The Effect of Temperature on Components of Seed Yield and on Hard Seededness in Three Cultivars of Red Clover (*Trifolium pratense* L.)[1]

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ABSTRACT

Two experiments were carried out to investigate the effect of temperature (12, 16 and 20 C) during inflorescence development on floret morphology of cvs Sabtoron (2n) and Hungaropoly (4n) and on components of yield and hard seededness in Sabtoron, Hamua (2n) and Hungaropoly, florets having been pollinated by hand.

Of the floret characteristics only nectar height was influenced by temperature being slightly lower at 12 C than at 20 C. Sabtoron had significantly smaller floral parts than Hungaropoly, the corolla tube length being 8.75 mm and 9.43 mm, respectively.

Hungaropoly had no more than 50% of the inflorescences of Sabtoron or Hamua at any time. Increasing temperature from 12 C to 20 C resulted in a 4 to 5 fold increase in the rate of inflorescence appearance in all cultivars, a 68% increase in seeds per inflorescence and a corresponding increase in seed setting. At the higher two temperatures there were more seeds in the later formed inflorescences. Thousand seed weight was affected little by temperature, Hungaropoly producing seeds 46% heavier than the mean of the other two cultivars.

Hungaropoly had significantly fewer hard seeds than the other cultivars at all temperatures, the content declining from 47.3% at 12 C to 26.5% at 20 C. In contrast hard seed content of Sabtoron was relatively unaffected by temperature and that of Hamua decreased slightly from 16 C to 20 C.

It is concluded that yield, principally because of low inflorescence numbers and poor seed setting, and quality of red clover seed are likely to be adversely affected by low temperatures in cool temperate areas. This should be taken into account when considering potential areas for red clover seed production.

Additional index words: controlled environments, floret morphology, time of flowering.

INTRODUCTION

The seed yield per unit area of red clover is the product of number of inflorescences per unit area, florets per inflorescence, seed setting and unit seed weight. These are referred to collectively as components of seed yield (Dennis, 1980).

The influence of temperature on seed production is the sum effect on the components and the response will differ between components for a given change in temperature. For example *Stylosanthes humilis* grown at 24 C, as compared to 17 C, had 3.6 times more inflorescences, 1.6 times more florets per inflorescence, 5.8 times greater seed setting and 5% lighter 1000 seed weight (Skerman and Humphreys, 1973). Thomas (1961) recorded 3.7 times more inflorescences formed over a 60 day period on white clover grown at 20 C than at 10 C. However, the number of florets per inflorescence did not differ markedly between temperatures. Thomas also found that corolla tubes were on average 1 mm shorter at 20 C than at 10 C.

The length of the corolla tubes is important in pollination of insect pollinated crops. In inflorescences where the corolla tube is narrow, the distance from the mouth of the corolla tube to the level of the nectar at the base of the floret (nectar depth) has to be short enough to allow the tongues of pollinators to reach the nectar otherwise pollination will be inefficient (Bond and Fyfe, 1968). Therefore the effect of temperature on the length of the corolla tube, especially on depth of nectar level, is worthy of attention as the effect of temperature on pollination efficiency may not only have a direct effect on the activity of the pollinator but also on the accessibility of nectar to the pollinator.

Although red clover is grown in a wide range of climatic areas, no reports of studies on the effects of temperature on seed yield components or floret morphology have been found.

Temperature during seed development is known to influence hard seed content in some forage legumes. Dexter (1955) found a relationship between the content of hard seed in lucerne and the altitude of the site at which the seed was produced, and concluded that low temperatures induced hard seededness. Quinlivan (1965) showed that low temperatures induced hard seeds in subterranean clover, and this was confirmed by Taylor and Palmer (1979) although the degree of effect was lower.

Two experiments were carried out, therefore, in N Ireland to quantify the influence of different constant temperatures during flowering and seed development on (1) floret morphology, and (2) the components of seed yield and hard seed content in red clover (*Trifolium pratense* L.)

MATERIALS AND METHODS

Experiment 1. Effect of temperature on red clover floret morphology.

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Table 1. Details of procedures and measurements taken in Experiment 2.

Treatment	Inflorescence number (weekly) weeks	Inflorescences tripped (weekly) weeks	Harvested (days after tripping ceased on all inflorescences)	Bulked seed samples for 1000 seed weight and germination (weeks)
12 C	1 - 20	1 - 17	40	1-3, 4-6, 7-9, 10-12, 13-15 and 16 and 17
16 C	1 - 12	1 - 10	26	1-3, 4-6, 7-9 and 10
20 C	1 - 11	1 - 9	20	1-3, 4-6 and 7-9

Red clover cultivars, Sabtoron (2n, United Kingdom) and Hungaropoly (4n, Hungary), grown in micro-swards (21 spaced plants per 17.5 cm diameter pot) in a 1:1 soilless compost crushed gravel mixture, were transferred from a heated greenhouse (10 − 25 C, 16 h daylength) to growth cabinets at 12, 16 and 20 C (± 0.5 C) constant temperature when internodes were elongating. Relative humidity ranged between 45-55%, daylength was 16 h and irradiance at pot height was 120 W m^{-2}. One pot of each cultivar was in each of 4 blocks within each cabinet.

Fifteen florets on each of 4 inflorescences at full bloom per pot were measured when the plants at each temperature had reached a similar stage of development viz. 90, 45 and 35 days after transfer to 12, 16 and 20 C, respectively.

Height of nectar level (distance of the level of nectar from the base of the corolla tube) and nectar depth were measured to the nearest 0.5 mm (Fig. 1). The sum of these two characters represented corolla tube length.

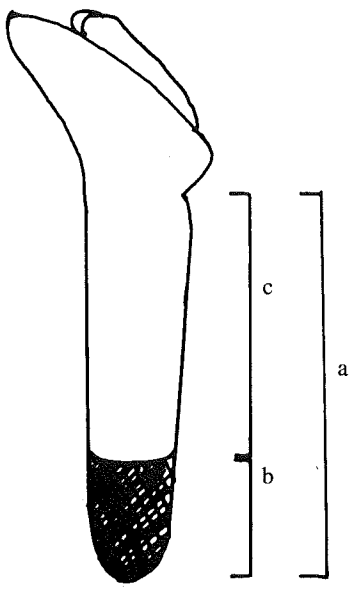


Fig. 1. Diagram of a floret of red clover. (a = corolla tube lengh, b = height of nectar level and c = depth of nectar level below corolla tube mouth).

Experiment 2. Effect of temperature on components of red clover seed yield and hard seed content.

Red clover cultivars Sabtoron, Hamua (2n, New Zealand) and Hungaropoly were grown in pots of 25 cm diameter (32 plants per pot) under the same conditions and transferred to growth cabinets as described for Experiment 1. One pot of each cultivar was in each of 4 blocks in each cabinet.

Inflorescences were grouped according to date of blooming by tagging with a ring of PVC coated wire of a different color for each week and tripped as required with a mounting needle when most of the florets on an inflorescence had opened.

The number of inflorescences per pot was recorded, inflorescences were tripped and florets and seeds counted on inflorescences (4 per pot), weekly, for the periods specified in Table 1. Time of harvesting and samples taken for 1000 seed weight and germination are also stated in Table 1.

Percentage germination was assessed after 10 days. Hard seeds were considered to be those in each batch of 300 seeds (12 lots of 25) which germinated between 10 and 35 days under conditions specified by the International Seed Testing Association (1976). Seeds which had not germinated after 30 days were scarified.

Means of inflorescence number, and florets and seeds per inflorescence, were statistically analyzed from week 9 at 12 C and week 3 at 16 and 20 C as Hungaropoly did not produce inflorescences before that time.

Thousand seed weight was assessed on samples bulked over the 4 replicates.

Statistical comparisons could not be made between the means at each temperature as only one growth cabinet for each temperature was used. However, the standard errors derived from the comparison between cultivars and between inflorescences appearing during each week at each temperature were taken as a measure of within treatment variation and used as a basis for drawing conclusions about large differences between means at the different temperatures.

RESULTS

In Experiment 1 the corolla tube and nectar depth were longer and nectar level higher in Hungaropoly than Sabtoron (Table 2). However, the differences between temperature treatments were small, there being only a slight trend in which the nectar level was higher and nectar depth shorter at 20 C relative to those at 12 and 16 C.

In Experiment 2 inflorescence rate of appearance was

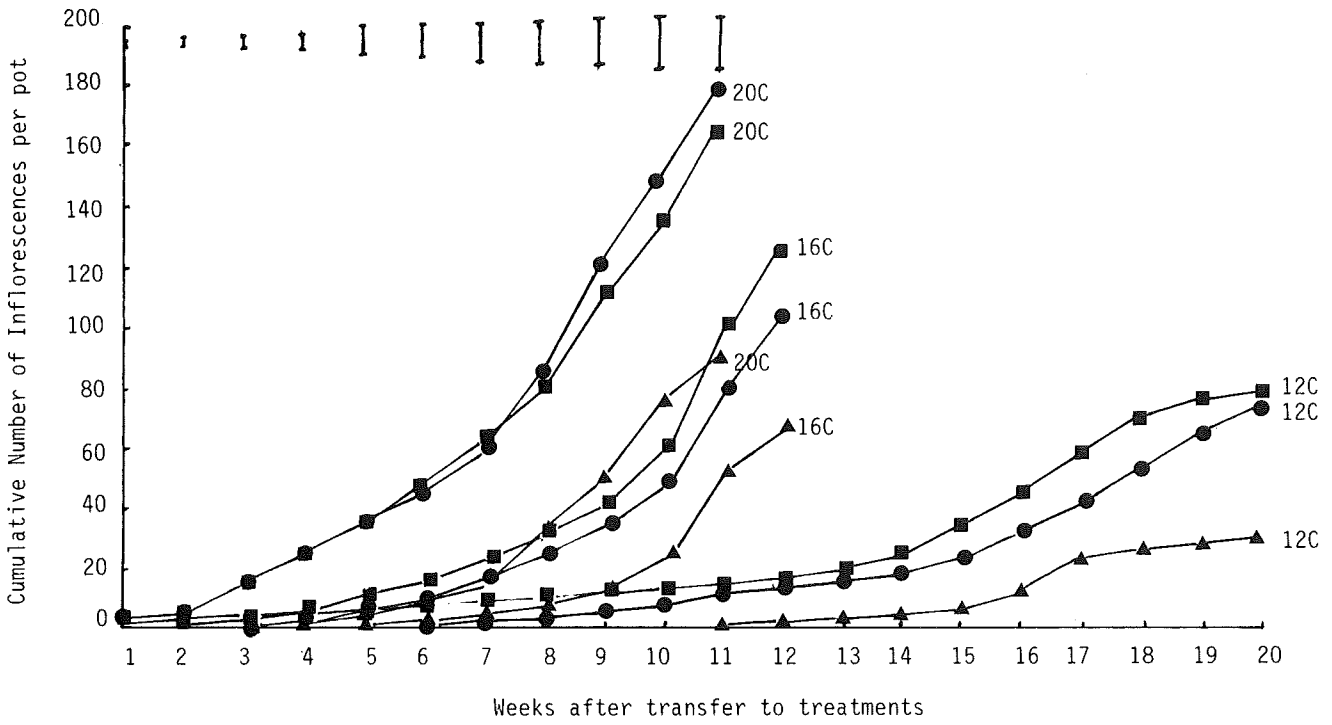


Fig. 2. Mean cumulative number of inflorescences produced per pot in Sabtoron (■), Hamua (●) and Hungaropoly (▲) at intervals after transfer to temperature treatments.

Table 2. Corolla tube length and height and depth (mm) for each cultivar over all temperature treatments and for each temperature measured over both cultivars.

	Corolla Tube	Height of nectar	Depth of nectar
Sabtoron	8.75	1.37	7.38
Hungaropoly	9.43	1.54	7.89
s.e.m.	0.075***	0.022***	0.077***
12 C	9.10	1.38	7.72
16 C	9.11	1.40	7.71
20 C	9.06	1.58	7.49
s.e.m.	0.092 NS	0.027***	0.094 NS

greater in the two diploid cultivars than in Hungaropoly at all three temperatures, Hungaropoly rarely having more than 50% of the inflorescences of Sabtoron (Figure 2). Over the full period during which numbers were recorded, the weekly rate of production per pot for Sabtoron, Hamua and Hungaropoly was respectively 4.1, 3.8 and 2.2 at 12 C, 11.5, 10.3 and 7.3 at 16 C and 16.7, 17.7 and 11.3 at 20 C.

Due to the differing rates of inflorescence production at the various temperatures, comparison of means of characteristics of inflorescences produced at any one time could result in erroneous conclusions being drawn. Vescovi (1977) has shown that temperature does not affect the physiological stage at which inflorescences are produced on red clover but influences the time at which that stage is reached. Consequently the means for components of seed yield over the periods during which similar inflorescence numbers appeared, as described in the previous paragraph, were compared to those for the full period measured. As means were similar for the two periods only means for the full periods are presented in tables. However, where relevant, reference is made to means of components in the period during which similar numbers of inflorescences were produced at the different temperatures.

The effect of temperature on floret number per inflorescence was not marked (Table 3). However, the number of florets per inflorescence tended to increase with later formed inflorescences, this being significant at 20 C. Cultivar differences were not significant. Time of inflorescence appearance had a significant effect on number of seeds per inflorescence at both 16 and 20 C. Only data for seed numbers on heads formed over the period at 20 C are presented (Fig. 3) but the trend was similar for 16 C. The significant interaction at 20 C was due to the increase in seeds per inflorescence of Hungaropoly relative to the diploid cultivars becoming greater in later formed inflorescences, (Fig. 3).

Seed setting followed a similar trend to seeds per inflorescence, with the earliest formed inflorescences at 16 C and 20 C having the lower seed setting percentage (Fig.

Table 3. Mean number of florets and seeds per inflorescence and seed setting (%) for three cultivars of Red Clover at 3 temperatures. (Data for significant effects of time of inflorescence appearance and interaction with cultivars are presented in Fig. 3)

Florets per inflorescence

	12C		16C		20C	
Sabtoron	99.6		107.6		107.7	
Hamua	97.7		98.8		99.3	
Hungaropoly	98.8		103.1		99.0	
Mean	98.7		103.2		102.0	
	Sign.	s.e.m.	Sign.	s.e.m.	Sign.	s.e.m.
Cv	NS	4.02	NS	4.47	NS	4.59
Time	NS	5.12	NS	3.23	***	4.23
Cv × Time	NS	7.85	NS	5.47	NS	7.37

Seeds per inflorescence

	12C		16C		20C	
Sabtoron	13.8		20.5		24.2	
Hamua	11.9		18.3		22.0	
Hungaropoly	19.3		31.2		32.3	
Mean	15.0		23.3		26.2	
	Sign.	s.e.m.	Sign.	s.e.m.	Sign.	s.e.m.
Cv	NS	2.03	NS	3.13	*	1.86
Time	NS	1.19	***	0.89	***	1.44
Cv × Time	NS	1.83	**	1.51	**	2.47

Seed setting

	12C		16C		20C	
Sabtoron	13.9		19.0		22.4	
Hamua	12.2		18.2		22.3	
Hungaropoly	19.7		30.9		33.2	
Mean	15.3		22.7		26.0	
	Sign.	s.e.m.	Sign.	s.e.m.	Sign.	s.e.m.
Cv	NS	1.74	NS	3.51	NS	2.84
Time	NS	1.35	***	1.16	*	1.70
Cv × Time	NS	2.06	**	1.97	NS	2.93

3). Inflorescences formed at 16 C had a 60% improvement in seed set over those formed at 12 C, an increase of a further 4 C to 20 C resulting in only a slight additional improvement in seed setting (Table 3).

Hungaropoly had consistently the heaviest seeds being 0.8 to 1.0 g (i.e. over 30%) heavier per 1000 than the mean of the diploids, taken over all periods and temperature treatments (Table 4). Differences between temperatures were small, although there was an increase of 21% in Sabtoron seed weight at 16 C relative to 12 C.

All treatments resulted in eventual germination of 93% or higher; however there were differences in hard seed content between cultivars. Table 4 contains the mean weighted hard seed content for number of seeds per pot produced over each 3-week period. At all temperatures Hungaropoly had a significantly lower content of hard seeds than the other two cultivars, the content at 16 and 20 C in Hungaropoly being less than half that in the two diploid cultivars.

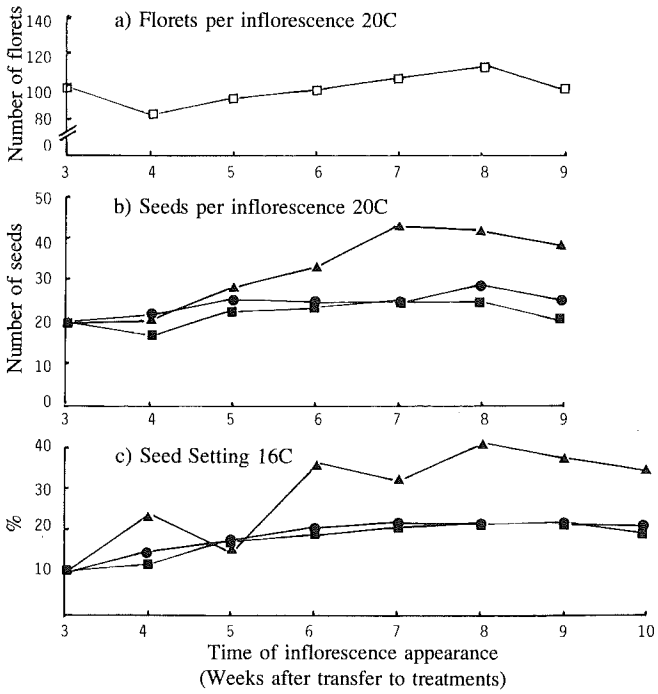


Fig. 3. Effect of time of inflorescence appearance on: a) floret number per inflorescence at 20 C (mean of 3 cultivars) b) seeds per inflorescence for each of three cultivars at 20 C and c) seed setting for each of three cultivars at 16 C. (Relevant statistical data are presented in Table 3) (■ Sabtoron, ● Hamua, ▲ Hungaropoly)

Table 4. Mean thousand seed weight (g) and hard seed content (%) for three cultivars of Red Clover at three temperatures.

Thousand seed weight

	12C	16C	20C
Sabtoron	1.92	2.43	2.33
Hamua	1.91	1.96	1.88
Hungaropoly	2.97	3.12	2.99
Mean	2.26	2.50	2.40

Hard seed content

	12C	16C	20C
Sabtoron	68.1	65.4	65.1
Hamua	70.5	70.9	55.5
Hungaropoly	47.3	30.9	26.5
Mean	62.0	55.7	49.0
	Sign. s.e.m.	Sign. s.e.m.	Sign. s.e.m.
Cv	*** 1.79	*** 1.23	*** 1.58

The overall effect of temperature was to reduce the content of hard seeds in Hungaropoly from 47.3% at 12 C to 26.5% at 20 C whereas the hard seed content of Sabtoron was relatively unaffected by temperature. Hamua had less hard seed at 20 C than 16 C.

DISCUSSION

The comparatively small effect of temperature on depth of nectar in Sabtoron and Hungaropoly would be of little practical significance in improving pollination. The lowest, i.e. 7.49 mm at 20 C, is still in the upper end of the range of nectar depths measured by Bond and Fyfe (1968) in contrasting lines of red clover. High temperatures, however, could have a beneficial effect on pollinator efficiency by increasing the population of pollinators on the seed crop and increasing the activity of the individual pollinator. The lower depth of nectar in the diploid Sabtoron relative to that of the tetraploid Hungaropoly has also been found in field studies in which the mean length of the diploid corolla tubes was 9.9 mm compared to 11.0 for those of the tetraploids (Julén, 1956).

The effect of temperature on components of yield was greater for inflorescence production than for seeds per inflorescence. Floret number increased only between 12 and 16 C. Skerman and Humphreys (1973) also found inflorescence number to be the most sensitive component to temperature in *Stylosanthes humilis* with floret number, although increasing with temperature, being less sensitive than inflorescence production.

Under field conditions where there was a high population of bees during warm periods in summer, natural pollination has been shown to give rise to seed setting as high as 109% (Puri and Laidlaw, 1983). The low seed setting percentage in this experiment (21.3%) would suggest that the manual pollination method adopted was inadequate to efficiently transfer pollen between plants. The fact that the seed setting percentage was higher in the tetraploid than the diploids supports this as Laczynska-Hulewiczowa (1961) found ten times more compatability between closely related florets in tetraploids than diploids. The lower temperatures may have resulted in higher pollen abortion (Thomas, 1961) accounting for the lower seed set at 12 C than 16 and 20 C. This might only be important in the field when pollen availability is low, e.g. due to low pollinator activity. Nevertheless, it is an additional adverse effect of low temperatures on red clover seed production to be taken into account.

The combined effect of temperature on the components of yield per inflorescence resulted in a calculated seed yield over the periods of similar inflorescence development of 209, 442 and 455 kg ha^{-1} at 12, 16 and 20 C, respectively. The differences in the rate of inflorescence production at the various temperatures would markedly increase the differential between the crops at 12 C and those at the other two temperatures and also contribute to the seed yield at 20 C being higher than that at 16 C.

The temperatures used were intended to represent the daily means of climates ranging from cool temperate to warm temperate conditions during the growing season. Relating the effect of constant environmental conditions to the field, however, could result in misinterpretation. There is inadequate information available to establish whether a reliable relationship exists between the effect of constant temperatures and diurnal fluctuation, as well as day-to-day fluctuation. Indeed, the temperature of one part of the day may be more important to seed yield components than mean temperature. Skerman and Humphreys (1973) found night temperatures overrode day temperatures unless the latter were lower than the former. Independent of the problems in relating these temperatures to field conditions the data demonstrate the importance of temperature in governing the magnitude of seed yield components and that a difference of 4 C can have a marked effect on red clover seed production.

Red clover seed quality can also be affected by temperature during seed formation, Hungaropoly being clearly the most sensitive in this respect. The fact that there are genotypic differences in the induction of hard-seededness with low temperatures might have accounted for the differing degree of effect in subterranean clover *(Trifolium subterraneum)* found between the studies of Quinlivan (1965) and Taylor and Palmer (1979). Although mechanical harvesting of red clover seed is likely to reduce the content of hard seeds, the process cannot be relied upon to eradicate the condition (Rincker, 1954). Consequently the temperature during seed formation in some production areas may be responsible for the induction of hard seeds in commercial seed crops. As 12 C induces the condition in Hungaropoly, this would suggest that cool temperate areas may be unsuitable for red clover seed production in terms of seed qualtiy in cultivars which are prone to hard-seededness.

In a field trial in Northern Ireland when the mean daily temperature for the period June to September was 13.9 C, Puri (1982) found Sabtoron and Hungaropoly to have 65% and 75% hard seed contents, respectively. This confirms that hard seed content can be high in red clover grown for seed under cool temperate conditions.

In conclusion although these data cannot be directly extrapolated to field conditions, they do show that differences of only a few degrees of temperature, within the range of mean temperatures experienced in temperate regions, have a marked effect on red clover seed production and quality and that the sites suitable for optimum red clover seed production may be limited because of the crop's sensitivity to temperature.

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