Subterranean clover response to different herbicide applications

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Abstract
Successful establishment of subterranean clover in the year of sowing is essential for the productivity and persistence of the pasture. With appropriate management, success is ensured for future years. At present, there are no recommendations for sub clover safe herbicides in New Zealand, and no advice for appropriate application time. An experiment was established in autumn 2016 of sub clover monocultures treated with various herbicides at the 1-2 or 4-6 trifoliate leaf stage. Herbicide application at the 1-2 trifoliate leaf stage produced higher sown sub clover yields than an application at the 4-6 trifoliate leaf stage for 'Antas' and 'Narrikup'. 'Narrikup' treated with imazethapyr or flumetsulam at the 1-2 trifoliate leaf stage had the highest sown clover dry matter yields with 1850-2600 kg ha^{-1}. These were 1.60 times higher than controls, and had the greatest broadleaf weed reductions, with <300 kg ha^{-1}. Early application of these effective herbicides can improve sub clover establishment success by controlling weeds before they impact sub clover growth providing longer term broadleaf weed control than 2,4-DB and MCPB.

Keywords
Flumetsulam, imazethapyr, dryland pasture, *Trifolium subterraneum* L.

Introduction

A focus on input reduction, while maintaining high quality feed in year-round grazing systems has led to the widespread adoption of legume-grass mixtures on pastoral farms (Evers et al. 1993). However, sown clover species are especially vulnerable to competition for resources in their establishment phase (Smetham, 2003). Herbicides are often required for adequate weed control when establishing a new pasture. Subterranean (sub) clover, (*Trifolium subterraneum* L.) is the most commonly sown annual clover species in New Zealand, and is considered the most important annual species adapted to summer dry environments (Monk et al. 2016). Sub clovers are particularly vulnerable when germinating in autumn as the emerging seedlings cannot grow as fast as competing weeds (Evers et al. 1993). Current management protocols are adapted from Australian systems, and there is presently no available information on suitability of commercially available herbicides for sub clover in a New Zealand environment. A glasshouse experiment in Australia suggested that application time and growth stage has little influence on phytotoxic effect of herbicides to sub clover when applied at the 3-4 and 8-10 trifoliate leaf stage (Sandral et al. 1997). However, due to the shortened growing season in New Zealand, applications can rarely be delayed to the 8-10 leaf stage, and weed control is often required before plants are at the 3-4 leaf stage.

Appropriate management practices in the establishment year for sub clover are crucial to ensure persistence of this species in the pasture. High productivity at establishment ensures a high seed set for regeneration in subsequent years (Smetham 2003). With appropriate information about the impact of available herbicides for sub clover pastures, more comprehensive management protocols can be recommended for farmers.This paper reports the effect that herbicides and their application times had on seedling performance of 'Narrikup' and 'Antas' sub clovers treated with eight commercially available herbicides when applied at two different growth stages, 1-2, and 4-6 trifoliate leaves.

Methods

Site and experimental design

An experiment was conducted at the Lincoln University Ashley Dene Research Farm, at Springston, Canterbury, New Zealand. Two lines of subterranean clover, 'Narrikup', subspecies subterraneum and 'Antas', subspecies brachycalycinium were sown in a split-strip plot design on 22 March 2016. Resident broadleaf weed species were chickweed (*Stellaria media*), fathen (*Chenopodium album*), shepherds purse (*Capsella bursa-pastoris*), speedwell (*Veronica persica)* and storksbill (*Erodium moschatum*).

The experimental area was sprayed out with Buster® (a.i. 200 g/l glufosinate-ammonium) prior to direct drilling of bare non inoculated seeds in two sets of five cultivar strips 30 m in length. Herbicide treatments (Table 1) were applied to half the experiment on the 14.06.16 when plants were at 1-2 trifoliate leaf stage. The second application occurred on the 12.07.16 when plants were at the 4+ trifolitate leaf stage. 0.2 m^2 quadrat pasture cuts were taken from the centre of each plot in September and November 2016. The site was grazed for seven days following the September harvest, and topped with a Fieldmaster TM twin belt drive pasture topper to remove remaining seed heads from ungrazed weeds. Cut samples were mixed and a sub-sample of approximately 50 g fresh weight was separated and sorted into sown clover and broadleaf weed categories. Components were then dried and quantified. Data were analysed by ANOVA using Genstat 16.1. When significant, Fisher's protected least significant difference (LSD) test was used to separate means.

Table 1. Summary of herbicides applied to sub clover monocultures at Ashley Dene in autumn 2016.

Herbicide trade name	Active ingredient	Rate applied ha^{-1}
2,4-DB	*400 g/L 2,4-DB*	8.0 L
Basagran®	*480 g/L bentazone*	3.0 L
Weedmaster G360®	*360 g/L glyphosate*	1.0 L
Headstart®	*50 g/L flumetsulam*	1.0 L
Jaguar®	*250 g/L bromoxynil + 25 g/L diflufenican*	1.0 L
MCPB	*385 g/L MCPB*	7.5 L
Pulsar®	*200 g/L MCPB + 200 g/L bentazone*	6.0 L
Spinnaker®	*240 g/L imazethapyr*	400 mL

* All treatments were applied with Hasten™ (704g/L ethyl and methyl esters of vegetable oil) as an adjuvant.

Results

Visible phytotoxicity was not different between the two application times. The earlier application had faster symptom development than the later application, but symptoms by 63 days after application were the same across both times of application.

'Narrikup' plots had a herbicide x application time interaction (P=0.022) for total biomass (sown clover + broadleaf weeds). Untreated, unweeded controls for both application timings had total biomass of 2600 kg/ha which indicates the biological potential of the site for the experimental period. Total biomass from early applications (1-2 trifoliate leaves) of flumetsulam, imazethapyr, MCPB, and bromoxynil + diflufenican, along with late application of MCPB, bentazone + MCPB, and bromoxynil + diflufenican showed no differences to the untreated unweeded controls, while all other times and treatments had reduced total biomass (P=0.022) by >1000 kg/ha. Sown clover yields were higher (P=0.002) when herbicide was applied at the 1-2 trifoliate leaf than the 4-6 trifoliate leaf stage for all treatments except 2,4-DB, bentazone, and bentazone + MCPB. 'Narrikup' treated with imazethapyr at the 1-2 leaf stage had the highest (P<0.001) yield of 2500 kg DM ha^{-1} sown clover (Figure 1A). Broadleaf weed control was higher (P=0.009) from the earlier treatment application, with broadleaf weed biomass <700 kg/ha for all treatments, compared with 1500 kg/ha in untreated unweeded controls (Figure 1A). In the later treatment application, only bentazone, flumetsulam, and imazethapyr reduced (P<0.001) broadleaf weeds compared with the untreated unweeded controls (Figure 1B).

Total biomass for 'Antas' was not different (P=0.233) between times of application, only among the herbicide treatments. Bentazone and MCPB were the only herbicides applied at the 1-2 trifoliate leaf stage with total biomass the same as the 3000 kg/ha produced in the untreated unweeded control, all others were reduced. All herbicides applied at the 4-6 trifoliate leaf stage resulted in reduced (P<0.001) total biomass compared to the untreated unweeded control (4000 kg/ha). Sown clover yields for bentazone and imazethapyr applied at the 1-2 leaf stage were the highest yielding, with 1700-1800 kg/ha, which was greater than the untreated unweeded control (1000-1500 kg/ha). All herbicides applied at the 1-2 trifoliate leaf stage yielded at least 200 kg/ha greater (P<0.001) sown clover than the later application, except 2,4-DB (Figure 2). There was no sown clover yield difference (P<0.001) at the later application time for any of the treatments. There was no difference (P<0.001) in broadleaf weed biomass between application time in 'Antas', only differences among herbicides (Figure 2). Imazethapyr, flumetsulam, and bromoxynil + difluefenican had the greatest (P<0.001) broadleaf weed control with less than 400 kg/ha. All other treatments were reduced (P<0.001) with 700-1200 kg/ha, while the untreated unweeded controls had 2400 kg/ha.

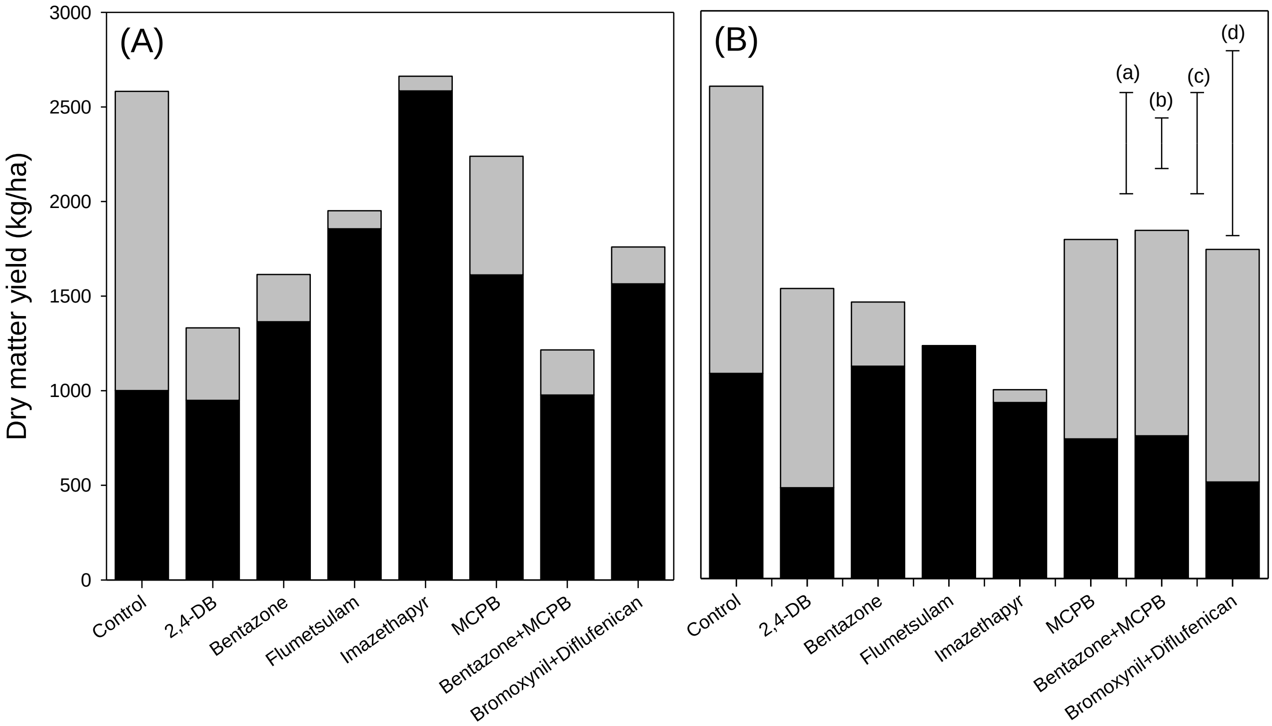

Figure 1. Sown clover (■) and broadleaf weed (■) yields for 'Narrikup' sub clover from 1 March to 12 November 2016 at Ashley Dene C9BS, Springston, Canterbury, New Zealand. Applied at the 1-2 trifoliate leaf stage on 14 June 2016 (A) or at the 4-6 trifoliate leaf stage on 12 July 2016 (B). (a) LSD for sown clover yield treatment x cultivar interaction (P=0.002); (b) LSD for broadleaf weed yields between experiments (P=0.009); (c) LSD for broadleaf weed yield differences between treatments (P<0.001); (d) LSD for total yield treatment x application timing interaction (P=0.022).

Discussion

This investigation into herbicides for control of broadleaf weeds during sub clover establishment showed that greater establishment success occurred with an early application time. No visible difference in phytotoxicity symptoms between the treatment times was observed, which suggests that the yield depressions seen in the later application was a result of increased weed competition preventing establishment, rather than greater phytotoxicity. The earlier application had the advantage of earlier broadleaf weed control (Figures 1 and 2), when conditions were warmer, allowing clover seedlings to take advantage of the gaps created by the herbicide treatments before growth was limited by cooler temperature.

While a minimum leaf stage is often specified for herbicide applications, we found no phytotoxicity evidence to suggest a later herbicide application caused less damage to sub clover seedlings. This is supported by findings that sub clover has a lack of major interaction between cultivar and leaf stage or spraying time, and earlier applications of some herbicides will not result in increased damage to the clover component of the pasture (Sandral et al. 1997).

The difference in response to herbicide treatments of 'Antas' compared to 'Narrikup' was shown by the cultivar x herbicide interaction for sub clover. 'Antas' treated with bentazone or MCPB at the 1-2 trifoliate leaf stage were the only two treatments with total dry matter yields not different to the untreated unweeded controls. However, these two treatments failed to provide the same level of broadleaf weed control obtained by imazethapyr and flumetsulam. Imazethapyr and flumetsulam as ALS inhibiting herbicides have the same mode of action, but the higher yields produced in the imazethapyr treatments, and no difference in weed control, suggest imazethapyr was the preferred option. 2,4-DB and MCPB had less successful weed control, which is to be expected due to the lack of residual action, showing flumetsualm and imazethapyr to be superior options for long-term control of broadleaf weeds due to their longer residual control of weeds.

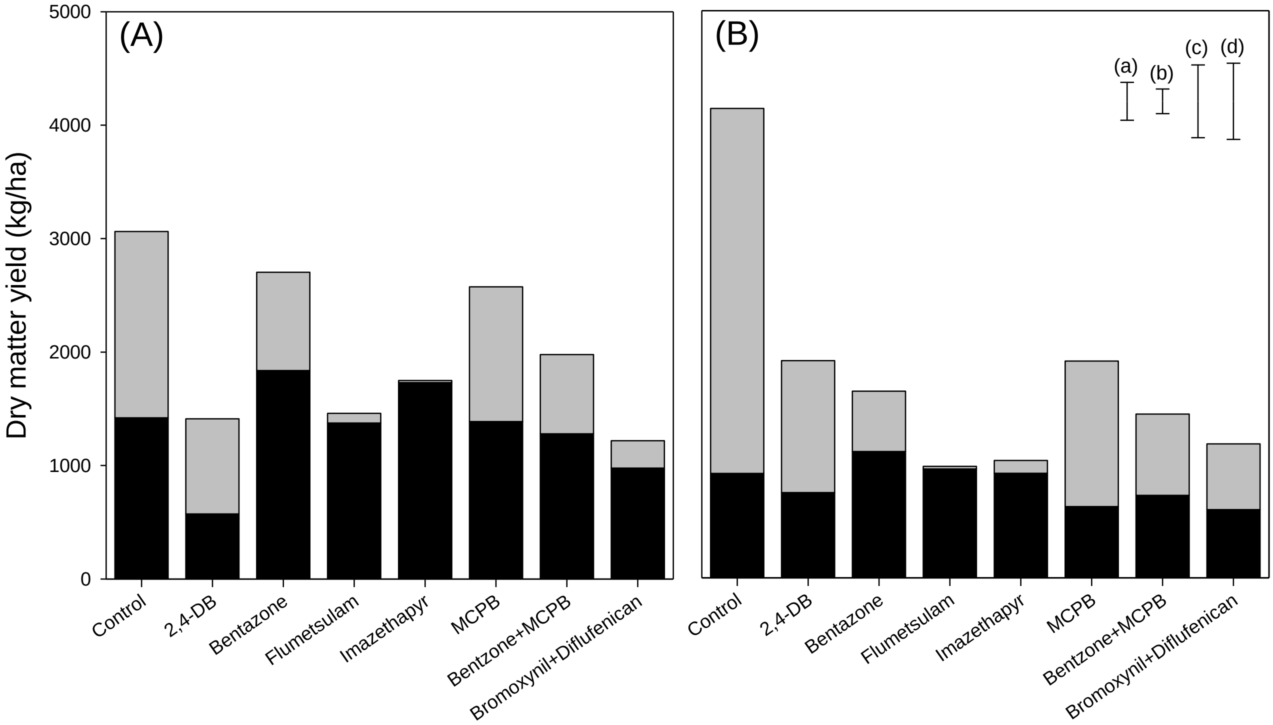

Figure 2. Sown clover (■) and broadleaf weed (■) yields for 'Antas' sub clover from 1 March to 12 November 2016 at Ashley Dene C9BS, Springston, Canterbury, New Zealand following herbicide application at the 1-2 trifoliate lead stage (A) or at the 4-6 trifoliate leaf stage (B). (a) LSD for treatment effect on sown clover yield (P=0.013); (b) LSD difference in sub clover yields between experiments (P=0.009); (c) LSD for broadleaf weed yield differences between treatments (P<0.001); (d) LSD for treatment effect on total yield (P<0.001).

Conclusion

Application of herbicides to sub clovers at the 1-2 trifoliate leaf stage increased sown sub clover yields compared to a later application at the 4-6 trifoliate leaf stage. The higher clover yields in imazethapyr and flumetsulam treatments, with their reduction of broadleaf weeds by >95% compared to the untreated unweeded controls, suggest they were more effective than 2,4-DB and MCPB and could be used safely to aid in the establishment of sub clover.

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