BIOSECURITY SA
PIRSA

Buffel Grass Herbicide Trials

A SUMMARY OF RESULTS AND RECOMMENDATIONS

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

Buffel Grass Herbicide Trials

Information current as of 15 November 2016

© Government of South Australia 2016

Disclaimer

PIRSA and its employees do not warrant or make any representation regarding the use, or results of the use, of the information contained herein as regards to its correctness, accuracy, reliability and currency or otherwise. PIRSA and its employees expressly disclaim all liability or responsibility to any person using the information or advice.

Produced by

Dr Jane Prider and Troy Bowman with funding through the Native Vegetation Councils Significant Environmental Benefits Grants Scheme and the South Australian Arid Lands NRM Board.

Native Vegetation Council

All enquiries

Ross Meffin- Principal Biosecurity Officer, Weeds, Biosecurity SA (PIRSA)
Building 1, Soil Water Environs, Entry 4, Waite Rd, Urrbrae, SA
GPO Box 1671, Adelaide SA 5001
T 08 83039620 F 08 83039555 M 0484587217
E ross.meffin2@sa.gov.au

Table of Contents

ABSTRACT

Buffel grass herbicide trials were conducted at four sites within South Australia. The main trial compared the effectiveness of Roundup™, Taskforce™, a mixture of these and Graslan™ for buffel grass control. Fusilade™ and Weedmaster Duo™ were included at some sites. Other trials compared different rates of Roundup Biactive™ and Roundup™ following tussock burning. The aim of the trials was to identify which herbicides controlled mature buffel grass tussocks and suppressed the recruitment of juveniles whilst minimising the effects on non-target vegetation.

Roundup™ and Taskforce™ mixtures resulted in the highest mortality to mature buffel grass tussocks across all sites and these herbicides alone were also effective in at least two sites. Applications of Taskforce 3 L ha^{-1} provided some pre-emergence control of buffel grass seedlings up to 20 months after application at two sites. Juvenile recruitment was not suppressed by Roundup™ at any of the sites and was higher than all other treatments in the year following herbicide application at two sites. Graslan™ killed all mature tussocks at two sites but not at the third site. This herbicide provided two years of pre-emergence control of juveniles. Weedmaster Duo™, was not as effective as Roundup™ in killing tussocks. Most mature tussocks survived in Fusilade™ treatments but juvenile recruitment was suppressed, however recruitment was also suppressed in untreated plots. Tussock mortality was highest (80%) when Roundup Biactive™ was applied at rates of 5 L ha^{-1}. Spraying burnt tussocks with Roundup Powermax™ six weeks after burning resulted in the highest mortality although overall buffel cover was reduced in all burnt plots regardless of herbicide application. In all herbicide treatments, except Roundup™ high rate treatments where there was high juvenile recruitment, buffel grass cover was depleted from 40% before treatment to less than 10% a year following herbicide application. After two years, with recruitment of new plants, buffel grass cover in all herbicide treated plots was comparable to untreated plots. The cover of native vegetation at all sites was low and varied between years. This limited the conclusions that could be made about the impacts of herbicide treatments. Roundup™ was detrimental to shrubs but had no evident impact on regenerating grasses and forbs. The inclusion of low rates of Roundup™ in mixture with Taskforce had no detrimental impact on shrubs. The abundance of the grass *Aristida holathera* was reduced in Taskforce™ treatments but no other negative effects of this herbicide on other native grasses and forbs was detected. Graslan™ had the most severe off-target effects with very few species surviving in plots treated with this herbicide.

Management recommendations

Roundup™ and Taskforce™, or mixtures of these two herbicides, offer several control options for buffel grass when used in conjunction with other management measures. Choice will depend on the native species present, the size of the buffel grass infestation and their growth stage and the amount of follow-up spraying that can be done. Any of these herbicides can be used for spot-spraying although fast-acting Roundup™ should be used if plants are about to set seed. This herbicide is also only effective if plants are actively growing. Slow-acting Taskforce™ is a better choice for plants that may not be actively growing. Addition of Roundup™ to Taskforce™ provides some long-term control and prevents seed set. For large core populations, boom-spray with Roundup™ is only suitable for stands where other native perennial species are infrequent. Taskforce™ is a more suitable choice where there are native species although some native grasses may be adversely affected. Roundup™ applications require frequent follow-ups but re-treatment after Taskforce™ applications may not be necessary for up to 18 months. This herbicide is therefore more suitable for remote or isolated infestations. Due to its severe impacts on other native species, Graslan™ is not recommended.

INTRODUCTION

Herbicides are one of a number of tools that can be used in a control program for the management of buffel grass. Herbicides with the active ingredients glyphosate and flupropanate, that have been effective in controlling other perennial tussock grasses, are recommended for buffel grass control but their efficacy has not been formally tested. Flupropanate is a slow-working residual herbicide. Residues in the soil can provide pre-emergent control of weed species for one to two years after application. Glyphosate is more effective when applied to freshly, growing foliage and is fast-acting but has no residual activity. These herbicides may be used alone or in mixture. Although buffel grass is primarily a summer-active grass, it may rapidly grow to reproductive maturity at any time of the year given a suitable rainfall event. Flupropanate may not prevent seed set in rapidly developing plants. Glyphosate may kill mature tussocks but as there is no residual activity recruitment may not be suppressed.

The main trial was designed to test the following hypotheses:

1. Glyphosate will provide control of mature tussocks but juvenile recruitment will not be suppressed and this non-selective herbicide will have negative impacts on desirable vegetation.
2. Flupropanate will control mature tussocks and suppress juvenile recruitment and the selectivity of this herbicide will reduce the negative impacts on desirable vegetation.
3. Glyphosate and flupropanate mixtures will prevent seed set and control mature tussocks but the glyphosate in the mixture will have negative effects on desirable vegetation, although lower rates of glyphosate in the mixtures will reduce these impacts as compared to glyphosate alone.

Other herbicides were included that could offer specific control strategies in different situations. The aim of the trials was to identify the herbicides and application rates most effective in:

- killing mature buffel grass tussocks
- suppressing buffel grass germination
- reducing buffel grass cover
- controlling buffel grass whilst minimising impacts on native vegetation

METHODS

The results for three separate trials are described in this report:

1. Main trial
2. Roundup Biactive™ trial
3. Burn trial

Four trial sites were established across the latitudinal range of buffel grass in South Australia. The sites north to south respectively are named APY, NW, BB and ALBG.

At each site for each trial, three replicate plots were laid out within uniform well-established buffel grass stands. Herbicide treatments were applied to a single 30 m × 10 m subplot within each plot (Figure 1). Herbicides were applied with a calibrated knapsack sprayer. Graslan™ was applied by first mixing with 1.5 L of sand and hand-broadcasting over the subplot.

The main herbicide trial was conducted at BB, APY and ALBG and included the herbicides in Table 1.

At the NW site, treatments were different rates of Roundup Biactive™ (active ingredient glyphosate 360 g L^{-1}), Fusilade™, the physical removal of tussocks and untreated controls. Roundup Biactive™ was applied at rates of 2, 3, 4, and 5 L ha^{-1} and Fusilade™ treatments as in Table 1.

For the burn trial, Roundup™ was applied at a rate of 3 L ha^{-1} to tussocks either 2, 4 or 6 weeks after they had been burnt. Controls were also burnt but not treated with herbicide. Monitoring of this trial occurred before burning and herbicide application, two weeks after application of the 6 week treatment and 8 months later.

Table 1. Herbicide treatments in the main trial at ALBG, APY and BB. Note that the Roundup treatments had the same rate of product applied per hectare but the high rate had more added water to improve coverage. In the Roundup™ + Taskforce™ mixtures the same rate of Taskforce™ was applied and only the amount of Roundup varied between the high and low rate treatments.

Treatment L = low rate H = high rate	Herbicide	Active ingredient	Rate/ha	Application rate /ha	Site
C	Nil	Nil	Nil	Nil	all
RL	Roundup Powermax™	Glyphosate (540 g L^{-1})	4 L	300 L	all
RH	Roundup Powermax™	Glyphosate (540 g L^{-1})	4 L	600 L	all
RTL	Roundup Powermax™ + Taskforce™	Glyphosate (540 g L^{-1}) Flupropanate (745 g L^{-1})	250 ml 3L	1000 L	all
RTH	Roundup Powermax™ + Taskforce	Glyphosate (540 g L^{-1}) Flupropanate (745 g L^{-1})	415 ml 3L	1000 L	all
TL	Taskforce™	Flupropanate (745 g L^{-1})	1.5 L	1000 L	all
TH	Taskforce™	Flupropanate (745 g L^{-1})	3 L	1000 L	all
GL	Graslan™	Tebuthiuron (200 g kg^{-1})	2 kg	2 kg	all
GH	Graslan™	Tebuthiuron (200 g kg^{-1})	4 kg	4 kg	all
FL	Fusilade™	Fluazifop-P (212 g kg^{-1})	3.3 L	300 L	BB, ALBG
FH	Fusilade™	Fluazifop-P (212 g kg^{-1})	6.6 L	300 L	BB, ALBG
WL	Weedmaster Duo™	Glyphosate (470 g L^{-1})	4.5 L	300 L	APY, ALBG
WH	Weedmaster Duo™	Glyphosate (470 g L^{-1})	4.5 L	600 L	APY

Plant responses were measured at three times during the main trial and Roundup Biactive trial; prior to herbicide application in the winter of 2014 and again in winter 2015 and 2016. In all trials, data were collected from three, 1 m^2 quadrats placed in the same location within each subplot at each monitoring time. The numbers of juvenile and mature buffel grass plants were counted in each quadrat and the cover of buffel grass, other grass and forb species and bare ground were assessed by counting their presence in each of 100 divisions of the quadrat (Figure 1). The number of live and dead tussocks were also counted in each subplot. Other subplot variables were visual estimates of the % cover of other species and counts of shrubs.

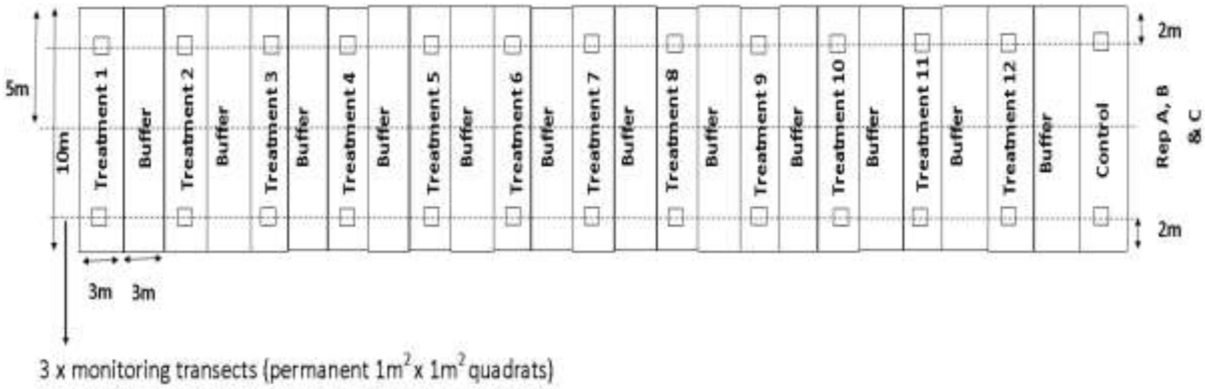

Figure 1. Layout of treatment plots replicated three times at each site. Within each plot, herbicide treatments were randomly assigned to subplots. Response variables were measured in these subplots or from three quadrats located within each subplot.

Statistical analyses

Quadrat data were summed for the three quadrats in each replicate. Summed data were used for analyses but converted to mean values for the charts in this report. For the main trial, the analysis included herbicide treatments that were common across all three sites unless otherwise stated.

Control of mature tussocks

Changes in numbers of tussocks, were assessed by examining the difference between the pre-treatment values and the two post-treatment values. Analysis of variance (ANOVA) was used to check that all treatment subplots and sites had the same number of tussocks prior to herbicide application. Repeated measures multiple analysis of variance (rmMANOVA) was used to test for differences between the two post-treatment monitoring times, among herbicide treatments, sites and all interactions between these in the main trial. Site and treatment interactions were removed from the fitted models if not significant. The effect tests for the Roundup Biactive™ and Burn Trial included time, treatment and their interaction (no site effect). Contrasts were used to test for differences between controls and all other herbicide treatments in rmMANOVA ($\alpha < 0.05$). Student's t test was used for multiple pairwise comparisons following ANOVA with significance levels $\alpha < 0.05$.

The percentage of surviving tussocks was calculated from the subplot data:

$$\%\,survival = \frac{mature\ tussocks\ t1 - dead\ tussocks\ tn}{mature\ tussocks\ t1} \times 100$$

where *t1* is monitoring time 1 (2014) and *tn* is monitoring time 2 or 3 (2015 or 2016). Dead tussocks were only recorded at BB in 2015. Tussocks were not counted at the APY site in 2014 so the number of tussocks in 2015 plus the number of dead tussocks in 2016 was used as the *t1* value for this site. All tussocks were recorded as dead in 2016 if no mature tussocks were recorded from that subplot in that year. All herbicides used at each site were included in this analysis as sites were examined separately. Subplots that received herbicide treatments with numbers of tussocks greater than zero were compared to untreated control plots using Dunnet's tests. Zero values were not included in these tests.

Suppression of juveniles

Numbers of juveniles per quadrat varied greatly among sites and years therefore separate analyses were conducted for each site and year following treatment (2015 and 2016). These data sets had a strong positive skew therefore a negative binomial Generalised Linear Model (GLM) with a log link was fitted. Models were fitted with and without the Site and Treatment terms and the model fits compared using a Log-Likelihood Ratio test. Differences between each site were also tested using negative binomial GLMs. P-values for these tests were adjusted to account for multiple test on the same data set using a Bonferroni correction (α / n). Over-dispersion in these models was checked by examining the ratio of the residual deviance and the residual degrees of freedom. The 2016 ALBG data were not analysed as there were only four seedlings found in two subplots in this year. All herbicides used at each site were included in these analyses.

Buffel cover

Changes in buffel grass cover in quadrats were assessed using the same method as changes in tussock numbers.

Non target effects

Shrubs were only present in sufficient numbers for statistical analysis at the ALBG site. ANOVA was used to assess whether the number of shrubs differed between the subplots prior to the application of treatments in 2014. A negative binomial model with a log link was used to assess whether the number of shrubs differed between treatments after herbicides were applied (separate tests for 2015 and 2016).

To examine the effects of herbicide treatments on other grasses and forbs, non-parametric analysis of similarities (ANOSIM) was used to test for differences in species composition between treatments for each year of monitoring at each site. Due to a lack of statistical power, data from high and low rate treatments for each herbicide were pooled to increase the number of replicates. Subplots with no plants in that year were removed from the analysis and for each test only species present in at least 3 subplots were included. The Bray-Curtis index was used to construct a dissimilarity matrix, and the rank-based test on dissimilarity scores used 999 random permutations. Treatments were compared using boxplots but there was insufficient replication to determine whether differences between treatments were significant. The most common species in sites and years where differences between treatments were observed were examined further. Data from ALBG were square-root transformed and the effect of Treatment was tested with Welch ANOVA as variances were not equal. Negative binomial GLMs were used to test the effect of Treatment on the abundance of two common grasses from APY. These analyses were restricted to herbicide treatments common across all sites.

Changes in cover of plants other than buffel grass and bare ground cover were assessed using the same method as changes in tussock numbers.

Software

The JMP statistics platform (Ver. 13.0.0, SAS Insitute 2016) was used to fit rmMANOVA and ANOVA models. Negative binomial GLMs were fitted in the R platform (Ver. 3.1.2, The R Foundation for Statistical Computing, 2014). The statistic software R (ver 3.1.2, R Development Core Team 2014) with the package vegan (ver 2.3-5, Oksanen et al 2009) was used for the multivariate analysis of community dissimilarity. Reported are test statistics, their associated degrees of freedom and p-values.

RESULTS

Control of mature tussocks

At the start of the main trial there was a higher density of mature buffel grass tussocks at the BB site than at APY which had a higher density of tussocks than ALBG (Site effect, ANOVA, $F = 13.11_2$, $p < 0.0001$). Mean tussock densities (tussocks m^{-2}) were BB: 2.8, APY: 2, and ALBG: 1.5. There were no significant differences in tussock density between the treatment subplots across the sites prior to the application of treatments (Treatment effect, ANOVA, $F = 0.41_8$, $p = 0.91$).

In all subplots there was a decrease in the number of mature live tussocks following herbicide treatments (Figure 2). There was a significant difference between treatments ($p = 0.0014$, Appendix Table 4). The herbicide treated plots had a significant reduction in the number of mature tussocks compared with untreated control plots (Contrast $p = 0.0002$).

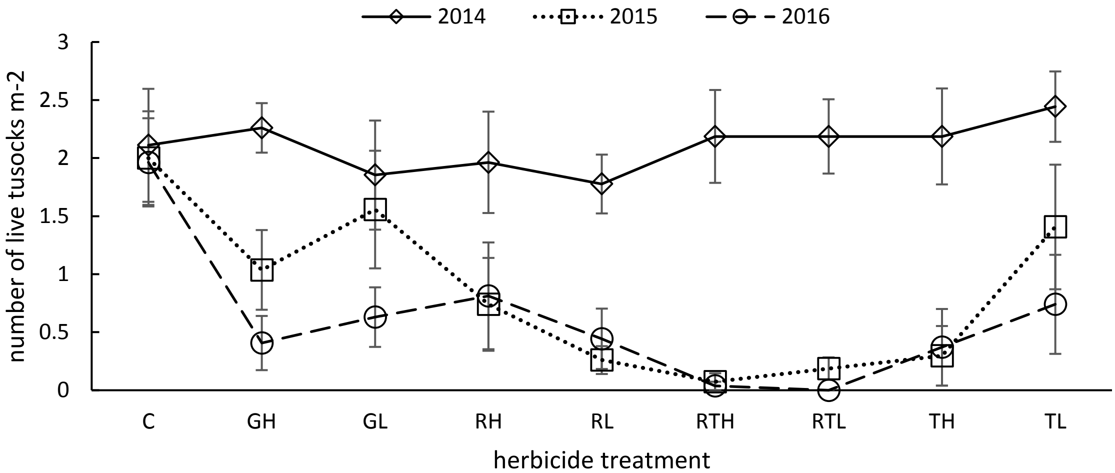

Figure 2. Number of tussocks in each monitoring year in different herbicide treatments in the Main Trial. Points are mean ± 1 standard error, *n* = 9. See Table 1 for full names of herbicide treatments. (Connecting lines are for ease of interpretation and do not indicate a relationship between the x axis terms.)

There was a further decrease in tussocks from 2015 to 2016 (Time effect, $p = 0.028$, Appendix Table 4) but there was a Time X Site interaction therefore a reduction did not occur at all sites ($p = 0.028$, Appendix Table 4). At the APY site there was no further reduction in the number of tussocks after 2015 as there was at BB and ALBG.

To examine differences between herbicide treatments, the reduction in tussocks between treatments in 2016 was compared. By 2016, all herbicide treated subplots had a greater reduction in number of live mature tussocks than the untreated controls. Roundup™ + Taskforce™ plots had the largest decrease in numbers of tussocks but this only differed significantly from the Roundup™ H treatments (Figure 3).

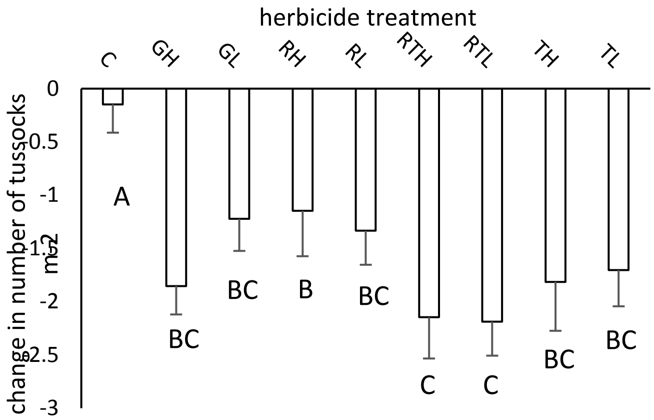

Figure 3. Change in number of tussocks from pre-treatment levels in 2014 to final tussock numbers in 2016 in plots treated with herbicides in the Main Trial. Bars labelled with different letters were significantly different (Student's t test, $\alpha < 0.05$). Bars show means and 1 standard error, $n = 9$. See Table 1 for full names of herbicide treatments.

Tussock mortality

Overall, Roundup™ and Taskforce™ (3 L ha^{-1}) and the mixture of these products were effective in killing mature tussocks. Survival was significantly less in these herbicide treatments than untreated controls (Table 2). Roundup™ + Taskforce™ gave the most effective control across all sites. Roundup™ alone was inconsistent and killed most tussocks at two sites but was less effective at the APY site. Taskforce™ alone was also inconsistent and tussock survival at the low rate (1.5 L ha^{-1}) at the BB site did not differ significantly from controls. Graslan™ also performed poorly at BB. Survival of tussocks treated with this herbicide at BB did not differ from controls. Conversely, Graslan™ (4 kg ha^{-1}) killed all tussocks at APY and ALBG. Fusilade™ did not kill a significant number of tussocks at either the ALBG or BB sites. Weedmaster Duo™ killed approximately half of tussocks but the number of dead tussocks in this treatment only differed significantly from controls at the APY site at the high application rate.

At the BB site where there was data for 2015 and 2016, there was only an increase in mortality from 2015 to 2016 in the Taskforce™ 3 L ha^{-1} treatment (Table 2).

Roundup Biactive™ applied at the rate of 4 or 5 L ha^{-1} significantly reduced the number of mature buffel grass tussocks within subplots (ANOVA, $F = 7.83_6$, $p = 0.0008$,Figure 4).

Spraying plants 4 to 6 weeks after burning gave better control of mature tussocks than spraying only two weeks after burning (ANOVA, $F = 4.11_3$, $p = 0.049$, Figure 5).

Table 2. % survival of mature buffel grass tussocks in each herbicide treatment plot in 2016 and the BB site in 2015 (mean ± 1 standard error, n = 3). Values in bold within a site were different to controls at that site (values > 0, Dunnet's Test, $\alpha < 0.05$).

		Site			
Treatment	rate	BB 2015	BB 2016	ALBG 2016	APY 2016
Control		71 ± 30	91 ± 3	82 ± 14	96 ± 6
Fusilade™	low	100	92 ± 8	99 ± 1	-
Fusilade™	high	100	93 ± 6	98 ± 2	-
Graslan™	low	45 ± 24	45 ± 1	**0**	**21 ± 10**
Graslan™	high	67 ± 21	62 ± 14	**0**	**0**
Roundup™	low	**0**	**0**	**1 ± 1**	**54 ± 10**
Roundup™	high	27 ± 27	**24 ± 24**	**0**	**36 ± 6**
Roundup™ + Taskforce™	low	**0**	**22 ±11**	**0**	**1 ± 1**
Roundup™ + Taskforce™	high	**0**	**0**	**0**	**5 ± 5**
Taskforce™	low	96 ± 4	88 ± 11	**0**	**8 ± 6**
Taskforce™	high	67 ± 33	**37 ± 24**	**0**	**1 ± 1**
Weedmaster	low	-	-	55 ± 29	52 ± 29
Weedmaster	high	**-**	**-**	-	**46 ± 9**

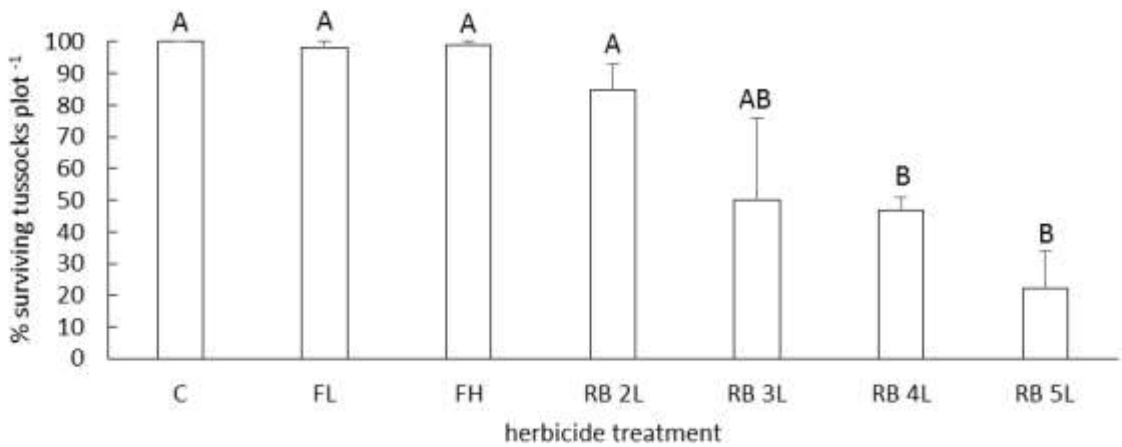

Figure 4. Survival of mature buffel grass tussocks treated with different rates (L) of Roundup Biactive™ (RB) or Fusilade™ (F) and untreated controls (C). Bars are means + 1 Standard Error, n = 3. Bars labelled with different letters are significantly different (Student's t test, $\alpha < 0.05$).

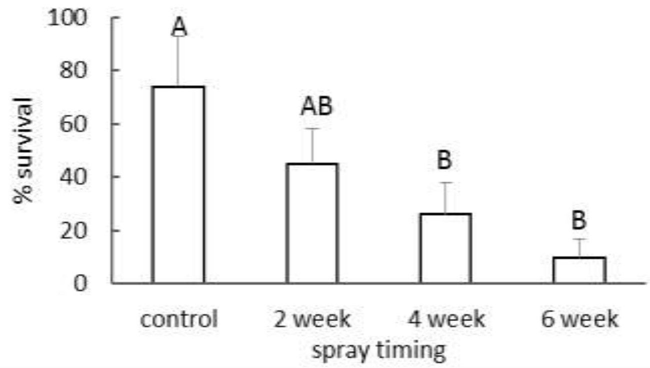

Figure 5. % survival of mature buffel grass tussocks treated with Roundup™ at different time intervals after burning. Bars are means + 1 standard error, n = 3. Bars labelled with different letters are significantly different (Student's t test, $\alpha < 0.05$).

Suppression of juveniles

There were differences between sites and treatments in the number of juveniles in quadrats in 2015 however, there was an interaction between Site and Treatment, meaning the differences among treatments were not consistent across sites (Site X Treatment interaction, $p < 0.0001$, AppendixTable 5). Each site was therefore analysed separately and all herbicides used at each site were included.

At the ALBG site the number of juveniles differed between herbicide treatments in 2015 ($LR = 36.76_{11}$, $p < 0.0002$). There were more juveniles in Roundup™ treatments and the Taskforce™ L treatments in 2015 than in other subplots (Figure 6). Several treatments had no juveniles in 2015.

At BB, the number of juveniles also differed between treatments in 2015 ($LR = 29.94_{11}$, $p < 0.002$). The Roundup™ L treatment and one replicate in the Roundup™ H treatment had more juveniles than all other subplots in 2015 (Figure 6).

At the APY site there was no difference in the number of juveniles between herbicide treatment subplots in 2015 (ANOVA, $F = 0.90_{10}$, $p = 0.55$, Figure 6).

At the BB and APY sites there was recruitment in 2016. There were differences between treatments (Treatment effect, $p < 0.01$, Appendix Table 5) that were consistent between these two sites. In 2016 at BB and APY, juvenile recruitment was less in the controls and Graslan™ H subplots than those treated with Roundup™, Taskforce™, and Roundup™ + Taskforce™ (Student's t test on log transformed data, $p < 0.006$, Figure 7). The plot data collected at BB also supports this trend of fewer juveniles in Taskforce™ subplots in 2015, compared with Roundup™ alone, but not in 2016 (Figure 8). However these differences were not statistically significant.

The number of juvenile plants was very low at the NW site (Roundup Biactive™ trial). There was no significant difference in the number of juveniles per quadrat among treatments in 2015 or 2016. There was an average of 0.6 ± 0.2 juveniles m^{-2} in 2015 and 4.6 ± 0.9 m^{-2} in 2016.

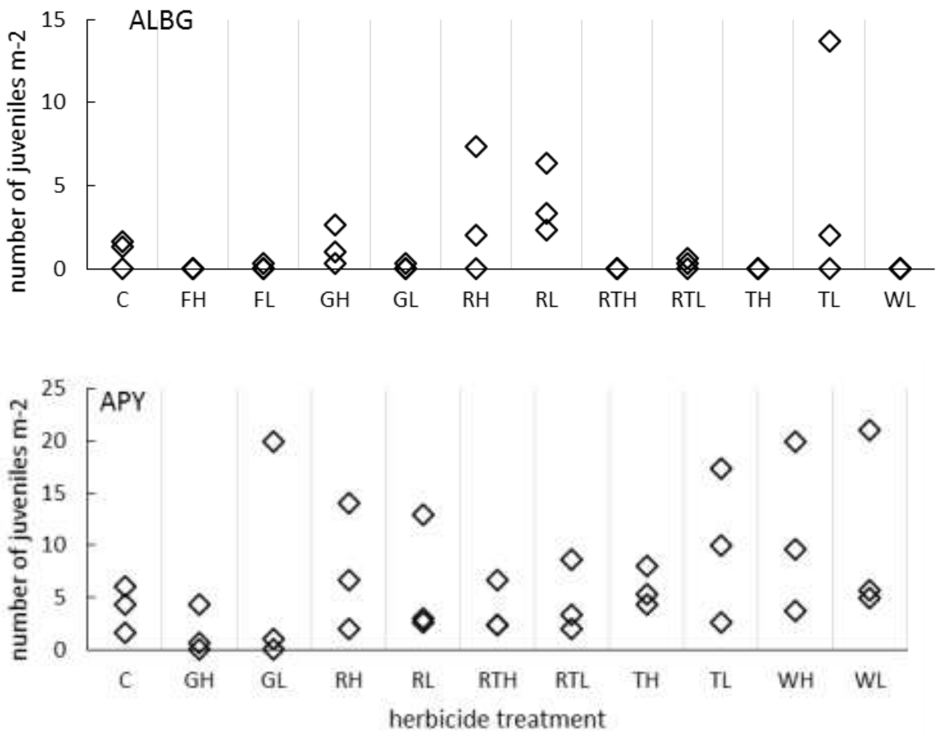

Figure 6. Scatterplots of number of juveniles in each herbicide treatment in 2015 at each site. (*n* = 3). See Table 1 for full names of herbicide treatments.

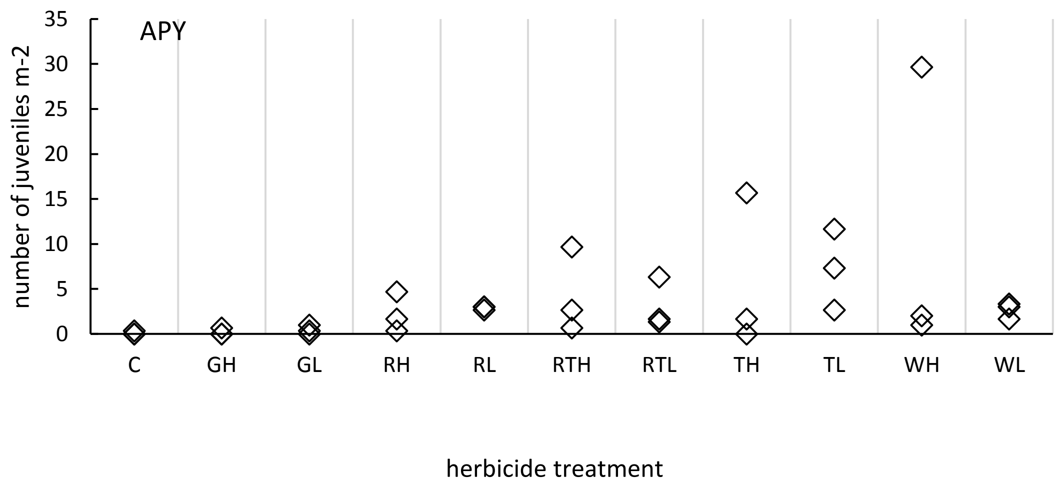

Figure 7. Scatterplots of number of juveniles in quadrats in 2016 at the BB and APY sites. See Table 1 for full names of herbicide treatments.

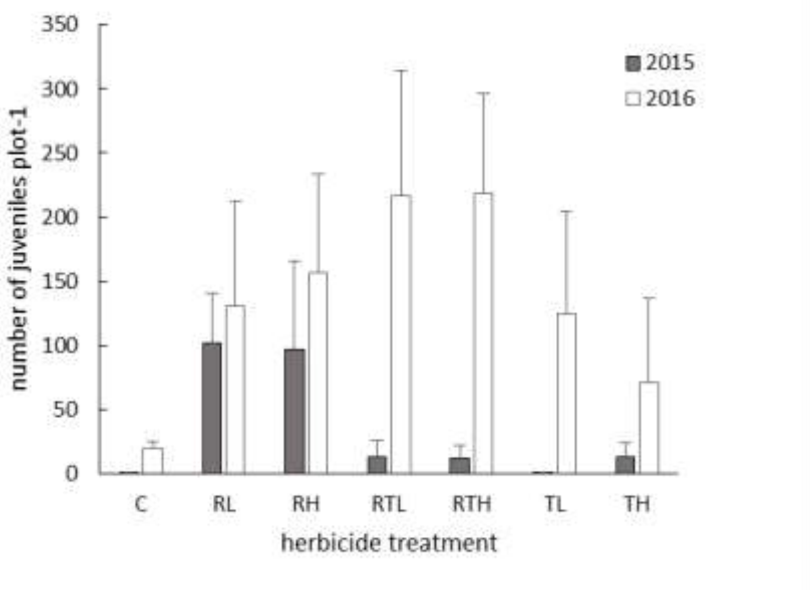

Figure 8. Counts of juveniles in plots with Roundup™ and Taskforce™ at the BB site. Bars are means and 1 standard error, n = 3. See Table 1 for full names of herbicide treatments.

Buffel cover

There were significant differences in buffel cover among sites before treatments were applied in 2014 (ANOVA, $F = 3.5_2$, $p = 0.04$). The ALBG site had the highest mean buffel cover (45%), followed by APY (35%) and BB (33%). All subplots within sites did not differ significantly in buffel cover before herbicide treatments were applied (ANOVA, $F = 0.88_8$, $p = 0.53$).

The ALBG site had the greatest decrease in buffel cover after herbicide treatment (Site effect, $p = 0.015$, Appendix Table 6). In all sites there was a loss of buffel cover in the year following herbicide application (2015) in herbicide treated subplots. Buffel cover decreased in untreated control subplots in 2015 and in 2016 (Time X Treatment interaction, p = 0.017, AppendixTable 6) but in herbicide treatments buffel cover increased or maintained 2015 cover in 2016 (Figure 9).

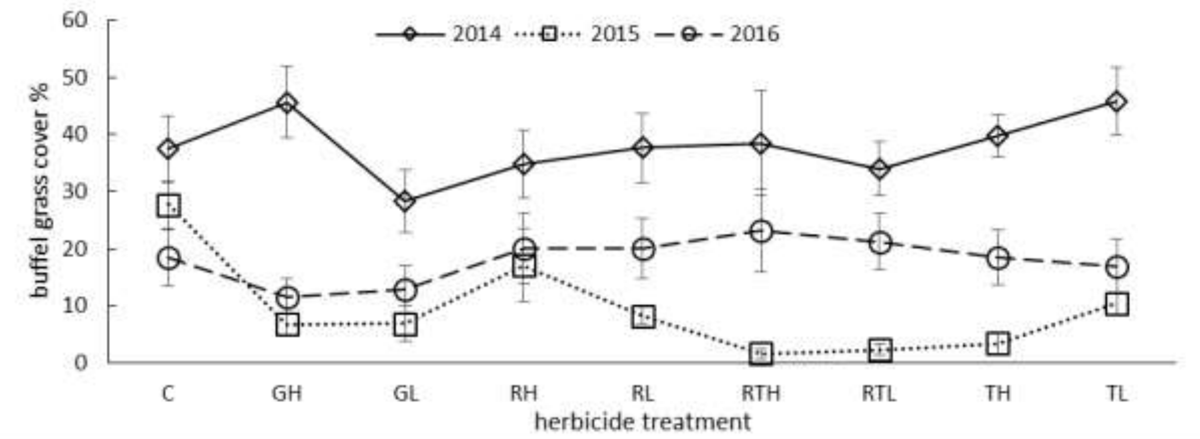

Figure 9. % buffel cover in plots in the three years of monitoring combined across sites in the Main Trial (means ± 1 standard error, n = 9). See Table 1 for full names of herbicide treatments. (Connecting lines are for ease of interpretation and do not indicate a relationship between the x axis values.)

In the Burn Trial, buffel cover in herbicide treated subplots did not differ from burnt control subplots (rmMANOVA, Treatment effect, Exact $F = 0.55_{3,8}$, $p = 0.66$). There was a significant decrease in buffel cover in all subplots (rmMANOVA, Time effect, Exact $F = 26.8_{1,8}$, $p = 0.0009$), from a mean of 53% before burning and applying herbicides to 0.4%, eight months later.

Non-target effects

Shrubs

At the ALBG site the number of shrubs did not differ significantly among subplots in 2014 (ANOVA $F = 0.73_{11}$, $p = 0.7$). Following herbicide application, there were significant differences among treatment subplots in the number of shrubs in 2015 ($LR = 21.83_{11}$, $p = 0.026$) and 2016 ($LR = 21.82_{11}$, $p = 0.026$). There were 13 *Maireana pyramidata,* and *Nitraria* sp. shrubs in Graslan™ subplots in 2014 and only 1 of these 13 shrubs present was alive in 2016. The surviving shrub was *M. pyramidata*. The number of shrubs also decreased in Roundup™ plots by 2016. There were 19 shrubs present in 2014 and 3 shrubs in 2016. The *M. pyramidata* and *Atriplex* sp. in the plot in 2014 were all killed and the 3 shrubs present in 2016 were *Nitraria* sp.

For the Burn Trial at this site, large variance in the numbers of shrubs between plots reduced the power of statistical tests to detect differences. Only one of the 26 shrubs in the plots treated with Roundup™ 6 weeks after burning was alive in 2016. Only 2 of the 15 shrubs in the plots treated with Roundup™ 4 weeks after burning were alive in 2016. Conversely, up to half of the shrubs in the 2 week and control treatments were alive in 2016. Therefore there is evidence that the Roundup™ treatments killed shrubs when herbicides were applied 4-6 weeks after burning.

At BB none of the five shrubs in the Graslan™ plots survived. Two out of two shrubs died in the Taskforce™ L plot and one of five shrubs in the Taskforce™ H plot. There were insufficient shrubs present for a statistical analysis.

There were no shrubs at the APY site.

Species composition (forbs and grasses)

Species composition did not differ significantly among any of the subplots at any site before herbicide treatments were applied in 2014.

At ALBG in 2015, a year after herbicide application, there was a significant difference in species composition among subplots (ANOSIM, $R = 0.3$, $p = 0.001$). Graslan™ subplots were different from all other treatments and two Graslan™ subplots had no plants present. Fusilade™ subplots were also dissimilar to other treatments in 2015 (Figure 10). The difference among subplots persisted into 2016 (ANOSIM, $R = 0.418$, $p = 0.001$) but by this year only Graslan™ subplots were of a different composition to other treatments (Figure 10).

There was a significant difference in species composition among treatments at APY in 2015 (ANOSIM, $R = 0.226$, $p = 0.001$). All Graslan™ H subplots had no plants in 2015 and were not included in the analysis. The Graslan™ L subplots were also different from some other treatments but not from untreated controls. Roundup™ and Taskforce™ subplots were dissimilar to controls in 2015 (Figure 11). By 2016, only one Graslan™ subplot had plants present so this treatment was removed from the analysis. There was a significant difference in species composition among the other treatments (ANOSIM, $R = 0.41$, $p = 0.001$). In 2016, the Roundup™ + Taskforce™ and Taskforce™ subplots had a different species composition to the other treatments (Figure 11).

The BB and NW (Roundup Biactive™ Trial) subplots did not differ significantly in species composition during any of the monitoring years following herbicide application.

Variability in species abundance among sites and years and small replicate size limited the conclusions that could be made about the effects of herbicides on general patterns of species composition. Individual analyses were undertaken for some common species that changed in abundance.

Aristida holathera (Erect Kerosene Grass) is a perennial grass that was found across all plots at the APY site in 2014. The abundance of *Aristida* decreased across all subplots in 2015, including controls. The grass reached 2014 levels in 2016 in controls and subplots treated with Roundup™ alone. *Aristida* abundance remained low in subplots that had been treated with Taskforce™ or Roundup™ + Taskforce™ (Table 3).

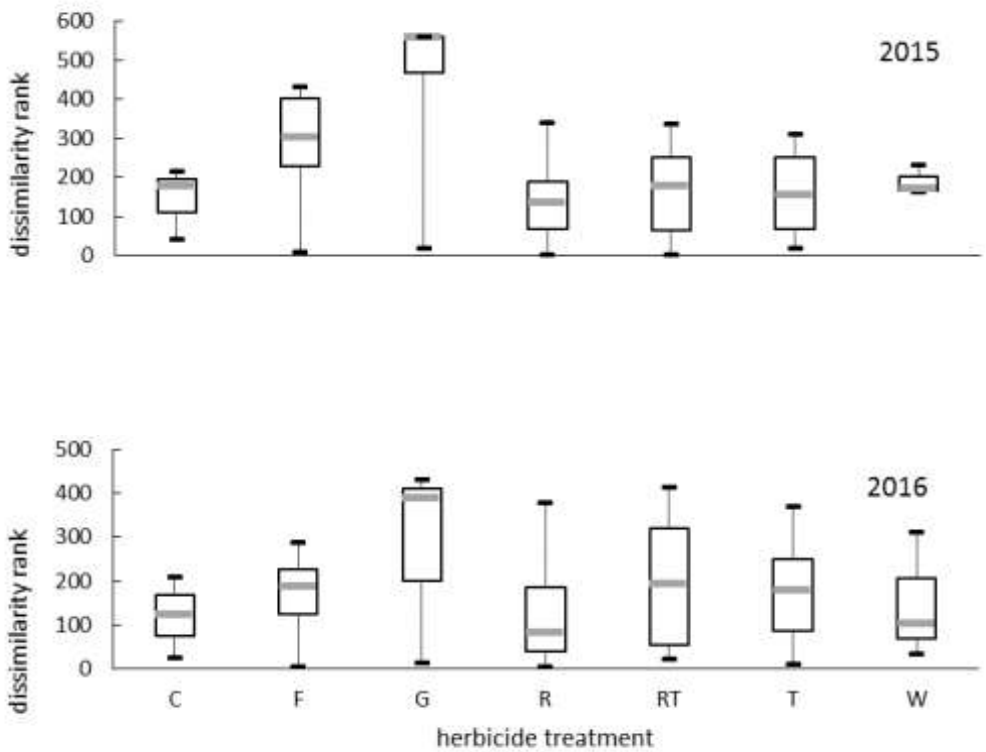

Figure 10. Boxplots of dissimilarity scores from ANOSIM analysis of species composition at ALBG in the two monitoring years after treatment. The grey bars within boxes are the median, the boxes enclose the 25 % and 75 % quantiles and the whiskers are minimum and maximum dissimilarity rank scores. See Table 1 for full names of herbicide treatments.

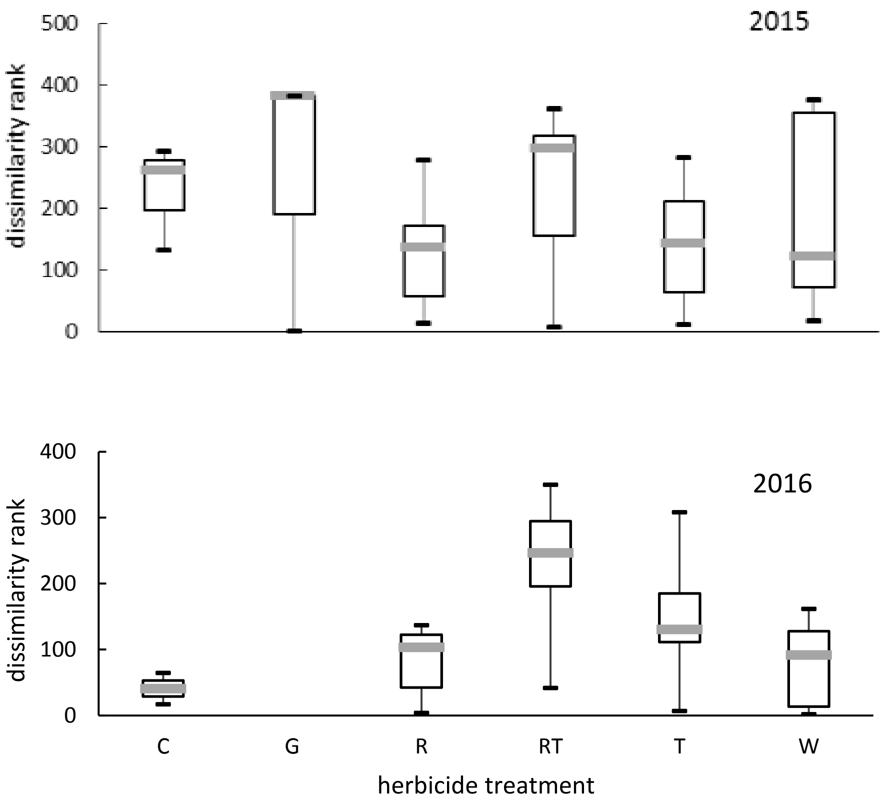

Figure 11. Boxplots of dissimilarity scores from ANOSIM analysis of species composition at APY in the two monitoring years after treatment. The grey bars within boxes are the median, the boxes enclose the 25 % and 75 % quantiles and the whiskers are minimum and maximum dissimilarity rank scores. Graslan™ excluded from 2016 analysis. See Table 1 for full names of herbicide treatments.

Another annual or short-lived perennial grass at APY, *Digitaria coenicola* (Finger Panic), was abundant in all plots prior to spraying in 2014. Grass abundance was very low in 2015 and 2016, including untreated control subplots. Taskforce™ subplots had more plants than other treatments in 2016 (Table 3).

The annual herb *Erodium cygnorum* occurred at the ALBG site. This species had higher abundance in Taskforce™ subplots in 2015 than Roundup™ or Roundup™ + Taskforce™ subplots. Abundance in Roundup™ treatments did not differ from controls (Table 3). This species was absent from Graslan™ subplots.

The small annual herbs, *Calandrinia* sp. and *Crassula* sp. were mostly absent at ALBG in 2015 but numbers increased in the following year and were more frequent in the Taskforce™ and Roundup™ + Taskforce™ subplots (Table 3).

Carrichtera annua (Ward's Weed) and *Medicago minima* (Medic) were abundant across all plots at ALBG prior to spraying in 2014. These species decreased in abundance the following year. Abundance remained low in subplots treated with Graslan, Taskforce and Roundup + Taskforce into 2016 (Table 3).

Table 3. Number of species per m^2 quadrat in treated plots one and two years after spraying (rates combined) where species compositions showed significant differences with ANOSIM tests (means ± 1 standard error). Values labelled with a different letter were significantly different $\alpha < 0.05$; values without letters were not included in tests. ALBG data, Welch ANOVA on square-root transformed data, $\alpha < 0.025$. APY data, negative binomial GLM $\alpha < 0.025$.

Site/Year	Species	Treatment				
		Control	Graslan	Roundup	Roundup + Taskforce	Taskforce
APY 2015	*Aristida holathera*	2.2 ± 2^{A}	0.3 ± 0.3^{B}	1.2 ± 0.2^{AB}	0.7 ± 0.5^{AB}	0
APY 2016	*Aristida holathera*	11.9 ± 2^{A}	1.6 ± 1.6^{B}	20.7 ± 4.2^{A}	1.4 ± 0.6^{B}	2.4 ± 0.7^{B}
APY 2015	*Digitaria coenicola*	2 ± 2	0.1 ± 0.1	0.2 ± 0.2	4.2 ± 3	1.7 ± 1.7
APY 2016	*Digitaria coenicola*	0	0.2 ± 0.2^{A}	0	1.1 ± 0.4^{AB}	3.4 ± 0.9^{B}
ALBG 2015	*Erodium cygnorum*	23 ± 5^{AB}	0	39.2 ± 9.5^{A}	12.1 ± 3.8^{B}	15.3 ± 5^{B}
ALBG 2015	*Carrichtera annua*	11.6 ± 3.1^{A}	0.2 ± 0.2	11.2 ± 1.1^{A}	28.3 ± 4.6^{B}	24.9 ± 4.9^{B}
ALBG 2016	*Carrichtera annua*	43.4 ± 6.4^{A}	0.2 ± 0.2	44.8 ± 6.9^{A}	6.7 ± 4.9^{B}	20.3 ± 1.6^{C}
ALBG 2016	*Calandrinia* sp.	3.9 ± 2.3^{A}	0.7 ± 0.3^{A}	4.8 ± 1.4^{A}	27.9 ± 8.5^{B}	27.8 ± 6^{B}
ALBG 2016	*Crassula* sp.	5.8 ± 4.6^{AB}	2.4 ± 1^{A}	6.1 ± 2.3^{AB}	17.2 ± 4.7^{BC}	21.1 ± 5.9^{C}
ALBG 2016	*Medicago minima*	36.8 ± 6.8	0.7 ± 0.3	0	0	0

Cover of bare ground and plants other than buffel grass

Before herbicide application, there was a significant difference in the amount of bare ground among sites (ANOVA, Site effect, $F = 8.25_2$, $p = 0.0006$). ALBG had the least mean bare ground (6%) and APY and BB had 11 – 12%. There was no significant difference in the amount of bare ground between treatments pooled across sites in 2014 (ANOVA, Site effect, $F = 1.15_8$, $p = 0.34$).

ALBG had the greatest increase in mean bare ground (30%), BB had a 14 % increase in bare ground and APY had a 7% increase in bare ground (Site effect, $p < 0.0001$, Appendix Table 7. The greatest increase occurred in Graslan™ subplots which had an average 15% increase in bare ground from pre-treatment levels by 2015 and 40% bare ground by 2016 (Time X Treatment interaction, $p = 0.003$, Appendix Table 7). In the other treatments (including controls) bare ground did not increase between the two monitoring periods or there was only a non-significant small increase (range from 6% increase in controls in 2015 to 16% in Taskforce™ H subplots by 2016).

There was no significant difference in the cover of plants other than buffel grass among sites or treatments in 2014 (ANOVA, Site effect $F = 1.53_{2}$, $p = 0.22$; Treatment effect $F = 0.93_{8}$, $p = 0.5$). Overall cover was 16 ± 2 % (mean ± 1 standard error) across all sites and treatments.

The cover of other plants increased in ALBG plots in 2015 and again in 2016. At BB and APY the cover of other plants decreased in 2015 with no change in 2016 (Time X Site interaction, $p = 0.011$, Appendix Table 8). There were significant differences between herbicide treatments (Treatment effect, $p = 0.0001$, Appendix Table 8). The cover of other plants increased in all subplots except those treated with Graslan™, where there was decrease in the cover of other plants.

Burning increased bare ground cover in all subplots, including controls, in the Burn Trial (rmMANOVA, Treatment effect, Exact $F = 0.05_{3,8}$, $p = 0.99$). Bare ground increased from a mean of 1 % in 2014 to 49% in 2015 following burning and application of herbicides and decreased to 7% by 2016. Changes in bare ground cover were related to changes in the cover of other plants in plots. The mean estimated cover of other plants in plots was 57% in 2014 before burning. This was reduced to 23% in 2015 following burning but increased to 81% by 2016. There was no significant difference between treatments (rmMANOVA, Treatment effect, Exact $F = 1.24_{3,8}$, $p = 0.36$). Therefore buffel grass cover at the site was largely replaced by the cover of other species by 2016, irrespective of herbicide treatment.

DISCUSSION

Control of mature tussocks

Herbicide applications, with the exception of Fusilade™, resulted in a reduction grass tussocks. This reduction was evident one year after application of Roundup™, Taskforce™ at the high application rate (3 L ha^{-1}) or Roundup™ + Taskforce™ (3 L ha^{-1}) mixtures. Graslan™ and lower rates of Taskforce™ (1.5 L ha^{-1}) were slower acting, and tussock numbers decreased further from one to two years after spraying.

Taskforce™, applied alone at 3 L ha^{-1} or in mixture with Roundup™, was the most phytotoxic to mature and killed up to 95% of tussocks across two sites. At the BB site 37% of tussocks survived in the Taskforce™ (3 L ha-1) treatment. This site was a sloping roadside and the herbicide may have been transported away from the site due to heavy overnight rains, reducing effectiveness. Taskforce™ applied at the rate of 1.5 L ha^{-1} was less effective in controlling mature tussocks and more than half of sprayed tussocks that appeared dead had regrowth.

Roundup™ was inconsistent in its effects on tussock mortality. All tussocks were killed at two sites when Roundup™ (4 L ha^{-1} was applied at the lower rate of 300 L ha^{-1} but not at the higher rate of 600 L ha^{-1}). Effectiveness of this treatment at APY was reduced which may be due to the age structure of the population resulting in significantly more dry leaf matter intercepting the foliar applied Roundup™. Roundup Biactive™ killed up to 80% of mature tussocks when applied at the rate of 5 L ha^{-1}. This formulation is recommended for areas in proximity to drainage lines to minimise potential risk to aquatic species. Roundup™ can also be used to kill mature tussocks after they have been burnt. The best results were obtained when plants were sprayed 4 – 6 weeks after burning. At this time tussocks had 15 – 20 cm of regrowth. Timing depends on seasonal conditions and plants may reach this level of regrowth before 4 weeks or after 6 weeks.

Graslan™ applied at the rate of 2 kg ha^{-1} was also less effective than Roundup™ and Taskforce™ in reducing tussock numbers with approximately 25% of tussocks re-sprouting 12 months following control. Graslan™ applied at the higher rate of 4 kg/ha killed all tussocks in two sites but not at the BB site where more than half of tussocks survived. The Graslan™ granules may have been transported away from this site by heavy overnight rains at this sloping roadside site.

Suppression of juveniles

The bare ground remaining after mature tussocks are killed, provides opportunities for the invasion of new plants from the seed bank or dispersal from untreated areas. Where tussocks were not killed, in control and Fusilade™ plots, there were very few seedlings, suggesting allelopathic or competitive effects of mature tussocks. Seasonal conditions were also important. More seedlings were found in plots before herbicides were applied in 2014. Control plots had fewer seedlings in 2015, indicating unsuitable conditions for recruitment in this year. There was site variation in recruitment as well with a recruitment event at BB and APY in 2016 but not at ALBG.

Graslan™ and Taskforce™ provided some pre-emergence control. Juvenile recruitment was also lower in plots that had been treated with these herbicides than in subplots treated with Roundup™ alone. Graslan™ was effective at both rates but Taskforce™ was only effective at suppressing recruitment at the higher rate of 3 L ha^{-1}. Roundup™ had no pre-emergence effects on buffel grass. Juvenile recruitment occurred in Roundup™ treatments where the death of tussocks created opportunities for seedling establishment.

Recruitment in plots at BB in 2016 occurred after a rainfall event prior to the 2016 monitoring. Although suitable rainfall for recruitment occurred up to 20 months after herbicide application, only one buffel grass seedling was observed in subplots that had received Roundup™ + Taskforce™. This indicates that in this trial the residual effect of Taskforce™ persisted for up to 20 months in the soil.

Buffel grass cover

All herbicides (excluding Fusilade™) reduced the cover of buffel grass in the year following monitoring compared to untreated control plots. However, recruitment of juveniles before the 2016 monitoring resulted in buffel grass cover being similar to controls two years after treatment. Buffel grass biomass was not measured. Due to the presence of juveniles in Roundup™ plots in 2015 and the less effective control of tussocks at some sites, buffel cover in these treatments was higher than in plots treated with Taskforce™ at 3 L ha^{-1}. Roundup™ treatments would therefore require follow up treatment in less than 12 months, depending on seasonal conditions that promote seedling germination or tussock regrowth. The trial results suggest that applications of Taskforce™ at 3 L ha^{-1} or in mixture with Roundup™ would require follow up spraying after one year.

Although buffel grass cover was measured as the presence of plants in 10 cm square cells, this does not provide a measure of buffel grass biomass. A reduction in biomass, through the death of mature tussocks, not only provides opportunities for the recruitment of buffel grass but also native species. In one of the sites (ALBG), there was an increase in the cover of other plants in the year following herbicide application and again in the following year.

Off-target impacts

Almost all shrubs were killed in subplots where Graslan™ was applied and there were significant impacts on forbs and grasses at the low application rate. Most subplots treated with the high rate of Graslan™ had no plants. Applications of Roundup™ were also detrimental to shrubs but where coverage of the herbicide was not complete using the knapsack sprayer, some shrubs later re-grew.

Changes in the species composition of the vegetation at a site may be the result of either direct death of plants following herbicide contact, destruction of the seeds in the soil by residual herbicides or release from competition or other allelopathic effects when the buffel grass tussocks are destroyed. For most native species, there were no significant differences in abundance between control and treated subplots. Abundance was probably more affected by seasonal conditions than herbicide treatments. Most species were too infrequent to compare treatment effects. For common species, Taskforce™ had a negative effect on the abundance of the native grass *Aristida holathera* two years after herbicide application, but not the grass *Digitaria coenicola*. The abundance of some introduced species was lower in plots treated with herbicides although at least three native annual species increased in abundance after herbicide application. Grasses were observed to be absent from Roundup™ + Taskforce™ subplots up to 20 months after treatment with some grasses observed during the 2016 monitoring.

Management recommendations

Integrated weed management is important and should be applied to the treatment of all buffel grass infestations and management choices will depend on the size, density, location and native species present at priority control sites. There are a combination of treatment approaches to prevent the spread of the weed and to control existing populations. Herbicides are only one of the control tools that may be used in conjunction with manual removal, mulching and fire. The herbicides Roundup™, Taskforce™ and mixtures provide a variety of control options for buffel grass.

Roundup™ was effective at controlling mature tussocks but results in off target damage to other species. It is suitable for spot-spraying in small infestations so that contact with non-target species can be avoided. Regular follow-up treatments are required to control new plants or tussocks that may have re-sprouted. Treated areas require checking after significant rainfall events that could promote germination of new seedlings from the soil seed bank. This herbicide is suitable for use after tussocks have been burnt and best results were obtained when plants had achieved from 15 – 20 cm of regrowth.

Roundup™ + Taskforce™ mixtures can be used as a spot spray so that sensitive native species, especially native perennial grasses, can be avoided. This herbicide could also be used for boom-spraying large infestations where there is no significant native component. The fast-acting Roundup™ in this mixture can prevent seed set and the slower-acting Taskforce™ will kill tussocks and provide some residual activity in the soil, reducing the emergence of new seedlings in the next season. Follow up control will be required after 18 months.

High density core populations can be controlled with Taskforce™ at 3 L ha^{-1}. This treatment will kill mature tussocks and prevent germination of seedlings during the following season. Several native grass species are sensitive to this herbicide and spot spraying may be required where there is a high density of native perennial grasses or boom spraying where native species are at low density. This herbicide is a better choice than the Roundup™ + Taskforce™ mixture where there are non-sensitive native species present.

Graslan™ is effective in controlling buffel grass tussocks and preventing the germination of new seedlings for up to two years. However this herbicide had severe negative impacts on other species, and killing shrubs in subplots and prevented the germination of most other forbs and grasses for up to two years after application. This herbicide is a potential option for small isolated, outlier populations where follow up controls are not feasible for up to two years. This herbicide is only suitable for use by experienced weed control practitioners.

APPENDIX

Statistical test results

Table 4. Results of rmMANOVA of difference in pre-treatment and post-treatment number of mature live tussocks per quadrat testing the effects of Site and Treatment in the Main Trial.

	Value	Exact F	Num df	Den df	P> F
All between	0.47	3.29	10	70	0.0015
Intercept	2.17	151.70	1	70	<0.0001
Site	0.06	1.99	2	70	0.145
Treatment	0.41	3.62	8	70	0.0014
All within	0.29	2.00	10	70	0.046
Time	0.07	5.05	1	70	0.028
Time X Site	0.11	3.77	2	70	0.028
Time X Treatment	0.18	1.56	8	70	0.153

Table 5. Results of ANOVA testing the fit of negative binomial GLMs with or without the effects Site and Treatment. ALBG site not included in 2016. Bonferonni adjusted significance level α < 0.25.

	df	Likelihood Ratio	P
2015			
Site	2	22.98	<0.0001
Treatment	8	26.06	0.001
Site X Treatment	16	45.27	<0.0001
2016			
Site	1	12.63	0.0004
Treatment	8	35.2	<0.0001

Table 6. Results or rmMANOVA of difference in pre-treatment and post-treatment cover of buffel grass per quadrat testing the effects of Site and Treatment.

	Value	Exact F	Num df	Den df	P> F
All between	0.31	2.20	10	70	0.027
Intercept	2.28	159.41	1	70	<0.0001
Site	0.13	4.46	2	70	0.015
Treatment	0.19	1.64	8	70	0.129
All within	0.31	2.15	10	70	0.031
Time	0.28	19.84	1	70	<0.0001
Time X Site	0.02	0.60	2	70	0.549
Time X Treatment	0.29	2.54	8	70	0.017

Table 7. Results of rmMANOVA of difference in pre-treatment and post-treatment cover of bare ground per quadrat testing the effects of Site and Treatment.

	Value	Exact F	Num df	Den df	P> F
All between	1.31	9.14	10	70	<0.0001
Intercept	2.06	144.33	1	70	<0.0001
Site	0.76	26.81	2	70	<0.0001
Treatment	0.54	4.73	8	70	0.0001
All within	0.45	3.15	10	70	0.002
Time	0.18	12.26	1	70	0.0008
Time X Site	0.07	2.58	2	70	0.08
Time X Treatment	0.38	3.3	8	70	0.003

Table 8. Results or rmMANOVA of difference in pre-treatment and post-treatment cover of other plants per quadrat testing the effects of Site and Treatment.

	Value	Exact F	Num df	Den df	P> F
All between	1.26	8.85	10	70	<0.0001
Intercept	0.03	1.89	1	70	0.17
Site	0.78	27.24	2	70	<0.0001
Treatment	0.54	4.73	8	70	0.0001
All within	0.49	4.25	10	70	0.0003
Time	0.31	23.65	1	70	<0.0001
Time X Site	0.14	4.8	2	70	0.011