ESTABLISHING WHITE CLOVER (*TRIFOLIUM REPENS*) IN HIGH PEST DENSITY REGIONS

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ABSTRACT

Four field trials were conducted on commercial dairy farms in Waikato and Manawatu to develop guidelines for re-establishing white clover into vigorous grass pastures. Treatments were designed to (1) determine the minimum 'clover fallow' period needed to minimise clover pest populations and (2) identify options for re-introducing white clover. Tribenuron proved damaging to grass and results for thifensulfuron were variable. Dicamba was effective in removing clover and the length of 'clover fallow' required to remove clover root weevil larvae appeared to be 6-8 months. Glyphosate applied at sowing reduced pasture cover significantly for up to 10 weeks, but seedling establishment was improved by 50–100%. The re-introduction of clover was better in autumn than spring, but no treatment resulted in increased long term clover content of the pasture. This was due to both poor emergence and survival of clover seedlings facing severe competition from resident well established grass plants. **Keywords:** white clover, *Trifolium repens*, clover root weevil, *Sitona lepidus*, 'clover fallow', dicamba, thifensulfuron, tribenuron, seedling establishment, oversowing, nematodes.

INTRODUCTION

Maintaining white clover (*Trifolium repens*) in high fertility, high production mixed pastures is under increasing pressure as a result of new technologies and changing management systems. Pasture pests and diseases add to the problem, and in recent years the predation by clover root weevil (*Sitona lepidus*) has caused considerable damage to clover in many high producing dairy pastures (Gerard & Hackell 2005; Gerard et al. 2007; White & Gerard 2006). This has raised the question of whether white clover can be re-introduced into pastures with a strong, productive grass base, without the need for traditional cultivation or renovation and their associated production losses.

As clover root weevil, root knot nematodes and clover cyst nematodes are obligate pests of white clover, it is possible, by significantly reducing or completely removing residual clover, to create a period of 'clover fallow' to eliminate these pests (Bell et al. 2004). Clover could then be re-introduced by direct drilling to capitalise on the high growth potential of its tap rooted plants in their first year (Brock & Tilbrook 2000) to increase the clover content of pastures. Four field trials on dairy farms in the Waikato and Manawatu regions were carried out to test this hypothesis, investigating suitable herbicides, the length of the 'clover fallow' and direct drilling techniques. The specific objectives of the project were to develop a best management practice for re-introducing white clover into established dairy pastures, in order to improve production and reduce reliance on mineral nitrogen for dairy farmers.

MATERIALS AND METHODS

Four trials were conducted on productive dairy pastures, on sites with a history of high clover root weevil damage. A split-plot design with three replicates was used in all trials with over-sowing treatments as main plots (7 m wide to accommodate a double pass of a conventional drill) and factorial combinations of 'clover fallow' treatments as 5 m long sub-plots. 'Prillcote' white clover seed (a mixture of cultivars Tahora, Bounty and Kopu II) was sown at 5 kg/ha (equivalent to 3 kg/ha bare seed). All herbicide treatments were applied by a CO₂ powered backpack sprayer with a 2 m boom fitted with 4 nozzles to deliver 200 litres of water/ha at 210 kPa. Details of sowing dates, equipment and chemicals used are given in Table 1.

TABLE 1: Site and treatment details of four trials conducted in the Waikato and Manawatu over 2006-08.

Trial no., site and soil type	'Clover fallow' treatments	Sowing details
1. Keith Holmes, Tauhei, Morrinsville, Waikato; Kaipaki peat loam.	Tribenuron 30 g/ha, applied 12, 8, 4 or 0 weeks before sowing	11 January 2006; John Deere air mix direct drill; glyphosate at 0 or 150 ml/ha
2. Craig McKie, Hoe-O-Tainui, Waikato; Hamilton clay loam	Thifensulfuron 30 g/ha, applied 12, 8, 4 or 0 weeks before sowing	30 September 2006; John Deere air mix direct drill; glyphosate at 0, 75 or 150 ml/ha
3. Craig McKie, Hoe-O-Tainui, Waikato; Hamilton clay loam	Dicamba 0.3 kg/ha, applied 6 weeks before sowing; thifensulfuron 30 g/ha, at sowing	17 April 2007; direct drill or broadcast sow by Aitchesen Turf Drill; glyphosate at 0 or 150 ml/ha
4. Hopkins Farming Group, Tiakitahuna, Manawatu; Kairanga silt loam	Dicamba 0.3 kg/ha, 12 or 6 weeks before sowing; thifensulfuron 30 g/ha, 12, 6 or 0 weeks before sowing	10 April 2007; direct drill or broadcast sow by Aitchesen Seedmatic 3000C; glyphosate at 0 or 200 ml/ha

White clover performance was assessed by visual estimate of clover abundance (% strikes in ten, 15 cm diameter quadrats/sub-plot), seasonal botanical composition (Trials 3 and 4), and through pasture growth measured by rising plate meter (10 readings/sub-plot). Seedling density was estimated 4 to 8 weeks after sowing by counting ten, 25 × 10 cm quadrats/sub-plot. Survival of seedlings was monitored in Trial 4 by mapping 140 plants in 20 re-locatable quadrats across several sub-plot treatments. Seedlings were identified by their taproot, which normally remains intact for 12-18 months after sowing (Brock & Tilbrook 2000).

Insect sampling was conducted on four occasions in both regions: Manawatu in January, May and November 2007 and April 2008; and Waikato in July 2006, February, June and November 2007. Two 10 cm diameter cores were taken from each plot to 12 cm depth and hand sorted either in the field or laboratory to assess the insects present. For the Manawatu site, at each sampling date, clover root weevil adult abundance was monitored in pasture adjacent to the trial plots. A modified blower vacuum with a collection sleeve was used to sample weevils along a randomly selected 30 m transect.

Soil nematodes were sampled on 24 January 2007 and again on 1 April 2008 from control plots and from plots treated with dicamba 12 weeks before sowing. Soil cores (2.5 cm diameter) were taken to 10 cm depth, placed in plastic bags and stored at 4°C

until processing. Cores were hand-crumbled, mixed and nematodes extracted from 100 g sub-samples per plot using the tray method described by Bell & Watson (2001). Total nematode numbers were assessed using a Doncaster dish and plant parasitic nematodes identified to genus using a Zeiss Stemi microscope at ca 80× magnification. For species identification, nematodes were mounted on temporary slides, heat killed and examined under an Olympus BX51 microscope at 400× magnification.

The data generated by the trials had variable replication of the main factors of interest, which made it difficult to apply conventional data analysis. Therefore data were analysed by Microsoft Excel pivot tables to generate means and standard errors of the means.

RESULTS AND DISCUSSION

Pest populations

The trial sites in both regions were selected for their past history of damaging clover root weevil populations and had larval populations in the range of 170–200/m² in October 2005, but for the 3 years of these trials, levels were consistently low (<50 larvae/m²). Host-prey relationships follow a cyclic pattern and after several years of high pest pressure, there may have been a decline in clover root nodule production and thus low larval recruitment. Clover root weevil was first recorded in Manawatu in 2004 (Hardwick et al. 2004) and it was thought that populations would be high at the Trial 4 site in 2007/08 if the invasion pattern followed that in other regions. This did not occur and larval densities were low, despite there being considerable adult foliar feeding (average of 9.3 adults/m² recorded in the area adjacent to trial plots in May 2007). Nevertheless, numbers were sufficient to derive some measure of the effectiveness of creating a ‘clover fallow’.

Of the herbicides tested, only dicamba was successful in removing white clover (Table 2, Figs 1 & 2) with no damage observed to the grass. Thifensulfuron did not completely remove the clover although it was more effective in Waikato than Manawatu (Table 2). Clover root weevil density tended to parallel these effects and, larvae were removed only in the dicamba treatments, completely in Manawatu and partially (87% reduction compared to pre-treatment populations) in Waikato (Table 3). Estimates of the length of fallow required would appear to be 6–8 months. Even if clover can be removed and clover root weevil eliminated, the following results and other recent research (Brock & Kane 2003) would suggest re-introduction of the clover may not be easily achievable.

FIGURE 1: The abundance (% strike/ten quadrats) of white clover in pasture in Trial 3 after treatment with dicamba applied 8 weeks before sowing and thifensulfuron at sowing (arrow).

FIGURE 2: The abundance (% strike/ten quadrats) of white clover in pasture in Trial 4 after treatment with dicamba applied 6 or 12 weeks before sowing and thifensulfuron applied at 6 or 12 weeks before sowing or at sowing (arrow).

TABLE 2: Mean clover content of pastures (% of DM) following autumn sowing with pre-sowing dicamba or thifensulfuron treatments, and glyphosate at sowing.

Herbicides at:		Trial 3 Waikato		Trial 4 Manawatu		
Fallow	Sowing	Winter	Spring	Winter	Spring	Summer
None	None	4.5	9.3	3.5	8.9	15.3
	glyphosate	10.6	10.2	6.9	8.6	14.9
dicamba	None	0.0	0.0	0.0	0.2	1.5
	glyphosate	0.0	0.1	0.1	1.4	3.7
thifensulfuron	None	1.0	0.7	2.8	8.4	14.3
	glyphosate	1.9	0.8	3.4	7.9	15.3
SEM		0.91	0.96	0.34	0.75	1.13

There was no difference between control and dicamba-treated plots in the abundance of total nematodes (5560 cf. 5250/100 g soil) or plant feeding nematodes (2240 cf. 1960/100 g soil). The high levels of plant feeding nematodes (40% of total population) were likely to be having a deleterious effect on plant root function (Yeates 1976). Lesion nematodes (1375/100 g soil) were the most abundant plant damaging genus, followed by spiral nematodes (665/100 g soil), which have yet to be shown to cause major plant production losses. The root knot nematodes observed in these samples were identified as *Meloidogyne naasi* (grass-feeding nematodes) and as their numbers were very low (3/100 g soil), damage by them can be considered inconsequential.

TABLE 3: Mean clover root weevil larvae (no./m²) following herbicide treatment with dicamba or thifensulfuron in the pre-sowing 'fallow' period in Trials 3 and 4.

'Fallow' herbicide	Applied	9.2.07	18.6.07	27.11.07
Trial 3				
Control	-	21.2	45.1	0
dicamba	20.2.07	26.5	5.3	5.3
thifensulfuron	17.4.07	10.6	10.6	5.3
SEM		7.06	7.92	1.84
Trial 4		12.1.07	8.5.07	19.11.07
Control	-	21	84	55
dicamba	11.1.07	17	58	0
dicamba	22.2.07	23	28	5
thifensulfuron	11.1.07	64	40	30
thifensulfuron	22.2.07	12	91	115
thifensulfuron	5.4.07	25	31	42
SEM		6.3	13.7	12.3

'Clover-fallow'

Finding a selective herbicide to remove clover for a short period without significant residual carryover has been difficult. The first product tested was tribenuron (Granstar) used at 30 g/ha. While effective in removing clover, it had damaging soil residual effects on clover germination (Table 4) and also affected the ryegrass to an extent that was unacceptable in the context of maintaining a strong productive grass base. The next herbicide tested, thifensulfuron (Harmony) at 30 g/ha, had no effect on the ryegrass, but was variable in removing the clover. For instance, in Trial 3 (Waikato), thifensulfuron in autumn reduced clover by 78% for up to a year, whereas in Trial 4 (Manawatu), the reduction was only 20% and had disappeared by spring (Table 2). In Manawatu, rainfall (185 mm January to April) was only 50% of normal during the period of herbicide application, which may have reduced its effectiveness (Table 5, Fig. 2). According to the label, thifensulfuron should not be applied when pasture is stressed by dry conditions, which occurs frequently in the autumn, thus thifensulfuron may not be the most suitable product for autumn use. Nevertheless, in Waikato with 250 mm of rainfall, thifensulfuron was more effective (Table 4, Fig. 1). In contrast, Trial 2 showed the winter application of thifensulfuron 8 weeks before sowing was the most effective at reducing clover by sowing (Table 4). In order to ensure a 'clover-fallow' would be created and allow the re-introduction of new clover from seed to be properly assessed, dicamba (Banvel), known to be highly effective against clover, was included in Trials 3 and 4. Dicamba does have some residual soil activity (Rahman et al. 1981), but soil samples taken at sowing to bioassay for phytotoxicity on germinating clover seed found no damaging residues (data not presented).

TABLE 4: Clover abundance (% strike/ten quadrats), seedling emergence (no./m²) and pasture DM (kg/ha) 6 weeks after sowing in two spring trials in Waikato with herbicide treatments at 0, 4, 8 or 12 weeks pre-sowing to create 'clover fallow' and glyphosate at sowing to reduce competition.

Herbicide:	Trial 1 – tribenuron			Trial 2 – thifensulfuron		
'clover fallow'	Clover	Seedlings	DM	Clover	Seedlings ¹	DM
0 weeks	91	112	1790	88	4	3730
4 weeks	20	2	2220	67	6	3480
8 weeks	29	3	1570	4	19	2820
12 weeks	29	15	1570	34	4	3160
at sowing						
none	40	33	2290	47	5	3330
glyphosate	70	97	1390	55	11	3190
SEM	4.3	11.3	41.5	5.1	2.5	27.3

¹Results from one replicate only that was re-over sown 1 month after the original sowing.

Clover re-introduction

Results between trials were variable and seedling establishment was low overall, apart from Trial 1. Here there was a major influence of buried clover seed with high numbers emerging in the control (no 'clover-fallow') plots resulting in increased clover content 6 months later (Table 4). This was not seen in the tribenuron treatments, indicating a considerable residual effect, which decreased seedling emergence as the 'clover-fallow' treatment approached the sowing date. Band spraying combined with sowing was evaluated in this trial, but in the absence of commercially configured machinery was unreliable, which was more due to the inadequacy of the available equipment, than the failure of the technique.

The subsequent three trials gave similarly poor results. In Trial 2, less than 1 seedling/m² could be found 3 months after direct drilling in the spring. This site is located in an area with traditionally high clover flea (*Sminthurus viridis*) population densities and pest monitoring showed that clover flea populations increased shortly after sowing, which may have contributed to poor clover establishment. Broadcast oversowing of one replicate a month later gave better results, of which thifensulfuron 8 weeks before sowing was the best (Table 4). The autumn trials (Table 5) gave better seedling establishment, with thifensulfuron at sowing being the best in both trials and dicamba 8 weeks before sowing being intermediate in the Waikato trial. In the Manawatu trial emergence was better than in the Waikato trial (Table 5), with thifensulfuron at sowing again being the best. Direct drilling produced nearly three times more seedlings than broadcast oversowing (66 cf. 23 seedlings/m²) in the Manawatu trial, but no differences were found in the Waikato trial.

In all four trials, chemical topping with glyphosate at sowing reduced pasture DM and consistently increased clover seedling densities by 50-100% (Tables 4 & 5) indicating the importance of reducing plant (grass) competition at emergence. Despite this, no treatment resulted in significantly increased clover in pastures in the longer term, except in Trial 1, which could be attributed to buried clover seed rather than over-sowing.

TABLE 5: Clover abundance (% strike/ten quadrats), seedling emergence (no./m²) and pasture DM (kg/ha) 6 weeks after sowing in two autumn trials with herbicide to create 'clover fallow' and glyphosate at sowing to reduce competition.

Herbicide: 'clover fallow'	Trial 3 (Waikato)			Trial 4 (Manawatu)		
	Clover ¹	Seedlings	DM	Clover	Seedlings	DM
Control (no herbicide)	70	4	3020	85	37	2580
thifensulfuron–0 weeks	42	31	2540	59	70	2360
thifensulfuron–6 weeks	– ¹	–	–	78	41	2540
thifensulfuron–12 weeks	–	–	–	69	42	2480
dicamba–6 weeks	3	15	2800	5	41	2600
dicamba–12 weeks	–	–	–	8	41	2600
at sowing						
Control (no herbicide)	38	17	2790	47	35	2610
glyphosate	45	25	2440	59	54	2390
SEM	5.3	2.5	46.3	3.9	4.6	25

¹Treatment not included in this trial.

GENERAL DISCUSSION

While the 'clover fallow' did reduce the clover root weevil populations, clover re-establishment was rather poor in these trials. With a sowing rate of 5 kg coated seed/ha (400 seed/m²), these emerging seedling densities translate into very low proportions of seed sown (2–10%), in line with results achieved in over-sowing hill country or natural reseeded (Edwards et al. 2005). Although the highest number of seedlings in Trial 4 (thifensulfuron at sowing, direct drilled with glyphosate) was 134/m² (33% of seed sown), similar to average results for many new sown pastures (Brock & Kane 2003), the difference here is that seedling mortality was initially very high (Fig. 1). Mapped plants showed losses in excess of 60% by the end of the following summer from competition with the strongly growing grass base under full farm production management (Brock et al. 2008). The few surviving plants, while large [mean 187 mm spread (range 46–495); 21 growing points (4–64); 555 mg DW (140–1360)] and comparable to plants rotationally grazed by sheep (Brock & Tilbrook 2000), were insufficient in number to increase clover content of the pasture in the first year when new plants are at their greatest growth potential.

Soil type may contribute to such variability between sites. Clover establishment is usually greater in lighter soils with higher sand or organic matter content, falling as the proportion of silt and clay increases (Brock & Kane 2003). This would fit the pattern of better emergence results in the peaty soil of Trial 1, poor results in the clay soil of Trials 2 and 3, and the intermediate result for the silt loam of Trial 4. Soil type may also have contributed to the difference in activity of thifensulfuron between the Waikato and Manawatu trial sites.

Results of these trials support those of Bell et al. (2004) and indicate that the premise of 'clover fallow' to remove pests with a narrow host range, such as clover root weevil, is technically feasible. However, with an estimated 6–8 months needed to achieve complete removal of clover root weevil larvae, re-sowing at 12 weeks would not be advisable. This would mean that 'clover fallow' would need to be achieved in spring, the grass maintained through the summer with fertiliser nitrogen and the clover re-introduced in autumn.

Establishing clover, whether from new sowing or over-sowing, is fraught with problems and still is not well understood. However, clover re-establishment following a 'clover

fallow' will be difficult practically until reliable and consistent technology and farm management strategies have been developed to address the specific issue of minimising interspecific competition when clover plants are in the establishment phase.

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