Effects of spring management techniques on seed yield and yield components of two contrasting white clover varieties

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Abstract

White clover seed yield is primarily determined by the number of inflorescences produced per unit area, thus management practices should include a focus on concentrating and increasing inflorescence production. The use of plant growth regulators such as paclobutrazol is both expensive and has produced inconsistent results in New Zealand and overseas. In a field experiment at Lincoln, Canterbury, New Zealand the effects of three management techniques, including different herbicides (Ravensdown Glyphosate 360 (Glyphosate 360) - a.i. 360 g l^{-1} glyphosate; and Jaguar® - a.i. 25 g l^{-1} diflufenican and 250 g l^{-1} bromoxynil EC) and rates of application (1 & 1.5 l ha^{-1} Glyphosate 360), topping and the use of a plant growth regulator (PGR; Moddus®, a.i. 250 g l^{-1} trinexapac-ethyl EC) on 'Apex' and 'Kotare' white clover growth and development were examined. Seed yield was lower (630 kg ha^{-1}) for the 1.5 l ha^{-1} glyphosate 360 crops than the other treatments (838-990 kg ha^{-1}). There was no difference in seed yield between the two cultivars. Harvest index was similar across treatments at 0.12. However, there were more ($P<0.001$) inflorescences under topping than any of the other treatments. Topping also produced more stolons than all other treatments except the control crops. Time to canopy closure differed ($P<0.001$) with treatments, being earliest for the control followed by the Jaguar®, Moddus® and topping treatments. Both glyphosate treated crops failed to close their canopies and hence had the lowest leaf area indices (LAI).

Additional keywords: Glyphosate, harvest index (HI), indeterminate, inflorescence, Jaguar®, Moddus®, stolon density, *Trifolium repens*

Introduction

For many years New Zealand has produced about 38-50% of the world's white clover (*Trifolium repens* L.) seed requirements (Mather *et al.*, 1995; Pyke *et al*., 2004). It is estimated that white clover contributes NZ $3.1 billion to New Zealand each year, due to N fixation, improved forage yield quality and quantity, seed production and honey production. Of this, approximately NZ $25 million is related to the production of seed and 75% of this is from export (Caradus *et al.*, 1989; Statistics New Zealand, 2011). Data on white clover seed value (Statistics New Zealand, 2011), land under improved pastures (van Bunnik *et al*., 2007) and amount of N fixed by white clover per hectare per year (Lucas *et al*., 2010; Gerard *et al*., 2011) suggest that the contribution of white clover to New Zealand economy is unlikely to have changed in last 15-20 years. There are 33 registered white clover cultivars in New Zealand, 22 of these eligible for OECD certification (MAF, 2011). These are grown and also marketed locally and around the world.

White clover seed production is closely related to the number of inflorescences produced per unit area (Clifford, 1986; 1987; Lopes and Franke, 2009). Clifford (1985; 1987) reported that management practices should be directed at obtaining high numbers of uniformly distributed stolon tips at the time of closing the crop so as to produce a short and prolific flowering period. However, in practice white cover is indeterminate (Hollington *et al.*, 1989). Therefore, the extended period of flowering and resultant range of inflorescence ripeness makes it difficult to determine optimum harvest time. Thomas *et al*. (2009) have estimated seed yield loses at harvest of up to 30%. Furthermore, when the seed is harvested there will be seeds with a range of maturities and therefore seed quality will be variable. Pasumarty and Thomas (1990) and Thomas *et al*. (2009) have reported that shading of stolons by bulk vegetative crops in late spring can lead to reduced seed yield. A number of management techniques, including chemical and manual topping and irrigation management can be used to manipulate reproductive to vegetative ratios (Clifford, 1987) and therefore improve white clover seed yield.

Experimentally, the use plant growth regulators (PGR) such as paclobutrazol (Budhianto *et al.*, 1994a) have improved white clover yield components such as number of nodes and stolons, thus increasing inflorescence production (Budhianto *et al.*, 1994b) and potentially seed yield. However, results from the traditional white clover seed producing areas in Canterbury have not shown any benefit for use of paclobutrazol. This has led to the investigation of other PGR such as Moddus® (Thomas *et al*., 2009) in Canterbury. Moddus® application was expected to reduce internode length and therefore produce more flowers between the rows. Thomas *et al*. (2009) observed that the application of Moddus® depressed seed yield in an irrigated crop by approximately 170 kg ha^{-1} compared with the 570 kg ha $^{-1}$ for the control. The authors attributed the depressed yield to the late application of Moddus® resulting in the PGR having insufficient time to reduce internode length as most were already present. Consequently an earlier application date was used in this experimental work.

Herbicides have also been used to slow down vegetative growth and therefore reduce density and early growth rate in white clover. Thomas *et al*. (2009) investigated the effects of Gramoxone® (a.i. 250 g kg^{-1} paraquat dichloride salt; SC), Jaguar® and Tropatox® (a.i. 25 g l^{-1} MCPA and 375 g l^{-1} MCPB; SC) on white clover seed production at two sites in Canterbury and the results were inconclusive. Gramoxone® and Jaguar® treated 'Riesling' white clover crops produced lower seed yield at approximately 1,000 kg ha^{-1} compared to 1,200 kg ha^{-1} from the untreated and Tropatox crops at Leeston site. However, at Newlands, Jaguar® treated 'Tribute' white clover crops produced the least seed yield at 735 kg ha^{-1} compared with approximately 840 kg ha^{-1} for the other treatments.

Manual topping of white clover seed crops also gave inconsistent results (Thomas *et al*., 2009) at three sites in Canterbury (Leeston, Tai Tapu and Chertsey). Early topping at first flower (mid-November) had no effect on seed yield across the sites. Topping at mid-flowering, 14 days after first flower (end of November) increased seed yield at Chertsey and Leeston, while late topping (21 days after first flower; early December) substantially decreased yield at Chertsey and Tai Tapu. However, at one site, Leeston, the time of topping was important. Topping later than mid-flowering and double topping improved seed yield to approximately 785 kg ha^{-1} compared with approximately 700 kg ha^{-1} for the untreated and early topped crops.

The current experimental work evaluates the use of chemical topping (two herbicides; glyphosate (two rates) and Jaguar®), manual topping and PGR (Moddus®) on growth and development of 'Apex' and 'Kotare' white clover. 'Apex' white clover is a medium leaf variety (Woodfield *et al.,* 2003) bred for high stolon density, disease resistance and good drought tolerance and 'Kotare' white clover is a large leaf, high yielding variety, bred for increased stolon point density and therefore improved persistence (Agriseeds, 2011). 'Kotare' has excellent summer and autumn yielding ability. The spring management techniques were intended to check rapid spring growth and therefore reduce excess leaf production (Thomas *et al*., 2009). It was expected that the PGR used in this experiment would reduce petiole height but not penducle height (Marshall and Hides, 1986). Both the herbicide and manual topping are aimed at reducing the shading of the stolons and emerging flower heads. This will have the effect of elevating the flowers above the leaf canopy, resulting in a more favourable micro-climate for pollination. The main objective was to develop a management strategy that would increase seed yield and to quantify the mechanisms for the increase.

Materials and Methods

The experiment was situated on the AgResearch farm at Lincoln, New Zealand (43°39΄S; 172°28΄E), on a Wakanui silt loam.

The trial was a randomised complete block design with two white clover cultivars and three spring management treatments, replicated four times. These included using different herbicides ((Ravensdown Glyphosate® 360 (Glyphosate® 360)) - a.i. 360 g l^{-1} glyphosate; and Jaguar® - a.i. 25 g l^{-1} diflufenican and 250 g l^{-1} bromoxynil EC) and rates of application (1 & 1.5 l ha^{-1} Glyphosate® 360), topping and the use of a plant growth regulator (PGR; Moddus®, a.i. 250 g l^{-1} trinexapac-ethyl EC) (Table 1).

The trial plots were 2.7 m wide by 10 m long. 'Apex' and 'Kotare' white clover were direct drilled at 3 kg ha^{-1} on 24 March 2010 following an oat crop that was harvested on 3 March 2010. The site had been under ryegrass since 2005. All the treatments were applied on 15 October 2010. Another topping was carried out on the relevant plots on 17 November 2010. Average soil tests for the site were; pH 6.0, Olsen P 32, potassium (K) 380, calcium (Ca) 875, magnesium (Mg) 65, sodium (Na) 25 mg kg^{-1} soil and available N 60 kg ha^{-1}. The soil fertility levels were adequate for white clover production, with the background P levels more than twice the recommended (Clifford, 1985).

Table 1: Treatments details and dates of application.

Treatment	Rate (l ha^{-1})	Date of application
Control	Nil	
Moddus® (a.i. 250 g l^{-1} trinexapac-ethyl EC)	2.4	15 October 2010
Jaguar® EC (a.i. 25 g l^{-1} diflufenican and 250 g l^{-1} bromoxynil)	1.5	15 October 2010
Ravensdown Glyphosate 360 (a.i. 360 g l^{-1} glyphosate)	1.0	15 October 2010
Ravensdown Glyphosate 360 (a.i. 360 g l^{-1} glyphosate)	1.5	15 October 2010
Topping	MD[1]	15 October 2010 & 17 November 2010

[1]Mechanical defoliation.

Measurements

Sequential dry matter (DM) measurements were taken from each plot by harvesting a single 0.1 m^2 quadrat to ground level, monthly from 12 October 2010 (3 days before treatments were applied) to 12 January 2011. All harvested fresh weight (FW) was kept for DM determination. From November, about 10% of the harvested FW was sub-sampled for DM partitioning into leaves, petioles, inflorescences and peduncles. The remainder of the plant samples were dried in a forced air oven at 60°C to constant weight to determine DM. Sub-sample leaf area (cm^2) was determined from the partitioned leaves, using a leaf area meter (LiCor model LI-3100). Total leaf area per quadrat was obtained by dividing total FW by sub-sample FW and then multiplying the result by sub-sample leaf area, and 10 to give area m^{-2}. Total leaf area m^{-2} was them divided by 10,000 cm^2 (=1 m^2) to give leaf area index (LAI).

Percentage light transmission through the canopy was measured with a Sunfleck ceptometer (Model SF-80), which was placed in an open grove made below the ground, across the plant rows in each plot. Measurements of intercepted light ceased when 95% of the incoming radiation was being intercepted.

Crop was direct headed on 28 February 2011, following 2 applications of Reglone (a.i. 200 g l^{-1} diquat) at 3 litres per application. Seed yield was determined by hand harvesting a single 0.33 m^2 quadrat from each plot. Components of yield such as inflorescences harvested, seeds per inflorescence, stolon numbers and harvest index (HI) were determined on all plots at final harvest (after the second application of Reglone).

Data analysis

Canopy development, DM yields and yield components were analysed by analysis of variance (ANOVA). Significant interactions and main effects were separated using Fisher's protected least significant difference (LSD) tests (α=0.05). Where values show $P<0.1$ a trend is indicated in the text. Fitted curves used regression analyses of the form $y=a+bx$ fitted by SigmaPlot between individual data sets. The adjusted coefficient of determination (R^2) (Willmott 1982) was used to determine the relationship between data sets.

Meteorological conditions

The total rainfall for the experimental period was 673 mm with Penman potential evaporation of 880 mm. This was more than the long term seasonal average (LTA) of 640 mm (Widdup *et al*., 2004). A total of 100 mm of irrigation was split applied on 3 November 2010 and 6 December 2010. The average maximum temperature was 16.8°C and the average minimum temperature was 7.1°C, compared with an LTA of 12.2°C. The total sunshine hours were 1,877 for the experimental period compared with 2,054 for the year and LTA of 2,100 hours. There were 54 ground frosts during the trial period.

Results

Seed yield

Total seed yield was lowest (P=0.001) for the 1.5 l ha^{-1} glyphosate treatment compared with other treatments for both 'Apex' and 'Kotare' white clover (Table 2). None of the treatments increased seed yield. They had an average yield of about 900 kg ha^{-1}. There was also no yield difference (P=0.681) between the two cultivars across all treatments, with an average yield of 854 kg ha^{-1}.

The relative seed yield (RSY) was lowest for the 1.5 l ha^{-1} glyphosate treated crops at <70% of the control for both cultivars.

Table 2: Effects of spring treatments on seed yield (kg ha^{-1}) and relative seed yield (RSY; percentage of the control) for 'Apex' and 'Kotare' white clover in the 2010-11 season at Lincoln.

Treatments	Cultivar			
	Apex		Kotare	
	Seed yield	Relative Seed Yield	Seed yield	Relative Seed Yield
Control	993	100	865	100
Moddus®	890	90	925	107
Jaguar®	938	94	916	106
Glyphosate 1 l ha^{-1}	824	83	853	99
Glyphosate 1.5 l ha^{-1}	683	69	575	66
Topping	850	86	939	109
$LSD_{0.05}$ (33df)	205		205	
Significance	P<0.001		P<0.001	

Table 3: Mean total dry matter yield (DM; kg ha^{-1}) and seed yield (kg ha^{-1}) for white clover grown under six spring treatments in the 2010-11 season at Lincoln.

Treatments	Total DM	Average Seed Yield[1]
Control	7305	929
Moddus®	7616	908
Jaguar®	7798	927
Glyphosate 1 l ha^{-1}	6648	838
Glyphosate 1.5 l ha^{-1}	5586	629
Topping	6648	895
$LSD_{0.05}$ (33df)	962.4	147.3
Significance	P<0.001	P<0.001

[1]Both total DM and seed yield are averaged as there were no yield differences between cultivars.

The 1.5 l ha^{-1} glyphosate treated crops produced the lowest total DM yield (Table 3). There were no DM differences between the control, the 1 l ha^{-1} glyphosate and the topping treatments with the latter two having less DM (P<0.001) than the Moddus® and Jaguar® treated plots. There were no differences (P=0.649) in harvest index (HI) which averaged 0.12 across all treatments (Figure 1a).

There was a strong positive linear relationship between seed yield and LAI below 3 (R^2=0.92) and total DM (R^2=0.79) and to a lesser extent, in order of importance to stolon numbers up to approximately 600 m^{-2} (R^2=0.58) and flower number (R^2=0.40) (Figure 1). For example, seed yield increased from 630 kg ha^{-1} at about 560 kg m^{-2} DM to about 900 kg ha^{-1} of seed at DM yields above 780 kg m^{-2}. The same level of seed yield increase was also associated with the increase in number of stolons from about 331 to 580 stolons m^{-2}, respectively.

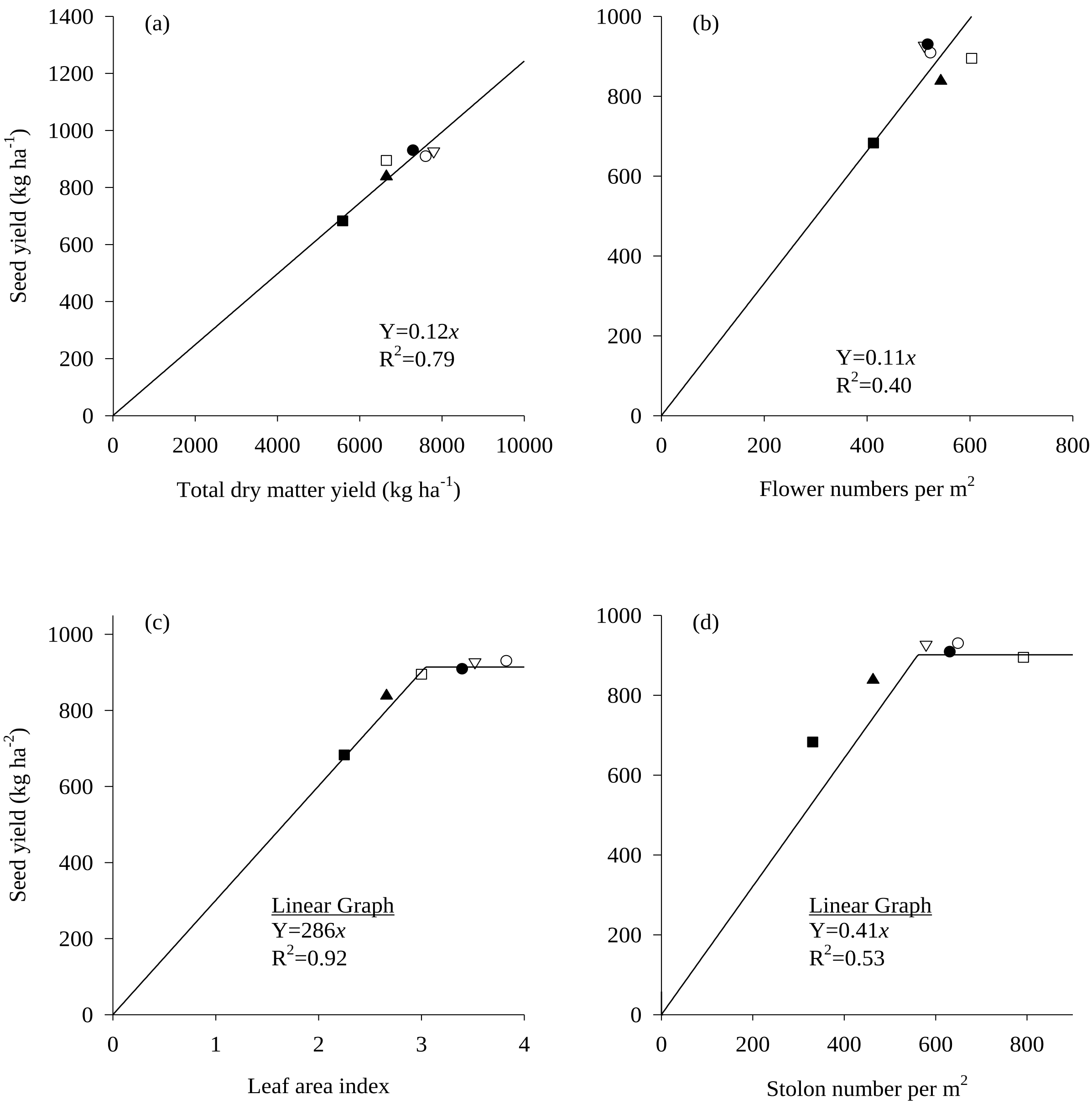

Figure 1: Interactions between seed yield (kg ha^{-1}) and (a) total dry matter per m^2 (b) total inflorescences per m^2 (c) leaf area index at the end of the season (12 January, 2011; Figure 2) and (d) stolon numbers per m^2 for white clover under different treatments, ○Control; ● Moddus®; ▽ Jaguar®; ▲1 l ha^{-1} Glyphosate; ■ 1.5 l ha^{-1} Glyphosate and □ Topping at Lincoln in the 2010-11 season.

Topping led to increased (P<0.001) inflorescences (flowers) (Figure 1b) and stolon (Figure 1d) production compared with all the other treatments. For example, topped crops produced about 600 flowers m^{-2} compared with 410 flowers m^{-2} for the 1.5 l ha^{-1} glyphosate treated crops and an average 520 flowers m^{-2} for all the other treatments. The topped treatments produced about 790 stolons m^{-2}, which was not different from the control but larger than the average of 560 stolons m^{-2} for the Moddus®, Jaguar® and 1 l ha^{-1} glyphosate treated crops and the 331 stolons m^{-2} for the 1.5 l ha^{-1} glyphosate crops. There were no differences in stolon numbers between the glyphosate treated crops.

Leaf area index increased (P<0.001) with time after application of treatments. Both glyphosate treatments reduced (P<0.001) canopy development as assessed by LAI earlier in the spring (Figure 2) but there were no differences in leaf area indices among the other treatments. The reduced LAI for the glyphosate treated white clover crops resulted in lower (P<0.001) light interception (Figure 3) over most of the season. None of the glyphosate treated crops attained full cover; they only attained 90% interception 3 weeks after all other crops had reached full cover.

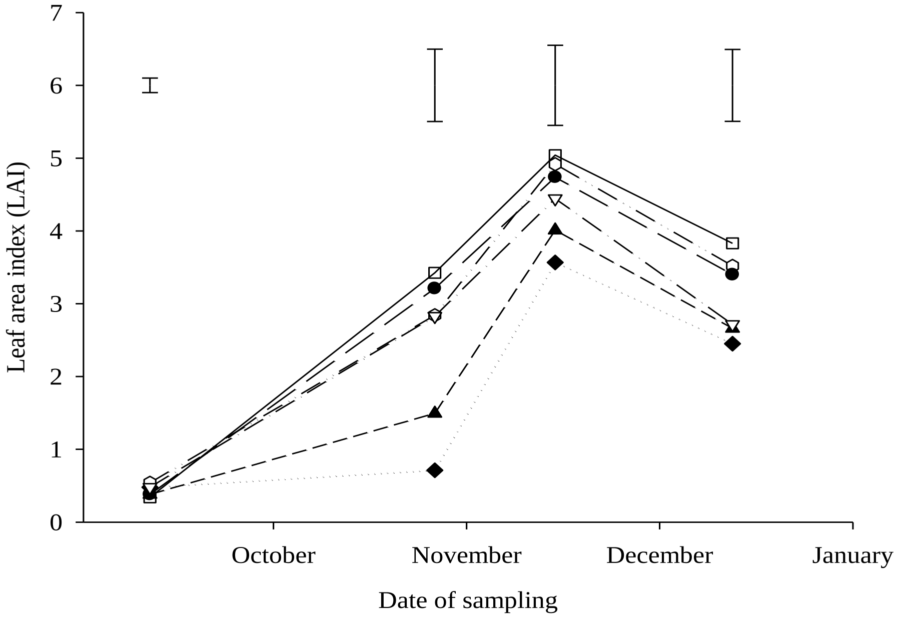

Figure 2: Leaf area index (LAI) for white clover grown under six treatments: ○Control; ● Moddus®; ▽ Jaguar®; ▲1 l ha^{-1} Glyphosate; ♦ 1.5 l ha^{-1} Glyphosate and □ Topping at Lincoln in the 2010-11 season. Bars represent the least significant differences ($LSD_{0.05}$; 33 df). Treatments were applied on 15 October 2010 (see Table 1 for more details).

LAI was highest in December (Figure 2) when the crops had reached full cover (Figure 3). The differences in LAI were evident throughout the season. However, LAI for all the treatments declined from the end of December to the final harvest in February, due to leaf senescence.

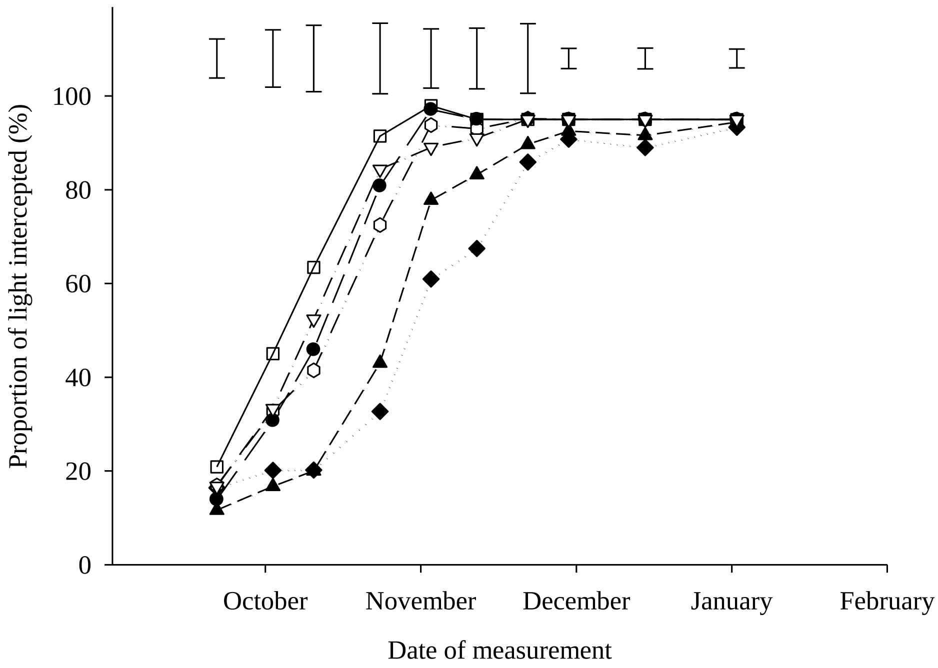

Figure 3: Proportion of light intercepted by white clover crops under different spring management systems: ○Control; ● Moddus®; ▽ Jaguar®; ▲1 l ha^{-1} Glyphosate; ♦ 1.5 l ha^{-1} Glyphosate and □ Topping at Lincoln in the 2010-11 growing season. Bars represent the least significant differences ($LSD_{0.05}$; 33 df). Treatments were applied on 15 October 2010 (see Table 1 for more details).

LAI had a strong linear relationship with total DM (R^2=0.80) and flower numbers (up to LAI 3; R^2=0.95) but a weaker relationship to stolon numbers (R^2=0.30) (Figure 4). Peak flower numbers of about 550 m^{-2} were attained at LAI of 3. At LAI >3, flower numbers fell to ≤500 flowers m^{-2}.

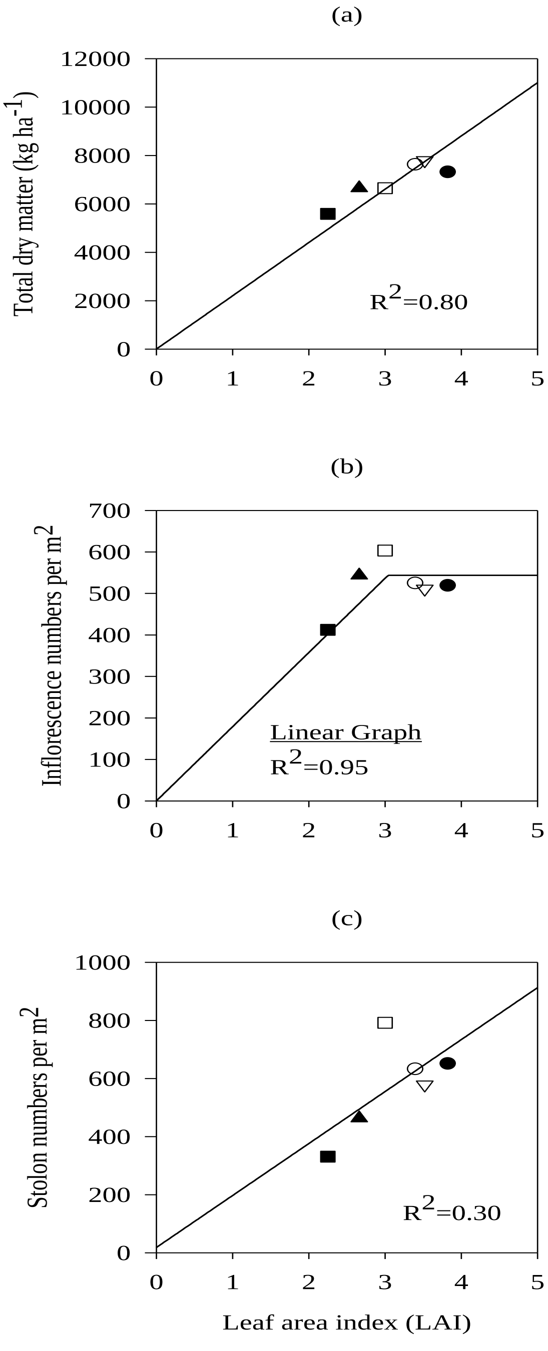

Figure 4: Interactions between (a) total dry matter (b) flowers per m^2 (c) stolons per m^2 and leaf area index (LAI) towards the end of the season (12 January, 2011; Figure 2) for white clover under different treatments: ○ Control, ● Moddus®, ▽ Jaguar®, ▲1 l ha^{-1} Glyphosate, ■ 1.5 l ha^{-1} Glyphosate and □ Topping at Lincoln in the 2010-11 season.

Discussion

The seed yield of 630-930 kg ha^{-1} in this experiment was similar to the 570-830 kg ha^{-1} previously reported in literature (Marshall and Hides, 1986; Clifford, 1987) and more than the 300-620 reported by Budhianto *et al*. (1994b) and Pyke *et al*. (2004). In the current experiment seed yield was not affected by the treatment, except for the 1.5 l ha^{-1} Glyphosate 360 which produced the lowest yield (Table 2).

The seed yield from the Jaguar® treated crops of approximately 930 kg ha^{-1} in the current experiment was similar to the 1000 kg ha^{-1} reported by Thomas *et al*. (2009) for same treatment, for 'Riesling' white clover. It was however, larger than the 730 kg ha^{-1} reported for 'Tribute' white clover by the same authors. However, the seed yield for Jaguar® treated crops were 14-21% lower than the control crops in Thomas *et al*. (2009) for both cultivars.

Seed yield of 895 kg ha^{-1} for the manually topped crops in this experiment was 12-24% more than the approximately 800 kg ha^{-1} for the mid- and late topped 'Riesling' white clover crops, and the 690 kg ha^{-1} for the early topping, reported by Thomas *et al*. (2009) for same treatment. Similarly, the 910 kg ha^{-1} seed yield for the Moddus® treatment was more than double the 400 kg ha^{-1} reported by Thomas *et al*. (2009) for same treatment. These inconsistent results across treatments suggest that response may differ depending on cultivar, site and season. Woodward *et al*. (2003) have reported that 'Apex' performed better than 'Tribute' white clover.

The reduction of seed yield under the 1.5 l ha^{-1} Glyphosate 360 may be attributed to the crops' failure to close their canopies (Figure 3) and therefore overall limited growth as shown by consistently low yield components (Figure 4).

The high Olsen P of 32 mg kg^{-1} soil in the current experiment coupled with a wetter than normal season (673 mm per year) and the additional 100 mm of irrigation may have encouraged excessive LAI (Figure 2) at peak flowering. Excessive canopy covers have been shown to reduce seed yield by up to 50% (Clifford, 1985). Clifford (1985) has reported that high soil P levels (Olsen P>15) and moisture resulted in excessive vegetative growth resulting in low seed yield. Martin *et al*. (2003) has also corroborated the sensitivity of seed yield to water application. Both too little and too much water during flowering period reduced seed yields. Replacement of potential soil moisture deficit (PSMD) by 100% reduced seed yield by approximately 37% compared with the non-irrigated crops and by approximately 110% compared with crops irrigated at 25-37% of the PSMD (Martin *et al*., 2003). These authors recommended Olsen P levels of 6-15 mg kg^{-1} and maximum PSMD of <50%, respectively.

Similar seed yield among the treatments in the current experiment could also be attributed to the timing of treatment application, mid-October in this experiment, as Thomas (1980, 1981) reported that axillary buds formed in September and October will generally produce flowers, while buds produced at any other time form secondary stolons. The application of treatments during periods of optimum flower production may have led to either promoting secondary stolon production at the expense of flowers or uniform flower production across treatment as was the case in this experiment, except for the severely stressed crops receiving 1.5 l ha^{-1} Glyphosate 360. Thomas *et al*. (2009) have shown that mid- to late topping treatments increased seed yield. The topping treatments in the current experiment were applied in mid-October and mid-November. These may have been discontinued too early or may not have been severe enough to open up the canopy (Figure 2 and 3). These crops could have benefitted from regular topping until early to mid-December as they were sown later than normal.

Seed yield increased with leaf area index in the current experiment which was similar to findings by Clifford (1987), up to LAI of 3. However, seed yield did not differ from the control in the current experiment, except for the treatment receiving 1.5 l ha^{-1} Glyphosate 360 which produced the lowest seed yield. These crops did not reach full cover. These results are not consistent with Clifford (1987) who reported that reduced stolon density leads to high reproductive to vegetative ratios and hence more seed yield. Thomas *et al*. (2009) also reported negative yield response when Jaguar® and Paraquat herbicides are applied. This could be due to the delayed flowering by glyphosate treated crops and to less mature seed at the final harvest as all crops across treatments were harvested on the same date.

Defoliation has been reported to enhance flower production (Pasumarty and Thomas, 1990) and other components of seed yield for white clover (Zaleski, 1961). In the current experiment, flower numbers m^{-2} increased with topping treatments but this was not translated into increased seed yield. The 400-600 flowers m^{-2} found in this experiment were within ranges previously reported (Zaleski, 1961; Marshall and Hides, 1986; Marshall *et al.,* 1986). The positive correlation between seed yield and flower numbers has also been reported (Clifford, 1986).

Clifford (1987) reported that white clover flowering potential will be fully utilised when stolon numbers are insufficient to completely utilise available space. Production of more stolons resulted in insufficiently developed stolons unable to produce flowers but still able to compete for space and resources with productive stolons resulting in reduced seed yield. The positive correlation to stolon numbers in the current experiment could help explain the relationship between seed yield and flower numbers. The effects of these treatments on seed quality, number of seeds per flower and thousand seed weight will be discussed in a future paper.

Conclusion

The higher concentration of glyphosate (1.5 l ha^{-1}) reduced both seed and total dry matter yield for white clover. Glyphosate treatments had large effects on canopy development and therefore light interception. Although the mid-October/mid-November topping increased flower numbers compared with all the treatments and stolon numbers compared with the herbicides and PGR treatments, this was not translated into increased seed production. The Jaguar® and Moddus® treatments also had no effect on seed yield in this experiment.

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