Relationships between vegetative and reproductive growth in a four year old stand of Caucasian clover (*Trifolium ambiguum*, M. Bieb) cv. Monaro

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Abstract

The potential of Caucasian clover as a perennial, stress tolerant pasture species has not been fully exploited in New Zealand owing to a shortage of available seed. This study was undertaken in order to increase our understanding of the reproductive strategy of this plant as a basis for improving management practices in the seed crop. The protracted flowering pattern of this clover largely results from the continuous production of reproductive shoots from crowns; rhizomatous shoots remaining vegetative during the first year or production. Two classes of crowns were identified, the larger, main crowns producing an average of 7.4 reproductive shoots per crown at peak flowering compared to smaller, secondary crowns producing only 1.7 shoots. Inflorescences produced from main crowns gave higher seed yields than those from secondary crowns. These results give preliminary indications that, because of the nature of crown development, stands of Caucasian clover may take several years to reach their maximum reproductive potential, and inter-row cultivation is an in inappropriate management practice for this seed crop.

Additional key words: *flowering pattern; rhizomatous shoots; crowns inflorescence; density, inter-row cultivation; reproductive potential.*

Introduction

Originating from the region encompassing Caucasian Russia, eastern Turkey and northern Iran, *Trifolium ambiguum* M. Bieb. has considerable promise as a persistent hill country pasture legume (Zohary, 1970; Bryant, 1974). Although slow to establish, trials in New Zealand (Paljor, 1973; Stewart, 1979; Gurung, 1991) have shown that the deep rooting growth habit of Caucasian clover lends this species both a drought and a low temperature tolerance not found in the more familiar clovers or in *Lotus corniculatus* (Scott *et al.*, 1985; Chapman and Macfarlane, 1985). Other attractive features of this species are its potential for soil erosion control (Pellet, 1945), its growth potential under low phosphate conditions (Barnard, 1972) and its disease and pest resistance (Stewart, 1979; Jones *et al.*, 1981).

Despite this evident potential, Caucasian clover has yet to be developed commercially. While this is partly due to problems of slow establishment, a major issue has been low or highly variable seed production (Hampton *et al.*, 1990). The preliminary investigation reported here was designed to address some of the basic gaps in our knowledge about the morphology of reproductive yield in this species, and to form a basis on which further research towards effective seed crop management could be developed.

Materials and Methods

Experimental site and crop management

The study was carried out in one growing season (1991-1992) on a four year old established stand of the hexaploid cultivar of *T. ambiguum*, cv. Monaro at the AgResearch Grasslands Research Farm, Aorangi, Palmerston North, New Zealand.

At the beginning of the experiment (15 October 1991), the sward was inter-row cultivated to provide a 60 cm row spacing. This type of management practice, designed to increase both production of successful reproductive shoots and uniformity of flower production is standard practice for cultivar change seed crops of white clover in New Zealand (Clifford, 1985) and has also been used successfully with *Lotus uliginosus* Maku lotus (Hare, 1983). No irrigation, fertilizer or pollinator

introduction was applied during the course of the study, but on 12 November, 1991 plots were sprayed with 0.3 kg ai/ha of fluazifop-P-butyl ("Fusilade"). Ten days previously the plots had been spot sprayed with a 1% solution of "Asulox" (asulam) to control *Rumex* species. Hand weeding was subsequently carried out as necessary.

Sampling strategies

The area was divided into five sub-plots for destructive and non-destructive sampling and randomly allocated 0.5 x 0.5m grids placed across rows were destructively sampled every two weeks for aerial component analysis. Each sample was cut to ground level and then separated into its different components so that numbers and dry weights of vegetative and reproductive shoots could be calculated. For the dry matter determinations, plant material was dried at 80°C for 24 h. The total number of expanded leaves were counted and the area of a subsample of the leaflets from 40 trifoliate leaves determined using a Hayashi photocell planimeter.

Dry matter of root material was assessed after harvesting aerial components by removing a 20 cm deep, 25 x 30 cm sod sample from each grid area. Sods were soaked in water for 30 minutes and then washed free of soil. Dry weight determinations were carried out as for the aerial components.

Flowering pattern and determination of reproductive efficiency

The start of flowering, flowering duration, peak flowering and completion of flowering were observed in 5 permanent quadrats of 2 x 1.5m rows by counting the number of newly opened inflorescences every 5 days. Generally it took 5 days for flowers to fully open. These inflorescence were mainly white when fully open but later changed to predominantly pink within 5 days. This was used as a criteria to prevent double counting. Another check was made by tagging 5 to 10 inflorescences which had been counted and had their florets open, and the development stage of these flowers was monitored when the next counting was undertaken.

On each of four sampling dates (1 November, 20 November, 10 December 1991 and 1 January 1992) 15 visible buds from both primary and a further 15 from secondary crowns were randomly identified in an allocated 4m row of each sub-plot by tagging their peduncles with different coloured plastic wires. This tagged population of approximately 600 inflorescences was used to determine the time required for the development of inflorescences emerging at different times and from different positions, from bud stage to open flower and maturity, and to examine the changing pattern of each seed yield component.

The number of florets per inflorescence were counted from three inflorescences of each type per subplot on each tagging date (total 3 x 5 x 4 x 2 = 120 buds), while number of florets per inflorescence was measured from a similar sample of open inflorescences obtained when more than 90% of tagged inflorescences were blooming (about 10 to 17 days from the bud stage). After all tagged inflorescences became dark brown (about 30 to 40 days after blooming stage), a further sample of inflorescences per treatment were taken for determination of pods per inflorescence. All inflorescences in each treatment were pooled over the five replicates and the number of seeds per pod (0, 1 or 2) was counted from 4 x 100 pods, randomly selected from these inflorescences.

Seed yield

From each sub-plot one $0.25m^2$ quadrat was harvested to determine seed yield components and final seed yield. Harvest timing (approximately 34d after peak flowering) was based on a visual assessment of when more than 90% of the inflorescences were dark brown and therefore mature. For each replicate, the number of inflorescences were separated into unripe inflorescences (including buds), open inflorescences (pink/white or light brown) and mature inflorescences (dark brown). Total inflorescences were used to calculate potential seed yield (PSY), and only ripe inflorescences were used to obtain potential harvestable seed yield (PHYS). After harvesting, samples from each replicate were kept in paper bags and dried at room temperature for 7 weeks. Ten ripe inflorescences were randomly taken from each replicate (total 5 x 10 inflorescences) to calculate the number of pods per inflorescence. The number of seeds per pod was counted from 100 pods per replicate (total 5 x 100 pods) dissected from these inflorescences.

Seed yield was calculated from the seed yield components recorded at harvest according to the following formula:

$$\text{Seed yield} = P \times E \times N \times S$$

Where P = the number of inflorescences/unit area; E = the number of florets/inflorescence; N = the number of seeds/floret; and S = individual seed weight.

The remaining harvested inflorescences per replicate were then hand threshed and total actual seed yield per 1m row was assessed. Thousand seed weight and seed yield were expressed at an adjusted moisture content of 10%.

Results

General patterns of growth

Figure 1 shows the overall pattern of growth of cv. Monaro during the experimental period. The high proportion of root relative to shoot material is immediately obvious, comprising between 70-80% of total plant weight throughout most of the experimental period. Inter-row cultivation was followed by substantial regrowth of aerial components at the expense of root dry weight until the end of November, after which root dry weights recovered substantially until they reached or even exceeded levels prior to inter-row cultivations. Maximum reproductive dry weight was achieved around mid-December, but was never more than 20% of total aerial dry weight, with new vegetative growth being observed throughout the reproductive season.

Before inter-row cultivation a closed canopy had been achieved with an estimated Leaf Area Index (LAI) of 5.9. Cultivation reduced this index to 2.2, but sustained leaf replacement at a rate in excess of 130 leaves per metre row per week, restored the LAI to original levels within six weeks (Figure 2).

Reproductive growth

The pattern of reproductive shoot production is shown in Figure 3. Throughout the study it was found that inflorescences were produced soon after reproductive shoot emergence, often at the first node on the stem. An analysis of the source of reproductive shoots conducted around the time of peak flowering indicated that nearly two thirds of reproductive shoots emerged from main crowns, which on average produced more than four times as many shoots per crown (Table 1). Reproductive growth was only found from crowns, daughter shoots arising from rhizome extension growth being purely vegetative.

Data on flower production are shown in Figure 4. Although a few inflorescences were observed in the field in early September, flowering did not get fully under way until early November (the first open flower being

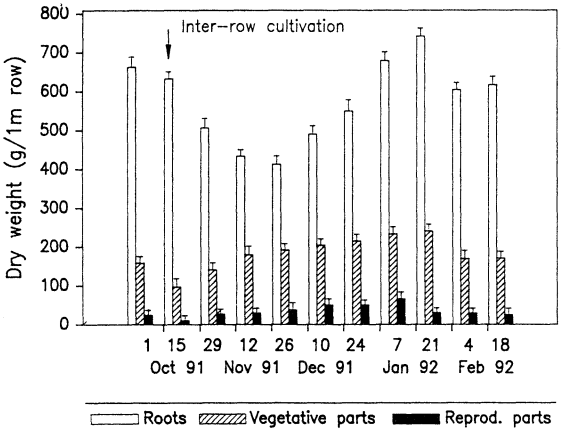

Figure 1. Dry matter accumulation in vegetative and reproductive components of Caucasian clover cv. Monaro during 1991-1992. Data are means of five replicates. Vertical bars represent individual standard errors.

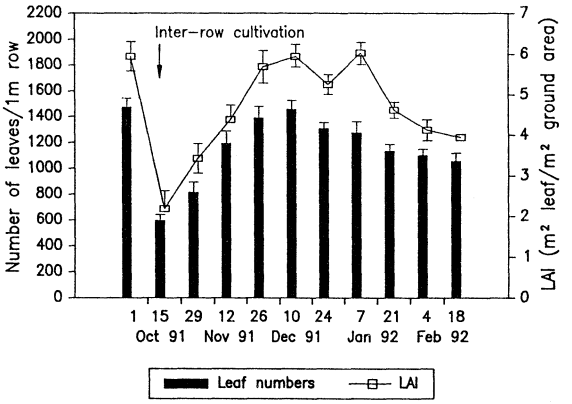

Figure 2. Leaf area index (LAI) and the number of leaves of Caucasian clover cv. Monaro on each sampling date. Data are means of five replications with individual standard errors shown.

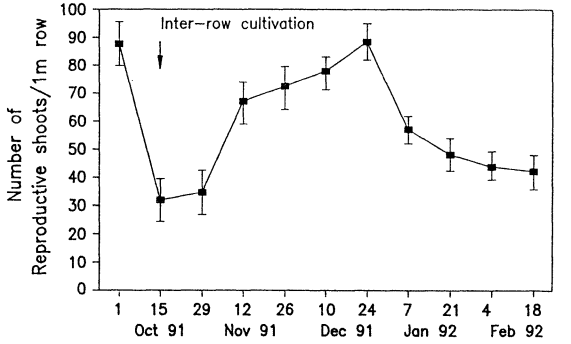

Figure 3. Reproductive shoot production in Caucasian clover cv. Monaro. Data presented are means of five replications with individual standard errors shown.

Table 1. The number of reproductive shoots originating from main and secondary crowns at peak flowering. Data are means of five replications (± S.E.M.)

Crown type	Crowns per 1m row	Reproductive shoots per crown
Main	7.6 ± 0.75	7.4 ± 0.44
Secondary	18.6 ± 2.13	1.7 ± 0.11

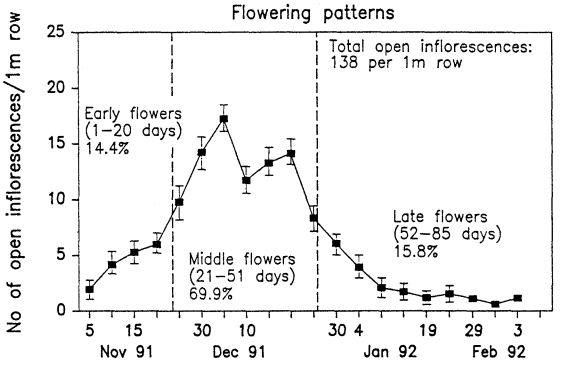

Figure 4. The flowering pattern of Caucasian clover cv. Monaro. Data produced are newly opened flowers and means of five replications with individual standard errors are shown.

observed in a permanent quadrat on 5 November). Mass flowering occurred over a period of 12 weeks, although nearly 70% of flowers were produced in a 30 day period 3 weeks into flowering.

Changes in floret production and survival with time and site are summarised in Tables 2 and 3. Inflorescences produced in early November tended to have more florets per inflorescence, but mature pod production was more successful from inflorescences emerging in mid-December. Inflorescences originating from main crowns tended to be larger than those produced from secondary crowns and as survival rates did not differ between the two categories, levels of pod production were almost 12% greater from inflorescences produced from main crowns (Table 3).

Most pods bore a single seed, although the number of empty pods (25%) was significantly higher in early flowers, falling to 10-12% from inflorescences opening around peak flowering. Inflorescences contributing to peak flowering had a higher proportion of two seeded pods (15% compared to 6.5% and 8.5% for early and late flowers respectively). Estimated seed yield, calculated as described in the methods was around 400 kg/ha gained from a calculated potential harvestable yield (based on the number of ripe inflorescences) of just over 700 kg/ha.

Discussion

Factors determining reproductive growth

The general growth data presented here highlight the extensiveness of root growth in *T. ambiguum*. The highest proportion of aerial components observed in this study was 32% which is similar to data reported by Spencer *et al.* (1975) for other cultivars. As a comparison, in white clover aerial components may comprise up to 90% of plant dry weight (Spencer *et al.*,

Table 2. Floret survival as a function of time of inflorescence production (data averaged over position of inflorescence; interaction NS).

	Sampling date				
	1 Nov	20 Nov	10 Dec	1 Jan	$LSD_{0.05}$
Floret buds per inflorescence	127	114	100	98	10.7
Open florets per inflorescence	115	99	95	87	12.8
Bud survival (as % of floret buds)	91	86	95	87	NS
Mature pods per inflorescence	60	69	81	59	11.9
Pod survival (as % of open florets)	52	70	85	69	8.9

Table 3. Floret survival as a function of site of inflorescence (data averaged over four harvest times; interaction NS).

	Main crown	Secondary crown	$LSD_{0.05}$
Floret buds per inflorescence	120	100	9.2
Open florets per inflorescence	109	89	14.6
Bud survival (as % of floret buds)	91	89	NS
Mature pods per inflorescence	73	62	6.7
Pod survival (as % of open florets)	67	71	NS

1975). The data also emphasise in various ways the importance of the interrelationships between root growth and reproductive growth within this species. Although this was a single season study, it is clear that extensive root growth and the development of crowns is an essential prerequisite for reproductive growth.

The first peak of flowering (Fig. 4) would appear to result from the massive increase in reproductive shoots from 29 October to 12 November as reproductive growth recovered from inter-row cultivation (Fig. 3). Extended flowering after this is likely to be a function of both extended reproductive shoot production from the crowns and, perhaps, continuing inflorescence production on existing stems, although this latter aspect was not analysed in detail. Note, however, that the rate of reproductive growth slowed substantially after mid-November as the roots began to recover their dry weight (Figs. 1 and 3). Differences in reproductive efficiency of inflorescences produced at different times (Table 2) are likely to be a function of competition for assimilate with recovering vegetative growth (Fig. 2) during early flowering and with renewed root growth during late flowering (Fig. 1).

Our data on the relative contributions of main and secondary crowns to reproductive growth are incomplete, but strongly suggest the importance of the former in generating reproductive yield (Table 1). Given that each reproductive shoot produces an inflorescence often at the first node, it is likely that much reproductive development is driven by root reserves rather than local photosynthesis, and this places inflorescences originating from main crowns at an advantage (Table 3). However, the fact that the survival rates of flowers to the pod stage do not differ between the two classes of inflorescences, would suggest that any differences in reproductive yield between inflorescences are likely to be a function of differences in the size of the floral meristem. This notion would seem to be supported by the findings of Daly *et al.* (1993) that seed yields in a first year crop of cv. Monaro were minimal and increased six-fold in the second year.

Based on these data we can question the appropriateness of an inter-row cultivation regime for this species. Inter-row cultivation strategies are designed in other clover species to variously encourage reproductive growth by reducing both above and below ground competition and enhancing light penetration of the canopy to promote flower head development (cf. Clifford, 1980; Thomas, 1980). As, however, reproductive growth in this crop is dependent largely on prior crown development - and, in particular, the numbers of main crowns - the net effect of this management practice in *T. ambiguum* is likely to be deleterious in that main crowns are inevitably going to be destroyed and this will negate any other potential benefits. The preliminary research reported here has demonstrated the need for more intensive work in this area and presently a detailed time course study is under way at Massey University aiming to determine how root growth in this species drives subsequent reproductive growth through the first two years of crop establishment.

Seed yield

One interesting facet of this study is that it did not indicate any major problems with seed production in this mature crop. Yield was high compared to that reported by many other authors (e.g., Kannenberg and Elliot, 1962; Townsend, 1970; Stewart and Daly, 1980), but comparable with those reported in a second year crop by Daly *et al.* (1993). Of course, yield estimates based on small sample hand-harvesting are likely to be high.

The high percentage of florets surviving to the pod stage noted in this study implies that pollination was not a limiting factor in seed production. In contrast, however, the second ovule in the overwhelming majority of pods failed to set seed. This type of phenomenon has been noted by other workers with similar species (e.g., Stephenson, 1984; Pasumarty *et al.*, 1993). Whether it

is a pre- or post-fertilization problem or the result of competition for root reserves remains to be investigated.

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