Effect of Inflorescence and Floret Number on Floret Site Utilisation of White Clover

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ABSTRACT

In white clover (*Trifolium repens* L.), low floret site utilisation limits potential seed yield, as in general, less than half the ovules in each floret develop into mature seeds. This paper discusses two experiments conducted to determine the effect of competition between reproductive organs on floret site utilisation and the extent to which competition is influenced by the availability of phosphorus. Reducing the number of inflorescences per plant influenced reproductive development including the number of seeds per floret. Plants with one or four inflorescences had significantly more seeds per floret and a higher seed weight per inflorescence than plants with eight inflorescences but there was no effect on individual seed weight. Reducing the number of florets to 20 per inflorescence had a positive effect on the number of seeds per floret but had no effect on individual seed weight. Florets from different parts of the inflorescence differed significantly in the proportion of ovules that produced a mature seed. Florets from the lower part of the inflorescence had significantly more seeds than those from the middle and upper parts of the inflorescence. Increasing the amount of phosphorus in the basal nutrient solution had no effect on floret site utilisation, other seed yield components, pollen viability or the number of ovules per floret.

Additional index words: **competition, floret site utilisation, reproductive growth, *Trifolium repens*.**

INTRODUCTION

Under optimal conditions for white clover pollination, less than half the ovules in each floret develop into mature seeds (Dessureaux, 1951; Zaleski, 1961; Van Bogaert, 1977; Hollington, Marshall and Hides, 1989). A floret can have up to seven ovules but rarely more than two or three develop into mature seeds. Poor floret site utilisation is therefore one of the primary causes of low seed yield of white clover (Hides, Lewis and Marshall, 1984). Identification of the factors contributing to low floret site utilisation is therefore important if techniques are to be developed to increase seed yields.

Several factors may contribute to low floret site utilisation. Florets may be unpollinated because of unfavourable weather during the pollination period and/or lack of suitable insect pollinators (Hides *et al.*, 1984). Pollination may occur but incompatible pollen deposited on the stigma or conditions inappropriate for pollen germination and pollen tube growth will lead to poor fertilisation. Fertilised embryos or developing seeds may abort due to lethal gene combinations in the embryo or to postzygotic incompatibility (Wiens, 1984). The limited evidence available suggests that in white clover the majority of ovules are successfully fertilised but abort when they have almost doubled in size (Robbie, 1989). Abortion of this type has also been demonstrated in self-fertile white clover plants, in which their own pollen affects fertilisation and the question of incompatibility being the cause of reduced seed set is removed (Robbie, 1989). Factors influencing early embryo abortion appear to be critical in determining the number of seeds reaching maturity.

In self-fertile white clover plants there is a higher seed set at basal compared to apical ovary positions, regardless of whether flowers have been self or cross-pollinated (Robbie, 1989). There is also some evidence that the position of the white clover ovule influences its subsequent development, ovules located in the central part of the ovary having more opportunity to develop to mature seeds than those located near the style (Cebrat, Sobtka-Mioduzewska and Kobierzynska-Golab, 1980). This suggests that nutrition may be an important factor in limiting the number of seeds per floret (ie the seeds nearest the source of nutrition develop at the expense of those further away) (Robbie, 1989). Abortion of developing seeds might therefore arise as a result of competition for assimilates between reproductive organs, either within an inflorescence (ie between florets) (Atwood, 1944; Clifford, 1986), between inflorescences or between reproductive and vegetative organs (Kawanabe, Yoshibhara, Okada, Veno and Hidaka, 1963).

The availability of external supplies of nutrients may also influence floret site utilisation. Limitations in nutrient availability to the plant have been shown to influence reproductive growth in white clover (Khrbeet, 1990), and therefore the extent to which nutrient availability may influence competition between reproductive organs may also be important. Phosphorus has been shown to have the largest effect on the number of seeds per floret (Khrbeet, 1990).

The objective of the present experiments was to determine the extent of the competition between reproductive organs of white clover, the effect on floret site utilisation and the extent to which competition is influenced by limitations imposed by the availability of phosphorus.

MATERIALS AND METHODS

Experiment 1

Experimental Procedure

The experiment was laid out as a complete randomised block with 10 replicate plants in each of 5 treatments as

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shown in Table 1. Scarified seed of the large leaved white clover cultivar Olwen was sown in trays containing John Innes No. 3 compost (with a 50% reduction in nitrogen) on 12 February 1987, and placed in a heated glasshouse, maintained at approximately 16°C. No *Rhizobium* inoculum was added. At the first trifoliate leaf stage, 200 seedlings were transplanted, singly, into 15 cm diameter pots containing the same compost. No nutrients were added and water was applied as required. At the onset of flowering (middle of May), 100 plants of uniform growth were selected, placed in an insect proof isolation chamber to prevent pollination by natural pollinators, and treatments applied. The pots remained in the isolation house until the end of the experiment when the inflorescences were harvested. During flowering the photosynthetic photon flux density was measured with a quantum sensor (185 B, LICOR, Lincoln, Nebraska, USA). It ranged from 350 to 820 umol $m^{-2}s^{-1}$ depending upon climatic conditions.

Table 1. Experimental treatments (Expt 1).

Treatment	Inflorescences per plant	Florets per inflorescence	Florets per plant
1	1	20	20
2	1	40	40
3	4	20	80
4	4	40	160
5	8	40	320

Treatments

The inflorescences began to bloom on 23 May. The first one, four or eight inflorescences to appear on each plant were left to flower according to treatment. Any inflorescences subsequently appearing were removed before they had reached full bloom. In practice this meant that all the inflorescences in the various treatments were on different primary stolons. Inflorescences were then reduced to 20 or 40 florets prior to hand pollination. This was achieved by removing any florets which had begun to reflex and any immature florets. A vertical section of florets was removed from each side of the inflorescence, dividing the inflorescence into two parts containing equal numbers of florets. As insufficient plants produced eight inflorescences, inflorescences were only reduced to 40 florets. Plants therefore received one of five treatments (Table 1).

Inflorescences were hand pollinated when more than 95% of the florets had opened. All florets on an inflorescence opened over a 3-4 day period allowing them to be pollinated at the same time.

Biological measurements

Each inflorescence was harvested when all the florets were brown and 3-4 cm of peduncle below the inflorescence had dried and developed a yellowish colour. The number of seeds per floret from different parts of the inflorescence was determined by removing at harvest five florets from each of the top, middle and lower sections of the inflorescence. Each individual floret was carefully threshed by hand to determine the number of seeds per floret and the mean number of seeds per floret at each position determined. Seeds from the remaining florets of each inflorescence were carefully removed, counted and weighed. A sub-sample of 100 seeds was used to determine 100 seed weight, except in Treatment 2 where insufficient seeds meant only 50 seed sub-samples could be used.

Experiment 2

Experimental procedure

Partially dry soil, classified for phosphorus (P) content as being of soil index 0 (MAFF, 1979) was sieved through a 6 mm screen and 1400 g added to 15 cm diameter pots. Basal nutrients, micronutrients and two levels of P were then applied according to Table 2. The two levels of P were 132.5 and 265 mg pot^{-1} (referred to as P1 (75 kg ha^{-1}) and P2 (150 kg ha^{-1}) respectively).

Three seeds of cv. Olwen were sown per 15 cm diameter pot on 4 January 1989 at a depth of approximately 0.5 cm and reduced to one plant per pot at the first trifoliate leaf stage. The pots were arranged in a controlled environment cabinet in which the day/night temperatures were maintained at 20/15°C at the beginning of the experiment and increased to 25/15°C on 7 March, 1989. The light regime of 16 h photoperiod, provided a flux density of 530 umol $m^{-2}s^{-1}$ supplied by a bank of warm white fluorescent tubes. A vapour pressure deficit of 0.6 kPa was maintained in the cabinet. The pots were placed on saucers and watered daily until the end of the experiment. Flower buds were visible on 20 March and the first inflorescence was at full bloom on 29 March. Removal of inflorescences and florets was as described in Expt 1 but in this experiment only the one and four inflorescence treatments were included. The experiment was a split-split plot (2 (P) levels x 2 inflorescence treatments x 2 floret treatments x 8 replicates) with a total of 64 plants. Data were analysed by analysis of variance.

Table 2. Level of P and basal nutrients.

Treatment	mg per pot	kg ha^{-1}
P1, P2	132.5, 265 as $CaHPO_4$	75, 150
Basal applications:		
N	106 as $NaNO_3$	60
K	180 as KCI	102
Lime	3153 $CaCO_3$ 530 MgO	2000
Nicronutrient[1]:		
Manganese sulphate	16.8	10
Copper sulphate	16.8	10
Boric acid	8.4	5
Cobalt sulphate	3.4	2
Zinc sulphate	3.4	2
Sodium nolybdate	0.6	0.5

[1] Applied in solution (g l^{-1}), sulphate group added separately, iron added according to soil weight = 5 g l^{-1} as solution.

Biological measurements

Five florets were removed prior to hand pollination from the top, middle and lower parts of the first inflorescence on each plant and the mean number of ovules per floret at each position determined. Pollen viability was assessed prior to treatments being applied on twenty plants from each level of P in the manner described by Heslop-Harrison, Heslop-Harrison and Shivanna (1984). Approximately 200 pollen grains from each treatment were evaluated using this method.

Each inflorescence was harvested when ripe as described in Expt 1. At harvest, five florets were selected at random from the upper, middle and lower parts of each inflorescence and the mean number of seeds per floret at each position determined. Seeds from these florets were then added to the seeds which had been removed from the remaining florets on each inflorescence and seed weight per inflorescence and 100 seed weight determined as described in Expt 1.

RESULTS

Experiment 1

In general the number of seeds per floret declined as the number of florets per plant increased (Table 3), although floret number had no effect on 100 seed weight. The number of florets did, however, influence the number and weight of seeds per inflorescence. Plants with one and four inflorescences, but reduced to 20 florets per inflorescence, had similar and not surprisingly, significantly fewer seeds and a lower seed weight per inflorescence than all other treatments (Table 3). In contrast, a comparison of treatments with different inflorescence numbers but reduced to forty florets shows that plants with one inflorescence had significantly more seeds per inflorescence and a higher seed weight per inflorescence than those with four and eight inflorescences, which did not differ significantly $P < 0.05$ (Table 3).

Table 3. Effect of inflorescence and floret density on reproductive growth characters (Expt 1).

Inflorescences per plant	1	1	4	4	8	SED (36 d.f.)
Florets per inflorescence	20	40	20	40	40	
Florets per plant	20	40	80	160	320	
Seeds per floret[1]	3.1	2.8	3.4	2.3	2.2	0.24
100 seed weight (mg)	62.6	60.2	60.5	60.8	58.4	3.61
Seeds per inflorescence	62.0	112.4	68.8	91.1	86.4	5.99
Seed weight per inflorescence (mg)	38.0	67.8	40.9	55.4	50.7	4.43

[1] Calculated from the bulked seeds of each inflorescence

The removal and examination of florets from different parts of the inflorescence also revealed that plants with eight inflorescences and forty florets (320 florets per plant) had significantly fewer seeds per floret than all other treatments, which did not differ significantly (Table 4). However, florets from different parts of the inflorescence differed in the number of seeds per floret. Lower florets consistently had significantly more seeds than the middle florets and both had significantly more than the top florets. There was no significant treatment x floret position interaction (Table 4).

Table 4. Effect of inflorescence and floret density on the number of seeds per floret (Expt 1).

Treatments (T)			Floret position (FP)			
Inflorescences per plant	Florets per inflorescence	Florets per plant	Low	Middle	Top	Mean
1	20	20	4.2	3.2	2.7	3.4
1	40	40	3.6	3.5	2.5	3.2
4	20	80	3.9	3.4	3.1	3.4
4	40	160	3.5	3.3	2.4	3.1
8	40	320	3.0	2.3	2.1	2.5
	Mean		3.6	3.1	2.5	

SED (126 d.f.) T = 0.17; FP = 0.13; TxFP = 0.30

Experiment 2

Pollen viability was not influenced by floret removal or phosphorus and was generally higher than 90%. The number of seeds per floret was not influenced by phosphorus, floret number per plant, inflorescence number per plant or florets per inflorescence. The position of the floret on the inflorescence, however, significantly influenced the number of seeds per floret. Lower florets had significantly more seeds than the middle and top florets which did not differ significantly (Table 5). Overall, 28.4% of ovules failed to produce a mature seed although a significantly higher proportion of ovules in lower florets produced a seed compared to those in the middle or top florets (Table 5).

Plants with one inflorescence had a significantly higher seed weight per inflorescence than those with four inflorescences (Table 6). Reducing the number of florets from forty to twenty per inflorescence significantly reduced seed weight per inflorescence by 30% (Table 6). In all four treatments, phosphorus increased seed weight per inflorescence, though not significantly so (Table 6). One hundred seed weight was not influenced by phosphorus, the number of inflorescences per plant or florets per inflorescence, and there were no significant interactions.

Table 5. Effect of floret position on number of ovules and seeds per floret and percentage of ovules that failed to produce a mature seed (Expt 2). Data are a mean of the 5 treatments.

Floret position	Ovules per floret	Seeds per floret	% ovules failing to produce a mature seed
Top	5.1	3.5	30.9
Middle	5.2	3.6	30.7
Low	5.3	3.9	23.5
S.E.D.	0.05 (76 d.f.)	0.08 (114 d.f.)	2.02 (114 d.f.)

Table 6. Effect of phosphorus (P), number of inflorescences per plant and florets per inflorescence on seed weight per inflorescence (mg).

	Inflorescences per plant (Inflor)						
	One			Four			
	Florets per inflorescence (FI)			Florets per inflorescence (FI)			
P levels (kg ha^{-1})	20	40	Mean	20	40	Mean	Mean
P1 (75)	53.6	72.6	63.1	37.3	57.4	47.4	55.3
P2 (150)	56.8	75.3	66.1	41.4	64.9	53.2	59.7
Mean	55.2	74.0	64.6	39.4	61.2	50.3	
Florets Mean		FI_{20} = 47.3			FL_{40} = 67.6		

S.E.D.	(7 d.f.)	P	3.87
	(14 d.f.)	Inflor	4.35
	(28 d.f.)	FI	4.69
	(14 d.f.)	P x Inflor	5.82
	(28 d.f.)	P x FI	6.08
	(28 d.f.)	Inflor x FI	6.40
	(28 d.f.)	P x Inflor x FI	8.83

DISCUSSION

These experiments have examined the extent to which competition between reproductive organs can affect the floret site utilisation of white clover and whether the application of phosphorus can influence this characteristic. These factors were examined by reducing the number of reproductive sites (ie inflorescences per plant and florets per inflorescence) and assessing the effect on the number of seeds per floret and seed weight.

In Expt 1, varying the total number of florets per plant both by inflorescence and floret removal influenced floret site utilisation. Seed numbers per floret were significantly reduced above a total number of 80 florets per plant. Reducing the total number of inflorescences per plant affected the number of seeds per floret as well as some other seed yield components. Plants with eight inflorescences produced significantly fewer seeds per floret than those with one or four inflorescences per plant, resulting in a lower seed weight per inflorescence. These treatments, however, had no effect on individual seed weight. In Expt 2 varying the number of inflorescences had no effect on these seed yield components. However, the maximum number of inflorescences per plant in this experiment was only four compared with eight in Expt 1 suggesting that the number of inflorescences in Expt 2 was insufficient to produce measurable competition. Reducing the number of florets per inflorescence had some positive effects on the number of seeds per floret in Expt 1, but had no effect on individual seed weight in either experiment, although not surprisingly there was a reduction in seed weight per inflorescence. There were therefore some differences in the results of the two experiments. Several likely reasons for the discrepancies can be proposed. The possible effect of the different numbers of inflorescences in the two experiments has already been discussed. Other factors may also be important. For example, light intensity was more variable in Expt 1 (glasshouse) than in Expt 2 (growth room) and during flowering may have been lower. Light intensity is likely to have reduced the supply of assimilate and consequently have increased the competition for assimilates within the plant. In addition there is some evidence that low light intensity can influence floret site utilisation by increasing ovule sterility (Pasumarty and Thomas, 1990). These results do however show that competition among reproductive organs has an effect on the number of seeds per floret, the total number of florets per plant and inflorescence number being particularly important. Extrapolation of these results to the field is difficult. However, in the field situation white clover plants may have up to thirty inflorescences (Marshall and James, 1988) with floret number reaching a maximum of eight (Hollington *et al.*, 1989). Consequently the competitive effects could clearly be of a greater magnitude than those demonstrated here.

The position of florets within the inflorescence also influenced floret site utilisation. More ovules from florets in the lower part of the inflorescence produced a seed compared to those in the middle and top part of the inflorescence. Clifford (1986) suggested that competition for nutrients may occur between florets on an individual inflorescence, but did not determine the pattern of this competition. Since lower florets are located near the material resources they may have more opportunity for nutrient uptake. These lower florets are also the first to open and be pollinated and could have a competitive advantage over those higher on the inflorescence and which are pollinated later. Harvey (1970) found the strength of reproductive sinks was dependent on the stage of development, and more assimilates were imported during inflorescence development and seed filling than during fertilisation. In this controlled environment the whole inflorescence opened over a 3-4 day period, enabling all the florets to be pollinated at the same time. In the field where temperatures are likely to be lower, the whole inflorescence requires 7-8 days to open (Erith, 1924) and ovules in the lower florets can be fertilised before the top florets have opened. The period over which the florets open and are pollinated may therefore have some effect on the extent of this competition and this needs further investigation.

Increasing the amount of phosphorus applied to each plant had no effect on floret site utilisation as the two levels applied in Expt 2 had no effect on any of the measured seed yield components, pollen viability or the number of ovules per floret. There was some evidence of an effect of phosphorus on seed weight but the increases were not significant. The lowest level of phosphorus used in this experiment was lower than in most soils. It is therefore unlikely that varying phosphorus will increase floret site utilisation, but it may increase individual seed weight. It is clear, however, from other experiments that phosphorus does have an effect on reproductive growth via inflorescence production (Khrbeet, 1990).

In these experiments where hand pollination was used, 71.6% of potential seed sites (ovules) produced a seed. This is a higher figure than previously reported from field experiments (Dessureaux, 1951; Van Bogaert, 1977; Cebrat *et al.*, 1982). This may have been because of the greater efficiency of hand compared to insect pollination but may also in part be due to low overall levels of florets per plant compared to the field situation which enabled more seeds to reach maturity. While these factors clearly require further investigation, these studies have shown that competition between the reproductive organs of white clover has a significant effect on floret site utilisation.

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