RESEARCH NOTE

The Reproductive Behaviour of Individual Plants from within Caucasian Clover (*Trifolium ambiguum* Bieb.) cv. Monaro.

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ABSTRACT

Thirteen individual plants selected at random from a four year old field of Caucasian clover (*Trifolium ambiguum* Bieb.) cv. Monaro varied widely in their ability to flower and hence produce seed. Four of the plants failed to flower. Vegetatively propagated plants from three of the original thirteen were further investigated in a field trial. Inflorescence number per plant differed significantly (from 1.1 to 41.6) as did seed yield per plant (from 0.18 g to 3.65 g). The reason for this variable flowering response is not known, but there is obviously scope for selection to improve both inflorescence number and seed yield.

Additional index words: inflorescence number, genotype, seed yield, daylength.

EXPERIMENTAL AND DISCUSSION

Caucasian clover (*Trifolium ambiguum* Bieb.) has long been recognised as a pasture plant with potential for New Zealand's dry, low fertility hill and high country environments (Lucas, White, Daly, Jarvis and Meijer, 1981) and is currently being investigated for use in summer dry lowland regions and irrigated pastures (Widdup, Knight and Hunt, 1996). However, the reliable production of quality seed has been a limiting factor (Guy, 1996; Widdup *et al.*, 1996) with cultivars such as Monaro showing variable seed yield (Efendi, 1993).

Morphologically Caucasian clover is a very diverse species (Townsend, 1970) varying not only with ploidy level (Widdup *et al.*, 1996) but also among individual plants of the same cultivar (Efendi, 1993; Widdup *et al.*, 1996). For example Widdup *et al.* (1996) found that inflorescence number per plant in the hexaploid cultivar Endura varied from 25 to 510.

In 1992 thirteen randomly chosen individual plants were dug up from a seed production field of Caucasian clover cv. Monaro established four years previously (M.P. Rolston, pers. comm. 1992). These plants were grown on in large (40 x 40 x 60 cm) containers kept outside the Seed Technology Centre at Massey University and inflorescence production and seed yield were determined in the 1993/94 season by counting open inflorescences (50% florets open) every 5 days from November to January and hand harvesting seed heads when they were ripe. Four of the thirteen plants did not flower, while total inflorescence number per plant for those plants which did flower ranged from 4 to 46 (Table 1). Seed yield per plant was also highly variable, ranging from 0 to 6 g per plant (Table 1).

To further investigate this reproductive variability, three of the 13 plants were chosen for a field trial at Massey University in the 1994/95 season ie. plant 2 (apparent good flowering ability), plant 12 (apparent medium flowering ability), and plant 9 (did not flower).

Fifty young crown segments were cut from the rhizomes of each plant on 2 September 1994, potted up and maintained in a glasshouse until mid October, by which time plants were 15-20 cm high. Forty plants from each original plant (= genotype) were hardened off outside for 3 weeks before being transplanted into the field on 5 November.

The experiment used a randomised block design with four replicates (each of 10 plants) for each genotype. Plot size was 3 x 1.2 m and plants were spaced 60 cm apart in both directions. The site had previously been in pasture and had undergone conventional cultivation to prepare a seed bed. Trifluralin (800g a.i. ha^{-1}) was applied and surface incorporated before transplanting. Plants were irrigated (600 l h^{-1}) as required during the season, and a compound (N:P:K:S) fertiliser (12:10:10:2) applied at 200 kg ha^{-1} on 5 December.

The seed production potential of individual plants was assessed beginning from the date of first flowering, and inflorescence numbers were counted every 5 days, with a final count on 5 March, the day of seed harvest. Plant diameter (mean of north/south and east/west axis) was measured before plants were hand cut at the base and leaf number, stem number and inflorescence number recorded, and leaf size visually assessed using a key developed by Fu (1998). Inflorescences were hand removed from each plant and placed in paper bags for ambient air drying. The remaining plant material was oven dried at 60°C for 48 h to determine plant dry matter. Two months after harvest five inflorescences were removed from each bag and floret and seed number recorded. The remaining florets

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Table 1. Inflorescence number and seed yield of 13 individual plants selected at random from within a field of Caucasian clover cv. Monaro.

Plant Number	Inflorescence number per plant[1]	Seed yield per plant (g)[2]
1	32	4.36
2	46	5.97
3	0	0
4	7	1.14
5	5	0.48
6	8	0.98
7	6	0.61
8	0	0
9	0	0
10	4	0.33
11	5	0.79
12	29	2.71
13	0	0

[1] *assessed 30 January*
[2] *determined 25 February*

Table 2. A comparison of morphological and reproductive characters among three genotypes of Caucasian clover from within cv. Monaro.

	Genotype			
Plant morphology	2	9	12	LSD P<0.05
leaves per plant	160	132	159	NS
stems per plant	12.0	1.0	15.6	4.7
leaf size[1]	1.5	1.1	2.8	0.5
plant diameter (cm)	36.3	25.9	35.6	2.1
plant dry matter (g)	19.1	9.9	22.9	7.3
Flowering behaviour				
date of first flowering[2]	15 Jan	20 Feb	17 Jan	-
flowering plants per plot[3]	8.5±0.6	3.3±0.7	8.0±0.5	-
Seed yield and components				
inflorescences per plant	34.7	1.1	41.6	13.8
florets per inflorescence	80.8	86.5	87.6	NS
seeds per inflorescence	49.4	60.0	56.7	NS
thousand seed weight (g)	2.58	2.13	1.90	0.065
seed yield per plant (g)	3.65	0.18	2.99	1.48

[1] *visual assessment based on a key (Fu, 1998) where 1 = small and 5 = large*
[2] *date first inflorescence observed*
[3] *each plot had ten plants*

were then hand threshed, sieved to remove trash, and pure seed obtained by air blowing the samples at 345 rpm for 30 seconds. Germination and thousand seed weight (TSW) were determined using internationally agreed methodology (ISTA, 1996).

Plants of genotypes 2 and 12 were larger than those of genotype 9 in that they had more leaves and stems, a greater diameter and hence greater dry matter (Table 2). Leaf size was greater for genotype 12 than for the other two genotypes.

Genotype 9 was almost one month later in commencing flowering than the other two genotypes (Table 2) and although some plants of all three genotypes did not produce inflorescences , only around one-third of genotype 9 plants flowered, and therefore the average number of inflorescences per plant was only 1.1 (Table 2). In contrast genotypes 2 and 12 produced 35 and 42 inflorescences per plant respectively.

Neither florets nor seeds per inflorescence were affected by genotype but seed yield was significantly greater for genotypes 2 and 12 than for genotype 9 (Table 2). Thousand seed weight differed among all three genotypes, being highest in genotype 2 and lowest in genotype 12. Germination (90 + %) did not differ among the genotypes (data not presented).

Although only three genotypes were field evaluated in this study, there were significant differences in their morphological and reproductive characteristics. Most important for seed production was the ability to flower and the time that flowering began. The fact that some genotypes, and some plants within a genotype, remained vegetative over the entire growing season suggests that floral induction requirements may differ among genotypes and for some were not met. Kannenberg and Elliott (1962) reported that Caucasian clover required a 17 h daylength and 4.5°C night temperatures for induction, but while these conditions may not have been met in the field trial, they certainly were for the individual plants in containers. In a further field trial in the 1995/96 season (Fu, 1998) where once again the daylength/temperature conditions for induction were met, three genotypes (8, 9 and 13) failed to flower, and a further three genotypes (5, 7 and 10) failed to flower when plant population was greater than 11 plants m^{-2} (Fu, 1998).

The reason for this varying flowering response among genotypes from within this one cultivar remains to be determined. However, in Caucasian clover, inflorescence number is a major determinant of seed yield (Widdup *et al.*, 1996; Fu, 1998) and therefore selection for plants that are strong inflorescence producers would improve seed yield. This has already been achieved from within cultivar Monaro, whereby two cycles of selection for improved seed yield per plant (not solely inflorescence numbers) have resulted in cultivar Endura (Norriss, 1995; Widdup *et al.*, 1996) which has a significantly greater seed yield than the parent cultivar. It is also probable that further selection gains will be achievable (Widdup *et al.*, 1996).

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