Effect of cultivar, timing of establishment and cutting interval on yield and seed set of arrowleaf clover

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Abstract
Arrowleaf clover is an erect hard-seeded annual clover with potential to improve the typically low legume content of dryland hill country. A number of small plot experiments were undertaken to better understand arrowleaf management. Cultivar maturity impacted on single-cut yields, with later maturing cultivars ('Arrotas' and 'Zulu 11') having significantly higher yields than an early maturing cultivar ('Cefalu'). When autumn sowing in April was practised, the slow growth of arrowleaf during winter caused weed issues. Delaying sowing of arrowleaf clover from April to winter (July) did not affect dry matter yield, seed set or seed viability and avoided the need for a weed spray as the vigorous spring growth of arrowleaf out-competed weed species. Arrowleaf oversown on hill country and allowed to set seed to build a large bank of hard seed resulted in a bulk of fibrous trash that was difficult to clean-up with grazing stock. Four mowings to simulate grazing encouraged weed invasion and reduced legume yields (and trash). Whilst two mowings were optimal for seed yield, up to three mowings provided a compromise between setting seed, utilising feed for grazing and reducing trash after flowering.

Keywords: arrowleaf clover, cultivar, yield, grazing management, seed set

Introduction
Increasing frequency of droughts in North Island hill country provide challenges for perennial legumes and have led to a greater focus on annual clovers. Arrowleaf clover (*Trifolium vesiculosum*) is native to the Mediterranean but persists in areas of Australia with rainfall of only 500–600 mm (Evans 2006). Arrowleaf clover is an erect, aerially seeding species which is hard seeded and has the potential in New Zealand to produce significantly more spring dry matter than either white (*T. repens*) or subterranean (*T. subterraneum*) clovers (Evans & Mills 2008). An initial study, on dryland hill country sown with a mix of plantain (*Plantain lanceolata*) and other clovers, showed that an early closing date (fenced off from grazing) resulted in seed yields of 500–700 kg/ha (MacFarlane et al. 2015). This work was extended to two other dryland hill sites where arrowleaf clover was established as a sole crop with the objective of maximising seed set. The intention was to generate a large seed bank that could be managed as a regenerating and grazeable stand over a number of years as has occurred in cropping areas in Australia (Evans et al.2003; Zhang et al. 2004). High dry matter yields (~ 10,000 kg DM/year) and good seed set (425 kg/ha with cultivar 'Arrotas' and 1390 kg/ha with 'Zulu 11') were achieved on both properties (Muir *unpublished data*). These on-farm studies were undertaken on uncultivatable hill country (slope ~ 45°) and the cleaning up of crop residue (mature arrowleaf clover stems) by stock following seed set was difficult with the resulting mulch hindering plantain establishment the following autumn. Thus, in the establishment or seed-set year, there needs to be a compromise between grazing, achieving reasonable seed set and reducing the trash resulting from seed set.

In dryland areas (particularly on hill country), autumn sowing is traditional as spring sowing means limited time for root development, and seedling survival is often compromised by the onset of summer drought. However, the slow winter growth of arrowleaf means that establishing plants are likely to be more susceptible to competition from more actively growing grass and weed species. Nori et al. (2015) also suggested that low temperatures restrict photosynthesis more in arrowleaf than in other erect annual clovers (balansa, *T. michelianum*; gland *T. glanduliferum*; and Persian *T. resupinatum*). This theory is supported by on-farm observations of difficulties with weed control with autumn-sown arrowleaf clover. Nori et al. (2015) found that winter sowing reduced yields because plants had less time to accumulate dry matter before reproductive maturity occurred. However, these authors used an early-maturing cultivar (cv. 'Cefalu') and later-maturing cultivars may have provided a greater opportunity to accumulate more grazeable dry matter.

This study examined: 1) the trade-off between early grazing of arrowleaf clover and its effect on yield and seed set; and 2) the effect of delaying autumn sowing on spring dry matter yield and seed set. Arrowleaf cultivars with different maturity times were also compared.

Materials and Methods
A trial was conducted at the Poukawa Research Farm in Hawke's Bay on a Matapiro silt loam. The area is

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winter-warm and summer-dry with an annual rainfall of 770 mm. The site was previously in permanent pasture and was sprayed out with Weedmaster (a.i. 360 g/litre glyphosate, Nufarm, Auckland) in November 2017 and in March 2018. Prior to each sowing, the plot area was rotary hoed and harrowed. Individual plots were 2 m × 2 m, hand sown and then lightly harrowed. Germination tests were conducted prior to each trial by placing 100 seeds in an incubator at 20°C for 7 days. Sufficient seed was sown so that each plot had the equivalent of 6 kg of viable seed/ha. All seed was inoculated with fresh Group C inoculant (*Rhizobium leguminosum* bv. *trifolii* WSM 1325). Slugbait (Metarex, 50 g/kg metachloride; De Sangrosse SA, France) was applied at sowing at 6 kg/ha. Three separate experimental areas were established to examine: (1) cultivar; (2) sowing date; and (3) effect of mowing (to simulate grazing) on dry matter yield and seed set. Broadleaf weed spray was applied to all April-sown plots on 25 June (Thistrol: a.i. 25 g/L 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 375 g/L 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB); Nufarm Ltd, Auckland). A grass spray was applied on 11 September with Gallant NF (a.i. 104 g/L haloxyfop-R; Dow, New Zealand) at 600 L/ha to all plots sown up until 3 July.

1. Cultivars

Three arrowleaf cultivars with differing maturity dates were used ('Cefalu' early, 'Zulu 11' medium, 'Arrotas' late). 'Cefalu' and 'Arrotas' seed was supplied by Kiwi Seed Company and 'Zulu 11' seed was supplied by Barenbrug. Six replicates of each cultivar were sown in three blocks with two replicates per block and using a randomised complete block on 12 April 2018. On 17 December 2018, half of each plot (2 m × 1m) was mown to 4-cm height using electric clippers (Makita DUM604; Anjo, Japan) and weighed. Dry matter was determined by drying a 200 g sub-sample at 100°C for 48 hours. No dissections were necessary as the plots were 100% arrowleaf. Flowerheads were counted using four 30 cm × 30 cm quadrats in the un-harvested half of the plot. On 8 February 2019, 30 intact flowerheads were harvested and seed threshed by passing through a mill (Retsch 5657 HAAN, Dettingen-Teck, Germany) with no sieve attached. A fan was used to separate the seed from the remaining vegetable matter. Seed was stored at room temperature and in April 2020, 100 seeds from each plot were germinated in an incubator at 20°C. The percentages of germinated (soft), swelled but not germinated (non-viable) and non-swelling seeds (hard) were determined after 7 days of incubation.

2. Sowing date

Three replicates of 'Zulu 11' were allocated to each of five sowing dates (9 April, 15 May, 3 July, 16 August and 2 October 2018) in a randomised complete block design. Half of each plot was harvested by mowing to 4 cm on 18 December 2018 and flowerheads counted. On 8 February 2019, 30 intact flowerheads were harvested, and seed weights and subsequent germination determined using the procedure described above.

3. Effect of mowing to simulate grazing

Grazing was stimulated using a lawn mower with the blade height set at 6 cm. The design was a randomised complete block design with three blocks sown with 'Zulu 11' on 4 April 2018. The four treatments were mown either once, twice, three or four times during 2018. The four treatments were 1 cut (18 December), 2 cuts (12 September, 18 December), 3 cuts (12 September, 11 October, 18 December) and 4 cuts (12 September, 11 October, 9 November, 18 December). At the first 3 cuts, the whole plot was mown and fresh weight recorded. At the final harvest on 18 December, half of each plot was cut and weighed. One sub-sample of 200 g was dried at 100°C for 48 hours and a further 200 g sample dissected into arrowleaf and "other" before drying at 100°C for 48 hours. Flowers were counted on the un-cut half of each plot. On 8 February 2019, 30 intact flowerheads were harvested and seed weights and germination determined using the procedure described above.

Environment

Rainfall was recorded on-site and mean daily temperature was recorded at the Hastings automatic weather station, 14 km from the experimental site. At 940 mm, annual rainfall for the 2018 calendar year was above the long-term average of 770 mm over 30 years, largely as a result of above average rainfall in June and

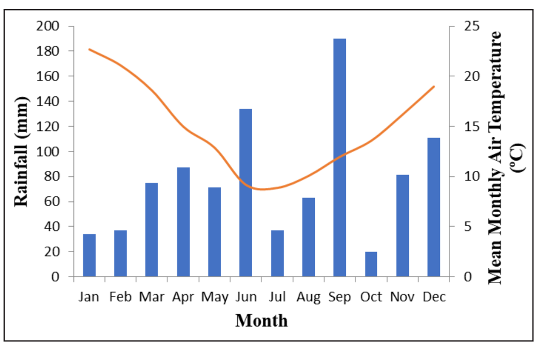

Figure 1 Monthly rainfall (mm; solid bars) recorded at Poukawa and mean monthly air temperature (°C) recorded at the Hasting automatic weather station in 2018.

September. Mean monthly daily temperature was at its lowest in July (9.2°C) increasing to 12°C in September and 19°C degrees in December (Figure 1).

Statistical analyses

Each individual experiment was analysed separately using a generalised linear model and Tukey's test used to compare between treatments in Minitab for Windows (version 14).

Results

1. Effect of cultivar

Plot effects were highly variable, but there were significant differences ($P<0.01$) in yield between 'Cefalu' and the other two cultivars 'Zulu 11' and 'Arrotas' (4857, 8885 and 9891 kg DM/ha, respectively; Table 1). There were no significant cultivar effects on numbers of flowerheads/m^2 but there were differences in amount of seed set per flowerhead ($P<0.001$), with 'Zulu 11' producing more seed/ha than both 'Cefalu' and 'Arrotas'. There were no differences in the viability of collected seed among cultivars with 83% of threshed/stored seed germinating in each case (Table 1).

2. Sowing date

Cultivar 'Zulu 11' was harvested on 18 December 2018 and yields from the earliest sowing (9 April) were highly variable (9076 ± 4753 kg DM/ha). The highest yields occurred from the May and July sowings (12354 and 10004 kg DM/ha respectively). Seed sown in August yielded 7012 kg DM/ha but only the last sowing (2 October) resulted in a significantly lower yield (3107 kg DM/ha; Table 2). Calculation of daily growth rate from sowing to harvest on 18 December showed that 'Zulu 11' grew at 55–59 kg/ha/day whether sown in May, July or August (Table 2). The earliest sowing produced the slowest overall growth rate (35 kg DM/ha/day) but it was only significantly different from the

Table 1 Effect of arrowleaf clover cultivar on dry matter yield, flower number, seeds per flower and proportion of soft, hard and non-viable seeds that germinated following milling and storage for 15 months. Means with different letters in the same row indicate a significant difference ($P<0.05$).

	Cefalu	Zulu 11	Arrotas	SEM	P
Seedlings/m^2 §	156	295	211	54.4	0.23
DM (kg/ha) §	4857a	8885b	9891b	914	0.004
Flowers/m^2 §	561	571	522	22.3	0.29
Seeds/flower (g) §	0.392a	0.558b	0.345a	0.018	0.001
Weight of 100 seeds (g) †	0.167b	0.170b	0.142a	0.0043	0.02
Soft seed (%)†	83.3	83.3	83.0	2.64	0.99
Hard seed (%)†	6.7	6.0	6.7	1.43	0.93
Non-viable seed (%)†	10.0	10.7	10.3	1.75	0.97

§ Data from harvest on 18 December 2018
† Data from harvest on 8 February 2019

Table 2 Effect of time of sowing on dry matter yield, flower production, seeds per flower and the proportion of soft, hard and non-viable seeds that germinated following threshing and storage for 15 months in arrowleaf clover (cv 'Zulu 11'). Means with different letters in the same row indicate a significant difference ($P<0.05$).

	Sowing date						
	9 Apr	15 May	3 Jul	16 Aug	2 Oct	SEM	P
DM (kg/ha)§	9076ab	12354b	10004b	7012ab	3107a	1456	0.01
Growth rate (kg DM/ha/day) §	35.0a	56.7ab	58.7b	55.7ab	39.3ab	4.48	0.02
Flowers/m^2 §	701c	741c	824c	316b	0a	48.4	0.001
Seeds/flower (g) †	0.590	0.513	0.553	0.473	-	0.027	0.09
Weight of 100 seeds (g) †	0.154	0.163	0.160	0.153	-	0.005	0.09
Soft seed (%)†	75.0	80.0	80.1	72.0	-	3.15	0.32
Hard seed (%)†	7.7	4.3	3.4	11.3	-	2.7	0.30
Non-viable seed (%)†	17.3	15.7	16.9	16.7	-	3.3	0.70

§ Data from harvest on 18 December 2018
† Data from harvest on 8 February 2019

growth rate from the July sowing (58.7 kg DM/ha/day). Delaying sowing date from 9 April to 3 July 2018 had no impact on number of flowers/m^2 in December 2018 nor on the weight of seed set per flower when harvested in February 2019. Delaying sowing from 3 July to 16 August 2018 significantly reduced flower production in December 2018 by around 60%; however, there was no reduction in seed weight per flower, in 100-seed weight or in the viability of threshed and stored seed following harvest in February 2019 (Table 2). There were no flowers produced by the last sowing (2 October) by time of the 18 December harvest or by seed harvest the following February.

3. Effect of mowing

Repeated mowing did not significantly affect the total dry matter of cultivar 'Zulu 11' produced but it did increase the amount of weed and grass invasion. Thus, the single-cut plots contained 100% clover at final harvest, but this proportion had reduced to 61% clover for plots that had been mown four times. A combination of reduced sward vigour and increased weed content meant that cutting frequency reduced clover DM yield in almost a linear fashion, from 6072 kg DM/ha for a single cut to 2698 kg DM/ha when four cuts were made (Table 3). Multiple cuts did not affect the number of flowers produced but the seed weight per flower and the actual seed weight (100-seed weight) when harvested in February 2019 declined significantly with increasing number of cuts. There was, however, no effect of number of cuts on the subsequent germination percentage of the threshed/stored seed (Table 3).

Discussion

1. Cultivar

Arrowleaf cultivars 'Arrotas' and 'Zulu 11' produced the highest amount of dry matter (9891 kg and 8885 kg DM/ha), values similar to the 9800 kg DM/ha reported by Evans & Mills (2008) for 'Arrotas' grown in Canterbury between 23 May and 20 December 2007. 'Cefalu' produced significantly less DM/ ha than the other two cultivars at a single harvest. These differences may be related to flowering date, since 'Cefalu' matures 50 to 60 days before 'Arrotas' (Thompson 2005). One of the advantages of arrowleaf clover is its deep taproot, which has been measured at up to 1.5 m in freely drained, deep gravel soils in Australia (Thompson 2005) and 0.5 m on mudstone at Castlepoint Station in New Zealand (Muir, *unpublished data*). This taproot enables arrowleaf clover to access moisture at greater depths than the typical root depth of other resident pasture species. For example, Troughton (1957) reported that species such as ryegrass have 80% of their roots in the top 10–15 cm of soil. However, early-flowering arrowleaf cultivars such as 'Cefalu' do cease vegetative growth at flowering. This suggests that, in the longer growing season experienced in New Zealand, deep soil moisture may be best exploited by later-maturing cultivars, resulting in higher yields.

All seed heads were counted at the December 2018 harvest but only a selection of mature intact seed heads (i.e. the best seed heads) were collected on 8 February 2019. When calculated out on a per hectare basis, these seed weights would translate into extremely large and unlikely seed yields (up to 3000 kg/ha) and need

Table 3 Effect of number of mowings on arrowleaf clover DM yield, flower production, seeds per flower and the proportion of soft, hard and non-viable seeds that germinated following milling and storage for 15 months (cv 'Zulu 11'). Means with different letters in the same row indicate a significant difference (P<0.05).

	Number of mowings					
	1	2	3	4	SEM	P
Total DM yield (kg DM/ha) §	6072	6564	7274	5454	819.3	0.37
Legume % §	100b	87ab	65ab	61a	9.2	0.03
Legume DM yield (kg DM/ha) §	6072	5085	4190	2698	921	0.07
Flowers/m^2 §	678	530	400	423	72.3	0.07
Seed weight/flower (g) §	0.280c	0.260bc	0.197ba	0.140a	0.017	0.001
Weight per 100 seeds (g) †	0.145bc	0.150c	0.133b	0.128a	0.003	0.001
Soft seed (%)†	74.2	72.6	72.6	72.6	4.51	0.99
Hard seed (%)†	10.7	4.7	4.7	5.7	3.1	0.50
Non-viable seed (%)†	15.1	23.2	22.3	21.7	2.62	0.18

§ Data from harvest on 18 December 2018
† Data from harvest on 8 February 2019

to be viewed with caution as they are likely a result of collecting the “better” seed heads (as opposed to average seed heads) from within each plot. Flowering rates were similar among the three cultivars test but the number of seeds/flower collected from ‘Zulu 11’ were significantly greater ($P < 0.001$) than those from the other two cultivars. High seed yields with ‘Zulu 11’ are consistent with on-farm studies with ‘Zulu 11’ where yields of 1390 kg/ha have been measured in stands where oversown arrowleaf has been managed to maximise seed yield (Muir *unpublished data*). At final harvest, none of the plots required dissection analysis as samples collected were 100% arrowleaf.

2. Sowing date

Yields of ‘Zulu 11’ from the earliest sowing date (April 2018) were highly variable, probably because of the dry conditions and variable germination that occurred around sowing. Weeds were a problem in plots with early-sown arrowleaf, which necessitated a broadleaf weed spray on 25 June, but no weed control was required for the later sowings (3 July, 16 August and 2 October). At final harvest, none of the plots required dissection analysis as samples collected were 100% arrowleaf. Arrowleaf clover grows slowly in cooler temperatures but its vigorous growth in spring means it can out-compete other grass and weed species. Delaying sowing from 9 April to 3 July had no significant effect on yield of ‘Zulu 11’ nor on seed yield or amount of viable seed produced. Sowing on 2 October resulted in significantly lower yields ($P<0.01$) at the December 2018 harvest and no flowers nor seed were produced by the final harvest in February 2019. Thus, sowing as late as July provides a tool for farmers to obtain grazing further into the winter from resident pasture while reducing the need for weed control.

3. Mowing date

Mowing was undertaken to simulate grazing because of the difficulties in achieving even and non-selective grazing within a small plot trial. Increasing the number of mowing events had no effect on the total dry matter yield but did result in a greater weed invasion and a reduction in the amount of arrowleaf present at final harvest. At maturity, arrowleaf clover plants that have been left to set seed have a hard, fibrous stem. Animals will not eat this material so repeated mowings/grazings provide a means to reduce the amount of trash produced and enables better germination of any subsequent crop. Whilst the mowing regime employed may have resulted in a different result to that which might be achieved with sheep grazing, it does seem likely that early grazing of arrowleaf will impact on total yield, presumably because of damage to the growing points. Successive mowings did not affect flower production although moving from one to four cuts did reduce the seed weight per flower by around 50%. Mowing as late as 11 October did reduce seed weight but seed viability was not affected. Thus, a grazing/cutting regime can be employed to utilise available dry matter and reduce the amount of herbage remaining as trash after seed set, whilst still producing a viable seed crop.

Collected seed was threshed and stored for 15 months, and subsequent germination rates were very high with rates of soft seed (72–83%) measured across all cultivars and treatments. By comparison, MacFarlane et al. (2015) measured very low rates of soft seed in arrowleaf clover (~2%). The difference may be due to the method whereby seed was separated from the flowerheads. Whilst MacFarlane et al. (2015) used soft rubber pads to rub the seed from the flowers, the current study used a mill and this may have caused some damage to the seed coat and encouraged germination. Moreover, the germination tests were undertaken 15 months after harvest and although the seed was stored at room temperature, it is possible the extended time period may have contributed to a breakdown in the hard seed coat. Arrowleaf is a hard seeded annual and it appears the milling process used to thresh the seed may have scarified the seed and led to high germination rates. However, this approach had the advantage that it demonstrated arrowleaf was still capable of producing viable seed even with late sowing and/or repeated mowing and, therefore, a shortened period for producing seed. Collecting hard seed with subsequent low germination would not have provided important information on seed viability.

Practical implications

A deep tap root enables arrowleaf clover to extract moisture from a greater depth than other resident hill-country pastures. Selection of late-maturing arrowleaf cultivars will enable a longer growth period and produce more dry matter. There were significant differences among cultivars in seeding ability and this is likely to be important if arrowleaf is sown as a crop with the intention of maximising seed set and generating a large seed bank of hard seed for future years.

While autumn sowing is traditional in summer-dry areas, the slow winter growth of legume species like arrowleaf can result in weed management problems in autumn and early winter. Sowing of arrowleaf as late as July had no effect on dry matter yield, seed set and viability of seed. Adopting this approach would provide a tool for farmers to achieve winter grazing of resident pastures and achieve later oversowing of arrowleaf without jeopardising yield or seed set.

Previous on-farm attempts to oversow arrowleaf clover on hill country and use it as a crop to set large quantities of hard seed have been successful but have resulted in a large amount of trash that has been difficult to remove with grazing animals (Muir *unpublished data*). In this study, consecutive mowings (to simulate grazing) through to late September were successful in reducing the amount of trash while still achieving good seed set. However, these extra 'grazing' events appear to have resulted in weed invasion and compromised legume yield, and may be due the effects of mechanised mowing on the growing points of this erect annual clover. It needs to be noted that careful grazing with stock may have had less impact on the growing points and on overall yield.

The hard seeded nature of arrowleaf clover means that there is generally very poor germination in Year 2 so a cover crop has to be established on hill country. East Coast hill-country farmers have had success with oversowing of plantain in the autumn of Year 2. This provides high-quality feed in Year 2 as well as providing a more suitable (less competitive) environment for the germinating arrowleaf seedlings in Year 3.

Whilst this work was aimed at understanding the management of arrowleaf clover on uncultivatable hill country, the difficulties in running grazing trials on steep hill country meant that this work was conducted on flat land using a mower to simulate grazing. Thus, whilst the results obtained are insightful, actual results might differ in oversown hill-country environment.

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