BIOSECURITY SA
PIRSA

Compendium of branched broomrape research

Section 12. Herbicide residual and non-target effects

A COMPILATION OF RESEARCH REPORTS FROM THE BRANCHED BROOMRAPE ERADICATION PROGRAM SOUTH AUSTRALIA

JANUARY 2014

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

Compendium of branched broomrape research

Information current as of 17 January 2014

Disclaimer

Table of Contents

1. The effects of metsulfuron methyl on broomrape seed viability

Jane Prider and Andrew Craig

Branched Broomrape Eradication Program

February 2011

Introduction

Group B herbicides (acetolactase synthase-inhibiting herbicides) are widely used in the quarantine area (QA) for the control of branched broomrape. These herbicides can persist in soils, particularly dry soils with high alkalinity and low organic matter which are characteristic of the QA. This may have consequences for the subsequent growth of sensitive crops but herbicide residues may also have a residual effect on the branched broomrape seed bank.

The literature describes one experiment that examined the effects of Group B herbicides on the early stages of broomrape development. Sulfonylurea herbicides were applied to *Orobanche aegyptiaca* seeds in petri dishes at the conditioning stage and the germination stage (after the addition of a germination stimulant) in a series of dose response trials (Hershenhorn *et al.* 1998). Many of the herbicides tested had an effect on the elongation of the radicle although the herbicides had limited effects on germination. Chlorsulfuron was the most effective herbicide, causing necrosis in 80% of broomrape radicles when applied at the germination stage.

Aims

The Group B herbicide, Ally™ (a.i. metsulfuron methyl), is applied routinely in the QA. Although the herbicide is not recommended as a pre-emergent herbicide as it does not provide a commercially acceptable level of residual weed control, the effects of residues on *O. ramosa* seeds is not known.

In this experiment we screened the herbicide Ally to determine its effects on *O. ramosa* seed. The experiments addressed the following questions:

1. Are herbicides commonly used to control broomrape able to kill seeds or affect radicle elongation?
2. Are concentrations of these herbicides likely to persist in soils as residues able to affect early broomrape development (pre-attachment phases)?
3. Does seed conditioning affect the activity of the herbicide on the seed?

Method

Broomrape seed in the soil seed bank may be affected by Ally herbicide residues at several different pre-attachment stages. Seed may be affected in the dormant stage (when seed is dry), at the conditioning stage when soils become moist, or at the germination stage following conditioning. The effect of Ally on seeds at these three developmental stages was tested in the laboratory by adding the herbicide in separate treatments for each stage.

We used a range of Ally concentrations that included the concentrations of Ally that may be expected to occur as soil residues up to the concentration of applied Ally, including controls with no Ally. These concentrations were determined from previous bioassays of paddock soils where Ally had been applied (Section 11.3). We used the serial dilution developed for our bioassays for concentrations of 0, 0.039, 0.078, 0.156, 0.316, 0.625, 1.25, 2.5, and 5 g a.i. ha^{-1}.

The seed lot used for all experiments had a viability of 90 %. Seed was surface sterilised with 1% sodium hypochlorite prior to placing 100-200 seeds on 20 mm diameter filter papers placed in 6 cm petri dishes. Six replicates were prepared for each herbicide concentration in each treatment.

For the unconditioned seed treatment, GR24 stimulant was added to dry seeds and 200 µl of herbicide was applied to a separate filter paper that was placed over the seeds. The dishes were sealed and placed in a 20 °C incubator for two weeks.

For the conditioned seed treatment, filter papers with seeds were moistened with water and a second filter paper with the herbicide solution was placed over the top. Plates were sealed and incubated at 20 °C for 20 days and then the herbicide filter papers were removed and the seeds were rinsed to remove any residual herbicide. Seeds were placed on fresh filter papers and GR24 added and plates were incubated for two weeks.

For the germination stage, filter papers with seeds were moistened with water and plates were incubated for 20 days. After this time, GR24 was added and the filter papers covered by a second filter paper with the herbicide solution. Seeds were incubated for a further two weeks at 20 °C.

For all treatments, germination was scored two weeks after GR24 was added and 20 germinated seeds were photographed so that radicle length could be measured. Ungerminated seeds were placed in eppendorf tubes with 200 µl of tetrazolium solution. Tubes were incubated for two weeks in the dark at 30 °C. Viable seeds were counted as those seeds that had stained red after two weeks in tetrazolium.

We scored the proportion of and viable seed (includes germinated seed and seed that did not germinate but was viable), germinated seed only and radicle length (mean of up to 20 seeds for each replicate).

Analysis

To determine the effect of Ally on germination and viability we tested whether these proportions differed between Ally concentrations separately for each developmental stage. A dose response analysis was not warranted so a one-way analysis of variance test was conducted.

To compare between developmental stages, we calculated the germination and viability of different Ally concentrations as a percentage of controls (where no Ally was added). This gave a standardised score that enabled comparison between treatments. A two-way analysis was used to test for differences between herbicide dose levels and developmental stages.

Where there were differences between treatments or doses, Tukey HSD tests were used to determine which treatment levels were significantly different.

Results

Ally had no effect on the viability of broomrape seed when added at any of the pre-attachment stages. There was no dose response to increasing herbicide concentrations and none of the dose levels differed significantly from the controls which had no Ally added (Fig. 1).

There was a stimulation of germination in the unconditioned seeds where Ally was added at a concentration of 5 g a.i. ha^{-1} (Fig. 2). This stimulation did not occur if Ally was added to conditioned seeds in the other two treatments.

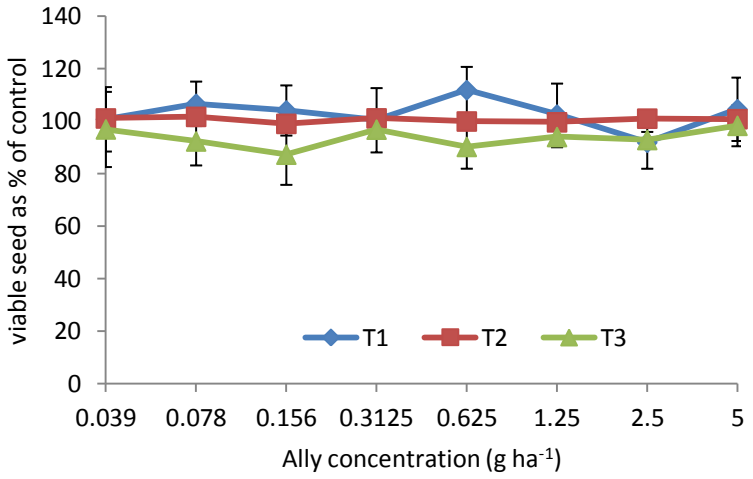


Figure 1. Dose response of broomrape seeds to Ally as a % of controls (no Ally), with the herbicide added at the conditioning stage (T1), at the germination stage (T2) or to unconditioned seeds (T3). Mean ± 1SE, *n*=6.

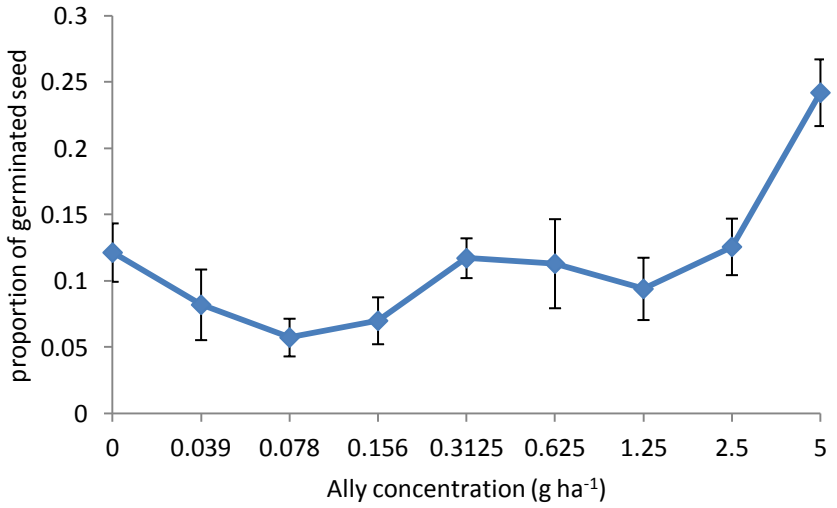


Figure 2. Germination response of broomrape seeds to increasing concentrations of Ally. Mean ± 1SE, *n*=6.

Conclusions

Any residues of Ally remaining in the soil are not likely to have any effect on the viability of broomrape seed in the soil seed bank.

2. Residual herbicide effects on medic

Ray Correll

Rho Environmetrics Pty Ltd

Prepared for Keith Bolto

Rural Solutions

June 2009

Introduction

Herbicides are being used extensively in the control of branched broomrape in the quarantine area. Concerns have been raised that the sprays have a residual effect that adversely affects subsequent crops. An experiment was planned to quantify any such effects.

The experiment was concerned with the following questions.

1. What is the effect of rate of application and time from application of Group B herbicides used on non-tolerant medic, e.g. Herald medic, production? Can Group B herbicides be used to control broomrape with minimal effect on agricultural production?
2. Are there any management factors which interact with the effect of group B herbicides, e. g. Does a high level of root disease increase the effect of group B residues?
3. Are there effects of soil fertility, soil texture and soil organic matter?
4. What effect does type of herbicide used have?
5. What are the most important criteria for medic production?
6. Does location and management have bigger impact on production than group B residues?

Experiment design

The experiment initially involved selecting paddocks that had varying histories of spray use, ranging from some that have been regularly sprayed to others that are 'organic' and have received no chemical formulations. Four samples of soil were taken from these paddocks and a composite sample was made from them. Ten subsamples were taken from each composite and placed in pots. Soil was collected from across the Quarantine Area.

Fertility and root disease tests were taken standard 0-10cm soil corer. At least 40 samples were taken from the same location as the bulk soil sample.

Five 180 mm pots from each soil type were then sown to a strain of medic that is susceptible to Group B herbicides (Herald medic) and the remaining five were sown to a tolerant variety (Angel medic). Growth of the medic was then monitored and the following information was recorded:

- Germination number
- Disease score (in particular of Herald relative to Angel)
- Overall score
- Nutritional status of the medic

Data

Data were available on 25 paddocks typically going back to 1998. The data included:

- Quantity and formulation used on each paddock in each year
- Crop history
- Disease status
- Soil texture and soil nutrition

Subsequently data were provided on plant growth (rating) and plant nutrient status.

Statistical Methods

The active ingredient for each herbicide formulation was taken from the label information as supplied by the client. Properties of the active ingredients were obtained from the PPDB (2008) database. Those data were used to form a small database of the active ingredients and the formulations used in the trial reported here.

The pots from each paddock had been formed from a single composite sample and therefore have to be considered as such in the analysis. The assessment was therefore over the average scores from each site.

There were a total of nine active ingredients each scored over several years. There were therefore far more potential variables than paddocks, making it difficult to answer questions concerning them all. Some simplification approximations were therefore made.

The trial was designed effectively so that a key parameter of interest was the comparison of the effect of pesticide on the herald versus angel medic. This variable, together with the overall rating of herald have been analysed in the first instance. Because this is a difference, other variables such as soil texture, fertility and disease status would affect both Herald and Angel medic, thus (at least to a first approximation) cancelling out those effects.

A useful approximation arose in that the data were presented as scores on a 0 – 5 scale, which had been averaged over 5 replicates. Because of the central limit theorem the mean score was effectively normally distributed, thus enabling standard regression techniques to be applied.

Most of the pesticides had moderate half lives, with longest being imazapic (120 d), followed by metsulfuron-methyl (66 d). In the 2008 application imazapic did not appear to have been used, so the longest half-life was apparently 66 d. Little residue would be expected therefore after two seasons. Initially the analyses have been concentrated on the 2008 spray data.

The 2008 spray program included a variety of mixtures and rates. These were converted to application rates of active ingredient. Two active ingredients, MCPA and imazapic, were not included in the 2008 data, so no information was available on those chemicals. Furthermore, imazapyr and imazamox, were used together in the same proportions, so it was impossible to segregate the effect of these two compounds; a result is given for imazapyr but this will include a contribution for imazamox.

The regression coefficients represent the effect of the chemical expressed as rating point per g/ha. That is an appropriate scale for significance testing. An alternative scale was formed by multiplying the regression coefficients by a typical application rate so the units were then rating per application.

Univariate results

In this section correlations between medic growth and single parameters are examined using correlation coefficients. Theses analyses are used to identify large effects and provide input into a method for selecting variables that can be used in more complicated models.

Relationship between soil nutrients and plant growth

Data were available on nutrient levels of the soil for 16 parameters as shown in Table 1.

The relationship between soil nutritional status and plant growth was assessed using a Pearson's correlation coefficient. The Pearson's correlation is moderately robust against departures from normality, but may give false positives due to outliers. The resulting correlations are shown in

Table 2. The only significant correlation between nutritional status and growth of Herald was with P in 2 June readings when there was a strong correlation with K, and positive correlations with P and pH but a negative correlation with Cu. Angel medic showed strong correlations with nitrate N, P, K, pH, Mn and Fe but negative correlation with conductivity and Cu.

Relationship between plant nutrients and plant growth

Data were available on nutrient levels of the plant tissue for 20 elements. Data for Mo, Cd, Pb and Se were mainly below quantifiable limit and were excluded. Al, Ti, Ni and Cr are not considered essential for plant growth and so were also excluded from the analyses.

Table 1. Pearson correlation between medic growth and soil nutrient content. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic.

Nutrient	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Nitrate N	0.14	-0.09	0.21	0.40*	0.49*	0.61**
Ammonium N	-0.32	-0.18	-0.03	0.19	-0.10	-0.14
Phosphorus	0.04	0.00	0.39*	0.53**	0.53**	0.53**
Potassium	0.19	0.15	0.60**	0.57**	0.59**	0.73**
Sulphur	0.19	0.07	0.29	0.42*	0.44*	0.44*
Organic Carbon	0.34	0.12	0.18	0.05	0.37	0.43
Conductivity	0.20	0.06	-0.29	-0.59**	-0.32	-0.43*
pH $CaCl_2$	0.17	0.14	0.37	0.64**	0.72**	0.91**
pH H_2O	0.09	0.10	0.47*	0.62**	0.60**	0.65**
DTPA Cu	0.14	0.00	-0.46*	-0.55**	-0.16	-0.06
DTPA Zu	0.19	0.29	0.28	0.24	0.36	0.32
DTPA Mn	0.23	0.04	0.36	0.42*	0.64**	0.63**
DTPA Fe	-0.08	-0.09	0.01	0.22	-0.13	-0.07
PBI	0.20	0.03	0.36	0.39*	0.52**	0.66

*Significant at $p < 0.05$
** significant at $p < 0.01$

The relationship between plant nutritional status and plant growth was assessed using a Pearson's correlation coefficient. The Pearson's correlation is moderately robust against departures from normality, but may give false positives due to outliers. The resulting correlations are shown in

Table 2. The only significant correlation between nutritional status and growth of Herald was with P in 28/5 although there was a suggestion of a correlation with P before that reading. No correlation would be expected for the first two measures as they were assessments relative to Angel medic. Angel medic showed negative correlations with Na, especially for the last two readings. There were positive correlations with Mn and K and single correlations with Fe and Ca.

Table 2. Pearson correlation between medic growth and plant nutrient content. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic.

Nutrient	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Fe	-0.04	-0.15	-0.03	0.40*	0.08	0.15
Mn	0.10	0.16	0.36	0.62**	0.50**	0.42*
B	-0.11	0.04	0.04	0.05	-0.02	-0.11
Cu	-0.08	-0.24	-0.22	0.06	-0.11	-0.09
Co	-0.08	-0.14	-0.16	0.26	0.21	0.24
Zn	-0.02	0.03	0.17	0.34	-0.12	-0.14
Ca	-0.14	-0.08	0.10	0.05	0.32	0.50**
Mg	0.03	0.23	0.37	0.48*	0.16	0.09
Na	-0.19	-0.13	-0.33	-0.32	-0.67**	-0.83**
K	0.14	0.15	0.37	0.33	0.45*	0.64**
P	0.34	0.30	0.39*	0.21	0.10	0.12
S	-0.11	-0.08	0.02	0.03	-0.21	-0.08

*Significant at $p < 0.05$
** significant at $p < 0.01$

Overall some 72 correlations have been considered in this section, so it would be expected by chance for 3 or 4 to be significant if there was no effect. It would not be unexpected to have one significant at the p>0.01 level when 72 correlations are considered.

Relationship between plant pathogens and plant growth

Data were available on pathogen levels of the plant tissue for 15 diseases. Data for Gga, *Pratylenchys thornei,* stem nematodes and *Phoma koolunga* were all below detection limits and so their effect could not be assessed.

The relationship between plant disease status and plant growth was assessed using a Pearson's correlation coefficient with the disease being on a log scale. The Pearson's correlation is moderately robust against departures from normality, but may give false positives due to outliers. The resulting correlations are shown in Table 3. The only significant negative correlation between disease status and growth of Herald was with *Fusarium pseudograminiarum* (2) in 2 June. There was also a negative correlation between *Fusarium pseudograminiarum* and growth of Angel medic at all three measurement times.

There was a positive correlation between *Rhizoctonia solani* and Angel medic in the 2 June data. This would seem counter-intuitive.

Overall some 66 correlations have been considered in this section, so it would be expected by chance for 3 or 4 to be significant if there was no effect. It would not be unexpected to have one significant at the p>0.01 level when 72 correlations are considered.

Relationship between paddock herbicide history and plant growth

A summary of the active ingredients of the formulations is given in **Table** . Curiously there was little relationship between the half life and the replanting interval (

Figure 1).

The effect of imaxamox and imazapyr could not be separated in the regression.

There appeared to be little effect of pesticides on Angel medic (**Error! Reference source not found.**, **Error! Reference source not found.** and **Error! Reference source not found.**). It was noted that there was a significant (p = 0.02) relationship between pesticide half life and the effect on Angel medic (**Error! Reference source not found.**).

Table 3. Pearson correlation between medic growth and plant disease status. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic. Cells highlighted in yellow exceed the p<0.05 critical value and the cell highlighted in red exceeds the p<0.01 critical value

Pathogen	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
CCN	-0.32	-0.29	-0.22	-0.10	-0.17	0.08
Take all	0.07	-0.08	0.13	0.05	0.08	0.21
R.solani	-0.14	-0.21	0.15	0.23	0.31	0.47*
Crown rot (*F. p*. log scale)	0.15	0.07	0.06	-0.02	0.13	0.06
F pseudograminiarum	0.30	0.31	0.16	0.03	0.09	0.05
F.culmorum	0.19	0.11	0.09	-0.02	-0.09	-0.25
Bipolaris	0.27	0.06	0.06	-0.18	-0.18	-0.02
Pythium clade f	-0.04	-0.14	-0.14	0.07	0.18	0.24
Pr. neglect	0.22	0.10	-0.01	-0.06	0.11	0.06
Black spot	0.04	0.02	-0.24	-0.29	-0.15	-0.21

*Significant at p < 0.05

Table 4. Active ingredients in formulations considered in this report, together with some properties of the active ingredient

Active ingredient

Formulation	Butafenacil	Imazapyr	MCPAester	Triasulfuron	Imazamox	Clopyralid	Flumetsulam	Metsulfuron-methyl	Imazapic
Ally								0.6	
Broadstrike							0.8		
Intervix		0.015			0.033				
Logran				0.75					
Logran B	0.2			0.52					
Lontrel liquid						0.3			
Lontrel powder						0.75			
Midas		0.0073	0.285						0.022
OnDuty		0.175							0.525
Property									
K_{oc}	365	125	74	16	67	5	28	92	137
pK_a	0	1.9	3.73	4.64	2.3	2.01	4.6	0	2
$t_{0.5}$	1.5	11	15	23	25	34	45	66	120
EC_{50} Algae	0.0025	71	79.8	0.035	0.037	30.5	10.68	0.2	0.051

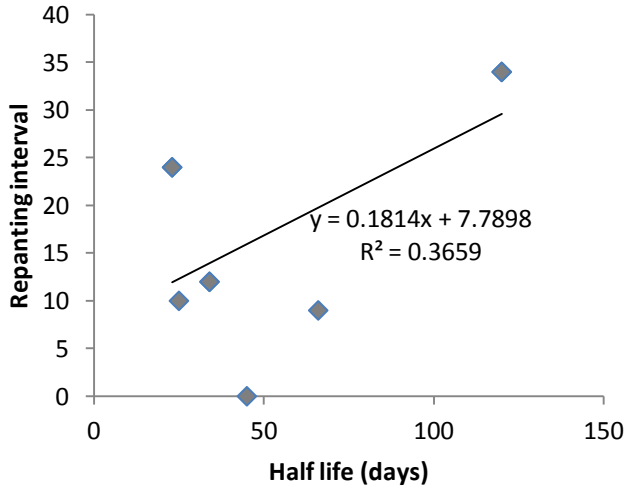


Figure 1. Comparison of half life of herbicides and the replanting interval.

There was little relationship between medic growth herbicide applications. There was a suggestion of suppression by metsulfuron-methyl application in the previous year (Table 5), there was no significant effect from applications in the year before but there was a significant ($p<0.05$) between the total metsulfuron-methyl applied and the relative growth of Herald scored on 13 May (Table 6). For comparison the correlations with potassium were included in Table 7.

The results of the regression analyses are shown in **Error! Reference source not found.** and **Error! Reference source not found.**. Ideally the intercept should have reflected the score of 5 **Error! Reference source not found.** or 10 in **Error! Reference source not found.** that would have occurred if there was no pesticide addition. The departure from this represents some deviation from the model assumptions

such as additivity of effects or that the application rates were known exactly. There were also some positive effects of herbicides and these would contribute to the bias.

The coefficients (estimates) for each pesticide are shown in the tables. It was anticipated that all the effects would have been negative so it would be appropriate to use a single sided test. For scores relative to Angel medic there was a marginally significant depression of the score by clopyralid.

The overall score effects are shown in **Error! Reference source not found.**. Clopyralid again showed up as significant, and there was a significant effect of metsulfuron-methyl. There was a comparable effect on triasulfuron but this was not statistically significant.

Table 5. Pearson correlation between medic growth and herbicide application in previous year. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic.

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Butafenacil	-0.11	-0.25	-0.24	0.16	0.29	0.31
Clopyralid	-0.20	-0.23	-0.16	0.06	-0.02	0.02
Flumetsulam	0.33	0.25	0.10	-0.20	-0.18	-0.16
Imazamox	-0.01	0.09	0.13	0.02	0.20	0.16
Imazapyr	-0.01	0.04	0.09	-0.01	0.18	0.17
Metsulfuron-methyl	-0.30	-0.22	-0.37*	-0.21	-0.26	-0.30
PMA08	-0.19	-0.24	-0.20	0.14	0.19	0.18

*significant at p < 0.1

Table 6. Pearson correlation between medic growth and herbicide application two years before. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic. Cells highlighted in yellow exceed the p<0.05 critical value and the cell highlighted in red exceeds the p<0.01 critical value

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Clopyralid07	-0.24	-0.33	-0.21	-0.04	-0.05	0.12
Flumetsulam07	-0.08	-0.17	-0.17	0.16	0.26	0.16
Imazamox07	0.00	0.06	0.02	-0.02	0.02	-0.11
Imazapic07	0.17	0.23	0.12	-0.01	0.06	-0.07
Imazapyr07	0.08	0.14	0.07	-0.01	0.03	-0.09
MCPAester07	0.32	0.32	0.17	0.01	0.08	0.02
Metsulfuron-methyl07	-0.37**	-0.06	-0.23	-0.19	-0.31	-0.20
Triasulfuron07	-0.22	-0.10	-0.10	-0.07	0.00	0.12

** significant at p < 0.05

Table 7. Pearson correlation between medic growth and total herbicide application in previous two years. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic. Cells highlighted in yellow exceed the p<0.05 critical value and the cell highlighted in red exceeds the p<0.01 critical value

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Butafenacilb	-0.11	-0.25	-0.24	0.16	0.29	0.31
Clopyralidb	-0.25	-0.29	-0.18	0.03	-0.03	0.08
Flumetsulamb	0.19	0.07	-0.04	-0.01	0.08	0.02
Imazamoxb	0.00	0.12	0.11	0.02	0.17	0.07
Imazapicb	0.17	0.22	0.12	-0.01	0.06	-0.08
Imazapyrb	0.07	0.18	0.12	0.00	0.13	0.00
MCPAb	0.32	0.32	0.17	0.01	0.08	0.02
Metsulfuron-methylb	-0.43**	-0.16	-0.37*	-0.22	-0.32	-0.25
Triasulfuronb	-0.31	-0.20	-0.18	0.05	0.13	0.22
POTASSIUM	0.26	0.47**	0.57***	0.53***	0.60***	0.76***

*significant at p < 0.1
** significant at p< 0.05
*** significant at p < 0.01

Multivariate analyses

In the previous section, the dominant effects were found to be soil nutrition, in particular soil pH and soil potassium. One approach is to consider each pesticide individually after correction for the nutritional effect. This approach required approximately 10 pesticides, for six measurements, with the possibility of spray applied in 2007, 2008 or both together, making some 180 regressions. In each case the regressions included potassium in the soil.

The results are tabulated for 2008 spraying in Table 8, the 2007 spraying in Table 9 and the total of both years in Table 10. All the significant or near significant effects were negative and occurred in the Herald strain. There was a suggestion of a negative effect of butafenacil, flumetsulam, metsulfuron-methyl and trisulfuron. The effect of imaxamox and imazapyr could not be separated in the regression.

Table 8. Students 't' value for testing effect of herbicide applied in 2008 on medic growth corrected for effect of potassium. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic.

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Butafenacil	-0.99	-1.73*	-2.09**	0.48	0.89	0.67
Clopyralid	-0.82	-1.32	-0.93	0.41	0.13	0.55
Flumetsulam	1.84*	1.52	0.67	-1.46	-1.05	-1.57
Imazamox	-0.02	0.48	0.71	0.15	1.49	2.01*
Imazapyr	-0.02	0.48	0.71	0.15	1.49	2.01*
Metsulfuron.methyl	-1.10	-0.75	-1.40	-0.58	-0.59	-0.73
Triasulfuron	-1.23	-1.92*	-1.81*	0.71	0.86	1.20

*significant at p < 0.1
** significant at p< 0.05

Table 9. Students 't' value for testing effect of herbicide applied in 2007 on medic growth corrected for effect of potassium. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic. Cells highlighted in grey exceeded the p< 0.1 level, yellow exceed the p<0.05 critical value and the cell highlighted in red exceeds the p<0.01 critical value

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Clopyralid07	-1.20	-1.87*	-1.26	-0.21	-0.20	0.95
Flumetsulam07	-0.56	-1.38	-1.69	0.75	1.26	0.75
Imazamox07	0.15	0.49	0.41	0.11	0.42	-0.37
Imazapic07	0.95	1.31	0.76	0.01	0.47	-0.49
Imazapyr07	0.51	0.88	0.59	0.06	0.46	-0.44
MCPAester07	1.55	1.66	0.75	-0.16	0.21	-0.32
Metsulfuron.methyl 07	-1.72	-0.14	-0.87	-0.82	-1.65	-1.22
Triasulfuron07	-1.20	-0.95	-0.88	-0.78	-0.33	0.67

*significant at p < 0.1

Table 40. Students 't' value for testing effect of herbicide applied in 2007 or 2008 on medic growth corrected for effect of potassium. Herald measures on 13/5 and 28/5 were relative to the corresponding performance of Angel medic. Cells highlighted in grey exceeded the p< 0.1 level, yellow exceed the p<0.05 critical value and the cell highlighted in red exceeds the p<0.01 critical value

Herbicide	Herald 13/5	Herald 28/5	Herald 2/6	Angel 13/5	Angel 28/5	Angel 2/6
Butafenacil	-0.99	-1.73*	-2.09**	0.48	0.89	0.67
Clopyralid	-1.15	-1.82*	-1.25	0.12	-0.04	0.85
Flumetsulam	0.90	0.09	-0.70	-0.50	0.14	-0.57
Imazamox	0.11	0.70	0.79	0.18	1.28	0.89
Imazapic	0.95	1.31	0.76	0.01	0.47	-0.49
Imazapyr	0.48	1.02	0.83	0.12	0.99	0.27
MCPA	1.55	1.66	0.75	-0.16	0.21	-0.32
Metsulfuron.methyl	-1.92*	-0.51	-1.43	-0.93	-1.54	-1.31
Triasulfuron	-1.85*	-1.99*	-1.85*	-0.29	0.18	1.30

*significant at p < 0.1
** significant at p< 0.05

Discussion

Use of Angel as a control

The concept of the trial is interesting but is dependent on Angel medic being tolerant to a broad range of Group B herbicides. This includes metsulfuron-methyl as well as herbicides like imazapic. Data were not available to determine whether this is so.

There was an apparent effect of pesticide on Angel medic noted in the raw data supplied. This would imply that Angel medic may not be a good control. On the other hand, the effects of nutrients on Herald relative to Angel were all very small (Table 1).

From the data there was some suggestion that Angel medic was affected by the herbicides, but not as much as was Herald (although there are no absolute data on this). An alternative control may have been to use a different type of plant (e.g. wheat). Other crops may have been considered that were known to be very sensitive to Group B herbicides (e.g. Faba beans).

Composite sampling

In the current trial field several samples from each paddock were bulked into a composite sample, which was subsequently sub-sampled. In the current context little has been lost by this procedure but in general it is preferable to keep field samples together so that the pot samples are independent. In the current case it would have been preferable to have had 5 field samples that were subdivided into pairs for the two medic varieties.

Chemical residues

Techniques do exist for assaying very small concentrations of Group B herbicides. If funds were available it would be useful to have the soil samples assayed. The cost of this would be approximately $200 per sample – some $5000 in all. Such information would give good field data on the breakdown/removal rates of the Group B herbicides.

Nutritional effects

As was anticipated the largest effect was nutritional. However, the two elements nominated (priv. comm.) were phosphorus and zinc. The largest effect was due to potassium and the correlation with plant score was consistently higher than for phophorus. Soil pH was also important.

Root pathogens

The array of plant pathogen tests was large and surprising. Crown rot would normally not be associated with medic – indeed medic is used as a break crop. Less clear was the use of *Rhizoctonia solani,* which has numerous strains, some more pathogenic than others. Overall, it was not clear whether a positive or negative association would be expected with the root pathogens as it is known that more fertile soil is likely to have larger plants that in turn can carry more disease, while on the other hand, the presence of a pathogen would intuitively reduce plant growth.

Conclusions

The concept of the trial is interesting, and the lack of correlation between the relative Herald scores and the soil nutritional data give support for the idea.

There was no clear evidence of any particular herbicide having a carry-over effect, but the consistent negative effect is strongly suggestive of there being a carry-over effect.

The correlation between soil nutritional effects and growth was much stronger than that between herbicide history and growth.

The results provide raise questions as to the effective field half-life of the Group B herbicides.

References

PPDB (2008). FOOTPRINT Pesticide Properties DataBase http://sitem.herts.ac.uk/aeru/footprint/

3. The effect of Broadstrike on medic pasture productivity

Steve Lamey and Jane Prider

Branched Broomrape Eradication Program

November 2009

Aim:

This survey examined whether applications of Broadstrike to medic pasture affected pasture productivity.

Methods:

Medic pastures were sampled at 3 locations that had been sprayed by farmers with Broadstrike at a rate of 25 g ha^{-1} as part of their regular management. Two pastures were on sandy soils; Paddock WS was sprayed in late July and Paddock HS in mid July. Paddock HL was on a loamy soil and sprayed in mid July. Comparable areas in unsprayed parts of the paddock were selected for comparison. At HL and HS the sprayed and unsprayed plots were 20 m apart. At the WS site they were 200-300 m apart.

Twenty quadrats (10 sprayed and 10 unsprayed), 0.1 m by 0.1 m were harvested by cutting the above-ground biomass at ground level. Data was collected on the amount of feed or dry matter, the number of medic pods and the medic pod weight (per 20 pods). At the WS site data was collected on the number of medic plants.

A feed test analysis of bulked medic samples was conducted by Agrifoods Technology, Werribee Victoria.

Analysis

Each variable for each site and in each soil type were tested separately for differences between sprayed and unsprayed sites. Data for each variable were first checked for normal distributions and variance homogeneity so that an appropriate test could be used. Distributions were checked using quantile plots and a Shapiro Test, and homogeneity of variances with Barlett's and Fligner's tests. Where these tests indicated that there was a significant departure from normality or variance homogeneity for a variable, the data were transformed using a natural log or a square root transformation. The transformed variables were retested but the transformations did not improve normality or variance homogeneity, therefore a Wilcoxon test was used for these data. This test does not assume a normal distribution or homogeneous variances. In other cases a t-test was used. All tests compared means and variances between sprayed and unsprayed plots. R software (version 2.8.0) was used for the analysis.

Results

There were no differences in total feed between sprayed and unsprayed plots at any of the sites (Table 1, Fig. 1).

In the HL paddocks on the loamy soil, there were more medic pods in sprayed plots (Fig. 2). At the HS and HL paddocks there was more pod biomass in sprayed sites (Fig. 3), so although there was no difference in pod number at the HS paddock, the pods were larger.

There were no significant differences between sprayed and unsprayed plots in the WS paddock. The boxplots indicate that there was more variability in medic feed quantity in unsprayed plots at this site (the

boxplots have more spread) (Fig. 1). The number of medic plants at this site did not differ between sprayed and unsprayed plots (Fig. 4).

Table 1. Results of tests for differences between sprayed and unsprayed plots (n=10). P-values in bold are significant at α <0.05. Values of means and standard errors for U, unsprayed and S, sprayed plots. Pod weights are for single pods.

Site	Soil type	variable	Mean ± standard error	Test statistic	P value
HS	Sand	Feed weight	U: 14.4 ± 1.9 g 0.1m^{-2} S: 16.8 ± 2.4 g 0.1m^{-2}	58^{w}	0.579
		Number medic pods	U: 81 ± 14 pods 0.1m^{-2} S: 96 ± 10 pods 0.1m^{-2}	0.849	0.408
		Medic pod weight	U: 19.3 ± 1.3 mg S: 24.4 ± 1.2 mg	2.280	**0.012**
HL	Loam	Feed weight	U: 14.2 ± 1.3 g 0.1m^{-2} S: 14.9 ± 1.19 g m^{-2}	0.520	0.610
		Number medic pods	U: 134 ± 15 pods 0.1 m^{-2} S: 199 ± 16 pods 0.1 m^{-2}	2.929	**0.009**
		Medic pod weight	U: 29.4 ± 1.5 mg S: 36.5 ± 1.8 mg	2.977	**0.008**
WS	Sand	Feed weight	U: 13.2 ± 1.1 g 0.1m^{-2} S: 16.5 ± 1.24 g 0.1m^{-2}	1.947	0.068
		Number medic pods	U: 90 ± 18 pods 0.1 m^{-2} S: 80 ± 9 pods 0.1 m^{-2}	44^{w}	0.684
		Medic pod weight	U: 25.5 ± 2.1 mg S: 28.5 ± 1.1 mg	59.5^{w}	0.496
		Number medic plants	U: 20 ± 2 plants 0.1m^{-2} S: 15 ± 2 plants 0.1m^{-2}	-2.067	0.054

^{W} Wilcoxon tests, all others were t-tests

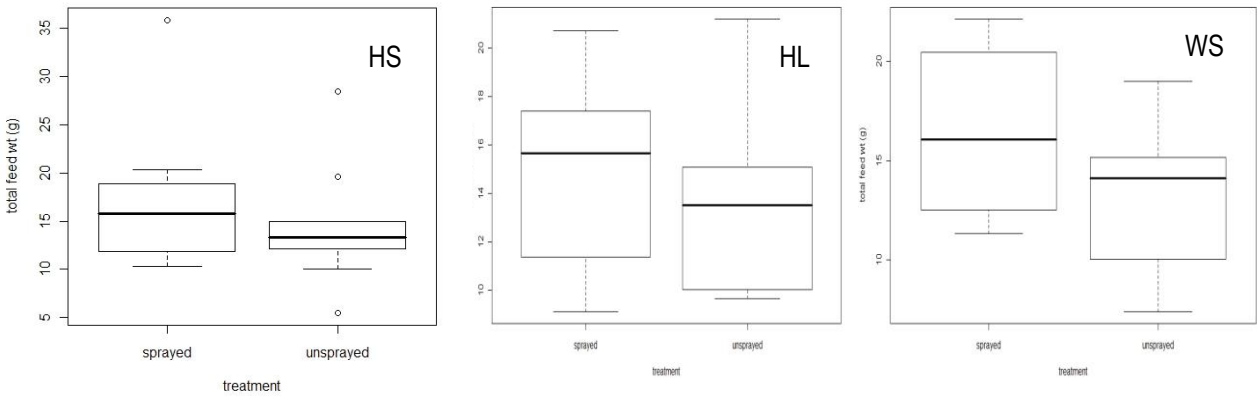


Figure 1. Box plots of total feed weight per 0.1 m^{-2} harvested from unsprayed medic pasture plots or plots sprayed with Broadstrike at three sites, n = 10.

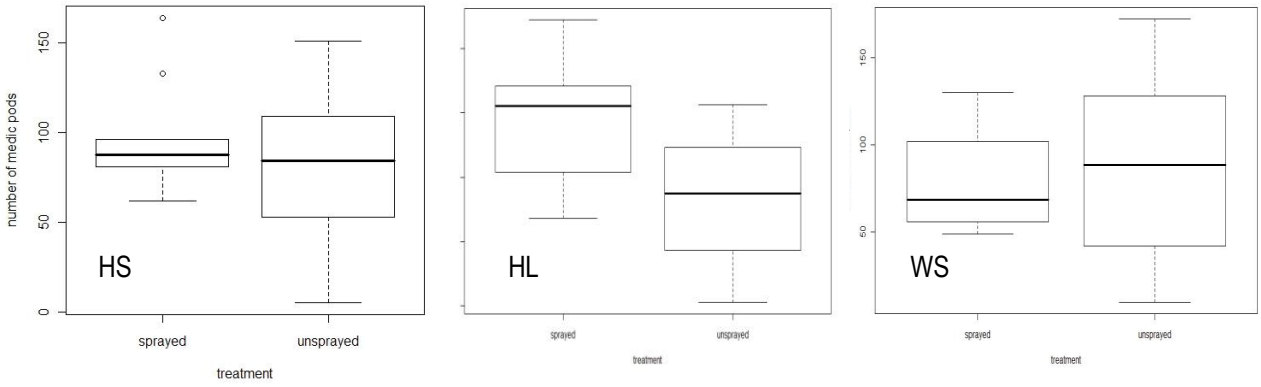


Figure 2. Box plots of number of medic pods per 0.1 m^{-2} harvested from unsprayed medic pasture plots or plots sprayed with Broadstrike at three sites, n = 10.

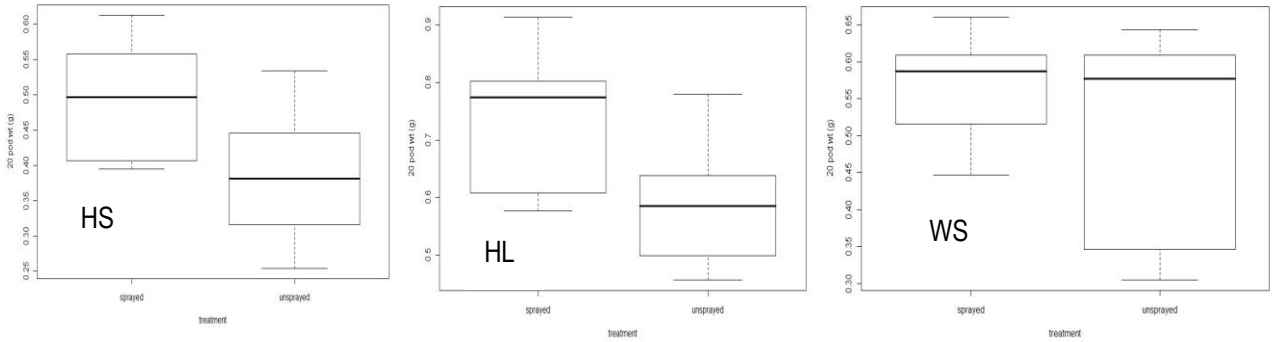


Figure 3. Box plots of weight of 20 medic pods harvested from 0.1 m^{-2} unsprayed medic pasture plots or plots sprayed with Broadstrike at three sites, n = 10.

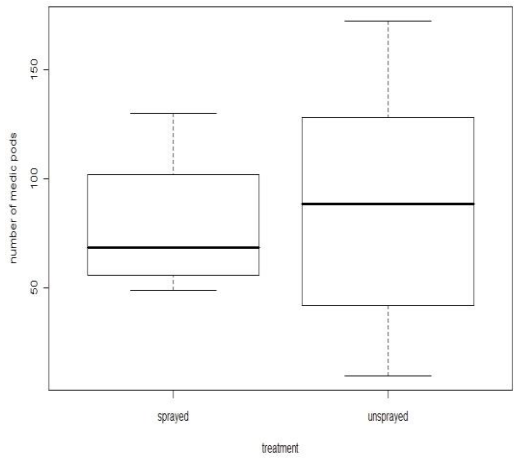


Figure 4. Box plots of number of medic plants harvested from 0.1 m^{-2} unsprayed medic pasture plots or plots sprayed with Broadstrike at the WS site, n = 10.

Feed test

The feed analysis showed higher values for crude protein content, digestibility and metabolisable energy in medic from sprayed plots in Paddock HS and HL (Table 2).

Table 2. Feed analysis report. Data is missing for WS unsprayed site.

	HS		HL		WS
test	unsprayed	sprayed	unsprayed	sprayed	sprayed
crude protein (% of dry matter)	9.8	11.5	14.0	15.2	11.0
digestibility (% of dry matter)	46.7	51.3	51.4	54.9	63.3
digestibility(calculated) (% of dry matter)	46.41	50.29	50.34	53.31	60.41
dry matter (%)	88.2	89.7	90.2	90.5	90.0
est. metabolisable energy (MJ kg^{-1} DM)	6.4	7.2	7.2	7.8	9.3
moisture (%)	11.8	10.3	9.8	9.5	10.0
neutral detergent fibre (% of dry matter)	56.4	50.6	52.5	53.1	42.5

Discussion

The inferences that can be made from the data are limited to these paddock locations at these sites. The treatment, i.e. the spraying, has not been replicated, so these differences may be due to some other site-specific effects. It can be concluded that there is no evidence that the spraying applied to these areas in these two paddocks this year has decreased feed yield.

This sampling was conducted following a wet spring. Under these conditions there was no evidence that applications of Broadstrike to medic pastures resulted in a decrease in the productivity or feed value of medic at these sites. These results may differ under drier conditions and in other sites.

In years with adequate spring rains, set-backs to medic growth following spraying can recover with the added advantage of the removal of many weeds. In the HS and HL paddocks, unsprayed areas had large populations of *Brassica tournefortii* that were reduced by about 75% in sprayed areas, thus reducing competition with medic.

4. Bioassay for the detection of Group B herbicide residues

Jane Prider

Branched Broomrape Eradication Program

2009

Summary

The aim of this project was to develop a method to determine whether the application of Group B, acetolactate synthase inhibiting or ALS , herbicides by farmers within the quarantine area persisted to detectable levels in soils prior to crop sowing in the subsequent year. A literature review identified three common methods that could be used for assessing herbicide residues; ELISA, HPLC and bioassay. The bioassay technique was evaluated as the most cost-effective method for assessing herbicide residues for this project. A bioassay protocol was developed that used lentil root length at 14 days as a response to metsulfuron methyl concentrations between 0 and 10 ng active ingredient g^{-1} soil. Initially, a component of the project was to use the bioassay technique to determine herbicide residues from paddocks in the quarantine area. The collected paddock soils were not assessed as they had been subjected to uncontrolled storage conditions for several months after collection whilst the bioassay method was developed. This document describes the development of the bioassay protocol, and an application of the bioassay method.

Introduction

Group B herbicides are widely used within the quarantine area for the control of branched broomrape and its broad-leaf weed hosts. These herbicides can persist, particularly in dry soils with high alkalinity and low organic matter content, where the rate of degradation of herbicide residues is reduced (Hollaway *et al.* 2006; Sarmah *et al.* 1998). If herbicides persist at levels that are phytotoxic, this has implications for the growth of subsequent sensitive rotation crops, such as legumes and oilseeds (Noy and Hollaway 2001).

Group B herbicides are difficult to detect in soil residues as the herbicides are typically applied in concentrations that are below the level of detection of most chemical techniques. An enzyme-linked immunosorbent assay (ELISA) and high pressure liquid chromatography (hplc) are two techniques that have been developed for sulfonylurea detection in water and soil samples (Degelmann *et al.* 2004; Hollaway *et al.* 1999; Seiden *et al.* 2000). These techniques are relatively fast and require small soil samples although they are expensive. DuPont did produce an ELISA kit for testing sulfonylureas but these are no longer available. It would be possible to develop a technique after purchasing the appropriate antibodies but this may require some expertise. HPLC requires specialised equipment and technical expertise. There has been substantial development in the procedure recently and very low herbicide concentrations can now be detected using advanced techniques (Rai Kookana, pers. comm.). The price is very restrictive and is not economical for large numbers of samples or routine sampling.

Bioassays are the most common method used for analysing Group B herbicide residues in both experimental studies (Stork and Hannah 1996) and for routine screening (Streibig *et al.* 1995). Compared to other analysis techniques, they are economical without the loss of sensitivity and do not require special equipment or technical expertise. In this method a test plant species is grown in soils dosed with the herbicide and a plant response is measured that can be correlated to the concentration of herbicide present. Residues in test soils are assessed by predicting herbicide concentration from the plant response, typically using the formula for a dose response curve (Stork and Hannah 1996). Legume species are

frequently used for analysis of Group Bs as they are highly sensitive (Hollaway *et al.* 1999; Hollaway *et al.* 2006; Kaushik and Inderjit 2006; Stork and Hannah 1996). Plant responses can be measured after varying amounts of time, ranging from 6 days to 28 days, depending on growth conditions, culture method and the growth rate of the test species. Roots appear to be the most sensitive to Group B herbicides, and root length is a common response that is measured (Stork and Hannah 1996) although other responses include root or shoot biomass (fresh or dry), and shoot height (Streibig *et al.* 1995).

The disadvantages of the bioassay method are that it requires a lot of soil and comparable control soils that have had no herbicide application, as the technique is not specific to the target herbicide. Other herbicides or contaminants in the soil and soil nutrient levels can also affect the growth of the bioassay species. The procedure is also time-consuming and depending on growth time, may take several weeks. The bioassay technique was selected as the most feasible method to use given the budget and other constraints. The aim of this project was to develop a protocol for a bioassay for detecting Group B herbicide residues in soils.

Development of bioassay

An initial trial identified the most suitable test species. Three test plants were trialled, Herald medic, Angel medic and lentil. Lentil had the most consistent germination so this species was used for all bioassays. The only herbicide tested was Ally (active ingredient (a.i.) metsulfuron methyl). The bioassays used a commercially available control soil (Burdett sand, Eichlers Earthmovers, Mannum).

Trial 1

For the initial bioassay, metsulfuron methyl (using Ally) was prepared to give final soil concentrations of 0, 0.1, 0.5, 1, 5, and 10 ng a.i. g^{-1} soil. The prepared metsulfuron methyl solutions were thoroughly mixed into the sand and then placed in 50 ml tubes. A single lentil seed, which had been imbibed in water overnight, was sown into each tube and tubes were watered. Five replicate tubes were planted for each concentration. Tubes were kept in the glasshouse and watered as the top soil dried out. After seven days, the sand was gently washed from the roots. Measures were made of plant height, number of leaves, root length, fresh and dry root and shoot weight. Dry weights were obtained after oven-drying for 24 hours at 75°C.

This assay gave highly variable results, with no clear pattern of response to metsulfuron methyl concentration (Fig. 1A). One of the reasons for this could have been that the plants were not grown for long enough for a response to develop. Fresh weights were very similar to dry weights and duplicated effort so fresh weights were not measured in subsequent trials.

Trial 2

For the second bioassay trial, the same method was replicated but plants were harvested after 14 days and 24 days. The number of root branches was counted for the 14 day harvest and leaf area was calculated for the 24 day harvest. For these bioassays, excess tubes were prepared so that tubes could be discarded where the lentils had delayed emergence, but five replicates per dose were used for the final analyses.

There were more consistent responses to metsulfuron methyl in this bioassay and the most sensitive measure was root length (Fig. 1 B, C). The roots of plants at 0 ng a.i. g^{-1} soil growing in the tubes for 24 days had coiled around the base of the tube, therefore the 14 day harvest time was more suitable for the size of the tubes used. Plants were showing a response to the lowest concentration of metsulfuron methyl (0.1 ng a.i. g^{-1} soil) with root length less than 80% of that of plants grown with no herbicide (Fig. 1 B, C). It was important to detect the lowest level at which a response was occurring so it was necessary to look at lower concentrations. As it is very difficult to measure out very low amounts of active ingredient, the next trial used a different method for producing the herbicide solutions. There was also very little difference between concentrations between 0.5 and 5 ng a.i. g^{-1} soil. This may also be due to difficulties in measuring

out the small amounts of active ingredient. There was a large difference between 0.1 and 0.5 ng a.i. g^{-1} soil, therefore more concentrations were needed between these ranges.

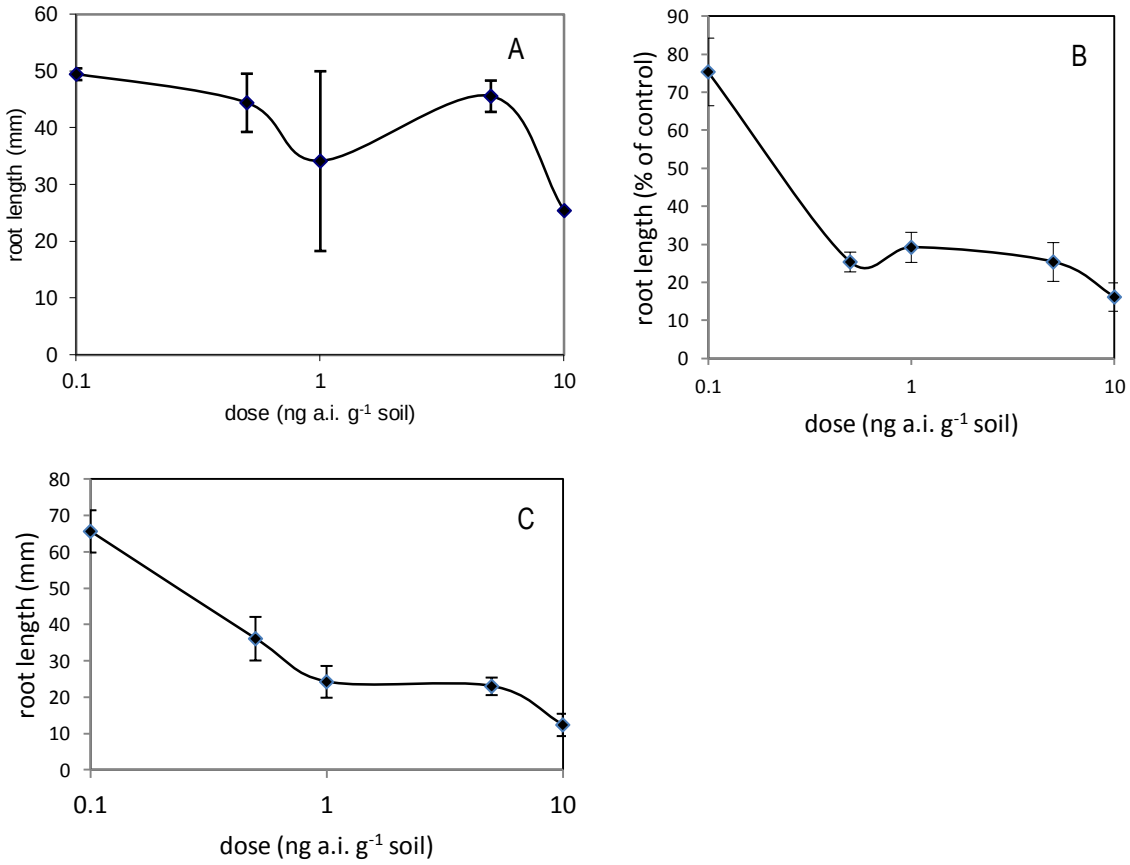


Figure 1. Lentil root length as a percentage of root length of control plants growing in absence of herbicide, in response to metsulfuron methyl concentration at three harvest times; A) Trial 1 at 7 days, B) Trial 2 at 14 days, C) Trial 2 at 24 days.

Trial 3

For the third bioassay trial, a dilution series was prepared so that responses could be measured at very low metsulfuron methyl concentrations. A 0.5 dilution series was prepared giving concentrations of 0, 0.039, 0.078, 0.156, 0.316, 0.625, 1.25, 2.5, 5 and 10 ng a.i. g^{-1} soil. This method also produced more data points so that non-linear curve fitting procedures could be used for data analysis. Tubes were prepared and grown as previously and root length was measured on freshly washed-out roots harvested after 14 days. Excess tubes were prepared so that plants that germinated late could be discarded but five replicates were used for the final analysis.

The package drc from the statistical program R (ver. 2.8.0, R Development Core Team 2008) was used to fit a curve to the data (Ritz and Streibig 2005). The program was also used to predict estimated effective doses (ED) for a 10%, 50% and 90% reduction in response. Responses less than ED10 (or a 10% reduction) are routinely used as a no observable effect level for herbicides. The model can also be used to predict herbicide concentrations and root lengths for unknown variables. For the bioassay data, the best model fit was a three parameter logistic model with a boxcox transformation (Fig.2). The boxcox transformation improves the model fit when the variances are not normally distributed. Lentil was highly

sensitive to metsulfuron methyl. Concentrations as low as 0.015 ng a.i. g^{-1} soil (equivalent to 0.15 g ha^{-1}) resulted in a 10% decrease in lentil root length. For the range of metsulfuron methyl concentrations used in the bioassay, the model was not able to accurately predict the herbicide level that would result in complete inhibition of lentil root growth (i.e. no lower plateau or zero response in the model).

Given that the development of an appropriate bioassay protocol for only one herbicide took several months, it was decided not to proceed with testing paddock soils, as the soils had been kept in conditions that were not comparable to those occurring under field conditions. In addition, the use of a commercial soil for the dose responses is not likely to be comparable to the paddock soils. However, the technique was applied to soils collected for native vegetation monitoring.

Full details of the final protocol are in the Appendix.

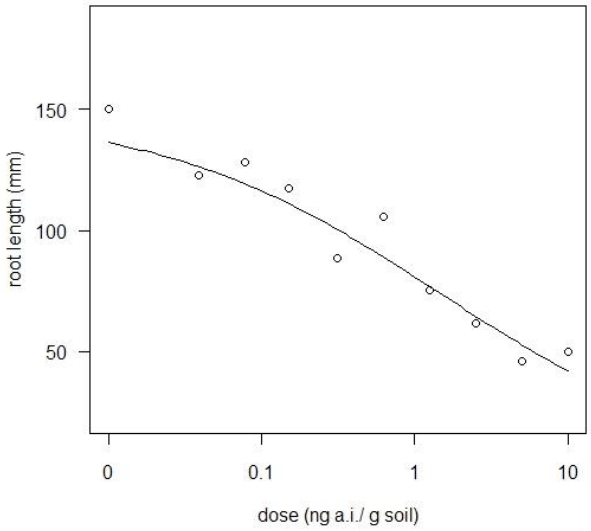


Figure 2. Dose response curve for activity of metsulfuron methyl on lentil root length. The curve is a three parameter log logistic curve with a boxcox transformation.

Application of bioassay to native vegetation monitoring

Method

The bioassay protocol was used to assess the persistence of Group B herbicide residues in soils subject to regular aerial spraying of the herbicide Ally. Soils were collected before and after spraying from five sites within the sprayed area and from an unsprayed control site north of the sprayed area (5 replicate soil samples from each site). Control soils were used to construct separate dose response curves for pre-spray and post-spray soils as described above. A four parameter logistic curve was fitted to the pre-spray data and a four parameter Weibull curve to the post-spray data. The formula for the pre-spray curve was used to estimate herbicide residues in soils collected from the sprayed area using the lentil root lengths. Each replicate in a site was used to calculate a mean and standard error for that site. A Wilcoxon signed rank test was used to determine if these values differed significantly from zero. The post-spray data was not used.

Results

Dose response curves for control soils were different for pre-spray and post-spray soils (Fig. 3). Lentil root growth was less in soils collected in spring than soils collected in winter. Aerial spray records showed that the sites where control oils were collected were not sprayed nor were weather conditions likely to result in substantial drift of spray onto the collection sites. There are other unexplained factors that resulted in reduced root growth in the second measurements. Due to the uncertainty of the reliability of the post-spray data, it was not used for further analyses.

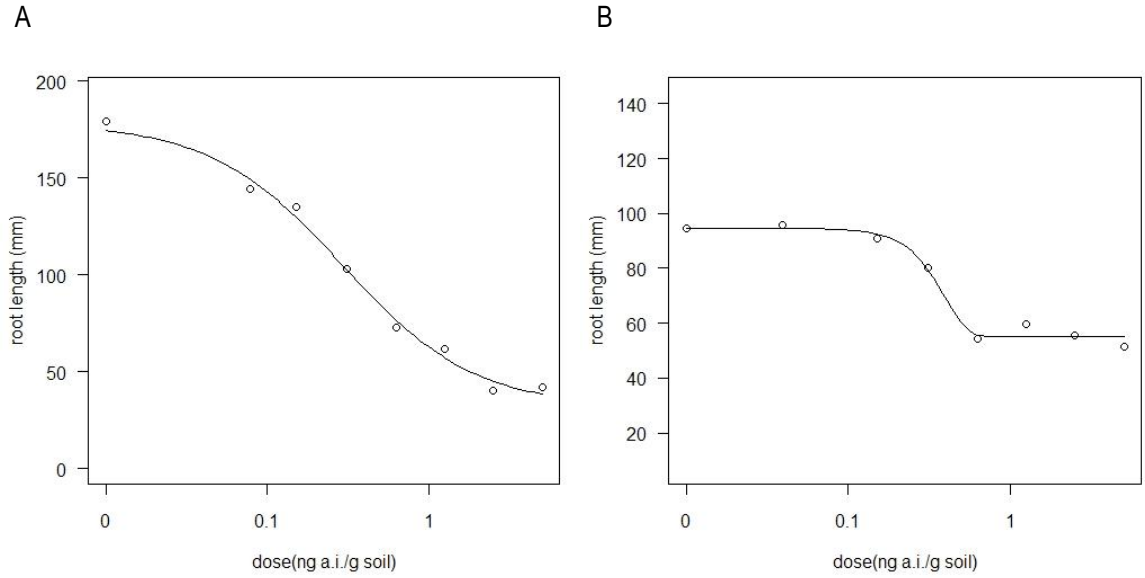


Figure 3. Dose response curves for metsulfuron methyl applied to control (unsprayed) soils sampled before spraying in winter (A) and after spraying in spring (B). Curve A is a four parameter logistic function and B is a four parameter Weibull function.

The estimated herbicide concentrations for replicate samples at each site in the sprayed area were not significantly different from zero (Table 1). This indicates that there were no detectable herbicide residues in soils in the sprayed area.

Table 1. Measured root lengths (mean ± standard error, n = 5) and predicted herbicide residues (mean ± standard error) from the model shown in Figure 3A. Mean root lengths from lentils grown in soils from five sites in the sprayed area sampled before aerial herbicide application. P-values are results of Wilcoxon signed rank tests, testing for a significant difference from zero.

Site	Mean root length measured in bioassay (mm)	Metsulfuron methyl concentration predicted from model (ng a.i. g^{-1} soil)	p-value
1	147 ± 7	-0.29 ± 0.16	0.313
2	172 ± 40	-0.26 ± 1.67	0.875
3	132 ± 14	0.07 ± 0.32	0.813
4	167 ± 8	-0.84 ± 0.25	0.063
5	153 ± 32	0.19 ± 0.56	0.875

Conclusions

Lentils were very sensitive to metsulfuron methyl and bioassays need to span a broad range of active ingredient concentrations. We found that lentil root growth was rapid and 14 day bioassays were required for the small amounts of soil that were used. Careful measurement of herbicide doses and thorough mixing into soil samples is required for accurate bioassay results.

Responses to soil nutrients can be as great or sometimes greater than responses to herbicides, particularly when trying to detect minor concentrations of herbicide residues. It is therefore important to construct dose response curves from the same soil type as the soils to be tested for residues. This is often difficult on farms where the history of herbicide use is not clear. In some cases there may be no comparable soils that are herbicide-free. Dose responses and test soils must be processed at the same time so that growing conditions are identical.

Bioassay is a sensitive technique and does not require specialised equipment or expertise but is time-consuming to set up. It would not be very cost-effective if many samples needed to be processed as a dose response curve would need to be made for each paddock soil. Additionally, as the bioassay is not specific to a single herbicide, the spraying history of the paddock would need to be known. For routine testing, the technique would require a dedicated laboratory service.

References

Degelmann P, Wenger J, Niessner R, Knopp D (2004) Development of a Class-Specific ELISA for Sulfonylurea Herbicides (Sulfuron Screen). *Environmental Science & Technology* **38**, 6795-6802.

Hollaway, Kookana, McQuinn, Moerkerk, Noy, Smal (1999) Comparison of sulfonylurea herbicide residue detection in soil by bioassay, enzyme-linked immunosorbent assay and hplc. *Weed Research* **39**, 383-397.

Hollaway KL, Kookana RS, Noy DM, Smith JG, Wilhelm N (2006) Persistence and leaching of sulfonylurea herbicides over a 4-year period in the highly alkaline soils of south-eastern Australia. *Australian Journal of Experimental Agriculture* **46**, 1069-1076.

Kaushik S, Inderjit (2006) Phytotoxicity of selected herbicides to mung bean (Phaseolus aureus Roxb.) *Environmental and Experimental Botany* **55**, 41-48.

Noy D, Hollaway K (2001) Metsulfuron-methyl residues and potential recroppping damage in Victorian cropping soils. In 'Proceedings of the 10th Australian Agronomy Conference'. (Australian Society of Agronomy: Hobart).

Ritz C, Streibig J (2005) Bioassay analysis using R. *Journal of Statistical Software* **12**, 1-22.

Sarmah AK, Kookana RS, Alston AM (1998) Fate and behaviour of triasulfuron, metsulfuron-methyl, and chlorsulfuron in the Australian soil environment: a review. *Australian Journal of Agricultural Research* **49**, 775-790.

Seiden P, Rossana B, Rossana, Streibig JC (2000) Applicability of ELISA for determination of metsulfuron-methyl in soil samples. *Pest Management Science* **56**, 637-643.

Stork P, Hannah M (1996) A bioassay method for formulation testing and residue studies of sulfonylurea and sulfonanylide herbicides. *Weed Research* **36**, 271-281.

Streibig J, Walker A*, et al.* (1995) Variability of bioassays with metsulfuron-methyl in soil. *Weed Research* **35**, 215-224.

Appendix

Bioassay for metsulfuron methyl

Aim: to determine the unknown concentration of metsulfuron methyl in a soil sample using a dose response curve

Known concentrations of metsulfuron methyl are added to previously untreated field collected soil. A susceptible plant species is planted into each dosed soil and root length measured after 14 days. The data is used to generate a dose response curve. The formula for this curve is used to calculate the unknown herbicide concentration of a soil sample by using the root length of lentil grown in the test soil sample.

For the dose response curve:

1. Make a stock solution of metsulfuron methyl. For Stock Solution A, dissolve 1.66667 g of Ally in 1 L of water. Ally has 600g/kg of metsulfuron methyl. This will yield a concentration of 1 mg a.i 1 ml water.

2. Prepare Stock Solution B from Stock Solution A by diluting 10 ml of Stock Solution A in 1190 ml water to yield a concentration of 10 ng a.i. 1 µl water.

3. Prepare a 0.5 serial solution of metsulfuron methyl using Stock Solution B. For 1 kg of soil for each dose prepare the following:

Solution	Target concentration (ng a.i. g^{-1} soil)	Starting solution	water
1	10	1ml Stock Solution B	9ml
2	5	5 ml of Solution 1	5 ml
3	2.5	5 ml of Solution 2	5 ml
4	1.25	5 ml of Solution 3	5 ml
5	0.625	5 ml of Solution 4	5 ml
6	0.3125	5 ml of Solution 5	5 ml
7	0.156	5 ml of Solution 6	5 ml
8	0.078	5 ml of Solution 7	5 ml
9	0.039	5 ml of Solution 8	5 ml
10	0		10 ml

4. Add water to make each solution up to 20 ml. This is the optimum amount to mix into 1 kg dry sandy soil.

5. Prepare soil by sieving and allowing to air dry. The soil must be free of herbicide residues and match as closely as possible the test soil with unknown herbicide concentration.

6. Thoroughly mix prepared herbicide dose into soil by placing in a plastic bag and mixing thoroughly.

7. Fill 50 ml labelled tubes with dosed soils, compacting well by tapping the tube as it is filled. One kg of soil is enough to fill about 12 tubes but replicates of 5 should suffice. A few extra tubes are

useful as lentils may not all germinate at the same time and late seedlings can then be discarded. Alternatively, two seeds could be sown and any superfluous late seedlings removed.

8. Sow a lentil seed that has been imbibed overnight in water into each tube and cover with soil. Water tubes to ensure soil is evenly moist.

9. Keep tubes moist but not wet for 14 days.

10. Wash soil away from roots and measure root length to nearest millimetre.

For test soils:

1. Prepare soils by sieving and air drying.

2. Sow lentil seeds in replicates of 5 tubes for each test soil, water and harvest as above. Test plants should be grown at the same time and in the same conditions as the dose response plants.

Curve fitting procedure

Data is analysed by fitting non-linear models using the R program drc (Ritz and Streibig 2005).

Examples of the commands used in the program are in square brackets.

1. Prepare text file with doses as one column and responses as second column.

2. Load drc library and import the text file.

3. Various models should be fitted and the model chosen that gives the best fit. To fit a log logistic four parameter model [m1<-drm(rootl~dose,data=dr,fct=LL.4())]. This model gives best fit if there is a flattening of the curve at both ends of the distribution. If not, use a three parameter model. Some other models are a three parameter logistic (LL.3), a Weibull four parameter (W2.4) and a Weibull three parameter (W2.3). They can all be found by looking in the help file [?drm].

4. To check which model gives the best fit, check the variance for each of the fitted parameters and the residual variance. This can be found by looking at the summary for each model [summary(m1)]. Models can be compared using anova [anova(m1,m20)]. If the test is not significant it means that no model is a better fit than another.

5. Also check the model fit by comparing it to an ANOVA model [modelFit(m1)]. If the p value for this is significant it means that an ANOVA model provides a better fit to the data (i.e. a non-linear model should not be used).

6. The models assume that the variances are homogeneous. The data can be transformed and the models refitted. This is done by applying the boxcox transformation to the model [m2<-boxcox(m10)]. This transforms both the x and y values. The fit of these transformed models can then be compared to the models with untransformed data.

7. Estimated effective doses can be given [ED(m1,c(10,50,90)]. It is also possible to predict a root length from a given dose with standard errors, confidence or prediction intervals(predict x from y) but I can't see a way to predict y from x [predict(m1,dose=2,interval="confidence")]. Ray Correll has prepared a spreadsheet that can calculate y from x for a four parameter logistic model.

8. To make a plot in R [plot(m1,xlab="dose (ng a.i./g soil",ylab="root length (mm)")]

5. Monitoring of native vegetation sprayed aerially with metsulfuron methyl herbicide

Jane Prider, Anna Williams and Darryl Miegel

Branched Broomrape Eradication Program

November 2011

Summary

The Group B herbicide, metsulfuorn methyl (Ally®), is sprayed aerially over some areas of native vegetation for the control of branched broomrape (*Orobanche ramosa*) in the Murray Mallee region. The program has monitored a site at Tailem Bend for the past five years to assess the effects of aerial herbicide application on native plant species. Permanent sites were sampled twice annually, prior to, and following spraying. The semi-woody perennial climber, *Clematis microphylla*, declined in condition following spraying and this was significant in 2011 when herbicide application rates increased. The other seven monitored woody perennial species did not decline in condition following spraying. There were no immediate differences in ground-layer species composition when plots were compared prior to and after spraying. However, there have been long-term changes in the composition of ground-layer species communities over the five years of monitoring. The abundance of the herbaceous perennial species, *Senecio pinnatifolius* and *Podolepis rugata,* declined over the five years and these species had visual signs of herbicide damage. Stands of dead plants were observed following the application of higher herbicide rates in 2011.

Introduction

The Group B, acetolactate synthase inhibiting or ALS, herbicides are becoming more widely used for environmental weed control in native vegetation in Australia due to their selectivity and ease of application (Pritchard 1993, Vitelli and van Haaren 2001, Brown et al. 2002, Broese van Groenou and Downey 2006). Group B herbicides are very effective for the control of branched broomrape (*Orobanche ramosa*) in agricultural regions in the Murray Mallee of South Australia and this is currently the only viable option for branched broomrape control in infested native vegetation. Current practice for many of the larger areas of native vegetation in the region, is to apply the selective Group B herbicide, Ally® (a.i. metsulfuron methyl), aerially at a rate of 3 g ha^{-1}. Due to the translocation of the herbicide from host foliage into *O. ramosa* attached to host roots, control can be achieved at very low application rates with minimal detrimental effects on the host plant. However, even at low application rates, the off-target effects of herbicide application to native plant species in the region, is not known. Observations of the effects of aerial spraying on native vegetation in New South Wales, as part of the Bitou Bush control program, found very few native species were affected by metsulfuron methyl although further controlled studies are required (Broese van Groenou and Downey 2006, Toth and Winkler 2008). In addition, Group B herbicides can persist in the soil, particularly in dry, alkaline soils with low organic matter content, where the rate of degradation of herbicide residues is reduced (Sarmah et al. 1998, Hollaway et al. 2006). These residues may affect the germination and growth of sensitive annual species.

A monitoring program was established to investigate the effects of aerial herbicide application on the abundance of native ground-layer species (excluding grasses) and the condition of dominant woody perennial species of different life forms. This report presents the results of five years of monitoring from 2007 to 2011.

Methods

Site

Native vegetation monitoring was conducted at a single site near Tailem Bend (Figure 2). The vegetation comprises a mosaic of open mallee woodland and *Lomandra effusa* grassland. Open areas have introduced grasses and annuals. Monitoring is conducted twice annually; in winter, prior to spraying and in spring, following herbicide application. There can be no unsprayed control as the entire site must be sprayed to control branched broomrape.

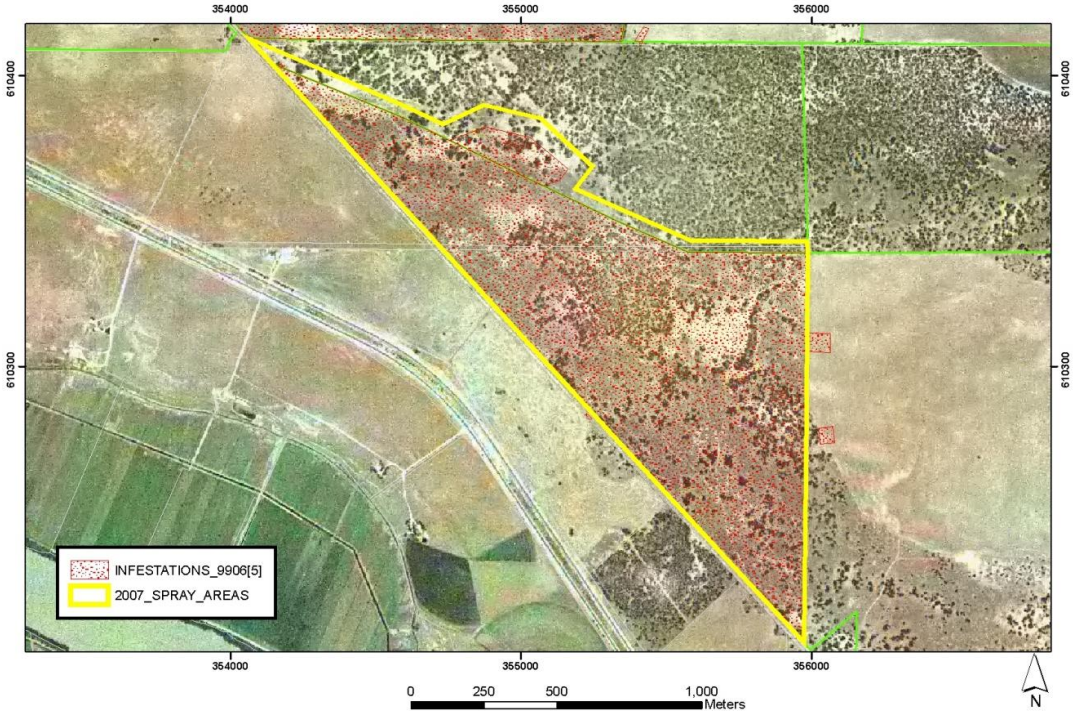


Figure 2. The monitoring site at the commencement of the monitoring period showing the broomrape infested area and the target spray area for 2007. This has been the minimum sprayed area for the monitoring period.

The site was sprayed by aircraft annually as part of the Branched Broomrape Eradication Program's aerial spray project. From 2007 to 2010 the site was sprayed with 3 g ha^{-1} of Ally® herbicide (600g metsulfuron methyl kg^{-1}) plus adjuvant, usually in early July. In 2011, the site was sprayed twice with Ally at the rate of 5 g ha^{-1} in late June and 3 g ha^{-1} in late August.

Perennial species

For perennial species monitoring, five plants of each of the following species were selected in 2007:

- *Eucalyptus socialis* (Mallee)
- *Callitris gracilis* (Murray Pine)
- *Pittosporum phyllaerioides* (Native Apricot)
- *Melaleuca lanceolata* (Dryland Teatree)
- *Clematis microphylla* (Old Man's Beard)
- *Enchylaena tomentosa* (Ruby Saltbush)
- *Maireana brevifolia* (Small-leaved Bluebush)

On each monitoring occasion, each plant was photographed from a marked photopoint and its condition scored between 0 and 10, with 0 for a dead plant and 10 for a plant in good condition (see Appendix A for details of scoring system). Plants that died during the course of monitoring were replaced by another individual. In 2010, monitoring of *Enchylaena tomentosa* was discontinued and five *Acacia pycnantha* (Golden Wattle) plants were selected for monitoring.

Ground-layer species

For monitoring ground layer herbs, ten by 1m^2 permanent quadrats were established in 2007. At each monitoring time, plots were photographed and the number of each native species occurring in the plot was counted. Counts did not include native grasses.

Analysis

For perennial species, pre-spray condition scores were compared to post-test scores for each monitoring year, and differences were tested using paired t-tests. Ground-layer species abundance was assessed using ANOSIM (analysis of similarities), to test for differences between pre and post-spray species composition for each sampling year. To assess whether there were any long-term declines in species composition, pre-spray abundances were tested across all sampling years. The data set used for ANOSIM analyses included all species that occurred in at least three plots across the monitoring period. The common winter-active plant *Ophioglossum lusitanicum* (Adder's Tongue) was not included in the analyses as it had usually become dormant by the spring post-spray monitoring.

Results and Discussion

Perennial species

The majority of woody perennial species had no change in condition score following aerial spraying (scores and analysis results in Appendix B). The trees *Eucalyptus socialis* and *Callitris gracilis* consistently had condition scores of 9 or 10. The small tree *Pittosporum phyllaerioides* scored between 8 and 10, with the exception of one plant that died after a fungal infection. The large shrub *Melaleuca lanceolata* also scored between 8 and 10 with the exception of one plant that was affected by borers. The small chenopod shrubs *Maireana brevifolia* and *Enchylaena tomentosa* had lower condition scores (between 6 and 10) but lower scores were not recorded following spraying.

The semi-woody climber *Clematis microphylla* appeared to be affected by the herbicide. *Clematis microphylla* condition significantly decreased following aerial spraying in 2011 and there was a trend for reduced condition scores in other years after spraying (Figure 3). Three dead plants were replaced in 2009. Although plants displayed a decrease in condition over time to 2009 (comparison of pre-spray scores), this trend was not present in 2010 and 2011, *i.e.* plants recovered condition prior to spraying the subsequent year. Possible herbicide symptoms in this species were dead reproductive panicles and discolouration of the leaves (

Figure 4).

It was decided to include a legume in later monitoring as these plants are relatively sensitive to Group B herbicides. *Acacia pcynantha* showed no decline in condition following spraying and flowered following spraying. One plant had complete flower drop following spraying in 2011 but this may not have been a result of the herbicide as it was not observed for other individuals.

Ground-layer species

With the exception of 2008, there was no difference between species composition prior to and following aerial spraying in the monitored plots (see Appendix C). In 2008, the post-spray monitoring was conducted at the end of October when most plants in plots had died due to the dry conditions. In all other years the monitoring was done at the end of August or early September, whilst plants persisted. Differences pre and post-spray in 2008 are therefore attributable to the timing of the second monitoring.

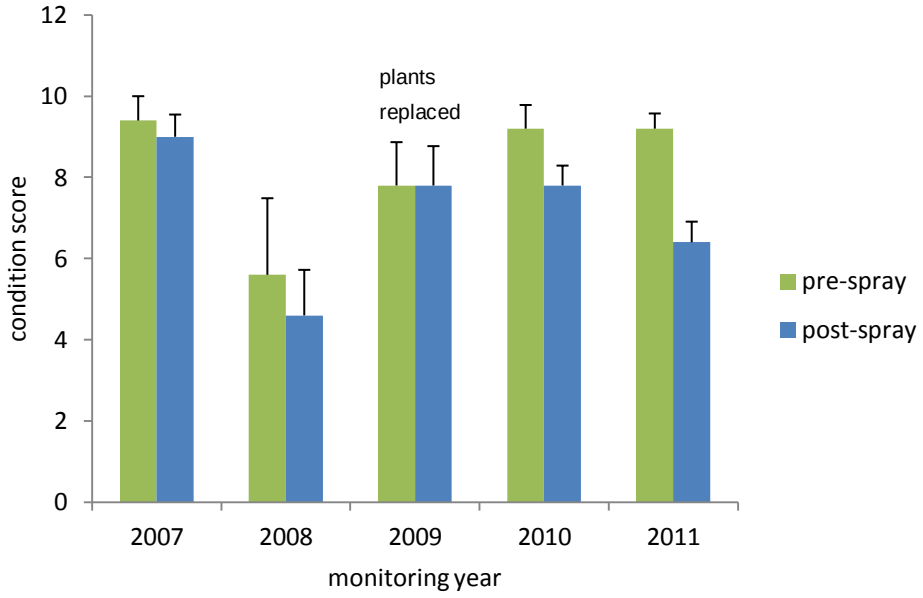


Figure 3. Condition scores for *Clematis microphylla*, prior to and following aerial spraying with the herbicide Ally. There was a significant difference between pre and post-spray condition scores in 2011 (t-test, $t = 4.8$, $p = 0.008$).

Figure 4. Discolouration of leaves and dead flower panicles of *Clematis microphylla* – image taken after spraying.

There was a significant difference in species composition over the course of monitoring. Plot composition in 2008, 2009 and 2010 was different to other years (

Figure 5). Without unsprayed control plots for comparison these compositional changes cannot be attributed to herbicide application. In addition, plots monitored in 2011 did not differ significantly from other years so compositional differences were not sustained.

The daisy species *Senecio pinnatifolius* and *Podolepis rugata* were common in 2007 but were rarely found in plots in 2010 and 2011 and their abundance has declined significantly over the course of monitoring (Figure 6). Observations of these plants following spraying and in an adjacent unsprayed area indicated that the loss of these plants may be due to the affects of spraying (

Senecio pinnatifolius in sprayed area September 2011

Senecio pinnatifolius in adjacent unsprayed area September 2011

Figure 7).

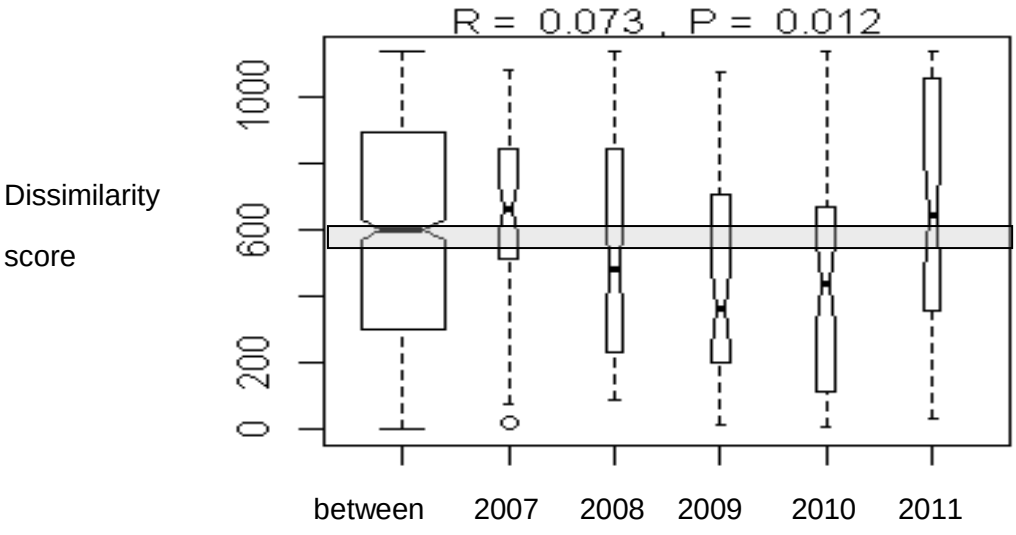


Figure 5. ANOSIM plot for pre-spray species composition comparisons across the five years of sampling. The mean dissimilarity scores for each year (indicated by the notch and bar in each column) differs from the mean dissimilarity scores between all plots (the column on the left) when the difference between years is significant. The area of non-significance is indicated by the shaded rectangle. The notches for years 2007 and 2009 do not overlap with the notch for the between plots comparison, so there is a significant difference between years.

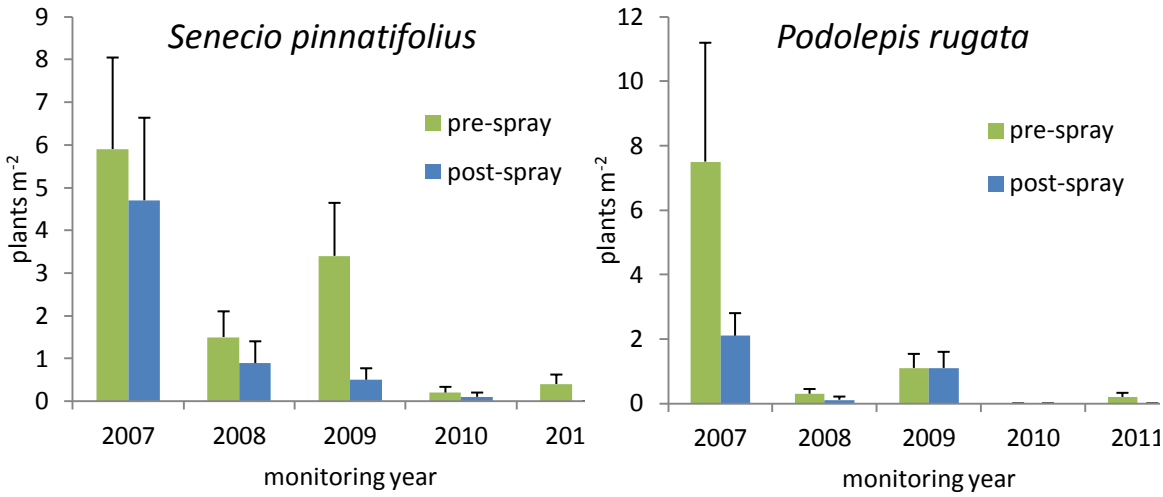


Figure 6. Pre and post-spray abundance of the species *Senecio pinnatifolius* and *Podolpeis rugata*. Each bar represents mean + 1SE, n = 10 plots.

Podolepis rugata prior to spraying June 2011

Podolpeis rugata after spraying September 2011

Podolepis rugata in adjacent unsprayed area September 2011

Podolepis rugata after spraying September 2010

Senecio pinnatifolius in sprayed area September 2011

Senecio pinnatifolius in adjacent unsprayed area September 2011

Figure 7. Potential spray damage to stands of the native daisies, *Podolepis rugata* and *Senecio pinnatifolius*.

Senecio pinnatifolius is sensitive to high applications of Group B herbicide, with plants dying at application rates of metsulfuron methyl at 9 g ha^{-1} and 27 g ha^{-1} (Yates 1997). However , no visual symptoms of herbicide toxicity were observed in plants where metsulfuron methyl was applied aerially at lower rates (Broese van Groenou and Downey 2006). Our data suggests that at rates of 3 g ha^{-1}, the herbicide may affect this species. However, the decline may be due to other factors as we did not measure abundance in sites that were not sprayed for comparison. Ally at the rate of 3 g ha^{-1} appears to have sub-lethal effects on *Podolepis rugata* but the higher rate or double spray of a total of 8 g ha^{-1} in 2011 appears to be lethal to this species.

Other common ground-layer species found in plots have not shown a decline in abundance over the monitoring period. This includes the geophytes *Hypoxis glabella* and *Wurmbea dioica,* the herbaceous perennials *Arthropodium milleflorum* and *Convolvulus remotus*, the fern *Ophioglossum lusitanicum* and the annual *Crassula* sp. Other plants were present in too few numbers to assess any trends in change in abundance over time.

Other observations

Several native grasses are sensitive to rates of metsulfuron methyl from 4.8 to 9.6 g ha^{-1} (Cole et al. 2003). Although grasses were not monitored in plots, several of the monitored plots were dominated by native grasses (mostly *Austrostipa* spp.) which showed no obvious decline over the monitoring period. *Austrostipa* flowered after the higher rate of metsulfuron methyl applied in 2011 (

Figure 8).

The introduced species *Schinus molle* (Pepper Tree) and *Asclepias rotundifolia* (Cotton-bush) also showed signs of herbicide damage following the higher rate of application in 2011 (

Figure 9). Suspected damage to old *Schinus molle* trees following aerial spraying has previously been reported near Mannum (Kym Bond, pers. comm., 23/3/2011).

Figure 8. Flowering native grasses after herbicide application in September 2011.

Conclusions

The monitoring program has no control sites in which to compare plant responses with sprayed sites and there is no site replication. There is therefore limited scope to infer that any changes in vegetation are attributable to herbicide use and to apply this over the entire quarantine area. However the monitoring

program can be used as an indicator for species that may be detrimentally impacted by the aerial application of low rates of the herbicide metsulfuron methyl.

Figure 9. *Schinus molle* (Pepper Tree) showing potential signs of herbicide damage following spraying in September 2011.

Aerial spraying of the herbicide metsulfuron methyl at the rate of 3 g ha^{-1} had no visual effect on the condition of several common woody perennial species in open mallee woodland. The perennial climber *Clematis microphylla* may have been affected by spraying as the condition of this species was lower following spraying than prior to spraying. This was significant in 2011 when a higher rate of metsulfuron methyl was applied.

There have been changes to the non-grassy ground vegetation during the course of monitoring. Compositional changes, i.e. the identity and abundance of species, have been driven by the decrease in abundance of the common species *Senecio pinnatifolius* and *Podolepis rugata*. These species have shown visual signs of damage that were sub-lethal in years when low rates of herbicide were applied, but lethal in 2011 under the higher herbicide rates. These were the only two short-lived non-woody native species that showed a significant decline in abundance post-spray.

References

Broese van Groenou, E. A. and P. O. H. Downey. 2006. Best Practice Guidelines for Aerial Spraying of Bitou Bush in New South Wales. Department of Environment and Conservation (NSW), Hurstville, NSW.

Brown, K., K. Brooks, S. Madden, and J. Marshall. 2002. Control of the exotic bulb, Yellow Soldier (*Lachenalia reflexa*) invading a Banksia woodland, Perth, Western Australia. Ecological Management & Restoration **3**:28-36.

Cole, I., T. Koen, J. Metcalfe, W. Johnston, and M. Mitchell. 2003. Tolerance of *Austrodanthonia fulva*, *Microlaena stipoides* and *Elymus scaber* seedlings to nine herbicides. Plant Protection Quarterly **18**:18-22.

Hollaway, K. L., R. S. Kookana, D. M. Noy, J. G. Smith, and N. Wilhelm. 2006. Persistence and leaching of sulfonylurea herbicides over a 4-year period in the highly alkaline soils of south-eastern Australia. Australian Journal of Experimental Agriculture **46**:1069-1076.

Pritchard, G. 1993. Evaluation of herbicides for the control of the environmental weed bridal creeper (*Asparagus asparagoides*). Plant Protection Quarterly **8**:40-43.

Sarmah, A. K., R. S. Kookana, and A. M. Alston. 1998. Fate and behaviour of triasulfuron, metsulfuron-methyl, and chlorsulfuron in the Australian soil environment: a review. Australian Journal of Agricultural Research **49**:775-790.

Toth, J. and M. A. Winkler. 2008. Bitou bush aerial spraying in New South Wales - what have we learned? Plant Protection Quarterly **23**:43-44.

Vitelli, J. and P. van Haaren. 2001. Chemical control of harungana (*Harungana madagascariensis*) shrubs in Queensland. **16**:41-43.

Yates, E. J. 1997. Integrated management of Bridal Creeper (*Asparagus asparagoides*) in the Coorong region. Honours Thesis, University of Adelaide, Adelaide

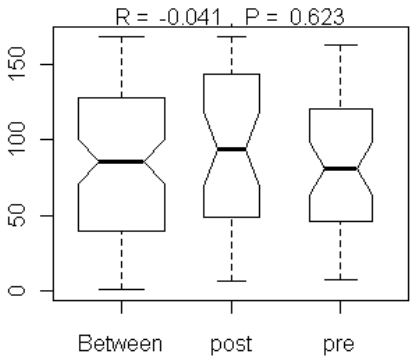
Appendix A . Condition scores used for assessing perennial species

Score	Description
0	Plant dead
1	Most of plant newly dead, remaining live portions of plant in poor condition and not likely to survive
2	Most of plant newly dead, remaining portions of plant are likely to survive
3	Most of plant diseased or has discoloured foliage, some newly dead biomass
4	At least 30% of plant alive but there is no strong growth or reproductive structures present
5	At least 50% of the plant is newly dead, although the live parts of the plant have potential for new growth
6	A substantial part of the plant (25-50%) has diseased or newly dead material and there is no new growth
7	Plant mostly as 8, but there is no strong new growth
8	Some minor discolouration of leaves or disease to stems or a little new dead material present, but there is still shoot growth
9	Plant mostly as 10, but there may be a very minor amount of diseased material or dead matter present
10	Mature leaves dark green with no signs of discolouration, new shoot growth evident, reproductive structures present

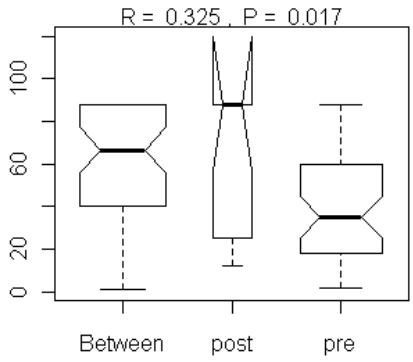
Appendix B. Perennial species condition scores

Species	Year	Pre-spray condition score (mean ± se)	Post-spray condition score (mean ± se)	T statistic	p-value (two-tailed)
Callitris gracilis	*2007*	9.8 ± 0.2	10 ± 0	-1	0.374
	2008	10 ± 0	9.8 ± 0.2	1	0.374
	2009	10 ± 0	9.4 ± 0.24	2.449	0.070
	2010	9.8 ± 0.2	10 ± 0	-1	0.391
	2011	9.8 ± 0.2	10 ± 0	-1	0.374
Pittosporum phyllaerioides	*2007*	10 ± 0	9.2 ± 0.4	2.138	0.099
	2008	9.4 ± 0.2	10 ± 0	-2.449	0.07
	2009	8.4 ± 1.1	8.6 ± 1.4	-0.535	0.621
	2010	9.6 ± 0.2	9.4 ± 0.4	0.535	0.621
	2011	9 ± 0.3	9 ± 0.4	0	1
Eucalyptus socialis	*2007*	9.6 ± 0.2	9.6 ± 0.2	no change	
	2008	9.4 ± 0.6	9.4 ± 0.6	no change	
	2009	9 ± 0.6	9.8 ± 0.2	-1.63	00.178
	2010	9.6 ± 0.2	9.6 ± 0.2	no change	
	2011	9.6 ± 0.2	8.6 ± 0.5	2.236	0.089
Melaleuca lanceolata	*2007*	10 ± 0	9.4 ± 0.6	1	0.374
	2008	9.8 ± 0.2	9.8 ± 0.2	no change	
	2009	8 ± 0.5	8.8 ± 0.8	-1.372	0.242
	2010	8.8 ± 0.6	8.4 ± 0.7	1	0.374
	2011	9 ± 1	8.8 ± 1.2	1	0.374
Maireana brevifolia	*2007*	7.4 ± 0.5	7 ± 0.4	1	0.374
	2008	7.8 ± 0.4	7.6 ± 0.2	0.535	0.621
	2009	7.8 ± 0.2	8.4 ± 0.2	-2.449	0.07
	2010	8.3 ± 0.3	8.2 ± 0.6	0.349	0.741
	2011	6.8 ± 0.5	7.7 ± 0.4	-1.19	0.289
Clematis microphylla	*2007*	9.4 ± 0.6	9 ± 0.55	0.492	0.648
	2008	5.6 ± 1.9	4.6 ± 1.1	1.118	0.326
	2009	4.6 ± 2.1	7.8 ± 1.1	plants replaced	
	2010	9.2 ± 0.6	7.8 ± 0.5	1.429	0.226
	2011	9.2 ± 0.4	6.4 ± 0.5	4.80	0.008
Enchylaena tomentosa	*2007*	8.2 ± 0.6	9.2 ± 0.6	-1.581	0.189
	2008	8.2 ± 0.5	8 ± 0.4	0.301	0.778
	2009	5 ± 0.4	5.8 ± 1	-0.873	0.432
Acacia pycnantha	*2010*	10 ± 0	10 ± 0	no change	
	2011	10 ± 0	10 ± 0	no change	

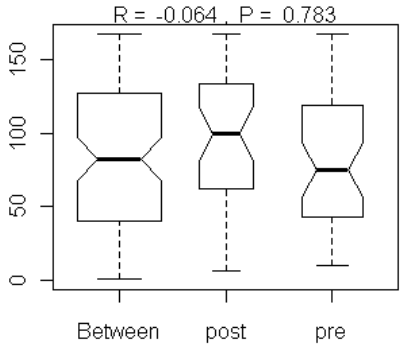
Appendix C. Annual species analysis



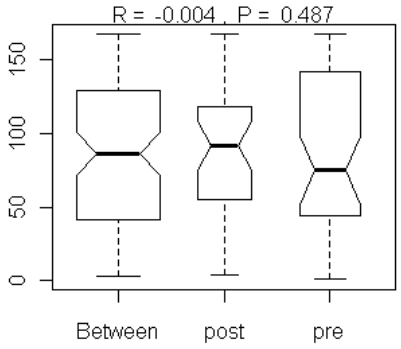
2007 ANOSIM plot comparing pre and post-spray annual species composition. Plots were not significantly different



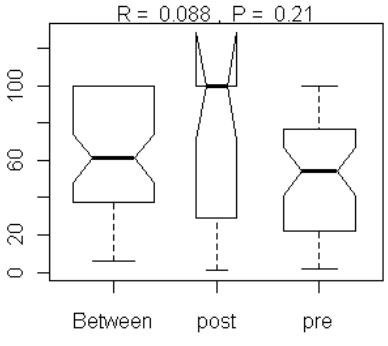
2008 ANOSIM plot comparing pre and post-spray annual species composition. Plots were significantly different



2009 ANOSIM plot comparing pre and post-spray annual species composition. Plots were not significantly different



2010 ANOSIM plot comparing pre and post-spray annual species composition. Plots were not significantly different



2011 ANOSIM plot comparing pre and post-spray annual species composition. Plots were not significantly different