DEPARTMENT OF
PRIMARY INDUSTRIES

Weed Sciences

FINAL REPORT

June 2009

Effects of Flupropanate on Non-Target Species - Glasshouse

Glasshouse study for Victorian Serrated Tussock Working Party

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Final report – Effects of Flupropanate on Non Target Species – Glasshouse, June 2009

(Data published as part of Honours thesis produced by Holly Bennett, supervised by Roger Cousens (The University of Melbourne) and Charles Grech (Department of Primary Industries).

Published by the Victorian Government Department of Primary Industries

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Printed by DPI Attwood

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SUMMARY

Chilean needle grass (*Nassella neesiana; CNG*) and serrated tussock (*Nassella trichotoma; ST*) are Weeds of National Significance. Flupropanate is considered a selective herbicide used in the control of these weeds, however its effects on non-target species such as important pasture varieties and native species has not been truly determined. To determine the effects on a number of non-target species, an experiment was set up in a glasshouse where a number of variables, which effect herbicide uptake, could be controlled.

Ten species including serrated tussock and Chilean needle grass were grown from seeds and then treated with a range of flupropanate rates up to 2.0 L/ha to determine their dose response. The growth and survival of these plants was then monitored for three and half months. At the end of the experiment all plants underwent a destructive harvest to gather detailed growth information.

It was found that one of the target species serrated tussock was very sensitive to flupropanate, while the other target species, Chilean needle grass, was less susceptible to the herbicide application. One of the non-target species, subterranean clover was significantly affected by flupropanate. However, due to unusual response curve, it is unclear as to exactly how and why subterranean clover is affected. All other non-target species in the experiment were found not to be significantly affected by the application of flupropanate.

INTRODUCTION

Chilean needle grass (*Nassella neesiana*) and serrated tussock (*Nassella trichotoma*) have been determined to be Weeds of National Significance (Thorp and Lynch 2000). As part of the Department of Primary Industries (DPI) Regional Best Management Practice Projects, experiments have been set up to discover the best integrated control management practice for Chilean needle grass, and serrated tussock. Two herbicides have been used in the experiments, glyphosate and flupropanate. Glyphosate is a knockdown herbicide, while flupropanate is a slow acting selective herbicide that impacts of plant lipid synthesis. However, evidence from DPI experiments have shown that flupropanate has an affect on the non-target native grasses and improved pasture grasses and legumes (Snell *et al*. 2007). The full extent of the effects of flupropanate on native and improved pasture grasses and legumes is unknown as there are many conflicting journal papers and minimal non-target species have been examined in relation to flupropanate effects. In Victoria, native grasslands are one of the most threatened ecosystems with less that 0.05% of pristine grasslands remaining (Craigie 1998). As serrated tussock and CNG are serious threats to these grasslands, land managers are wishing to control these noxious weeds through label recommended herbicides such as flupropanate. Anecdotal reports have suggested that some large areas of native grassland have been decimated by inappropriate use of boom spraying of flupropanate. The Environmental Protection and Biodiversity Conservation ACT (EPBC Act) prevents inappropriate weed control measures that may impact on rare and endangered native grasslands. This project explores the effects of flupropanate on a range of native and improved pasture species commonly found in areas containing both Chilean needle grass and serrated tussock. To what extent are the non-target species affected at different rates of herbicide application and can we understand the reasons for the differential selectivity. This information can then be used to help inform landholders and government authorities when considering using flupropanate as part of their integrated weed control management strategy, of the possible non target damage.

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MATERIALS AND METHODS

There are many environmental factors that may influence the uptake of flupropanate such as temperature, rainfall, life stage of the plant and animal grazing pressures. To minimise the number of variables affecting the results, the current project was run in the controlled environment of a glasshouse with regular watering, rather than as a field experiment.

Design

The experiment consisted of four replications. Each replication consisted of 12 species undergoing treatments of 5 application rates of flupropanate. Five application rates were used to allow a dose response curve to be fitted for each species with estimations of the "effective dose" for a 50% kill of the population (ED50). The list of treatments for each species can be seen in Table 1. The list of species can be seen in Table 2. The replications were set up as randomised complete blocks, with one block per glasshouse bench.

Table 1: Experiment Treatments

Treatment Number	Treatment
1	0.0 L/ha (0 g a.i./l)
2	0.5 L/ha (372.5 g a.i/l)
3	1.0 L/ha (745 g a.i./l)
4	1.5 L/ha (1117.5 g a.i/l)
5	2.0 L/ha (1490 g a.i./l)

Table 2: Species

Number	Common Name	Scientific Name
1	Windmill Grass	*Chloris truncata*
2	Kangaroo Grass	*Themeda triandra*
3	Poa Tussock	*Poa labillardieri*
4	Wallaby Grass	*Austrodanthonia duttoniana*
5	Spear Grass	*Austrostipa bigeniculata*
6	Lemon Beauty Heads	*Calocephalus citreus*
7	Perennial Rye Grass	*Lolium perenne*
8	Subterranean Clover	*Trifolium subterraneum*
9	Cocksfoot	*Dactylis glomerata*
10	Phalaris	*Phalaris aquatica*
11	Chilean needle grass	*Nassella neesiana*
12	Serrated tussock	*Nassella trichotoma*

Data Collection

Data was collected as growth measurements (leaf number, tiller number, plant height and length of the newest fully unfolded leaf) taken every two weeks and through destructive sampling (leaf areas measured with a planimeter, plant dry weights and root length) collected at the beginning (date of treatment application) and at the conclusion of the experiment.

The glasshouse experiment consisted of growing each of the test species in pots made from PVC pipe (90 mm in diameter and 350 mm in height) that were sealed at the base with fly screen mesh to hold in the soil while enabling drainage (Figure 2). The soil used to fill the pots, was collected from Oaklands Junction, a site where both Chilean needle grass and serrated tussock can be found. The soil was steam sterilised and then sieved before being placed in the pots. The steam steriliser was set to 80^{o}C for 3 days to ensure that the seed bank within the soil was destroyed. Once all of the pots were filled with the sterilised and sieved soil, they were placed in the glasshouse, each with a saucer to capture run-off. 240 pots and saucers were used to hold the plants used in the treatments. The pots were kept upright using wire mesh racks. Where possible, pots were rearranged at random on a fortnightly basis to reduce glasshouse bias.

Figure 1 & 2: Bench and Rack with pots

Two seedlings of the individual test species were allocated to each pot. Windmill grass and lemon beauty heads failed to germinate so these species were excluded from the experiment while kangaroo grass and serrated tussock had poor germination rates resulting in all kangaroo grass and most of the serrated tussock pots only being allocated one seedling per pot. The potted test plants were then grown for a six weeks in the glasshouse before the treatments were applied. The test plants were sprayed with Taskforce® (745 g a.i./L flupropanate) using a mechanical track sprayer in a spray cabinet with a standard flat nozzle (SS11002), to deliver a spray volume of 150 L/ha. After treatment, the plants were returned to the glasshouse for four months. Data was collected every two weeks to monitor the growth or death of the plants. At the end of the four months, starting on the 3/3/09 (Day 149), all plants were destructively harvested. The plants had reached full growth potential by four months given the size of the pots. Destructively harvested plants were removed separately from their pots, where each plant had their foliage assessed. Foliage was divided into the 'live' and 'dead' sections and each leaf was put though a planimeter (Paton Electroplan - Electronic Planimeter for Area Measurement Type T2) and then the foliage of the individual plant was placed in a labelled paper bag and dried in an oven for two weeks at 70^0C for two weeks before being weighed.

RESULTS

Survival

All non-target species tested in this trial were not significantly affected (Table 3) by any rate of flupropanate tested during this trial. Survival of Kangaroo Grass tended to decline at the 2l/ha rate but was not significant compared to the control. Serrated tussock was significantly (P = 0.00) affected by the application of flupropanate at rates as low as 0.5 l/ha. Plants sprayed with more than 1.5 l/ha ha all died (Table 3). Chilean needle grass survival declines with increasing flupropanate rate until 2.0L/ha where the survival rate increased.

Table 3: Percentage Survival

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	100	100	100	100	33.3	*0.071*
Poa Tussock	100	100	100	100	87.5	0.438
Spear Grass	100	87.5	75	100	87.5	0.679
Perennial Rye	100	100	87.5	100	100	0.438
Sub Clover	87.5	87.5	85.7	85.7	75	0.971
Phalaris	87.5	100	87.5	100	100	0.573
CNG	100	100	50	37.5	75	0.146
Serrated tussock	100	83.3	40	0	0	**0.000**

ED50

An ED50 can only be calculated for Serrated tussock. The high survival rate of the other species does not allow an ED50 to be calculated. Serrated tussock's ED50 was found to be at 0.75 L/ha in the glasshouse.

Plant Height (Day 149)

Chilean needle grass and serrated tussock have been significantly (P=0.00, P=0.00) stunted by flupropanate (Table 4). Kangaroo Grass tended to have taller plants when treated with flupropanate, although this was not significant. Subterranean Clover tended to have a 'quadratic' type height response for the given rates (P = 0.087). All other non-target species did not have significant responses to the application of flupropanate.

Table 4: Average Plant Height (cm) at the end of the experiment

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	68.62	90.57	93.70	82.32	80.40	0.502
Poa Tussock	46.85	45.93	50.28	51.05	60.82	0.530
Wallaby Grass	52.12	59.26	52.96	51.65	42.50	0.670
Spear Grass	61.62	49.63	46.26	53.81	43.62	0.542
Perennial Rye	28.80	24.23	38.78	28.05	24.91	0.333
Sub Clover	43.92	20.51	18.33	33.15	35.03	*0.087*
Cocksfoot	49.05	50.97	47.71	43.87	48.17	0.934
Phalaris	38.88	60.93	55.78	40.90	68.42	**0.012**
CNG	64.96	54.47	28.40	18.60	22.10	**0.000**
Serrated tussock	56.58	65.11	62.40	0.00	0.00	**0.000**

Dry Weight

Dry weight is a measurement of all above ground plant material including any dead material, which was still attached to the plant. Flupropanate treated Subterranean Clover had significantly less dry weight than untreated plants (P=0.025)(Table 5). Chilean Needle grass and Serrated tussock were significantly lighter at the high rates of flupropanate (Table 5). All other non-target species tended to have a slight decline in weight as application rate increased.

Table 5: Average Total Dry Weights (g) and P Values

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	6.91	5.70	10.70	7.86	2.47	0.159
Poa Tussock	5.17	5.82	5.86	3.35	3.30	0.256
Wallaby Grass	5.71	7.04	4.92	4.82	4.08	0.247
Spear Grass	4.43	5.23	4.41	4.30	3.09	0.464
Perennial Rye	5.36	4.53	4.62	3.95	5.11	0.461
Sub Clover	2.17	0.57	0.56	2.05	1.55	**0.025**
Cocksfoot	6.43	6.98	6.26	5.10	6.03	0.740
Phalaris	3.81	3.97	5.11	3.16	3.79	0.557
CNG	3.35	3.14	2.39	3.27	1.20	**0.043**
Serrated tussock	3.70	4.65	2.08	0.00	0.00	**0.000**

Chilean needle grass that had been treated with flupropanate had significantly less live/green plant material making up the total dry weight than untreated plants (Table 6). The amount of green plant material in Subterranean clover tended to increase with increasing flupropanate rate. All other species were not affected.

Table 6: Percentage of Average Live Plant Material in the Total Dry Weight and P Values

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	89.31	81.01	90.19	79.04	93.84	0.462
Poa Tussock	81.27	79.84	77.78	74.49	78.14	0.851
Wallaby Grass	69.06	62.47	60.10	56.27	61.26	0.460
Spear Grass	76.92	73.96	71.96	75.56	80.54	0.327
Perennial Rye	50.50	54.04	49.58	40.71	53.62	0.418
Sub Clover	60.60	74.12	80.12	78.97	89.78	0.174
Cocksfoot	81.83	76.49	79.17	73.15	79.11	0.831
Phalaris	40.60	35.32	42.33	48.75	46.21	0.655
CNG	76.66	73.51	56.88	68.34	63.67	**0.025**
Serrated tussock	96.61	97.75	94.71	-	-	0.564

Leaf Area

The leaf area is a measurement of all of the above ground plant material, including any dead material that was attached to the plant. The leaf area of serrated tussock was significantly affected by the flupropanate, at rates as low as 1.0 l/ha (P=0.001) (Table 7). Subterranean Clover was significantly affected at the low rates (0.5, 1.0 L/ha). Poa tussock and Chilean needle grass tended to be affected by higher rates of flupropanate.

Table 7: Average Total Leaf Area (cm^2) and P Values

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	5118.70	4177.67	7349.13	5167.12	2383.80	0.265
Poa Tussock	2478.87	2939.33	2788.55	1335.40	1805.33	*0.054*
Wallaby Grass	2109.37	2647.97	2270.58	2039.37	2150.21	0.767
Spear Grass	1455.52	2241.20	1876.33	1556.61	1150.85	0.328
Perennial Rye	2841.60	3608.30	3486.97	2905.23	2521.21	0.312
Sub Clover	1227.32	423.01	575.72	1464.87	1202.28	**0.050**
Cocksfoot	5276.46	5378.81	4513.72	3963.56	4827.38	0.433
Phalaris	2937.71	3251.86	5476.81	3086.45	2792.65	0.337
CNG	1902.23	1867.10	1447.07	1258.40	692.40	*0.083*
Serrated tussock	1094.25	1417.00	191.30	0.00	0.00	**0.001**

CNG that was treated with flupropanate had significantly less live material than untreated Chilean needle grass, P=0.021 (Table 8). No other species had any significant differences in the percentage of live material making up the leaf area. Note: Serrated tussock plants were completely dead at rates above 1.5l/ha.

Table 8: Percentage of Live Material in the Total Leaf Area and P Values

Species	0 L/ha	0.5 L/ha	1 L/ha	1.5 L/ha	2 L/ha	P Value
Kangaroo Grass	91.13	82.19	92.25	84.19	93.47	0.393
Poa Tussock	78.35	75.37	75.67	67.71	80.24	0.714
Wallaby Grass	68.20	65.75	80.33	58.93	65.02	0.332
Spear Grass	70.78	71.58	71.41	72.34	75.45	0.488
Perennial Rye	51.66	57.54	56.46	49.49	54.32	0.871
Sub Clover	81.97	72.00	85.00	85.35	93.35	0.162
Cocksfoot	83.14	79.22	76.10	78.45	80.62	0.875
Phalaris	48.06	33.11	44.32	50.48	35.16	0.423
CNG	82.13	76.92	61.20	69.34	62.51	**0.021**
Serrated tussock	98.17	96.30	97.07	-	-	0.708

Survival and ED50 -

The survival results for the majority of the species were unexpected. It was expected that Kangaroo Grass, Poa Tussock, Cocksfoot and Phalaris may have survived flupropanate treatments of up to 2 L/ha. However, all of the species except serrated tussock survived, though some showed obvious stunting and foliage effects. The fact that serrated tussocks dose response to the flupropanate treatments is as expected shows that the treatments were done correctly. It is thought that because the plants were healthy and were under minimal stresses, that the plants had a higher tolerance of flupropanate in the glasshouse situation.

Serrated tussock has been found to be very sensitive to flupropanate and the survival of the plants depends upon the rate of flupropanate (Figure 3). Chilean needle grass (CNG) has a large amount of variation in the survival rate when treated with flupropanate. At 1.5 L/ha 37.5 % of the plants survive, however, at the rate of 2.0 L/ha, 75% of the population survives. This result implies that CNG is not as sensitive as it is believed to be. Statistically, there is no difference between the survival responses of all the other species in the experiment.

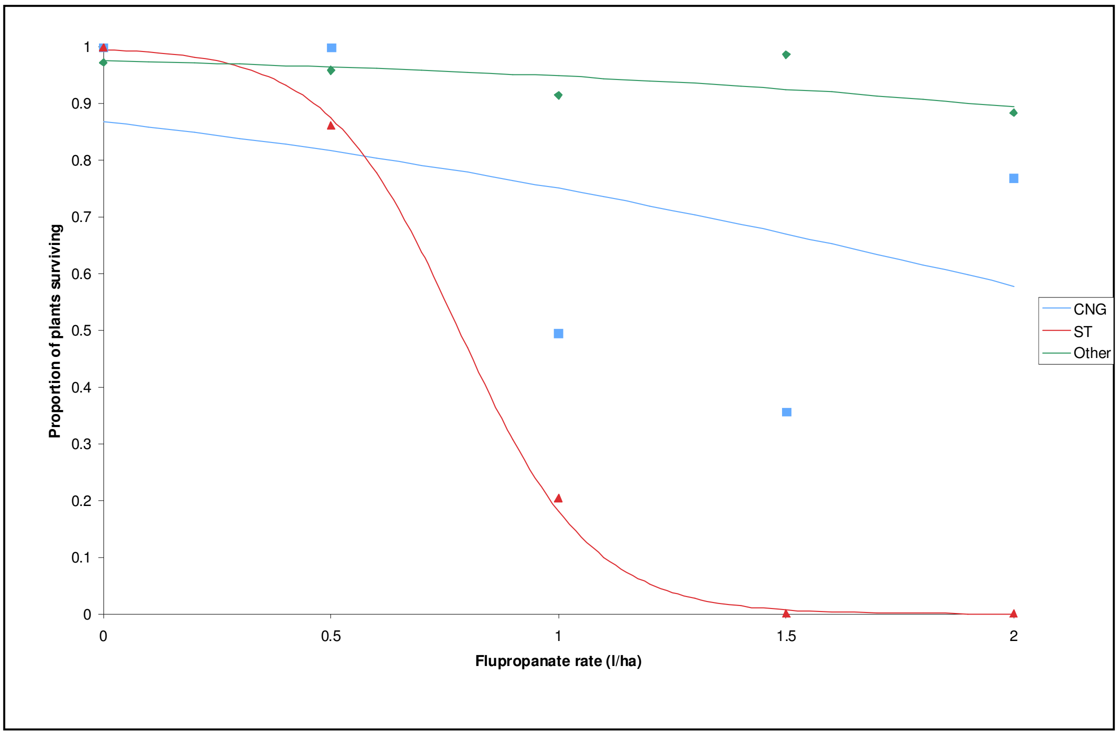

Figure 3: Survival of serrated tussock Logit(p) = γ + α + β×rate

The ED50 for serrated tussock was calculated using the model **Logit(p) = γ + α + β×rate** and was estimated at 0.75 L/ha (Figure 3). Given that the recommended rate for serrated tussock is 2.0 L/ha (Vee Dri (Aust.) Pty Ltd Undated), this is relatively low e ED 50 was thought to be at 1.0 L/ha rather than the 0.75 L/ha it was found to be. This ED50 cannot be compared to the other species as no other ED50's could be calculated. To estimate the ED50 for serrated tussock, the model used in Figure 3 was used to find the ED50.

Species Responses to Flupropanate

Kangaroo Grass

Kangaroo Grass had an unexpected response to flupropanate. Although the results were not always statically significant, they cannot be ignored. The low survival rate of Kangaroo Grass at 2.0 L/ha may be significant due to a relatively low P value (0.071). This is contrary to what the published past experiments have shown.

The flupropanate affected the growth of Kangaroo Grass at 2.0 L/ha. This can be seen in Figure 4. Figure 4 shows the growth of Kangaroo Grass over the duration of the experiment. It can be seen that from Day 61 to 92, the 2.0 L/ha plants were significantly stunted compared to the other treatments. However, by the end of the experiment, these plants had recovered and all plants treated with flupropanate were taller than the control plants (without herbicide). Although this is not statistically significant, it should be noted that there might be a hormesis effect occurring in response to the flupropanate.

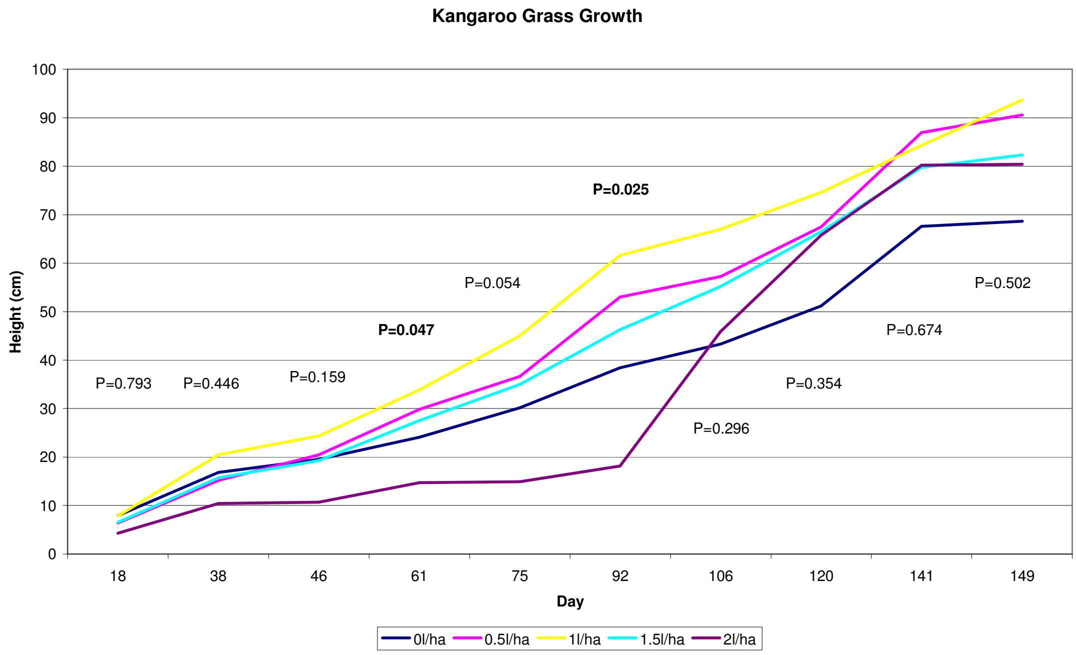

Figure 4: Kangaroo Grass Average Plant Height (cm) During the Experiment (Day 149 Error: 9.93)

Further evidence of the possibility of hormesis can be found in the dry weights of Kangaroo Grass. At 1.0 L/ha the plants are roughly 3.5g heavier than the control with no herbicide applied (Figure 5). The leaf area also follows the same pattern, suggesting that the plants treated at 1.0 L/ha are bigger than the plants with no flupropanate (Figure 6). Refer to the appendix for further graphs on the response of Kangaroo Grass to flupropanate.

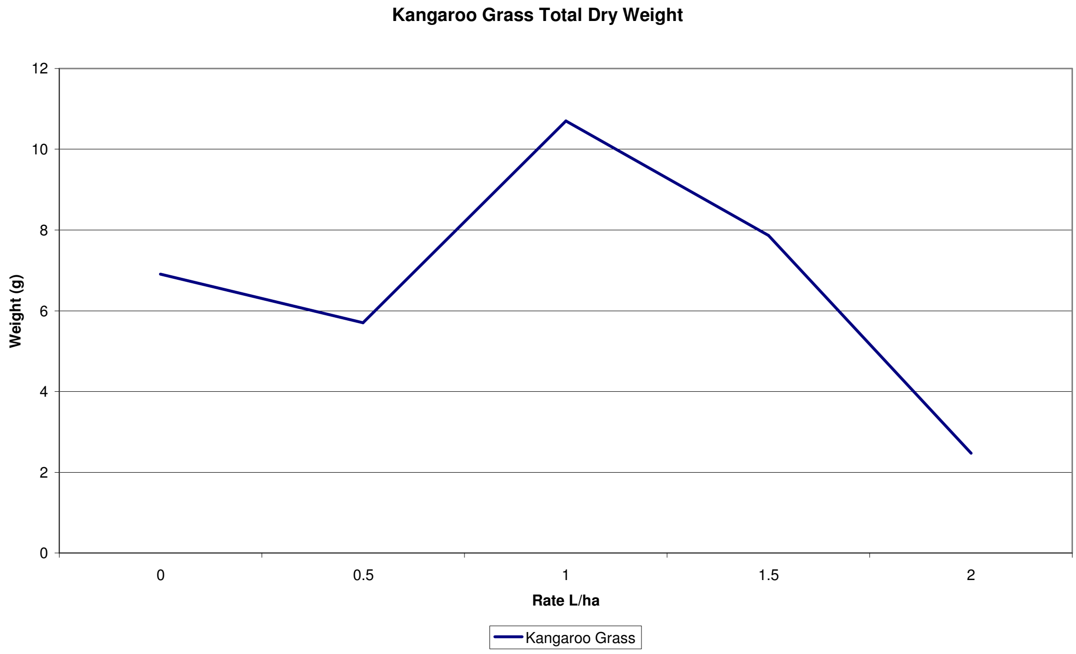

Figure 5: Kangaroo Grass Average Total Dry Weight

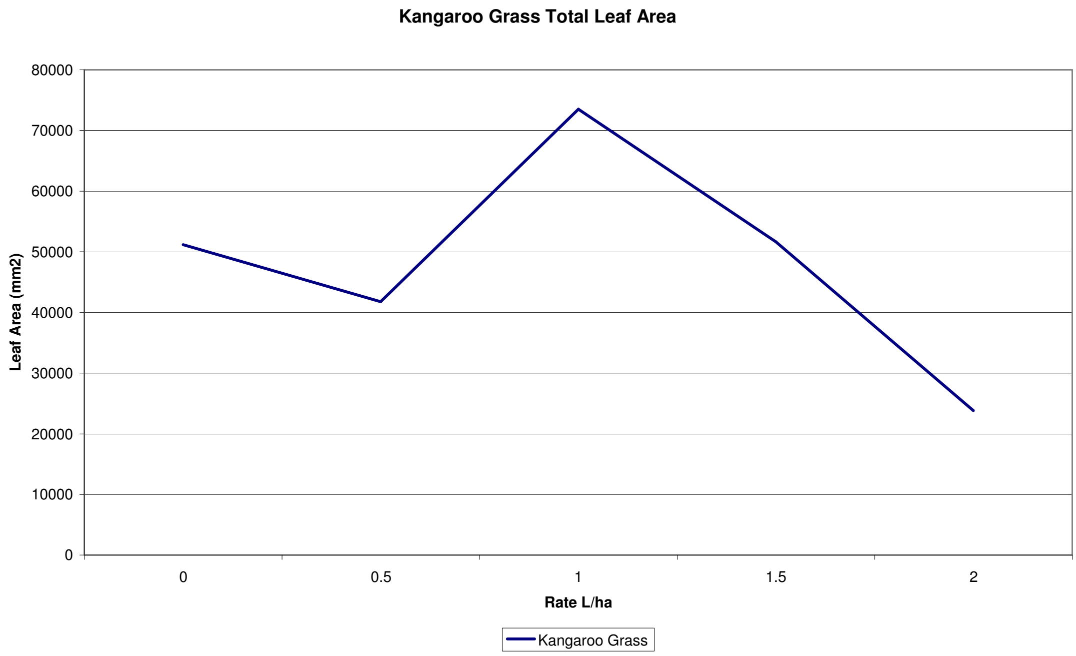

Figure 6: Kangaroo Grass Average Total Leaf Area

Poa Tussock

At the conclusion of the experiment, Poa Tussock did not show any signs of being significantly affected by flupropanate. However, on days 75 and 92, the plants treated with 1.5 L/ha and 2.0 L/ha were significantly (P = 0,045, P = 0.012) stunted compared to the other treatments (Figure 7). Like the majority of species, the dry weight and leaf area decreased with the higher rates of flupropanate. Refer to the appendix for further graphs on the response of Poa Tussock to flupropanate.

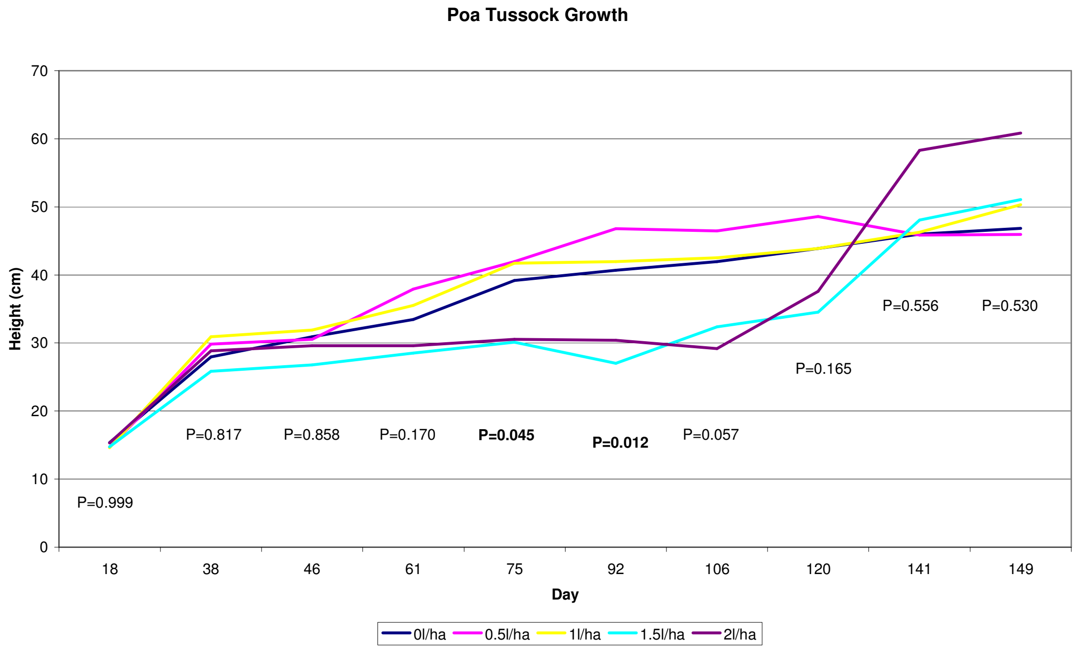

Figure 7: Poa Tussock Average Plant Height During the Experiment (Day 149 Error: 6.57)

Wallaby Grass

Wallaby Grass did not show any significant signs of being affected by flupropanate. Figure 8 shows that there was little variation in the plant heights of the different treatments throughout the experiment. The decrease in plant height shown in Figure 8 was due to the seed heads of the wallaby grass dying off after the reproductive stages. Although the dry weight of the wallaby grass was decreased at the higher rates, the leaf area was not (Figures 9, Figure 10). Refer to the appendix for further graphs on the response of Wallaby Grass to flupropanate.

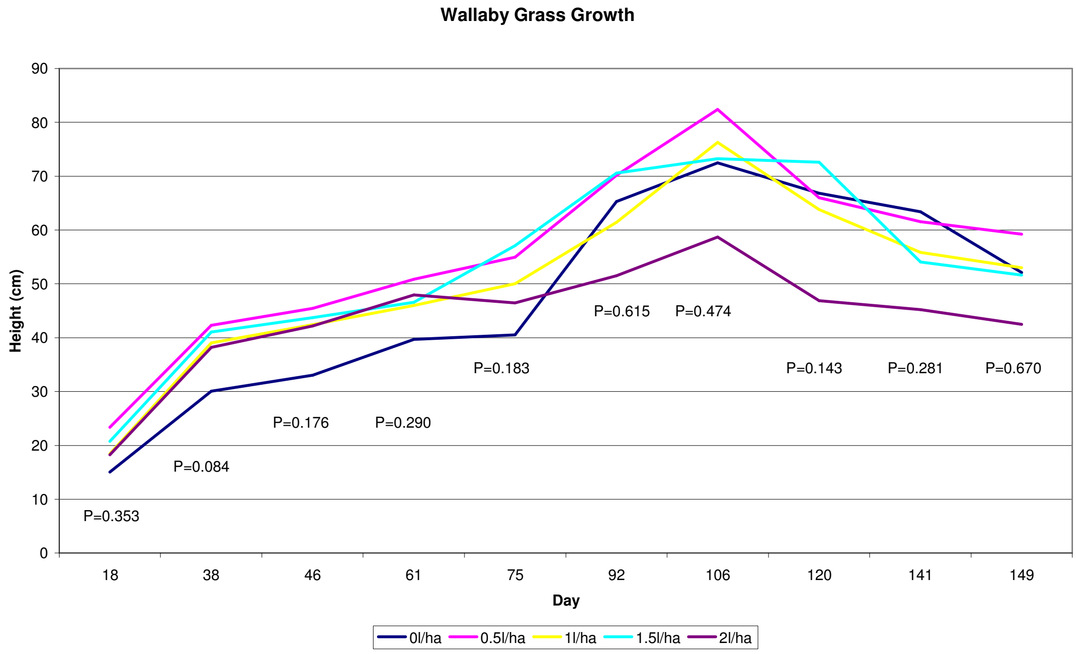

Figure 8: Wallaby Grass Average Plant Height During the Experiment (Day 149 Error: 7.12)

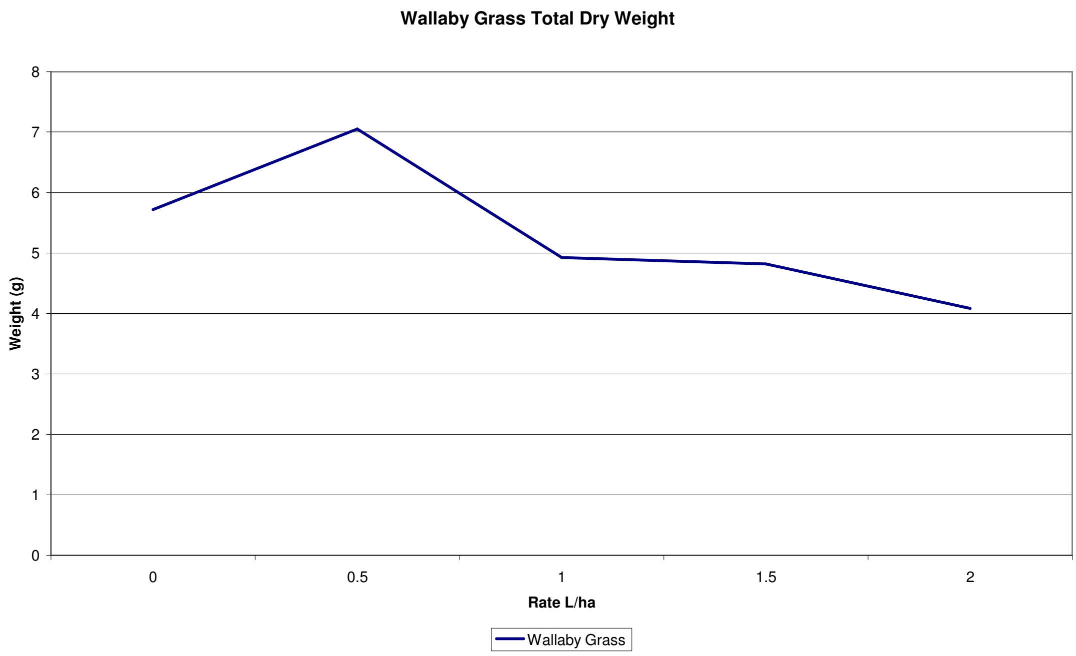

Figure 9: Wallaby Grass Average Dry Weight per plant.

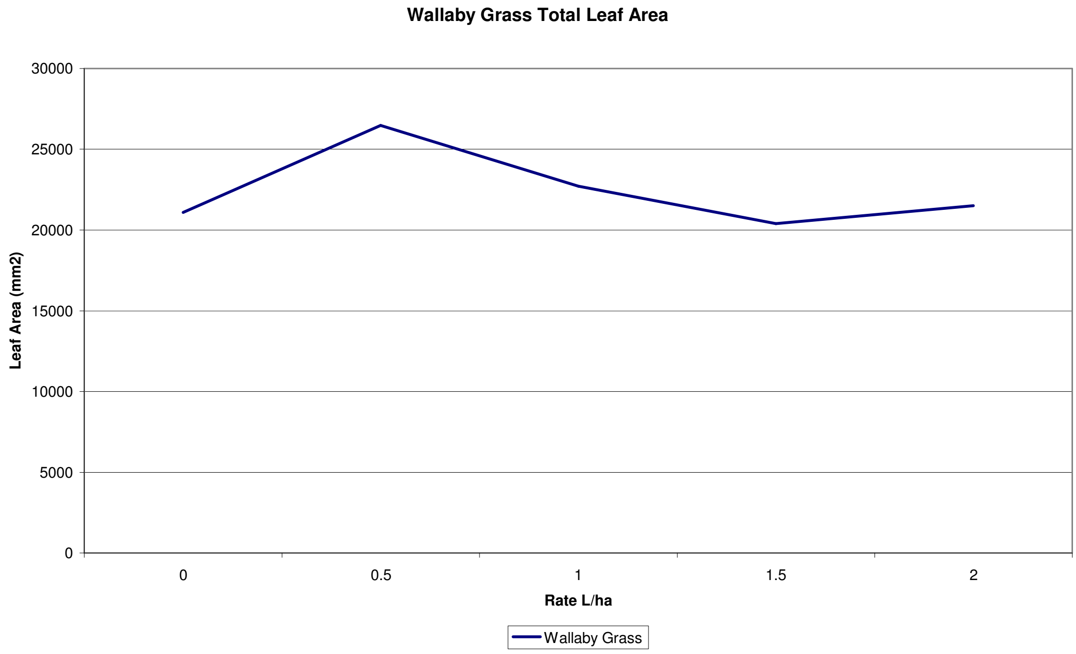

Figure 10: Wallaby Grass Average Leaf Area

Spear Grass

During the experiment, Spear Grass was affected by the flupropanate at the higher rates. This was noted on days 106 and 120 when the plant height was stunted at the higher rates (Figure 11). Along with most other species, the dry weight and leaf area of spear grass was reduced at the higher rates of flupropanate.

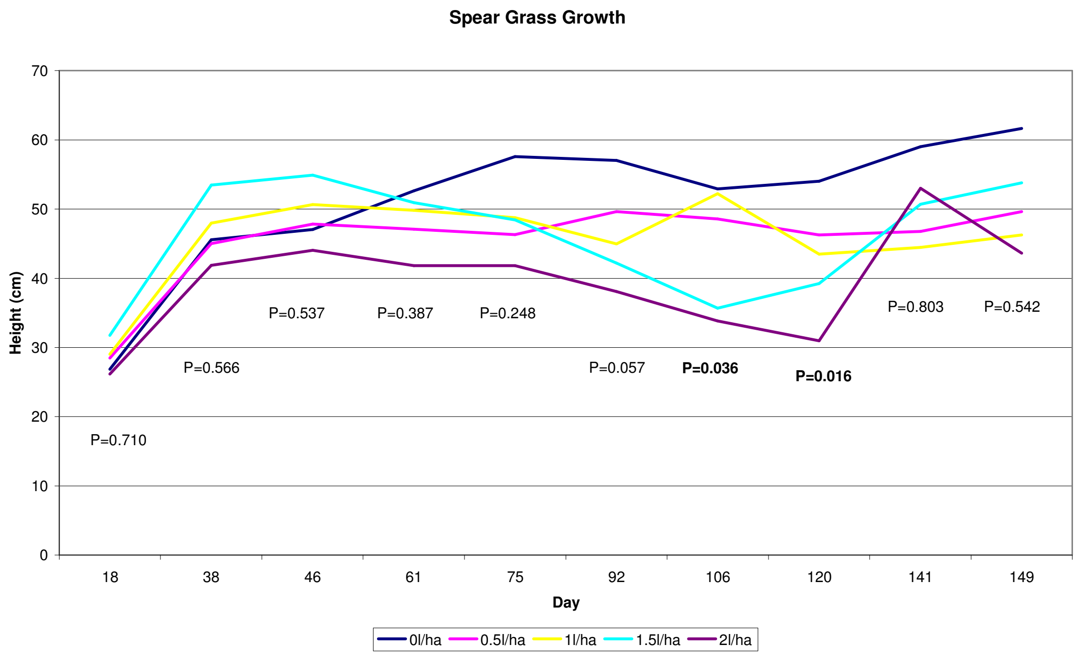

Figure 11: Spear Grass Average Plant Height During the Experiment (Day 149 Error: 7.74)

Perennial Rye Grass

Perennial Rye Grass was not significantly affected by flupropanate. During the experiment, the overall plant height was reduced but this was not caused by flupropanate as the control plant's height was also reduced (Figure 12). It is thought that the reduction in height was caused as the plants reached the 3-leaf stage and started natural senescence. Refer to the appendix for further graphs on the response of Perennial Rye Grass to flupropanate.

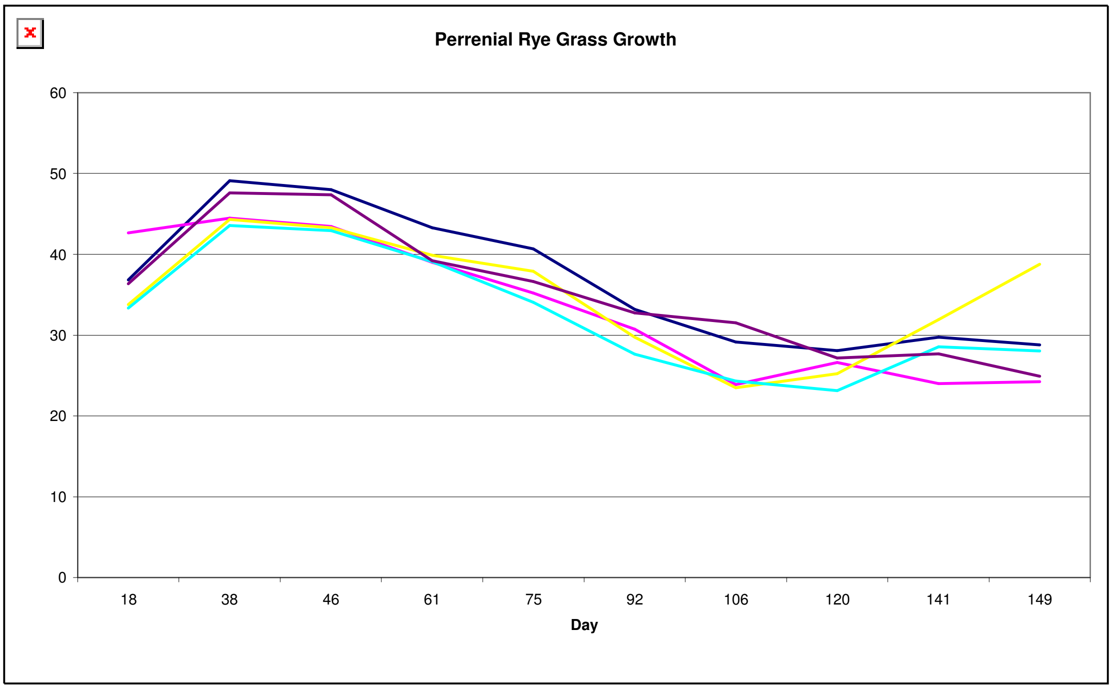

Figure 12: Perennial Rye Grass Average Plant Height During the Experiment (Day 149 Error: 5.22)

Subterranean Clover

Subterranean Clover was affected by flupropanate. For the rates studied, Subterranean Clover follows a quadratic model response to the rate of flupropanate. Figure 13 shows the 'quadratic' type response in the plant height. Quadratic models are not appropriate for use in biology, as plant growth cannot continue on to infinity as the quadratic models suggest. In this case another model would be required to describe the complete dose response, as at a high enough dose, herbicides will kill all plants. However, for the rates studied, the quadratic shaped curve describes the trend seen in Subterranean Clover.

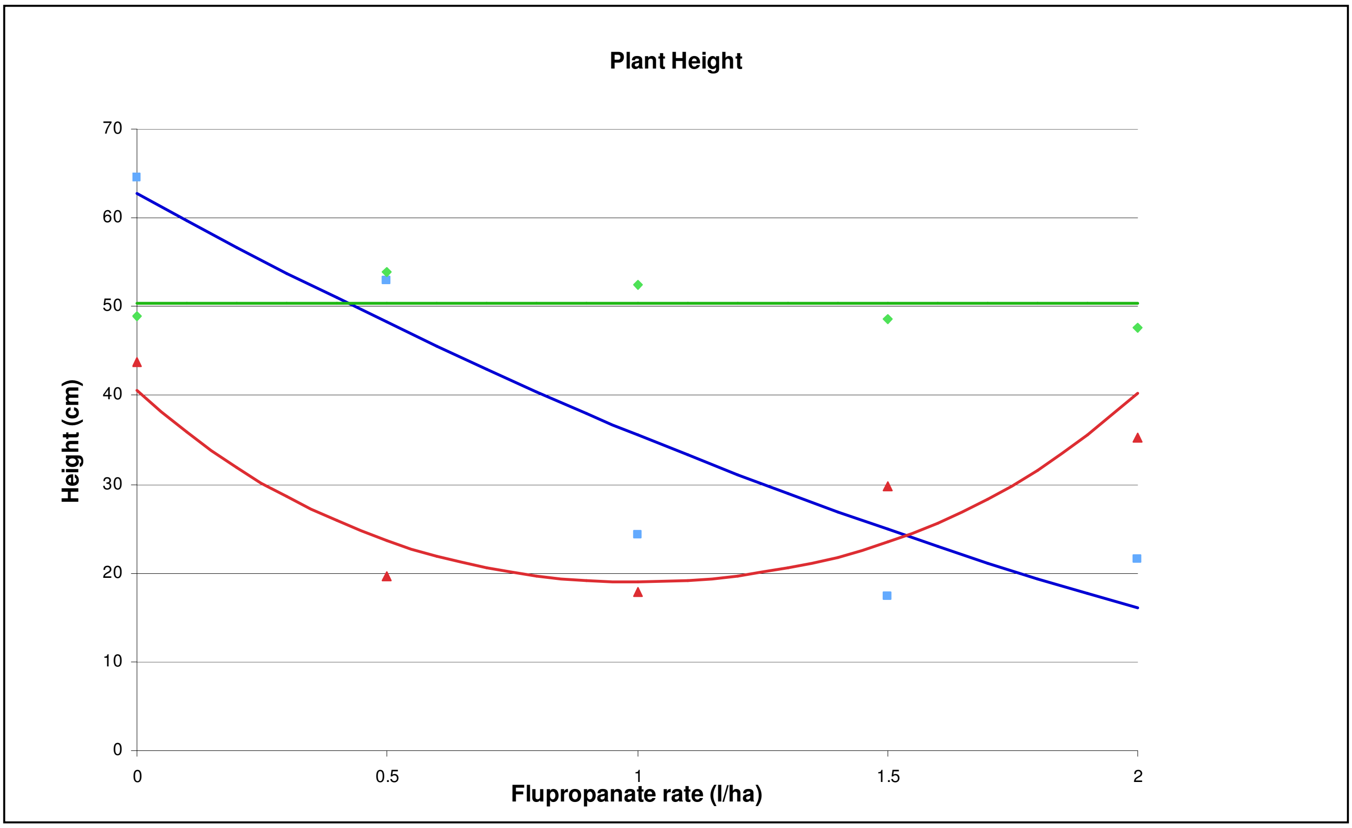

Figure 13: Height Response to Flupropanate

$$\sqrt{height} = \begin{cases} \rho + \alpha_1 + \beta_1 \text{Rate} \textit{ for} \text{ chilean needle grass} \\ \rho + \alpha_2 + \beta_2 \text{Rate} + \gamma_2 (\text{Rate})^2 \text{ for sub clover} \\ \rho + \alpha_s \text{ for other species} \end{cases}$$

The results show that at the low levels of flupropanate Subterranean Clover is affected. This can be seen in the plant height, dry weight and leaf area (Figures 14, Figure 15, Figure 16). It is unclear as to why the plants should only be affected at the lower rates of flupropanate, whilst the amount of live material increases with herbicide rate. (Figure 17). Refer to the appendix for more graphs relating to the response of Subterranean Clover.

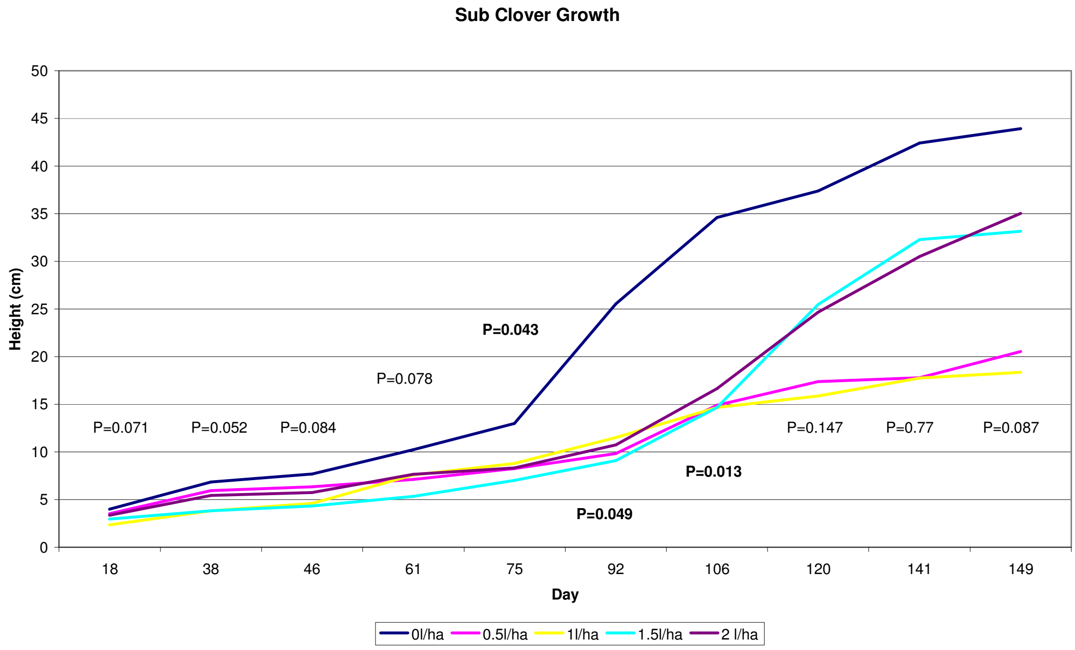

Figure 14: Subterranean Clover Average Plant Height During the Experiment (Day 149 Error: 6.65)

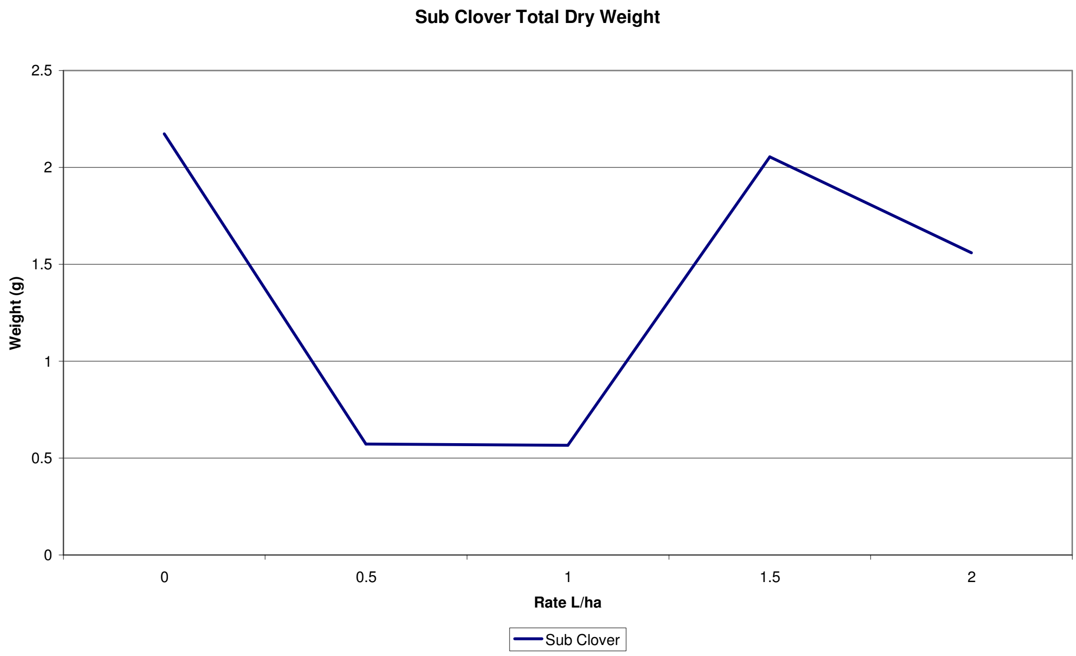

Figure 15: Subterranean Clover Average Dry Weight

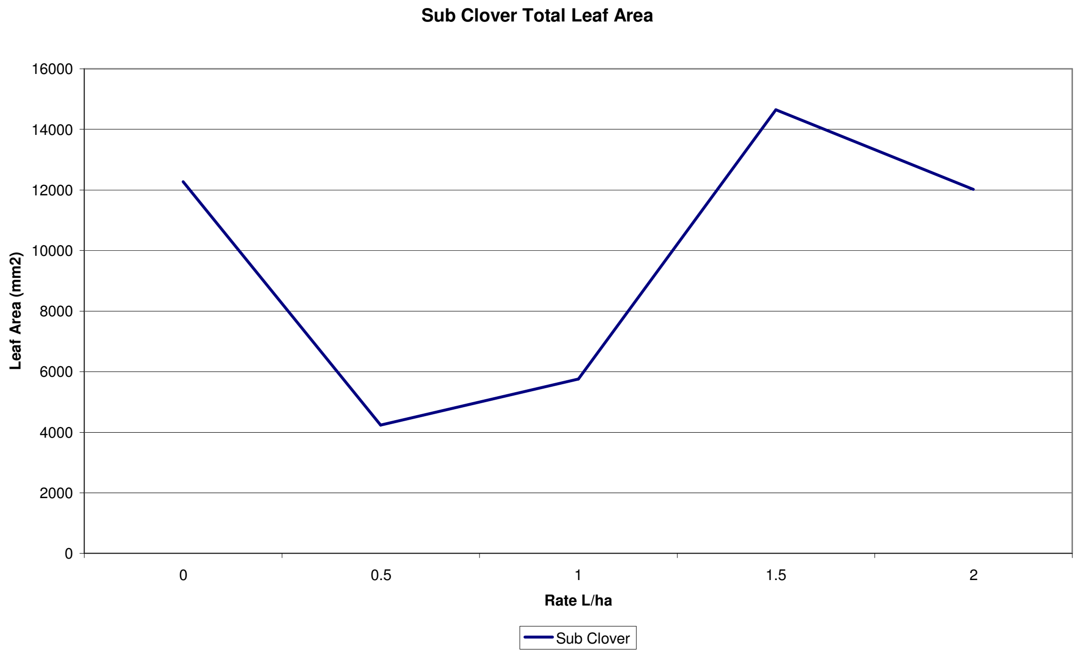

Figure 16: Subterranean Clover Average Total Leaf Area

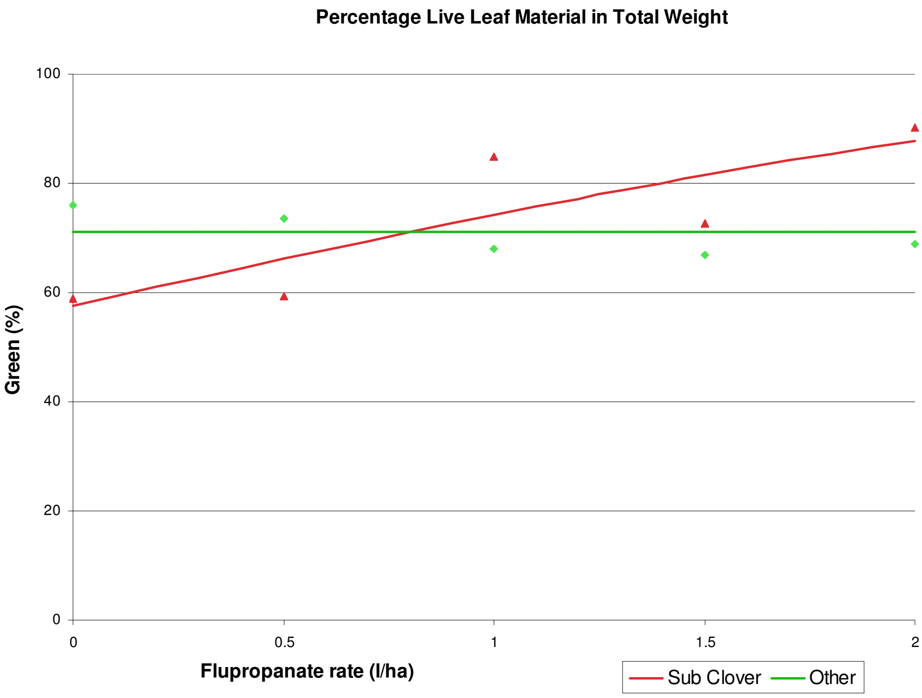

Figure 17: Percentage of Live Material in the Total Dry Weight, (Graph KB)

$$\sqrt{\%green} = \begin{cases} \rho + \alpha_1 + \beta_1 \text{Rate } for \text{ sub clover} \\ \rho + \alpha_s \end{cases}$$

Cocksfoot

Cocksfoot does not appear to be significantly affected by flupropanate. At no point during the experiment were there any significant differences between the treatments (Figure 18). Refer to the appendix for further graphs on the response of Cocksfoot to flupropanate.

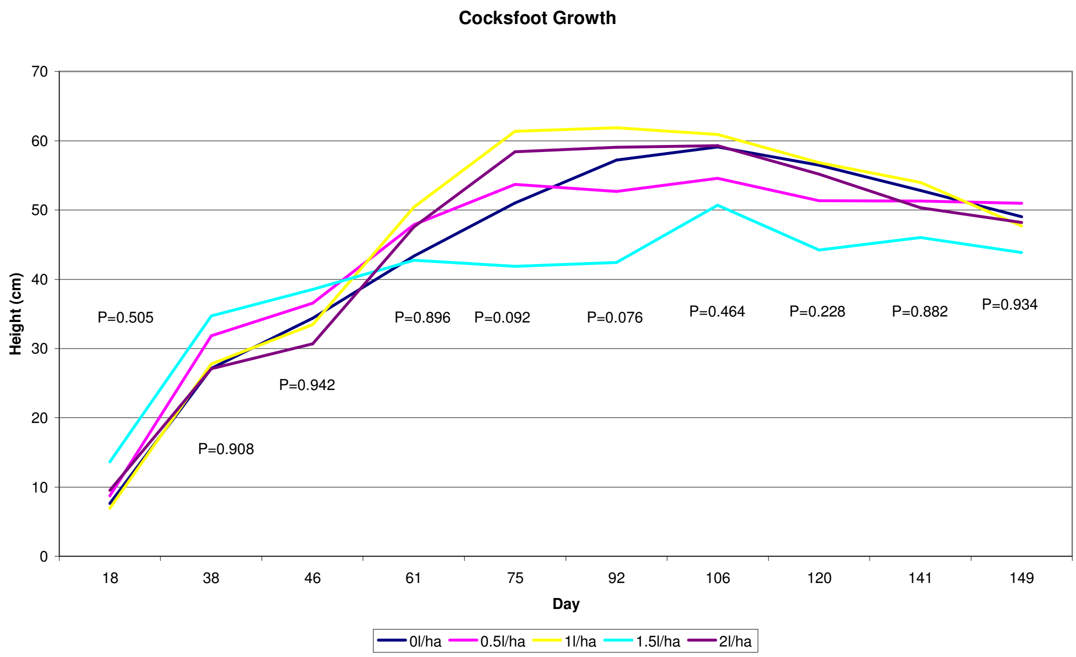

Figure 18: Cocksfoot Average Plant Height During the Experiment (Day 149 Error: 5.72)

Phalaris

Phalaris plant height and leaf area was generally not affected, although plants sprayed at 1.5 L/ha appear to be stunted compared to the plants at 0.5, 1.0 and 2.0 L/ha (Figure 19, Figure 20, Figure 21).. Refer to the appendix for further graphs on the response of Phalaris to flupropanate.

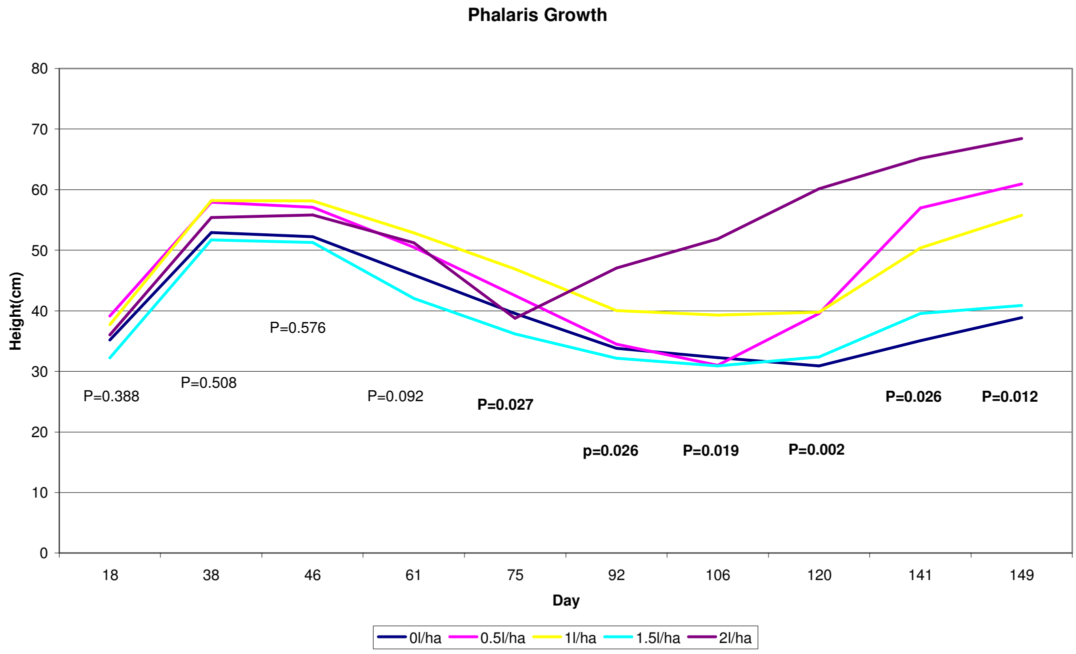

Figure 19: Phalaris Average Plant Height During the Experiment (Day 149 Error: 5.93)

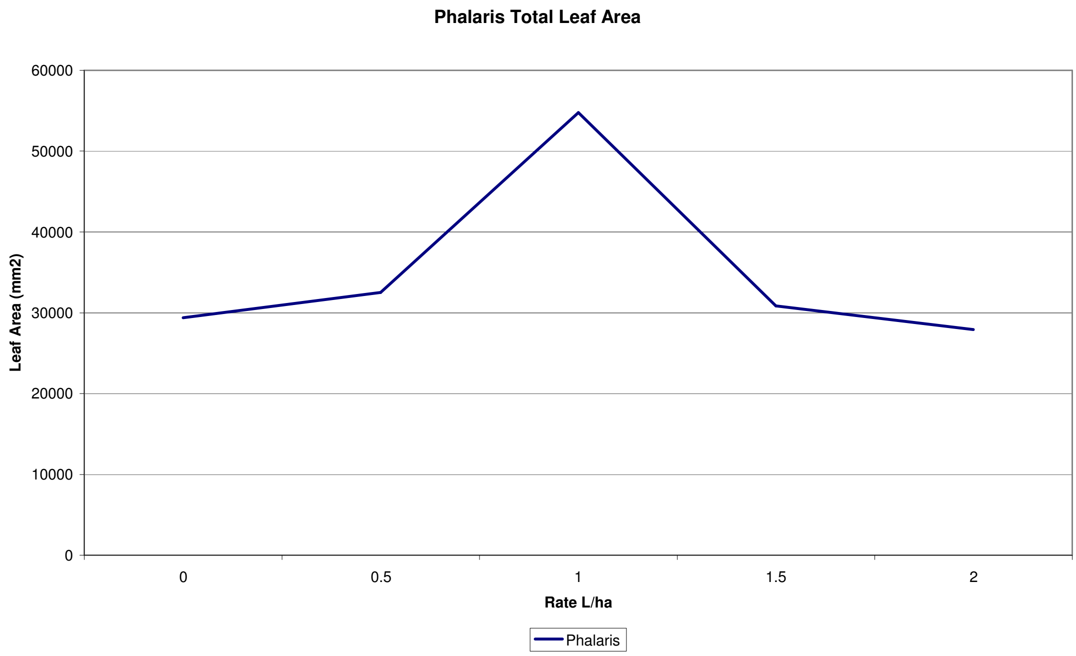

Figure 20: Phalaris Average Total Leaf Area

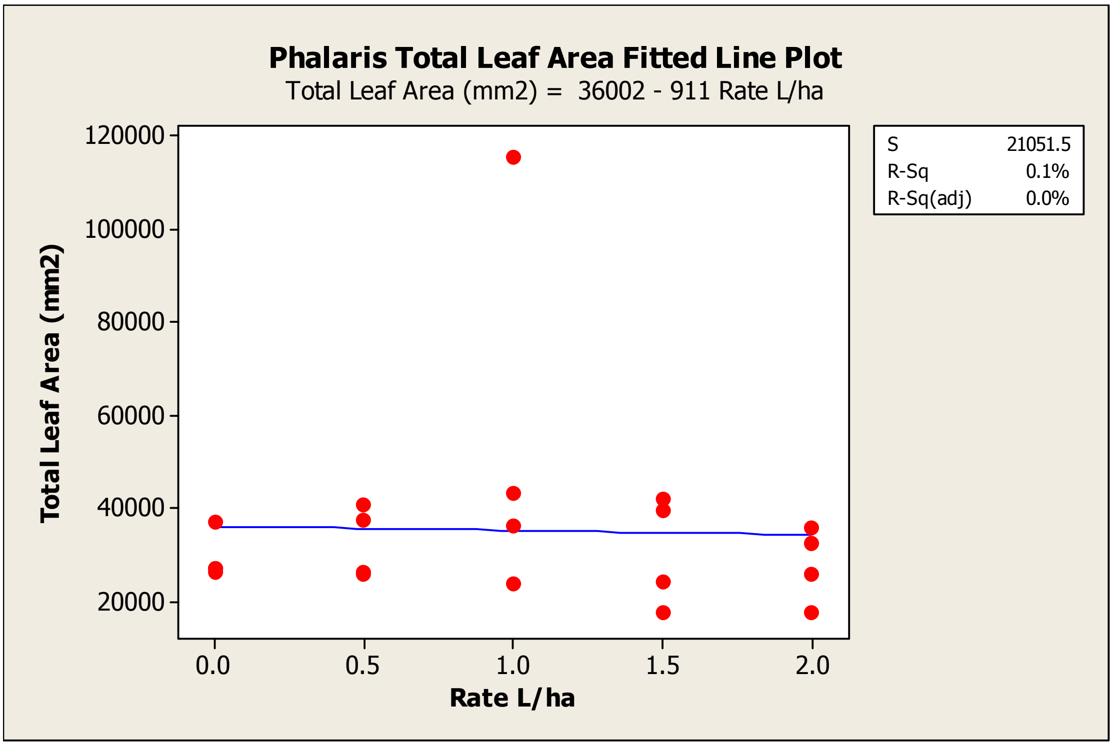

Figure 21: Phalaris Total Leaf Area Dot plot with Fitted Line

Chilean Needle grass

Chilean Needle grass (CNG) plants that were sprayed with more than 1.0l/ha were significantly shorter than untreated plants.(Figure 22).

The dry weight and leaf area of CNG are significantly reduced with an increasing rate of flupropanate (Figure 23, Figure 24). This response was expected, as CNG is a target species for flupropanate. Figure 25 shows that compared to all other species, CNG had a stronger rate of decrease in dry weight.

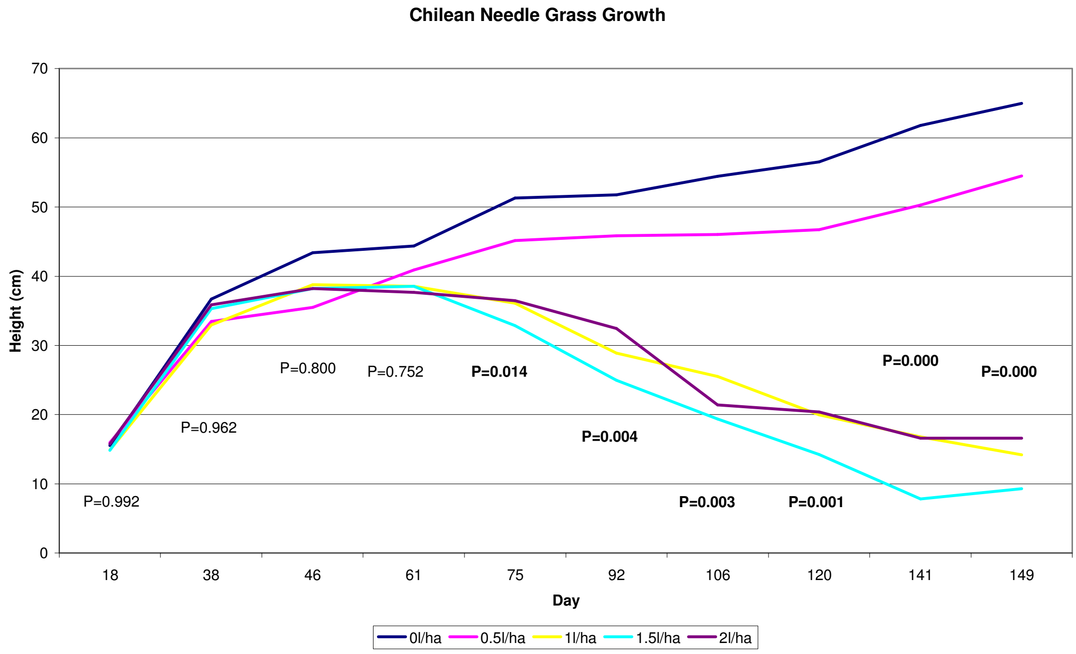

Figure 22: Chilean needle grass Average Plant Height During the Experiment (Day 149 Error: 8.35)

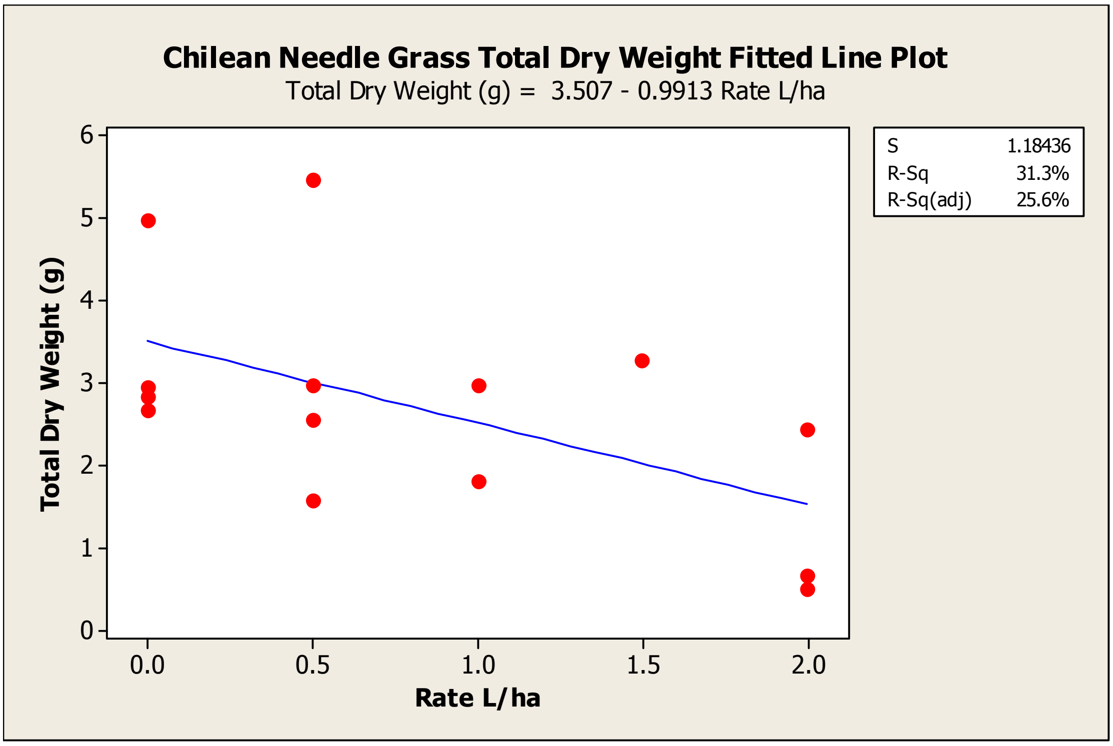

Figure 23: Chilean Needle grass Total Dry Weight Dot Plot

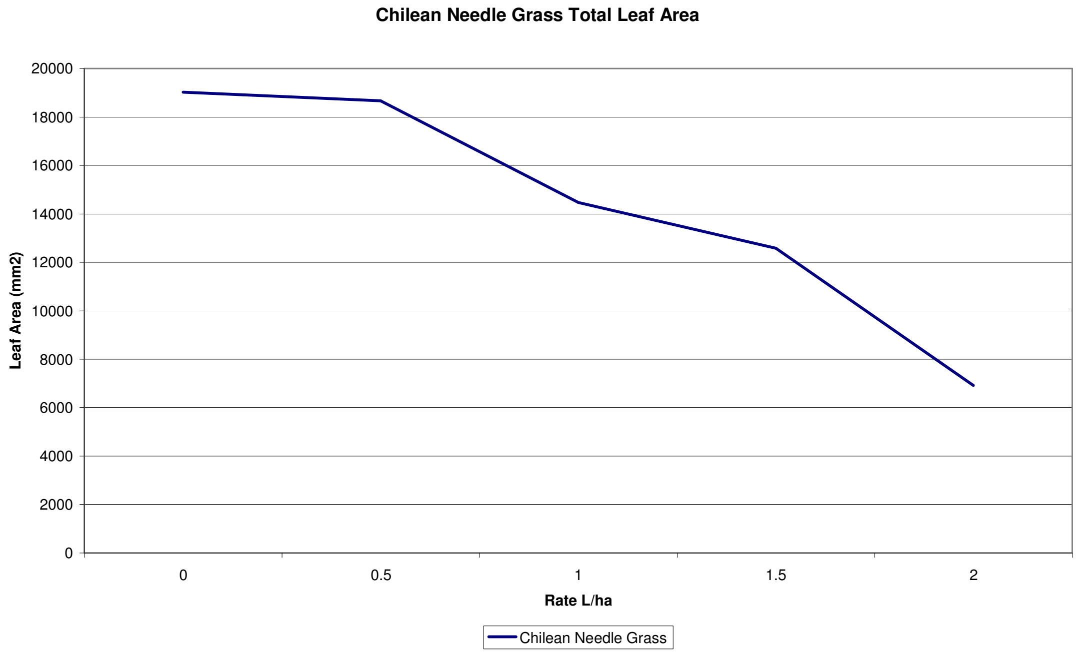

Figure 24: Chilean Needle grass Average Total Leaf Area

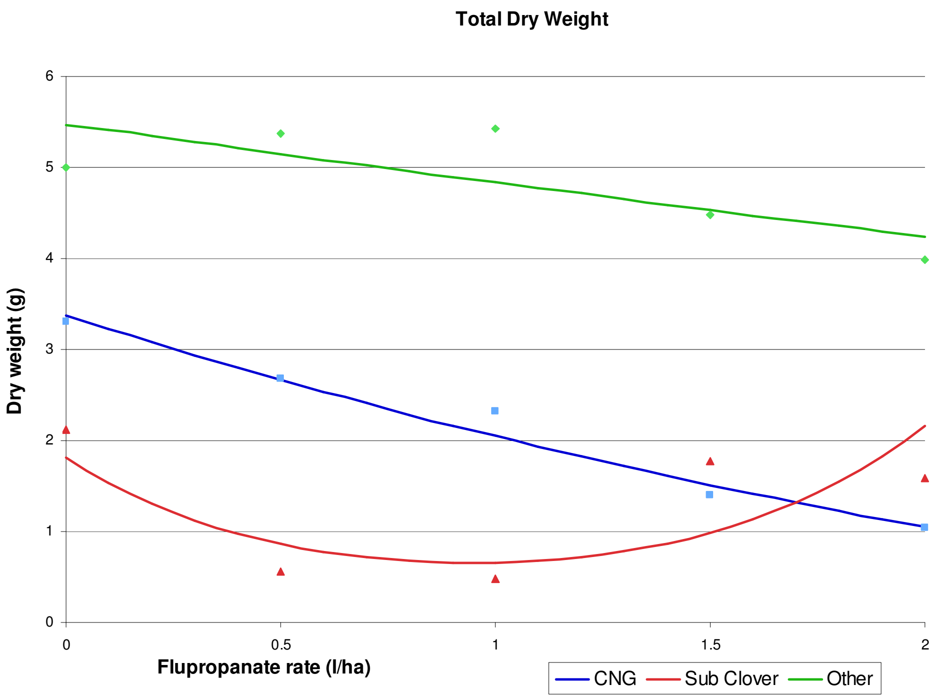

Figure 25: Total Dry Weight Vs Rate of Flupropanate (Graph KB)

$$\sqrt{dryweight} = \begin{cases} \rho + \alpha_1 + \beta_1 \text{Rate } \textit{for} \text{ chilean needle grass} \\ \rho + \alpha_2 + \beta_2 \text{Rate} + \gamma_2 (\text{Rate})^2 \text{ for sub clover} \\ \rho + \alpha_s + \beta_s \text{Rate for other species} \end{cases}$$

Flupropanate also significantly reduced the amount of live material, and leaf area of sprayed CNG plants (Figure 26, Figure 27). As the flupropanate rate increases, the percentage of live material making up the plants decreases. Observations from the glasshouse of the CNG plants dying off after the treatments were applied are consistent with these results, the height and dry weight results.

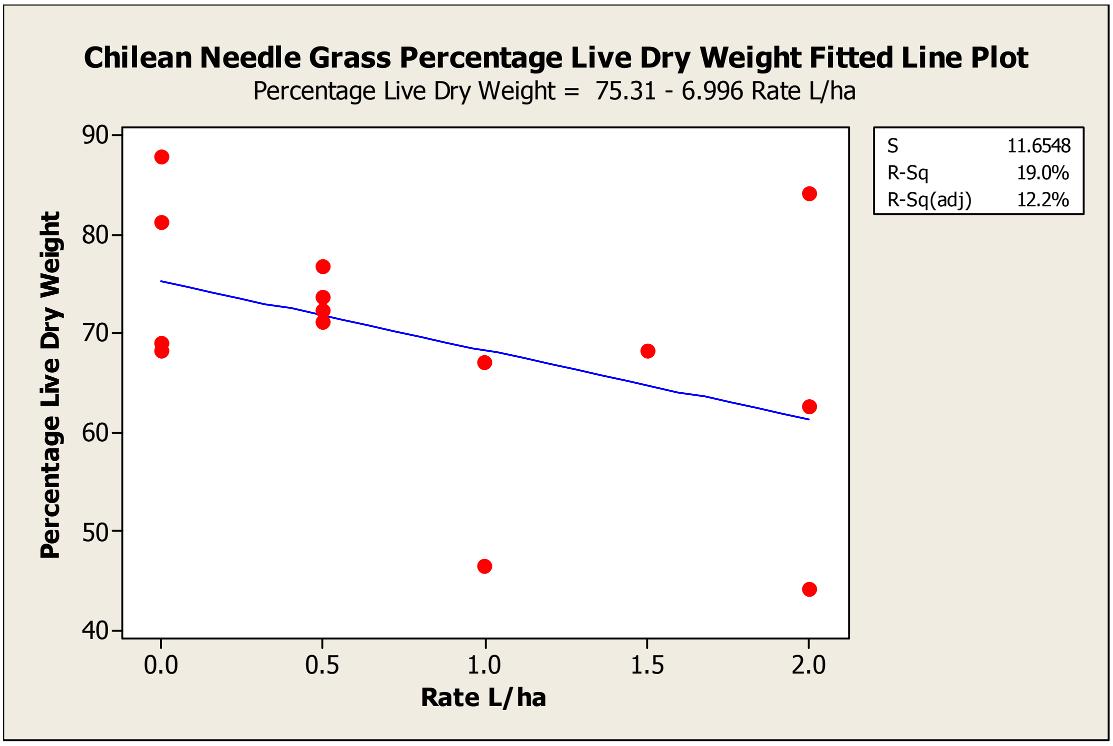

Figure 26: Chilean Needle grass Percentage Live Material in the Total Dry Weight Dot Plot

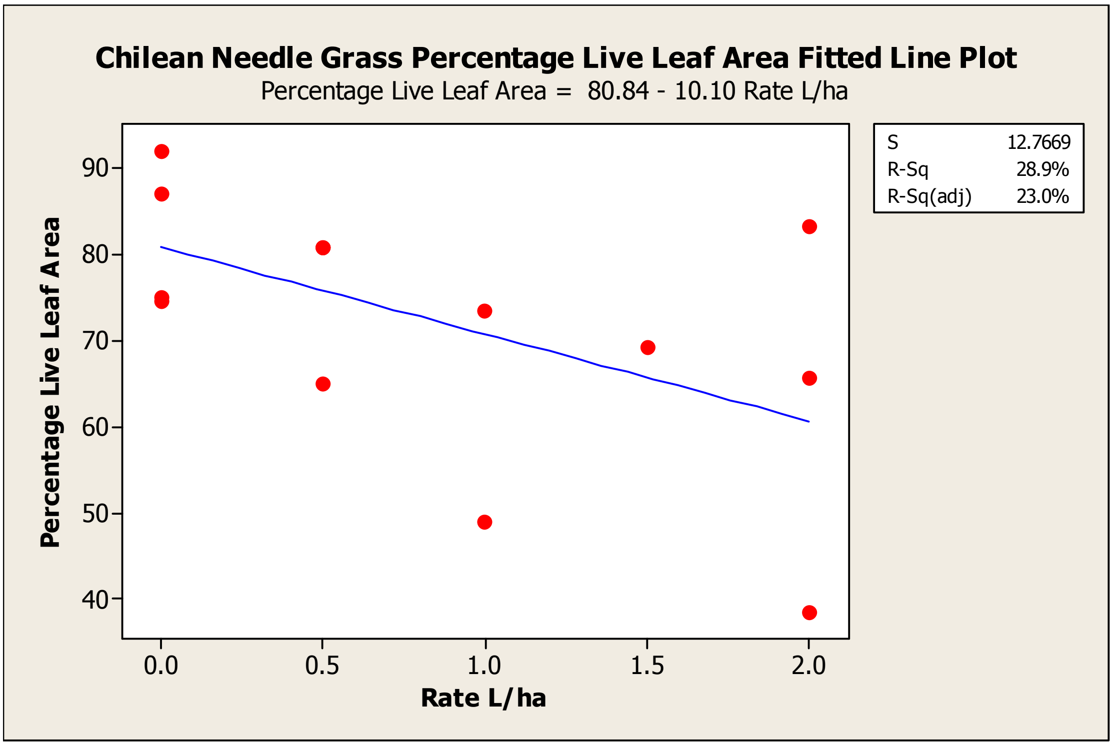

Figure 27: Chilean Needle grass Percentage Live Material in the Total Leaf Area Dot Plot

Serrated tussock

Serrated tussock plants that were sprayed with rates higher than 1.5l/ha had died approximately one month after application (Figure 28). This is consistent with the field expectations of 3-12 months for effective control listed on the herbicide label (Vee Dri (Aust.) Pty Ltd Undated). Those that were sprayed with the 1l/ha rate only differed from the control plants in terms of dry weight and leaf area. These plants had significantly less leaf area and dry weights than the controls (Figure 29, Figure 30).

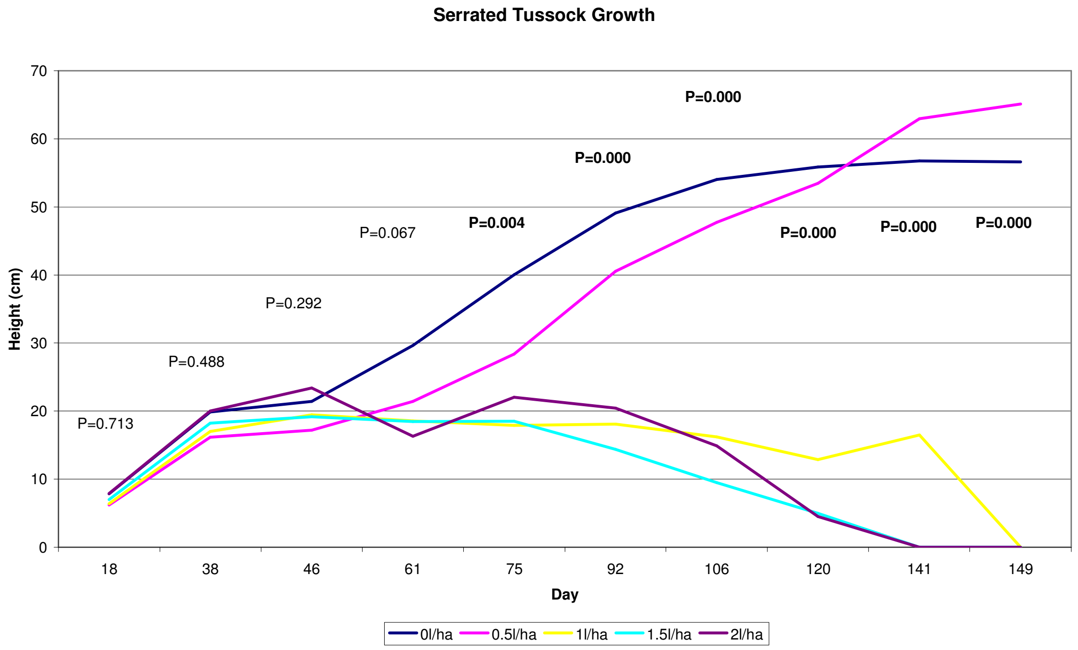

Figure 28: Serrated tussock Plant Height During the Experiment (Day 149 Error: 7.69)

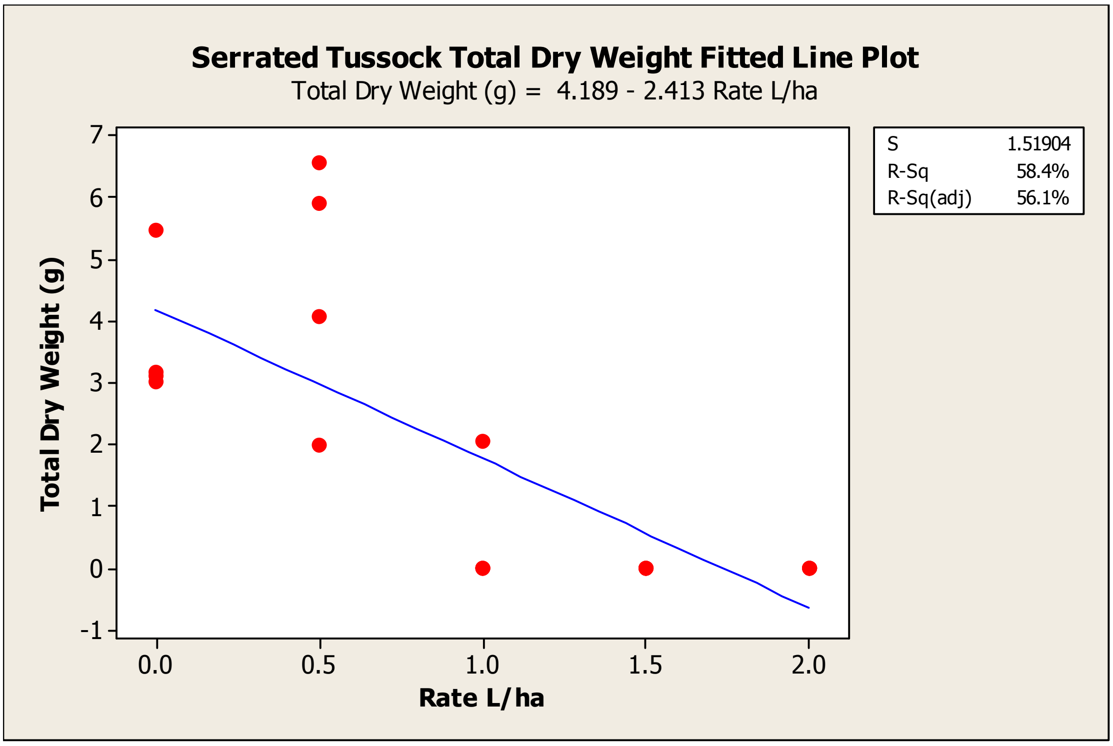

Figure 29: Serrated tussock Total Dry Weight Dot Plot

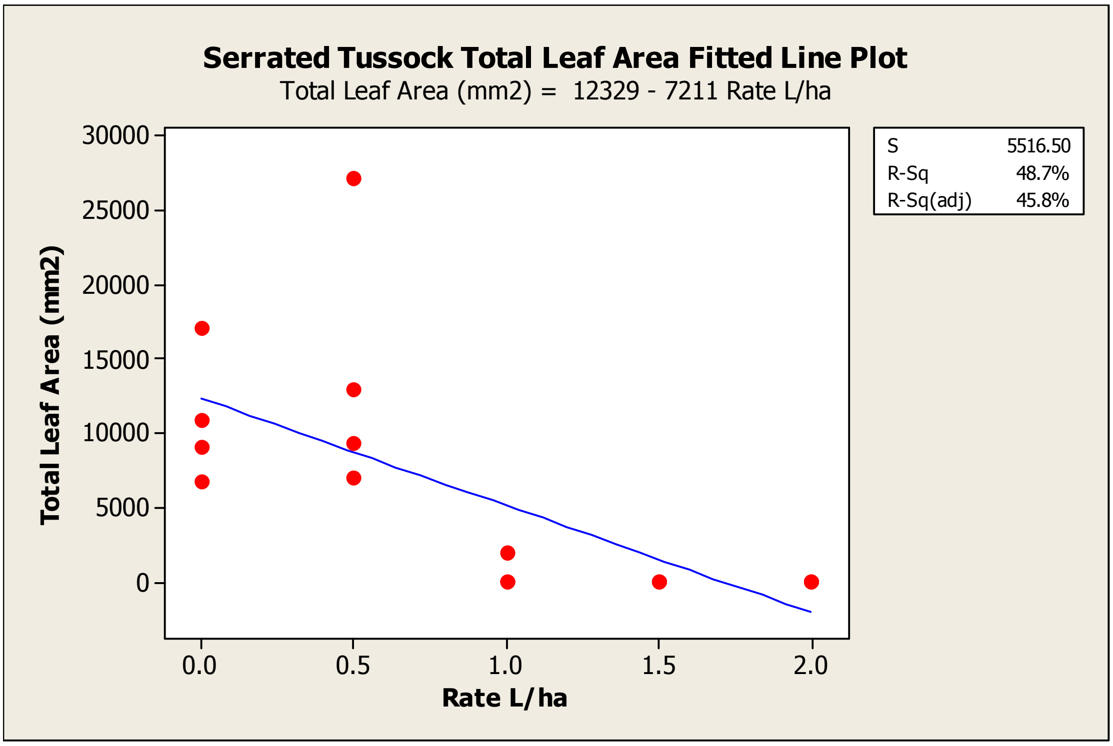

Figure 30: Serrated tussock Total Leaf Area Dot Plot

DISCUSSION

The results showed that one of the target species, Serrated tussock, is very sensitive to flupropanate and has an ED50 of 0.75 L/ha which supports the recommended application rate of up to 2l/ha. Chilean Needle grass, the other target species, was found to not be effectively controlled in the glasshouse situation, within the four month duration of this trial. This is unexpected as previous field results in similar soil types have shown 1.5l/ha to be effective (Grech 2007D).

Native species

Very high rates of flupropanate (5.0 and 10.0 L/ha) have been used as an effective herbicide for *Poa labillardieri,* where it has been noted that up to 100% of the tussocks are killed (Dellow and Campbell 1979, Campbell et al 1986). At the recommended rate of flupropanate, *Poa labillardieri* has been noted as being tolerant to flupropanate (Bray and Officer 2007). More detailed studies have showed that *Poa labillardieri* will tolerate rates of 2.0 – 4.0 L/ha of flupropanate (Campbell 1997).

These results are also supported in the current study where *P. labillardieri* was not significantly affected by flupropanate.

Mortality of *Themeda triandra* was not significantly affected by flupropanate in the current study, though plants were quite stunted at higher rates compared to untreated controls Literature on the tolerance of *Themeda triandra* suggests that it is tolerant of flupropanate to up to 3 L/ha. Bray and Officer (2007) state that *Themeda triandra* is tolerant of flupropanate at the recommended rate. Campbell and Viljoen (2006) state that *Themeda triandra* will tolerate flupropanate up to a rate of 3.0 L/ha. However, another study showed that *Themeda triandra* would tolerate 2.0 – 4.0 L/ha of flupropanate (Campbell 1997). The current study supports these observations.

The results show that there is the possibility of hormesis occurring as a result of the flupropanate application. This was mainly observed in Kangaroo Grass but other species such as Poa Tussock may have shared the possible hormesis response. Further research is required before it can be determined if hormesis was occurring.

Flupropanate affects native grasses to different extents, depending upon the rate of application and the species. However, *Austrodanthonia* species are severely affected by flupropanate (Badgery et al 2008, Bray and Officer 2007). Campbell (1997) found that *Austrodanthonia* species are killed at low rates (1.0 L/ha) of flupropanate. This was not supported in the current study where *Austrodanthonia* survived up to 2 L/ha over the 4 month study. Similarly, its growth was not significantly different from untreated controls.

Austrostipa species in the current trial were not significantly affected by flupropanate at rates up to 2 L/ha. This observation is in contrast to (Badgery *et al.* 2003) that state that spear grasses are highly susceptible to flupropanate.

Improved Pasture Species

Subterranean Clover (*Trifolium subterraneum*) is known to be highly susceptible to flupropanate (Vee Dri (Aust.) Pty Ltd Undated, Roux and Howe 1985, Campbell et al 1979). However, it has been found that large subterranean clover plants can tolerate flupropanate at rates of 1.0 L/ha but seedlings are severely damaged at 0.25 L/ha (Campbell 1997). In the current study, *Trifolium subterraneum* was severely affected from 0.5 to 1.0 L/ha but unexpectantly had better survival and growth at the higher flupropanate rates. Further research needs to be conducted on the response of Subterranean Clover to flupropanate. The response is unclear and needs to be fully researched to determine the full extent of the off-target damage that could be occurring as a result of flupropanate application.

Phalaris aquatica was not affected by flupropanate compared to the untreated control. This is in agreement with the literature that states that *Phalaris aquatica* has been found to not suffer adverse effects at 0.25 – 1.5 L/ha of flupropanate when applied to established pastures (Campbell and Viljoen). However, at high rates (4.0 L/ha) Phalaris is susceptible to flupropanate (Campbell and Ridings 1998). Flupropanate has been found to cause a reduction of green leaf area in Phalaris from which it has been found to recover (Campbell 1987, Campbell et al 1979).

Cocksfoot (*Dactylis glomerata*) will recover from being sprayed with rates of 2.5 L/ha (Campbell et al 1979). This species has also been found to not suffer adverse effects at 0.25 L/ha – 1.5 L/ha when applied to established pastures (Campbell and Viljoen). This is in agreement with the current study where *Dactylis glomerata* was not significantly affected by flupropanate.

According to the literature, perennial ryegrass (*Lolium perenne*) is moderately affected at 1.2 L/ha and severely affected at 1.8 and 2.3 L/ha by flupropanate (Viljoen and Erasmus 1996). In the current study this was not apparent as it was not affected by the flupropanate treatments applied.

Flupropanate has very little effect on the majority of the non-target species in this glasshouse study. There is a slight decline in vigour of the non-target species at the higher rates. However there is no threat to the survival of the species. It is therefore thought that flupropanate could be used more confidently up to a rate of 2.0 L/ha. It was found that minimal damage will be caused to the majority of the non-target species, although this should be verified in natural field conditions. When pastures contain Subterranean Clover, careful consideration needs to taken before treating the pasture with flupropanate, as the response to flupropanate is unclear. For the rates studied, it is known that in a glasshouse situation, Subterranean Clover is sensitive at low levels of flupropanate and as sensitive at the higher rates. These results of non-target damage occurring are consistent with the herbicide label, which states that Subterranean Clover and other legumes may be damaged if exposed to flupropanate spray or germinate before the residual affects dissipate (Vee Dri (Aust.) Pty Ltd Undated).

Verification of these results should be undertaken using field trials over a range of different soil types (light, heavy) to confirm these findings at different times of the year.

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