with Systat'. (Thomas Nelson Australia, Melbourne).

Kleinschmidt, H.E. and Johnson, R.W. (1979). 'Weeds of Queensland'. (Government Printer, Brisbane).

Neter, J., Wasserman, W. and Kutner M.H. (1990). 'Applied linear statistical models, regression, analysis of variance, and experimental designs', 3rd edition. (Irwin, Homewood, Illinois).

Swarbrick, J.T. and Skarratt, D.B. (1994). 'The bushweed 2 database of environmental weeds in Australia'. (University of Queensland Gatton College, Lawes, Queensland).

Tabachnick, B.G. and Fidell, L.S. (1989). 'Using multivariate statistics', 2nd edition. (HarperCollins, New York).

Wilkinson, L. (1994). Systat for DOS: Advanced applications. Version 6 edition. (Systat Inc., Evanston, Illinois).