Title

THE INFLUENCE OF ENVIRONMENT ON GROWTH AND DEVELOPMENT IN THE FORAGE LEGUME SAINFOIN (*Onobrychis viciifolia* Scop.)

A thesis presented in partial fulfilment
of the requirement for the degree
of Master of Science in Botany
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Frontpiece

Sainfoin (*Onobrychis viciifolia* Scop.)blossoming after long day treatment.

ABSTRACT

The effect of environment on growth and development in the forage legume sainfoin is examined. A comparative study has been made between five sainfoin cultivars (Melrose, Giant, Italian, Krasnodar, Common) and also between genotypes within each cultivar.

The effect of plant age on growth and development of cv. Melrose is examined. Results show that this cultivar had a juvenile phase of approximately 60 days when plants were grown in long days (LD) in a warm glasshouse (GH).

When Melrose sainfoin was grown in small pots reproductive development was inhibited. After data on root-and shoot growth and development had been obtained various hypotheses are presented that might explain this result.

The effect of gibberellic acid (GA_3) on growth and development of Melrose in a warm GH is examined. Low concentrations of GA_3 caused plants with a prostrate leaf and rosette habit to change to a erect leaf and rosette habit in short days (SD). Application of higher concentrations of GA_3 to plants in SD made plants elongate stems, although, inflorescence initiation did not occur. GA_3, when applied to warm LD grown plants, decreased the juvenile period, lowered the critical photoperiod for inflorescence initiation and possibly helped to overcome this observed root growth/small pot size inhibition on reproductive development, however, GA_3 did not cause an increase in the percentage of plants with macroscopically visible inflorescences (mvi) with only 30-50% having mvi.

Results on the effects of low temperature on growth and development are presented for all cultivars. Those plants that did not flower in long days when held in the warm glasshouse did so after low temperature treatment. Plants held in natural cool short days initiated inflorescences, although, these inflorescences did not develop to anthesis while held under these environmental conditions. Results on the effect of critical day length on continued inflorescence development after SD low temperature treatment show that a daylength of approximately 14 hours was necessary for inflorescences to reach anthesis. Data is also presented on inflorescence initiation and development for Melrose plants grown in the natural cool environment with various extended photoperiods.

The relative importance of these environmental and hormonal factors, and the interactions between them, in controlling growth and development is discussed.

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CHAPTER 1

INTRODUCTION

1.1 History of cultivation of sainfoin

The forage legume sainfoin (Onobrychis viciifolia Scop.), also known as French grass, esparcet or esparsette (Piper, 1924; Whyte et al, 1953), is especially adapted to dry calcareous soils and is used in Central Europe, Great Britain, the Mediterranean countries, Canada and the USA as a pasture, hay and forage plant (Thomson, 1938a; White et al, 1953; Bland, 1971; Hanna and Smoliak, 1968; Eslick, 1968).

Its name which is of French origin and was originally written Sain foin, means "wholesome hay". Clark and Malte (1913) note that in some of the early literature the name has sometimes been erroneously written Saint Foin and has thus led to the misconception that it means "Holy hay".

In Great Britain sainfoin is native from Somerset and Kent to Shropshire and Norfolk, Glamorgan, Carmarthen and Flint. It is also native to Central Europe north to France and Germany, Siberia, east to the Baikal region, Caucasus, Persia and Asia Minor (Clapham et al, 1962).

Piper (1924) notes that the culture of sainfoin probably dates back about 400 years. The first definite record of its cultivation being recorded by Vianne in 1582 in Southern France (quoted by Piper, 1924). In addition Piper (1924) notes that sainfoin was grown in Germany in the seventeenth century and in Italy in the eighteenth century. Sainfoin also has a long history of cultivation in southern England (Bland, 1971; Spedding and Diekmahns, 1972). The earliest reference to be found was in 1733 where Jethro Tull described sainfoin culture in England. Clark and Malte (1913) suggested that the cultivation of sainfoin first began in France, from there spreading to other European countries especially Italy, England, Switzerland and Germany. Bland (1971) suggested that the Giant and Common form of sainfoin in Britain may also have originally originated from France. Various strains of sainfoin have been introduced to North America since about 1820 (Clark and Malte 1913) but it has only in the last few years gained importance in North American agriculture climaxing in 1968 in the first sainfoin symposium at Montana State University (Cooper and Carleton, 1968) followed by the production of an information booklet by the Canada Department of

Agriculture in 1972 (Hanna et al, 1972).

1.2 Potential of sainfoin in New Zealand

In New Zealand agriculture heavy emphasis is placed on grassland farming. Today with the high cost of artificial fertilizers, and with the knowledge that they are a limited resource, the need for legumes is becoming more apparent; due to this we are entering a new grassland era where the legume component will be the limiting factor determining production. To meet this need the breeding and re-evaluation of new legume species will be necessary to fulfil all roles in our grassland farming so as to maintain present production levels.

In many areas of New Zealand summer production is limited, in some cases severely, by summer drought. To overcome this problem lucerne has been grown mainly for summer feed. However, this forage plant has many disadvantages for the complete role it is meant to fulfil. For example, it causes bloat among ruminant animals; therefore its use on 'town supply' dairy farms is limited. In addition this forage crop is a monoculture crop and is always under the continual threat from pests and diseases. This latter factor is of national importance. For example the recent aphid problem on lucerne has highlighted the need for a new legume species that will occupy a similar role to lucerne. With more than one monoculture legume species in use the risk of a major production loss from the introduction of a new pest or disease is greatly decreased.

A plant species that could show great promise under the New Zealand farming situation is the forage legume sainfoin. As very little is known about this plant a full agronomic programme will need to be undertaken so that its full potential can be realized. However, before a study of agronomic aspects begin an understanding of the plants growth characteristics and the environmental factors that control the flowering process are essential. Too often in the past there has been a poor understanding of a plant's physiological growth characteristics while undergoing an agronomic evaluation. Often in the past the tendency has been to agronomically evaluate a species, then by the time the new cultivar has been released for commercial seed distribution physiological aspects are examined because prior obtained agronomic aspects can not be fully interpreted without this fundamental knowledge. This often leads to a field trial needing to be repeated at a later date.

1.3 Agronomic features of sainfoin

The main agronomic features of sainfoin are its ability to fix nitrogen, a very high nutritive value and most importantly that it does not cause bloat in ruminant livestock.

In addition sainfoin has a very deeply penetrating root system that confers drought resistance to the plant. In fact Percival (1921) notes that sainfoin roots penetrate deeper than lucerne roots. Sainfoin can grow on a range of soil types from shallow soils to clays (Orsi, pers. comm.). In Canada sainfoin grows best on Black, Dark Brown, and Brown soils (Hanna et al 1972); in England it is found on the chalky soils of the south and east (Thomson, 1938a). Sainfoin seems to prefer a soil containing free chalk. Baker et al (1952) postulated that it might grow better than lucerne on dry chalky soils because it might be less sensitive to the generally low potash status of such soils. Badoux (1965) found that the yield of Giant sainfoin did not increase after nitrogen fertilizer application. Roath and Graham (1968) found no response in sainfoin to phosphorus fertilizer in a three-year trial on dry land (Western Montana USA), and in a one year irrigation trial. However, analysis of hay from these two trials showed no difference in phosphorus content between sainfoin, lucerne, and red clover. From this result Roath and Graham (1968) concluded that sainfoin might have a greater ability to grow on low phosphorus soils than lucerne or red clover.

Information on the yields of sainfoin under modern husbandry conditions is very scanty. Under a favourable management regime sainfoin has yielded, in Britain, up to 12000 kg dry matter/hectare/year with an organic matter digestibility of approximately 65% (Spedding and Diekmahns, 1962). In a sheep grazing trial over a five year period Smoliak and Hanna, (1975) working in Canada found that the amount of dry matter produced by lucerne and sainfoin did not differ significantly during the study. Most importantly these authors found that sainfoin was more palatable than lucerne and that throughout the five year period no deterioration in "cover" was observed. Whyte et al (1953) also noted that sainfoin"..... is at least as good as lucerne and probably better" in its ability to withstand and survive grazing.

For a more detailed description of the agronomic characteristics of sainfoin reviews of what is known about the plant are given by Spedding and Diekmahns (1972), Hanna et al (1972), Cooper and Carleton

(1968) and Bland (1971).

1.4 Physiology of sainfoin

To date there have been few studies on physiological aspects of sainfoin. Seed studies have been carried out by Thomson (1951b, 1952) and Carleton et al (1963). The latter authors found that the optimum temperature for germination was 15 - 20°C and that for seedling growth was 20 - 30°C. Carleton et al (1963) also noted that pods surrounding the seed reduced the speed of germination and seedling elongation. They found that this inhibitor effect was due to a water soluble inhibitor in the pod.

Studies on flowering have mainly involved observations made during agronomic trials. For example cultivars Remont (Cooper, 1972) and Common (Fagan and Rees, 1930; Thomson, 1938a) do not flower until their second year of growth then they only flower once per season. Cultivars Eski (Cooper, 1972) and Giant (Thomson, 1938a) do flower in their first year of growth with up to three flowering times per growing season. Koreisia (1935) suggested that the reason why Common did not flower in its first season of growth was that it may ".... have a long thermo-stage and must pass through winter before it can flower".

Thomson (1951a) noted that cv. Common blossomed five days earlier than cv. Giant. However, Percival (1949) observed that cv. Common blossomed five to ten days later than cv. Giant. Badoux (1965) explained this by suggesting variability in the origin of seed. However, differing environmental conditions of these two studies might also have affected the time of flowering.

Badoux (1965) noted that different cultivars of sainfoin showed variability in the time of flowering. This author mainly looked at Onobrychis species from a plant breeder's viewpoint.

In 1966 Bawolski published a paper in Poland on aspects of development in two local cultivars of sainfoin. He noted that vernailization did not exert any significant influence on the reproductive development of sainfoin and that sainfoin was a short-long day plant. On examination of his methods a low temperature treatment* effect, however, cannot be ruled out as opposed to a true vernalization* effect.

* Definition on page 5.

Thus from a review of the literature on sainfoin it is evident that the study of flowering, and the environmental factors that control flowering, appears to have excited little interest.

1.5 The present study

In the light of possible agronomic importance of sainfoin and our general lack of information on this plant the present study was made to examine in more detail effects of enviroment on growth and development from germination to anthesis.

This information will enable the plant breeder and agronomist to design field trials with more confidence and efficiency, and allow a more reliable interpretation of their results. In addition this study will provide additional physiological information on a 'new' legume, thus comparisons/contrasts can be made with information already obtained from other closely related legume species.

* Vernalization involves subjecting plants to low temperature conditions (usually around 5°C) for a period of time so that they become "ripe-to-flower", then on transfer to long photoperiods inflorescence initiation will occur. The 'low temperature effect' is a different response in that low temperature treatment will hasten inflorescence initiation even when plants are kept in short days, i.e. this is a direct effect of temperature as against the indirect effect of vernalization.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant Material

Sainfoin has in the past been variously referred to as Onobrychis viciaefolia Scop. (Hanna et al, 1970), O. sativa Lam. (Thomson, 1938a), and O viciifolia Scop. (Spedding and Diekmahns, 1972). The latter name (O. viciifolia Scop.) is now accepted as botanically correct.

In this thesis five different cultivars of sainfoin were used as experimental material. Two cultivars were selected from England (Eastern Counties Giant and Cotswold Common), latitude 52°N; one from the Causcasian region of the USSR (Krasnodar) latitude 45°N; one from Italy which is thought to have originated from regions around Florence (Italian), latitude 43°N; and finally one from Canada (Melrose), latitude 52°N. The Melrose cultivar was originally selected from plant material obtained from the Caucasian region (Hanna, pers. comm.).

To allow easier presentation, in this thesis each plant type will be referred to as a cultivar with names shortened to: Melrose, Italian, Giant, Krasnodar and Common.

Due to the low percentage germination in the cultivars used seed weight/1000 seeds has not been presented. It was decided that seed weight data would not be truly representative of each cultivars viable seed weight as many seed lots contained visibly non-viable withered seeds. However, the weight of milled seed/1000 is given by Percival (1936) and Mercer (1948) as 16 grams, the Official Seed Testing Station, Cambridge (UK) as 15.232 grams, and by Thomson (1951b) for Common as 15.69 grams and for Giant as 15.26 grams.

N.B. For comparison the 1000 seed weight for lucerne is 2.61 grams, red clover 1.77 grams and white clover 0.60 - 0.70 grams (Spedding and Diekmahns, 1972).

2.2 Growing conditions

2.2.1 Pot size

Plastic pots of sizes 5, 10, 13 and 15 centimetres were used. In addition large industrial food containers of volume 4800 cm^3 were adapted as pots; water release holes were drilled at the bottom of each pot.

Plate 2.1

Pot sizes used in this thesis. From left to right: 4800 cm^3 industrial containers, 15 cm (volume = 1400 cm^3), 13 cm, 10 cm (volume = 400 cm^3), and 5 cm. Length of ruler is 30 cm.

All pot sizes are shown in Plate 2.1.

2.2.2 Soil: Nutrient mixes used

For experiments in Chapters 3 to 11 a potting medium mix of 50:50 peat/perlite was used. For experiments in Chapters 12, 13 and 14 a mixture of 50:25:25 peat:perlite:pumice stone (grade B) was used. This latter mix allowed better drainage and aeration.

Fertilizer mixes added to the soil mix included:

(a) Modified Osmocote Nutrient Mix.

	mls./bushel of potting medium
Long term Osmocote granules (18N - 2.6P - 10K)	100
Superphosphate	70
Uramite	14
Dolomite Lime	35
Ground White Lime	400

pH = 6.2

For experiments in Chapters 12, 13 and 14 a trace element mix (Fetrilon Combi, Henry York Co. Ltd) mixed in fine perlite was added.

(b) Modified U.C. Nutrient Mix

	mls./bushel of potting medium
Superphosphate	75
Uramite	35
Dolomite Lime	18
Ground White Lime	18
Potassium nitrate	5
Potassium sulphate	5

pH = 6.5

(c) Modified Hoaglands Nutrient Solution (after Conger, 1964).

Stock Solution (gms/litre)		c.c. of stock solution used per litre of water (cc/litre)
115.1	$NH_4H_2PO_4$	1
101.1	KNO_3	6
164.1	$Ca(NO_3)_2$	4
120.4	$MgSO_4$	2

Minor element solution: use one cc/litre of final nutrient solution: 2.86 gms/litre H_3BO_3, 1.81 gms/litre $MnCl_2:4H_2O$, 0.22 gms/litre $ZnSO_4:7H_2O$, 0.08 gms/litre $CuSO_4:5H_2O$, 0.02 gms/litre $H_2MoO_4:H_2O$.

Preparation of iron salt: Dissolve 5 gms NaOH in 800 ml. distilled water, add 33.2 gms EDTA (tetra-sodium salt), stir until dissolved, add 24.9 gms $FeSO_4$, stir. Make up to 1000 ml. and aerate the solution overnight using an aquarium air pump. Use 2 ml. to 100 litres of water.

Hoaglands solution was used for experiments in Chapters 3 to 11 mainly as a source of trace elements. However, experiments in Chapters 7 and 8 only Hoaglands was used as the nutrient source.

2.2.3 Environmental conditions

All glasshouse experiments were carried out at the Massey University Botany Department's glasshouse which was heated so that the minimum temperature did not fall below 15°C. In mid-summer the afternoon temperature within the glasshouse reached 30 - 35°C, however, on very sunny days the maximum temperature was slightly higher than this.

Plants grown in the outside 'natural' environment were grown in pots in a fairly sheltered (from wind) area between the glasshouse and laboratory block.

Plants grown in the growth room and growth cabinets (Chapter 6, Appendix II) received light from 10 x 40 watt cool white fluorescent reflector tubes and 8 x 15 watt incandescent bulbs. Within the cabinets air was circulated and the sides were lined with Aluminum foil.

Watering was by hand (i.e. watering can or in the glasshouse with a sprinkler attached to the hose), or in the glasshouse by sub-irrigation for 30 minutes once or twice daily depending on the amount of water loss from pots.

For the best control of mites under glasshouse conditions a mixture of Yates' White Summer Oil (30 mls./litre) and Kelthane AP (Rohm and Haas, USA) (1 teaspoon/2 litres) was used. Sprinkling the foliage with water 30 minutes prior to spraying overcame the problem of leaf damage.

2.2.4 Temperature

The average outside daily maximum and minimum temperatures are provided below for 1976. Figures were obtained from the Massey

meteorological station. These figures are provided so that the reader has an indication of the seasonal variations in temperature experienced in Palmerston North.

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Av. Max.	20.6	18.9	21.2	18.0	14.3	11.8	11.9	13.2	14.1	15.9	17.0	19.9
Av. Min. (^{o}C)	13.7	10.1	11.0	9.5	6.2	4.4	4.5	7.0	6.9	7.7	8.5	13.1

In the growth room and growth cabinets the temperature fluctuated between 21 - 25^{o}C.

2.2.5 Light

The light intensity in the glasshouse was slightly lower than that in the natual environment outside. This is due to dust, dirt etc. on the glass plus an effect of the glass itself. Over the summer period the glasshouse roof and sides were white washed so that a greater control over temperature within the glasshouse could be obtained; this also decreased the light intensity. To give an indication of light intensity differences values are provided below. Each value was obtained at the same time on a clear sunny day.

	PAR (microeinsteins/m^2/sec.)
Outside	= 4100
In glasshouse	= 3000
In glasshouse with whitewashed walls and roof	= 2500

Plants receiving artificial short days were placed into 30 cm. deep boxes. At 4.30 p.m. a lid was placed on the box, over this was pinned a black fabric to ensure no light filtered through cracks, gaps etc. At 8.30 a.m. the lid and fabric covering were removed. Plants remained inside the boxes during the day (Plate 2.2).

In experiments receiving artificially extended long days special curtained off sections were set up in the glasshouse (Plate 2.3) with light being supplemented in both morning and evenings. At 8.30 a.m. curtains were opened (or in some cases lifted) and at 4.30 p.m. curtains were allowed to hang with joins pegged allowing no light to escape into

Plate 2.2

Short day cabinet with lid removed.

Plate 2.3

Curtained sections within the glasshouse for long day treatment. The above photograph shows the curtained section of the glasshouse along the full glasshouse bench; note how the curtains are secured during the day. The lower photograph shows the apparatus that was used for both 17 hour and 24 hour photoperiods.

areas of the glasshouse.

For experiments in Chapters 13 and 14 special photoperiod boxes were adapted from previously used dark boxes (Plate 2.4). Each box was partitioned inside with black plastic so as to give two compartments each of measurement 70 x 55 x height 70 cm. Inside each box were fitted two 40 watt incandescent tungsten filament lamps tied on to a bamboo stick nailed on top of each compartment. On each lamp an aluminum dish (20 cm. diameter) was used as a shade. All lights were controlled by timer clocks. Electricity passing through the lights in the photoperiod boxes passed through an isolating transformer.

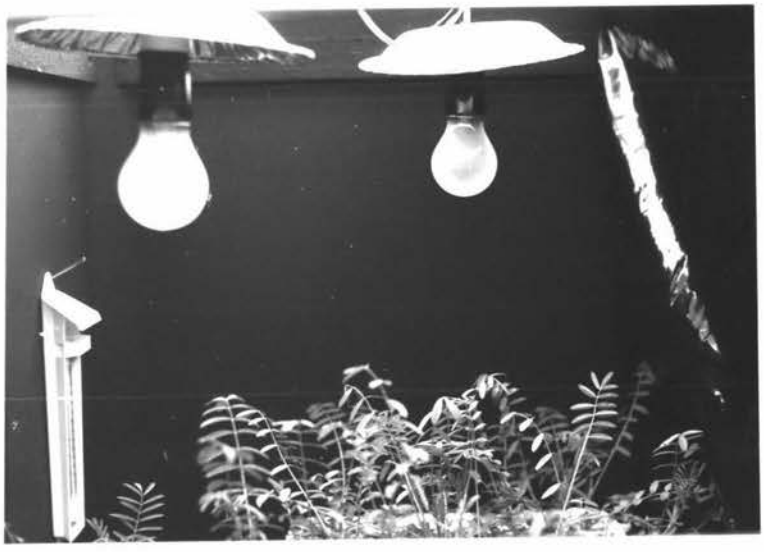
At 8.30 a.m. each day plants inside the photoperiod boxes were placed, depending on the experiment, into either natural outside conditions or onto the glasshouse bench. At 4.30 p.m. plants were replaced into the photoperiod boxes. In all photoperiod treatments in these boxes lights came on at 4.30 p.m. for the evening supplement and went off at 8.30 a.m. at the end of the morning supplement. For the photoperiod boxes within the glasshouse the bench area they occupied was also curtained off during the night. Due to the black paint within all boxes very little light was reflected out.

For all long day photoperiod treatments supplemented light has been expressed as photosynthetically active radiation (PAR). Values are expressed below.

Type of light supplement		PAR at plant height (Av.) (μeinsteins/m^2/sec)	Used for experiments in Chapters -
1.	100w ITFL* as shown in Plate 2.3a	120	8, 11, 12
2.	5 x 40w ITFL as shown in Plate 2.3b	100	6, 7
3.	2 x 150w BG Atlas Flood Lamps	450	9
4.	2 x 40w ITFL in Photoperiod box	90	13, 14
5.	Growth cabinets	320	Appendix II
6.	Natural sunny day in outside environment (summer)	4100	

* ITFL = incandescant tungsten filament lamps.

To provide an indication of light supplement variability PAR values are provided for various regions under the 100w lamps (plate 2.3a).



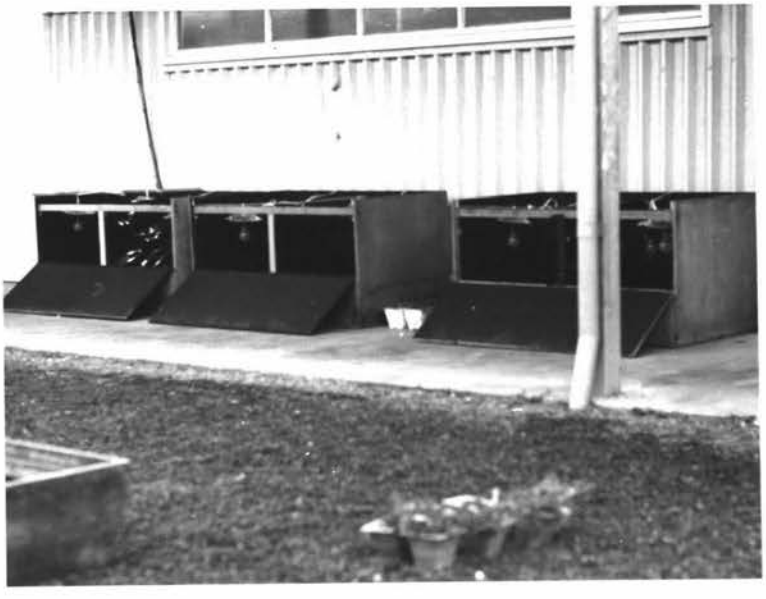


Plate 2.4

Photoperiod boxes. The upper photograph provides a view within an individual photoperiod box.

	PAR (μeinsteins/m^2/sec)
Directly below lamp at pot level	= 125
20 cm. above pot surface, directly below a lamp	= 150
Edges of bench furtherest away from lamps at pot surface level	= 50
A position mid-way between two lamps but at pot level	= 124

For an indication of the natural photoperiodic conditions at Palmerston North (latitude 40° 30' S) seasonal day length figures are provided in Figure 2.1. This Figure is often referred to when discussing results in the following chapters.

2.3 Plant Dissection

All plant dissections carried out in this thesis involved hand dissection with a Gillette sabre sterile disposable scalpel (shape E/11) using an Olympus sterioscopic 200m microscope, model III (magnification 7x to 40x).

2.4 Dry Matter Determinations

Procedure for dry matter determinations in Chapter 7 involved placing root and shoot samples into individual brown paper bags. These were then evenly distributed on three trays within the oven (Watvic Model, forced air circulation). At 5.00 p.m. the oven was turned on (100 $\pm$ 3°C) and left overnight. When the roots and shoots were weighed, beginning at 8.30 a.m., only small groups of root and shoot samples were removed from the oven at each time so as to avoid an atmospheric moisture gain effect. The same drying and weighing methods were used in Chapter 8 except 'pyrex' beakers and not paper bags were used.

2.5 Statistical Methods

To give an indication of variability standard errors of the means (SEM) values are provided. Where significance is noted the 5% level has been used. In many cases the 1% level of significance has been indicated (Student's T test, chi^2 test).

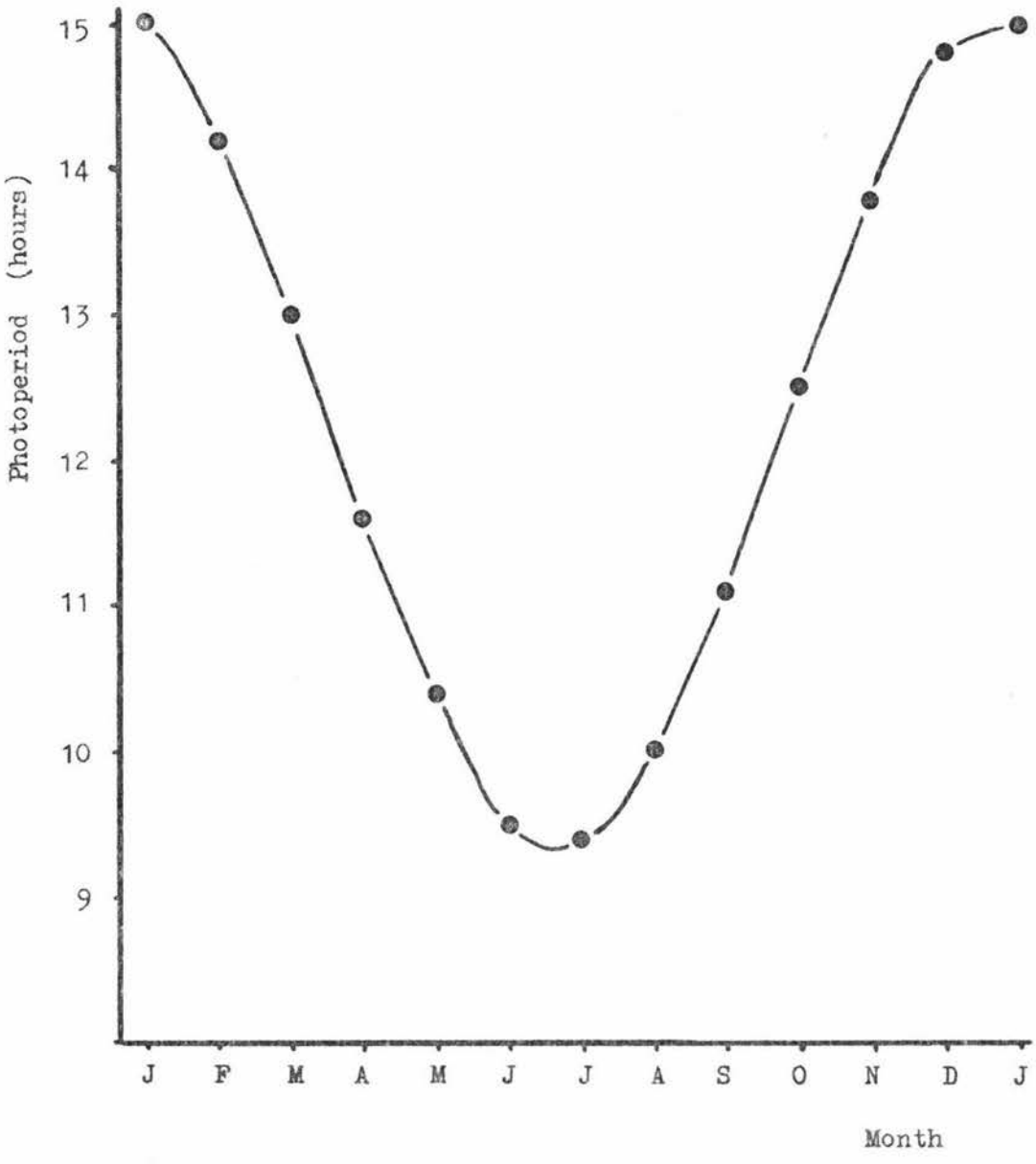


Figure 2.1

Graph to show seasonal variation in daylength for 40°S latitude. The latitude for Palmerston North, New Zealand is 40° 30'S.

N.B. Civil twilight has not been added.

SECTION I

ONTOGENY: SEEDLING GROWTH AND FLORAL DEVELOPMENT

Introduction

To understand fully any aspect of a plant under study one must be familiar with growth phenomena relating to that aspect. Thus when the flowering physiology of a plant species is examined an understanding of the plant's ontogeny from germination to anthesis is very important. This allows easier understanding of results as data can be interpreted with regard to the growth form and physiology of the whole plant.

Lack of familiarity with sainfoin necessitates a study of the pattern of vegetative development of the seedling plant and floral development of an 'adult' plant.

CHAPTER 3

SEEDLING GROWTH FORM

3.1 Introduction

Knowledge of the morphological development of sainfoin from seedling to maturity is important so as to allow a greater understanding of the plants overall physiology. Thomson (1938a), working in England with cultivars Giant and Common, was the first to describe sainfoin development in any detail. He noted that germination is epigeal, upon reaching the soil surface the cotyledons are thick and fleshy, kidney shaped, with a flat upper surface and a convex lower surface, turning dark green on exposure to light. Due to the very short internodes the first six foliage leaves all arose from the same region of the stem. The first leaf was usually simple, but a number of seedlings were found with two, three or four leaflets. In the majority of seedlings grown under spring environmental conditions outside, and in an unheated glasshouse, the second leaf was trifoliolate; third leaves with three or five leaflets were most frequent; the fourth leaf was usually similar to the third. From the fifth to the sixth leaf the number of leaflets per leaf varied reaching a maximum on some plants of fifteen. In Common sainfoin the internodes remained short throughout the growing season and new leaves continued to be formed on the short main stem. Soon after the formation of the sixth leaf basal buds became visible producing numerous leaves but remaining short. The internodes of Giant sainfoin plants elongated after the production of the sixth leaf. Elongation took place in the upper internodes; the first four to six, lowest, internodes remaining short. Basal buds developed in the axils of the lower leaves. The number of visible basal buds in three month old plants varied from one to sixteen in Giant and from two to twenty-one in Common, the most frequent number in the former being six and in the latter nine.

The aim of the present investigation was to compare the early morphological development of the Melrose cultivar to that found by Thomson (1938a) in Giant and Common. Thus an indication of conformity of variability was obtained. In addition this study was designed to provide more information on axillary bud development associated with plant growth and also to examine the initiation and growth of leaf primordia.

The results of this study provide an understanding of early plant growth thus enabling a more adequate understanding and interpretation of data obtained in future chapters.

3.2 Materials and Methods

On December 8 1975 Melrose seed was sown into 10 centimetre plastic pots containing an Osmocote soil/nutrient mix. Seedlings were grown in the glasshouse.

Beginning one week after seedling germination six plants per week were dissected until the eighth week of growth. Dissections involved a complete binocular microscopic examination of the growing regions of the plant. In addition some other vegetative parameters were measured.

The terminology associated with this type of study is often confused, with the reader unable to interpret exactly what the author is trying to say. Because of this a diagram, (Figure 3.1), has been provided to show clearly what I mean when reference is made to a particular structure.

3.3 Results

Axillary and basal bud development over the first eight weeks of seedling growth is shown in Figure 3.2 and 3.3. The first node at which active axillary bud development takes place is the node associated with the first leaf. By the eighth week 12 - 13 nodes on the primary stem have actively growing buds. Continued axillary bud development usually occurred first at node 2. (Figure 3.1).

Figure 3.2 shows that there is active axillary bud development in the axil of all visible leaves even in those that have senesced. In the group sampled at week eight on average the first to third leaves had senesced.

The first sign of bud development was the initiation of a primordium in the leaf axil. The next stage involved the initiation of a leaf primordium in a similar manner to that of the primary stem apex. This occurred on the next oldest node (i.e. node number four from the apex). Associated with the first axillary bud leaf primordium were two stipules. These stipules remain membraneous and often fully enclosed the basal bud up until the fourth to fifth leaf primordium had been initiated within it. Often the leaf primodium associated with these stipules

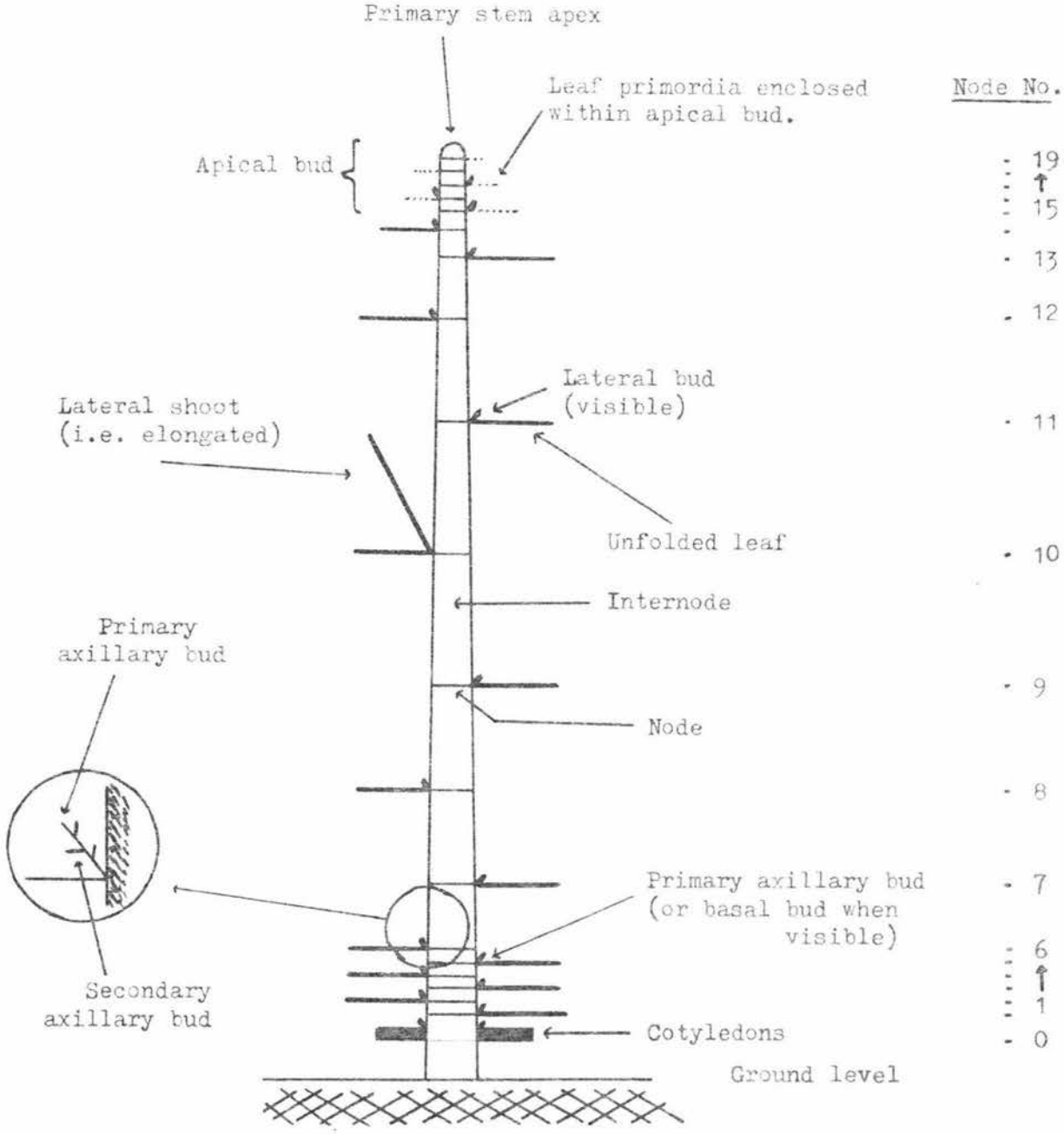


Figure 3.1

A diagram of sainfoin showing the various organs and illustrating the meaning of certain terms.

N.B. A secondary stem is defined as a basal bud with elongated internodes.

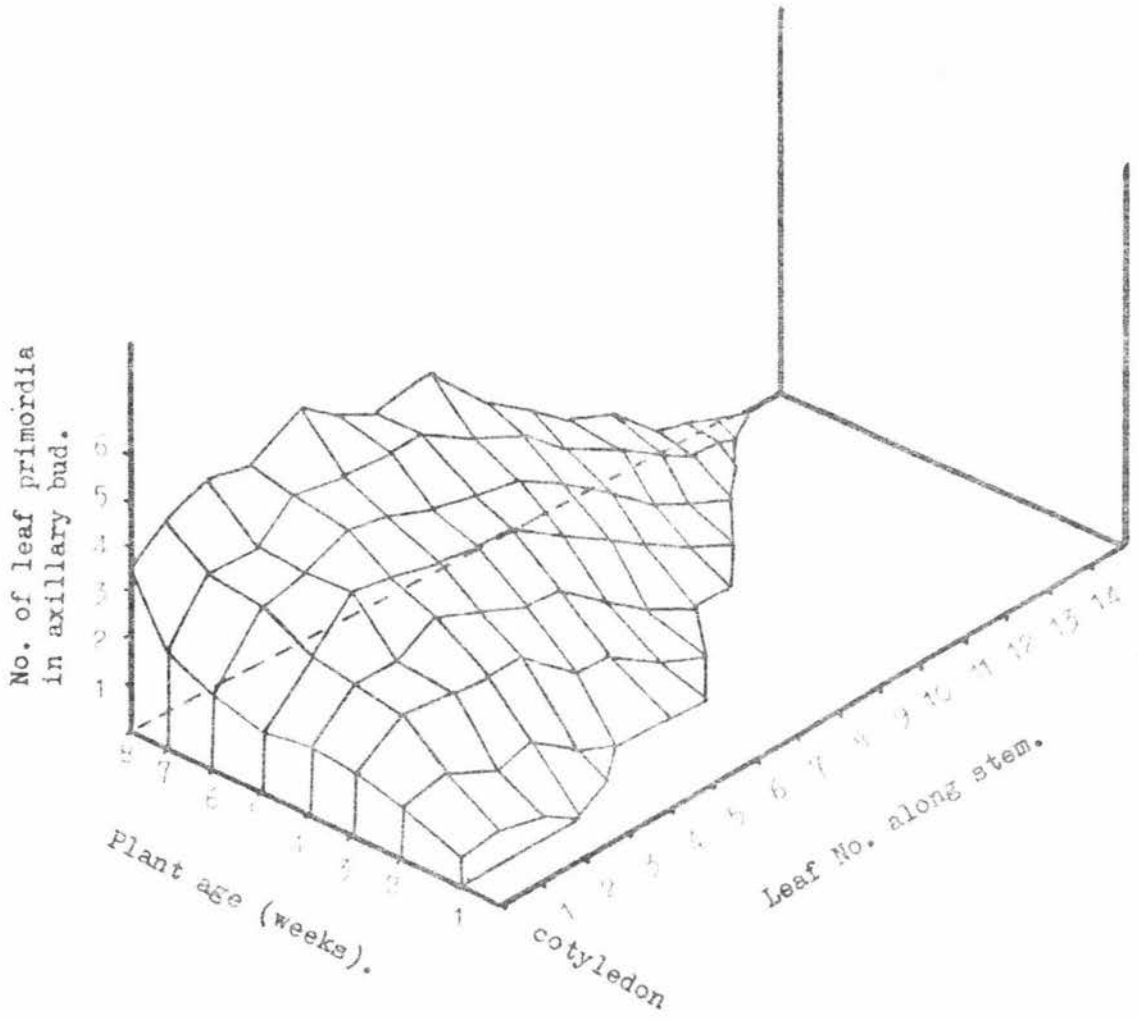


Figure 3.2

A diagram to show axillary bud development along the primary stem with increasing plant age.

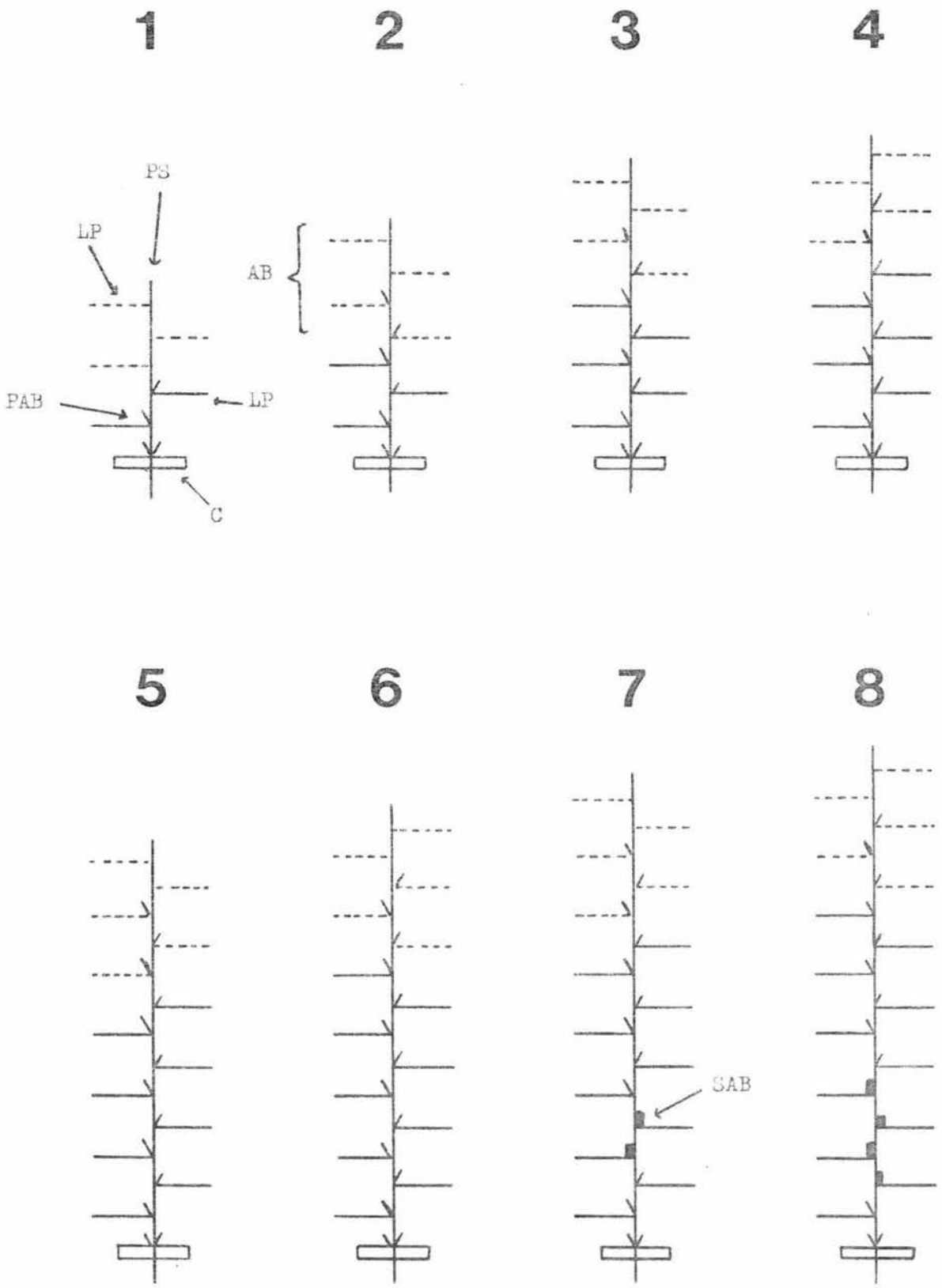


Figure 3.3

Diagram to illustrate the initiation and development of axillary buds in cv. Melrose. L = leaf, PAB = primary axillary bud, LP = young leaf primordium enclosed within the apical bud, PS = primary stem, C = cotyledon, SAB = secondary axillary buds on PAB, AB = leaf primordia enclosed in apical bud. NB. Numbers indicate plant age (weeks).

later decay leaving what appeared to be an axillary bud fully enclosed within this membraneous covering. The oldest leaf to emerge from within the axillary bud ruptured this covering. Just before the axillary bud becomes visible it extens the oldest leaf primordium out of the axil between the older leaf attached to the node, and the internode.

While dissections were being carried out secondary axillary bud development was also noted. These did not develop until the seventh week of seedling growth. These secondary buds occurred on the primary axillary buds on nodes three to seven but most frequently on the buds at nodes three and four. At no time did any of the secondary axillary buds become visible during the period of this experiment.

The total number of leaves and leaf primordia initiated on the primary stem is shown in Figure 3.4. There was a steady increase reaching an average of 15.5 $\pm$ 0.22 leaves and leaf primordia per plant by week eight.

The number of leaf primordia enclosed within the apical bud of the main stem is shown in Figure 3.5. There was a large increase between week one and two, with a smaller increase at week three. Following this there was a constant number of leaf primordia enclosed within the apical bud, although, in larger and more mature shoots it has been observed (from other dissections) that the apical bud often has six enclosed leaf primordia.

The average number of leaflets per leaf was noted (Figure 3.6). The first leaf initiated usually had one leaflet but sometimes there were three, the second leaf always having three leaflets. Older leaves had an increasing number of leaflets. When comparing plants, leaflet number was variable especially after node number 5 as shown in Figure 3.6.

Average maximum leaf length was also measured (Figure 3.7). When plotted against seedling age the line takes the form of an asymptotic curve with only small increases in leaf length between nodes one and two, and nodes seven and eight. Between nodes three and six a steady, but greater, increase in maximum leaf length occurred.

3.4 Discussion

In this Chapter it has been observed that the pattern of growth for Melrose is similar to that described by Thomson (1938a) for Giant and Common.

In the first three weeks of growth there was a faster rate of leaf initiation on the primary stem than leaf appearance (i.e.

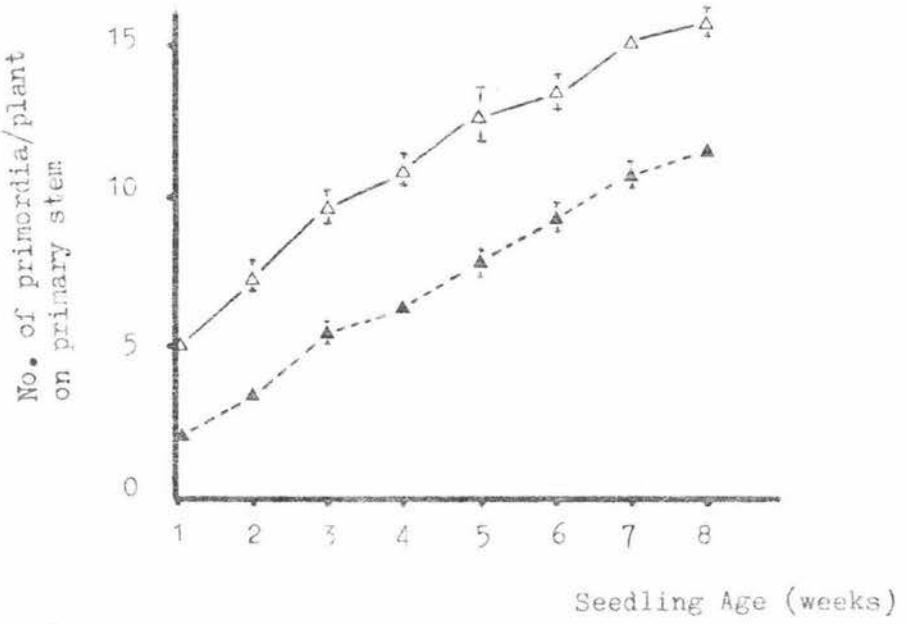


Figure 3.4

Average number of leaves and leaf primordia initiated on the primary stem per plant with increasing seedling age (solid line). Broken line refers to the number of unfolded leaf primordia on the primary stem. Vertical lines indicate 2x SEM.

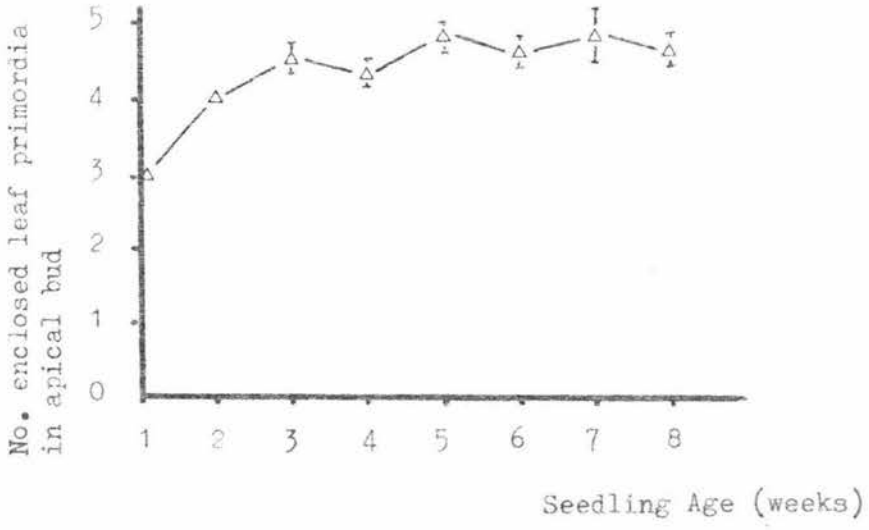


Figure 3.5

Number of enclosed leaf primordia in apical bud (i.e. attached to the primary stem) with increasing seedling age. Vertical lines indicate 2x SEM.

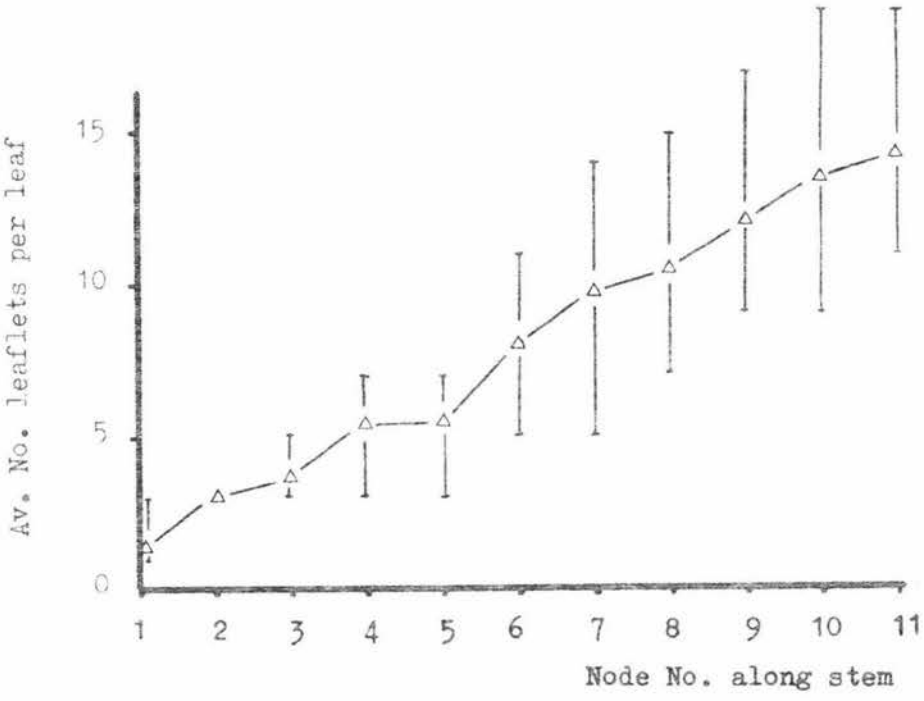


Figure 3.6

The average number of leaflets per leaf plotted against node number along stem. Vertical lines show range in leaflet number.

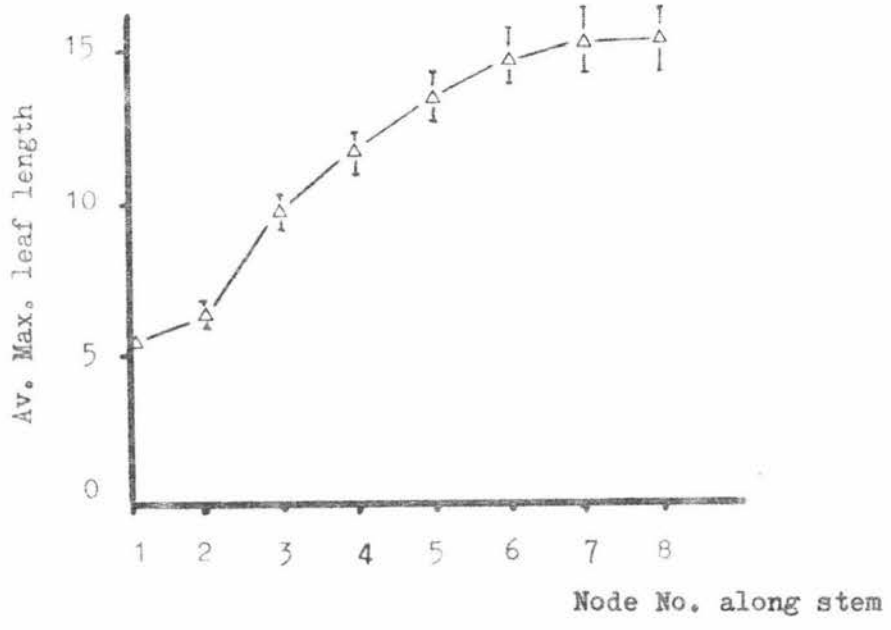


Figure 3.7

Average maximum leaf lengths plotted against node number along the stem. Vertical lines indicate 2x SEM.

unfolding and unfolded leaves) (Figure 3.4). This was due to an increase in the number of enclosed leaf primordia in the apical bud (Figure 3.5). After the third week of growth the rates of leaf initiation and leaf appearance, on the primary stem were similar (Figure 3.4).

The average number of leaflets per leaf showed a steady increase with increasing leaf number along the stem (Figure 3.6). When comparing this to Thomson's results for Common and Giant, Melrose had a similar number of leaflets per leaf on the first three leaves. Melrose leaves after this have more leaflets than do leaves for Common or Giant plants. The variability in leaflet number per leaf also increased with increaing leaf number along the stem. This is in agreement with the results obtained by Thomson (1938a). Thomson also visually observed that leaflet number could be correlated with leaflet area in Common and Giant. In Melrose the same trend occurs only for the first few leaves, in latter formed leaves leaflet size was more variable especially between different genotypes within the cultivar.

The smaller increase in leaf length between nodes number seven and eight (Figure 3.7) was due to (a) the fact that the leaves were beginning to reach their maximum size, and, (b) the leaf at node number eight may not have reached its maximum length when the measurements were obtained at week eight.

No plants that were dissected showed any internode elongation. In Giant Thomson (1938a) noted that internode elongation began after the appearance of the sixth leaf (i.e. comparable to a four to five week old Melrose seedling). In the first season of growth Common plants showed no internode elongation.

Axillary buds were initiated in an uniform manner beginning usually at the third node from the primary stem apex. Thomas (1962) observed a similar trend in white clover.

From examination of Figure 3.2 it is observed that after the fifth week of growth basal bud development in the axils of the node associated with the cotyledons greatly increased. It is interesting to note that it was just after the fourth week of growth that the cotyledons began to dry-up and senesce. From this correlation it appears that the cotyledons might exert an inhibitory influence on basal bud development in the axil between the cotyledon and the stem. Dostal (1967) observed a similar inhibitory effect in the garden pea (Pisum sativum) finding that the bud in the axil of an amputated cotyledon grows larger than

the opposite bud in the axil of a non-amputated cotyledon.

In the plants used in this experiment axillary buds became visible in some plants after the seventh week of growth. For Common and Giant plants Thomson (1938a) found that basal buds developed "..... soon after the appearance of the sixth leaf". For Melrose this corresponds to about the fourth to fifth week of growth (Figure 3.4). Thus in Melrose basal buds did not become visible until the plants are slightly older than either Giant or Common. However, the different environmental conditions experienced by the plants in these two studies may have partly contributed to this difference in rate of basal bud appearance.

Secondary axillary bud development occurred on nodes of the primary stem associated with active primary axillary bud development. These secondary buds began developing before the primary axillary buds became visible.

CHAPTER 4

FLORAL ONTOGENY

4.1 Introduction

The first visible sign of flowering in sainfoin occurs when a small inflorescence primordium is initiated in the axil of a leaf primordium at the stem apex. However, this inflorescence primordium is enclosed within developing leaf primordia; thus it is inconspicuous and cannot be observed except by microscropic examination. In sainfoin there can be up to six developing leaf primordia enclosed within the stem shoot hence a period of time is required before an inflorescence can be observed without performing a destructive analysis of the stem.

In some plants the development of the primordia to a mature inflorescence can be affected by environmental conditions, for example, temperature and sometimes photoperiod (Lang, 1965), and in some cases these conditions are different from those favourable for flower initiation. Thus differences in the time of appearance may reflect, at least partly, the action of these conditions and not the conditions determining initiation. Therefore, reliable information as to the time of occurrence of inflorescence initiation can only be obtained by dissection.

It is usual to measure inflorescence initiation by measuring later stages of inflorescence development (Lang, 1965). In some instances this method may not be the most useful. For example, Salisbury (1955) wanted to study the developmental condition of the first formed inflorescence of Xanthium after a definite period of time. In some of these time spans there was not sufficient time for a visible inflorescence to occur. To overcome this problem he developed a system of describing inflorescence bud development. This involved eight stages, the earliest of which could be determined by dissection. Thomas and Forde (1967) used a similar system to describe stages of inflorescence development in Lotus pedunculatus for recording results.

When a plant flowers it is usually at its maximum size, thus the problem of apace can arise especially when large populations are needed for each treatment. To overcome this a system can be developed for describing stages of inflorescence development (i.e. similar to Salisbury, 1955; and Thomas and Forde, 1967). Thus the experiment could be ended much earlier than would otherwise be possible.

The stage and score methods suffer basically from the same potential error as the time method, that is, they may reflect differences in the development of the initiated inflorescences rather than their initiation.

The aim of this present investigation was to describe the ontogeny of the inflorescence and flower of sainfoin. In addition the inflorescence developmental stages, as described in this Chapter, will be used throughout this thesis when discussing results.

4.2 Materials and Methods

Throughout this investigation cv. Melrose plants were used. Plant dissection methods have been described in Chapter 2.

In Table 4.1 measurements of inflorescence length and peduncle length are provided so as to indicate size differences between stages of development. For these two parameters a large amount of variation occurs between cultivars, genotypes within a cultivar, different environmental conditions and plant age. Measurements of inflorescence length does not include the peduncle length at any stages of development.

4.3 Ontogeny of the Inflorescence

The ontogeny of the inflorescence begins with the initiation of a reproductive growing point in the axil between the youngest leaf primordium and the vegetative apex (McLean and Ivimey-Cook, 1956; Aitken, 1960). When sainfoin plants receive strong inductive conditions after they have reached 'ripeness-to-flower', they produce inflorescence primordia on ten successive nodes by the time the first flower on the oldest inflorescence has turned pink and its petals have opened. Thus it was possible to describe ten stages of inflorescence development corresponding to those occurring at the ten successive flowering nodes immediately below the stem apex (Table 4.1, Plate 4.1).

Often some inflorescences aborted on the stem before reaching anthesis. Cessation of inflorescence development resulting in subsequent abortion was observed to occur mostly at stages 6, 7 and 8 (Tables 4.1, Plate 4.2). Inflorescences at stage 9 nearly always developed to anthesis. However, in some instances (see Chapter 5, Figure 5.10) florets on a stage 9 inflorescence would abort at the upper end of the of the inflorescence; those florets towards the lower half of the inflorescence usually developed to anthesis (Plate 4.3).

A.

B.

Plate 4.1

Stages of inflorescence development.

Photograph A. From left to right, inflorescence stage 10, 9, 8 (0.8x)

B. From left to right, inflorescence stage 7, 6, 5 (6x).

Plate 4.2

Aborted inflorescences.

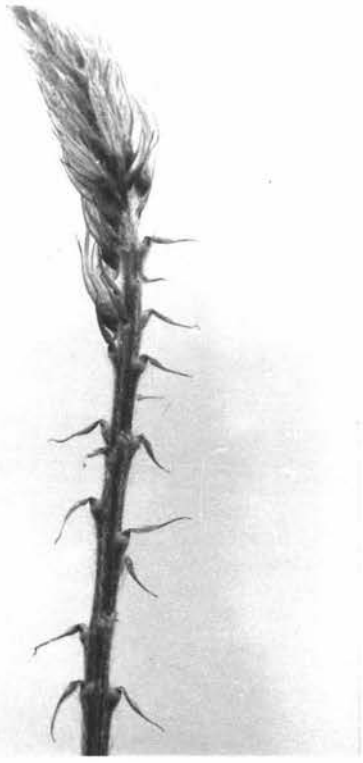


Plate 4.3

Partially aborted inflorescence. Lower bracts represent where mature florets have already abscised as pollination does not occur when plants are kept within the glasshouse. Note how the upper florets on the inflorescence have aborted before reaching maturity.

When an inflorescence showed signs of aborting the first areas to decay were in the carpel region of the oldest florets. Tissue decay usually progressed rapidly acropetally along the inflorescence. Just prior to decay aborting inflorescences lost their 'greenness' and became light yellow.

Table 4.1

Stages of Inflorescence Development

Stage 0: Vegetative. Height of apex is around 0.12 mm.

Stage 1: An inflorescence primordium is visible in the axil of the youngest leaf primordium. Both the inflorescence primordium and the associated leaf primordium are smaller than the main stem apex primordium. Surface of inflorescence primordium is smooth.

Length of inflorescence = 0.06 mm.

Stage 2: Inflorescence primordium is larger than the apex primordium. Bulges of future flower primordia are beginning to appear at the base of the inflorescence primordium. These floret primordia first appear on the side of the inflorescence furthest away from the main apical dome of the stem.

Length of inflorescence = 0.35 mm.

Stage 3: Three rings of floret primordia have been initiated at the base of the inflorescence primordium. A bract primordium at the base of the oldest floret primordia has been initiated. Peduncle is beginning to elongate raising the inflorescence away from the shoot.

Length of inflorescence = 0.5 mm.

Stage 4: Floret primordia cover approximately half of the inflorescence. The first formed bract primordia, each surrounding a developing floret, nearly equal the length of the inflorescence. The older bracts are becoming hairy.

Length of inflorescence = 0.8 - 0.9 mm.

Length of peduncle = 0.25 mm.

Stage 5: The young inflorescence is completely covered by bracts associated with the florets. Inflorescence is becoming very hairy. Inflorescence still has a smooth dome which is initiating floret primordia.

Length of inflorescence = 1.25 mm.
Length of peduncle = 0.5 mm.

Stage 6: The inflorescence is completely covered with developing florets and floret primordia. Inflorescence is emerging from the shoot thus becoming visible, without shoot dissection, for the first time.

Length of inflorescence = 2.0 mm.
Length of peduncle = 1.8 mm.

Stage 7: The inflorescence has now emerged from the enclosing stipules. The youngest floret primordia are surrounded by their bracts. The inflorescence does not have the appearance of being hairy; this is due to the fact that the distance between the hairs has increased while at the same time the hairs have not elongated any more.

Length of inflorescence = 10 mm.
Length of peduncle = 4 mm.

Stage 8: The inflorescence has been raised above the stem shoot by the elongating peduncle. The upper end of the inflorescence has a similar width to that of the lower end.

Length of inflorescence = 20 mm.
Length of peduncle = 80 mm.

Stage 9: The inflorescence has greatly enlarged in overall size. Basal florets are beginning to expand thus giving the inflorescence the shape of an arrow tip. The oldest florets' petals are beginning to become pink.

Inflorescence length = 35 mm.
Peduncle length = 150 mm.

Stage 10: The first formed floret has moved at right angles to the rachis; this has been caused by the growth of that floret's pedicel.

The petals are pink/red with the standard and keel clearly visible. Florets mature in an acroptel direction.

Throughout this thesis when a plant has reached this stage then it is defined as having blossomed.

Length of inflorescence = 50-60 mm.

Length of peduncle = 200 mm.

N.B. The final length of the inflorescence is determined by the number of florets on the inflorescence. This can vary between plants, and also between different inflorescences on the same plant. In addition peduncle length can also vary.

To provide the reader with a more clearer indication of when the main periods of inflorescence and peduncle growth occurred respective figures presented in Table 4.1 have been diagramatically represented in Figure 4.1.

4.4 Ontogeny of an individual Floret

The ontogeny of a floret on an inflorescence begins with a swelling on the lower end of an inflorescence primordium. This swelling always occurs on that side of the inflorescence primordium furthest away from the main stem spex. To describe the development of the floret, twelve main stages have been described (Table 4.2). In addition to this each stage has a bracketed number which corresponds to the stage earliest at which that floret would occur on an inflorescence in Table 4.1.

Table 4.2

Stages of Floret Development

Stage 0(1) Inflorescence primordium is dome shaped. There is no visible sign of floret primodia.

Stage 1(2) At the base of the inflorescence primordium floret primordia are clearly distinguishable.

Stage 2(3) A bract primordium is developing at the base of each floret primordium. Floret primordia are developing on the inflorescence primordium in an acropetal direction.

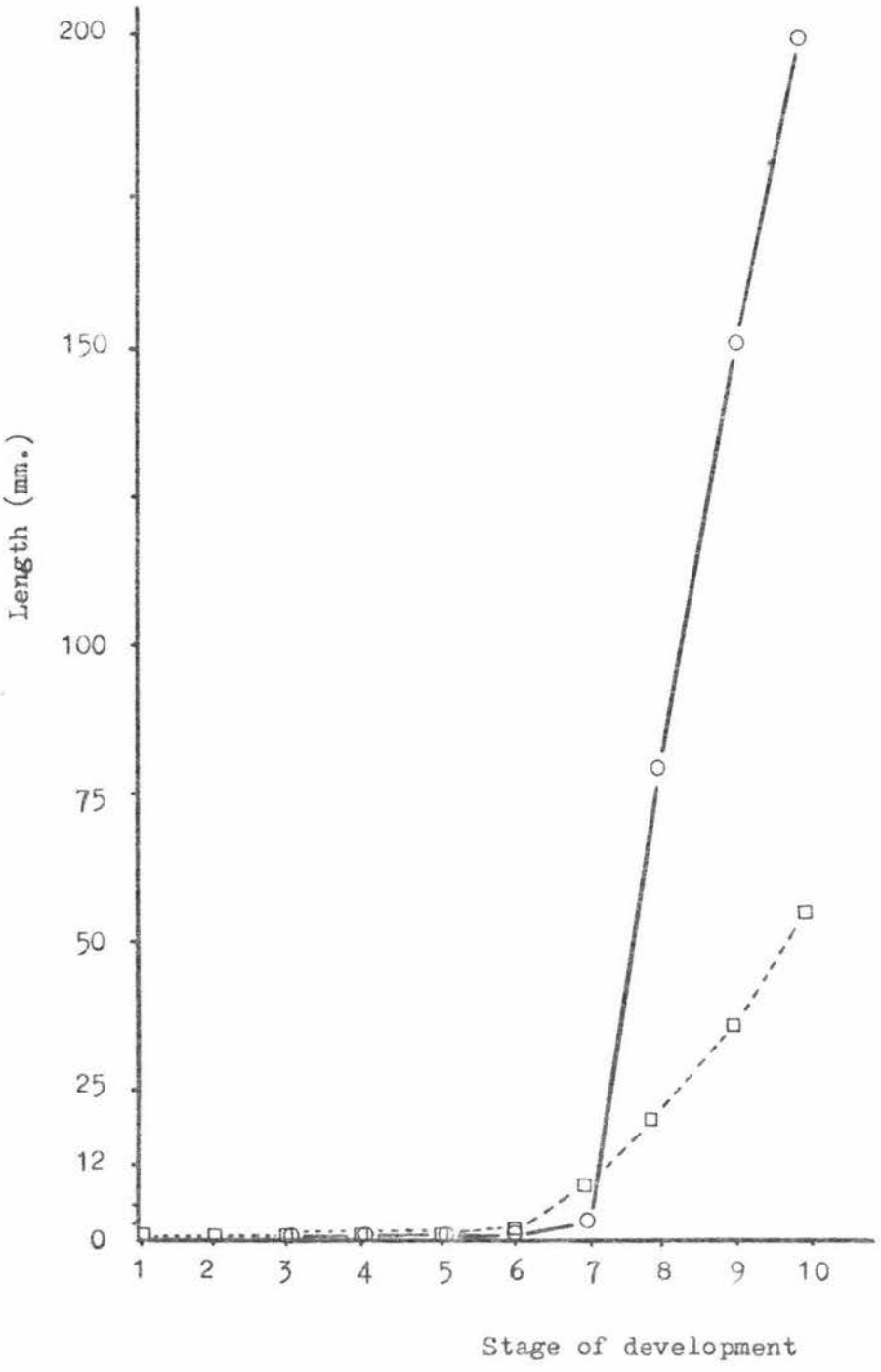


Figure 4.1

A diagram to show inflorescence length (broken lines) and peduncle length (solid lines) on ten successive nodes for a flowering stem of sainfoin. Each node corresponds to a developmental stage as described in Table 4.1.

Stage 3(4) Due to a greater growth in length the oldest bracts have completely enclosed each floret primordium against the inflorescence 'rachis'. At the base of each floret primordia one sepal primordium has been initiated.

Stage 4(4) Inbetween the sepal primordia and the apex of the floret primodium (in future this will be renamed the carpel primordium) are visible ten very small stamen primordia. The sepal primordia are beginning to take shape becoming flattish on their posterior and anterior sides.

Stage 5(4) The carpel primordium has developed into a carpel shaped organ with an elongated style and expanded ovary. The sepals nearly fully enclose the carpel. On the inside base of the sepals, small petal primordia are just discernible.

Stage 6(4, 5) Five stamen primordia have developed into a green coloured anther and filament. The other five stamen primordia are still distinguishable as hemispheres.

Stage 7(5, 6) All ten stamen primordia have developed an anther and filament. The anthers are taking on their mature shape. Sepals and petals are further developing, with the sepals much larger than the petals.

Stage 8(7) Anthers still green in colour. There still remain five filaments longer than the rest.

Stage 9(8) Anthers have turned yellow. Standard and keel petals are green and macroscopically visible. Stigma is clearly visible.

Stage 10(9) The 'tube' of filaments surrounding the style is beginning to elongate.

Stage 11(9) Sepals are now shorter than the petals. The first sign of petal colour is beginning to appear. Pedicel is developing in size and length.

Stage 12(10) Petals have become pink/red in colour (depending on genotype of plant), and have opened. However, the two wing petals have remained reduced in size (length 2 mm. compared to standard petal 12 mm.); they are however, still pink in colour. At this stage the stamens are clearly diadelphous, 9 being fused to form the incompletely closed tube and 1 remaining free. The floret axis has moved at right angles to the rachis; this movement has been caused by the growth of the pedicel.

SECTION II

GROWTH AND DEVELOPMENT UNDER WARM GLASSHOUSE ENVIRONMENTAL CONDITIONS.

In this section various aspects of growth and development are examined under glasshouse environmental conditions.

In Chapter 5 growth and development of five cultivars of sainfoin (Melrose, Giant, Italian, Krasnodar and Common) are examined under natural photoperiods. Detailed data were obtained and comparisons made between sainfoin cultivars and other closely related forage and pasture legumes.

In Chapter 6 the effect of plant age on flowering in Melrose is examined to determine if a juvenile phase is present in this cultivar.

In previous investigations it has been observed that when sainfoin plants are grown in small pots in long days fewer plants had elongated stems and fewer plants flowered when compared to plants that had been repotted into larger pots when transferred to long days. In Chapter 7 data are presented confirming this earlier observation of the effect of pot size on reproductive development. Experiments in Chapter 8 examine this pot size effect in more detail.

CHAPTER 5

A COMPARATIVE STUDY OF GROWTH AND REPRODUCTIVE DEVELOPMENT OF FIVE CULTIVARS IN RELATION TO SEASONAL CHANGES IN ENVIRONMENT

5.1 Introduction

The most quoted difference found in the literature between cultivars and strains of sainfoin is the ability to flower in the first or second year of growth. For example, Giant sainfoin flowers during its establishment year, whereas Common sainfoin does not flower until its second spring season of growth (Spedding and Diekmahns 1972, Baker *et al* 1952, and Thomson 1938, 1951).

The ability of sainfoin to regrow and flower twice or more in one season is also another difference between cultivars previously noted. Cooper (1972) described cv. Eski as a "one cut" cultivar and cv. Remont as a rapid regrowth "two cut" sainfoin. Baker *et al* (1952) noted that Giant sainfoin gives two and sometimes three flowering cuts for one season, whereas Common flowers only once a season.

Studies of ecotypic variation within legume and grassland vegetation indicate that climatic selection has yielded northern latitudinal communities with individuals that can grow and mature under long daylengths and short frost free periods. Progressively southward are communites containing individuals that mature under shorter daylengths and longer frost free periods. Ludwig *et al* (1953) showed that various strains of *Trifolium pratense* L. had different critical photoperiods for flowering. Some strains could flower normally in a 14 hour photoperiod whereas other strains required a 16 hour photoperiod. Williams (1945) noted two main forms of *Trifolium pratense*, an early and a late flowering form. This author showed that red clover, according to flowering time, could be distributed geographically according to latitude. Early flowering strains occur approximately south of 50 degrees N latitude. North of about 60 degrees N latitude the late flowering types are more prevalent whilst in the areas between 50 to 60 degrees N latitudes e.g. Britain, both early and late flowering red clovers are grown.

A low temperature requirement may also affect the time when flowering can occur. Leffel and Graham (1966) found one strain of *Trifolium pratense* that required vernalizing before it would flower. McKee *et al* (1972) found that crownvetch genotypes and cultivars differ in their

need for cold temperature pretreatment.

Often a photoperiod x temperature interaction may occur. For example, Knight and Hollowell (1958) (Trifolium incarnatum), Kasperbauer et al (1962) (Melilotus sp.), Clarkson and Russell (1975) (Medicago sp.) and Morley (1961) (Trifolium subterraneum) all noted that cold temperature pretreatment enabled plants to flower at a shorter photoperiod.

In addition to reproductive variation within species, variation in vegetative parameters can also occur between strains and cultivars of the same species. For example, Laude (1958) noted that different strains of lucerne (Medicago sativa) varied from those having a short and spreading growth habit to others being tall and upright.

As already noted variation within a plant species is very common. To describe the variation found in sainfoin five cultivars were selected on the basis of their climatic and latitudinal origins. Seed availability placed some limitations on final selections.

In addition this experiment was designed so that a comparative study of the developmental biology of the five cultivars under natural photoperiods in the glasshouse at Palmerston North could be made.

5.2 Materials and Methods

Five cultivars were selected (Giant, Common, Melrose, Krasnodar and Italian). Thirty seeds per cultivar were sown on September 2 1975 into 10 centimetre plastic pots containing a U.C. soil/nutrient mix (Chapter 2). Any seeds that failed to germinate were replaced with control spare seedlings. On November 18 1975 all plants were repotted into larger 15 centimetre pots, this time using an Osmocote soil/nutrient mix (Chapter 2).

At the beginning of November 1975 all plants received a 20 ml. dose of Hoagland's solution as measured with a MSD plastic cattle drenching gun.

As the plants increased in size the spacing between pots was increased to reduce any competitive effects, especially shading.

In some cultivars a few of the plants died thus slightly reducing the total.

(a) Leaf Angle Measurements

Nov. 4 1975 leaf angle measurements on individual plant's were measured for the first time. Each plants mean leaf angle was scored on a visual scale of one to five (Plate 5.1). Leaf angle measurements were obtained at 14 day intervals.

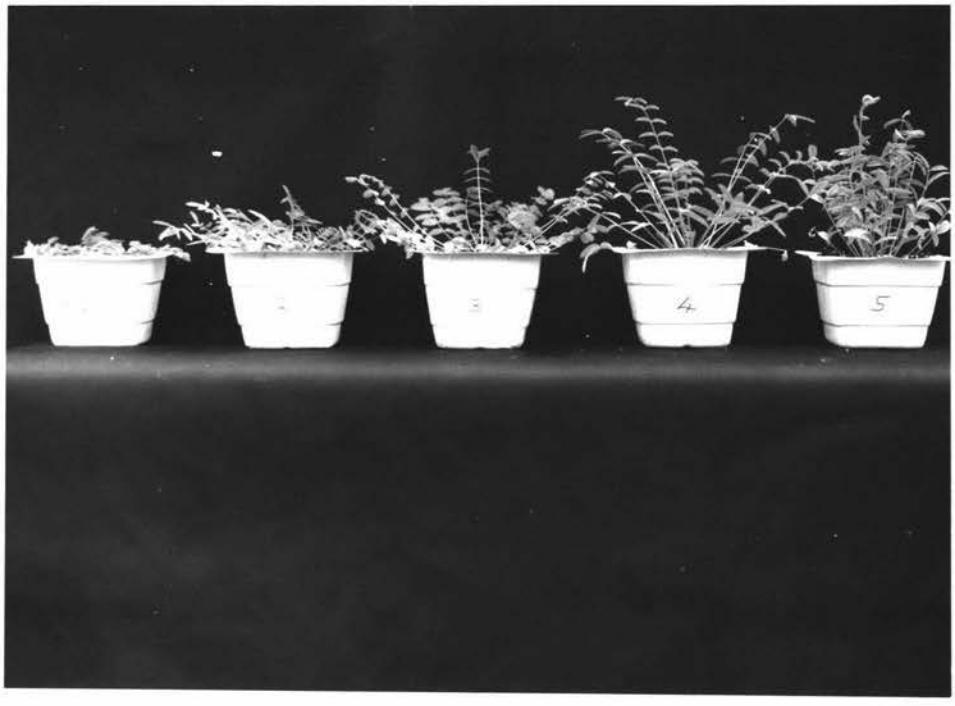


Plate 5.1

Representative plants of the leaf angle classes used for measuring leaf orientation. From left to right: Score 1, 2, 3, 4, 5.

Three observers were used to decrease any personal error that could be involved in any visual technique such as this. For each measurement five plants representing each leaf angle scale were used as indicators. These five plants were chosen on the basis of their very close resemblance to plants in the photograph (Plate 5.1).

During the Christmas-New Year period (1975-76) two measurements were made on Common. These were only scored by one observer - myself. From my previous scoring observations I tended to slightly overscore in comparison with the mean by approximately +0.1 to +0.2.

An extra two counts were made on Common as there was no stem elongation within the population being studied. Counts on the other four cultivars were discontinued when shoot elongation began.

(b) Bud Swelling

Beginning on November 18 1975 individual plants were scored for bud swelling. For this and the following parameters data were collected by me only. Reference to page 34 will help the reader interested what is meant by bud swelling.

(c) Stem Elongation and Flowering

Beginning on November 18 1975 individual plants were scored for stem elongation; again data were collected over 14 day intervals. Stem elongation was defined as an elongating shoot having one internode that was visually longer than three millimetres.

All flowering data were collected as defined in Chapter 2.

(d) Associated Vegetative Development

Counting the number of visible basal buds per plant began when the plants were 60 days old (November 18 1975) and was continued, at 14 day intervals, until the trial was ended on January 31 1976. At the first count basal buds would have been mainly primary basal buds; however, towards the end of the trial many secondary and tertiary basal buds were also present. No distinction was made between these bud types when data were recorded.

In all cultivars two counts were made on rate of leaf emergence. On December 2 1975 plants were tagged on the youngest leaf of a shoot that could hold a tag (i.e. an unfolding leaf). Two shoots per plant were tagged; a basal bud and the primary shoot bud. Some plants tagged had shoots with elongating stems. After 20 days the number of new leaves emerged was counted on each tagged shoot.

On December 22 1975 all plants were retagged so that another measurement could be obtained, again after 20 days.

For each cultivar three leaf parameters were measured on December 10 1975, these included; total leaf length, petiole length, and largest leaflet width. Leaf length was defined as the total petiole length including the rachis to which the leaflets were attached; the petiole was defined as the distance from the stem node to the first pair of leaflets. Leaflet width was defined as the distance between the leaflet tips of two opposing leaflets.

5.3 Results

(a) Leaf Angle Measurements

For each of the five cultivars the first response to the changing environment was an increase in the angle of the plants' leaves (Figure 5.1, 5.2). Associated with this was an increase in the length of the leaves; extension occurred both in the petiole region and the regions between leaflets. Individual leaves, including older as well as newly initiated ones, moved to a more erect position.

Figure 5.1 shows this increase in leaf angle which was especially apparent in Common, Giant and Melrose. When leaf measurements were first made Krasnodar, Italian, Giant and Melrose had higher mean leaf angle scores than Common.

For Common (Figure 5.1) the mean leaf angle increased, beginning on December 2 1975, then by mid-January 1976 the mean leaf angle began to decrease. It was noted that at the completion of this experiment the mean leaf angle of the Common population had continued to decrease. By mid-March 1976 all Common plants were, again, wholly prostrate.

A clearer picture of variability within each population is shown in Figure 5.2. These graphs show how Melrose, Giant, Italian and Krasnodar changed from an initial score class of mainly 2, 3 and 4; by December 16 1975 most plants were in either score class 3, 4 or 5. For Common, score class variability increased with time. The frequency in each group with score 5 greatly increased from graph 1 to graph 4.

(b) Bud Swelling

Bud swelling occurred rapidly following its onset and within approximately three weeks (November 18 to December 10 1975) nearly all Melrose, Giant, Krasnodar and Italian plants had swollen buds (Figure 5.3). It was not until 14 days later that the same response occurred in Common.

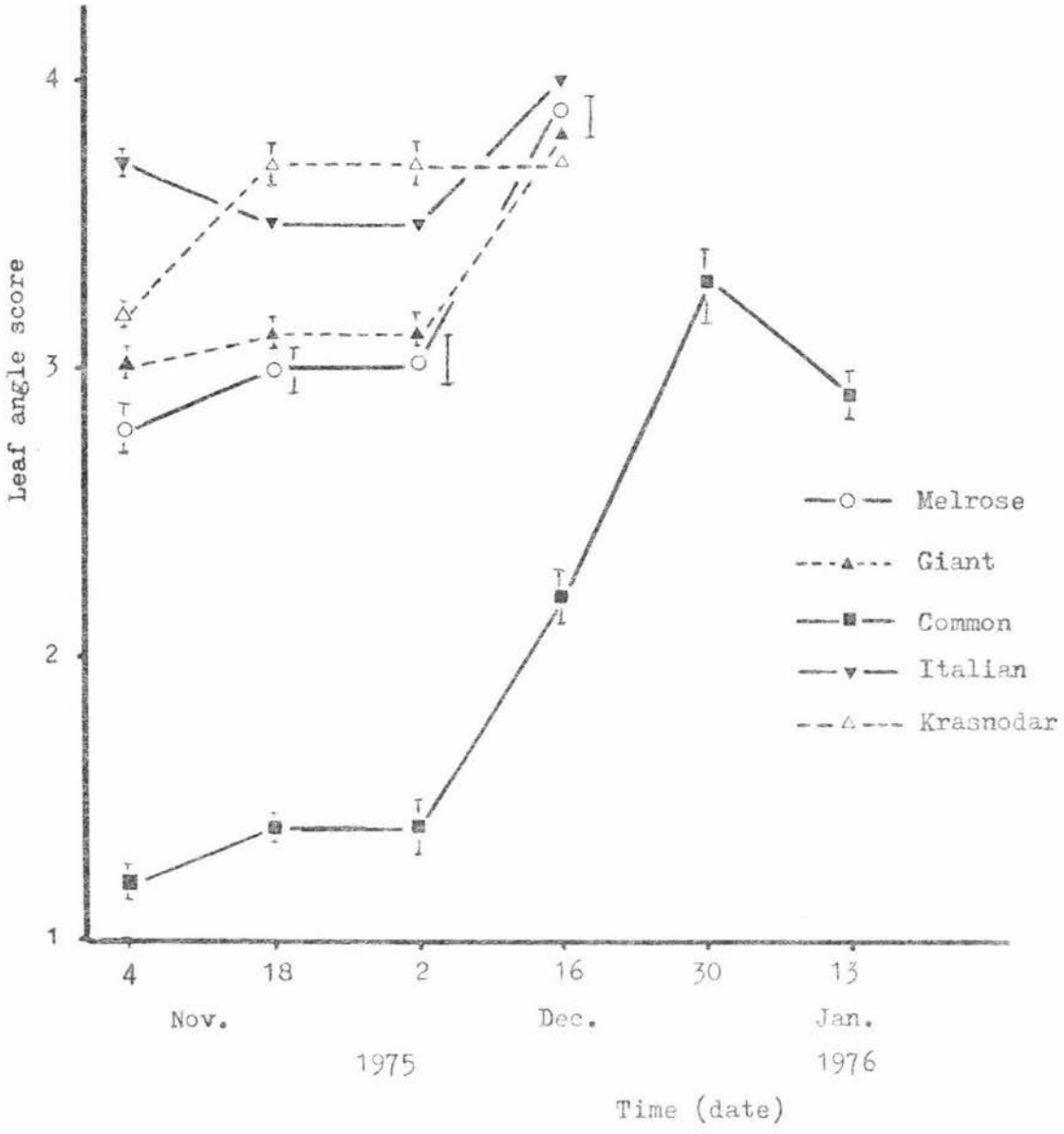


Figure 5.1

Change in average leaf angle per cultivar with time for Melrose, Giant, Italian, Krasnodar and Common. Vertical lines indicate SEM values.

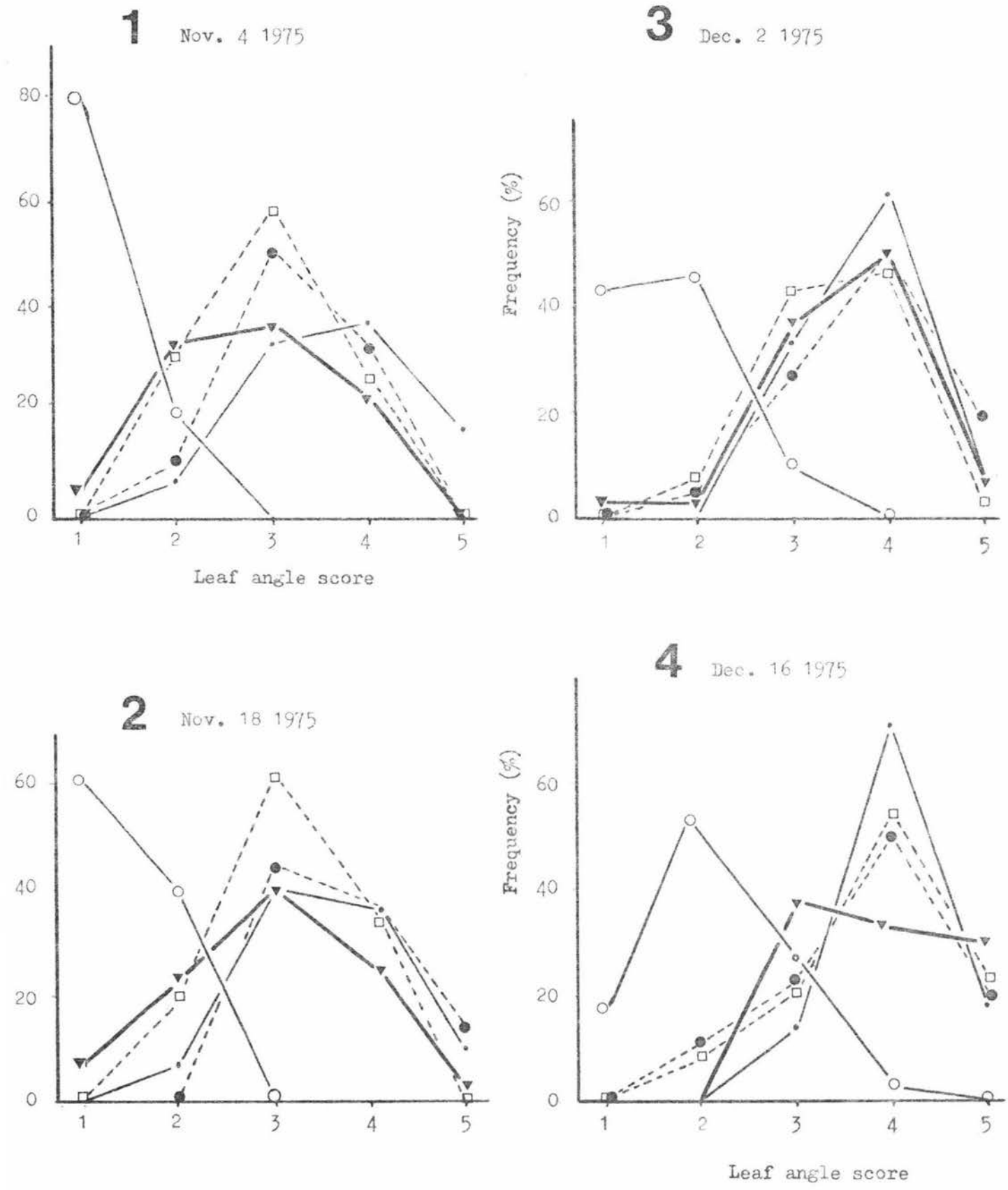
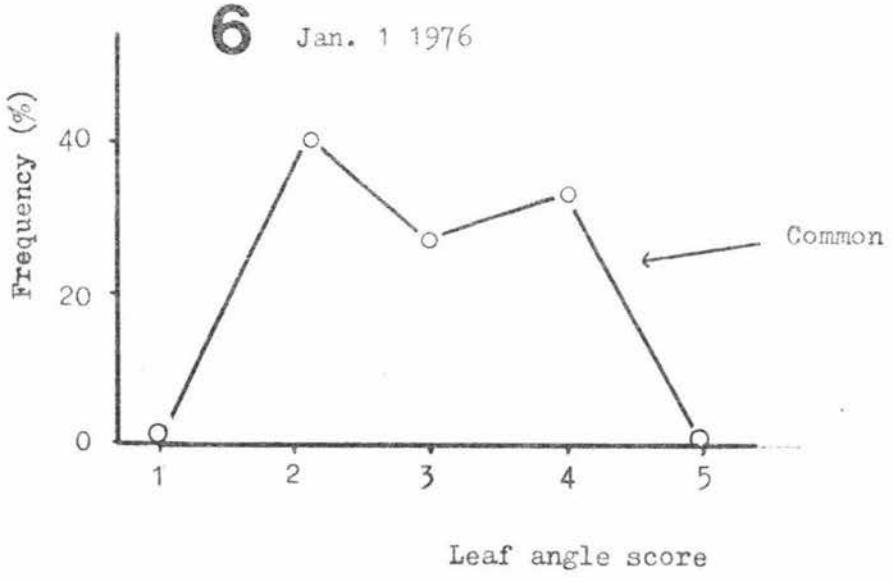


Figure 5.2

Variation in leaf angle at six observation times for ▼—▼ Melrose, □····□ Giant, •—• Italian, ●····● Krasnodar and ○—○ Common.

(Continued Overleaf)


5
Dec. 30 1975
60
40
20
0
Frequency (%)
Common
1
2
3
4
5
Leaf angle score


6
Jan. 1 1976
40
20
0
Frequency (%)
Common
1
2
3
4
5
Leaf angle score

The rate of increase in bud swelling was similar for all cultivars over their respective main periods of swelling (Figure 5.3).

(c) Stem Elongation

In cv. Common there was no stem elongation within the period of this experiment. Therefore in this section discussions will be centred only on the other four cultivars.

In Melrose, Giant, Italian and Krasnodar the primary shoot bud and basal buds elongated to produce primary and secondary stems respectively (Figure 5.4). The percentage of plants with elongating secondary stems was always greater than the percentage with elongating primary stems. For example, in the Melrose population 83% of the plants had secondary stem elongation whereas only 43% had primary stem elongation. In addition to this, comparison of plants within each population showed that secondary stem elongation tended to occur before primary stem elongation.

With the exception of one Melrose plant, all plants in each cultivar population that had primary stem elongation also had secondary stem elongation.

For all cultivars the main period of stem elongation began at about the same date (Figure 5.4). This was especially so for percentage secondary stem elongation.

(d) Flowering

Blossoming occurred in four of the cultivars, though in Krasnodar only 8% of the plants blossomed. In Common no plants flowered (Table 5.1). Within both Giant and Italian populations some plants had all their macroscopically visible inflorescences aborted. Thus these two cultivars had a higher figure for percentage inflorescence initiation when compared to the percentage of plants with blossoming inflorescences.

The main period of blossoming for the three flowering cultivars (Melrose, Giant, Italian) was towards the end of December (Figure 5.5). Italian and Giant were overall slightly earlier to blossom than Melrose. The two plants that blossomed within the Krasnodar population were much later (mid-January). Melrose, Giant and Italian all had a few very late blossoming plants (Figure 5.5).

Within the main flowering period for Melrose, Giant and Italian there were two main pulses of blossoming. These two pulses were related to the average number of sunshine hours per day over the blossoming period (Figure 5.6). This trend may have been exaggerated due to the rather unusual cool summer of 1975/76.

Corolla colour was noticeably different in some plants. This varied from pink to a deep red with prominent scarlet veins in the petals.

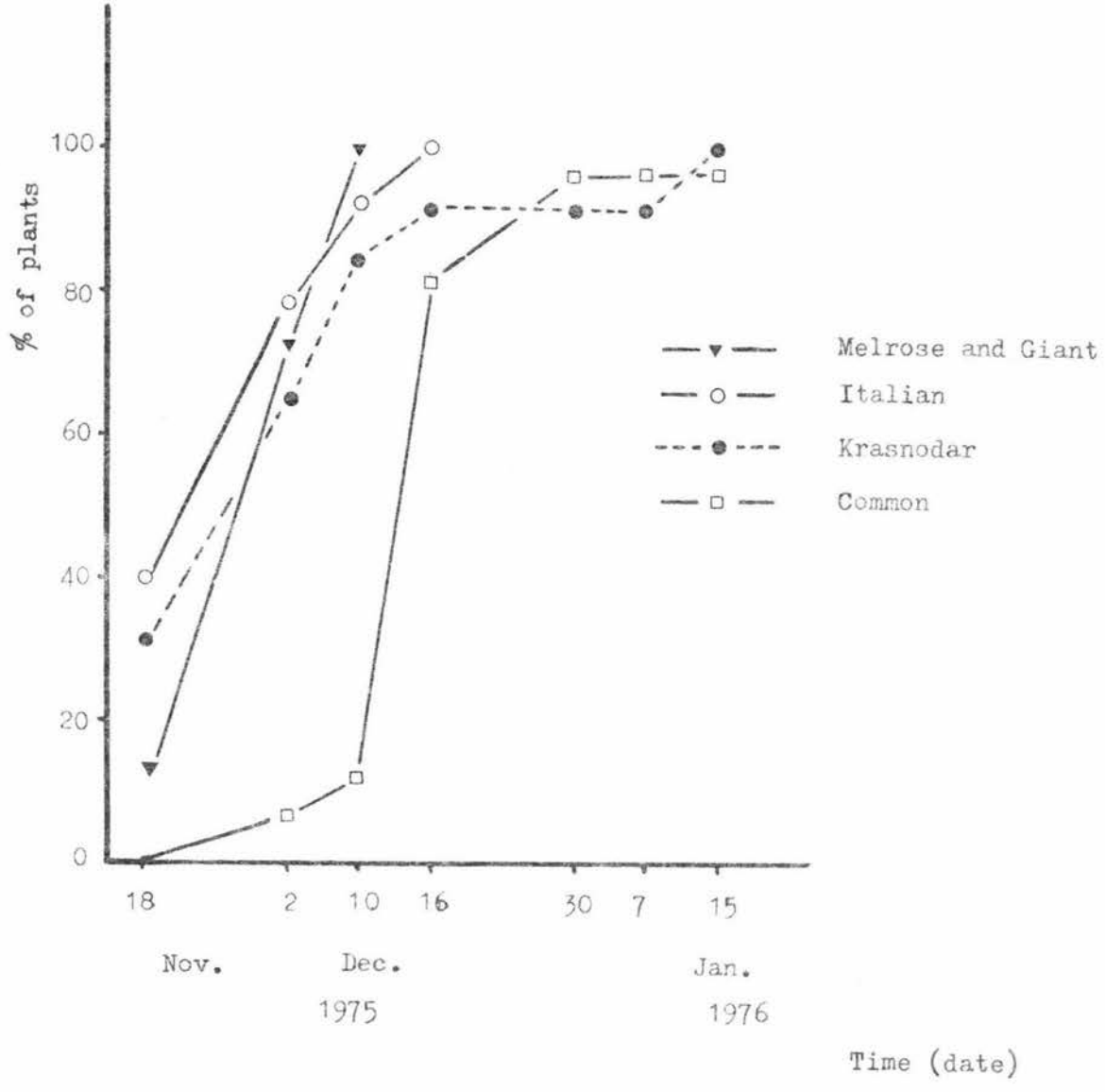


Figure 5.3

Percentage of plants with bud swelling for Melrose, Giant, Italian, Krasnodar and Common.

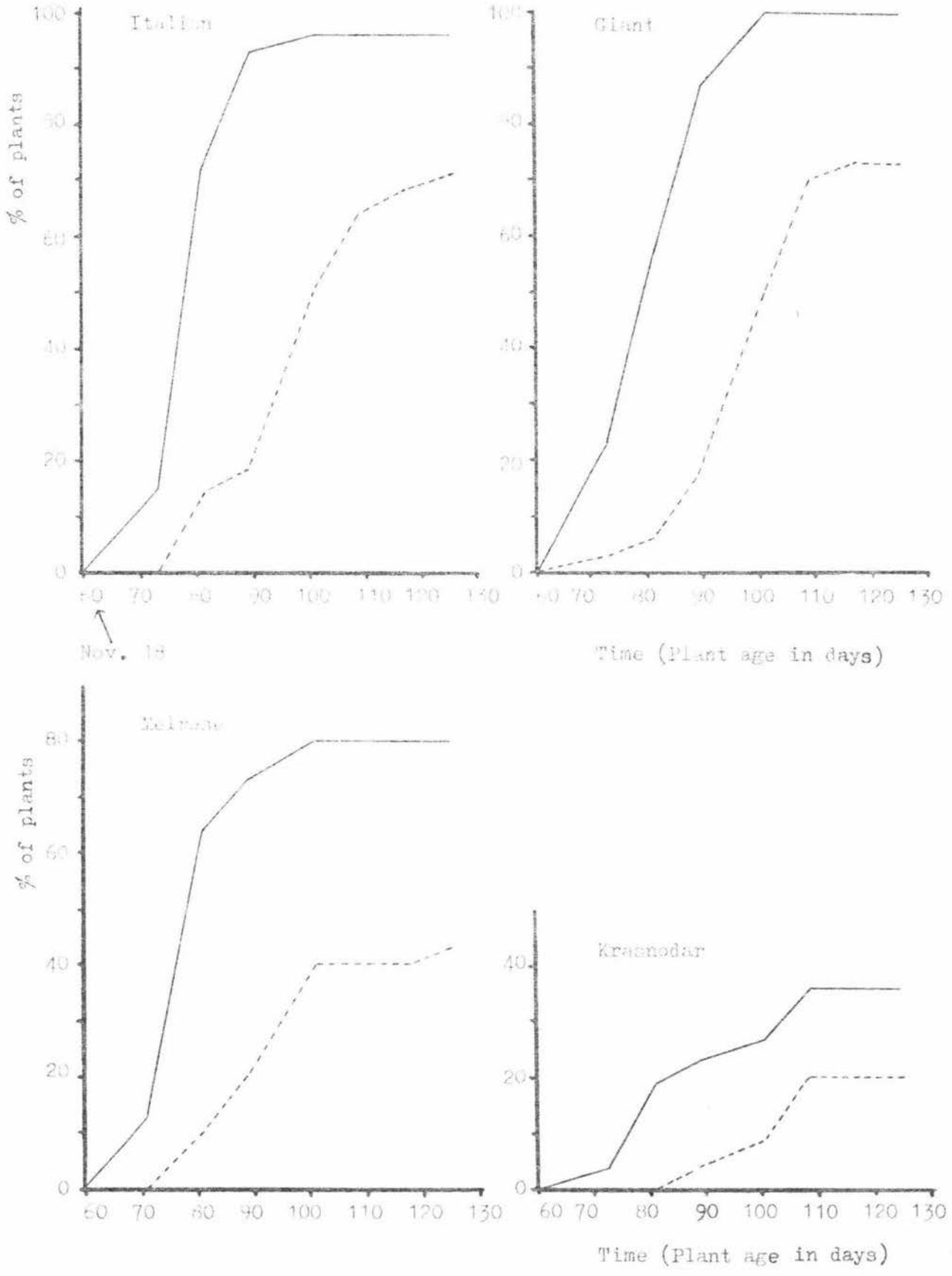


Figure 5.4

Percentage of plants with elongating primary stems (broken line) and secondary stems (solid line) for Melrose, Giant, Italian and Krasnodar.

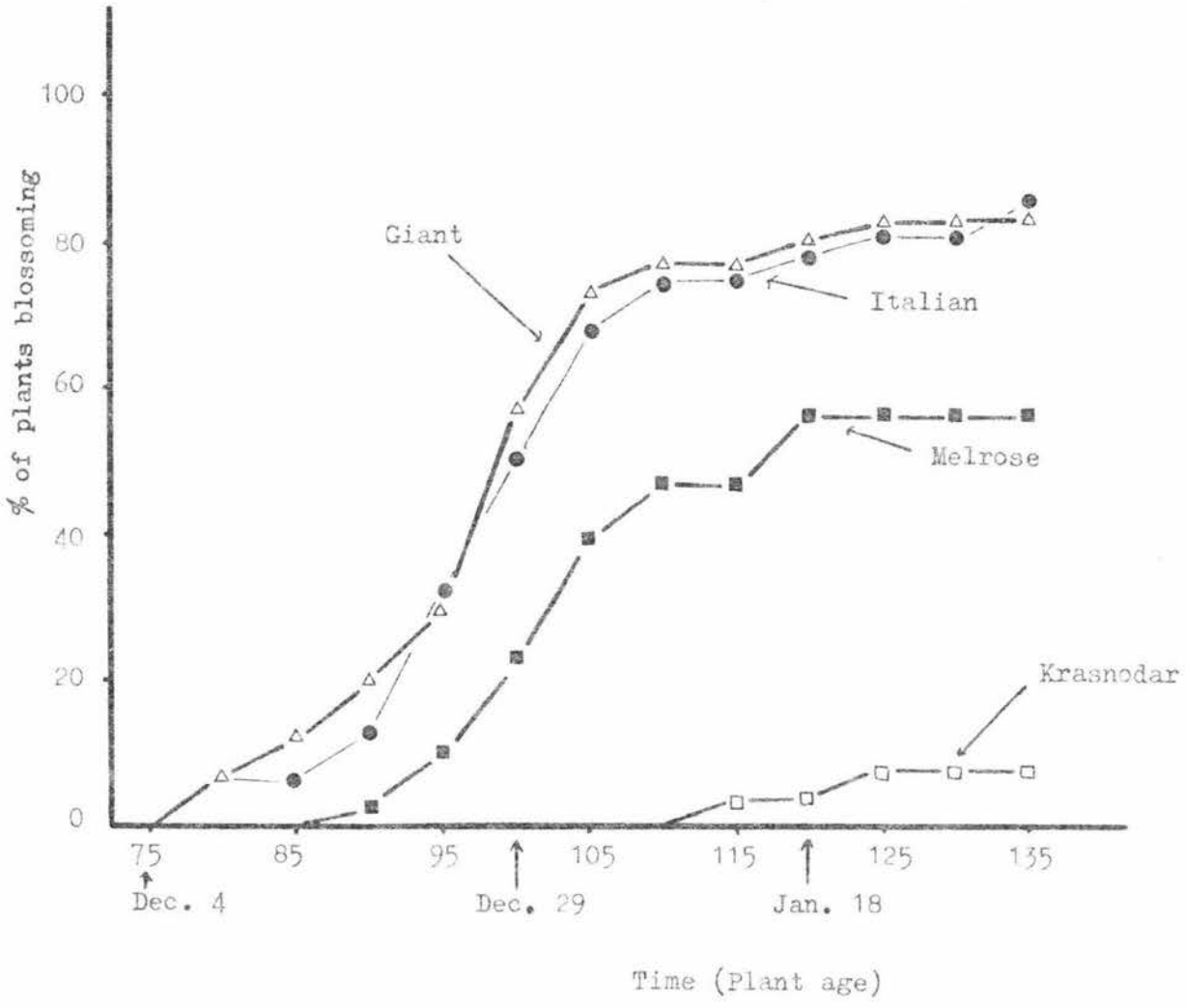


Figure 5.5

Cumulative percentage of blossoming for Melrose, Giant, Italian and Krasnodar. The average date of blossoming for each cultivar was: Melrose Jan. 5th, Giant Dec. 31st, Italian Jan. 1st, and Krasnodar Jan. 17th.

The greatest variation in corolla colour was in Melrose with individual plants having light pink coloured petals while other plants had flowers with a deep red petal colour.

Another difference between cultivars was that Melrose had fewer blossoming stems than Italian or Giant, but instead had more blossoming inflorescences on each stem (Table 5.1). It was observed also that blossoming stems in Melrose were taller than those of Giant or Italian.

In all cultivars the development of lateral shoots on elongating primary and secondary stems was very weak, hence very few inflorescences were to be found on lateral shoots (Table 5.1).

In Melrose, Giant and Italian there was a general trend for the number of florets per inflorescence to decrease acropetally (Figure 5.7). Melrose inflorescences had more florets than did those of Italian and Giant (5% and 1% level of significance respectively calculated from the first inflorescence on each stem).

As previously mentioned there were differences in growth and development between primary and secondary stems. Plants of Melrose, Italian and Giant that had blossoming inflorescences on the primary stem (as opposed to plants that did not have blossoming inflorescences on the primary stem) were analyzed. In Melrose there was a greater number (significant at the 5% level) of fully developed inflorescences per stem on the secondary stems than on the primary stem. There was no significant differences between primary and secondary stems in Giant and Italian (Table 5.2).

(e) Inflorescence Abortion

An outstanding feature noticed in all cultivars that flowered was the high percentage of aborted inflorescences (Table 5.3). Under the environmental conditions of this experiment the percentage of aborted inflorescences within cultivars ranged from 37% to 54%. In all cultivars inflorescences aborted at all stages of development. The first formed inflorescences on a stem tended to abort at a later stage of development corresponding to stages 8 and 9 (Table 4.1, Chapter 4) than those formed later. In other words inflorescences developed further before ceasing growth in these cases. Towards the apical end of the stem inflorescences aborted at a much earlier stage of development corresponding to stages 5, 6 and to a lesser extent stage 7 (Table 4.1, Chapter 4). It was also noted that a higher percentage of inflorescences aborted towards the apical end of the stem (Figure 5.8).

Examination of Figure 5.8 reveals what seems to be a definite abortion

Table 5.1

Flowering and associated parameters in cultivars Melrose, Giant, Italian, Krasnodar and Common.

	% plants having infl. initiation	% plants having blossoming infl.	Av. no. of infl. with complete floret development per flowering plant	Av. no. stems/ plant with blossoming infl.	Av. no. blossoming infl. per flowering stem	Av. no. of blossoming infl. on lateral stems
Melrose ± SEM	57	57	18.95 ± 3.63	3.5 ± 0.43	5.4 ± 0.420	1.05 ± 0.59
Giant ± SEM	93	83	19.25 ± 2.76	5.6 ± 0.53	3.4 ± 0.480	0.93 ± 0.39
Italian ± SEM	93	86	19.70 ± 2.78	5.4 ± 0.53	4.60 ± 0.275	0.54 ± 0.33
Krasnodar ± SEM	8	8	11.00 ± 6.00	2.5 ± 0.50	3.60 ± 1.40	-
Common	0	-	-	-	-	-

Table 5.2

Flowering characteristics of those plants that had inflorescence initiation on their primary stem.

		Primary stem		Secondary stems	
	No. plants with infl. initiation	Av. no. of blossoming infl./stem	Av. no. aborted infl./stem	Av. no. of blossoming infl./stem	Av. no. aborted infl./stem
Melrose	6	4.12	2.55	7.05	2.29
±SEM		± 1.12	± 0.648	± 0.921	± 0.458
Italian	13	4.77	3.54	3.28	2.94
±SEM		± 1.04	± 0.595	± 0.345	± 0.241
Giant	19	4.00	3.89	3.26	3.62
±SEM		± 0.721	± 0.389	± 0.267	± 0.187

Table 5.3

Inflorescence abortion and associated parameters in cultivars Melrose, Giant, Italian and Krasnodar.

					In those plants that flowered	
	% of infl. that aborted totally	Total no. of infl. per flowering plant	Av. no. of fully aborted infl./ flowering plant	% of blossoming infl. that partially aborted	% of flowering plants with poor flowering	% of flowering plants with vigorous flowering
Melrose ± SEM	37	29.95	11.00 ± 2.09	6.8	24	33
Giant ± SEM	54	41.85	22.60 ± 2.75	8.0	40	53
Italian ± SEM	50	36.80	17.10 ± 2.11	2.1	50	43
Krasnodar ± SEM	40	15.00	6.00 ± 5.00	10.5	4	4

N.B. Vigorous flowering = a plant having at least one stem with a minimum of six fully developed inflorescences. Poor flowering = a plant having no stem with more than 5 fully developed inflorescences.

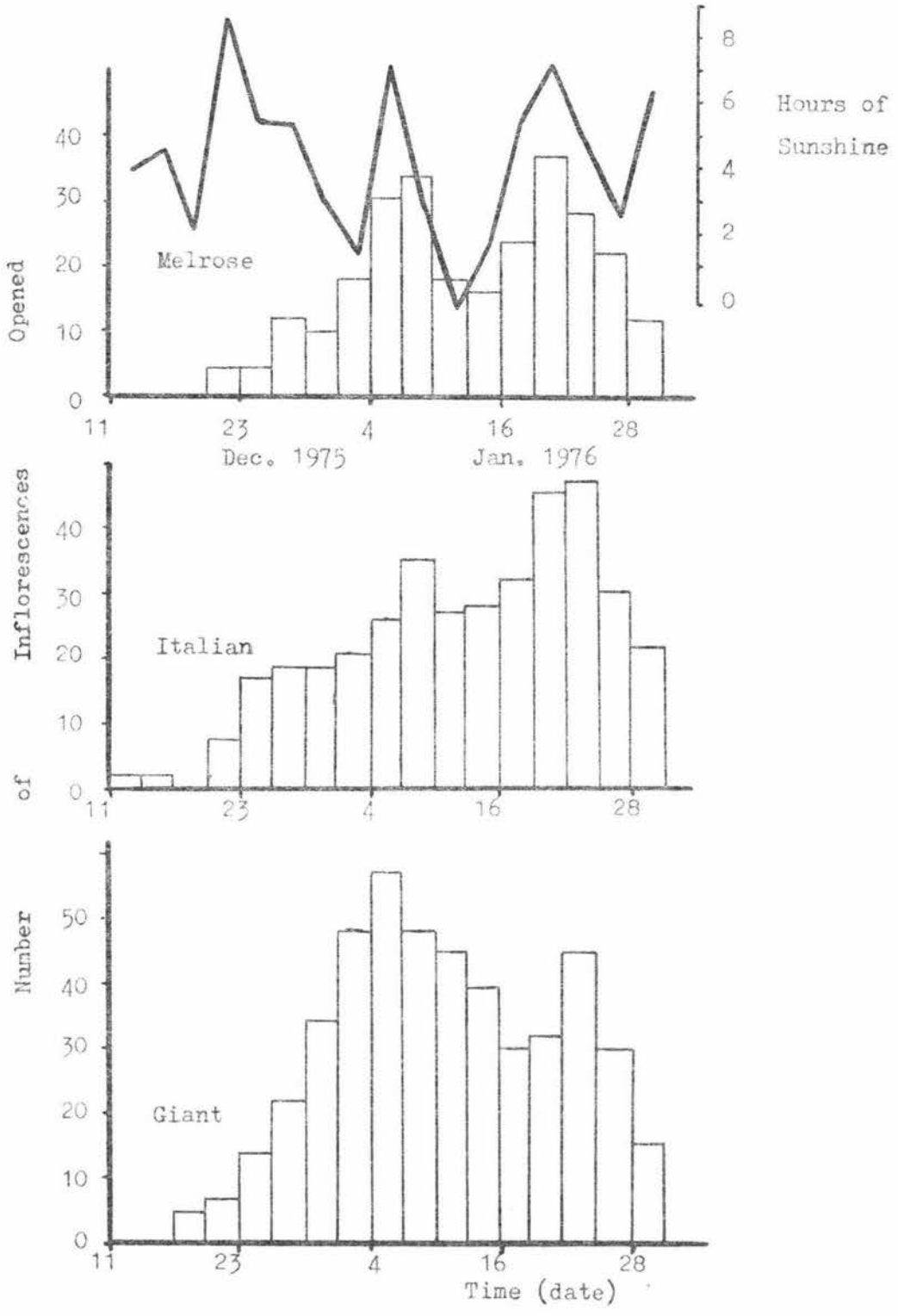


Figure 5.6

A frequency distribution to show when inflorescences first blossomed (i.e. Stage 10, Figure 4.1) for Melrose, Giant and Italian. Each bar represents the number of inflorescences that had started to blossom over a three day period.

The solid deep line associated with the histogram for Melrose represents the average number of sunshine hours per day over the three day time period associated with the calculation of inflorescence blossoming.

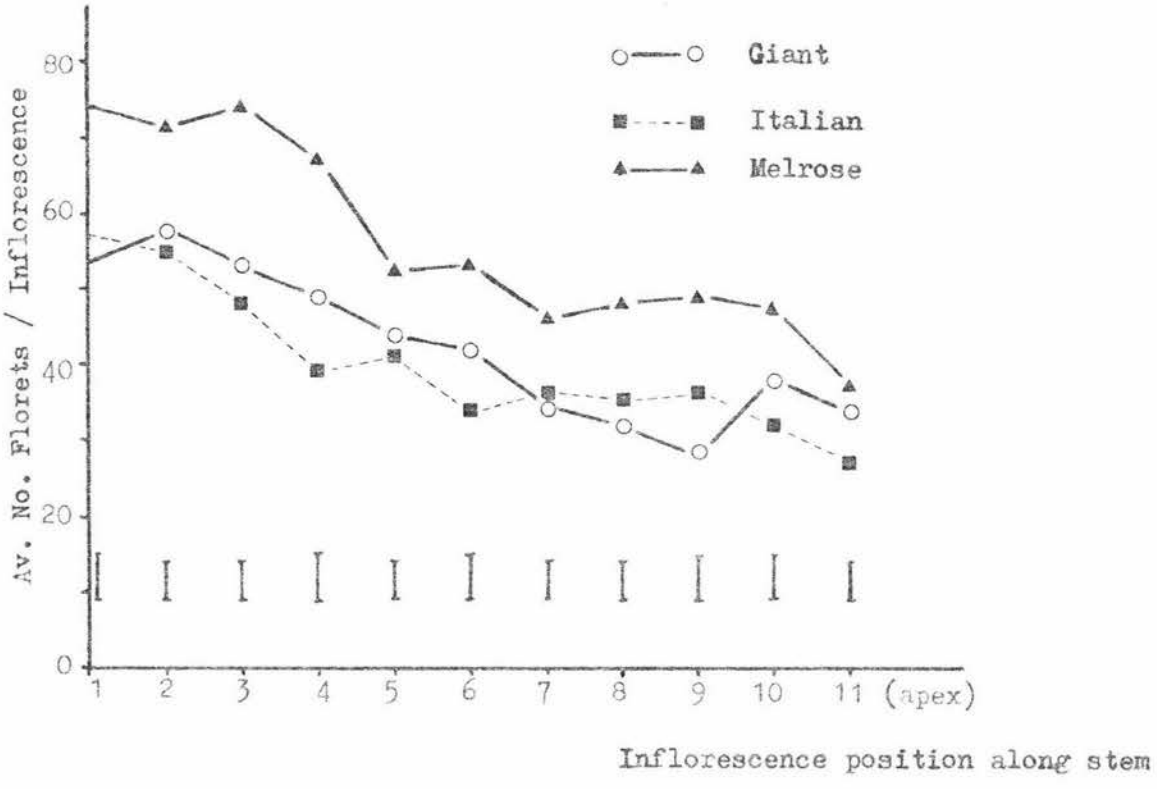


Figure 5.7

The average number of florets per inflorescence in relation to inflorescence position along the stem. Vertical lines indicate 2x SEM.

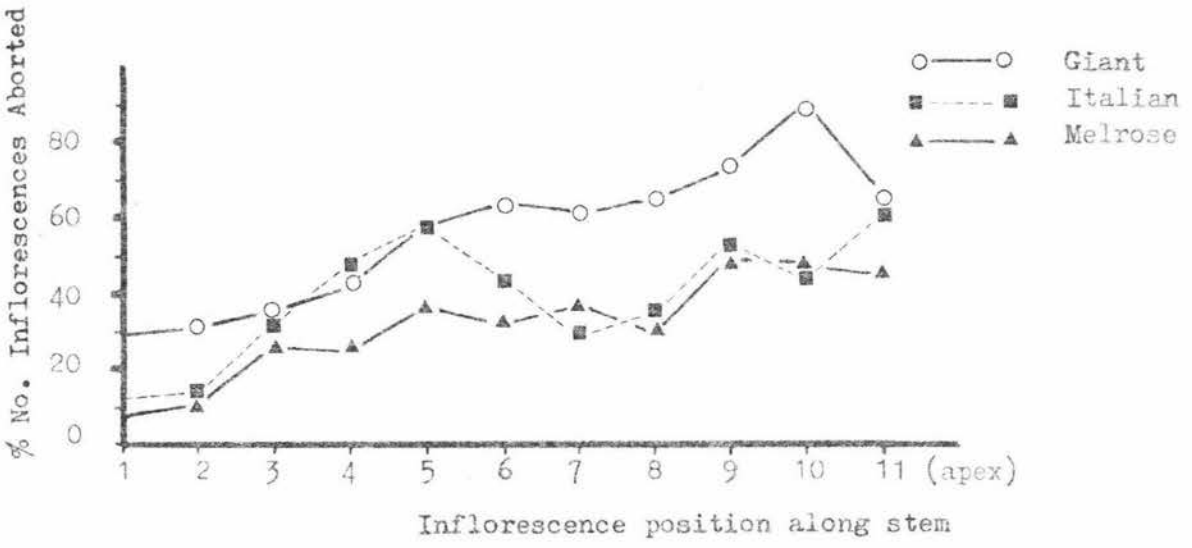


Figure 5.8

Average percentage of fully aborted inflorescences along the stem in Melrose, Giant, and Italian.

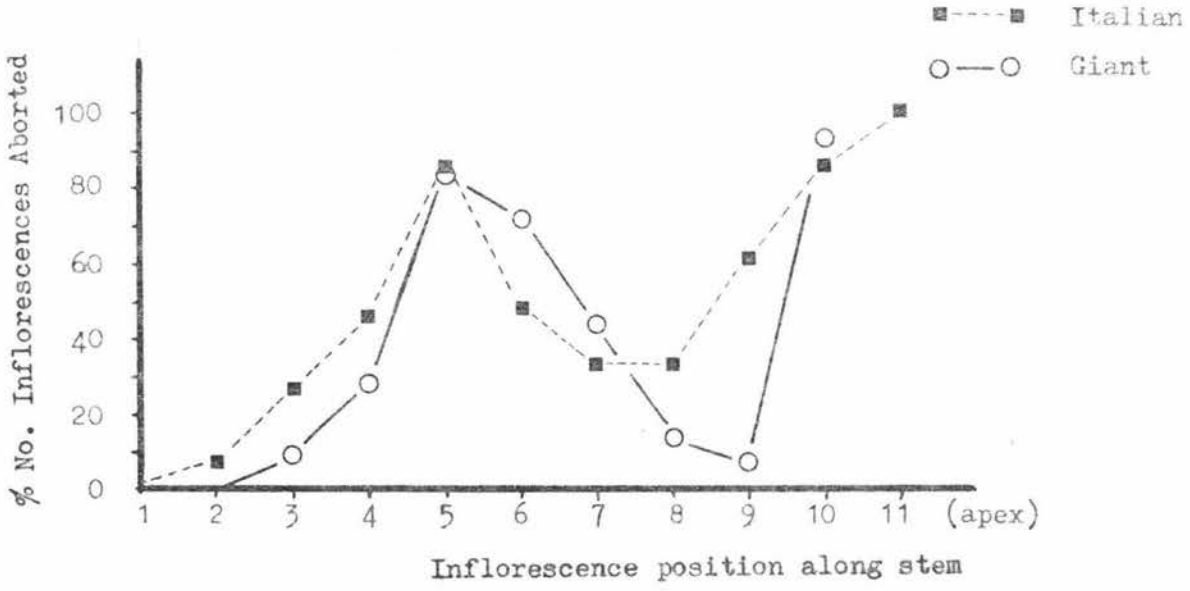


Figure 5.9

Percentage of fully aborted inflorescences along the stem in Italian (from 15 stems that blossomed at different times) and Giant (from 15 stems that blossomed at different times).

pattern along the stem, particularly in Italian and to a lesser extent Giant. Two peaks in the percentage of aborted inflorescences along the stem occur, the first occurring on the stem at about inflorescence number five with the second occurring towards the apical end of the stem.

To test whether this was an external environmental effect or an internal plant effect on inflorescence development 15 blossoming stems from both Giant and Italian were examined. All of these stems began blossoming at different time intervals. The results in Figure 5.9 show that two definite abortion peaks still appeared thus supporting the idea that this inflorescence abortion pattern first noted in Figure 5.8 was caused by an internal plant effect and not an environmental effect.

No inflorescence abortion pattern was observed in blossoming stems of Melrose, even when individual stems were examined. For the Melrose population there was a lower percentage of aborted inflorescences (Table 5.3). This was brought about by a lower percentage inflorescence abortion rate all along the stem (Figure 5.8).

Another interesting observation was that in some inflorescences the first formed florets blossomed while the later formed florets on the inflorescence aborted. This pattern of abortion occurred predominantly in Melrose and Giant (Table 5.3), especially on the first formed inflorescences (Figure 5.10). The figure in Table 5.3 for the percentage of blossoming inflorescences that partially aborted in the Krasnodar population will not be used for discussion because only two plants blossomed. Thus this figure may, or may not, be misleading. In some inflorescences only one or two florets reached maturity. When examining the frequency distribution (Figure 5.10) one should be aware that the percentage of partially aborted inflorescences in each cultivar is low in comparison to the number reaching full development (Table 5.3).

Associated Vegetative Development

(f) Basal Bud Formation

Basal bud formation is shown in Figure 5.11. All cultivars had a gradual increase in basal bud numbers during November and December, however, in the flowering cultivars (Melrose, Giant and Italian) there was a greater increase in basal bud numbers beginning at the start of January 1976. When comparing Figure 5.11 to Figure 5.5 it can be seen that this greater basal bud increase is associated with the end of the main period of blossoming. Common tended to have a steady rate of

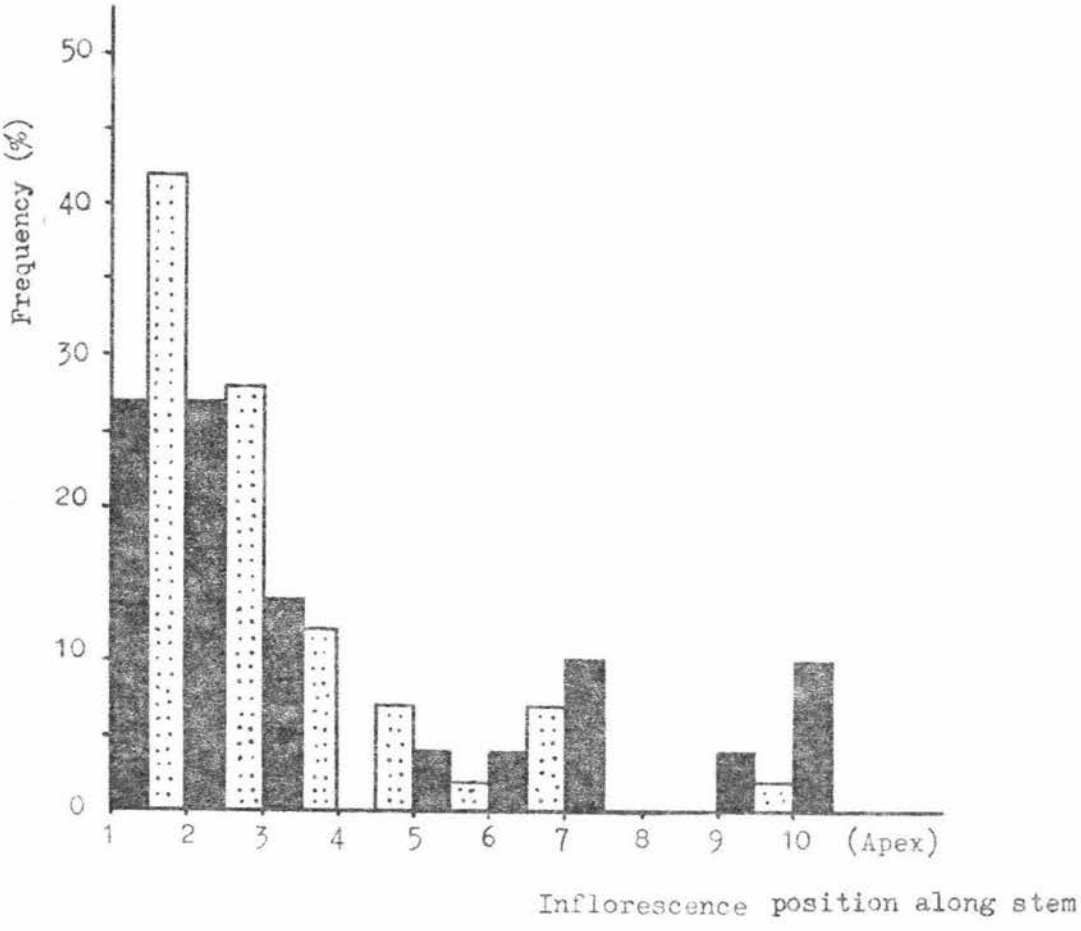


Figure 5.10

Frequency distribution of partially aborted blossoming inflorescences for Melrose (solid bars) and Giant (stippled bars) along the stem.

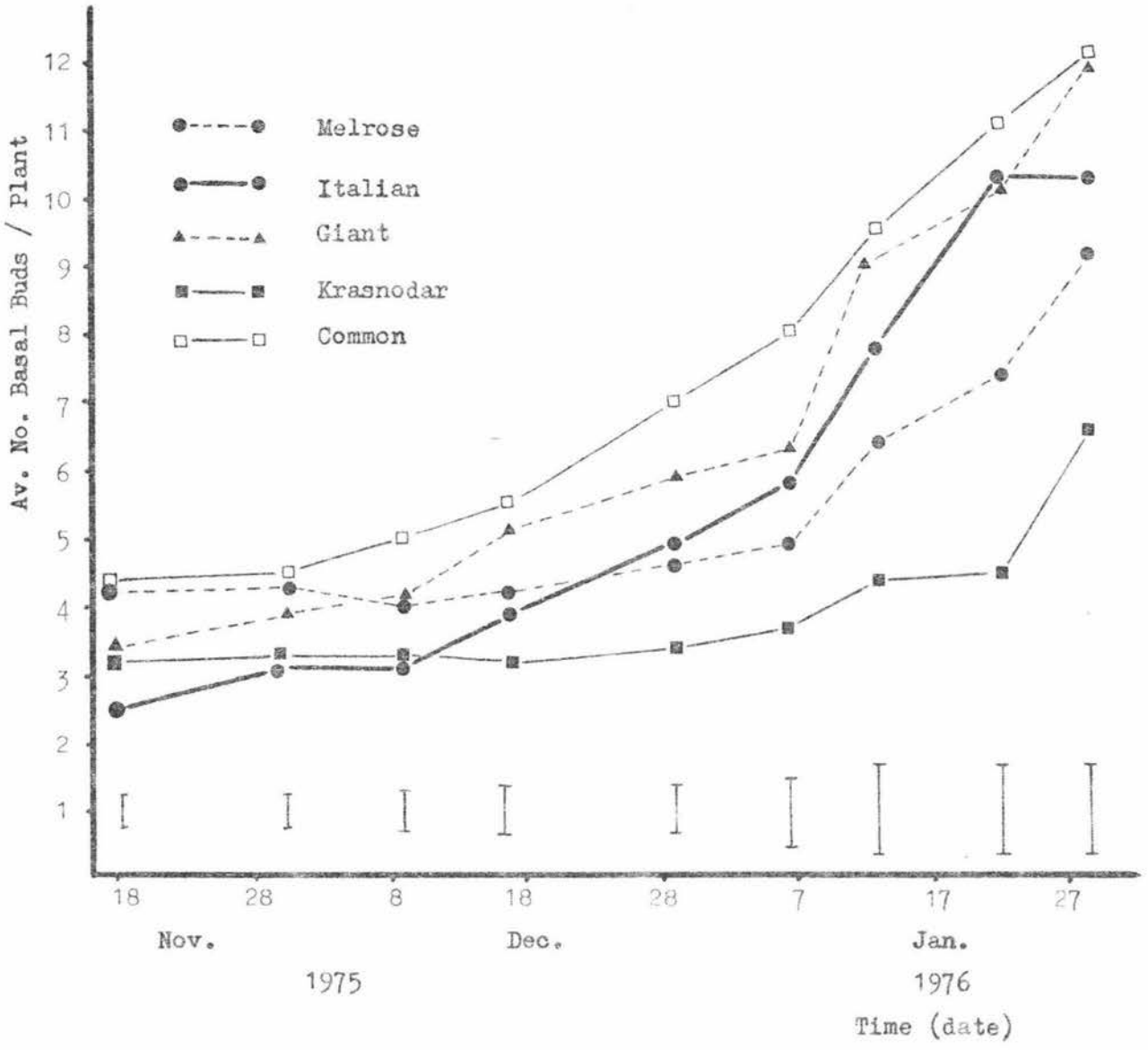


Figure 5.11

Basal bud development for Melrose, Giant, Italian, Krasnodar and Common. Vertical lines indicate 2x SEM.

basal bud increase beginning in late November 1975. Krasnodar, after initially having a period of comparitively low basal bud increase, had a greater increase in basal bud numbers beginning in late January 1976.

Of the cultivars that flowered Melrose had, at the end of the trial, fewest basal buds. When comparing the basal bud development of this cultivar in plants that flowered and those that did not flower (Figure 5.12) it can be seen that non-flowering plants followed a slight resemblance to the basal bud pattern of Krasnodar (Figure 5.11); flowering plants followed a pattern similar to Italian and Giant (Figure 5.11).

In Melrose the average number of basal buds per plant, at the end of the trial, was greater in the earlier flowering plants than in those flowering later (Figure 5.13). A similar trend occurred in Giant and Italian (Figure 5.14).

(g) Rate of Leaf Emergence

Two measurements were made of rates of leaf emergence. The first measurement was made during the period the flowering cultivars were beginning the phase of stem elongation (Dec. 2 to Dec. 22); the second measurement during the blossoming phase (Dec. 22. to Jan. 11).

The results, in Table 5.4, show that cultivars can be divided into two groups depending on their rates of leaf emergence particularly in the blossoming phase. Italian and Giant, and to a lesser degree Melrose, form one group that has a more rapid leaf emergence rate. Krasnodar and Common form a second group with a lower leaf emergence rate.

From Table 5.4 it could be argued that Melrose did in fact form another group that could be situated inbetween these two extremes. This was examined more closely. Plants from the Melrose population were divided into those having elongated versus non-elongated stems (Table 5.5). This shows that the average leaf emergence rate for elongated stems was similar to that found in Italian and Giant (Table 5.4). The average rate for non-elongated stems corresponded to the Common/Krasnodar grouping (Table 5.4).

There were no significant differences in Melrose for non-elongating or elongating stems with regard to primary versus basal buds (Table 5.5). There were, however, significant differences (1% level) between rates of leaf emergence in non-elongating versus elongating stems of a population from within the stem elongation and blossoming phase (vertical columns), and between the stem elongation phase for each population and the blossoming phase (horizontal columns) (Table 5.5).

Table 5.4

Rate of Leaf Emergence at two developmental stages in Melrose, Giant, Italian, Krasnodar, and Common. Each figure refers to the number of new leaves emerged per 20 days.

	Stem elongation phase (Dec. 2 to Dec. 22)			Blossoming phase (Dec. 22 to Jan. 11)		
	Overall av. / group	Av. for primary shoots	Av. for basal shoots	Overall av. / group	Av. for primary shoots	Av. for basal shoots
Melrose ± SEM	2.75 ± 0.12	2.63 ± 0.15	2.89 ± 0.18	4.56 ± 0.21	4.27 ± 0.30	4.78 ± 0.29
Giant ± SEM	3.08 ± 0.12	2.87 ± 0.14	3.30 ± 0.18	5.14 ± 0.19	5.17 ± 0.29	5.11 ± 0.24
Italian ± SEM	3.11 ± 0.13	2.75 ± 0.15	3.43 ± 0.18	4.97 ± 0.18	4.67 ± 0.26	5.28 ± 0.25
Krasnodar ± SEM	2.49 ± 0.12	2.74 ± 0.17	2.27 ± 0.16	3.92 ± 0.19	4.00 ± 0.17	3.83 ± 0.36
Common ± SEM	2.22 ± 0.08	2.43 ± 0.11	2.00 ± 0.08	3.61 ± 0.09	3.83 ± 0.12	3.38 ± 0.13

Table 5.5

Leaf emergence rates for elongating and non-elongating shoots of Melrose from the stem elongation and blossoming phases of growth. Results are expressed as the number of new leaves emerged in twenty days. The stem elongation and blossoming phases were periods when the flowering cultivars were at these stages of growth.

		Stem elongation phase	Blossoming phase	Increase
Shoots with elongated internodes	Primary shoot	3.71 ± 0.184	6.00 ± 0.365	2.29
	Secondary shoot	3.47 ± 0.174	5.63 ± 0.219	2.16
Shoots with no elongated internodes	Primary shoot bud	2.30 ± 0.132	3.35 ± 0.196	1.05
	Basal bud	1.92 ± 0.148	3.00 ± 0.289	1.08

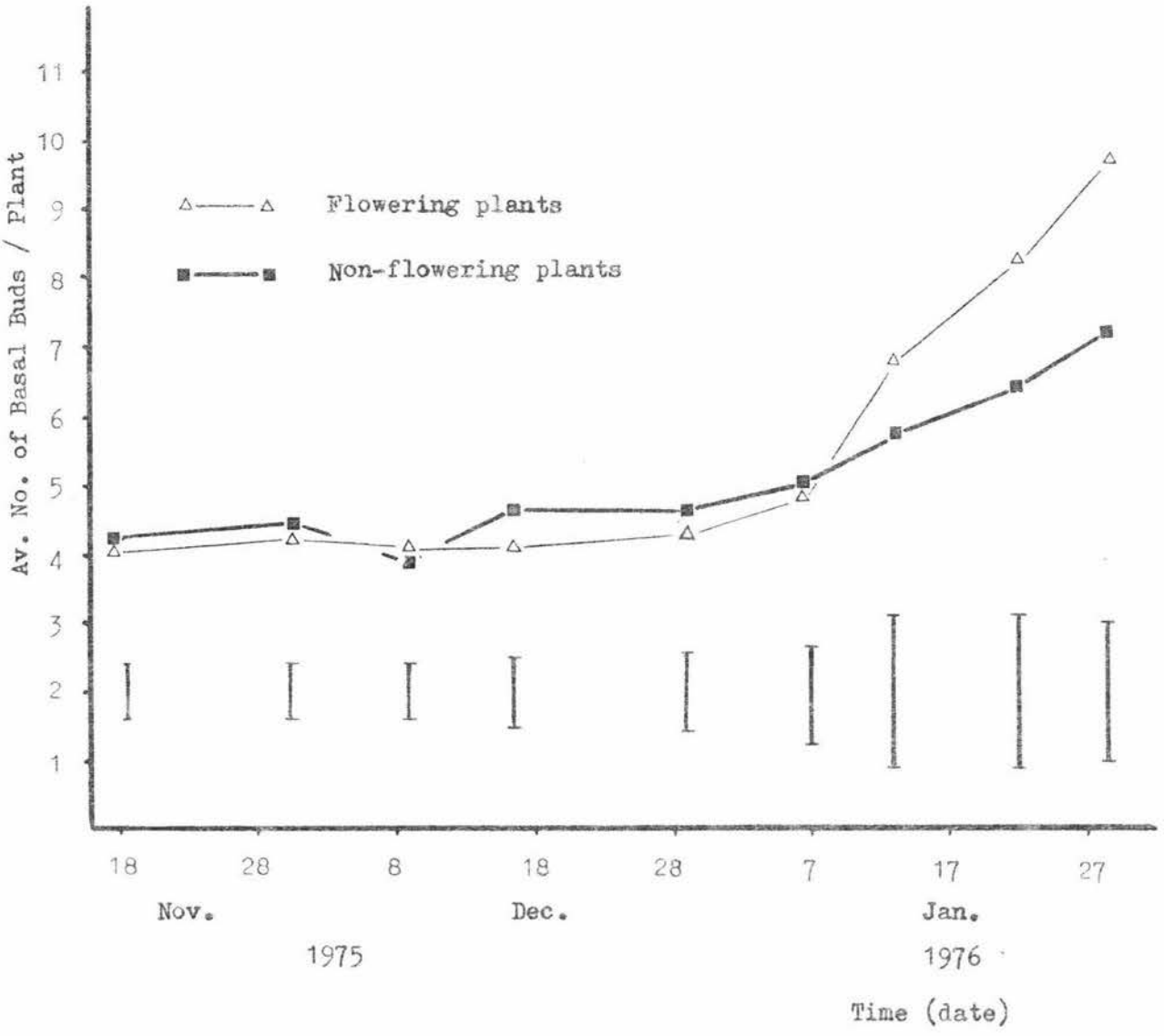


Figure 5.12

Basal bud development in Melrose for flowering and non-flowering plants. Vertical lines indicate 2x SEM.

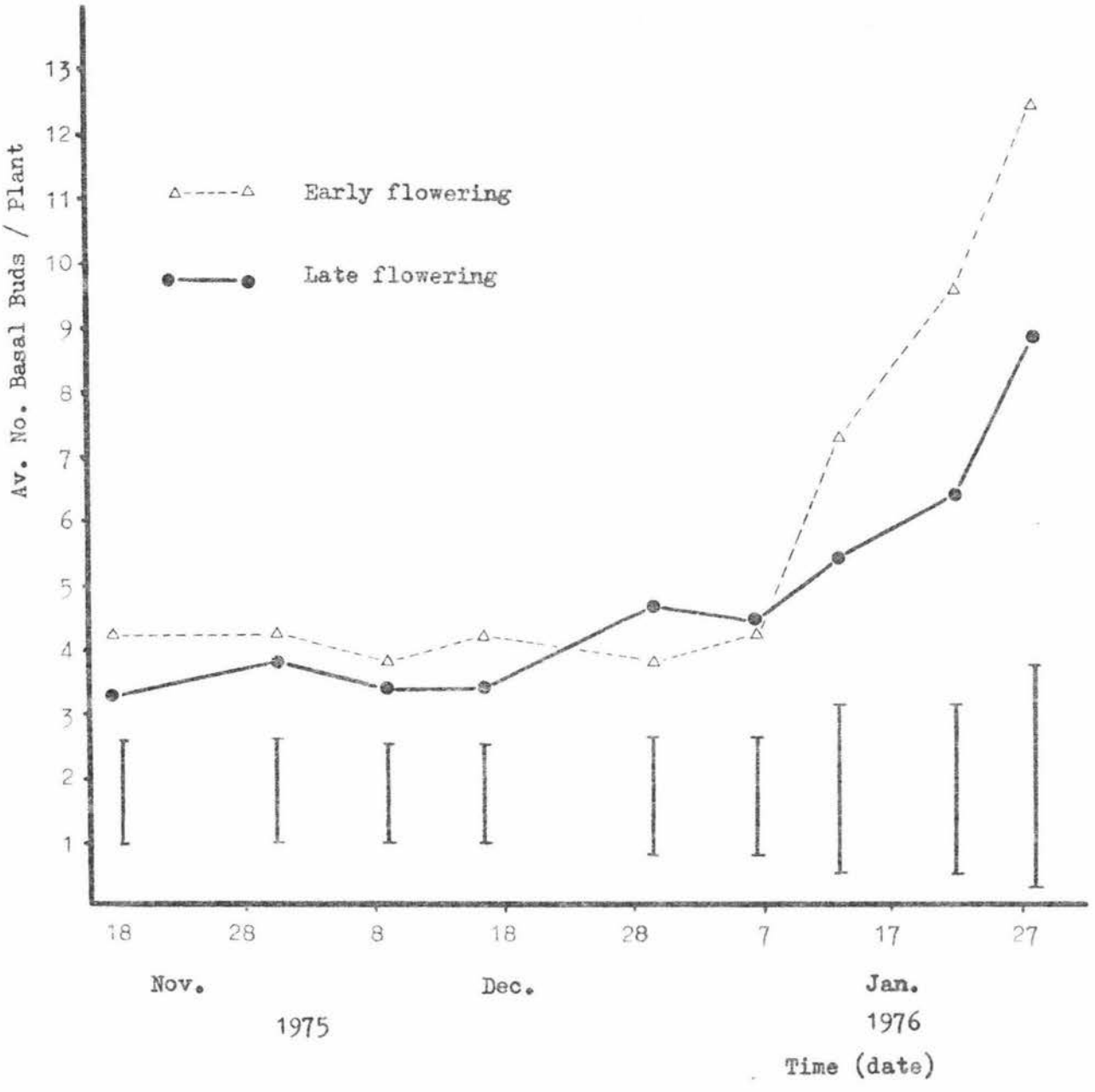


Figure 5.13

Basal bud development in Melrose for 'early' and 'late' flowering plants (early flowering = the first five plants to blossom, late = the last five plants to blossom). Vertical lines indicate 2x SEM.

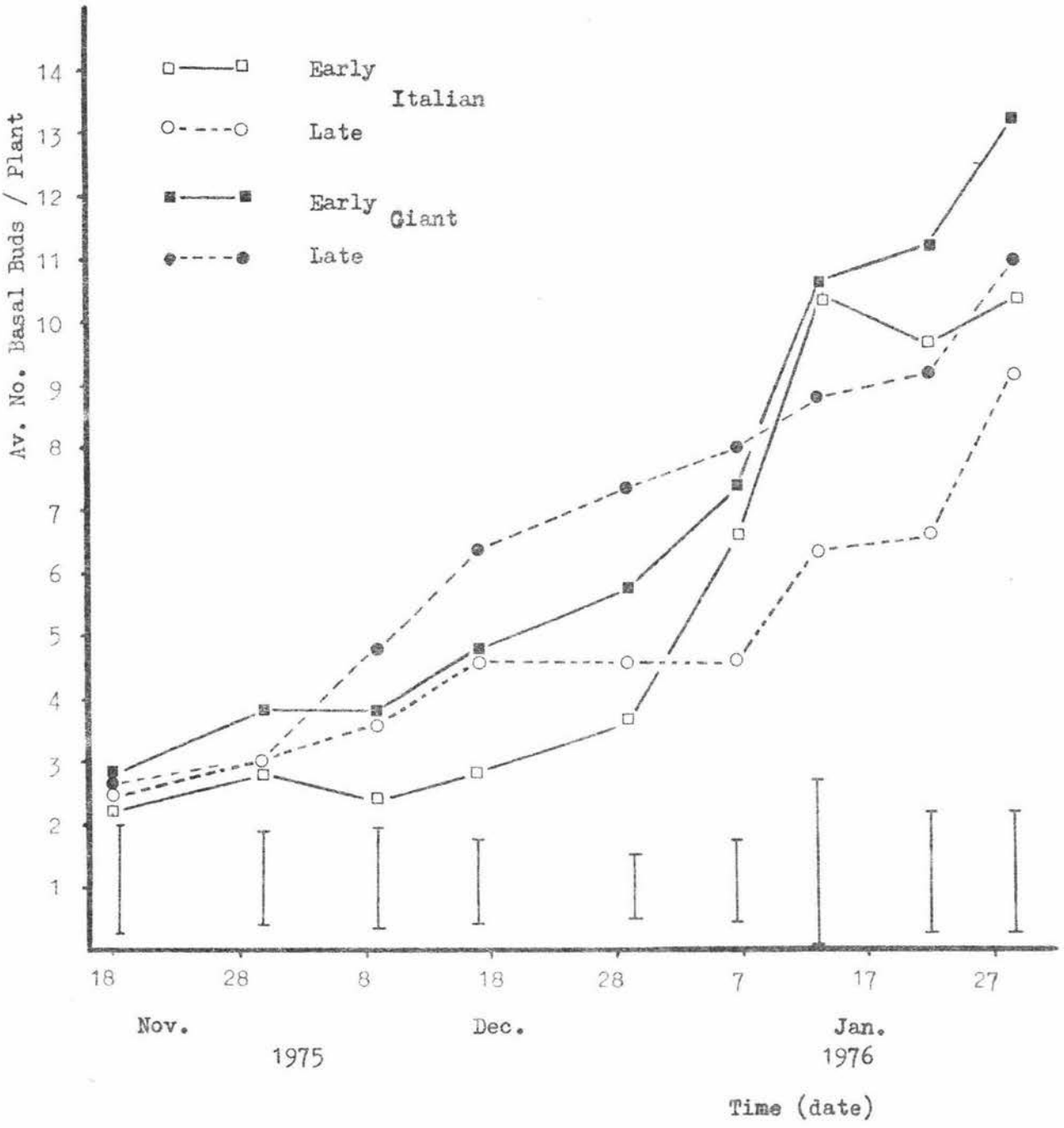


Figure 5.14

Basal bud development in Giant and Italian for 'early' and 'late' flowering plants (early flowering = the first five plants in each population to blossom, late = the last five plants in each population to blossom). Vertical lines indicate 2x SEM.

(h) Leaf Measurements

Results are presented in Table 5.6. The main cultivar differences were that Common and Giant had significantly (1% level) smaller leaves and petioles, Common had significantly (1% level) smaller leaflets.

From these leaf measurements a number of relationships appeared. Using Student's t-test these were not significant but when all five cultivars followed the same pattern this in itself is worth noting. These observations include: basal buds and secondary stems in all cultivars have longer leaves and petioles than the primary shoot (except Krasnodar re petioles), whereas leaflet widths of primary shoots/basal buds and secondary stems were all similar within cultivars.

5.4 Discussion

(a) Leaf Angle

In all cultivars of sainfoin the first visible response to the changing natural photoperiod was that of leaf erectness and bud swelling. Zeevaart (1971) found also that the earliest visible response of spinach plants transferred from short days to long days was a more erect leaf orientation and increased elongation of the petioles. In Trifolium pratense L. cv. Emerson and cv. Mammoth were prostrate under a 10 hour photoperiod, were at an intermediate stage uner 14 hours, while in an 18 hour photoperiod they were fully erect (Keller and Peterson, 1950). Ludwig et al (1953) also showed a similar effect of photoperiod in red clover, there being a gradual increase in leaf angle that corresponded to the 1 to 5 scale (Plate 5.1) as used in this thesis i.e. score 1 was equal to a 8 hour photoperiod, score 2 was equal to 10 hours photoperiod etc.

In Figure 5.1 it can be seen that four of the cultivars (Melrose, Italian, Giant and Krasnodar) had reasonably high leaf angle scores when the first leaf angle measurement was made. As this was an observational trial parameters were measured as they were observed to occur. Because the increase in leaf angle was initially quite rapid, the earlier leaf angle increases were missed. For Giant and Italian this initial high leaf angle score might also have resulted from their natural semi-erect growth habit in short days (see Chapter 12).

When comparing the leaf angle response for Common (Figure 5.1) to the natural photoperiod curve for Palmerston North (Figure 2.1) it can be seen that as the photoperiod at Palmerston North peaked so did the mean

Table 5.6

Leaf length, petiole length and leaflet width in cultivars Melrose, Giant, Italian, Krasnodar and Common.

	Leaf length		Petiole length		Leaflet width	
	P.S.	B.B.	P.S.	B.B.	P.S.	B.B.
Melrose	17.78	20.33	7.02	8.05	4.50	4.52
± SEM	± 0.72	± 0.58	± 0.48	± 0.42	± 0.24	± 0.15
Giant	12.76	13.75	4.83	5.56	3.85	3.81
± SEM	± 0.33	± 0.32	± 0.20	± 0.20	± 0.13	± 0.12
Italian	15.78	16.30	6.51	6.64	4.33	4.26
± SEM	± 0.55	± 0.54	± 0.32	± 0.33	± 0.11	± 0.12
Krasnodar	19.10	20.21	7.64	7.54	4.42	3.93
± SEM	± 0.93	± 0.98	± 0.57	± 0.64	± 0.23	± 0.20
Common	10.25	13.45	3.52	4.92	3.51	3.62
± SEM	± 0.27	± 0.33	± 0.16	± 0.20	± 0.10	± 0.08

leaf angle score of Common, the mean leaf angle score decreasing with the photoperiod thereafter.

When interpreting these leaf angle data it must be kept in mind that a score of 5 does not necessarily mean that a plant has then reached maximum development for this reproductive growth phase. A plant with a score of 3 or 4 can proceed with the later developmental stages, as often appears to be the case for Giant and Italain (i.e. compare Figure 5.2, graph no. 4 to percentage inflorescence initiation data in Table 5.1).

(b) Bud Swelling

With increasing daylength the primary bud and the basal buds began swelling.

Bud swelling was found to result from the expansion of the petiole bases. In Melrose plants grown in short days the basal petiole width was 1.10 $\pm$ 0.16 mm, whereas plants grown in long days had a corresponding mean petiole width of 2.22 $\pm$ 0.23 mm. As the petioles surround and partially enclose the shoot then their increase in size gave the shoot bud a swollen appearance.

It appears that this bud swelling is closely associated with the more erect leaf habit and increasing petiole length as described in 5.3 (a).

(c) Stem Elongation

Following the phase of leaf erectness and bud swelling the plants moved into another growth phase - that of stem elongation. This occurred in both the primary shoot bud and basal buds.

One interesting feature was the development of basal buds on plants that had primary stem elongation. The first plants to show primary stem elongation only had a small number of visible basal buds at that time. At the count of Dec. 10 1975, Melrose plants with elongating primary stems had 1.67 $\pm$ 0.333 basal buds per plant. In comparison the mean number of basal buds and secondary stems for the rest of the Melrose population without primary stem elongation that had secondary stem elongation was 4.40 $\pm$ 0.271 (significant at the 1% level). In addition Italian plants with elongating primary stems had 1.0 $\pm$ 0 basal buds per plant whereas the mean number of basal buds for the rest of this population was 3.71 $\pm$ 0.329 (significant at the 1% level). It would appear that the early conversion of the primary shoot to the reproductive state might have prevented further basal bud development. Plants with high numbers of basal buds may have been faster growing genotypes thus producing more basal buds before the reproductive state was induced. A juvenility factor might have also influenced the time available for basal bud

development. For example, it would be expected that a plant with a long juvenile period would have more basal buds than a plant with a short juvenile period as the former would have a longer time interval in which to develop basal buds before reproductive development started.

(d) Flowering

The flowering behaviour within many legume species is not uniform. For example, differences between cultivars, and clones within them, have been observed in red clover (Williams, 1945; Keller and Peterson, 1950; Fergus and Hollowell, 1960; Bula, 1969), white clover (Britten, 1960; Thomas, 1961), and lucerne (Medler et al, 1955; Zaleski, 1954).

Sainfoin appears to be a heterogeneous species with regard to its flowering behaviour (Spedding and Diekmahns, 1972; Thomson, 1938, 1951; Cooper, 1972; Sinskaya, 1958). Table 4.1 shows this variability in the selected cultivars but in greater detail than that previously described.

In this investigation on development in sainfoin under glasshouse conditions, genotypes within cultivars could be divided on the basis of flowering into two main groups, one of which flowered (nearly all Giant and Italian plants, approximately half of Melrose and only two Krasnodar plants), the second of which did not (the rest of the Melrose population, nearly all Krasnodar and all Common plants). In Melrose, of those plants that did not initiate inflorescences 31% had internode elongation (i.e. 69% did not). In Krasnodar, of those plants that did not initiate inflorescences only 18% had internode elongation. In Common there was no inflorescence initiation or internode elongation.

Figure 5.5 shows that blossoming in Melrose, Giant and Italian began in early December, with Giant and Italian beginning slightly earlier than Melrose. For these three cultivars the main period of blossoming occurred at the same time (i.e. late Dec. to early Jan.) (Figure 5.5, Figure 5.6), with each having a few plants that did not start blossoming until late in the growing season (Figure 5.5). Also the two Krasnodar plants that blossomed did so late in the season.

Corolla colour was noticeably different in some plants. The greatest variation in corolla colour was in Melrose with individual plants having pale pink petals to other plants whose flowers had a deep red petal colour. Sinskaya (1958) noted that in Northern Caucasian species of Onobrychis the petal colour changed from a bright red in the upper altitudinal zones to pink on the lower stepps.

(e) Inflorescence Abortion

All cultivars that flowered had a high percentage of aborted inflorescences. This abortion phenomenon has often been noted in many

types of plants by various authors. For example, in Trifolium repens L. (Thomas, 1961), Lotus pedunculatus (Forde and Thomas, 1966); Lotus corniculatus, (Joffe, 1958); Medicago sativa L. (Thomas, 1967); Phaseolus vulgaris (Bentley et al 1975); and Arachis hypogea (Smith, 1954).

An interesting observation was that the average number of fully developed inflorescences per plant was similar between cultivars (Table 4.1) yet the average number of aborted inflorescences per plant (Table 4.3) varied. This suggests that a plant might only be able to support the development of a certain maximum number of inflorescences, as appears to be the case in cotton (Eaton and Ergle, 1957).

In Figure 5.9 it was shown that there is a definite internal rhythm in inflorescence abortion along the stem in cultivars Giant and Italian. Detailed discussion on this aspect will be presented in Chapter 15.

An unusual observation was the occurrence of partially aborted inflorescences in Melrose and Giant (Figure 5.10). When occurring, these formed predominantly on the first two inflorescences on the stem. It is difficult to interpret and explain this observation when also taking into account the observation in Figure 5.9 (i.e. abortion pattern along the stem). Again detailed discussion of this aspect of abortion will be presented in Chapter 15.

(f) Basal Bud Formation

In the flowering cultivars basal bud development was retarded during the onset of reproductive development. When blossoming was completed basal bud numbers again began to increase. In the non-flowering Common cultivar basal bud formation continued steadily throughout the period of this experiment (Figure 5.11). An interesting cultivar was Krasnodar which had significantly lower basal bud numbers per plant, even on plants remaining in the rosette phase. This appears to be a genetic effect rather than environmental. The non-flowering Melrose plants (Figure 5.12) also followed a basal bud formation pattern similar to that of Krasnodar (Figure 5.11). This aspect will be discussed in greater detail in Chapter 15.

When comparing flowering dates (Figure 5.5) and average number of basal buds per plant (Figure 5.11) it should be remembered that Melrose was slightly later to blossom than Giant or Italian. Thus it would not be expected that Melrose, at the end of the experiment, would have as many basal buds as Giant or Italian, as buds not yet visible might still have been developing . This has an important practical aspect in that

early flowering genotypes might possibly be able to develop new basal buds after the first blossoming period that could later elongate into flowering secondary stems in the one season.

In conclusion it could be stated that, on the pattern of basal bud development, cultivars can be divided into three groups. The first group follows a developmental pattern similar to Italian, Giant and the flowering Melrose plants; the second group follows that of Krasnodar and the non-flowering Melrose plants; and the third group follows a pattern as found in Common.

(g) Rate of Leaf Emergence

The data show that cultivars can be divided into two groups depending on their rates of leaf emergence in the blossoming phase (Table 5.4). Italian and Giant, and flowering Melrose plants form one group that has a rapid leaf emergence rate. Krasnodar, Common and non-flowering Melrose plants form a second group with a lower leaf emergence rate.

Results similar to these have been observed in other plants. For example, in the short day plant Chenopodium amaranticolor, Thomas (1961) showed that the rate of leaf initiation increased following flower induction from 0.47 to a maximum of 1.35 per day.

Langer and Bussell (1964) also noted in the day neutral plant, Fagopyron tartaricum Gaetn., that the rate of leaf initiation increased during the period coinciding with the appearance of flower initials.

Lang (1965) in his review noted that this increase in leaf initiation rate associated with floral induction may be more widespread and may in fact be a regular feature of the transition from vegetative growth to flower initiation.

The smaller increase in the rate of leaf emergence on vegetative non-elongating shoots at the time other plants are blossoming (Table 4.5) can be explained as due to higher temperatures, and an increased photoperiod with a corresponding increased light intensity as summer draws near. In other words this might be due to an overall increase in the plant's metabolism brought about by higher temperatures coupled with an increased photosynthetic effect due to longer days with a higher intensity light. In addition this small increase in leaf emergence rate could be due to a partial reproductive induction effect. That is, the reproductive process might have been blocked at an early developmental stage. In the present case this could have occurred after the stage of leaf erectness and bud swelling.

(h) Leaf Measurements Prior to Flowering

Leaf data have been provided to allow a greater understanding of the results presented in this chapter. Sinskaya (1958) noted that in Caucasian Onobrychis species those from high altitudes have narrower leaflets than those originating from lower altitudes.

(i) General Discussion

In the development of sainfoin to the period when blossoming occurred distinct reproductive stages were noted. These stages included (a) an increase in the angle to the horizontal of the leaves of each plant coupled with bud swelling, (b) stem elongation, (c) inflorescence initiation, and finally (d) development of the inflorescence to maturity. Previous authors, working with other plants, have often argued that stages before initiation are not distinct reproductive stages (Lang, 1965). To examine, in sainfoin, whether the stages of leaf erectness and bud swelling, and stem elongation, are distinct reproductive stages data from the Melrose population were analyzed. All plants from this population were divided into two groups: those that flowered and those having no floral initiation.

On December 16 1975 (the last count) the mean leaf angle for those plants that later flowered was 4.23 ± 0.182 whilst for those plants not flowering the mean leaf angle was 3.69 ± 0.237. This difference, although not statistically significant, was apparent on all occasions when leaf angle measurements were carried out.

From the count on December 16 1975 leaf erectness was correlated with earlier flowering ($r = 0.86$). That is those plants that first responded by raising their leaves were subsequently the first to flower.

88% of the plants that had bud swelling on the first bud swelling count (December 2 1975) later flowered. For the group that did not flower only 54% had bud swelling. Using the Chi^2 test this difference was significant at the 1% level. This shows that the first plants to have basal swelling later flowered during the period when blossoming occurred.

In Melrose shoot elongation was correlated with flowering. In fact only 31% of the non-flowering plants had any stem elongation and generally these stems had only a few elongated internodes.

There was thus strong correlation between earlier and/or more erect leaves, early bud swelling, stem elongation and flowering.

When examining the characteristics of each cultivar when grown in the glasshouse genotypes from within each could be divided into different groups. For example, nearly all the Giant and Italain plants within the populations examined, and the Melrose plants that flowered, formed one

genotype group. A second genotype group consisted of the non-flowering Melrose plants and nearly all of the Krasnodar plants. Finally all Common plants and possibly some Melrose plants that remained as rosettes form a third genotype group. Under the glasshouse conditions of this investigation evidence tends to suggest that the "non-flowering" cultivars Common and Krasnodar are not closely related with regard to growth and developmental control. They both have different patterns of basal bud development. In Krasnodar 8% of the plants flowered, 25% had elongated stems with no macroscopically visible inflorescence initiation, 67% remained in the rosette phase. In Common 100% remained in the rosette stage. Leaf measurements (Table 4.6) were also different with Krasnodar having longer leaves, petioles and wider leaflets than Common. In describing the vegetative characteristics of these two cultivars leaflet width does not show the full difference between the two. Leaflet area would have been a better parameter to use. Krasnodar had a much greater leaflet area than did Common.

CHAPTER 6

THE EFFECT OF PLANT AGE ON FLOWERING

6.1 Introduction

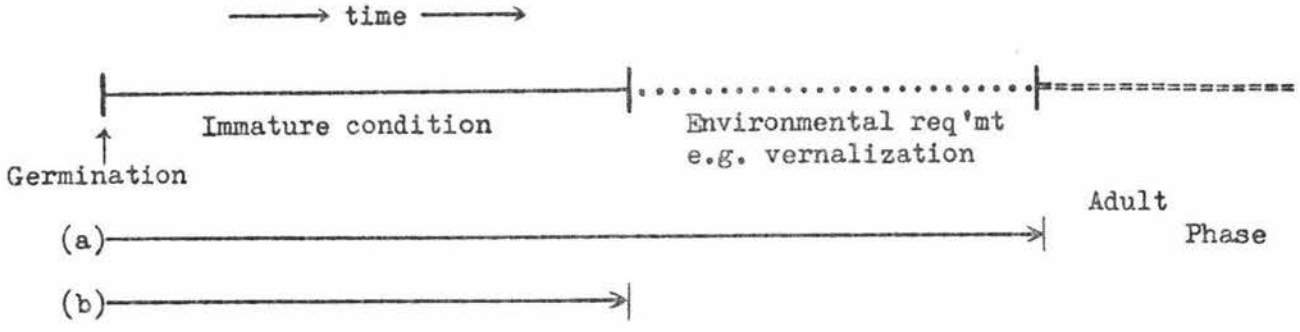
The failure of a plant to flower can be attributed to its immature state and/or the lack of favourable environmental conditions. The former refers to an internal characteristic within the plant whereas the latter refers to the external environmental conditions in which the plant is growing. Often it is difficult to distinguish which one of these two factors is actually responsible for inhibiting reproductive development, however, if no flowering occurs over a wide range of environmental conditions then the plant is probably still immature.

Most flowering plants pass through a series of developmental stages before reaching anthesis. That stage during which a shift from the vegetative to the reproductive state cannot be induced (even under conditions otherwise favourable for flower formation) is the juvenile phase (Wadi and Ram, 1967). Juvenility in plants was also defined by Schwabe (1971) as "..... the minimum growth requirement or delay, before sexual reproduction becomes possible ....." Higazy (1962) defined the juvenile phase as "... a period from seed germination during which no flower initiation can take place under conditions which are favourable at a later stage ..." The juvenile stage has also been called the minimum vegetative stage, immature condition or sterile stage and is often characterized by distinctive vegetative morphology. For example, in some plants juvenility has been discussed in terms of leaf shape and size, leaf abscission, phyllotaxy, thorniness, ease of rooting of cuttings and growth habit.

The stage at which a plant is able to flower has been called the mature or adult stage (Wadi and Ram, 1967) and the plant is referred to as having attained "ripeness-to-flower" (Klebs, 1918), "puberty" (Gregory, 1948) or "competence" (Cooper, 1952).

When defining the conceipt of juvenility some previous authors have included the plants requirement for specific environmental conditions before flowering can occur (Schwabe, 1971). In this study the juvenile phase will be discussed in terms only of the internal immature condition of the plant. This is diagramatically represented below.

Diagramatic Representation of Juvenility



(a) The juvenile phase as described by Schwabe (1971).
(b) The juvenile phase as defined for this study.

The duration of the juvenile phase varies between species. For example, the groundnut (Arachis hypogaea) actually carries flower initials in the seeds even before their germination (Wellensiek, 1957). Silene armeria L. has a juvenile period of around three months (Wellensiek, 1969). However, in some woody trees the juvenile phase lasts up to 30 to 40 years.

The fundamental causes of juvenility are unknown. However, various treatments have been shown to influence the length of the juvenile stage. Higazy (1962) noted that in plants of Silene armeria L. the juvenile stage was decreased under high light intensity. Wadi and Ram (1967) decreased the juvenile phase of Kalanchoe pinnata Pers. using gibberellic acid; similar results were obtained with Brassica oleracea using auxin (de Zeeuw and Leopold, 1955). Johnston and Crowden (1967) obtained earlier flowering in Pisum sativum by removing the cotyledons. Paton and Barber (1955), working with Pisum, obtained earlier flowering in late-flowering varieties by grafting late flowering stock to early flowering stock.

The objective of this present investigation was to determine if Melrose sainfoin has a juvenile phase and, if so, to determine the length of time this phase lasts before the plant can attain "ripeness-to-flower".

6.2 Materials and Methods

In this experiment cv. Melrose plants were used. Seed was sown on September 2 1975 into 10 centimetre plastic pots containing an U.C. soil/nutrient mix as described in Chapter 2. Each pot contained two plants. On November 29 1975 all plants were repotted into 15 centimetre plastic pots, still with two plants per pot. In this latter repotting an Osmocote soil/nutrient mix was used. For three weeks

prior to repotting all plants received a weekly 40 ml. dose of Hoagland's nutrient solution.

The experiment consisted of six treatments as shown on the next page, each of 30 plants. All plants in the glasshouse were grown in natural photoperiods; in some cases this was artificially lengthened or shortened. An important factor to consider is that as summer approached the mean daily temperature increased, the average light intensity per day increased and there was an increased daily duration of natural light. Figures demonstrating these changes are provided in Chapter 2. Because of this change in environment with time Treatment 6 was added thus a comparison can be made with Treatment 2 to determine, whether or not, temperature and light have any effect on the juvenile phase.

The reason for using the parameter of .20 percent of plants to have elongated stems per population (Table 6.1) to compare treatments was to enable a more reliable comparison to be made with the 12 week short day pretreated population as just after this period the plants in that population were defoliated by mites causing an inhibition of growth.

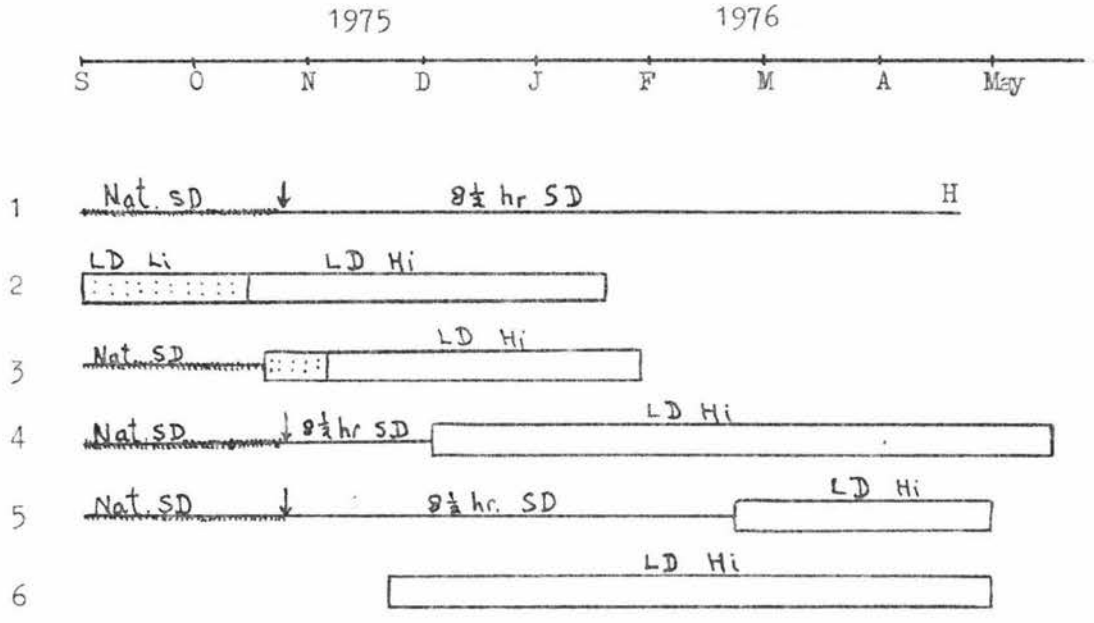
In Treatments 2 and 3 (i.e. 0/early sown, and 6 week SD pretreated populations respectively) the continuous light treatment was initially given in the controlled environment growth room (PAR = 320 microeinsteins/m^2/sec. at plant height). Due to the low intensity light and the difficulty in controlling mites, plants in these treatments were transferred into the glasshouse on October 18 and November 17 1975 (i.e. 0 and 6 week pretreated populations respectively) where a continuous light section had been curtained off. There the plants received natural light with a supplement of artificial light provided by 5 x 40 watt incandescent tungsten filament lamps (PAR = 100 microeinsteins/m^2/sec. at plant height) throughout the night. Treatments 4 and 5 (i.e. 12 and 24 week SD pretreated populations respectively) went straight into the continuous light conditions in the glasshouse.

Treatments receiving a 17 hour photoperiod all went into a curtained section of the glasshouse with supplemented morning and evening light provided by 5 x 40 watt incandescent tungsten filament lamps (PAR = 100 microeinsteins/m^2/sec. at plant height).

On October 27 1975 all plants not under long-day treatment were placed in artificially shortened 8½ hour photoperiods receiving natural light as described in Chapter 2.

On November 18 1975 more seed was sown (i.e. Treatment 6). Upon germination 30 plants (two per 10 centimetre pot) were placed immediately

EXPERIMENTAL TREATMENTS



Treatment

1. Short Day (SD) Control.
2. Early sown continual long days (LD)
   (a) 17 hour photoperiod (17hr).
   (b) Continuous light (CL).
3. 6 weeks SD followed by LD
   (a) 17 hr.
   (b) CL.
4. 12 weeks SD followed by LD
   (a) 17 hr.
   (b) CL.
5. 24 weeks SD followed by LD.
   (17 hr. photoperiod only).
6. Late sown continual LD treatment.

The above diagram is drawn to scale. Nat. SD = natural short day; 8½hr SD = artificially shortened natural day; LD Li = long days with low intensity light(i.e. controlled environment groth room); LD Hi = natural day with an artifically extended photoperiod; H = time when the final harvest was made.

into the 17 hour photoperiod. When required plants were repotted into 15 centimetre pots. The same soil/nutrient mixes as in the other treatments were used for respective pot sizes.

Treatments were kept in long photoperiods until flowering had been completed. The time of final harvest for each experimental group, shown on the diagram, was decided when no more new stem elongation was occurring on non-flowering plants. A stem was defined as having elongated when an internode had visibly elongated at least three mm.

The 17 hour and continuous light 12 week short-day pretreated populations (Treatment 4) received a heavy mite attack at the stage when stem elongation had just began. Because of this few parameters were measured in this treatment.

Often plants with elongated stems that did not have macroscopically visible inflorescences were dissected at the time of final harvest. At no time were inflorescences found.

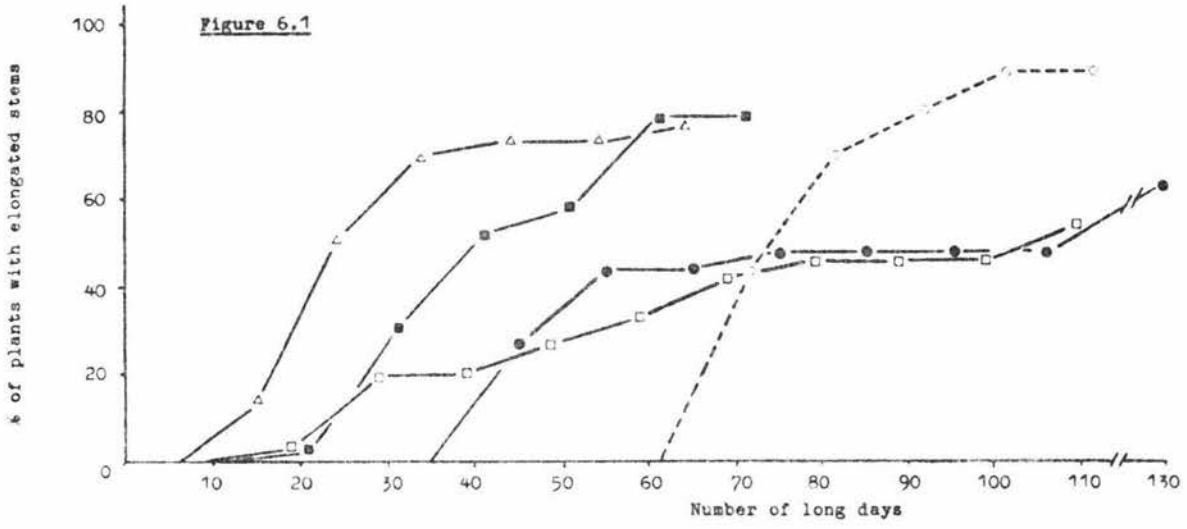
6.3 Results

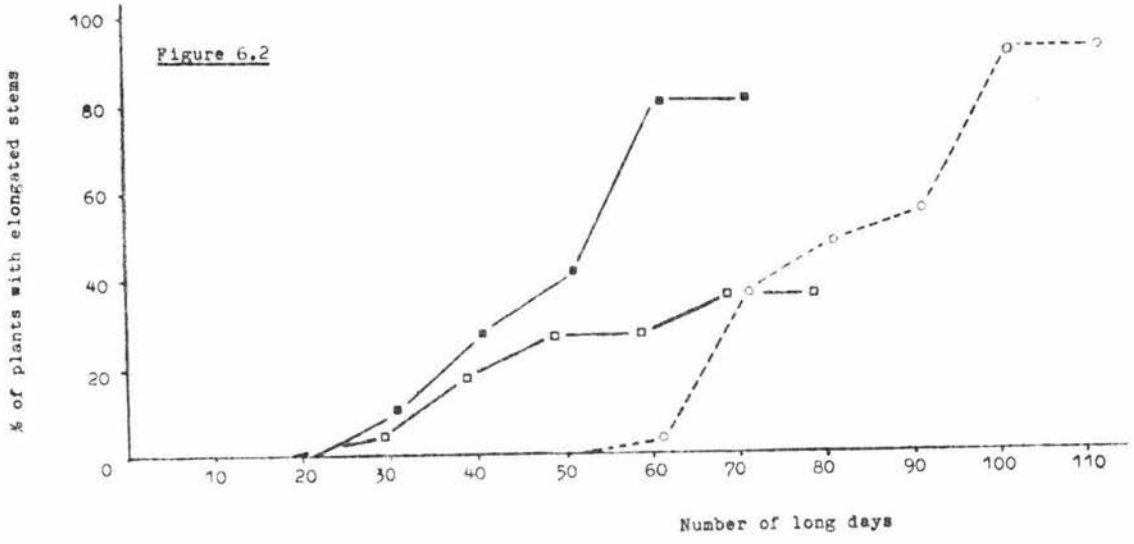
6.3.1 Stem Elongation

The percentage of plants to have elongated stems was recorded in the 17 hour photoperiod (Figure 6.1 and Table 6.1) and continuous light treatments (Figure 6.2 and Table 6.2). Treatments in the 17 hour photoperiod showed no significant differences. The same trend occurred in the continous light treatments. In the short day control treatment (Treatment 1) no plants had elongated stems or macroscopically visible inflorescence initiation throughout the period of this experiment.

As the plant age increased, the time required for stem elongation to begin, after transfer to both continuous light and the 17 hour photoperiod, decreased. There was no significant difference between the 6- and 12-week short day pretreatment populations in either the 17 hour photoperiod or continuous light treatments. For plants with no short day pretreatment the later sown population (Treatment 6) required fewer days for 20 percent of the plants to have elongated stems than did the earlier sown population (Treatment 2) (Table 6.1).

The length of elongated stems was also measured (Table 6.1, 6.2) but the large variation within each treatment makes it impossible to determine if any differences are significant. However, the differences within treatments of vegetative stems (i.e. stems without macroscopically





The effect of plant age at the time of transfer to a 17 hr photoperiod (Figure 6.1) and continuous light (Figure 6.2) on the percentage of plants with elongated stems. ○----- ○ 0 week short day (SD) pretreatment (early sown), ●——— ● 0 week SD pretreatment (late sown), □——— □ 6 week SD pretreatment, ■——— ■ 12 week SD pretreatment, and △——— △ 24 week SD pretreatment.

Table 6.1

The effect of plant age on stem elongation at final harvest for cv. Melrose in a 17 hour photoperiod: I percentage of plants having elongated stems, II number of long days required for 20% of plants within each population to have elongated stems, III average length of the longest vegetative and reproductive stem per plant. A vegetative or reproductive stem refers to whether or not a stem has a macroscopically visible inflorescence.

		Short Day Pretreatment (weeks)				
		0		6	12	24
		Early Sown	Late Sown			
Treatment No.		2	6	3	4	5
I % Stem Elongation		89	63	79	-	77
II No. days required to reach 20% stem elongation		66	42	27	29	17
III Av. length of longest stem per plant	Vegetative	18.85	28.46	*5.5	-	18.64
	± SEM	4.88	6.10			4.55
	Reproductive	53.31	53.50	58.91	-	66.31
	± SEM	6.77	3.59	6.61		4.73

*one plant only

Table 6.2

The effect of plant age on stem elongation at final harvest for cv. Melrose in continuous light, N.B. I, II and III are fully defined in Table 6.1.

		Short Day Pretreatment (weeks)		
		0	6	12
Treatment No.		2	3	4
I % Stem Elongation		92	81	-
II No. days required to reach 20% stem elongation		66	37	41
III Av. length of longest stem per plant	Vegetative	19.87	16.75	-
	± SEM	5.21	3.91	
	Reproductive	41.58	56.82	-
	± SEM	4.91	4.59	

visible inflorescences) compared to stems with inflorescence initiation are significant.

6.3.2 Flowering

Comparison within the individual population of Treatments 2, 6, 3 and 5 (i.e. 0 (early and late sown), 6- and 24-week SD pretreated populations respectively) of the percentage of plants that had inflorescence initiation and the percentage that blossomed, shows that only small differences occurred; thus few plants had 100 percent of their inflorescences aborting. However, there were differences between treatments. At the final harvest, Treatments 2 and 6 (i.e. no short day pretreatment) had a lower number of plants that had initiated inflorescences than did Treatments 3 and 5 (i.e. populations receiving short-day pretreatment). The same trend occurred for the percentage that had blossoming inflorescences (Table 6.3).

As the short day pretreatment increased, the number of long days required for the plants to reach the stage of blossoming decreased (Table 6.3). In Treatment 5 an average of 49.5 days was required for blossoming to occur in 17 hour long days whereas in Treatments 2 and 6 approximately 110 - 115 long days were required for blossoming to occur.

6.3.3 Growth Forms Associated with Long Days

For each treatment all plants were divided into one of three growth forms. At the final harvest some plants remained in the rosette form, other had elongated stems but did not flower, while others both elongated and flowered (i.e. initiated or blossomed) (Table 6.4).

When comparing the early sown and later sown populations which received no short day pretreatment, the later sown population had fewer plants in the rosette phase at final harvest.

6.4 Discussion

In this experiment there was an increase in the average daily temperature, an increase in light intensity throughout the day and an increase in the daily duration of natural light as summer approached. The later sown Treatment 6 population required fewer long days, for 20 percent of plants within the population to have elongated stems, than the early sown Treatment 2 population (Table 6.1). However, when

Table 6.3

The effect of plant age on reproductive development at final harvest in cv. Melrose. Parameters measured included: I the percentage of plants with inflorescence initiation, II the percentage of plants having blossoming inflorescences, III the number of long days required for the first and last plant to blossom, plus the average number of long days for all plants to blossom.

Short Day Pretreatment (weeks)

		0			6		24
		17 hr.		CL	17 hr.	CL	17 hr.
		Early Sown	Late Sown				
Treatment number		2	6	2		3	5
I	% of Plants with inflorescence initiation	21	25	20	39	38	30
II	% of Plants blossoming	18	17	20	36	34	20
III	No. LD's required to blossom — 1st plant	90	141	71	42	31	42
	last plant	129	147	131	96	100	64
	Average	112.6	144.3	114.6	69.2	71.9	49.5

Table 6.4

The effect of plant age and long day treatment on plant growth forms at final harvest for cv. Melrose. Three forms were noted; those plants that remained as rosettes, those plants having elongated stems but no flowering, and those that elongated and flowered. This latter group includes those plants that had all their visible inflorescences aborted.

			Growth Forms (%)		
			Rosette	Stem elongation no flowering	Flowering
0 week	17 hr.	Late sown	25	50	25
		Early sown	48	31	21
	CL		32	48	20
6 week	17 hr.		51	9	39
	CL		36	26	38
12 week	17 hr.		14	73	13
	CL		47	35	18
24 week	17 hr.		23	47	30
Control short day			100	0	0

averaging the number of long days required for all plants that blossomed to blossom it is observed that the 0 week short day late sown population (Treatment 6) required more long days to blossom than the early sown population (Treatment 2) (Table 6.3). This difference was caused by the late sown population receiving a mild mite attack that had the effect of slowing down the later stages of reproductive development.

The reason for presenting data on the various growth forms in each treatment is to provide some indication of how the non-flowering plants responded to long day treatment. When examining these data one must be careful not to 'over-analyze' as the variation displayed could be due to (a) the genetic variation within the cultivar and, (b) the fact that in some treatments a number of plants died; as the latter nearly always were in the rosette phase of growth the percentages of the other growth forms tended to increase in such populations in comparison to those in which no deaths occurred. What can be noted, however, is that there are large variations between figures within treatment populations for vegetative parameters whereas the flowering figures for each treatment population are relatively uniform (Table 6.4).

6.4.1 Flowering

(a) Number of days required to blossom.

If Melrose sainfoin does have a juvenile phase then one would expect that as the short day pretreatment period increased the time to blossoming, after transfer into long days, would decrease, until finally a standard time from initiation to blossoming was obtained. The results of this present study with Melrose sainfoin show that as the age of the plants increased the time required in long days for blossoming to occur decreased, with the greatest reduction occurring after plants have initially received six weeks of short day pretreatment (Figure 6.3). As the curve for blossoming in Figure 6.3 has levelled off by 24 weeks of short day pretreatment one can conclude that by this time the plants had attained 'ripeness-to-flower' before they received long days. If the plants in this latter population had no juvenility requirement then one can conclude that it takes approximately 50 days (Table 6.3) from the time of the first long day to blossoming. If this is so then one can also conclude that after six weeks of short day pretreatment the plants still had a juvenile

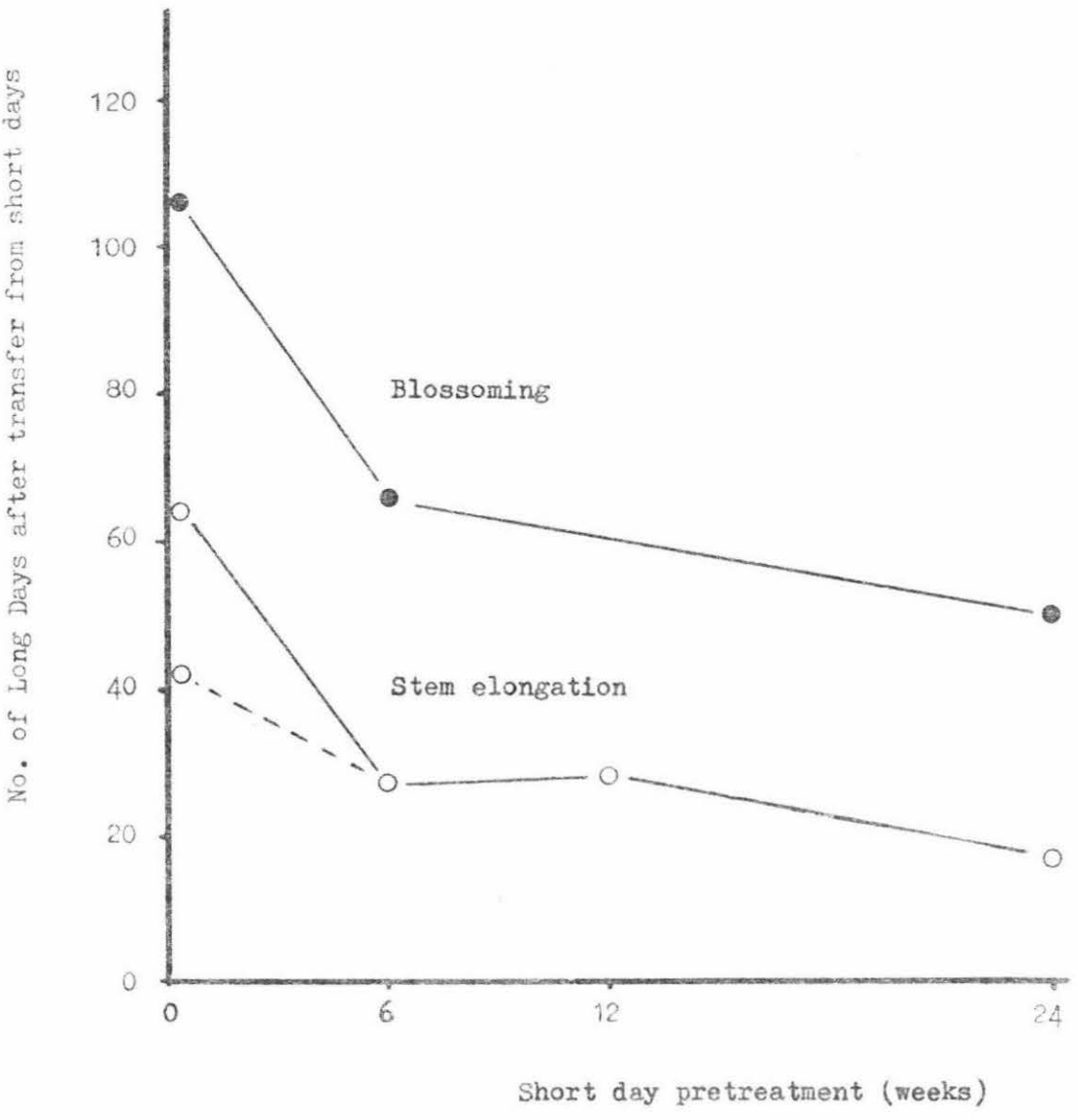


Figure 6.3

The effect of plant age on the number of long days (17 hr.) required for 20% of the plants in each population to have elongated stems (broken line refers to the late sown population); and the average number of long days (CL + 17 hr. data averaged) required to blossom.

period of approximately 20 days, while the plants that were transferred immediately into long days had a juvenile period of approximately 63 days.

Table 6.3 also shows that there were large variations in the blossoming dates within all treatment populations. For example, in the early sown 0 week short day pretreated population 90 long days were required for the first plant to blossom whereas 129 long days were required for the last plant to blossom. This could involve variability in the length of the juvenile phase between plants, different rates of inflorescence development, and varying responses to the environmental conditions experienced.

(b) Percentage of plants with inflorescence initiation.

An interesting feature these results show is that of percentage flowering between treatments (Table 6.3). In all the 0 week short day pretreatment populations total inflorescence initiation ranged from 20-25 percent. In populations that had either 6 or 24 weeks of short day pretreatment the average percentage inflorescence initiation was significantly higher. This suggests that a certain proportion of the plants within the Melrose cultivar may be short-long day plants. This observation is discussed in more detail in Appendix II.

6.4.2 Stem Elongation

Throughout this whole thesis study it has become obvious that there is a very close relationship between stem elongation and flowering. Even though previous authors have discussed juvenility in relation to inflorescence initiation I feel that the data produced in this study support the hypothesis that the juvenility phenomenon affects the whole reproductive process of sainfoin, including the first visible reproductive stage of leaf erectness and bud swelling. However, the time interval from the start of leaf erectness to blossoming for each treatment population was not obtained. There is however, a close relationship between stem elongation data and the time of blossoming data (Figure 6.3, compare Table 6.3 with Table 6.1 and 6.2). Because of this relationship with flowering I feel that the stem elongation data can be used to discuss aspects on juvenility in Melrose sainfoin.

The effect of plant age on stem elongation in the 17 hour photoperiod

is shown more clearly in Figure 6.3. After six weeks growth in short days there was a large decrease in the time required before stem elongation is shown in Figure 6.3. To reach the stage where 20 percent of the plants had elongated stems the earlier sown group required 24 days more than the late sown population. This demonstrates that the juvenile period is not a fixed character and can be influenced by the external environment. Studies with Silene armeria L have shown an effect of light intensity on juvenility. Plants grown under high light intensity (4773 compared to 942 $\mu W/cm^2$) required fewer days to budding (Higazy, 1962). Wellensiek (1969) has observed similar responses using the same species. This reported light intensity effect may not necessarily be due only to an increased photosynthetic assimilation rate. It might also be due to an increased metabolic rate (production of growth factors!) caused by an increase in temperature. These two envirnomental effects could possibly interact. Higazy (1962) provided no temperature data but it is possible, from examining his methods, that his low intensity treatments may have also been under a lower day-time temperature. Wellensiek (1969) provided no description of his experimental methods.

CHAPTER 7

THE EFFECT OF POT SIZE ON FLOWERING

7.1 Introduction

While initial experiments were being conducted on the growth and development of sainfoin it was noticed that fewer Melrose plants elongated when grown in small sized pots. In addition four three-month old Giant plants, in 10 centimetre pots, which had been held in short photoperiods, failed to elongate and flower when transferred to an artificially extended 17 hour photoperiod. In previous experiments nearly all Giant plants, when grown in larger pots, placed in long days elongated and flowered.

Upon examining the root system of each Giant plant from the small pots it was noted that there were only a few 'white' roots and many black/dark-brown roots, some of which might have been decaying. However, the root system could not be classified as being pot-bound in the sense that there was not extra soil space at the bottom of the pot for new root growth.

Few accounts of the effect of pot size on flowering have been published. Allard (1916) noted that tobacco plants grown in 10 centimetre clay pots had a smaller inflorescence than plants grown in 20 centimetre pots. However, the average number of nodes produced above the cotyledons to the inflorescence remained the same. Plants grown in the smaller pots did take longer to reach inflorescence maturity. Other plants grown in five centimetre pots remained as rosettes for periods as long as the normal life of the plant.

Because of the unusual results with Giant a more detailed observational study on the effects of pot size on flowering in Melrose was undertaken.

7.2 Materials and Methods

Melrose plants used in this experiment were sown on September 2 1975 into 10 centimetre plastic pots containing 400 cm^3 Osmocote soil/nutrient mix. At the start of October 1975 45 plants were placed into artificial short days for around four and a half months as described in Chapter 2.

On January 14 1976 plants were repotted into plastic pots of volume 1400 cm^3 and 4800 cm^3 using a 75:25 perlite: sand potting mix. Plants of one group were left in their original pots.

Thus there were three treatments of pot volume 400, 1400 and 4800 cm^3, each of 15 plants.

After repotting, plants were transferred to natural long days in the glasshouse (approx. 14½ hours). To make environmental conditions as uniform as possible periodically pots were repositioned around the bench.

Monday to Friday each pot received a daily application of Hoagland's nutrient (2 X normal strength) of 20 ml. On Saturday and Sunday water only was applied. Pot sizes 1400 and 4800 cm^3 had their nutrient solutions diluted with water to overcome any poor nutrient dispersal effect due to the larger pot size (i.e. all regions of the pot would receive nutrient). Water was added in proportion to pot volume. Hence each pot received the same amount of nutrient.

When the soil surface of the pots began to become dry, pots were hand watered using a plastic watering can (tap water).

From the start of the long day treatment the number of basal buds and elongated shoots per plant were counted and leaf lengths (the longest two per plant at each sampling time being measured) measured at 15 day intervals.

January 20 1976 and February 20 1976 all plants were tagged and the rates of leaf emergence recorded over the following 20 day periods. On each plant the youngest leaf on the primary shoot and one basal bud were tagged.

On March 15 1976 lights were erected (6 X 40 watt incandescent tungsten filament lamps providing an intensity at plant height of 100 microeinsteins/m^2/sec.) artificially increasing the photoperiod to 17 hours.

When the experiment was ended on April 12 1976 stem lengths, flowering data and root data were obtained. Root data included colour, length, number of main laterals and dry weight. Dry weights were carried out using brown paper bags, drying overnight at 100°C as described in Chapter 2. Root length was obtained by measuring the longest root regardless of whether it were a lateral branch or the main primary root. This involved spreading the root system out on a table and measuring the length from longest root tip to the base of the stem.

7.3 Results

7.3.1 Number of Basal Buds

The increase in the numbers of basal buds over the first 30 days

in all three treatments were similar. However after 30 days the plants in larger pots formed fewer new basal buds (Figure 7.1). This period of decreased bud production coincided with the period when inflorescence initiation was occurring.

It appears that the period when fewer basal buds were produced was shorter in the 1400 cm^3 treatment than in the 4800 cm^3 treatment. After 60 days the rate of basal bud formation in the 1400 cm^3 treatment increased again to become similar to its initial rate over the first 30 days (Figure 7.1).

7.3.2 Leaf Lengths

Leaf length was used to give an indication of the effect of treatments on plant vigour. The 400 cm^3 treatment had the lowest increase in maximum leaf length whereas the 1400 cm^3 and 4800 cm^3 treatments had greater increases in maximum leaf lengths (Figure 7.2). (N.B. This refers to the longest two leaves measured per plant at each sampling date). After 45 to 60 days the leaf growth began to level off. Figure 7.2 shows how the length of the longest leaves in all treatments increase with time yet the response is much greater for plants in larger pots.

7.3.3 Stem Elongation and Flowering

After 30 long days stem elongation had began in all treatments. However, the 1400 cm^3 and 4800 cm^3 treatments showed a much greater response than the 400 cm^3 potted plants (Figure 7.3). When the natural photoperiod was artifcially extended to 17 hours another stem elongation response was obtained in all treatments, especially so for the plants in larger pots.

The most notable feature in Table 7.1 is that in all parameters measured there is an increase in value with increasing pot size. In the largest pots 80% of the plants elongated whereas in the 400 cm^3 pots only 33% of the plants had elongated stems. In addition to this the length of flowering (includes stems with aborted inflorescences) and non-flowering stems was greatest in the large 4800 cm^3 pots. Those in the smaller 400 cm^3 pots had shorter stems (Table 7.1).

Exactly the same trend showed between treatments for percentage of plants with macroscopically visible inflorescence initiation, number of stems per treatment with inflorescence initiation (Table 7.1) and the number of elongated stems per treatment (Figure 7.3 and Table 7.1).

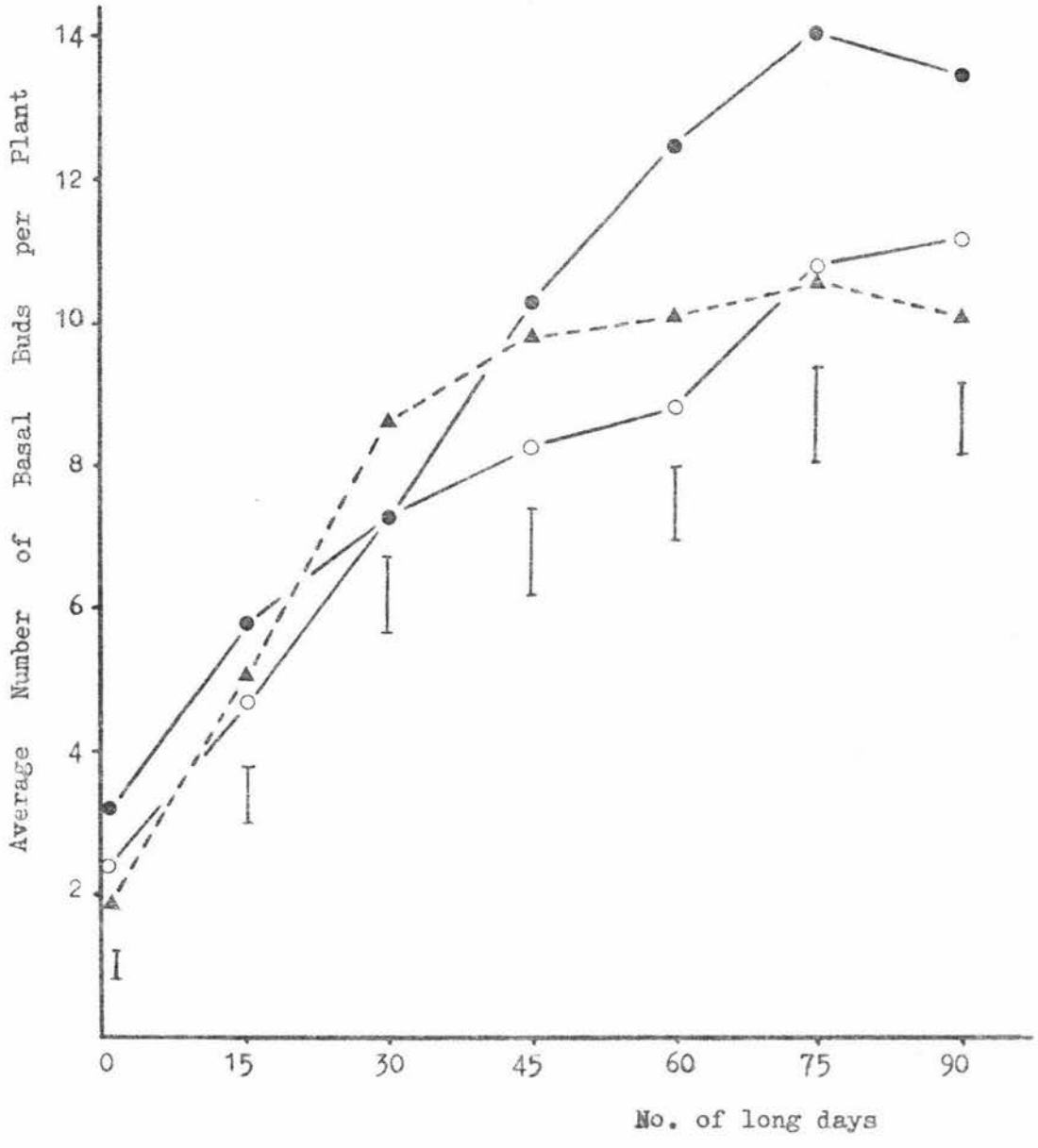


Figure 7.1

Average number of basal buds per plant for pot size treatments ●——● 400, ○——○ 1400 and ▲---▲ 4800 cm^3 in long days. Vertical lines indicate SEM values.

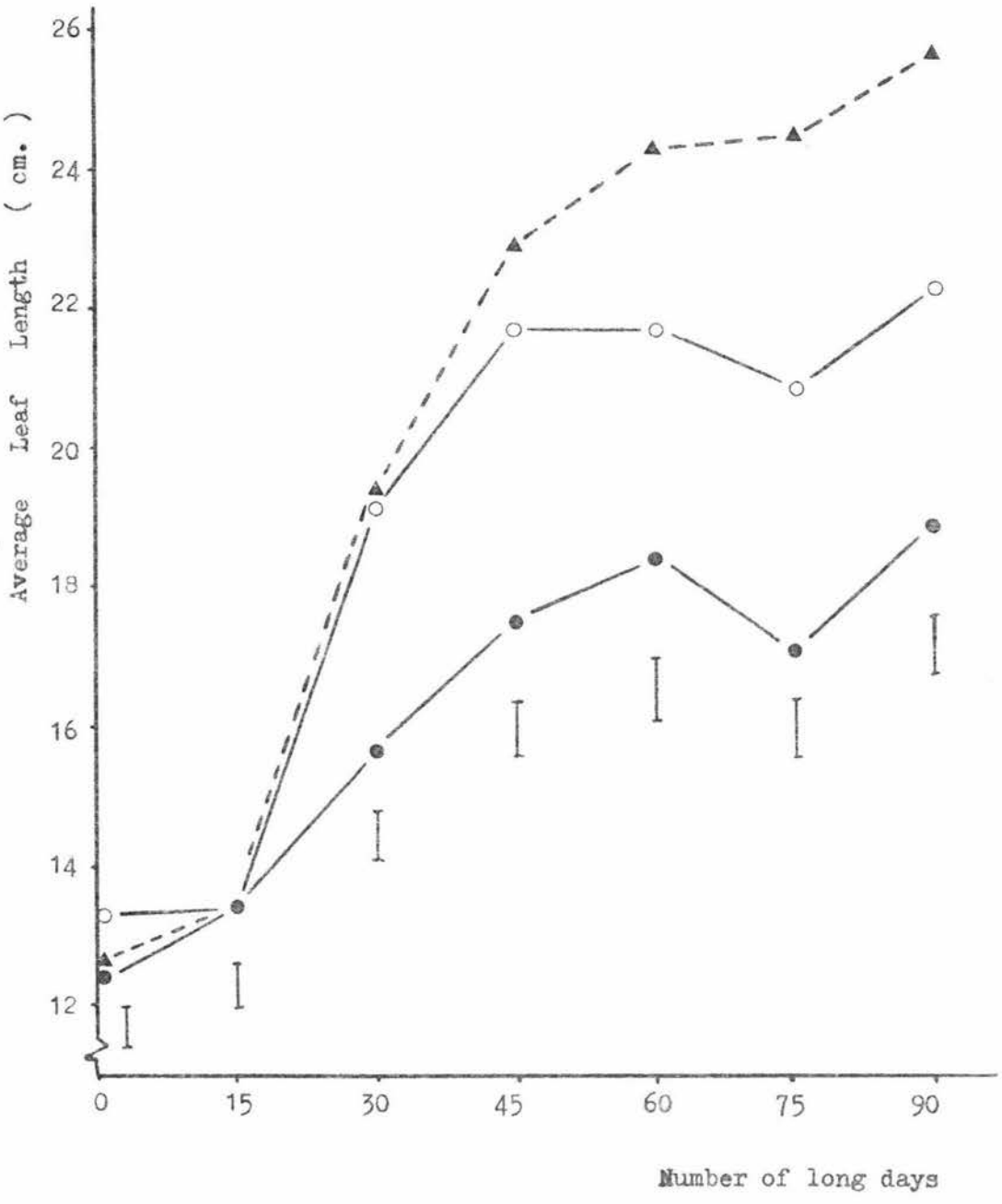


Figure 7.2

The average length of the longest two leaves on each plant at each measurement date for pot size treatments ●——● 400, ○——○ 1400 and ▲---▲ 4800 cm^3 in long days.

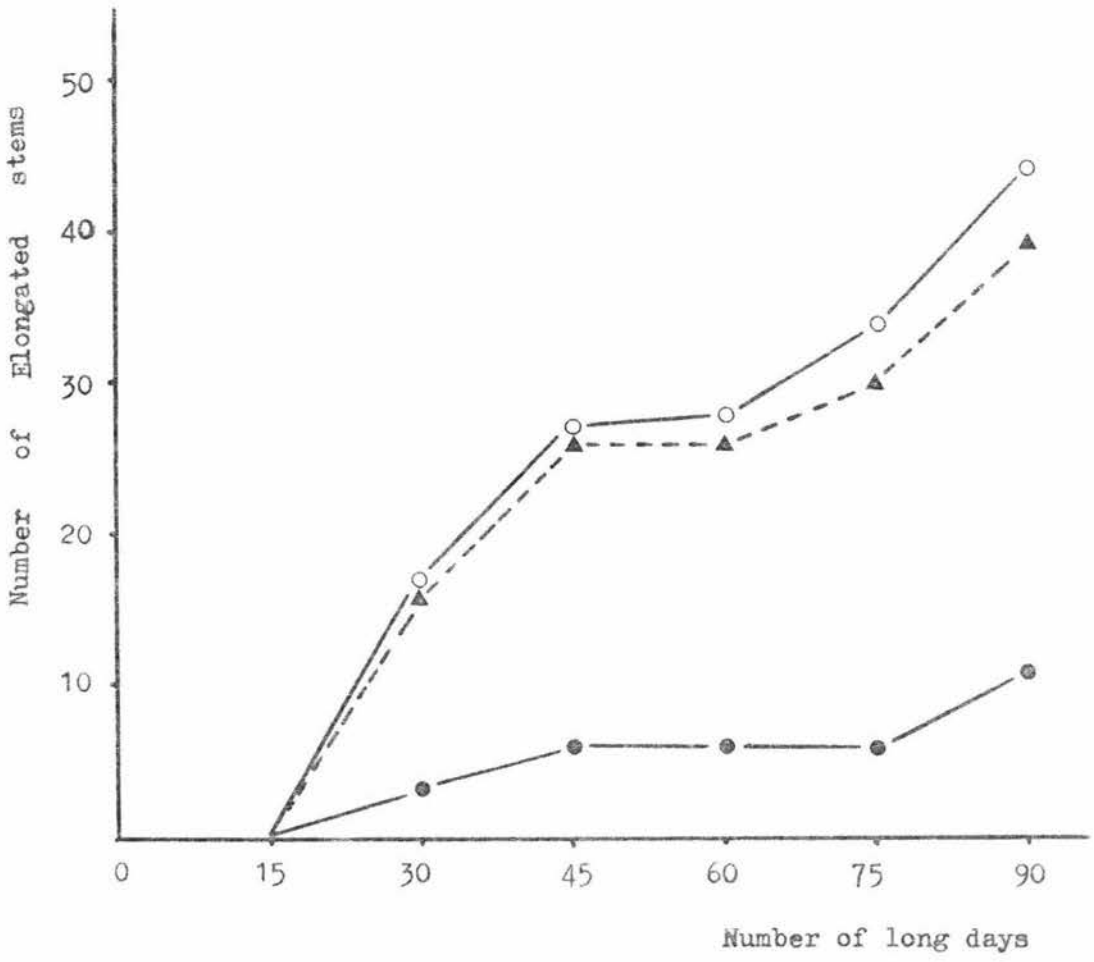


Figure 7.3

The total number of elongated stems per treatment for pot sizes ●——● 400, ○——○ 1400 and ▲---▲ 4800 cm^3 in long days. A stem was defined as elongated when one visible internode was greater than three mm. in length. NB. At the 60 day stage lamps were erected to provide an artificially extended 17 hour photoperiod.

It is shown that few of the elongated stems had inflorescence initiation.

Table 7.1

Effect of three pot sizes on stem elongation and flowering in Melrose after 90 days. SEM values provided. N.B. *i.e. macroscopically visible inflorescences.

	Pot Size (cm^3)		
	400	1400	4800
% of plants with elongated stems	33	73	80
Av. length of elongated non-flowering stems	3.45 ± 0.40	4.82 ± 0.94	7.27 ± 1.19
Av. length of elongated flowering stems	18.50 ± 4.33	25.14 ± 5.94	39.14 ± 6.55
% of plants with * inflorescence initiation	7	20	33
Total no. of stems per treatment with inflorescence initiation	3	5	14
No. of elongated stems per treatment	11	44	39

Few of the elongated stems had inflorescence initiation. For example, in the 4800 cm^3 treatment 36% of the elongated stems had inflorescence initiation, whereas in the 1400 cm^3 treatment only 11% of the elongated stems had inflorescence initiation. For plants in small pots 23% of the elongated stems had inflorescence initiation. However, this latter figure is misleading as there were only a few plants that showed elongation with one plant producing three stems with inflorescence initiation.

7.3.4 Rate of Leaf Emergence

Two counts were obtained which corresponded to the early shoot

elongation phase and the later inflorescence initiation phase in the plants' development.

The results show that for the early count there were no significant differences between treatments or between primary shoots and basal buds (Table 7.2).

On the count beginning February 20 1976 leaf emergence rates were lower than the first count beginning January 20 1976.

Table 7.2

Rates of leaf emergence from two growth stages in the plants development as affected by pot size. Results are expressed as the number of leaves emerged over 20 days. SEM values provided.

		Pot Size (cm^3)		
		400	1400	4800
Early Shoot Elongation Phase (Jan. 20 to Feb. 9)	PS	3.20 ± 0.24	3.60 ± 0.19	3.69 ± 0.21
	BB	3.00 ± 0.21	3.42 ± 0.23	4.00 ± 0.26
Inflorescence Initiation Phase (Feb. 20 to March 11)	PS	2.54 ± 0.21	2.00 ± 0.19	2.60 ± 0.19
	BB	2.54 ± 0.18	2.00 ± 0.26	2.71 ± 0.24

PS = Primary shoot bud or primary stem.
BB = Basal bud or secondary stem.

7.3.5 Root Growth and Development

Again data follow a similar pattern to that of above ground growth with the largest pot size plants having the highest values (Table 7.3). The length of the root system increased with pot size, although there was considerable variation between plants (note SEM values; Table 7.3). The number of large secondary roots also increased with pot size. There was a large difference (P less than 0.01) between the 400 cm^3 and 1400 cm^3 treatments. In the 4800 cm^3 treatment there was another slight increase over the 1400 cm^3 treatment.

Root colour was observed. In the 4800 cm^3 pots nearly all plants had healthy white/light-yellow roots. A similar situation occurred

in the 1400 cm^3 pots. However, in the 400 cm^3 potted plants the number of obviously healthy (i.e. white/yellow) roots was very much reduced; these being almost totally absent in some plants. Most roots were black with the appearance of dying or being dead in this group.

Table 7.3

Effect of pot size on the length of the root system, the number of main secondary lateral branches and root dry weight in Melrose. The main secondary lateral branches were clearly distinguishable as large laterals originating from the primary root. SEM values provided.

	Pot Size (cm^3)		
	400	1400	4800
Av. root length/plant (cm)	31.18 ± 4.52	39.27 ± 2.74	70.53 ± 8.79
No. of main secondary branches/plant	5.57 ± 0.68	10.13 ± 0.68	13.14 ± 0.93
Dry Weight (gms)	1.97 ± 0.36	3.19 ± 0.41	5.43 ± 0.59

7.4 Discussion

The results of this experiment show that the reproductive process in sainfoin can be affected by pot volume. Plants grown in small pots have their flowering process inhibited whereas those grown in larger pots appear to undergo normal development when placed in warm long days.

Shoot elongation and flowering data (Table 7.1) show a definite effect of pot size on growth. If the 4800 cm^3 treatment had the maximum percentage of plants with inflorescence initiation attainable under these glasshouse conditions, i.e. 33% (refer to Chapter 6 and 11) then it can be seen that the small 400 cm^3 potted plants had their reproductive process inhibited to such an extent that only 7% of the plants in that population had inflorescence initiation. All associated data in Table 7.1 show this same trend. It is difficult to determine if growth of the 1400 cm^3 potted plants was inhibited. Some parameters (number of stems with inflorescence initiation, shoot lengths) would suggest this, while other parameters (percentage shoot elongation, percentage inflorescence

initiation) would suggest little effect in comparison to the 4800 cm^3 potted plants.

Flowering shoots have stem lengths that are variable (Table 7.1). The reason for this is that in all treatments some stems had a few aborted inflorescences only with no fully developed inflorescences, and these stems had not elongated to any great length.

The root parameters measured (Table 7.3) also demonstrate differences between pot sizes. The length of the root system, lateral branching, and dry weight figures show the same trends as obtained for shoot elongation and flowering (Table 7.1). Root colour information was provided to give an indication of root health and vigour, white roots giving an indication of new and healthy growth.

The reason for adding a double strength Hoagland's nutrient solution was to obtain a good growth rate in all treatments. Initial tests showed that the smaller 400 cm^3 pots could only hold 20 cm^3 of solution; any in addition to this often drained out of the drainage hole in the pot. To overcome this problem the nutrient solution strength was doubled, thus effectively adding 40 cm^3 of Hoaglands solution to each pot. It should be realised that this experiment was carried out in mid-summer and considerable hand watering was necessary for the small pots. Due to this leaching effect a nutrient toxicity effect would be most unlikely

In the 1400 cm^3 and 4800 cm^3 pot volume treatments the basal bud formation pattern was similar to the Melrose plants in Chapter 5. That is, when stem elongation and flowering began, basal bud formation was reduced. The rate of basal bud formation in the 400 cm^3 pot volume treatment was similar throughout the long day treatment, although, on the last count (Figure 7.1) there was a decrease in the number of basal buds. At this period the small potted plants received a heavy mite attack.

The mite attack already mentioned also had an effect on leaf length measurements at day 75 (Figure 7.2.)

The lower rate of leaf emergence (Table 7.2) occurring in the second count could be due to the fact that the natural photoperiod was decreasing when these measurements were obtained. When individual plants were examined those that had blossoming inflorescences had higher leaf emergence rates than those that stayed at the rosette stage. For example, from the 4800 cm^3 pot treatment, plants with blossoming inflorescences had a leaf emergence rate of 3.20 per 20 days whereas plants in the rosette form had

a rate of only 2.30. However, the rate for the blossoming plants is still lower than the overall average leaf emergence rate for the plants when measurements were first made during the early stem elongation phase of development.

In Chapter 8 this effect of pot size on reproductive development in sainfoin will be discussed in more detail.

CHAPTER 8

SHOOT AND ROOT GROWTH IN INDUCTIVE PHOTOPERIODS AS AFFECTED BY POT SIZE

8.1 Introduction

In Chapter 7 data were produced showing that pot size can have an effect on reproductive development in sainfoin. However, in that experiment, because a destructive analysis was required for root parameters to be measured, all measurements made before the final harvest were associated only with characters of the plant occurring above ground level.

Few papers have been published on aspects related to this pot size effect on flowering. There have been, however, some papers published describing root and shoot growth when plants are progressing towards reproductive maturity. During the development of flowers and/or fruits in most plants there is an increase in shoot growth relative to that of the root (Troughton, 1956; Stuckey, 1941; Weaver and Himmell, 1929; Roberts and Struckmeyer, 1946; De Stigter, 1969).

De Stigter (1969) showed in cucumber that three to four days after pollination the root growth rate began to decrease; this decline continued until root growth stopped completely, while harvesting the fruit resulted in a gradual recovery of root growth. It appears that in the cucumber fruit development directly affects root growth.

Beever and Woolhouse (1975) followed the pattern of root and shoot growth of Perilla frutescens growing either in florally inductive photoperiods or in non-inductive photoperiods. Their results showed that by the 20th day of inductive treatment (i.e. around five days before the first flowers were pollinated) the rate of dry matter accumulation in the roots was slower in induced than in non-induced plants. Also the rate of root elongation became slower in induced plants than in non-induced plants, and the frequency of branching was greater in the roots of induced plants.

Factors other than flowering are known to be capable of modifying root/shoot ratios. These include: soil type, plant variety, light intensity, photoperiod, partial defoliation, girdling, growth substances (Roberts and Struckmeyer, 1946) and soil aeration (Kramer, 1949).

As detailed information on root and shoot growth is limited, an experiment was designed to study sainfoin development closely over a period of time after transfer into inductive photoperiods.

The experiment also allowed the study of the effects of pot size on root development and growth in Giant and Common as well as Melrose. Thus "flowering" and "non-flowering" cultivars were compared.

The data produced in this experiment assist understanding of the results that were obtained in Chapter 7 as well as providing an indication of what actually occurs to the roots when sainfoin plants are induced to flower.

8.2 Materials and Methods

Plants of sainfoin (cv. Melrose, Giant, Common) were grown from seed sown on April 10 1976 in the glasshouse in natural short photoperiods. The potting medium used throughout this experiment was fine gravel (as defined by Taylor and Pohlen, 1962; N.Z. Soil Survey Method) from which the finer particles had been removed by passing it through a three millimetre commercial metal crusher sieve; particles were of a uniform size and rounded rather than flat and knife shaped. Plants were initially sown into 200 cm^3 plastic pots (one plant/pot). When plants were well established (i.e. just prior to long day treatment) they were repotted into either 400 cm^3 or 4800 cm^3 plastic pots.

Throughout the experiment all plants received a daily application of 20 ml. double strength Hoagland's solution. Nutrient was applied to different regions of the pot at each application so that all regions of the pot, at some stage, received nutrient. After nutrient application each large sized pot was hand watered to ensure uniform nutrient mobility and dispersion.

Using tap water all large pots were watered twice daily, morning and evening, while the small pots were watered only once daily initially. As plants in the smaller pots became larger and the transpiration rate increased, watering twice daily was often required.

On June 30 1976 (i.e. after three months short day conditions to allow the juvenile period to pass) all plants were transferred into an artificial 17 hour photoperiod. This consisted of natural light within the glasshouse supplemented morning and evening with incandescent tungsten filament lamps providing an intensity at plant height of 120 microeinsteins/m^2/sec. (Plate 2.3a)

The experiment involved three cultivars (96 plants of both Giant and Common, and 160 plants of Melrose) in two pot sizes (volumes 400 cm^3 and 4800 cm^3). At seven day intervals after transfer to inductive

photoperiods six plants per cultivar were selected on a random basis for dry weight analysis of the roots and shoots. On the 35th and 49th days after transfer, however, 18 plants of Melrose were harvested, while on day 42 fourteen plants were harvested.

The easiest and most gentle method of separating the gravel from the roots was to fill a 44 gallon drum with water and immerse the whole plant, then invert the pot, at the same time drawing the pot away from the root system of the plant. Due to the effect of the water the gently falling stones did not damage the delicate young roots. A near perfect root/soil separation was obtained with virtually no root loss or damage at all.

Procedure for dry weight analysis involved placing individual roots and shoots in individual pyrex beakers, leaving them at the ambient laboratory temperature (approx. 20°C) for two hours, then transferring them to an air-circulated drying oven (Watvic Model) at 100°C (± 3°C) overnight.

In each cultivar for the first five harvests, two plant root systems from each treatment were preserved (70% Alcohol). At the end of the experiment these roots were photographed. In addition, a count, designed to give an indication of the number of root apices per plant was made for comperative purposes. This involved floating out the root system on a water filled tray and making a count of the number of apices four centimetres back from the tips of the main root ends. This also included secondary and tertiary etc. roots that were attached within the apical four centimetres of the main roots. When this had been completed the root system was placed into the beaker and a dry weight analysis made.

In addition to the treatments in which plants were grown in gravel six plants of each of Melrose and Common were grown in a 50:50 peat/perlite potting medium in both large and small pots similar to that used in the previous experiment. Nutrient was added in the same manner and same quantities as in the gravel treatments. At the end of the experiment data were obtained and compared to those from the gravel treatments. This peat/perlite group received the same photoperiod treatment as the gravel groups.

SEM values relating to Figures 8.1 and 8.2 are provided in Appendix III.

8.3 Results

8.3.1 Shoot Growth

Table 8.1 gives an indication of the developmental stage that the cultivars Melrose and Giant had reached when plants were harvested for dry weight determinations. These two cultivars seemed to begin reproductive development at the same time, although Giant reached the blossoming stage slightly earlier than Melrose. Common never passed the stage of leaf erectness.

Table 8.1

Time course of reproductive development in sainfoin exposed to long days.

Days of Inductive Treatment	Stages of Development	
0	I	Prostrate habit
7	II	Leaves becoming erect
14	III	Bud swelling by no completed
21	IV	Stems on some plants are beginning to elongate
28	V	Root decay first observed
35	VI	Small inflorescences becoming visible
42	VII	Inflorescences becoming larger
49	VIII	Blossoming just beginning. More advanced in Giant.

At stage IV stem elongation was just beginning in Giant and Melrose (Table 8.2); none of the differences between large and small pots were significant. In all populations those plants that did elongate did so at approximately the same time. In the populations sampled at the end of the experiment 70% of Melrose plants in both pot sizes had elongated stems, while all Giant plants in both pot sizes had elongated stems.

The average length of elongated stems in each population was measured on day 49 (i.e. stage VIII). Again no statistically significant differences between pot sizes or between cultivars were noted. What was noted, however, was that the small potted Giant plants had elongated stems

Table 8.2

The effect of pot size on: the percentage of plants with stem elongation, stem length, number of basal buds, flowering, and inflorescence abortion for Melrose, Giant and Common after exposure to seven weeks inductive long day treatment. Percentages of plants with elongated stems 21 days after start of inductive long day treatment are indicated to brackets.

	Melrose		Common		Giant	
	Small	Large	Small	Large	Small	Large
% of plants with stem elongation	78 (59)	72 (45)	0 (0)	0 (0)	100 (47)	100 (61)
Av. length of elongated stems (cm) ± SEM	23.3 ± 4.1	24.1 ± 4.9	0	0	27.2 ± 6.1	35.0 ± 7.3
Av. no. basal buds and secondary stems/plant ± SEM	2.00 ± 0.42	3.11 ± 0.78	4.00 ± 0.56	3.0 ± 0.45	4.00 ± 0.56	4.33 ± 1.26
% of plants with macroscopically visible inflorescences	17	11	0	0	83	67
% of inflorescences aborted	20 * ($\frac{1}{5}$)	62 * ($\frac{5}{8}$)	0	0	77 * ($\frac{10}{13}$)	45 * ($\frac{9}{20}$)

* Bracketed figures refer to the number of aborted inflorescences out of the total number of visible inflorescences.

that were much stronger compared to the large potted plants possibly due to early stem lignification. This strengthening is characteristic of a stem on a normal plant that has finished blossoming. The large potted Giant plants had soft stems. Because of this observation some significance should be attached to the stem lengths of large verus small potted Giant plants (Table 8.2). This early stem hardening was not observed on any Melrose plants.

On day 49 the average number of basal buds per plant was counted. The data indicate that pot size had no significant effect on basal bud development (Table 8.2).

The percentage of plants with healthy visible inflorescences was also calculated from the population analyzed on day 49 (Table 8.2). There were no significant differences in flowering behaviour between plants of Melrose and Giant grown in pots of different sizes although Giant had a significantly higher percentage of plants flowering than Melrose. In this last analysis (i.e. at Stage VIII) the percentage of inflorescences aborted was also calculated. There were fewer inflorescences macroscopically visible on Melrose because of slow inflorescence development; thus not too much notice should be placed on the abortion results presented for Melrose as these percentages were calculated from only a few inflorescences (Table 8.2). In Giant, inflorescence development was slightly more advanced. In this latter cultivar the percentage of inflorescences aborted was much greater in the small potted plants compared to that of the larger potted plants (Table 8.2).

In the peat/perlite control Melrose plants (Table 8.3) a pot size effect similar to that found in the previous experiment (Chapter 7) did show up for the percentage of plants flowering, percentage of inflorescences aborted and stem lengths on elongated plants. When the results in Table 8.2 are compared to Table 8.3 it is observed that the peat/perlite control plants had significantly higher figures for corresponding parameters measured (i.e. percentage flowering, average stem length). This was clearly noticeable visually also.

In Figure 8.1 shoot dry weights are presented for Melrose, Giant and Common. Except the large potted Common plants, all cultivars in all pot sizes showed a large increase in shoot dry weight after seven long days. For both large and small potted Melrose plants there was only a small increase in shoot dry weight from day 7 to day 42; after day 42 the shoot dry weights began to increase again at a greater rate with the large potted plants having a greater shoot dry weight increase than the

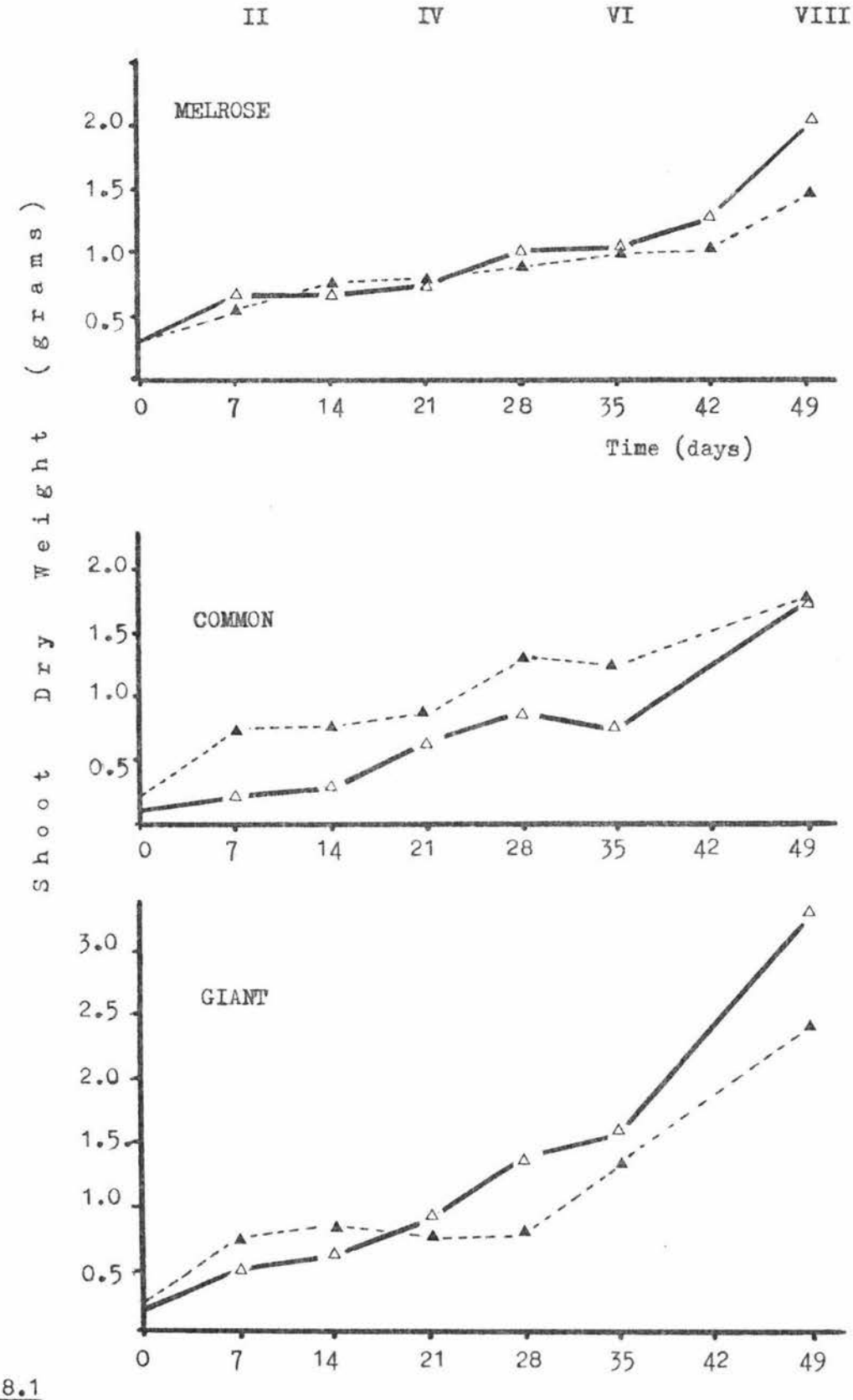


Figure 8.1

Shoot dry weights for Melrose, Common and Giant when transferred to long days. Solid lines refer to large potted plants, broken lines refer to small potted plants. Stages of reproductive development are noted (Table 8.1).

Table 8.3

The effect of pot size on the percentage of plants flowering, the percentage of inflorescences aborted, the average length of elongated stems and basal bud development for Melrose and Common grown in a peat/perlite potting medium (i.e. the control group).

	Melrose		Common	
	Small	Large	Small	Large
% of plants flowering	0	83	0	17*
% of inflorescences aborted		28		100
Av. stem length ± SEM	38.7 ± 7.90	49.2 ± 4.61	-	-
Av. no basal buds/plant ± SEM	5.5 ± 0.85	3.5 ± 0.56	3.3 ± 0.33	2.2 ± 0.31

* This consisted of one plant and was the only Common plant throughout this thesis study that had inflorescence initiation without prior low temperature pretreatment.

Table 8.4

The percentage of plants with root decay in Melrose, Giant and Common on days 28, 35, 42 and 49 for large (vol. = 4800 cm^3) and small (vol. = 400 cm^3) potted plants.

	Cultivar/Pot Size					
	Melrose		Common		Giant	
Stages of development	Small	Large	Small	Large	Small	Large
VIII Day 49	44	11	33	0	0	0
VII Day 42	62	15	0	0	0	0
VI Day 35	39	5	0	0	0	0
V Day 28	67	0	17	0	0	0
Overall Average (%)	53	8	12	0	0	0

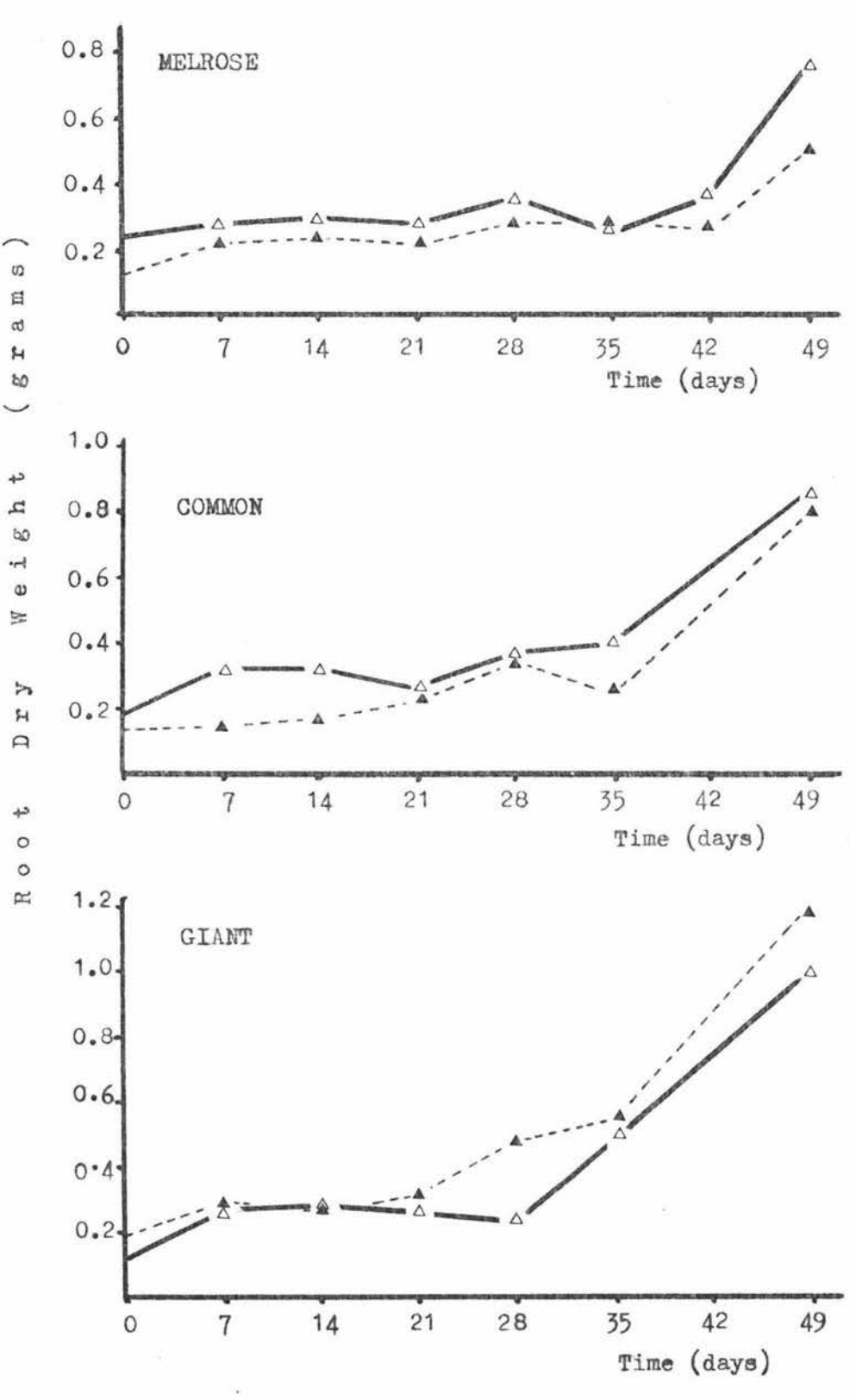


Figure 8.2

Root dry weights for Melrose, Common and Giant when transferred to long days. Solid lines refer to large potted plants, broken lines refer to small potted plants. Stages of reproductive development are noted (Table 8.1).

small potted plants. For small potted Giant plants there was a period of little change in shoot dry weight from day 7 to day 28; after this a larger increase occurred. For the large potted Giant plants there was a steady increase in shoot dry weight from day 0 to day 35; after day 35 a very large increase in shoot dry weight occurred. There was a steady rate of increase in shoot dry weight for both large and small potted Common plants from day 7 to day 49.

Initially for Giant, and throughout for Common, the small potted plants had a higher shoot weight than the larger potted plants whereas at the final dry weight measurement both Giant and Melrose had a higher shoot weight in large potted plants, especially so in Giant. Giant also had a much greater shoot dry weight at the end of the experiment than either Melrose and Common. In fact the small potted Giant plants had greater shoot dry weights than the large potted Melrose plants (Figure 8.1).

8.3.2 Root Growth

Root dry weights were determined at each harvest date (Figure 8.2). For all cultivars and pot sizes the main trend to show for root dry weight is an initial small increase between day 0 to day 7, a period of little root dry weight increase between day 7 to approximately day 35 then a large increase from day 35 to 49. However, for Melrose the main final period of root growth did not occur until stage VIII whereas in Giant an increase began at stage VI (i.e. day 35). Note that for Common and Giant no stage VII dry weight analysis was made. Because of this it is unfortunately not possible to determine when the increase for Common actually occurred i.e. whether Common was similar to Giant or similar to Melrose.

At the end of the experiment both large and small potted plants of Giant had a greater root dry weight than those of Melrose or Common. There were no significant differences in final weights for small versus large potted plants of Common and Giant. Large potted Melrose plants had a higher average root dry weight than the corresponding small potted plants.

Root decay was first noted at the stage IV analysis (Table 8.4). This involved the roots becoming a brown/black colour which could progress until finally no white roots were present. Melrose plants most frequently showed root decay, especially so the small potted plants

(Table 8.4). In Giant, root decay was never noted.

Plants having root decay could not be visually distinguished, prior to removal from the pot, from those plants with healthy root systems. In fact at stage VIII 47% of the Melrose plants that had root decay had elongated stems with an average length of 22.80 $\pm$ 5.36 cm., while those on plants with non-decayed roots had an average length of 23.50 $\pm$ 6.00 cm. At the last count no Melrose plants showing root decay but having elongated stems had visible developing inflorescences.

Pot size did affect the morphology of the root system of all cultivars (Plate 8.1). Plants in large pots had a root system that could be divided into two morphologically discrete parts, one at the bottom of the pot, and another towards the soil surface. Connecting these two areas of root development were larger primary and/or secondary roots. In the small pots the root system branched out so as to fill the whole pot.

The number of root apices per plant is shown in Figure 8.3 up until Stage V. For Common and Giant there was no significant change in the number of root apices per plant after transfer into inductive photoperiods. However, for Melrose there was a significant increase in the number of root apices beginning at stage IV for large potted plants, and stage V for small potted plants.

8.3.3 Root: Shoot Ratio

The root:shoot dry weight ratios for each cultivar are shown in Figure 8.4. For all cultivars the main trend noted was a gradual decrease in the root:shoot ratio with time. In addition the plants, for each cultivar, grown in large pots had a correspondingly higher root:shoot ratio values than the plants grown in the smaller pots.

For Melrose the root:shoot ratio decreased soon after plants were placed into inductive long days. With time the rate of decrease became less until finally levelling off at day 35. In the small-potted plants the decrease was not so great as in the large-potted plants mainly because of day 0 the small potted plants had a lower root:shoot ratio.

For the small potted Giant plants the root:shoot ratio curve began to increase again by day 49. This was due to a greater increase in the dry weight of the roots rather than a decrease in the shoots.

Common, that is the "non-flowering" cultivar, initially had a decrease in the root:shoot ratio (Figure 8.4); between day 21 to 35 there was a levelling off; and finally by day 49 the curve increased. This same graph shape occurred for both small and large potted plants.

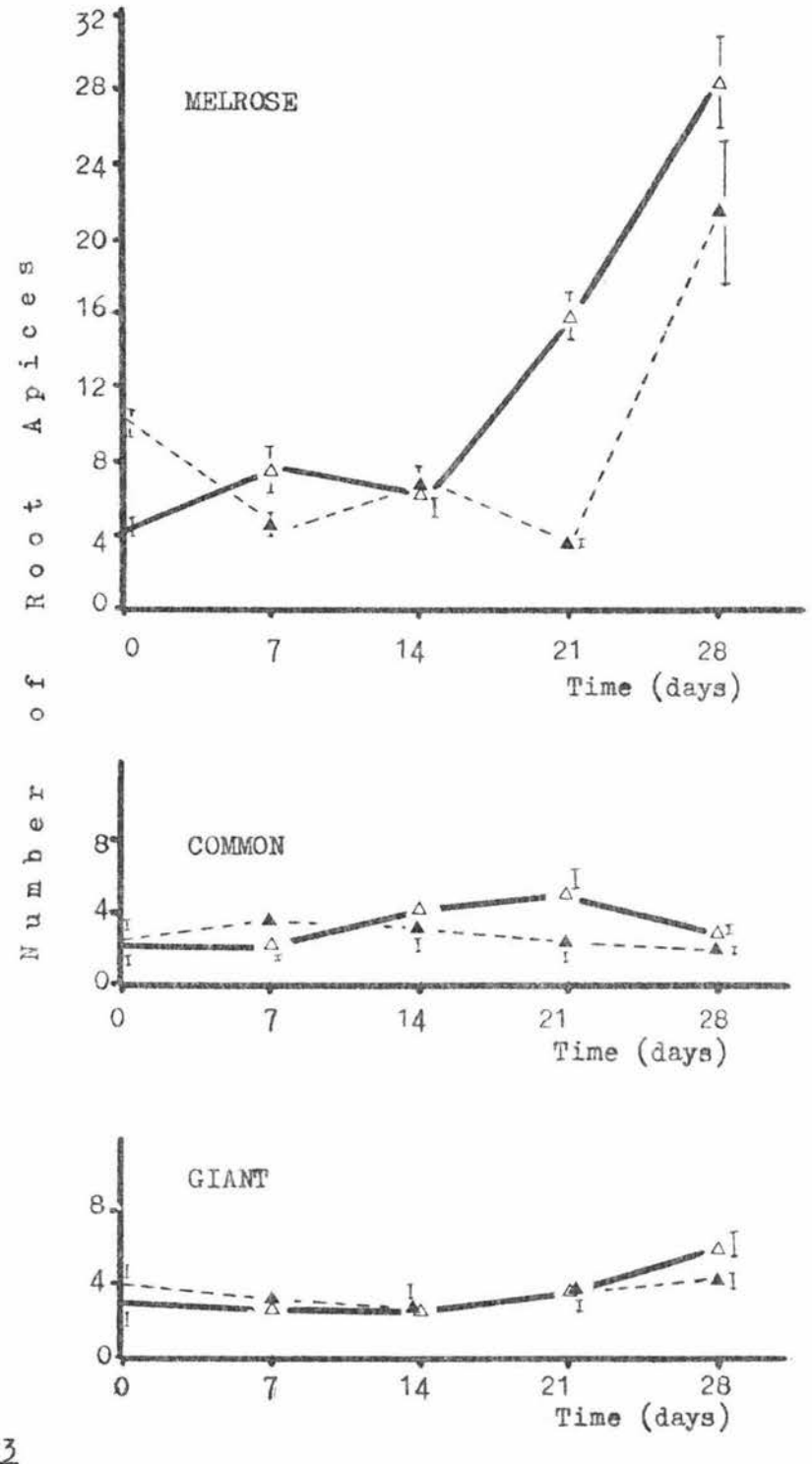


Figure 8.3

Average number of root apices (see section 8.2) for Melrose, Common and Giant when transferred to long days. Solid lines refer to large potted plants, broken lines refer to small potted plants. Vertical lines indicate SEM values.

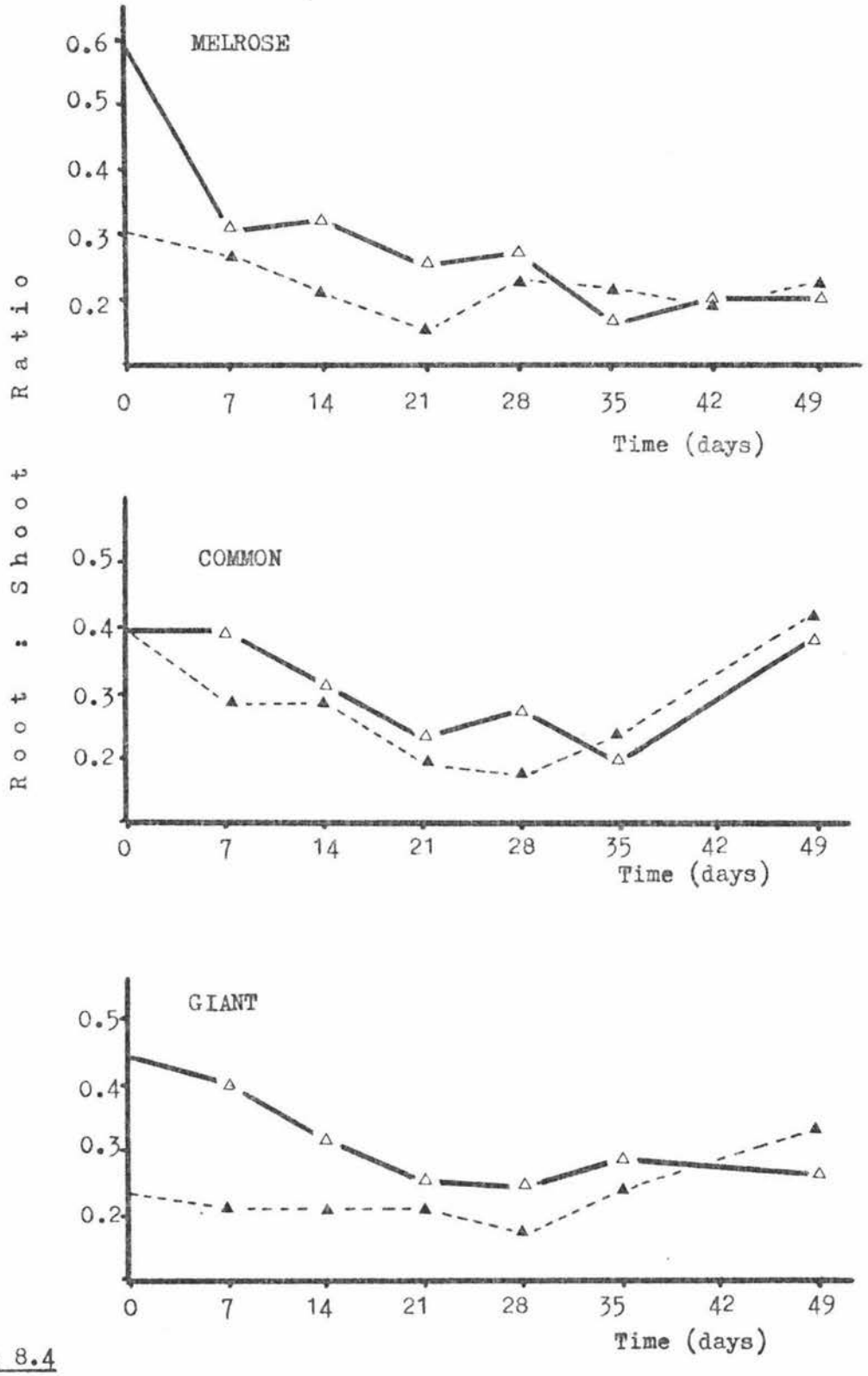


Figure 8.4

Changes in the Root:Shoot Ratio for Melrose, Common and Giant when plants were transferred to long days. Solid lines refer to large potted plants, broken lines refer to small potted plants.

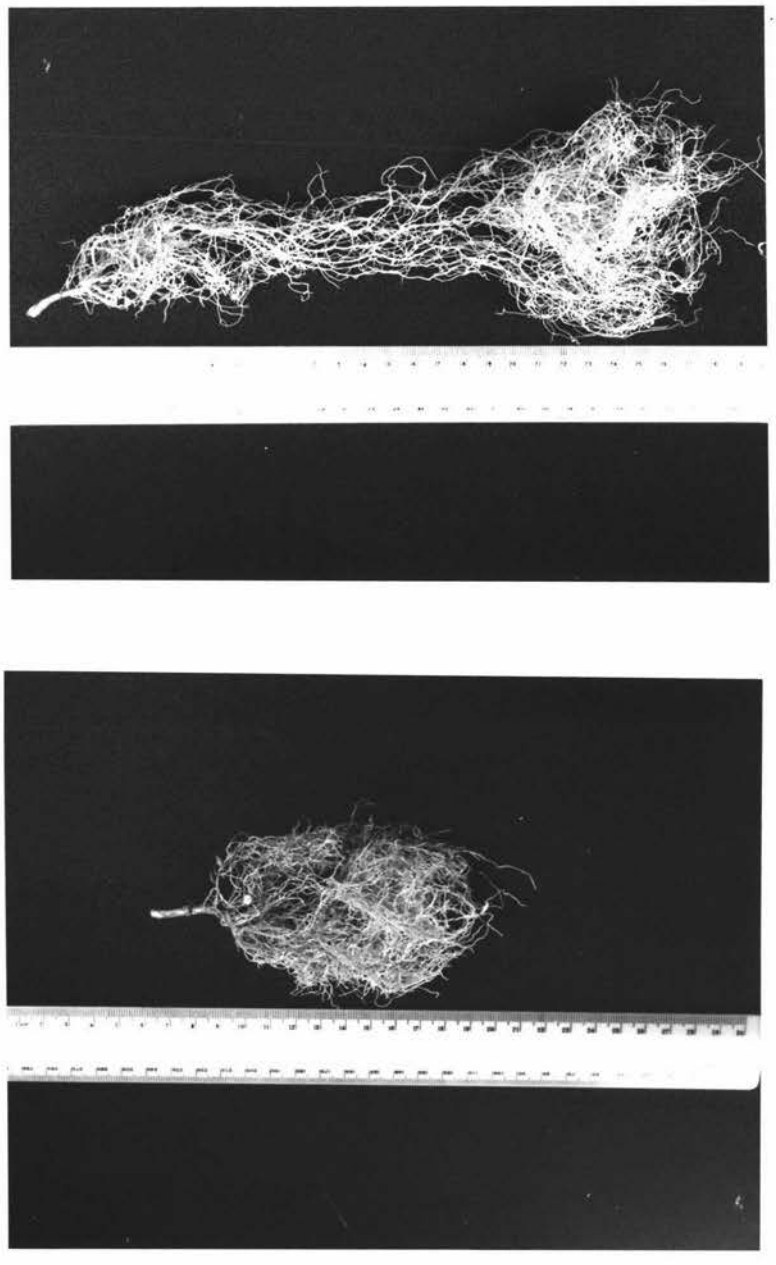


Plate 8.1

Root morphology of sainfoin plants when grown in 4800 cm^3 pots (upper photograph) and 400 cm^3 pots (lower photograph).
Ruler divisions equal 1 cm.

8.4 Discussion

To explain the results in Chapter 7 various hypotheses have been put forward that might explain why this observed pot size effect occurred. These include:

(a) The root system might have to reach a critical volume or size before reproductive development can proceed. Thus when a plant is grown in a small pot it might be unable to reach a critical root volume or size. The idea behind this hypothesis is that the root system might have to produce a certain amount of growth substances for full reproductive development to occur.

(b) For the plant to progress with reproductive development a critical root:shoot ratio might be required. Possibly growth in small pots could upset this ratio balance thus in effect inhibiting development.

(c) When a plant is transferred to long days root growth might need to occur at a certain rate so as to effect reproductive development. Possibly small pots do not allow the plant to have a normal root growth rate.

(d) It is possible that a change in the pattern of root growth might be responsible for reproductive development to occur with small pots inhibiting this pattern change. This could involve the root system initiating more lateral roots as against developing already present roots. This would then increase the amount/volume of root meristematic tissue. That is, the tissue thought to be responsible for hormone synthesis (Wilkins, 1969).

(e) Another factor to consider is the possibility of a self-poisoning effect caused by the secretion of allelopathic substances from the roots. Due to a small pot excreted substances from sainfoin roots that inhibit growth in other plants under natural conditions might become self-inhibitory if the inhibitor concentration reaches a certain level. This could occur because many roots are growing too close together. This might involve (i) the blocking of movement of "growth factors" that move from the stem to the root (i.e. possibly blocking the removal to the roots and/or breakdown within the roots of a stem produced inhibitor, or blocking the movement to the roots of a root growth promoter), (ii) it may inhibit the synthesis (directly or indirectly) of growth substances in the root, or (iii) it might inhibit the transport of growth factors from the root system to the shoot.

(f) Adequate oxygen is essential for root growth, and within the temperature limits for root growth, the greater the potting medium temperature the greater must be the concentration of soil oxygen for

for normal growth. Thus insufficient oxygen within the potting medium as the roots increase in size might inhibit the production of growth factors within the root.

(g) Finally it could be possible that the roots have no role to play in the reproductive process.

In this experiment there was not such a pronounced pot size effect as that noted in Chapter 7. However, a definite effect was observed in the small potted Giant plants (percentage abortion, stem height, stem hardening) and the small potted Melrose peat/perlite control plants (percentage flowering, stem length). This latter group plus the large potted Melrose control plants were unusual in that the size of the plants was much greater than that of their counterparts grown in gravel. This appears to be a potting medium effect and the reason for this might be the better retention and availability of nutrient in the peat/perlite medium than in the gravel.

De Stigter (1969) noted that reproductive development in the cucumber caused a decline in root growth until it finally stopped completely. Beever and Woolhouse (1975), however, noted in Perilla that there was still an increase in root dry weight with time even after flower buds had appeared. For Giant and Melrose sainfoin there was also an increase in root dry weight with time even after flower buds had appeared. For Giant and Melrose sainfoin there was also an increase in root dry weight as inflorescence development progresses. It is observed in Figure 8.2 that the increase in root dry weight for Giant is similar for both large and small potted plants. Thus it would appear unlikely that sainfoin requires a root growth rate above a critical level to effect inflorescence initiation as the Giant plants in large pots showed no effect of pot size whereas the plants in small pots did.

From these experimental results one can not rule out the possibility that a lack of oxygen around the roots caused this pot size effect. However, it does not seem unlikely as a pot size effect was observed in the small potted Giant plants when grown in a fine gravel medium. In addition the root system of plants affected by pot size, even when grown in a peat/perlite medium, produced no unusual odours usually associated with potting stagnation when the root system/potting medium were removed from the pot.

In Figure 8.1 and 8.2 the small potted Common and Giant plants had curves that are higher than those for the larger potted plants respectively. Because the total number of plants involved in this experiment occupied

more than one full glasshouse bench a smaller adjoining drop-side bench was raised. An extra set of lights was positioned over the drop-side bench area. All small potted plants were positioned on this region of the bench. There was a higher radiation level at plant height under the double spaced set of lights in this region (PAR = 145 microeinsteins/ m^2/ sec.) than for the other regions of the bench (PAR = 120 microeinsteins/ m^2/sec.). After six artificially extended long days this light difference was corrected by fitting lower intensity lamps at the region where the light intensity was originally greater.

This light intensity difference over the first six days does not, however, explain the large shoot, and to a less extent root, dry weight increases found for all cultivars after seven long days as the large potted plants were not positioned under this region of initial higher light intensity.

From the results of this investigation it is difficult to determine whether a critical root/shoot ratio is required for reproductive development to occur. The root/shoot ratio for both large and small potted Giant plants from day 21 to day 49 was similar (Figure 8.4), although, at the more critical time from day 0 to day 14 (i.e. the period when inflorescence initiation would have occurred) the root/shoot ratio for large potted Giant plants (i.e. this population showed no pot size effect) was much higher than for the plants grown in small pots (i.e. did show a pot size effect).

For sainfoin, Figure 8.3 shows that no change in the number of root apices for Common, and possibly also for Giant, occurred in response to long day treatment. However, in Melrose there is a sudden increase, for both large and small potted plants, in the number of root apices at the time when inflorescence initiation and development occurred. This was unexpected as the other more vigorous flowering cultivar, Giant, showed no change in root apex number. Due to this, interpreting Figure 8.3 becomes difficult. If a change in the pattern of root growth in sainfoin is responsible for the plant being able to initiate inflorescences then the graph for Melrose (Figure 8.3) fits perfectly with this theory. This could involve the added newly initiated root apices supplying a greater quantity of hormone/growth factor to the shoot that might play some role in reproductive development. To explain the curves for Giant (Figure 8.3) it would be necessary to hypothesize that this cultivar may have a different mechanism which effects inflorescence initiation; or that the mechanism may be similar to Melrose but the roots do not play

such a dominating role as in the latter cultivar. If this latter mechanism was correct then the small increase in root apex number for large potted Giant plants at day 28 might be significant.

The curves in Figure 8.3 could also be interpreted in another way, that is, a redistribution of growth factors caused by a sink effect. Long day produced growth factors might be channelled towards a dominating sink. In Giant, where inflorescence initiation did occur, the main sink would be the young developing inflorescences. However, in Melrose, which had a low percentage of plants with macroscopically visible inflorescence initiation but a relatively high percentage of plants with elongated stems (Table 8.2), long day produced growth factors might move to the root system because there was no dominating sink in the shoot. This might then affect the growth factor balance within the root system resulting in a significant increase in root branching. For Common, which had no elongated stems (Table 8.2) it could be argued that the shoot does not produce any, or not enough, long day factors to bring about this change in root growth that was noted in Melrose. Evidence to suggest that a plant can redistribute growth factors comes from the observation between Giant and Common. During the first three weeks of long day treatment the shoot dry weight increase was similar for both Giant and Common (Figure 8.1). In Giant at this period stems had begun to elongate whereas in Common no stem elongation occurred. This suggests that in Giant growth factors produced are channelled into stem growth whereas in Common no stem elongation occurred. This suggests that in Giant growth factors produced are channelled into stem growth whereas in Common the growth factors were channelled into extra leaf growth.

In this investigation any pot size effect observed only occurred in plants that were fast growing. The reason for this was most probably the size of the plants when they were induced to flower. All plants kept in natural short days where the temperatures in the glasshouse were often relatively low (i.e. minimum 15^{o}C) hence due to this plant growth was slow. By the time plants were placed into the 17 hour inductive photoperiod most small potted plants were perhaps not affected by their pot size. However, as the plants grew at a faster rate in long days, especially Giant, then the plants in small pots might have been affected by their pot size. In the small potted Giant plants it would appear that this inhibitory effect occurred just after inflorescence initiation had occurred (i.e. note abortion figures in Table 8.2). In the small potted plants this most likely occurred just before inflorescence initiation was about to occur. If all plants had been grown in warmer

and brighter short days then this pot size effect would most probably have occurred in all small potted plants.

The results in Chapter 7 and 8 would suggest that the roots do play a major role in the reproductive process of sainfoin thus eliminating the hypothesis that the roots play no major role in reproduction. Thompsett (1976) noted that if the distal portion of the root system of Andropogon gayanus was removed then the number of plants flowering was reduced by up to 50 percent.

To explain this pot size effect I feel that the most likely answer lies in the following observation. Becker-Dillingen (1929) noted in Germany that sainfoin can be successfully resown if a seedling stand fails, but establishment is very difficult or impossible on fields which have carried established sainfoin during the previous 10-12 years. Drawing on more modern knowledge this suggests that sainfoin actively excretes some allelopathic substance so as to help ward off any competitive effects from other plants. Thus when sainfoin is grown in a pot, and the roots fill a large volume of that pot, a self-poisoning effect may occur. The presence in soils of a wide variety of potentially phytotoxic chemicals, released by plants is well documented (Whittaker and Feeny, 1971). Glass (1973) noted that phosphate uptake was inhibited in barley (Hordeum vulgare) by naturally occurring phenolic acids and suggested that this inhibition is caused by (a) denaturation of specific membrance carriers; (b) uncoupling of mitochondrial electron transfer; (c) ulitization of ATP; or (d) alteration of membrance properties. Thus it is possible that growth aspects associated with flowering may also be inhibited. This could be either a hormonal or mineral factor, although plants affected by pot size did not appear unhealthy in any manner thus suggesting a missing hormonal factor might be responsible for this reproductive inhibition.

SECTION III

EFFECTS OF GIBBERELLIC ACID ON GROWTH AND DEVELOPMENT

INTRODUCTION

Previous studies reported by other authors have shown that gibberellic acid has a marked effect on plants that 'bolt' (Lang, 1965). In this type of plant gibberellin application, in some cases, can remove the low temperature pretreatment requirement and/or the photoperiodic requirement. As sainfoin, possesses this characteristic of 'bolting' it was decided that a series of gibberellic acid (GA) experiments be undertaken to determine if the results obtained for sainfoin conformed to those obtained for other plants of similar growth habit.

The results, from three experiments in this section, will also provide information helpful in understanding the mechanism of the internal regulation of flowering in sainfoin.

CHAPTER 9

EFFECT OF GIBBERELLIC ACID ON LEAF ANGLE

9.1 Introduction

In Chapter 4 it was noted that during progression towards the flowering phase the first response to increasing daylength was an increase in the angle of the leaves to the horizontal. That is, the leaves of each plant became erect. There was also an increase in the length of the leaves caused by elongation of the petioles.

Previous authors (Carlson et al, 1964 for lucerne; Fletcher and Martin, 1962 for white clover; and Stoddart, 1959 for red clover) have observed an increase in leaf angle to the horizontal and petiole elongation in response to GA treatment. However, these authors have not discussed these results in relation to the onset of the reproductive process. Zeevaart (1971) noted that the earliest visible responses of spinach plants transferred from short days to long days were an upright leaf orientation and increased elongation of the petioles. GA_3 applied in short days produced effects similar to the effect of long days in non GA-treated plants. He suggested that this observed response in spinach plants might be due to a higher rate of GA biosynthesis combined with an increased sensitivity to GA.

In this investigation sainfoin plants held in short days were treated with GA_3 to determine whether this hormone could substitute for long days and lead to an increase in leaf angle to the horizontal.

9.2 Materials and Methods

Cv. Melrose seed was sown on January 6 1975 into five centimetre plastic pots containing an osmocote potting mix as described in Chapter 2. Plants were kept at natural temperatures in a box outside as described in Chapter 2 to give a nine hour photoperiod.

On February 23 1976 experimental treatments began. All treatments in this investigation were under outside environmental conditions.

Treatments involved five concentrations of GA_3 (0.5, 1, 3, 5, 10 μg/ml. GA_3) plus a water control. In addition a long day treatment consisting of a water control with supplemented artificial light (two Atlas BG 150W Flood Lights, PAR = 450 microeinsteins/m^2/sec. at plant

height) to 17 hours was added.

Application of hormone and water at seven day intervals beginning on February 23 1976 and ending on March 15 1976 involved placing 1 ml. of solution on the apical region of the plant. This included all the young unfolded leaves plus the axils of the young unfolding leaves. A wetting agent (IWD Multi-film x 77 at 0.5%) was added to all treatment solutions.

Plants from each group were individually scored for leaf angle (see Chapter 5, section 5.2) each day. Each time leaf angle scores were obtained the scores for all plants in each treatment were summed and from this the initial leaf angle score (i.e. prior to the first application of hormone) was deducted. This gave the overall increase in leaf angle for each group (Figure 5.1). On March 22 1976 the last leaf angle score measurement was made.

The short day control, long day (17 hr), and 0.5 /ug/ml. GA_3 treatments had five plants each, the other treatments had ten plants each. In all treatments there were initially five or more plants that had a leaf angle score of 1 (Plate 5.1), some plants had a score slightly higher than this.

9.3 Results

Figure 9.1 shows that the response to hormone was very rapid in treatments receiving 1, 3, 5 and 10 /ug/ml. GA_3, with a gradual increase occurring throughout the first week. After the second application of hormone there was a large increase in leaf angle to the horizontal reaching a maximum by the second day. The leaf angle increase was much higher for the 5 and 10 /ug/ml. GA_3 treatments. For all treatments responding to GA there was an initial large increase in leaf angle to the horizontal which then gradually decreased for the rest of the week until the next GA application.

Plants not receiving GA but in artificially extended long days (17 hours) showed a leaf angle response after seven days. From this period onwards the groups' leaf angles showed a steady increase until nearly all plants had reached a score of 5. At the termination of this experiment some plants in long days were just beginning to show internode elongation. No plants in short days had begun elongation.

In the short day control and 0.5 /ug/ml. GA_3 treatment populations there was no increase in leaf angle during the period of this experiment.

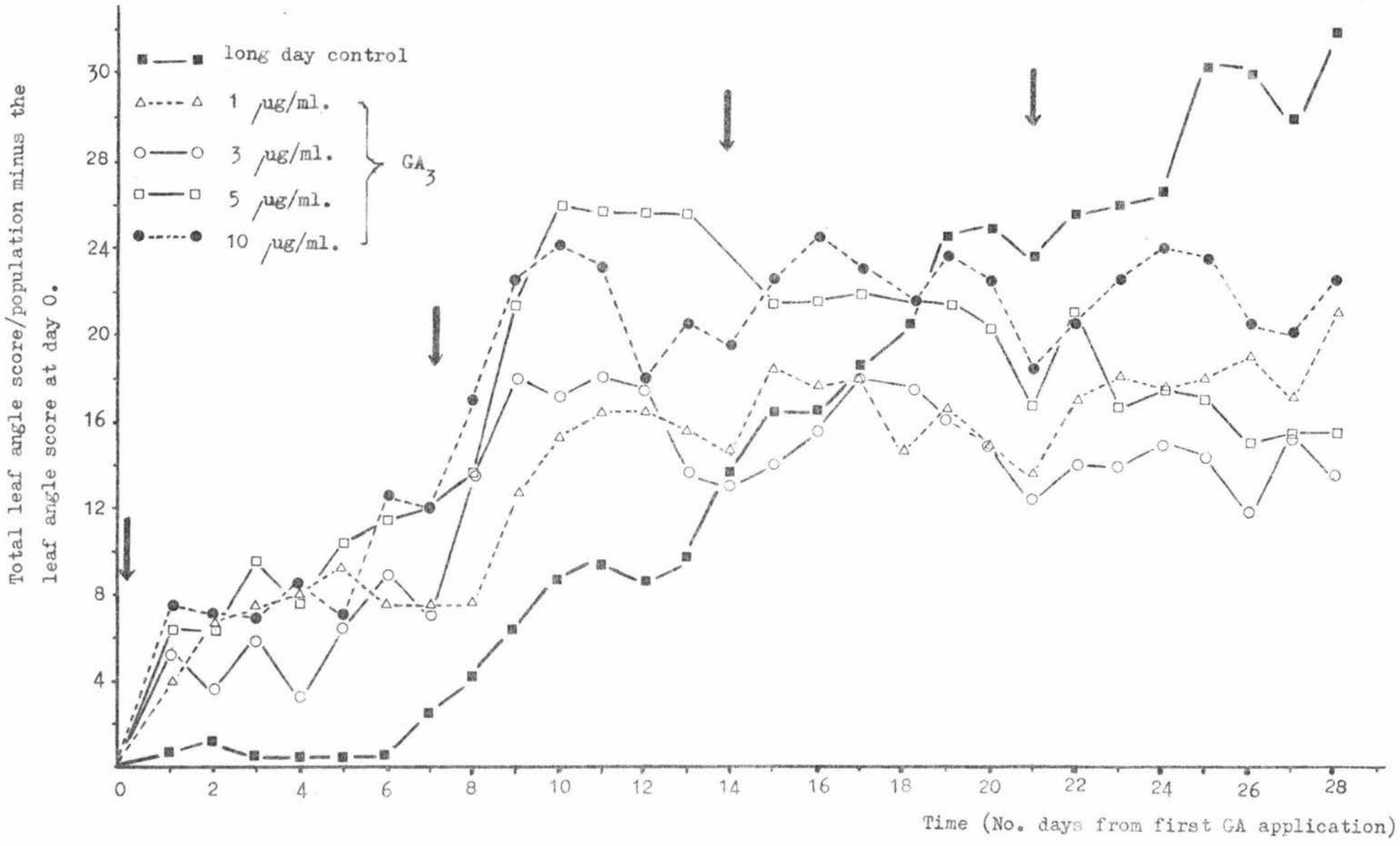


Figure 9.1

The effect of gibberellic acid (GA_3) on leaf orientation for cv. Melrose in short days. Arrows indicate GA application times.

9.4 Discussion

The reason for conducting this experiment outside was twofold. Firstly plants kept in the short day boxes in the warm glasshouse over summer were more liable to succumb to fungal attack. This was most probably due to the high temperatures and humidity within the boxes once the covers had been secured. Secondly it was noticed that plants kept in artificial short days in the glasshouse during the summer period tended to raise their leaves in a similar manner to plants in long days (Appendix I) which also might have been due to the relatively high temperatures within the boxes in the glasshouse.

When this experiment was ended plants receiving GA_3 did not have leaf angle scores as high as the long day control plants. This might have been due to differences in internal plant GA concentrations. With more regular applications of GA_3 treated groups might have had higher leaf angles. In addition, in the natural plant system another GA (i.e. other than GA_3) might be responsible for a more rapid increase in leaf angle.

In this experiment no plants receiving GA_3 treatment had elongated stems at the end of this experiment (i.e. after four weeks). However, by the end of the experiment plants in the 17 hour long day control treatment had begun elongating their stems.

CHAPTER 10

EFFECT OF GIBBERELLIC ACID ON FLOWERING IN SHORT DAYS

10.1 Introduction

Many previous studies have shown that GA can cause flowering in some short day and long day plants in non-inductive photoperiods (Lang, 1957, 1965; Michniewicz and Lang, 1962; Wittwer and Bukovac, 1957). In most long day pasture legumes application of GA has not been found to induce flowering under short day conditions. Pasture legume species tested include: Trifolium repens, Lotus pedunculatus (Thomas, 1967), Trifolium pratense (Stoddart, 1966).

However, in Trifolium subterraneum gibberellic acid application uner warm short day conditions had been reported to cause inflorescence initiation in Trifolium subterraneum (Evans, 1959).

The aim of this present investigation was to determine whether GA_3 could induce inflorescence initiation in short days in sainfoin.

10.2 Materials and Methods

On January 6 1976 Melrose seeds were sown into 5 centimetre plastic pots containing an osmocote soil nutrient mix as described in Chapter 2. Initially the plants were placed in short day boxes inside the glasshouse but due to unfavourable conditions (see Chap. 6) all experimental plants were moved into outside environmental conditions using the same boxes. A nine hour photoperiod was used throughout the duration of this experiment. On March 10 1976 all plants were repotted into 10 centimetre plastic pots.

On January 15 1976 hormone applications began (two applications per week). There were six treatments (0, 20, 40, 80, 160 and 500 /ug/ml. GA_3) of 12 plants each. GA_3 solution was applied to the apical region of each plant. GA_3 was last applied on March 11 1976.

Data in Table 10.1 were obtained on March 16 1976 and within all GA_3 treatments the most elongated stems were dissected to determine whether plants had initiated inflorescences.

10.3 Results

Shoot elongation occurred in response to all GA_3 treatments; the

Table 10.1

The effect of gibberellic acid on inflorescence initiation and vegetative development in short days for cv. Melrose. I the percentage of plants with inflorescence initiation, II the percentage of plants with elongated stems, III the average length of elongated stems, IV the V the average number of elongated internodes per plant greater than 2 mm. and 1 cm. respectively. Data were obtained on March 16 1976.

	GA_3 concentration (/ug/ml.)					
	0	20	40	80	160	500
I % of plants with inflorescence initiation	0	0	0	0	0	0
II % with elongated stems	0	100	100	82	100	80
III Av. stem length (cm.) ± SEM	0	5.14 0.937	4.15 0.662	5.61 1.125	6.19 0.847	4.60 0.619
IV Av. no. elongated internodes per plant (> 2 mm.) ± SEM	0	8.36 1.002	8.55 1.608	8.27 1.121	9.73 0.954	8.70 1.211
V Av. no. elongated internode per plant (> 1 cm.) ± SEM	0	0.36 0.203	0 0.0	0.54 0.390	0.64 0.388	0.10 0.100

first plants elongating nine days after the initial GA_3 application (Figure 10.1). Most plants that elongated did so over a 25 day period, beginning 21 days after the first GA_3 application, with all treatments being similar in response. All elongated stems were short and their elongated internodes also were short. Elongated stems were always vertical. The water treated control plants remained at the rosette stage.

In no GA_3 concentrations tested was there any flowering (Table 10.1). In no plant dissected was there any inflorescence initiation at or near the apex.

10.4 Discussion

The results of this investigation show that under short photoperiods GA_3 will not cause inflorescence initiation in sainfoin. However, stem elongation does occur although stems and elongated internodes tend to be short in comparison to long day GA_3 treated plants (Chapter 11, Table 11.5). This information is similar to that found for most other pasture and forage legumes (Stoddart, 1966). For a non-flowering strain of red clover Stoddart (1966) found that plants treated with GA_3 in short days had a mean stem length of 11.2 cm whereas plants treated with GA_3 in long days had a mean stem length of 43.0 cm.

These results will be discussed in more detail in Chapter 15.

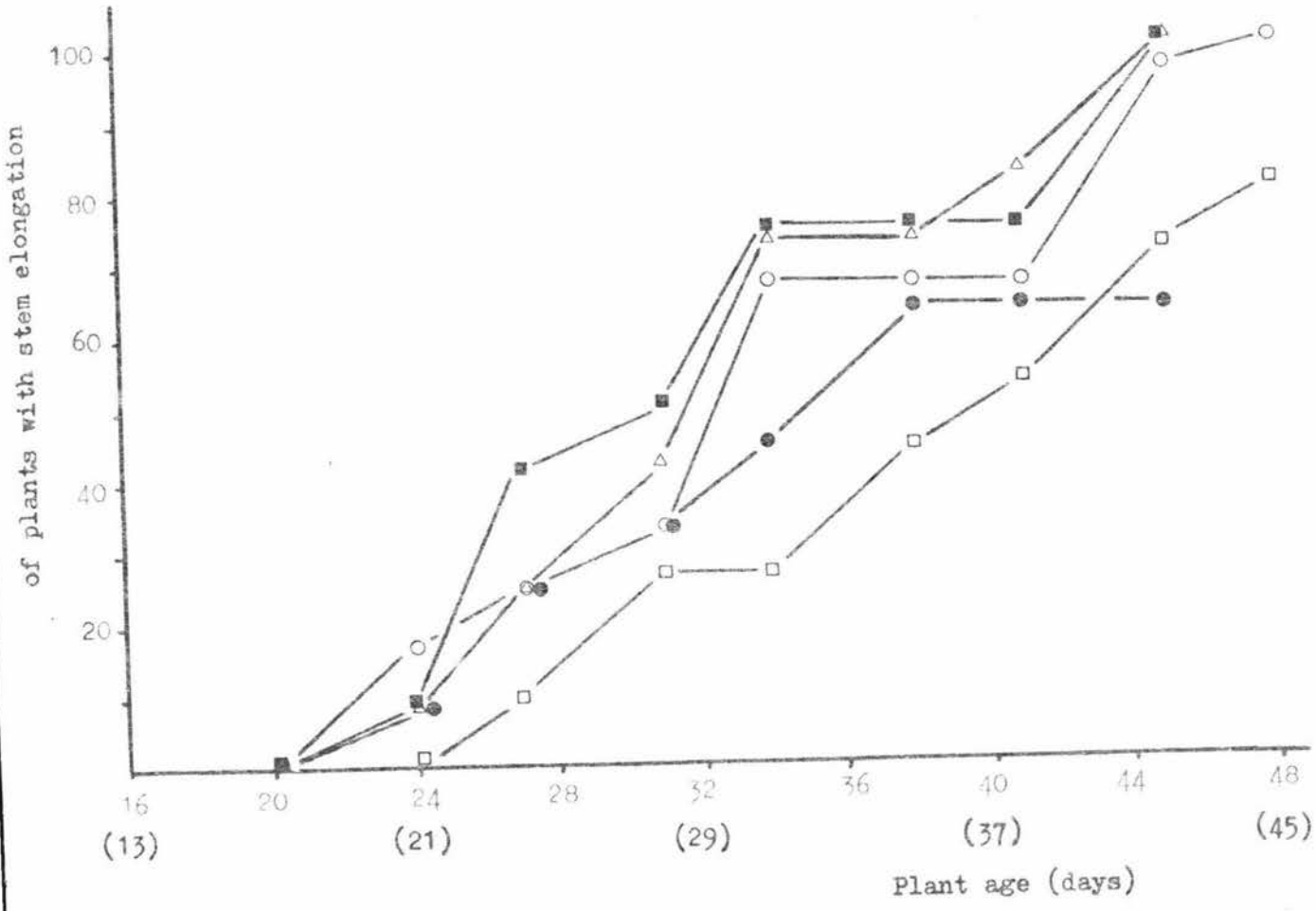


Figure 10.1

Effect of gibberellic acid (GA_3) on stem elongation for cv. Melrose plants in short days. In the water control population there were no plants with stem elongation. △——△ 20 /ug/ml. GA_3, ■——■ 40 /ug/ml. GA_3, ●——● 80 /ug/ml. GA_3, ○——○ 160 /ug/ml. GA_3, □——□ 500 /ug/ml. GA_3.

The 80 /ug/ml. treatment population had one plant that began elongation on March 15 1976 (i.e. by the end of the experiment 82% of the plants had elongated stems). This late stem elongating plant only elongated to 1.6 cm.

N.B. The first GA application was made nine days after germination. Figures in brackets refer to the number of days after the first GA application.

CHAPTER 11

EFFECTS OF GA_3 ON FLOWERING IN LONG DAYS

11.1 Introduction

Previous studies in this thesis have shown that a proportion of plants within a Melrose population does not flower in warm long days. The study in this Chapter was designed to test whether GA_3 would cause flowering in all genotypes of Melrose grown under warm long day conditions. From the results one might be able to speculate whether a lack of GA is responsible for some plants not flowering.

In addition, by taking detailed information, with as many characters being measured as possible the results of this study might lead to a greater understanding of the mechanism(s) that controls flowering in sainfoin.

Because of its reproductive variability Melrose was selected. It was hoped this variability might be representative of most genotypes of Onobrychis viciifolia Scop.

11.2 Materials and Methods

Seed of Melrose was sown on January 6 1976 into 5 centimetre plastic pots with an osmocote soil/nutrient mix. Six days later seedling emergence had begun. On March 10 1976 all plants were repotted into 10 centimetre pots.

At the start of the experiment the natural photoperiod was around 14.8 hours. It should be noted that the natural photoperiod throughout this experiment was decreasing with time.

The experiment consisted of six treatments involving five GA_3 concentrations (20, 40, 80, 160, 500 µg/ml. GA_3) with a distilled water control. All GA applications were made to the apical region of each plant with a plastic syringe (1 ml./plant/application). A wetting agent (IWD Multi-Film X 77, 0.5%) was added to all treatment solutions.

January 15 1976 treatment applications began twice weekly. At the first GA application plants were approximately three to four days old having two expanded leaves each. Each treatment group consisted of 12 plants, each in a separate pot.

Plants were measured and their reproduction development recorded on March 10 1976 when the natural photoperiod was 12.5 hours. After this had been completed all plants were transferred to a 17 hour photoperiod consisting of the natural photoperiod with a morning and night supplement as described in Chapter 2. Then on April 26 1976 the experiment was ended with another set of measurements and observations being recorded.

Epidermal cell length measurements in Table 11.7 were determined from an internode in the central region of an elongated stem, epidermal strips being obtained from the central region of an internode (Table 11.7, 11.8). 210 cells were measured from three epidermal strips (i.e. 70 cells counted from each) for each treatment (Table 11.7), and two internodes were measured per internode length class (100 cells/individual internode from three epidermal strips per plant) (Table 11.8).

Internode diameters were measured on elongated stems using calipers. ($\pm$ 0.05 mm.). The 'lower' internode was defined as the internode where elongation first began. The 'upper' internode was defined as that basal to the youngest fully expanded leaf.

To facilitate discussion, interpretation and understanding of the results this experiment has been divided into two sections (A and B). Firstly data obtained from each treatment under natural photoperiods are presented (Section A) and then follow the results obtained under the 17 hour photoperiod (Section B).

11.3 Results

SECTION A

11.3.1 Effect of GA_3 in Natural Long Days

11.3.1.1 Stem Elongation

The first response to GA_3 was that of stem elongation (Figure 11.1). Prior to elongation there was no swelling of the primary shoot bud (see Chapter 5). At the end of the experiment all plants had elongated primary stems in all GA_3 treatments. In contrast one plant only (8%) of the control group had elongated stems and this plant had only elongated one secondary stem that was just over two centimetres.

It required 11 days, after the first application of gibberellic acid,

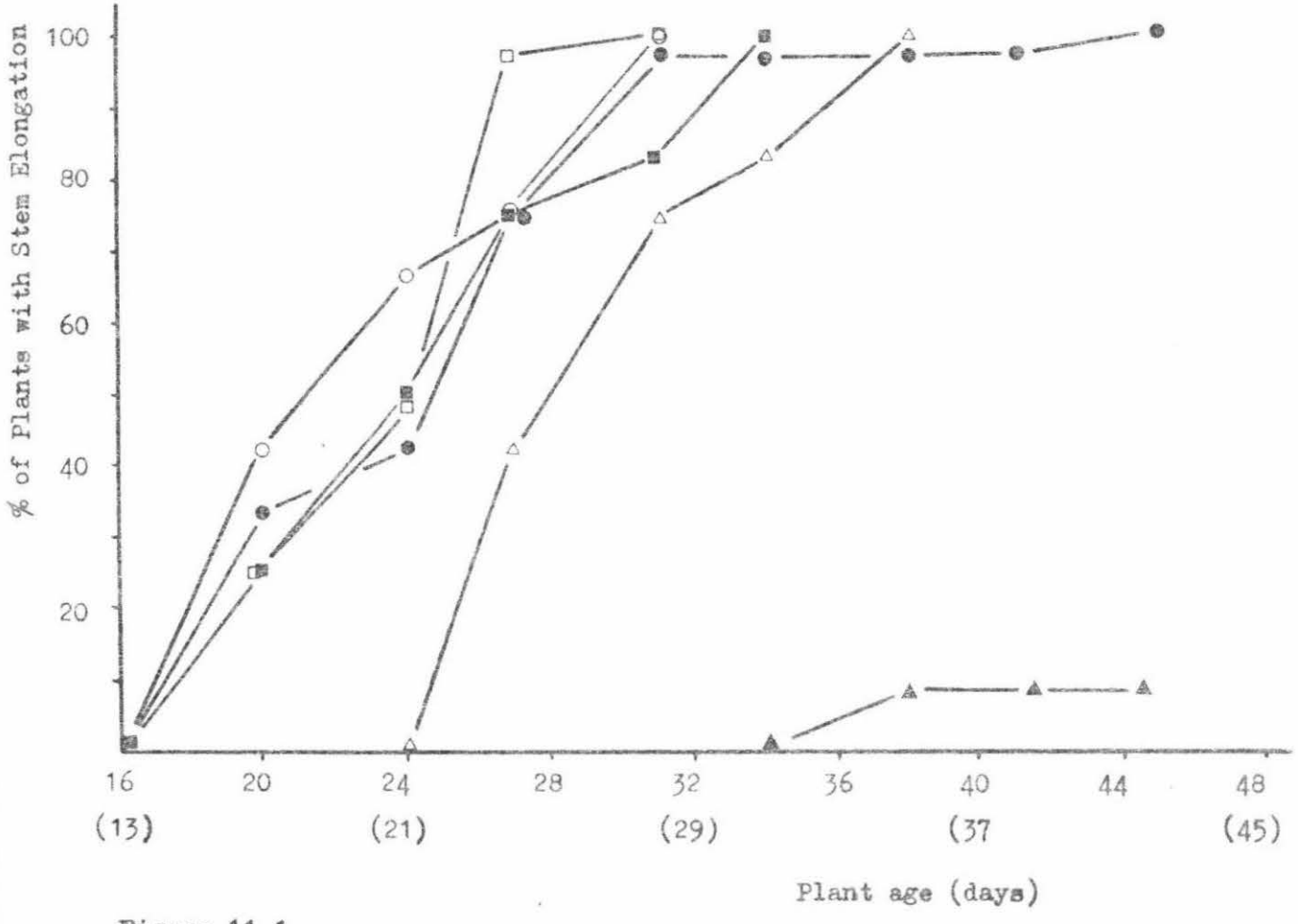


Figure 11.1

Effect of gibberellic acid (GA_3) on stem elongation for cv. Melrose plants in long days. ▲——▲ control, △——△ 20 /ug/ml. GA_3, ■——■ 40 /ug/ml. GA_3, ●——● 80 /ug/ml. GA_3, ○——○ 160 /ug/ml. GA_3, □——□ 500 /ug/ml. GA_3.

N.B. The first GA application was made nine days after germination. Figures in brackets refer to the number of days after the first GA application.

before stem elongation had began in plants receiving 40 to 500 /ug/ml. GA_3. In the 20 /ug/ml. GA_3 treatment stem elongation had not begun until 18 days after the first GA application. This represents a time difference of seven days between responses (Figure 11.1).

At the end of the first section of the experiment all elongated stems were measured. When averaging stem lengths on a treatment basis very little difference between treatments was observed. However, there was a large amount of variation within treatments with some plants having relatively long elongated stems with others being short.

The node at which elongation began was also recorded (Table 11.1). With increasing GA_3 concentration stem elongation began at a lower node number.

The average number of elongated internodes per plant was also measured (Table 11.1). In the GA_3 treatments the 20 /ug/ml. GA_3 group had fewer elongated internodes than the other groups. In fact there was a gradual increase with increasing GA_3 concentrations.

Measurements of elongated internodes (Table 11.1) showed that overall the 20 /ug/ml. GA_3 treated plants had the longest internodes. The other GA_3 treatments had similar average internode lengths. This observation will be discussed in more detail further on in this chapter as it has a relationship with flowering.

There were no significant differences between treatments in the diameter of the first elongated internode (measured at the centre of the internode) on each stem.

11.3.1.2 Flowering

One of the main features of this experiment was that although all plants in each GA treatment had elongated stems, macroscopically visible inflorescence initiation occurred in only one treatment (Table 11.2).

In the 20 /ug/ml. GA_3 group the three plants that did flower had the first inflorescence occurring on node number 10.66 ± 0.33. This corresponded to approximately the fourth to fifth node initiated after the first application of GA_3.

11.3.1.3 Associated Vegetative Characters

In addition to the previously described data the number of basal buds, rate of leaf emergence, seedling vigour, and the average length of

Table 11.1

Effect of GA_3 on stem elongation in natural long days ($\pm$ SEM values provided).

	GA_3 concentration (μg/ml. GA_3)					
	0	20	40	80	160	500
Av. elongated stem length per treatment (cm.)	0.20 $\pm$ 0.20	20.82 4.958	22.14 1.805	18.67 3.123	21.86 5.113	21.32 4.323
Average node at which elongation started	-	4.75 $\pm$ 0.250	4.42 0.312	3.58 0.193	3.58 0.417	3.45 0.366
Av. No. Elongated internodes/plant	0.08 $\pm$ 0.08	8.42 0.733	10.82 0.325	10.67 0.643	11.09 0.456	11.09 0.836
Av. length of elongated internodes	-	2.38 $\pm$ 0.272	1.81 0.164	1.58 0.177	1.79 0.178	1.57 0.160
Av. diameter of the first elongated internode	-	0.407 $\pm$ 0.021	0.361 0.027	0.369 0.042	0.414 0.036	0.343 0.023

Table 11.2

Effect of GA_3 on flowering in natural long days.

	GA_3 concentration (μg/ml. GA_3)					
	0	20	40	80	160	500
% of plants with blossoming inflorescences	0	25	0	0	0	0
% of plants with macroscopically visible inflorescences	0	25	0	0	0	0
Av. No. days to blossoming (From first GA application)	-	49 $\pm$ 1.33	-	-	-	-

leaves number four to twelve were noted (calculated from each plant, Table 11.3).

Table 11.3

Vegetative characters associated with reproductive development in natural long days (± SEM values provided).

	GA_3 Concentration (/ug/ml. GA_3)					
	0	20	40	80	160	500
Rate of leaf emergence over period of experiment (no. leaves emerged/week)	1.422 ± 0.075	1.650 0.059	1.641 0.049	1.622 0.058	1.740 0.060	1.740 0.106
Average length of leaves no. 4 to 12 (cm.)	17.39 ± 1.152	22.80 1.254	22.18 1.468	19.33 1.343	19.67 1.306	19.92 1.437
Av. no. basal buds/plant	3.75 ± 0.538	0.25 0.130	0.09 0.179	0.08 0.179	0.73 0.634	0.0 0.0

With GA_3 treatments the average number of basal buds per plant was markedly less than in control plants. Many GA_3 treated plants had no basal buds whatsoever whereas, with one exception, all control plants had basal buds.

The rate of leaf emergence showed an increase after GA_3 treatment (Table 11.3). However, this increase was only slight with no differences being observed between GA_3 concentrations. In the 20 /ug/ml. GA_3 group there were differences between plants that flowered (1.823/week) and those that did not flower (1.422/week, the latter having the same rate of leaf emergence as the control group. However, in comparison to the control, there was still a slight increase in rates of leaf emergence for the higher GA_3 concentrations that had no flowering (i.e. 40 to 500 /ug/ml. GA_3.

In each GA_3 treatment plants can be divided into two groups; those having stem lengths of less than 20 cm., and those having stem lengths greater than 20 cm. (Table 11.4). As all plants were individually numbered, leaf emergence rates could be calculated for each group. As a general rule plants with shorter stems had lower rates of leaf emergence than plants with longer stems.

Table 11.4

Rates of leaf emergence (per week) for GA_3 treated plants with stems (a) greater than 20 cm., and (b) less than 20 cm. in natural long days.

		Rates of leaf emergence ($\pm$ SEM)	
		(a) stems less than 20 cm.	(b) stems more than 20 cm.
GA_3 concentration (μg/ml. GA_3)	20	1.547 $\pm$ 0.065	1.794 $\pm$ 0.074
	40	1.641 $\pm$ 0.077	1.641 $\pm$ 0.069
	80	1.513 $\pm$ 0.077	1.732 $\pm$ 0.066
	160	1.719 $\pm$ 0.078	1.778 $\pm$ 0.103
	500	1.578 $\pm$ 0.098	2.105 $\pm$ 0.027

The leaf lengths, on each plant, for leaves on nodes number four to twelve were measured. GA_3 treatment caused only a slight increase in length in comparison with the controls. There appeared from a visual estimate to be no significant increase in leaflet area in response to GA application.

Seedling vigour, scored on degree of greenness, was recorded when the plants were beginning to elongate. The 20 μg/ml. GA_3 treatment group resembled the controls, having plants with deep green leaves whereas leaves in the other GA treatments were slightly yellow.

SECTION B

11.3.2 Effect of GA_3 in a 17 hour Photoperiod

As previously mentioned, plants from the natural photoperiod were placed into a 17 hour photoperiod in the same glasshouse on March 10 1976. On April 26 1976 another set of data was obtained.

11.3.2.1 Stem Elongation

Measurements associated with stem elongation are shown in Table 11.5.

The most significant feature was that there were large variations within treatment groups with regard to individual plant stem lengths. Stem elongation occurred in only two control plants and the stems on these were short. These two plants were left under a 17 hour photoperiod for another 14 days during which one plant elongated to about 12 centimetres, the other showed no more elongation. Even with this extended time no macroscopically visible inflorescence initiation occurred.

Table 11.5

Effect of GA_3 on stem elongation and internode diameter in a 17 hour photoperiod. In GA_3 treatments all plants had elongated primary stems (i.e. 12/treatment) with no elongated secondary stems. The two control plants each had only one elongated secondary stem with no elongated primary stem. The terms 'lower' and 'upper' are defined in section 11.2.

	GA_3 Concentration (/ug/ml. GA_3)					
	0	20	40	80	160	500
Av. stem length/treatment (cm.)	0.664	57.42	89.00	65.91	72.40	81.41
± SEM	± 0.446	11.17	9.08	12.31	12.99	7.80
Av. No. elongated internodes/ plant	-	18.82	21.20	19.89	22.70	22.10
± SEM	± -	1.181	0.712	0.934	0.667	1.622
Av. Internode Diameter: Lower	-	0.453	0.409	0.522	0.519	0.457
± SEM	± -	0.018	0.024	0.048	0.030	0.027
Upper	-	0.249	0.189	0.200	0.210	0.180
± SEM	± -	0.050	0.010	0.026	0.027	0.009

The number of internodes elongated per plant showed no differences between GA concentrations. When plant height was plotted against number of elongated internodes (Figure 11.2) three features were observed. Firstly in general there was a positive correlation between stem length and the number of elongated internodes. There were two exceptions to this rule. For plants that blossomed, elongated internodes tended to be longer than those in non-flowering plants, (circled dots). Another form of stem growth observed was that some plants with short stems had many short internodes (dots enclosed in squares).

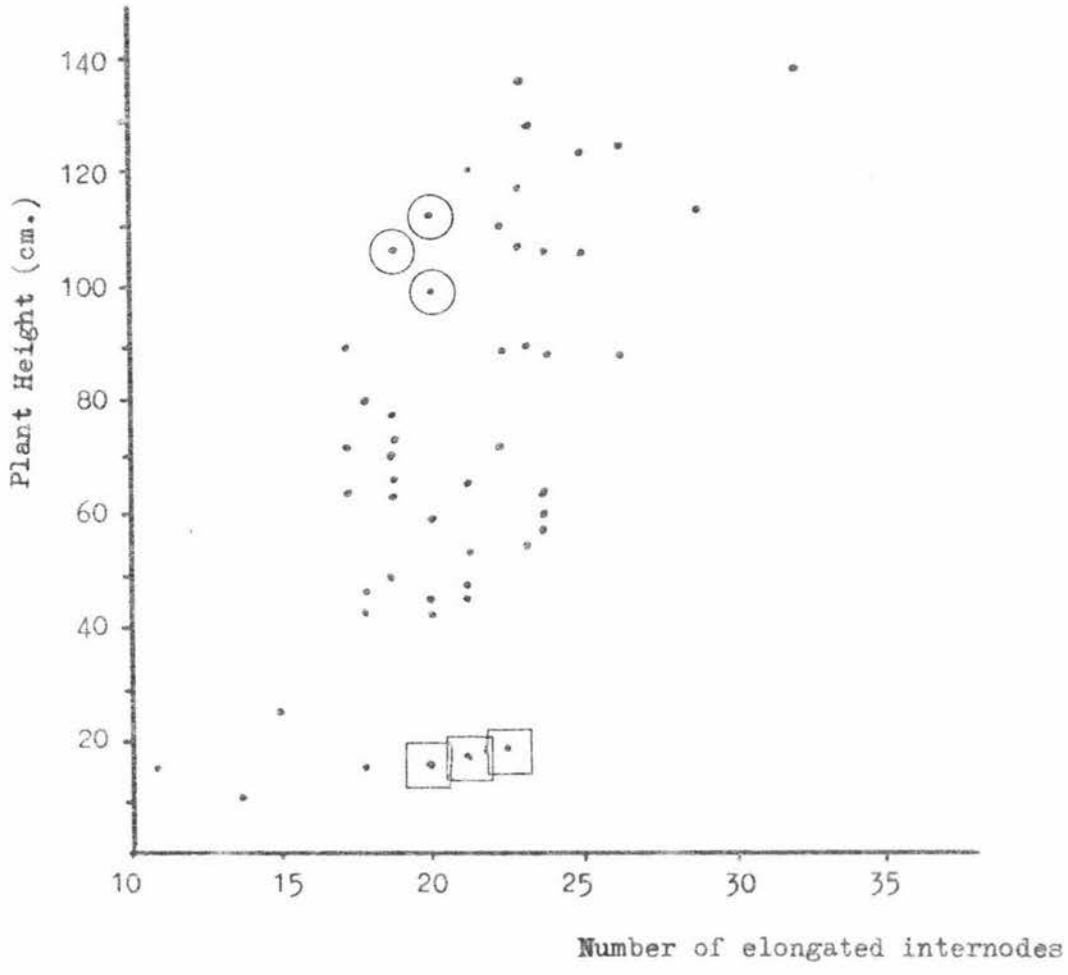


Figure 11.2

Plant height plotted against the number of elongated internodes per stem for plants treated with GA_3 (i.e. 20, 40, 80, 160 and 500 /ug/ml. GA_3 results are presented on this one graph).

A dot within a circle represents a flowering plant; a dot within a square represents a plant having a short stem with many elongated internodes; other dots represent a stem with no inflorescence initiation.

When measuring internode diameter, again these three stem growth forms can be noted. When averaging the highest measured internodes no differences were observed on a treatment basis (Table 11.5). However, within all treatments individual plants showed a large variation in the diameter of the highest measured internode. The lowest internode showed very little variation between plants (Table 11.6).

Table 11.6

Stem elongation and growth form of three individual GA_3 treated plants. Plant no. 1 short stem, non-flowering, many elongated internodes, thick upper internode; Plant 2 - long stem, non-flowering, upper internode slightly smaller than lower; Plant 3 - blossoming, upper internode very thin compared to basal internode.

	Plant Number		
	1	2	3
Stem length (cm.)	18.0	106.0	111.0
No. elongated internodes	21	24	20
Differences between diameters of lowest and highest internodes (cm.)	0.070	0.167	0.364
Rate of leaf emergence (No. emerged per week)	1.313	1.750	1.860

Plants having a short stem with many elongated internodes tended to have internodes all of a similar diameter (e.g. Plant No. 1., Table 11.6); those plants that flowered had very thin upper internodes in comparison to the basal internodes (e.g. Plant No. 3., Table 11.6); and finally those plants that elongated but did not flower, had upper internode diameters inbetween the other two stem growth forms (e.g. Plant No. 2., Table 11.6).

At the time of internode measurement Plant No. 3 had initiated 11 inflorescences on its primary stem.

Epidermal cell lengths on elongated stems are shown in Table 11.7. GA_3-treated stems had the same epidermal cell lengths as control non-

treated stems. However, epidermal cells on non-elongated internodes were significantly shorter (1% level).

Table 11.7

Epidermal cell lengths of non-elongated and elongated internodes in GA_3 treated and non-treated plants.

Type of Internode	Cell length (10^{-6} m.)
Non-GA_3 treated (but elongated)	61.68 ± 1.07
20 µg/ml. From a blossoming plant	60.32 ± 1.44
20 µg/ml. From a non-flowering plant	62.49 ± 1.00
500 µg/ml. From both a flowering and non-flowering plant	60.36 ± 1.72
From a non-elongated Internode (nontreated plant)	33.33 ± 0.93.

Within GA_3 treatments plants showed a variation in internode length. Six internode classes of various lengths were selected and their epidermal cell lengths were measured (Table 11.8).

Shorter internodes have slightly, but statistically significantly, shorter epidermal cells. However the cells are still longer than in non-elongated internodes (Table 11.7). The longer internodes were always found on plants having inflorescence initiation. These longer internodes result from more cells rather than longer cells (Table 11.8).

Basal bud development under a 17 hour photoperiod showed the same trends as in the natural photoperiods. Very little new bud formation occurred, but some basal buds elongated although none initiated inflorescences.

11.3.2.2 Flowering

Under the 17 hour photoperiod some plants from all GA_3 treatments either blossomed or produced aborted inflorescences (Table 11.9).

A maximum of 50 percent of plants initiated inflorescences within any GA treatment; the minimum was 25 percent. Within any treatment the maximum percentage of plants blossoming was 33 percent (Table 11.9).

The 20 µg/ml. GA_3-treated plants that flowered in the 17 hour photoperiod included the three that had earlier flowered in natural days.

Table 11.8

Epidermal cell lengths from internodes of different lengths. All plants measured are from the GA_3 treatments.

	Internode length (cm.) (± SEM)					
	11.20 ± 0.20	7.75 ± 0.25	5.5 ± 0	4.15 ± 0.35	3.35 ± 0.15	2.12 ± 0.12
Epidermal cell length (10^{-6} m.) ± SEM	57.33 1.58	62.72 1.70	61.03 1.88	54.13 1.46	52.82 1.27	52.48 1.45
Estimated no. of cells in a line from node to node	1953	1235	901	767	634	404

In the 17 hour photoperiod the average time required for blossoming to occur in all GA_3 treatments (i.e. with data bulked) was 41 days. This is comparable to the time required for the three 20 µg/ml. GA_3 treatment plants to flower in natural photoperiods, i.e. 49 days after the first GA application.

The node at which the first inflorescence was initiated in a 17 hour photoperiod is shown in Table 11.10, together with the final stem lengths attained. Stems of flowering plants were longer than for non-flowering plants.

11.3.2.3 Lateral Branching

The percentage of plants having lateral branches on elongated stems was low, although all plants with elongated stems that received GA_3 treatment had a few lateral branches (Table 11.11).

Table 11.9

I Percentage of plants with macroscopically visible inflorescences, II percentage of plants blossoming, III percentage of inflorescences aborted per treatment and IV the average number of inflorescences initiated per plant after transfer to a 17 hour photoperiod. 12 plants/ treatment).

	GA_3 Concentration ($\mu g/ml.$ GA_3)					
	0	20	40	80	160	500
I % of plants with visible inflorescences	0	33	33	25	25	50
II % of plants blossoming	0	33	25	8	0	17
III % of inflorescences aborted	0	47	53	76	100	81
IV Av. No. of visible inflorescences per flowering plant	0	10.25	4.25	4.33	1.75	3.14
$\pm$ SEM		2.49	0.479	0.333	0.250	1.12

Table 11.10

Stem lengths of non-flowering versus flowering plants, and the node number at which the first inflorescence occurred counting upwards from the first formed leaf in plants treated with GA_3.

	GA_3 Concentration ($\mu g/ml.$ GA_3)					
	0	20	40	80	160	500
Node at which the first inflorescence occurred (for plants that only flowered in the 17 hour photoperiod	-	28.0 ± 0.0	21.50 0.645	19.67 1.333	22.67 2.027	21.86 1.203
Stem lengths — Flowering plants ±	-	102.5 ± 5.31	115.6 1.45	107.6 13.86	113.6 12.33	95.7 13.45
Stem lengths — Non-flowering plants ±		34.87 ± 8.40	69.8 8.09	40.3 8.48	54.7 12.89	69.5 6.35

Table 11.11

Percentage of plants with lateral branching on elongated stems, and the average number of lateral branches per primary stem in GA_3 treatments.

	GA_3 Concentration ($\mu g/ml.$ GA_3)					
	0	20	40	80	160	500
% No. of plants with lateral branches (Longer than 1.5 cm)	0	33	8	33	42	25
No. of lateral branches/plant greater than 1.5 cm	-	3.67	2.0	2.25	3.0	3.0

11.4 Discussion

Under natural long days three plants from the 20 µg/ml. GA_3 treatment were the only plants to flower. Using the data provided below the approximate date for inflorescence initiation was calculated and at the same time compared to the average approximate date for inflorescence initiation for Melrose in the cultivar trial (Chapter 5).

	Melrose plants from the cultivar trial (Chapter 5)	Melrose plants in the present GA_3 experiment
Av. date of blossoming	Jan. 5th (Figure 5.5)	March 4th
Rate of leaf emergence (per week)	4.56/20 days (Table 5.4) = 1.60 per week	1.65 per week (Table 11.3)
Av. No. of inflorescences on a stem when the first inflorescence begins to blossom (Table 4.1)	10	10
Therefore the time from inflorescence initiation to blossoming equals	16 days	16.5 days
Therefore the average date for inflorescence initiation was	Dec. 19th	Feb. 17th
Photoperiod at this date (Figure 2.1)	14.9	13.5

From this comparison it would appear that GA_3 can lower the critical photoperiod for inflorescence initiation, under these environmental conditions, at least in those three plants that blossomed.

When all treatments were transferred to the 17 hour photoperiod some plants from all groups initiated inflorescences (Table 11.9). The percentage of plants with inflorescence initiation ranged from 25 to 50 percent. This represents a difference of only three plants, and taking into account the fact that Melrose is a heterogeneous cultivar regarding flowering, it is doubtful whether this difference is significant. When comparing these percentages to percentage inflorescence initiation values obtained in similar experiments where the daylength has been artificially

extended to 17 hours (Chapter 6), it is concluded that GA_3 does not influence the percentage of plants initiating inflorescences that one would expect in this cultivar under these glasshouse environmental conditions. It appears that if a Melrose plant has the potential to flower in the glasshouse then it will most probably do so whether GA is applied or not.

However, GA_3 does affect the time required before inflorescence initiation can occur i.e. the juvenile period. For the three 20 µg/ml. GA_3 treated plants that blossomed in natural photoperiods a period of only 52 days was required for blossoming to occur whereas in Chapter 6 young seedlings immediately placed into long days required approximately 110 - 112 days before blossoming occurred.

GA_3 may have an effect on inflorescence development. In Table 11.9 it is shown that with increasing GA_3 concentration the percentage of inflorescences aborted tended to increase. In addition to this it was observed that inflorescences tended to abort at an earlier stage of development at the higher GA_3 concentrations. As no flowering occurred within the control population no comparison can be made with the lower GA_3 concentrations to see if they also were having an inhibitory effect.

The failure of plants to flower within the control population was most probably influenced by two factors. Firstly, when working with smallish populations, there is always the chance that there will not be an even distribution of genotypes within the selected population under study. In this case under these environmental conditions a dominance of "rosette genotypes" may have been selected. Another possible explanation could be the previously observed effect of pot size (Chapter 7). The present experiment was conducted with the plants growing in 10 centimetre pots, which might be expected to inhibit reproductive development, especially towards the latter period of the experiment, when the root volume exceeded a certain value. This being the case it is possible that GA_3 might act by overcoming the inhibitory effect of pot size on reproductive development.

Internode elongation occurs mostly by cell division (Table 11.7, 11.8). It appears that GA_3 does not affect this process apart from increasing the rate of stem elongation. In greatly elongated internodes associated with inflorescence development the extra internode elongation occurs by cell division, not cell elongation (Table 11.8).

Another interesting feature related to the node at which lateral branching first occurred on elongated stems. For all GA_3 treatments

(i.e. data bulked) the average node was 9.37 $\pm$ 0.33 (lateral branches greater than 1.5 cm.). Comparison to the three plants that flowered under natural photoperiods shows that the first inflorescence initiations occurred at node number 10.66 $\pm$ 0.33. Could this suggest a relationship between inflorescence initiation and lateral bud development? Thomas (1962) provided evidence from white clover of the homologous nature of vegetative and reproductive axillary bud primordia. He suggested that the onset of flowering may be the result of decreased growth inhibition from the apex. The correlation, in the GA_3 treated sainfoin plants, between the node at which inflorescence initiation first occurred and the node at which lateral branches developed suggest that this apical inhibition, as suggested by Thomas (1962), is released at about the 10th node stage of growth under these environmental conditions. This would suggest that lateral bud development may be controlled by an endogenous process similar to that which controls inflorescence initiation. Further this could suggest that if the plant genotype does not have the potential to effect inflorescence initiation (e.g. lack of low temperature pretreatment), then the closely related lateral bud development phenomenon might be able to proceed.

All plants in the GA_3 treatments had elongated stems, from which it is concluded that GA_3 can effect stem elongation in all genotypes of Melrose, including those that normally remain as non flowering rosettes.

Figure 11.1 shows that stem elongation occurred soon after the first application of GA_3. For the 40-500 /ug/ml. GA_3 treatments 11 days passed after the first GA_3 application before stem elongation was first recorded. For the 20 /ug/ml. GA_3 treatment it was not until 18 days after the first GA_3 application that stem elongation was first noted. No reason can be given for this delay in the 20 /ug/ml. GA_3 treatment apart from the fact that this was the lowest concentration applied. It should be remembered also that this was the only treatment in which plants blossomed under natural photoperiods.

The node at which internode elongation began (Table 11.1) also shows this delayed effect (re stem elongation). When averaging all plants in each treatment it is shown that the 20 /ug/ml. GA_3 treatment plants began internode elongation later than the other treatments. This trend is more clearly observed when comparing the first plants that elongated in each treatment. In all early examples plants in the 20 /ug/ml. GA_3 treatment began stem elongation at least one node later than comparable examples

in the other GA_3 treatments.

In Chapter 3 it was noted from dissections that the number of enclosed leaf primordia in the parent shoot of a seven day old Melrose seedling averaged 2.66. In a two week old seedling the number had reached four. When the first GA application was made most plants would have had two expanded leaves, and these plants would also have had three to four enclosed leaf primordia i.e. a total of five to six leaves initiated. Comparing this to Table 11.1 one can conclude that upon the first GA_3 application internode elongation later began below a leaf primordium that had already been initiated.

When comparing treatments, average stem length showed no significant differences (Table 11.5). However, within all treatments separate stem growth forms (internode thickness and length) could readily be distinguished (Table 11.6, Figure 11.2). These growth forms do not appear to be influenced by GA_3, as non-treated plants in other experiments show the same growth forss.

In Table 11.1 it is shown that in the 20 /ug/ml. GA_3 treatment the average internode length was greater than in the other populations. The reason for this is that this group had three plants that blossomed and that inflorescence initiation is correlated with increased internode lengths. This effect is demonstrated in Figure 11.3. In all cases where a stem had blossoming inflorescences (also even aborted inflorescences) the internode basal to the node of attachment of the first inflorescence was longer than other internodes. This feature also occurred in non- GA_3 treated flowering stems in all cultivars. Booysen and Laude (1964) observed the same phenomen on Ladino white clover. These authors postulated that this extra internode elongation could be due to GA diffusing out from the developing inflorescence. Tcaczyk *et al* (1976) provided evidence that in *Avena* the developing inflorescence supplies gibberellins which cause the elongating growth response in next-to-last internodes. Kaufman *et al* (1976) found that in this *Avena* system the major GA of the inflorescence was GA_3.

In sainfoin, the experiments of this chapter show that applied GA_3 does not cause this extra internode elongation in non-flowering stems. This could be due to the following:-

(a) The GA_3 applied may not be entering the region within the plant where this extra-elongation growth response is controlled.

(b) Extra internode elongation could possibly be due to another GA (i.e. other than GA_3).

(c) Extra internode elongation could possibly be due to the effects of another hormone working independently of GA.
(d) There could be a synergistic effect of another hormone working with GA.

Internodes apical to the node of the first formed inflorescence do not show this extra-elongation even when higher nodes bear inflorescences (Figure 11.3). If the extra internode elongation were due to GA diffusing out of the developing inflorescence then it would be expected all internodes basipetal to a node bearing a developing inflorescence would be greatly elongated. Furthermore, in sainfoin the results obtained do not fit the model proposed by Booysen and Laude (1964), Tkaczyk et al (1976) and Kaufman et al (1976) suggesting that internode elongation in white clover and Avena is controlled by GA from the inflorescence. However, in the sainfoin example this GA mechanism could be working initially. For example, the first formed inflorescence, while at a young stage of development, could produce a growth promoting hormone(s) that might diffuse out into the stem in a polar direction only (this could involve only one of the hormones, if more than one is present). If there were a synergistic reaction then this would always occur basal to the node of the first formed inflorescence. As the first formed inflorescence developed in size it might next produce a stem growth inhibiting hormone that could move throughout the stem. This could possibly cancel the effect that would otherwise be produced by younger inflorescences above.

Shorter internodes apical to the first formed inflorescences might also be due in part to the fact that the apex initially has to supply cells to the developing inflorescence and the internode. Hence not as much internode growth may be able to occur as had occurred in previous internodes. Data for plant number 2 versus plant number 3 (Table 11.6) lend evidence to support this (i.e. comparing internode diameters, Bottom - Top).

GA_3 treatment does not appear to have had much influence on final leaf size (Table 11.3). These data appear to be in contrast to those obtained from other pasture and forage legumes (Fletcher and Martin, 1962; Finn and Nielsen 1959; Stoddart, 1959). In Wild White and Ladino Clover, Fletcher and Martin (1962) obtained increases in leaf area of 3.7 to 9.2 and 10.9 to 31.4 sq.cm. respectively. They obtained similar responses for petiole lengths also.

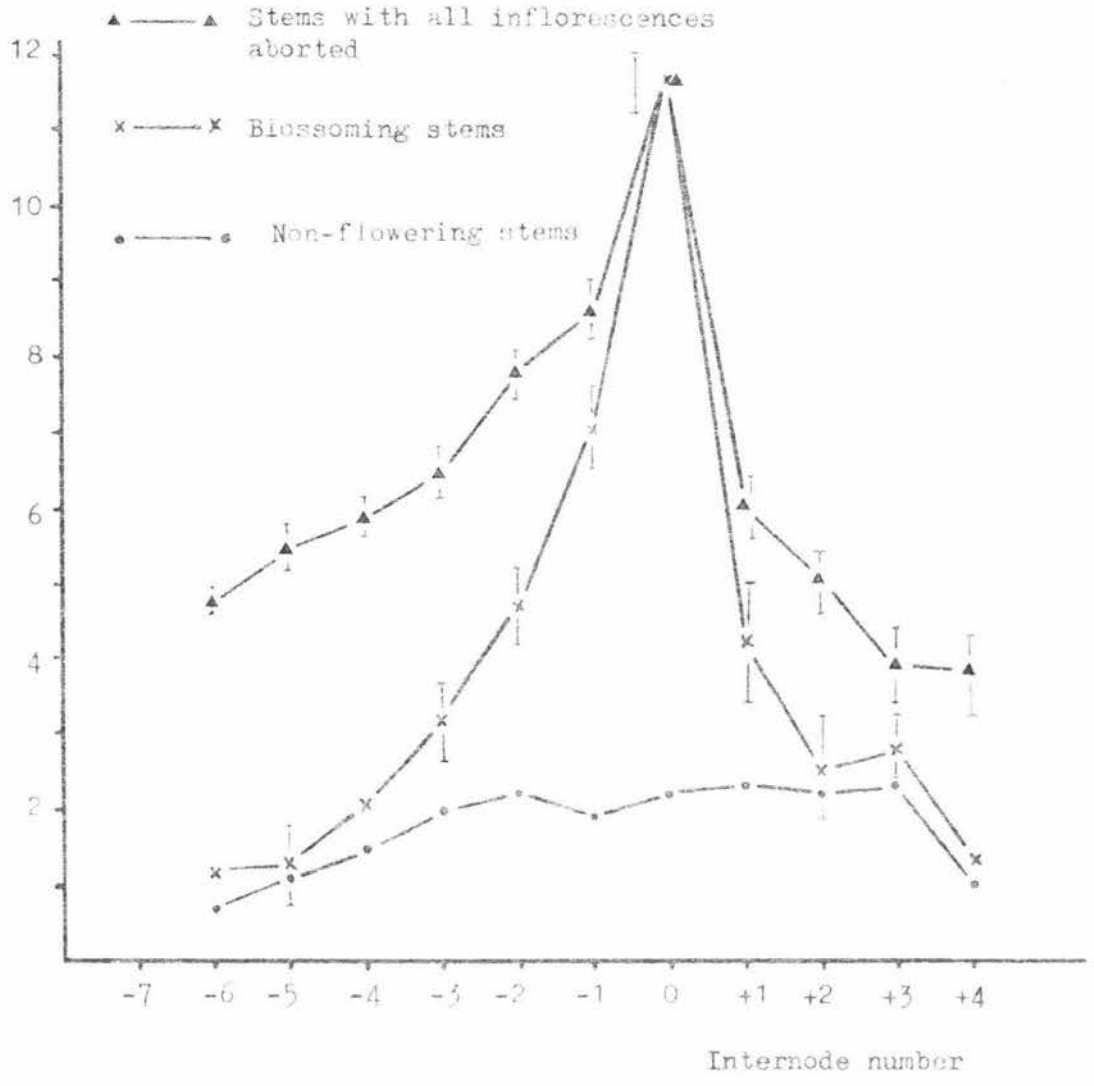


Figure 11.3

Internode elongation for blossoming stems, stems on which all inflorescences have aborted, and stems with no inflorescence initiation. 0 refers to the node number associated with the first formed inflorescence on the stem. For the blossoming stems nodes +1, +2, +3 and +4 had blossoming inflorescences. The non-flowering stems represent plants that had stem elongation but no inflorescence initiation. Vertical lines indicate SEM values. Symbols with no SEM lines indicate that SEM values were below 0.6.

When treatments were scored for leaf colour the 20 µg/ml. GA_3 population resembled the control group, having dark green leaves, the other GA_3 treatments had plants with light yellow leaves. From this observation it can be concluded that the higher GA_3 concentrations may have been inhibitory to some aspects of growth.

SECTION IV

GROWTH AND DEVELOPMENT UNDER COOL ENVIRONMENTAL CONDITIONS

Introduction

The experiments in this section have been designed to answer relevant questions regarding the effects of low temperature and low temperature pretreatment on reproductive development in sainfoin. From results of other experiments in this thesis it has been observed that all Common plants and a certain number of Melrose and Kranodar plants do not flower under long photoperiods when grown in the warm glasshouse. Thus a question that needs answering is whether these plants require low temperature pretreatment or even direct low temperature treatment before flowering can occur, and if so how much. The difference in the requirement for low temperature pretreatment between seedling plants and mature plants is also discussed.

Another question that will be answered is whether the low temperature pretreatment effect can be "held" in a glasshouse environment in short days until later favourable photoperiodic conditions occur.

Finally from the results obtained in this series of experiments one will be able to determine if there is any difference in the plants flowering characteristics after low temperature pretreatment (e.g. abortion and other flowering parameters).

CHAPTER 12

THE EFFECT OF LOW TEMPERATURE ON FLOWERING IN FIVE CULTIVARS OF SAINFOIN

12.1 Introduction

The results of the experiment described in Chapter 5 showed that different cultivars of sainfoin gave different flowering responses in their first year of growth when they were grown in natural environmental glasshouse conditions. Most Giant and Italian plants flowered; in Melrose only 57% of the plants had macroscopically visible inflorescences, in Krasnodar two plants (8%) had macroscopically visible inflorescences; and in Common no plants within the population studied had visible inflorescences.

Previous studies on sainfoin have shown that Giant will flower twice in its seeding year in a natural environment whereas Common will not flower at all (Thomson, 1951a; Gill and Vear, 1958). Cooper (1972) also described this ability to flower, or not flower, in the seeding year in cv. Eski and cv. Remont respectively. Spedding and Diekmahns (1972) for Common, and Cooper (1972) for cv. Remont noted that when these two cultivars did flower in the spring of their second season of growth there was no recurrence of flowering later in the season.

Fagan and Rees (1930) found that if Common was sown early in spring it would flower in its seeding year. Koreisa (1935) suggested that the difference in flowering behavior between Common and Giant might be due to a difference in the length of "..... the thermo-stage in development". He suggested that Giant has a short thermo-stage while Common has a long thermo-stage and must pass through winter before flowering can occur. Commenting on Koreisa's suggestion, Thomson (1938a) noted that temperature might be a factor determining the frequency of flowering, but to attribute all the differences in flowering between varieties to temperature requirement would be an over-simplification. Bawolski (1966) found that his vernalization treatments did not have any effect on development and flowering in Skrzeszowicka sainfoin and Ustrizyki Dolne sainfoin (Onobrychis viciaefolia Scop.).

In other pasture and forage legume species the effects of low temperature treatment have varied both between and within species.

For example, Rahman and Gladstone (1972, 1974) found that low temperature treatment was one environmental parameter that controlled floral initiation in Lupinus species. Clarkson and Russell (1975) and

Aitken (1955) for annual medics (Medicago spp.); Evans (1959) and Morley and Evans (1959) for Trifolium subterraneum; Knight and Hollowell (1958) for crimson clover (Trifolium incarnatum); and Kasperbauer et al (1962) for sweet clover (Melilotus albus Desr. and M. officinalis Lam.) also obtained similar responses. Beatty and Gardner (1961), for one clone of Minnesota white clover (Trifolium repens); and van Dobben (1964) and Bula (1969), for North European strains of red clover (Trifolium pratense), also noted a cold requirement in clones and cultivars of these two species.

From a brief survey of the literature on pasture and forage legumes which are taxonomically closely related to sainfoin it seems not unlikely that the fulfilment of a low temperature requirement might also determine whether or not some genotypes of sainfoin can flower.

The experiments described in this chapter were designed to assess the effects of various low temperature treatments on development and flowering in five cultivars of sainfoin. A comparison is made of the effects of low temperature treatment effect for 'adult' and 'seedling' plants of Melrose. In addition an experiment has been designed to test whether a low temperature treatment effect if found in short days can be "held" in the plant system after plants are placed into warm glasshouse short days which are followed by the increasing natural photoperiod culminating finally in potentially inductive long days. N.B. Many vernalized plants are 'devernalized' when transferred to an environment with higher temperatures (25-30°C), and it is often said that these types of plants are unable to "hold" the prior low temperature effect.

12.2 Materials and Methods

In this chapter the terms 'mature' and 'seedling' are used to distinguish between plants that have previously passed through a spring/summer environment (mature) as opposed to those that have not (seedling).

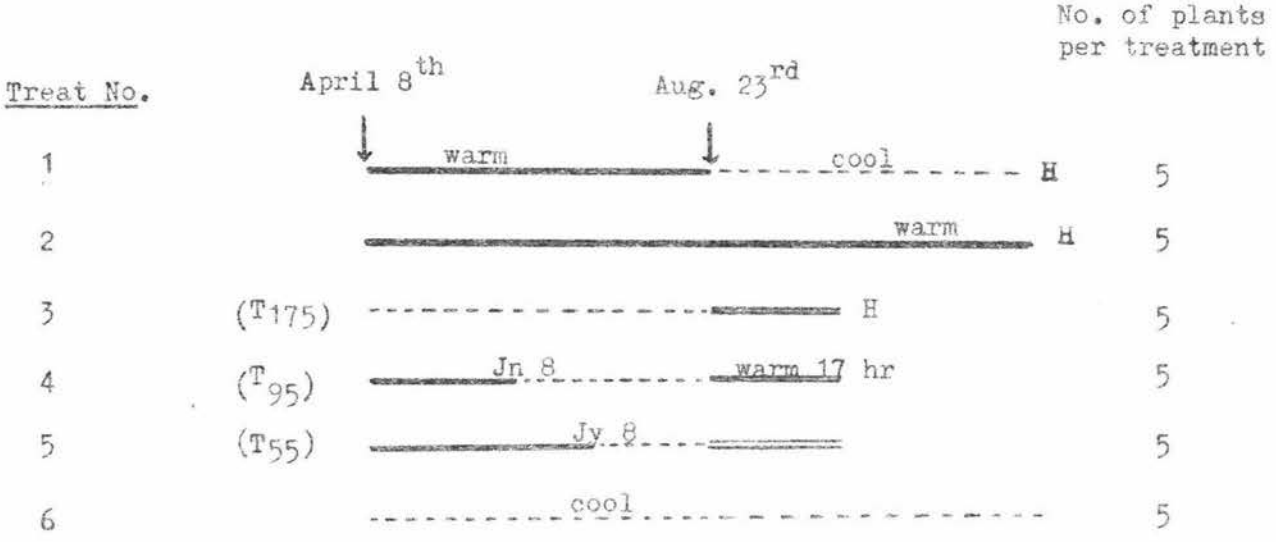
A. Mature plants

At the beginning of February 1976 all plants of cultivars Melrose, Giant, Italian, Krasnodar and Common used in the glasshouse experiment described in Chapter 5 were hand cut to five centimetres above the pot soil surface level then transferred from the glasshouse to the natural environment outside.

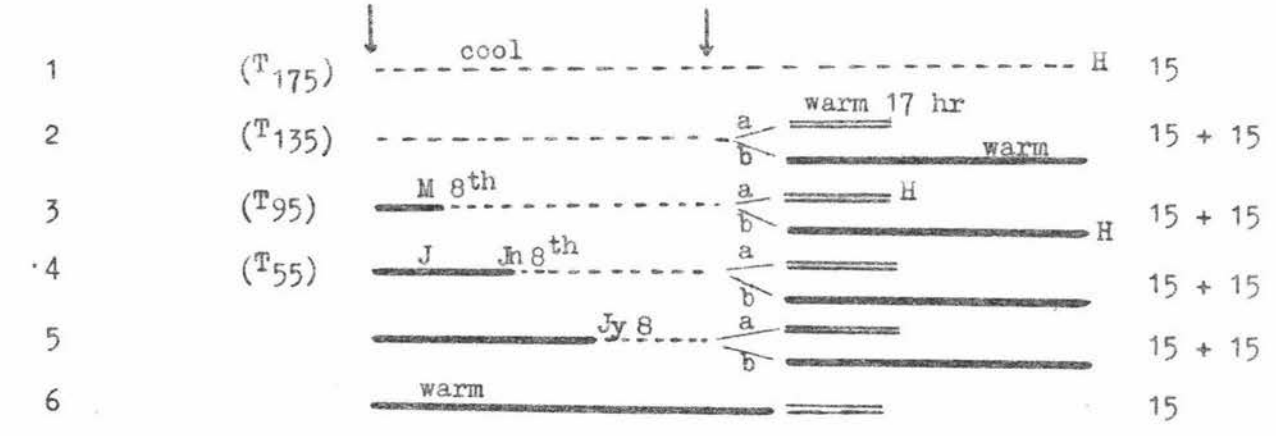
On April 8 1976 the experiment proper began. Experimental treatments are shown in Figure 12.1A. Where numbers allowed there were five plants per treatment, although in some treatments the numbers were reduced to

Figure 12.1 Experimental Treatments

A. Mature plants (5 cultivars).



B. Seedling Plants of Melrose (sown on March 8 1976)



KEY. warm = plants in glasshouse; cool = plants in natural outside environmen warm 17hr = plants in glasshouse under an artificially extended 17 hour photoperiod; T_{175} = indicates number of days plants were kept in the cool outside environment. H = date of final harvest.

four (i.e. those treatments with 4 plants. Fig. 12.1/A Treatment 1 = Italian, Melrose, Common, Krasnodar; Treatment 2 = Italian, Melrose, Krasnodar; Treatment 4 = Italian; Treatment 5 = Melrose, Krasnodar). Low temperature pretreatment consisted of leaving the plants under natural environmental conditions outside during the winter months. Plants not receiving low temperature pretreatment were kept in the glasshouse under natural photoperiods. For comparative purposes average monthly maximum and minimum temperatures are provided in Chapter 2.

On August 23 1976 plants in Treatments 3, 4 and 5 (Figure 12.1A) were placed into a 17 hour photoperiod. The same 17 hour photoperiodic conditions were used in this experiment as in Chapter 8, a detailed description being provided in section 8.2.

On November 15 1976 plants in Treatments 1 and 3 were repotted into 4800 cm^3 pots.

All plants received at various intervals a major element (NPKS) fertilizer application.

After transfer into the 17 hour photoperiod stem elongation measurements for Treatments 3, 4 and 5 were made at intervals of 10 days. The period when stem elongation began was also noted for the other treatments.

On October 13 1976 data were collected from plants in Treatments 3, 4 and 5. Due to no further plant growth these treatments were ended at this point. On December 21 and 29 1976 data from all other treatments were obtained. Data obtained on December 29 1976 are presented in Table 12.3.

B. Seedling Plants

Seed of cv. Melrose was sown in the glasshouse on March 8 1976 into 10 centimetre plastic pots containing an osmocote soil, nutrient mix as described in Chapter 2. When required, plants were repotted into 13 centimetre plastic pots.

Experimental treatments, each of 15 plants, are shown in Figure 12.1B. All plants received the same low temperature pretreatment, photoperiod and glasshouse environmental conditions as did the respective mature plants in section 12.2/A.

On November 1 1976 data from Treatments 2, 3, 4 and 5 (a and b) were obtained on December 21 1976.

Plants in this investigation of this chapter were not dissected to

determine if inflorescence initiation had occurred. When stem angle was noted the angle ($\pm$ 5°) to the horizontal of the apical five centimetres of each stem was measured.

12.3 Results

A. Mature Plants

A description of the plants' growth form and habit is provided in Table 12.1 to provide the reader with an indication of the growth form of the five cultivars after being held in natural glasshouse short days for five months.

Table 12.1

A description of adult plant growth form of plants in Treatments 3, 4 and 5 (Figure 12.1A) at August 5th after five months in the glasshouse under natural short days.

	Cultivar				
	Melrose	Giant	Italian	Krasnodar	Common
% of plants having elongated stems greater than 3.0 cm.	27	29	25	9	0
% of plants with a prostrate growth habit	100	64	54	100	100
% of plants with a semi-erect growth habit	0	14	19	0	0
% of plants with an erect growth habit	0	22	27	0	0

The main features shown in Table 12.1 are that a relatively high percentage of Italian, Giant and Melrose plants had stems that had elongated in warm short days and that also a relatively high percentage of Italian and Giant plants had an erect growth habit. All Common plants had a prostrate growth habit with no elongated stems. Melrose did have some plants with elongated stems, although all plants had a prostrate growth habit. It is also noted that on all plants of each cultivar elongated stems were prostrate.

In Figure 12.2 and Table 12.2, stem elongation, flowering and abortion data are presented for Treatments 3, 4 and 5. Figure 12.2 shows that within 30 days all plants in all populations of the five cultivars that had received prior low temperature pretreatment had begun stem elongation in the warm 17 hour photoperiod. The most interesting feature shown in this Figure was that there was overall for all cultivars (i.e. if data is bulked) a steady progression in time for all plants of each cultivar to have elongated stems as the length of the prior low temperature treatment, measured in days, decreased.

Table 12.2 shows that there was no significant difference in the percentage of plants with macroscopically visible inflorescences or with blossoming inflorescences in populations receiving 175 or 55 days of low temperature pretreatment. However the average number of days required to blossom, after transfer into the 17 hour photoperiod, decreased as the number of days of low temperature treatment increased for Melrose, Italian and Common, and to a lesser degree for Giant and Krasnodar. When comparing the T_{175} (i.e. 175 days of low temperature pretreatment) populations of each cultivar Common was the first cultivar to blossom followed by Melrose, then Italian, then Krasnodar, then finally Giant. When comparing the T_{55} treatments it is shown that Krasnodar was the first to blossom, next was Italian and Common, which was followed by Giant then finally Melrose. When T_{175} treated populations are compared to T_{55} treated populations regarding average number of days to blossoming it is shown that for Melrose and Common there is a large difference, for Italian a small difference, and for Krasnodar and Giant there is no significant difference in the average number of days required for plants within each population to blossom.

Table 12.2 also shows that there is no significant difference in the number of stems per flowering plant with macroscopically visible inflorescences within cultivars, although there are differences between cultivars. For example, Italian has less stems per flowering plant with macroscopically visible inflorescences than the other four cultivars.

For all cultivars, as the period of low temperature pretreatment increased there was an increase in the number of inflorescences per flowering stem. In the T_{55} populations there was more variation between cultivars in the number of inflorescences initiated per flowering stem when compared to the T_{175} populations. In the T_{55} populations Common and Melrose had the least, and Italian had the most inflorescences per flowering stem. For all cultivars there was the unexpected result that as the period of low temperature pretreatment increased the number of

Table 12.2

Flowering characteristics for low temperature pretreated plants of Melrose, Italain, Giant, Krasnodar, and Common, after treatment in a 17 hour photoperiod. T_{175}, T_{95}, T_{55} indicate the number of days of low temperature pretreatment. Measurements were made on October 13 1976. Where applicable SEM values are provided. Bracketed figures indicate the equivalent plants and their % inflorescence initiation when grown in the glasshouse over the 1975-76 season (Chapter 5).

		Melrose	Giant	Italian	Krasnodar	Common
% of plants having blossoming inflorescences	T_{175}	60	100	100	100	100
	T_{95}	100	100	75	80	100
	T_{55}	100	100	100	100	100
% of plants having inflorescence initiation	T_{175}	100(40)	100(80)	100(80)	100(0)	100(0)
	T_{95}	100(60)	100(50)	100(80)	80(0)	100(0)
	T_{55}	100(50)	100(100)	100(100)	100(0)	100(0)
Av. No. days to blossom	T_{175}	26.7	35.6	30.8	34.2	25.8
		±2.67	4.65	5.05	2.13	4.30
	T_{95}	36.0	35.5	-	38.7	33.2
		±1.87	1.55	-	1.67	2.92
	T_{75}	45.7	37.8	36.4	35.2	36.4
		±2.81	3.34	2.09	3.29	1.94
Av. No. stems per flowering plant with macroscopically visible inflorescences	T_{175}	7.0	7.2	5.0	7.0	7.4
		±0.89	0.73	0.71	0.32	1.33
	T_{95}	7.8	6.0	5.0	5.0	7.4
		±1.20	0.41	1.35	1.00	1.60
	T_{55}	7.5	5.4	4.6	6.6	7.6
		±1.55	1.12	0.68	1.63	1.29
Av. No. stems per flowering plant with blossoming inflorescences	T_{175}	3.4	2.4	2.8	2.8	5.4
		±1.60	0.93	0.66	0.73	0.60
	T_{95}	4.4	2.5	1.2	4.3	4.6
		±1.17	0.29	0.63	0.88	1.21
	T_{55}	4.8	3.6	3.0	5.2	6.2
		±1.70	0.75	0.55	1.46	0.92
Av. No. blossoms per blossoming plant	T_{175}	8.7	4.0	5.8	3.8	11.4
		±2.50	2.03	1.88	1.07	4.47
	T_{95}	7.2	3.0	2.0	7.0	8.0
		±2.67	1.11	0.87	2.87	2.77
	T_{55}	5.2	6.0	5.6	7.8	12.0
		±2.66	1.26	1.08	2.94	2.74
Av. No. blossoms per blossoming stem	T_{175}	2.56	1.67	2.07	1.36	2.11
	T_{95}	1.64	1.20	1.67	1.63	1.74
	T_{55}	1.08	1.67	1.87	1.50	1.93

(Continued overleaf)

		Melrose	Giant	Italian	Krasnodar	Common
Av. total number of visible inflorescences per plant	T_{175}	21.2	25.4	17.2	15.8	23.8
	T_{95}	18.0	16.2	15.0	15.6	19.4
	T_{55}	14.4	13.8	12.8	14.0	14.0
Av. Total No. of visible inflorescences per flowering stem	T_{175}	3.02	3.53	3.44	2.26	3.22
	T_{95}	2.31	2.70	3.00	3.12	2.06
	T_{55}	1.92	2.55	2.78	2.12	1.84
Av. stem length (Av. of longest per plant)	T_{175}	67.4	56.2	58.8	77.4	47.4
		±8.54	4.94	5.78	10.47	2.77
	T_{95}	70.8	52.2	57.7	73.0	40.6
		±10.05	4.77	5.02	12.79	1.86
	T_{55}	62.2	40.0	68.2	87.8	45.8
		±9.05	2.86	3.79	9.14	2.60
Overall Average		66.8	49.5	61.6	79.4	44.6
% of aborted inflorescences	T_{175}	75	84	66	76	52
	T_{95}	60	80	90	40	59
	T_{55}	64	56	56	44	33
Overall Average		66	73	71	53	48
Av. No. aborted inflorescences/ flowering stem	T_{175}	2.3	3.0	2.3	1.7	1.7
	T_{95}	1.4	2.2	2.7	1.3	1.5
	T_{55}	1.2	1.5	1.6	0.9	0.8

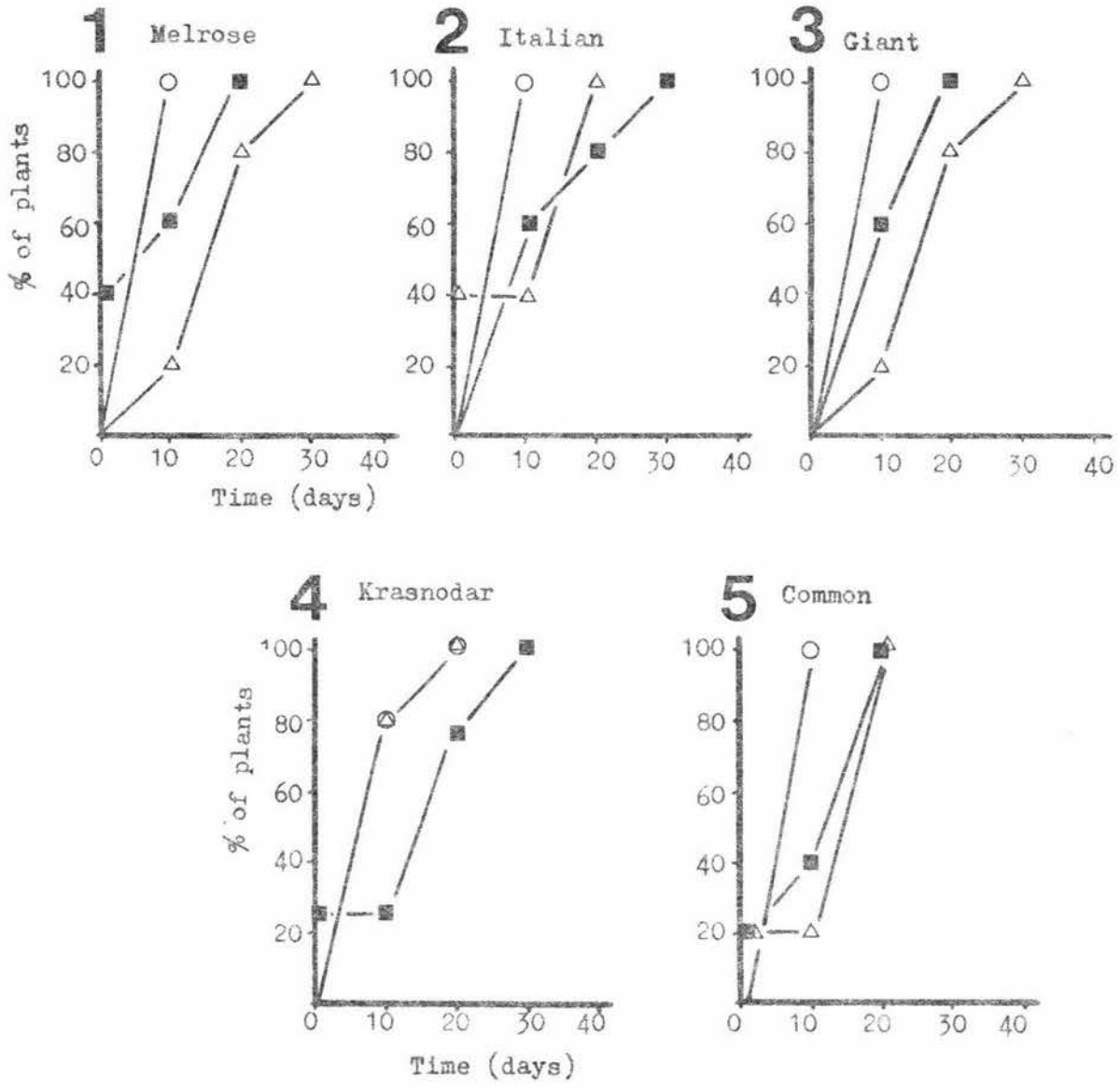


Figure 12.2

The effect of ○——○ 175, ■——■ 95 and △——△ 55 days of low temperature treatment on the percentage of 'adult' plants with stem elongation after transfer to a warm 17 hour photoperiod on August 23 1976.

stems per flowering plant with blossoming inflorescences decreased. When comparing the number of stems per flowering plant with visible inflorescences to the number of stems per flowering plant with blossoming inflorescences it is noted that there were many flowering stems in all cultivars that had no blossoming inflorescences i.e. all inflorescences had aborted.

Populations grown in the glasshouse had a low percentage of plants with macroscopically visible inflorescences when compared to the equivalent figures obtained with the same plants in the 1975-76 growth season. For example, in the previous growth season 60 percent of the Melrose plants within the GA treated population flowered, the next growth season, and under equivalent environmental conditions, no plants flowered (Table 12.3).

There was no significant difference in the time of blossoming between GH and GH/C treated populations for each cultivar, however, for Melrose, Italian and Common the C/C treated populations blossomed earlier than the GH/C treated populations.

The Giant and Italian plants that blossomed in the GH treatment had a low number of blossoms per plant whereas the low temperature treated populations had many more. There was little difference in the number of blossoms per blossoming plant for the GH/C and C/C treated populations of each cultivar.

B. Seedling Plants

(i) Seedling plants transferred on August 23 1976 into the 17 hour photoperiod plus the natural outside environment treated population.

For the above mentioned populations results are presented in Table 12.4 and Figure 12.3 and a description of treatments is given in Figure 12.1B.

Plants in each population had at least one elongated stem, except one plant in the warm pretreated 17 hour population; (Treatment 6, Figure 12.1B), in fact for those plants with stem elongation the average minimum number of elongated stems occurred in population T_{135}, which was 2.27 elongated stems; the highest number occurred in the natural cool environment treated population (Treatment 1, Figure 12.1B) which had an average of 3.86 elongated stems per plant with stem elongation. All elongated stems in the natural cool environment treated population had

Table 12.3

Flowering characteristics for Melrose, Giant, Italian, Krasnodar and Common populations in the glasshouse (GH) environment; in the glasshouse environment over the main period of winter then transferred into the natural outside environment on August 23 1976 (GH/C); and in the natural outside environment (C/C) throughout winter and spring. Data were obtained from individual plants on December 29 1976.

* Bracketed figures indicate the equivalent plants and their % inflorescence initiation when grown in the glasshouse over the 1975-76 season (Chapter 5).

		Melrose	Giant	Italian	Krasnodar	Common
% of plants with elongated stems	GH	20	67	50	0	0
	GH/C	100	100	100	100	100
	C/C	100	100	100	-	100
No. elongated stems on plants with stem elongation	GH	8.0	5.2	2.5	0	0
	GH/C	14.0	14.5	13.5	13.0	21.7
	C/C	14.5	34.2	15.5	-	17.6
Av. No. stems with inflorescence initiating per flowering plant	GH	0	5.5	3.0	0	0
	±SEM	-	1.12	0.86	-	-
	GH/C	14.0	14.5	13.5	13.0	21.75
	±SEM	2.12	1.71	2.22	3.05	2.59
	C/C	14.5	34.2	15.5	-	17.60
	±SEM	4.63	5.73	2.25	-	2.81
Av. length of longest elongated stem	GH	19.5	22.0	19.5	0	0
	±SEM	4.50	3.24	4.50	-	-
	GH/C	40.5	40.5	37.5	36.7	27.5
	±SEM	3.77	3.43	3.77	5.17	2.53
	C/C	23.2	35.6	35.7	-	15.2
	±SEM	6.24	2.46	6.21	-	3.81
% of plants with macroscopically visible inflorescences	GH	0*(60)	67 (83)	50 (75)	0 (50)	0 (0)
	GH/C	100 (75)	100(100)	100(100)	100(100)	100 (0)
	C/C	100 (50)	100 (80)	100(100)	- -	100 (0)
% of plants with blossoming inflorescences	GH	0	67	50	0	0
	GH/C	100	100	100	100	100
	C/C	75	100	100	-	80
Av. time when blossoming occurred	GH	-	Dec. 12	Dec. 10	-	-
	GH/C	Dec. 14	Dec. 10	Dec. 15	Dec. 19	Dec. 12
	C/C	Dec. 7	Dec. 11	Dec. 1	-	Dec. 30
Av. No. blossoms per blossoming plant	GH	-	8.0	3.0	-	-
	±SEM	-	2.55	2.00	-	-
	GH/C	17.7	31.2	28.25	15.0	28.25
	±SEM	5.66	3.09	4.11	2.64	5.23
	C/C	20.3	37.0	12.25	-	20.0
	±SEM	4.37	5.76	2.69	-	5.67

Table 12.4

Flowering characteristics for seedling Melrose plants pretreated with 175, 135, 95 and 55 days of natural low temperature environmental conditions and a population pretreated with warm glasshouse short day conditions (warm control) then transferred to the glasshouse 17 hour photoperiod. In addition a population was left in the outside environment over winter and spring 1976 (Natural cool). SEM values are provided. Data was obtained on October 20 1976.

	Treatment					
	Natural cool	17 hr. WARM CONTROL	T_{175}	T_{135}	T_{95}	T_{55}
Treatment No.	1	6	2a	3a	4a	5a
Stem Elongation						
% of plants having elongated stems	100	94	100	100	100	100
Av. No. elongated stems on plants with stem elongation	3.86 ± 0.34	2.53 0.29	2.53 0.29	2.27 0.21	2.87 0.26	3.06 0.27
Av. No. stems with infl. initiation per flowering plant	3.86 ± 0.34	2.10 0.23	1.93 0.22	2.14 0.18	2.53 0.26	2.44 0.30
Av. length of longest elongated stem per plant	41.0 ± 2.88	68.6 8.00	65.6 4.07	69.8 4.12	81.9 4.85	86.9 4.01
Av. length of the longest blossoming stem/flowering plant	41.0	82.3	70.3	73.2	81.9	86.9
Flowering						
% of plants having inflorescence initiation	100	62	93	93	100	100
% of plants having blossoming inflorescences	100	62	87	80	93	100
Av. No. days for first infl. on each plant to blossom	Nov. 22 ± 1.77	43.30 0.89	42.85 1.07	42.75 1.35	40.71 1.35	41.06 0.96
Av. No. blossoms/ blossoming plant	7.64 ± 0.82	4.70 0.73	4.31 0.91	4.42 0.61	7.64 1.88	7.69 1.59
Abortion						
Av. No. abortions/plant with inflorescence initiation	12.14 ± 1.71	2.67 0.53	2.70 0.95	2.18 0.30	2.77 0.53	1.73 0.25
% No. of aborted inflorescences	63	34	32	31	25	17
Av. No. aborted inflorescences per flowering stem	3.14	1.14	1.00	0.80	0.95	0.66

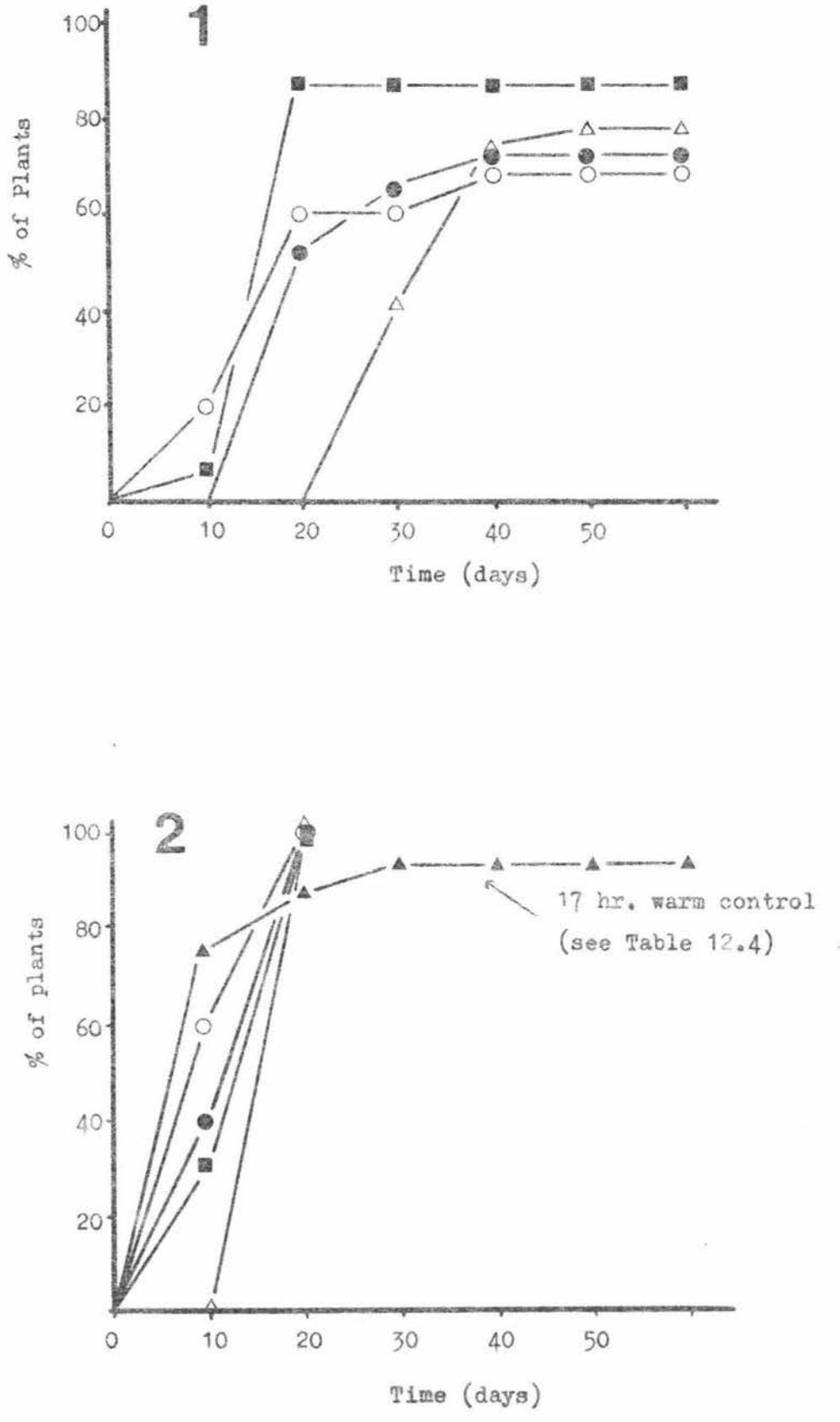


Figure 12.3

The effect of ○——○ 175, ●——● 135, ■——■ 95 and △——△ 55 days of low temperature treatment on the percentage of 'seedling' Melrose plants with stem elongation after transfer: Graph 1, to the natural environment within the glasshouse; and Graph 2, to an artificially extended photoperiod of 17 hours. All plants were transferred into the glasshouse on August 23 1976.

inflorescence initiation whereas the other low temperature pretreated populations, and the warm pretreated 17 hour treated population, did have some elongated stems with no macroscopically visible inflorescences.

Figure 12.3, Graph Number 2, shows the rate at which plants within each population began stem elongation when transferred into the 17 hour photoperiod. The main features of this graph are (a) the slower stem elongation response in the T_{55} population when compared to the other low temperature pretreatments and the control population and (b) the time taken to start elongating decreased generally with increased low temperature treatment.

With increasing low temperature pretreatment there was a decrease in the length of stems when compared to the warm control pretreated population (Treatment 6), the natural outside environment treated population having the shortest stems of all (Table 12.4).

All populations, except the warm control pretreated population, had a high percentage of plants with macroscopically visible inflorescences, as was the case for the percentage of plants having blossoming inflorescences. However, as the period of low temperature pretreatment increased the percentage of plants with blossoming inflorescences slightly decreased. There were no significant differences between populations treated with a 17 hour photoperiod in the average number of days required for plants in each population to blossom.

When comparing the average number of blossoms per blossoming plant it is shown that the T_{175}, T_{135} and warm pretreated/17 hour treated populations had a lower number of blossoms per blossoming plant than did the T_{55}, T_{95} and cool natural outside environment treated populations. All 17 hour treated populations had a low number of aborted inflorescences per stem when compared to the populations remaining outside. In fact the outside treated population had 63 percent of its inflorescences aborted whereas the warm SD pretreated/17 hour treated population had only 34% aborted. The most interesting feature here was that as the low temperature pretreatment period increased so did the percentage of aborted inflorescences.

(ii) <u>Seedling plants transferred, on August 23 1976, from the outside environment into the natural short day glasshouse environment.</u>

For the above mentioned populations results are presented in Table 12.5 and Figure 12.3, graph number 1. Treatments are described in Figure 12.1B.

The percentage of plants with stem elongation, when measurements were made on October 20 1976, varied between treatments, ranging from 67 to 87 percent. An increased number of plants with stem elongation did not necessarily correlate with a longer period of low temperature pretreatment. T_{135} and T_{175} treatments had fewer elongated stems on plants with stem elongation, and also shorter stems. From Figure 12.3 it is observed that the T_{55} treated population was much slower to respond to the warm glasshouse environment than the T_{95}, T_{135} and T_{175} treated populations. In addition the T_{55} and T_{95} treated populations had prostrate stems whereas the T_{175} and T_{135} treated populations had elongated stems that were more erect.

The T_{55} population had fewer plants with inflorescence initiation than the T_{175}, T_{135} and T_{95} populations, although, the T_{55} population had the highest number of plants with blossoming inflorescences: the fact there was a gradual increase in percentage blossoming from the T_{175} to the T_{55} population. The T_{175} treated population also had fewer flowering stems per flowering plant (Table 12.5). For the three populations that had plants that blossomed the average time required for the first inflorescences on each plant to open a flower did not differ significantly. The T_{135} population had the highest number of blossoms per blossoming plant (Table 12.5).

The percentage of aborted inflorescences increase with increasing low temperature pretreatment, although the average number of aborted inflorescences per flowering stem decreased as the low temperature pretreatment increased (Table 12.5).

When plants from the T_{175}, T_{135}, T_{95} and T_{55} populations were re-examined on December 21 1976 there was no increase in the percentage of plants with inflorescence initiation (Table 12.5). One plant in the T_{95} population was the only plant from all populations to have any more blossoming inflorescences. This plant did previously have inflorescence initiation but the inflorescences had aborted; when blossoming did occur the first flower opened on December 5 1976 then only three inflorescences blossomed.

12.4 Discussion

Results in Chapter 5 showed that 57% of Melrose, 8% of Krasnodar and no Common plants flowered when grown in the warm glasshouse, however, in this present study the data show that when plants have received low temperature treatment or low temperature pretreatment nearly all plants

Table 12.5

The effect of 175, 135, 95 and 55 days of low temperature pretreatment then transfer to natural warm short days on August 23, 1976 on reproductive development for Melrose seedling plants. The photoperiod in the natural warm glasshouse ranged from approximately 10.8 hours (late August) to 15 hours (end of December). Data was obtained on October 20 1976. SEM values are provided.

	Low Temperature Pretreatment (days)			
	T_{175}	T_{135}	T_{95}	T_{55}
Treatment Number	2b	3b	4b	5b
Stem Elongation				
% of plants having elongated stems	67	73	87	75
Av. No. elongated stems on plants with stem elongation	2.60	2.62	3.38	3.17
±	0.37	0.62	0.40	0.52
Av. No. stems with visible infl'ces per flowering plant	2.11	2.71	2.80	2.43
±	0.31	0.64	0.39	0.57
Av. length of longest elongated stem per plant	19.7	18.3	21.4	21.9
±	4.12	5.31	3.45	5.56
Av. length of longest blossoming stem per flowering plant	-	37.5	30.5	42.9
Av. angle to the horizontal of elongated stems (± 5°)	27.0	42.2	9.0	12.0
±	6.1	9.2	2.2	3.5
Flowering				
% of plants having inflorescence initiation	60	64	73	44
% of plants having blossoming inflorescences	0	18	27	31
Av. No. days required to blossom	-	41.0	39.2	42.0
±		2.00	2.36	1.56
Av. No. blossoms/blossoming plant	0	4.0	3.0	2.75
±		1.00	1.68	0.48
Abortion				
% No. of aborted inflorescences	100	83	73	77
Av. No. Aborted inflorescences/ flowering stem	1.94	2.00	2.07	2.23

within a population, in all cultivars, flowered.

In a study such as this it is difficult to totally rule out the possible effects on inflorescence initiation of environmental factors other than low temperature (e.g. light intensity, wind, humidity etc.); however, it would be most unlikely that these have any significant effects. The effect of lower intensity light during the winter months can at least be ruled out as plants kept in the glasshouse do not initiate inflorescences.

For all plants of each cultivar to flower in a 17 hour photoperiod only 55 days of natural outside low temperature pretreatment was necessary. However, if a longer period of low temperature pretreatment was allowed then plants, after transfer to the warm 17 hour photoperiod, will blossom earlier, this was especially so for Melrose and Common. After 175 days of low temperature pretreatment Melrose and Common blossomed earlier than Giant, Italian and Krasnodar. After only 55 days of low temperature pretreatment Melrose was the last, by a period of seven to eight days, to blossom (Table 12.2). Thus it can be concluded that although only 55 days of low temperature treatment are necessary for all plants to flower any further low temperature treatment will (a) hasten the time to blossoming when plants are transferred to long days and (b) increase the number of inflorescences per flowering stem and the number of blossoms per blossoming stem (Table 12.2).

In Table 12.3 it is shown that all cultivars that had low temperature treatment had elongated stems, whereas, some plants that were kept in the glasshouse over winter and spring had no elongated stems. All plants in each cultivar receiving low temperature treatment had inflorescence initiation whereas no Melrose, Krasnodar and Common plants and only 67 and 50 percent of Giant and Italian plants respectively had inflorescence initiation in those populations receiving no low temperature treatment. Figures within brackets in Table 12.2 and 12.3 represent the percentage of plants with inflorescence initiation for the same plants in each population that were grown in the natural environment within the glasshouse over the 1975 - 76 season (Chapter 5). From these figures it can be observed that, except for one Krasnodar plant from the T_{95} treatment in Table 12.2, all plants within each cultivar that did not have inflorescence initiation last growth season (1975-76) did so when each had received low temperature pretreatment or low temperature treatment. However, one observes in Table 12.3 that some plants that flowered last season (1975-76) in the glasshouse did not flower this season under equivalent conditions. One possible explanation for this decrease in the percentage of plants

with inflorescence initiation for Melrose, Giant, Italian and Krasnodar populations might have been a pot size effect as described in Chapters 7 and 8. To test if this was so individual plants from each cultivar were repotted into 4800 cm^3 pots on December 29 1976 and left in the glasshouse.

These plants were left until February 10 1977 and of these only one Krasnodar plant had initiated inflorescences, however, they all aborted. This result does not, however, totally rule out the possible effect of pot size as plants might have been initially inhibited when grown in the smaller pots and the effects of this were still blocking reproductive development when plants were repotted.

It would appear also that the GH/C and C/C treatments (Table 12.3) may also have been initially affected by pot size before plants were repotted into the 4800 cm^3 pots. For example the seedling Melrose plants (Table 12.4) blossomed on November 22 1976 whereas the equivalent Melrose adult plants (Table 12.3) did not blossom until December 7 1976. Because of the difficulty in maintaining an adequate soil moisture level within the pots under the outside, and often windy, environmental conditions plants in the GH/C and C/C treatments were transferred from the 1400 cm^3 to the 4800 cm^3 pots. In the glasshouse the plants root systems when kept in 15 centimetre pots (volume = 1400 cm^3) could be kept moist by using the sub-irrigation technique for watering.

In Table 12.3 it is shown that the C/C treated Melrose, Italian and Common plants blossomed earlier than the GH/C treated populations. However, for Giant all populations (GH, GH/C, C/C, Table 12.3) blossomed at a similar time. This suggests that Giant might not have any low temperature requirement, or low temperature interaction effect with photoperiod and that the main dominating influence that controls flowering is photoperiod. However it was noted on August 23 that Giant plants grown outside in cool short days did have aborted inflorescences on their stems. From this observation one can conclude that low temperature treatment does stimulate inflorescence initiation, however, it does not effect complete inflorescence development. It appears that in Giant the later stages of inflorescence development was controlled by photoperiod alone with no interaction effect with temperature. Where comparisons can be made it is shown that the other cultivars do respond to low temperature treatment and that a low temperature x photoperiod interaction brings about earlier blossoming.

In the later experiments of this Chapter, where only seedling Melrose plants were tested, it is shown that 55 days of low temperature pretreatment followed by warm 17 hour photoperiod treatment was enough to satify the

low temperature requirement for all genotypes within this cultivar. It can be noted (Table 12.4) that the T_{175}, and to a lesser extent the T_{135}, populations have relatively low figures for: the average number of stems with inflorescence initiation per flowering plant, the length of the longest elongated stem per flowering plant, the length of the longest blossoming stem per flowering plant, the average number of blossoms per blossoming plant and the percentage of plants with blossoming inflorescences, when compared to the T_{55} population. The only explanation that I can put forward for this result is that when the T_{175} and to a less extent the T_{135}, populations were placed outside plants were very small; under the cool outside environment growth was slow, while at the same time plants (e.g. T_{55}) still kept in the warm glasshouse were undergoing rapid growth. Due to this growth difference the response to low temperature treatment might have been lowered because of less vegetative development. Interacting with this, a plant age effect may have been affecting the results, that is, plants under ideal conditions for growth might reach the 'adult' stage earlier than slow growing plants. By the time all plants were brought into the glasshouse on August 23 1976 there was no visible difference in the size of plants in the T_{175} population compared to those in the T_{55} population.

In Table 12.5 the plant size/age effect, as described for results presented in Table 12.4, for the longer low temperature pretreated plants again showed up (e.g. percentage of plants having elongated stems, percentage of plants having blossoming inflorescences).

When low temperature pretreated plants were transferred to the glasshouse it was noted that the population having received only 55 days of low temperature conditions had only 44 percent of the plants with visible inflorescences whereas the equivalent but 95 day low temperature treated population had 73 percent of plants with visible inflorescences (Table 12.5) One can interpret from this that most of the T_{55} treated Melrose plants probably had just begaun initiating inflorescences when they were transferred to the glasshouse and because of this few plants were (a) not able to develop their inflorescences further in warm short days because they were at a very young stage of development at transfer or, (b) that not all plants had initiated inflorescences at the time of tranfer to the glasshouse. It would appear that after 95 days of low temperature pretreatment most plants would have initiated and developed inflorescences to at least stage 4 (Table 4.1) and so could be readily observed at a later stage even if they aborted immediately on transfer to the glasshouse.

There was no increase in the percentage of plants with inflorescence initiation or the percentage of plants having blossoming inflorescences

(Table 12.5) when these same plants were re-examined on December 21 1976.

Thus it can be concluded that Melrose plants can not "hold" the low temperature pretreatment effect in warm short days for later more favourable warm long day conditions. From this it appears that the changes taking place within the plant during low temperature treatment are not irreversible. Lang and Melchers (1947) for Hyoscyamus and Schwabe (1971) for chrysanthemum have observed a similar effect.

It was noted that the average angle to the horizontal of elongated stems in the T_{175} and T_{135} treated populations was higher than the T_{55} or T_{95} treated populations (Table 12.5). Elongated stems from 17 hour and natural long day treated populations were always erect. It appears that under warm short day conditions plants can elongate their stems only in a prostrate manner (as described for the elongated stems in Table 12.1); under long day conditions elongated stems are erect. However, if plants have up to 135 days of low temperature pretreatment then stems can elongate their stems only in a prostrate manner (as described for the elongated stems in Table 12.1); under long day conditions elongated stems are erect. However, if plants have up to 135 days of low temperature pretreatment then stems can elongate in a more erect manner under warm short day environmental conditions (Plate 12.1).

In Table 12.2 it is observed that the percentage of aborted inflorescences increase as the low temperature pretreatment increased. This trend showed up most clearly in those cultivars that flowered in the glasshouse (Chapter 5) over the 1975-76 growth season. For example, in the Giant T_{55} treatment 56 percent of the inflorescences aborted whereas in the T_{175} treatment 84 percent of the inflorescences aborted. The reason for this high percentage, and also increase with increasing low temperature treatment, of aborted inflorescences was due to the fact that sainfoin initiates inflorescence over the N.Z. winter period in short days (also refer to Chapter 13, section 13.1). However, under outside short day conditions inflorescence development cannot proceed past stage 5 (Table 4.1). When an inflorescence reaches this stage in development it aborts thus plants left outside for longer periods would be expected to have a higher percentage of aborted inflorescences.

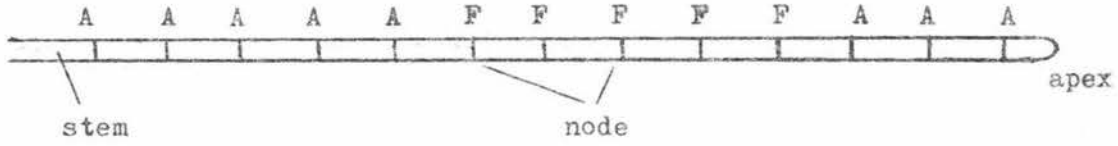
Within the same Melrose population (Table 12.4) the percentage of aborted inflorescences was highest in the T_{175} population and lowest

Plate 12.1

Stem elongation. The plant on the right with erect stems received a 17 hour photoperiod after low temperature pretreatment. The plant on the left received natural glasshouse short days (late August) after low temperature pretreatment. On this latter plant note the prostrate and semi-prostrate elongated stems.

in the T_{55} population. The same explanation as presented earlier accounts for this. Added weight for this explanation comes from the percentage of aborted inflorescences in the natural cool outside treated population (i.e. 63%). Stems from these plants showed a definite abortion pattern with the first formed inflorescences nearly always aborting.

This abortion pattern is described below.



A = aborted inflorescence

F = Blossoming inflorescence.

On some stems the first five to six formed inflorescences had aborted. This pattern and higher percentage is explained by the short photoperiod conditions when these inflorescences were initiated. Cool short days allowed plants to initiate inflorescences, but, there was a long day requirement for continued inflorescence development.

The results of this Chapter will be compared and contrasted to results obtained by previous authors for other closely related legumes in Chapter 15.

CHAPTER 13

THE EFFECT OF PHOTOPERIOD ON CONTINUED INFLORESCENCE DEVELOPMENT AFTER LOW TEMPERATURE PRETREATMENT

13.1 Introduction

Results presented in Chapter 12 indicate that certain genotypes of sainfoin require low temperature pretreatment before they can initiate and develop inflorescences, and that initiation can occur under cool environmental conditions in short days. The main photoperiodic treatment in that study was 17 hours, hence no indication was obtained on the effects of low temperature pretreatment on the critical photoperiod required for continued inflorescence development.

Previous studies for other plant species have revealed that in some cases the photoperiodic requirement is modified by low temperature or low temperature pretreatment. For example, the biennial sugarbeets require both thermo-induction and long days but with extended cold treatment become capable of flowering in short days (Owen *et al*, 1940). In other cases low temperature pretreatment can lower the critical daylength (Evans, 1959 a, b, *Trifolium subterraneum*; Rahman and Gladstones, 1972, *Lupinus* sp; Thomas, pers. comm., *Lotus pedunculatus*; Britten, 1960, *Trifolium repens*.

The initial aim of this present investigation was to determine the critical photoperiod of plants from within a low temperature pretreated cv. Melrose population, that is, to test the effects of vernalization (Lang, 1965). However, it has been demonstrated from results presented in Chapter 12 that sainfoin can initiate inflorescences while under short-day low temperature treatment. In addition, before photoperiod treatment began in this present experiment eight low temperature treated Melrose plants were dissected and inflorescence initiation was observed to have occurred with stages of development in each plant ranging from stage 1 to stage 5 (Table 4.1). One of those plants had initiated eight inflorescences. However, it was observed that any plants with inflorescences older than the youngest stage 5 inflorescences (Table 4.1) always aborted.

As sainfoin plants under low temperature treatment will initiate inflorescences this present investigation was aimed at determining the critical photoperiod for continued inflorescence development under warm environmental conditions.

13.2 Materials and Methods

On March 8 1976 Melrose seed was sown into 10 centimetre plastic pots containing an osmocote soil/nutrient mix. All seedlings were initially kept in the glasshouse. On April 8 1976 all plants were transferred into the outside environment (see Chapter 2). On August 23 1976 all plants were returned to the glasshouse.

On August 23 1976 photoperiod treatments began. These consisted of a natural photoperiod control treatment plus photoperiod treatments of 12¾, 13½, 14¼, 15 and 17 hours. Photoperiod treatments were given in the photoperiod boxes in the glasshouse (see Chapter 2 for a more detailed description) with each treatment having 15 plants. The photoperiod for the control population ranged from 10.7 to 13.2 hours during the period of this experiment.

At 10 day intervals, starting from when photoperiodic treatment began, stem elongation data were collected. Plants with stem elongation were defined as those having an internode with a visible length of three to four millimetres. The blossoming date for each plant was noted. On October 15 1976 measurements in Table 13.1 were obtained.

Throughout the duration of photoperiodic treatment daily maximum and minimum temperatures were recorded within one photoperiod box. The average daily maximum was 26.63°C and the minimum 15.54°C.

13.3 Results

(a) Stem elongation

For photoperiods of 14¼, 15 and 17 hours all plants produced elongated stems in photoperiods of 12¾ and 13½ hours 87% of plants in both populations had elongated stems. 27% of plants in the natural photoperiod control population had elongated stems (Table 13.1). For photoperiods 13½ and less the rate at which plants began elongating stems was lower than that for the longer photoperiods. Average rate at which plants begin elongating stems was similar for populations receiving photoperiods 14¼, 15 and 17 hours (Figure 13.1).

The length of the longest elongated stem per plant in each population and the average length of the longest blossoming stem per plant per population, increased with increasing photoperiod.

Except the control population which had prostrate stems, stems of all

Table 13.1

The effect of photoperiod on stem elongation, flowering and inflorescence abortion in low temperature pretreated Melrose plants. (± SEM values provided).

	Photoperiod treatment					
	Control	12¾	13½	14¼	15	17
Stem Elongation						
% of plants with elongated stems	27	87	87	100	100	100
Av. No. elongated stems/plant on plants with stem elongation	1.25 ±0.25	2.54 0.31	2.54 0.33	2.47 0.24	2.27 0.23	2.07 0.25
Av. No. stems with inflorescence initiation per flowering plant	1.25 ±0.25	1.78 0.28	2.17 0.27	2.27 0.19	2.14 0.25	2.00 0.27
Av. length of the longest stem on plants with elongated stems (cm)	20.8 ±5.99	18.8 3.88	28.6 4.23	27.5 3.61	39.6 3.56	41.3 5.13
Av. length of the longest blossoming stem per flowering plant (cm)	25.3	27.0	39.3	33.0	48.8	45.9
Flowering						
% of plants having macroscopically visible inflorescences	27	60	80	73	87	73
% of plants with blossoming inflorescences	20	40	47	53	87	73
Av. No. days required for first infl. on each plant to blossom	42.3 ±1.45	45.3 2.01	43.7 2.79	41.0 1.73	41.3 1.43	41.2 1.80
Av. No. blossoming inflorescences per blossoming plant	1.67 ±0.33	2.33 0.61	3.0 0.90	3.12 0.67	3.77 0.53	2.09 0.37
Av. total No. macroscopically visible inflorescences per plant with inflorescence initiation	3.92	5.77	7.25	7.76	7.44	4.81
Av. total No. macroscopically visible inflorescences per flowering stem	3.13	3.24	3.34	3.41	3.48	2.40
Av. No. blossoms/stem on plants with macroscopically visible inflorescences	1.33	1.81	1.38	1.37	1.77	0.82
Abortion						
% of inflorescences aborted	57	74	71	67	47	54
Av. No. aborted inflorescences/plant with visible inflorescences	2.25 ±0.63	3.44 0.93	4.25 0.62	4.64 0.73	3.67 0.59	2.72 0.74
Av. No. aborted inflorescences/ flowering stem	1.80	1.43	1.96	2.04	1.71	1.58

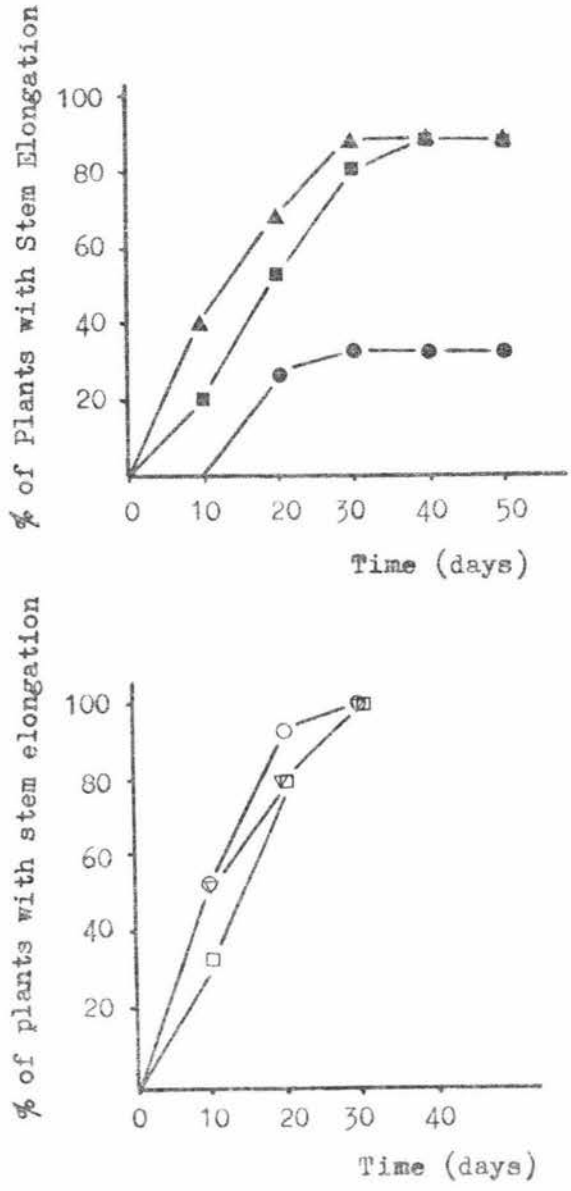


Figure 13.1

The effect of low temperature pretreatment : photoperiod on the percentage of plants with stem elongation. Treatments include: ●——● natural photoperiod, ▲——▲ 12¾ hours, ■——■ 13½ hours, □——□ 14¼ hours, ○——○ 15 hours, ▽——▽ 17 hours photoperiod.

plants with stem elongation in the various photoperiod treatments were erect.

(b) Flowering

Only 27% of the plants in the natural photoperiod control population initiated inflorescences; in photoperiods of 13½, 14¼, 15 and 17 hours the percentage ranged from 73% to 87%; in the 12¾ hour photoperiod only 60% of the plants had inflorescence initiation (Table 13.1).

In the shorter photoperiods (14¼ hours and less) some plants in each population with inflorescence initiation had all inflorescences aborted. In photoperiods of 15 and 17 hours, blossoming occurred in all plants that had macroscopically visible inflorescences (Table 13.1, Figure 13.2).

There was no difference in the average time required for plants to start blossoming in photoperiods, 14¼, 15 and 17 hours, but for photoperiods 13½ and 12¾ hours, progressively more time was required for blossoming (Table 13.1, Figure 13.3). In the natural photoperiod control population only three plants blossomed, one blossoming late in comparison to the other two (i.e. 45, 42, 40 days to blossoming). This had the effect of increasing the average.

There was an increase, with increasing photoperiod, in the total number of inflorescences per plant up to the 14¼ hour photoperiod. At the 15 hour photoperiod there were slightly fewer (i.e. compared to the 14¼ hour photoperiod), although, at the 17 hour photoperiod there were significantly fewer inflorescences per plant when compared to the other extended photoperiod treated populations.

On examination of the number of inflorescences per flowering stem for each population it is observed that only the 17 hour treated population had significantly fewer. Results for the number of blossoms per stem with visible inflorescences show the same trend.

(c) Inflorescence Abortion

Excluding the control population, the percentage of inflorescences aborted decreased as the photoperiod increased. There were fewer aborted inflorescences per plant in the 15 hour photoperiod treated population, becoming even lower in the 17 hour photoperiod treated population when compared to the 13½ and 14¼ hour treated populations (Table 13.1). In the control population only four plants had macroscopically visible inflorescences.

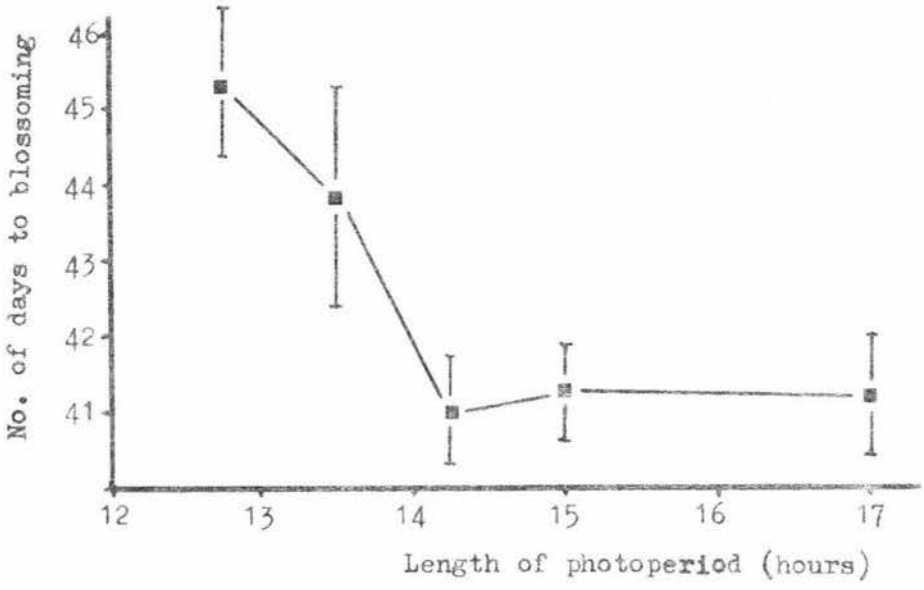


Figure 13.3

The effect of photoperiod on the number of days required for each plant that blossomed to begin blossoming. Vertical lines indicate SEM values.

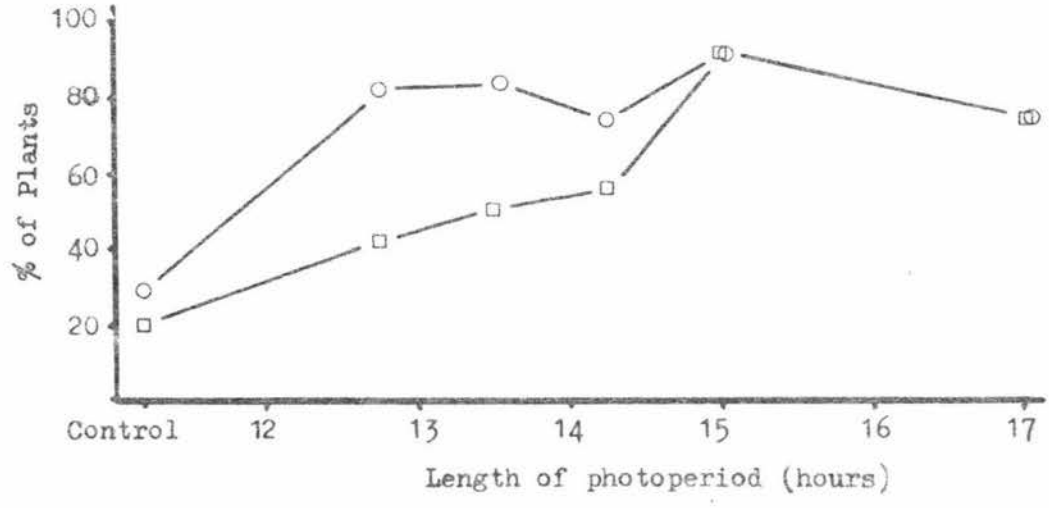


Figure 13.2

The effect of photoperiod on the percentage of plants with macroscopically visible inflorescences (circles) and the percentage of plants that blossomed (squares).

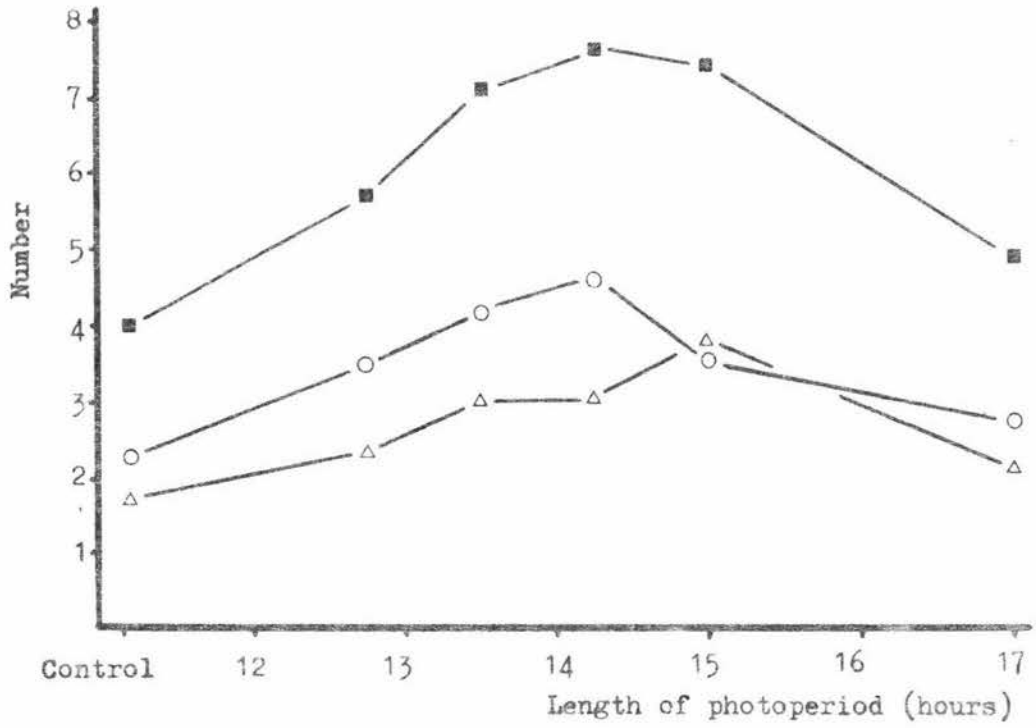


Figure 13.4

The effect of photoperiod : low temperature pretreatment on the (△———△) number of blossoms per blossoming plant, the (○———○) number of abortions per plant with inflorescence initiation, and (■———■) the total number of inflorescences per plant with inflorescence initiation. NB A few plants had inflorescences that were at stages 7 to 9 (Table 4.1). These were developing in a normal healthy manner and so were included with the blossoming inflorescences.

13.4 Discussion

To have at least half of the plants in a Melrose low temperature short day pretreated population reach the blossoming stage a photoperiod of 14¼ hours or higher was required, although, in the lower photoperiod treated populations some genotypes within each population did have blossoming inflorescences.

In the previous chapter (i.e. 12) it was noted that sainfoin plants initiate inflorescences during the cool short day winter season at Palmerston North. Therefore, when the plants in this present study were transferred into the glasshouse in late August nearly all of the plants would have already initiated inflorescences (refer also to section 13.1 paragraph 4). Therefore this study is examining the effects of photoperiod on continued inflorescence development to the stage of blossoming.

The reason why the control population had a low percentage of plants with macroscopically visible inflorescences (Table 13.1) is because many of the inflorescences on these plants probably did not develop further when the plants were transferred to the glasshouse, thus these inflorescences were too small to be observed and with time would have decayed.

Melrose plants when continually grown in the glasshouse began inflorescence initiation when the photoperiod was approximately 14.9 hours (see Chapter 11, section 11.4). When a population was left outside, blossoming occurred, on average, on November 22 (Table 12.4). The photoperiod at this time was approximately 14.6 hours. In this same population stem elongation began when the natural photoperiod was around 13.0 hours, and soon after this visible inflorescences began appearing. As already noted, a photoperiod of at least 14.0 hours was required for most low temperature short day pretreated plants to have blossoming inflorescences. Thus it would appear that sainfoin can initiate inflorescences in cool short days, however, longer warmer days of approximately 14 hours duration are required before inflorescences can reach maturity.

As Melrose is a heterogeneous cultivar with regard to reproductive development one would not expect a Melrose population to display an even critical photoperiod response as would be expected with clonal material. For photoperiods 12¾ hours and above most low temperature pretreated plants had elongated stems. For photoperiods 13½ hours and above (and to a lesser extent 12¾ hours) most plants (73 to 87%) had visible inflorescences. However, populations having a high percentage of plants with blossoming inflorescences occurred only in photoperiods 15 and 17 hours.

Thus one could conclude that in a low temperature pretreated population of Melrose under artificial photoperiod treatment in the glasshouse that: (a) stem elongation can occur in photoperiods of $12\frac{3}{4}$ hours and above; (b) continued inflorescence development to maturity will occur in most genotypes of Melrose at photoperiods of $13\frac{1}{2}$ hours and above with only some genotypes being able to develop mature inflorescences at $12\frac{3}{4}$ hours; (c) high percentages of plants with blossoming inflorescences occurred in photoperiods 15 and 17 hours with lower percentages occurring in $12\frac{3}{4}$ to $14\frac{1}{4}$ hours.

In summary the results of this investigation of the critical photoperiod for blossoming of Melrose following low temperature pretreatment show that with increasing photoperiod; the percentage of plants per population having inflorescence initiation increases, the number of plants per population having blossoming inflorescences increases, the number of blossoms per plant increases, the percentage of aborted inflorescences per population decreases, and the time required for the first inflorescence of each plant to blossom decreases.

However when examining Table 13.1 and the curves in Figure 13.4 it is noted that the 17 hour photoperiod, and to a lesser extent the 15 hour photoperiod, often deviate from the trend described in the above paragraph. This is most noticeable in Figure 13.4.

Two possible explanations can be suggested to explain this observation in Figure 13.4. Firstly plants receiving low temperature pretreatment may acquire a build up of a 'vernalization factor' and on transfer to warm long days this factor is lost (e.g. not synthesized, degraded, all used up).

Secondly, on transfer to warm long day photoperiodic conditions the production of a 'senescence factor' (S - factor) might be greater than the production of a 'flowering factor' (F - factor). This also would be determined by the length of the photoperiodic treatment.

These possibilities will be examined in greater detail in Chapter 15 using results from throughout this thesis.

It was also noted that those stems that elongated on plants in the extended photoperiod treatments were always erect, whereas, elongated stems on plants in the control natural daylength population were prostrate. It is difficult to determine why this difference occurred, however, it is probably related to the fact that the light supplement was of a different quality to that of natural light. Possibly the light supplement had more far red light than 'natural' light and this increased

the sensitivity of the plant tissue to a growth factor(s) that controls stem orientation. This aspect is discussed in more detail in Chapter 15.

CHAPTER 14

THE EFFECT OF LOW TEMPERATURE ON FLOWERING AT VARIOUS DAYLENGTHS

14.1 Introduction

Within any population of cv. Melrose grown in the glasshouse environment under natural photoperiods approximately 50 percent of the plants do not flower (Chapter 5). Low temperature pretreated Melrose populations receiving artificially extended photoperiods of 15 and 17 hours in the glasshouse had 87 percent and 73 percent respectively of of the plants within each population that had blossoming inflorescences (Chapter 13). However, all plants blossomed in equivalent populations of the same age that were left under natural environmental conditions over both winter and spring (Chapter 12). Thus the question that needs answering is whether a Melrose population requires direct low temperature treatment under long photoperiods for 100 percent of the genotypes within that population to blossom.

It has been found that in some plants with both a cold and long day requirement, cold treatment under long days (called photothermal induction, Owen et al, 1940) may promote flower formation and development more effectively than vernalization followed by warmer long days. This has been clearly shown in sugarbeets (Owen et al, 1940).

The aim of this present investigation was to determine the effects of long days on flowering of plants grown at low temperatures.

14.2 Materials and Methods

On March 8 1976 Melrose seed was sown into 10 centimetre plastic pots containing an osmocote soil/nutrient mix. Until photoperiodic treatments began on August 23 1976 all plants were kept in the glasshouse in natural photoperiods.

On that day plants were placed in the outside environment as described in Chapter 2. Treatments, each of 15 plants, consisted of photoperiods 12, $12\frac{3}{4}$, $13\frac{1}{2}$, $14\frac{1}{4}$, 15 and 17 hours plus a natural photoperiod control. To avoid rainwater coming in contact with the lamps, photoperiod boxes as described in Chapter 2 were positioned under a covered walkway (Plate 2.3). Twenty days after photoperiod treatments began, all plants were repotted into 13 centimetre plastic pots (osmocote mix). On November 13 1976, when the natural photoperiod had reached 14.3 hours, populations receiving

photoperiods of 12, 12¾, 13½ hours and the control population were left outside in the natural environment. Thus when each of these treatment populations was left in the natural environment outside, the natural photoperiod was higher than the photoperiod it initially received when it was kept in the photoperiod boxes. The 14¼, 15 and 17 hour photoperiod treated populations remained under artificial photoperiod treatment throughout.

At 10 day intervals starting from when photoperiodic treatment began stem elongation data were collected; in addition, the blossoming date for each plant was noted. On November 22 1976 measurements in Table 14.1 were obtained. At this date the control natural photoperiod plants were just beginning to respond to the natural increasing photoperiod by increasing the angle of their leaves to the horizontal (i.e. as described in Chapter 5 section 5.2 (a).

Because when measurements were first made on November 22 1976 the oldest inflorescences on some plants in the shorter photoperiods were only at stages 8 and 9 (Table 4.1) the figures for percentage of plants having blossoming inflorescences and the average number of days required for the first flower to open were obtained on December 2 1976.

For each elongated stem the angle ($\pm$ 5°) to the horizontal of the apical five centimetres was measured at the time of final harvest.

Throughout the duration of photoperiodic treatment average daily maximum and minimum temperatures, recorded within one photoperiod box, were 16.02°C and 8.24°C respectively.

The photoperiod for the control population ranged from 10.8 to 14.8 hours during the period of the experiment.

14.3 Results

(a) Stem Elongation

For longer photoperiods the time required for plants to begin stem elongation was shorter. For example, approximately 30 days were required for 20 percent of the plants in the 17 hour treated population to have elongated stems whereas approximately 85 to 90 days were required for 20 percent of the plants in the 12 hour photoperiod population to have elongated stems (Figure 14.1). In addition populations receiving shorter photoperiodic treatment had lower final percentages of plants with elongated stems (Figure 14.1, Table 14.1) and lower stem length

Table 14.1

The effect of photoperiod on stem elongation, flowering and inflorescence abortion in low temperature treated Melrose plants. SEM values are provided. Measurements were made on November 22 1976.

	PHOTOPERIOD TREATMENTS (hours)						
	Control	12	12¾	13½	14¼	15	17
Stem Elongation							
% of plants with stem elongation	20	33	40	67	73	87	100
Av. No. elongated stems on plants with stem elongation	2.33 ±0.88	2.40 0.51	3.33 1.05	2.80 0.54	3.09 0.47	3.38 0.42	3.33 0.30
Av. length of the longest elongated stem per elongated plant (cm.)	6.33 ±1.76	8.80 3.51	8.33 3.33	12.50 2.94	17.68 3.35	19.92 3.80	55.50 3.30
Av. length of all elongated stems (cm)	5.16 ±1.17	5.80 1.39	5.33 1.25	8.78 1.38	12.03 2.20	13.74 2.27	46.35 4.01
No. of flowering stems/treatment	6	10	13	26	31	30	42
Average stem angle (degrees)	5.71 ±3.69	8.33 4.10	37.78 3.57	7.68 3.28	13.91 4.16	28.93 5.55	86.55 1.44
Flowering							
% of plants with macroscopically visible inflorescences	13	27	13	60	60	60	100
% of plants having blossoming inflorescences	0	0	7	13	7	27	87
Av. No. days required for first inflorescence on each plant to blossom	+ - - -	98.0 -	95.0 -	92.0 -	92.0 4.26	90.43 2.61	82.30 2.02
Av. No. macroscopically visible inflorescences/flowering plant	6.50	4.50	14.5	13.05	13.04	15.04	11.21
Av. No. macroscopically visible inflorescences/flowering stem	1.08	2.22	1.11	1.92	2.38	1.99	3.75
Av. No. blossoming inflorescences/ blossoming plant	0 ± -	0 -	2.00 0	2.00 0	4.00 0	3.50 0.64	4.40 0.7
Abortion							
% of inflorescences aborted	0	0	0	23	15	26	32
Av. No aborted inflorescences/ flowering stem	-	-	-	-	-	4.40	3.4

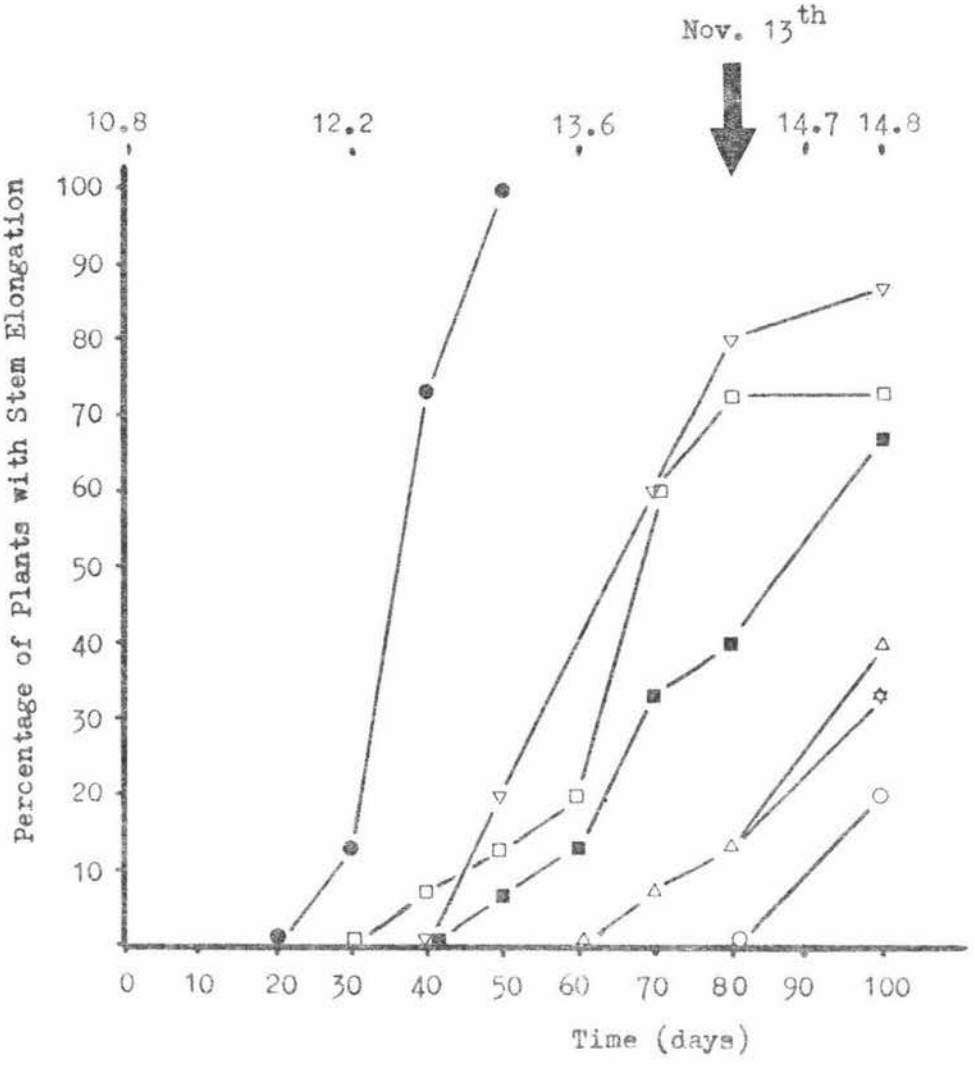


Figure 14.1

The effect of low temperature treatment : critical photoperiod on stem elongation. Day 0 (Aug. 23 1976) refers to when photoperiod treatment began. Treatments involved: ● ——— ● 17 hour, ▽ ——— ▽ 15 hour, □ ——— □ 14¼ hour, ■ ——— ■ 13½ hour, △ ——— △ 12¾ hour, ◭ ——— ◭ 12 hour, and ○ ——— ○ control natural photoperiod.

Figures above the graph show the length of the natural photoperiod during the time of this experiment. The arrow indicates the time of transfer of populations (12, 12¾ and 13½ hours) to the natural environment (see section 14.2).

measurements (Table 14.1). From Figure 14.1 it can also be observed that once the first plants in each population had begun to elongate their stems the rate at which the rest of the plants in the respective populations began to elongate their stems decreased with decreasing photoperiod. For example, in the 17 hour treated population only 30 days were required for all plants to elongate their stems, whereas, in the 15 hour treated population 60 days were required.

The total number of stems with inflorescence initiation per treatment increased as daylength increased, especially in photoperiods of 13½ hours and above (Table 14.1). Stem angle also showed an increase to the horizontal as photoperiodic treatment increased, with the 17 hour photoperiod treated population having an average angle of 86.55°.

(b) Flowering

As photoperiodic treatment increased there was an increase in: the percentage of plants with macroscopically visible inflorescences, and the percentage of plants with blossoming inflorescences. The average number of days required for the first inflorescence on each plant to blossom decreased with increasing photoperiod (Table 14.1, Figure 14.2).

On those plants the flowered, there was a general trend in that with increasing photoperiod treatment there was an increase in the number of macroscopically visible inflorescences per flowering stem. For the 17 hour treated population compared to the 15 hour treated population, both of which had more than one blossoming plant each, there was a large increase in the number of blossoms per blossoming plant.

(c) Inflorescence abortion

In comparison to other experiments (Chapter 5 and 13) the percentage of aborted inflorescences was low in this experiment (Table 14.1). When comparing the more vigorous flowering 15 and 17 hour photoperiod treatments the 17 hour treated population had fewer aborted inflorescences per flowering stem (Table 14.1). Apart from one plant in the 15 hour photoperiod treatment all plants that showed inflorescence initiation had some healthy developing inflorescences (i.e. not all aborted).

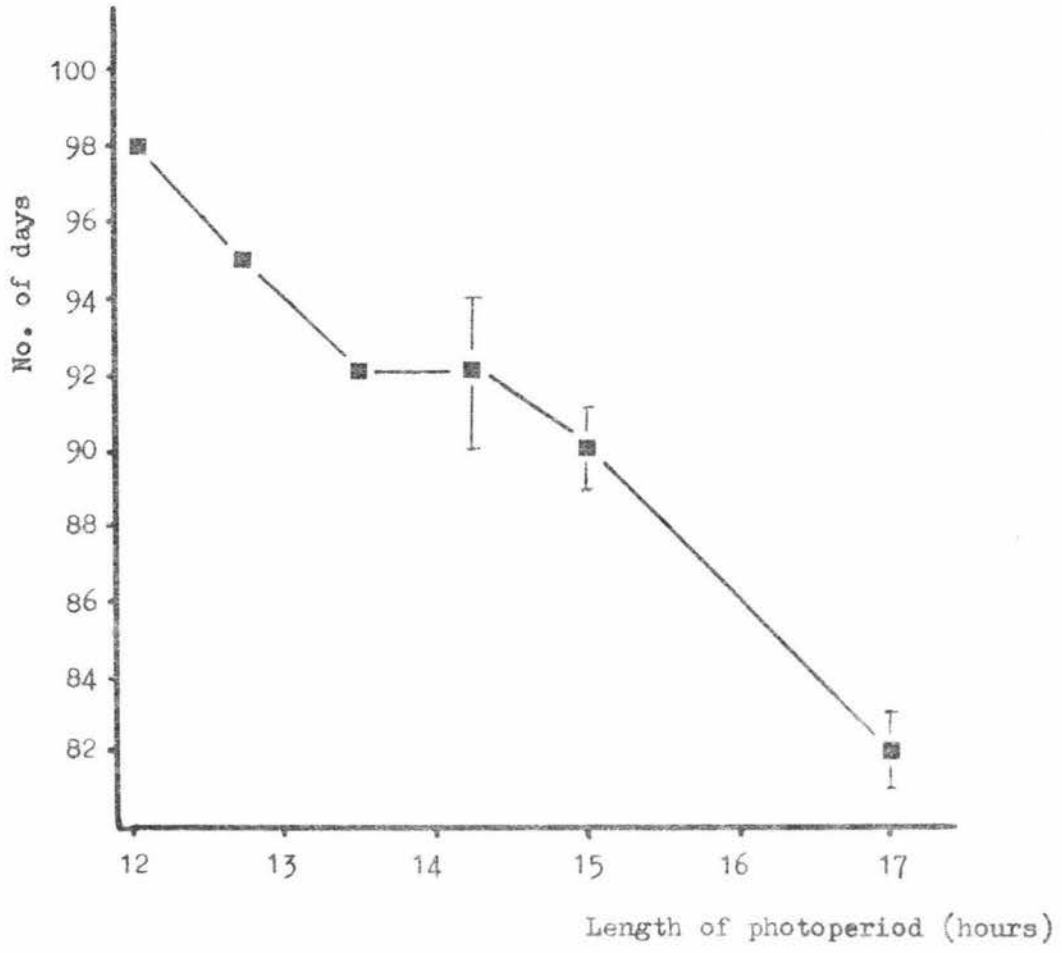


Figure 14.2

The effect of photoperiod on the average number of days required for each plant to begin blossoming. For photoperiods of 13½ or less only one or two plants per population (see Table 13.1) had blossoming inflorescences. Vertical lines indicate SEM values.

14.4 Discussion

The results of this investigation show a positive direct effect of low temperature on reproductive development in Melrose sainfoin. For example, in the main cultivar trial of Chapter 5 Melrose plants grown in the warm glasshouse began to elongate their stems at around November 30th (Figure 5.4); the natural photoperiod at this time was 14.8 hours. In this present investigation a considerable number of plants grown in the cool 13½ hour photoperiod had elongated stems, also some plants grown in the cool 12 and 12¾ hour photoperiods had elongated stems. Thus Melrose sainfoin would appear to be similar in response to low temperature treatment as sugarbeets (Owen et al, 1940).

It must be noted that it is difficult to determine whether the cool temperature response noted in this investigation had (a) a direct effect on development or (b) was a pretreatment effect, that is, a typical vernalization effect. For example, plants might have responded to the photoperiodic conditions only after a low temperature requirement had been fulfilled and thus this would not be a direct response to low temperature. However, low temperature pretreated Melrose populations receiving artificially extended photoperiod of 15 and 17 hours in the glasshouse had only 87% and 73% respectively of the plants within each population that had blossoming inflorescences (Chapter 13). In this present study, if further time had been allowed, 100% of the 17 hour treated plants would have blossomed as the two plants in this population that did not blossom had healthy developing inflorescences that would have blossomed later. Thus this indicates that there was a direct effect of low temperature on flowering in sainfoin.

The reader must be aware that this study only investigates the effect of photoperiod x cool temperatures on the rate of development of flowering, and not the final percentage of plants that would respond to these conditions, given enough time, by elongating and flowering. For example there was a relatively low percentage of plants blossoming in the 15 hour treated population when compared to the final percentage of plants that would have blossomed in a warm natural glasshouse treated population. Given more time many more plants would have blossomed in this population.

In this experiment it was advantageous, if possible, to leave plants outside instead of transferring them into and out of the photoperiod boxes each evening and morning, as less damage to the plants, from just physically moving each pot twice a day, occurred. This transfer did not

have any great effect on the experiment as when those treatments were left outside (i.e. 12, 12¾ and 13½ hour treated populations) plants had already begun to elongate their stems (see arrow in Figure 14.1).

In Table 14.1 it is shown that for those plants that blossomed there was not a great difference between treatments in the average time required for plants to blossom, especially in photoperiods of 15 hours and lower (Table 14.1, Figure 14.2). This appears to be due to particular genotypes that blossom under these cool environmental conditions. That is, these genotypes can blossom at low photoperiods when in low temperature conditions. Most genotypes within a population do not show this characteristic.

In Table 14.1 it is shown that there was an increase in the angle to the horizontal of elongated stems with increasing photoperiod. However, in the 12¾ hour treated population the average stem angle was higher than expected (i.e. compare with figures for the 12 and 13½ hour treated populations: Table 14.1). This is difficult to explain but it appears that the higher stem angle figure for the 12¾ hour treated population was due to a few genotype plants that tended to have more erect stems. As few plants had elongated stems in the lower photoperiod treatments this genotype difference showed.

CHAPTER 15

DISCUSSION AND CONCLUSIONS

15.1 Growth and development

A Melrose sainfoin plant that blossomed, when sown in early spring and grown in the warm glasshouse, passed through a series of distinct morphological stages from germination to anthesis. The young plant began development by initiating leaf and bud primordia in a manner as described in Chapter 3. After a few weeks of growth the young plant developed a prostrate leaf and rosette growth habit. Common and Krasnodar developed in a similar manner, although, within Giant and Italian some genotypes in each population had a semi-erect or erect leaf growth habit when in either long or short days (Table 12.1). After approximately 10 months of growth some genotypes within each cultivar population, especially in Common, had up to 30-40 basal buds per plant (Chapter 12).

In many species reproductive development can not proceed until a certain time after germination no matter what environmental conditions the plant receives. This growth phase is known as the juvenile phase For Melrose sainfoin it was shown in Chapter 6 that when plants are grown from seedlings in the warm glasshouse approximately 60 days were required before inflorescence initiation occurred.

When the juvenile condition had 'passed' and when the natural daylength began to increase, under environmental conditions described in Chapter 5, all genotypes from within each cultivar began the first morphological stage of reproductive development. Each plant responded to the changing environment by increasing the angle of its leaves to the horizontal. In effect plants moved from a prostrate (or semi-prostrate in the case of some Giant and Italian plants) to an erect leaf habit, but still rosette growth habit. In all cultivar populations, associated with this leaf angle increase the primary shoot bud and basal buds began to swell (Section 5.2b, 5.3b, Figure 5.3).

Soon after the buds had swollen and the leaves had become erect stem elongation began (Section 5.3c, 5.4c, Figure 5.4). For cultivars, and some genotypes within cultivars, that did not flower, stem elongation generally did not occur, although, some plants did have non-flowering elongated stems (Section 5.4). Final stem length depended on environmental conditions of growth and type of cultivar. For example, Krasnodar had the longest stems

(79.4 cm; Table 12.2) while Common had the shortest (44.6 cm; Table 12.2).

Elongating stems next began initiating and developing inflorescences. The stage when inflorescence initiation began on the stem varied with environmental conditions and between genotypes within cultivars, especially Melrose.

In Chapter 5 it was shown that in Melrose these observed developmental stages are correlated with flowering. Thus sainfoin passes through distinct morphological developmental stages before actual blossoming occurs. It appears that there is a gradual transition from one stage to the next (see Chapter 5), although the completion of one stage does not appear to be essential before a higher developmental stage can proceed. For example, natural outside cool temperature short day treated plants had initiated inflorescences before the leaves had become erect or had even elongated their stems (Chapter 12, 13). With increasing photoperiod and warmer spring conditions these plants went through, in the following order, the morphological developmental stages of,

inflorescence initiation ⟶ leaf erectness and bud swelling ⟶ stem elongation ⟶ inflorescence development

Under natural warm glasshouse conditions moving from short days to long days, plants passed through the reproductive stages in the following order

leaf erectness and bud swelling ⟶ stem elongation ⟶ inflorescence initiation ⟶ inflorescence development

Thus under natural conditions these stages are integrated into a distinct sequential pattern, and possibly even controlled by different growth processes. For example, with the application of GA_3 leaf erectness and stem elongation can occur in short days (Chapter 9, 10) although, inflorescence initiation does not occur. These reproductive stages described here are not analogous to the rigid system of developmental stages proposed by some workers (Mathon and Stroun, 1960).

Not all genotypes of sainfoin have the potential to carry out full reproductive development under glasshouse conditions. For example, in the Melrose cultivar when plants were germinated and grown wholly in long days (17 hour photoperiod) in the glasshouse four distict growth forms were observed: (a) there were those plants that remained as rosettes, (b) some plants had elongated stems but no inflorescence initiation, (c) some plants had elongated stems that blossomed relatively quickly

while finally (d) some plants had elongated stems but did not elongate and initiate inflorescences until a considerable period after the first blossoming genotypes. Possibly the reason for some plants not blossoming in a relative short time might be the presence of a juvenile factor. In Chapter 6 it was noted that when plants were grown wholly in long days the first plant in one population blossomed after 71 days of growth whereas the last plant in that population to blossom required 131 days, thus representing a difference of 60 days from the first to the last plant to blossom.(Table 6.3). In addition results from Chapter 6 indicated that Melrose plants when grown in the glasshouse have on average a juvenile period of around 60 days. A question that needs resolving now is would the results of Chapter 6 apply to plants when grown in the natural outside environment? For example, Fagan and Rees, (1930) noted that if Common sainfoin was sown early a high proportion of the plants would flower in that season. This raises the interesting question as to whether this is a direct low temperature response on inflorescence initiation associated with early spring conditions or a low temperature response affecting juvenility. Thus it is possible that low temperature conditions outside remove the juvenility condition earlier in some plants than do glasshouse conditions and thus the normal low temperature response leading to inflorescence initiation might then be able to be effected earlier than would otherwise be the case.

Results obtained by Waterschoot (1957) for Dianthus barbatus indicated that low temperature does not promote flowering unless given to plants of more than a certain minimum size. In sainfoin application of GA_3 does shorten the juvenile phase in at least some genotypes when grown in long days. For example in Chapter 11 the Melrose plants that blossomed in natural long days after GA_3 treatment did so after only 49 days, whereas in Chapter 6 a comparable population required 112.6 days before blossoming occurred (compare Table 11.2 to Table 6.3). In Chrysanthemum morifolium Thompsett and Schwable (1974) found that the level of endogenous gibberellins increased towards the end of vernalization treatment. From these latter results it would not be inconceivable that the juvenile period in sainfoin might be decreased when plants are grown from seedlings in cooler spring environmental conditions outside. Evidence to substantiate this comes from the observation by Thompson (1938a) where he noted that Giant seedlings, when grown in spring at lowish temperatures in the field, began elongation soon after the appearance of the sixth leaf. If stem elongation is associated with juvenility (see Section 6.4) then in Melrose

genotypes that flowered when grown in the glasshouse a considerable number of leaves (i.e. many more than six) had been initiated before stem elongation began.

When hypothesizing that GA_3 in sainfoin might decrease the juvenile phase it must be noted, however, that in Hedra helix application of GA_3 will induce the mature form to revert to the juvenile form of growth (Rogler and Hackett, 1975).

Those genotypes within a Melrose population that blossomed in a relatively short period of time when placed into long days might have a short juvenile period with no limiting low temperature requirement. In other words flowering in these genotypes is predominantly controlled by long photoperiods. In the previously mentioned genotypes (i.e. those requiring a longer period to blossom) it could also be possible that apart from a juvenile factor, a mild low temperature requirement might be affecting the time required for inflorescence initiation to occur. That is, a longer period in long days can overcome the low temperature requirement.

In the other growth form noted, where plants had only elongated stems, inflorescence initiation and development might be affected by some limiting factor in a process towards the end of reproductive development. This could involve a low temperature requirement or a requirement for short days to be followed by long days. There was always a low percentage of plants with inflorescence initiation in Melrose populations that received no short day pretreatment (Table 6.3) compared to populations that had passed through prior short days (Table 6.3, 5.1, 12.4, Appendix II). From this result it can be concluded that in Melrose there are some short-long day genotypes. Thomas (1961b) found that certain genotypes of white clover (Trifolium repens) failed to flower in long days unless they had previously received short days for at least four weeks. Thus in these genotypes ripeness to flower will only occur in short days and, once this condition is attained, long days are able to bring about inflorescence initiation and development.

The final growth form noted, that is, where the plant remained as a rosette under long day treatment, appears to be controlled by a low temperature requirement (Chapter 12). However, from the results of this thesis study one can not determine which genotypes that did not flower in the glasshouse when treated with only long days had a short-long day requirement or low temperature requirement.

15.2 The effect of photoperiod on growth and development

(a) Growth in short days

(i) Warm short days

In a population of Melrose held in warm short days for a period in excess of six months, at the end of this period no plants in this population had macroscopically visible inflorescences. In no cases when plants from within other experiments that had been held in warm short days were dissected, in no case was inflorescence initiation observed. However, in some plants the leaves became more erect in warm short days. This latter observation is described in more detail in Appendix I. On some plants, especially older plants with a dense foliage cover, limited stem elongation did occur, although, these stems were always short and prostrate (Table 12.1). I have suggested that this is a response to shading of the basal buds which in turn alters the quality of light reaching the basal bud; greater discussion on this aspect is provided in section 15.4.

(ii) Cool short days

In all cultivars examined it was noted that plants initiated inflorescences in cool short days, although, long days were required for these inflorescences to reach full development (i.e. blossom). Discussion on this aspect will be presented in section 15.4.

(b) Growth in long days

(i) Warm long days only

Here reference will only be made to Melrose. For Melrose plants germinated and grown wholly in long days a juvenile period of around 60 days was noted (Chapter 6). For plants in which the juvenile phase had passed (i.e. if plants had been held in a warm short photoperiod for around 60 days or more) approximately 40 to 50 long days were required from the start of the first long day to blossoming (Table 6.3). Melrose plants grown wholly in long days had a lower percentage of plants with macroscopically visible inflorescences (i.e. 20-25%) and a lower percentage with blossoming inflorescences (i.e. 17-20%) compared to those that had received prior short days (i.e. 30-50%: compare Table 6.3 to Table 5.1).

This demonstrates that some genotypes of Melrose sainfoin are short-long day plants. This aspect is discussed in more detail in Appendix II.

(ii) Growth in warm long days preceded by warm short days

Reference to Table 5.1 indicates the percentage of plants from each cultivar with macroscopically visible inflorescences and the percentage with blossoming inflorescences. There were large variations between cultivars: for example, the number of plants in each cultivar population that had blossoming inflorescences was: Melrose 57%, Italian 86%, Giant 83%, Krasnodar 8% and in Common no plants blossomed. Reference to section 5.4 and 15.1 describes in more detail reproductive development in Melrose under these conditions.

(iii)Growth in warm long days preceded by cool short days

Reference to Chapter 12 shows that nearly all plants from each cultivar blossomed after receiving these environmental conditions. A description of growth and development, under these conditions, is provided, in more detail, in section 15.4.

In conclusion, sainfoin appears to be a typical long day plant with some genotypes of at least Melrose having a short-long day requirement. This latter observation was also noted by Bawolski (1966) in two Polish sainfoin cultivars. Low temperature treatment will enable plants, however, to initiate inflorescences in short days. This is discussed further in section 15.4.

Melrose plants in their first year of growth, grown under natural warm glasshouse conditions had a critical photoperiod of around 14.9 hours (see section 11.4), although, low temperature treatment lowered the critical daylength of this cultivar (see section 15.4).

15.3 The effect of gibberellic acid on growth and development

In experiments with GA_3 it was noted that this hormone had a mild effect on inflorescence initiation, however, it had a substantial effect on vegetative growth. The early response of leaf erectness found after plants are transferred to long days (Chapter 5) could be reproduced in short days with the addition of gibberellic acid, even a concentration as low as 1.0 µg/ml. GA_3 being effective (Figure 9.1). In short days GA_3 could effect only limited stem elongation when compared to plants treated

with GA_3 in long days (i.e. compare Table 10.1 to Table 11.5). Stoddart (1966) found a similar response for red clover. A summary of the effects of GA_3 on growth and development for Melrose in different environmental conditions is provided below.

Environment	Response
1. Grown in artificially short days (SD) outside without GA.	Plants remain as rosettes. Leaves were prostrate.
2. SD in a warm glasshouse environment without GA.	Plants remain as rosettes, although the leaves are more erect (Appendix I).
3. SD and GA_3.	At low concentrations (1 to 10 µg/ml. GA_3) leaves become erect but the plant remains as a rosette (Chapter 9). At higher concentrations (20 to 500 µg/ml. GA_3) plants have stems that have elongated, although stems and internodes are short. Inflorescence initiation does not occur (Chapter 10).
4. Natural glasshouse long days (LD) without GA.	When plants are held in long days approximately 30-60% of the population have macroscopically visible inflorescences, with a slightly higher percentage of plants having elongated stems. The longest stems to elongated were around 70 cm.
5. Natural glasshouse LD and GA_3	At all concentrations tested (20 to 500 µg/ml. GA_3) all plants had elongated stems. Those plants having macroscopically visible inflorescences had an elongated stem as long as 140 cm. In all plants that had elongated stems only the primary stem had elongated. The percentage of plants with inflorescence initiation ranged from 33 to 50% (not significant). However, GA_3 lowered the critical photoperiod plus greatly decreased the juvenile period. GA_3 might also possibly overcome the inhibitory effect of small pot size on growth (Chapter 7).

It should be noted that only GA_3 was used throughout these investigations and the different results might have been obtained had other gibberellins been tested. For example, the long day plant Silene armeria could be induced to flower in short days by GA_7, with other

gibberellins being ineffective (Lang, 1965).

Another factor to consider is that the GA_3 concentrations tested might have been unfavourable for inflorescence initiation. However, this does appear unlikely as concentrations tested varied between 0.5 to 500 /ug/ml. GA_3. It could be possible that inflorescence initiation is dependent on more than one process or compound, all having a contributing effect to produce an inflorescence. If one substance is missing then the whole process might become blocked. In the case of the GA_3 experiments one substance has been added, although, without some other factor (s) GA is incapable of causing inflorescence initiation.

Under long day conditions it appears that even when GA_3 is applied reproductive development will occur only in those genotypes that would have had the potential to flower in warm glasshouse conditions without GA. Thus GA_3 seems not to play a major solo controlling role in reproductive development in sainfoin. Lang (1965), Schwabe (1971), Jones (1973) and Stoddart (1962) drew a similar conclusion. However, Stoddart (1966) demonstrated that in red clover floral initiation does require the presence of a specific level of free GA_3 during exposure to long days. Stoddart's (1966) results indicate that GA_3 is rapidly metabolized by the plant, thus providing evidence in support of his earlier suggestion (Stoddart, 1963) that GA_3 might be converted to another GA of which in turn exhibits an effect upon flowering. Michniewicz and Lang (1962) have shown that gibberellins A_1 to A_9 show remarkable differences in their ability to induce flowering in a variety of plants grown under non-inductive conditions. It is even possible that a combination of different forms of gibberellins have varying effects on plant development as opposed to when only one form of gibberellin is applied to a plant.

It was noted in Chapter 12 that when stems elongated in short days they were always prostrate whether under warm or cool conditions. Under natural conditions outside, stems began elongating in a prostrate manner then after a few centimetres of growth they became more erect. When plants were induced to flower when in long days, and when especially in the 17 hour photoperiod, stems always elongated in an erect manner. In strawberry clover (Trifolium fragiferum) Bendixen and Peterson (1962) suggested there was an interelationship between auxin and gibberellin in tropic behavior and also stem elongation. They suggested that erect genotype plants have supraoptimal auxin levels when compared to stoloniferous plants. When sainfoin was treated with GA in short- or long days, stems were always erect. Thus it is possible that in sainfoin

GA may influence or even control stem orientation. To explain why plants often begin stem elongation in a prostrate manner when outside then later elongate in an erect manner one could suggest that GA diffusing out of the developing inflorescence (Booysen and Laude, 1964, Tkaczyk et al 1976, Kaufman et al 1976) might bring about the more erect growth habit of the elongating stem. If this were so then it would appear that under low temperature treatment sainfoin inflorescences do not produce GA as very short stems do have inflorescence initiation. However, this could also be interpreted in another way by saying that under environmental conditions of low temperature stem tissue can not effectively respond to low concentrations of GA from the young inflorescences.

Results from Chapter 9 demonstrate that applied GA_3 can have an effect on leaf orientation in that GA_3 makes prostrate leaves become more erect. To bring about this increase to the horizontal in leaf angle the petiole would need to 'grow' on the basal lower side at a faster rate than the basal upper side. For many years it has been accepted that the appearance of a lateral differential in auxin concentration between the upper and lower sides of a plant organ is part of the mechanisim whereby a geotropic growth curvature occurs, (Went, 1925, Wilkins, 1966). If gibberellins are important regulatory factors in stem elongation then it is feasible that gibberellins might also be related with growth curvatures such as those involved in geotropic reactions. In experiments with Helianthus annuus and Phaseolus multiflorus seedling Philips (1972a) found approximately ten times more gibberellin diffusing out of the lower than upper tissues of horizontal shoot tips, whereas approximately equal quantities were obtained from the two halves of upright tips. Philips (1972b) obtained similar results for phototropic responses. This pattern of diffussible gibberellin distribution was correlated with the more rapid elongation growth of the lower tissues and the normal regulatory effects of apically synthesized gibberellins upon internode extension growth (Philips, 1972a). Rose (1974) showed that GA could stimulate cell extension in young excised wheat coleoptiles.

When sainfoin plants did elongate stems in short days (Table 12.1) it was noticed that these always occurred on plants with dense foliage. Thus this particular form of stem elongation might be a response to shading of the basal buds. Under the natural forest situation when light passes through a canopy of vegetation the proportion of far-red (FR) light increases to that of red (R) light (Richards, 1957). Thus in the sainfoin example a similar situation might be occurring with the result that the

ratio of FR/R light increases. In dwarf varieties of Pisum sativum growth in FR light caused plants to elongate their stems whereas growth in red light inhibited stem elongation (Lockhart, 1956). Addition of GA_3 on Pisum caused stems to elongate when grown in red light conditions (Jones, 1973). The FR light and GA responses might be closely related in the natural plant system FR light and GA might work independently on the same gene, or gene complex, that is responsible for stem elongation; or FR light might increase the sensitivity of plant tissues to endogenous gibberellins. From results in Appendix I it would appear that an increase in temperature increases the sensitivity of the plant tissue to endogenous gibberellin.

15.4 The effects of low temperature on growth and development

In this section only aspects not examined in section IV will be discussed. For a complete and full discusssion on aspects of low temperature the reader would be advised to read the discussion sections of Chapters 12, 13 and 14 in addition to this section.

It was noted in Chapter 5 that when plants were grown in the warm glasshouse only two cultivars (Giant, Italian) had a high percentage of plants with blossoming inflorescences. In Melrose 57%, Krasnodar 8% and in Common no plants had blossoming inflorescences. However, in Chapter 12 it was observed that populations treated with cool short days followed by warm long days always had a high percentage of plants with blossoming inflorescences. Results in Chapter 5, 12 and 13 confirmed that plants had a typical vernalization type requirement, and results in Chapter 14 confirmed that, for Melrose, direct cool temperature treatment in long days promoted inflorescence initiation and development.

The low temperature response in sainfoin allowed plants to initiate inflorescences even when plants were in short photoperiods. For lucerne, Roberts and Struckmeyer (1939) found that inflorescences were produced in short days of 9½ to 10 hours, and Van Dobben (1958) noted that buds held over winter "..... are completely or partially induced". Thomas (pers. comm.) for certain clones of white clover and Evans (1959) for Trifolium subterraneum have observed that inflorescence initiation does occur under short day winter conditions. This low temperature/short day inflorescence initiation effect is very interesting and leads one to ask the question whether it is dependent of daylength or whether low

temperature just greatly lowers the critical photoperiod. For Trifolium subterraneum Aitken (1955) found that the early variety Dwalganup, if vernalized for eight weeks, would initiate flower primordia in the dark. Roberts and Struckmeyer (1938, 1939) found that many higher plants the photoperiodic requirement decreased with decreasing night temperature. For subterraneum clover, flowering time in the field appears to depend on the interaction of vernalization with daylength (Morley, 1961). A similar growth response has been found for crimson clover (Trifolium incarnatum) (Knight and Hollowell, 1973).

In Melrose the average date of first blossoming in a population held over the spring/summer of 1975-76 was January 5 1976 (Figure 5.5). However, in the same cultivar but a different experimental population, blossoming occurred on November 22 1976 (Table 12.3) when plants had been left in the outside environment. This low temperature effect on growth represents a difference of 49 days for blossoming for the population as a whole. When examining data for individual plants, Melrose plants held in the outside environment (Table 12.3), that could be compared to the blossoming dates of the same plants in the 1975-76 season when held in the glasshouse, blossomed on December 28th 1976. When held in the glasshouse in the spring/summer of 1975-76 these plants blossomed on December 8 1976, i.e. a 20 day difference. When making a similar comparison for Italian plants there was a 27 day difference in the time of blossoming when comparing low temperature treated versus warm glasshouse treated plants. However, for Giant plants there was only a difference of eight days in the blossoming date for low temperature treated plants compared to plants kept with the warm glasshouse; this time difference would not be significant as these comparisons were made between two seasons. Examining the blossoming dates for the Giant populations in Table 12.3 it is again noted that low temperature treatment does not affect the time of blossoming. Thus in this Giant cultivar it appears that blossoming time is controlled solely by photoperiod. However, in the Giant population tested in the glasshouse (Table 5.1) four plants did not blossom, although they did so when low temperature treated. As this represents a very small percentage of the total Giant plants tested it would appear that this represents a genotype that is in the minority in a normal Giant population.

Again when comparisons could be made on an individual plant basis Giant blossomed 16 days, and Italian blossomed 18 days, earlier in the 1976-77 season compared to the 1975-76 season when plants were held in the glasshouse. To explain this result one would need to postulate

that a size/age effect has affected the date of blossoming. For Dactylis glomerata Calder (1964) noted that the critical photoperiod decreased as the plant aged.

In experiments in Chapter 13 and 14 on the effect of daylength on flowering it was observed that a photoperiod of 14½ hours was sufficient to enable over half of the Melrose low temperature pretreated plants to blossom (Table 13.1) and that in the low temperature treated population photoperiods of 13½ hours and above were sufficient for inflorescence initiation to occur in many plants within a population. In the natural situation this photoperiod would occur in late October early November, corresponding favourably with the time of blossoming for the natural outside treated population (i.e. Nov. 22). Thus it would appear that in the Palmerston North environment inflorescence initiation can occur over the winter period, although continued inflorescence development is determined by long photoperiods. However, low temperature treated plants of Melrose can blossom earlier than when held in the warm glasshouse, thus representing a photoperiod x temperature interaction. Giant differed in this respect as on August 23 1976 (Chapter 12 re adult plants) it was noted that Giant and Italian plants had clearly visible but aborted inflorescences. This then demonstrates that Giant can initiate inflorescences under low temperature/short day conditions but cannot proceed with continued inflorescence development until around the normal period for initiation in plants held in the warm glasshouse. Thus there appears to be no temperature x photoperiod interaction for Giant for continued inflorescence development even though plants can initiate in cool short days.

One interesting and rather unusual observation was that when low temperature pretreated plants were transferred to the warm glasshouse in either natural short days (Chapter 12,) or artificially extended long days (Chapter 12, 13) elongating stems after a period of growth tended to stop further development rather abruptly. After stems had stopped elongating leaf initiation and development still occurred, resulting in the formation of a "leaf rosette" on the end of an elongated stem (Plate 15.1). As the photoperiod increased, this growth inhibition tended to be brought about sooner (Figure 13.1, Figure 13.4, Table 13.1). In the discussion of Chapter 13 I mentioned the possibility that a senescence factor (S-Factor) or a vernalization factor (V-Factor) might have something to do with this inhibitory effect.

It could be possible that when plants are in low temperature

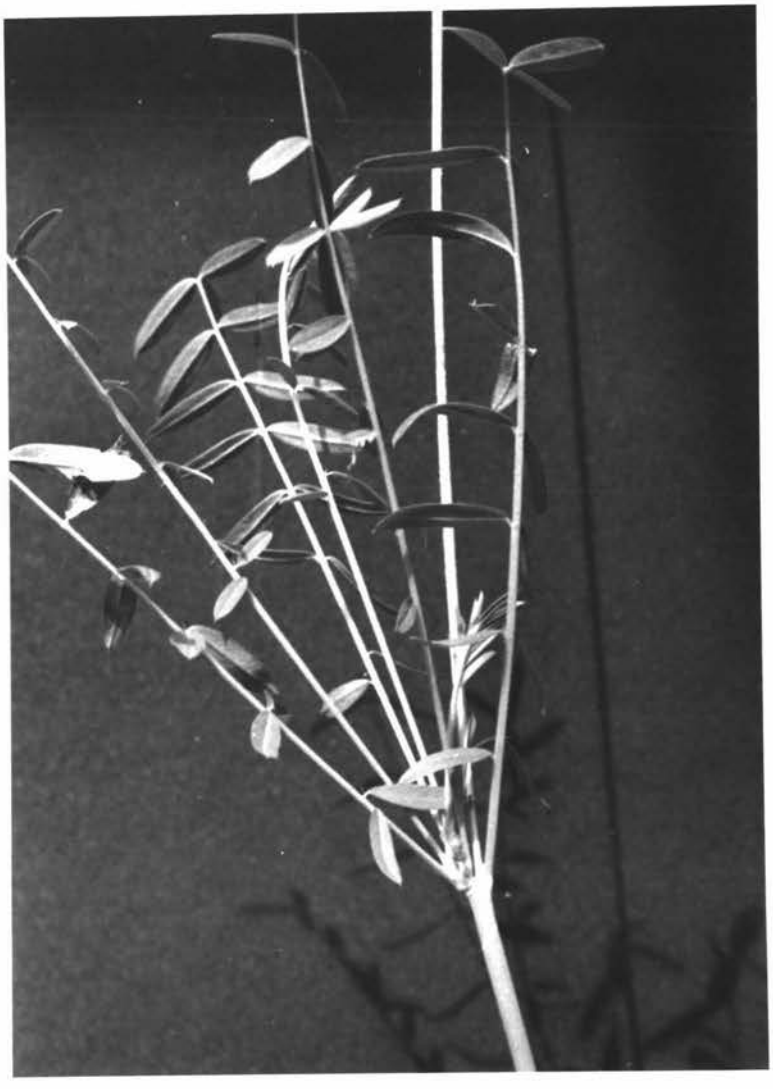


Plate 15.1

The formation of a leaf rosette on the end of an elongated stem. Note the peduncle of an inflorescence attached to the lowest visible node.

environmental conditions a V-Factor might be synthesized and that when plants are then transferred to warm long days, or warm short days this V-Factor is used in reproductive development. In a warm environment it would not be expected that this V-Factor is still produced. If this hypothetical V-Factor is 'used up' during reproductive growth one would expect that a given amount of V-Factor would produce the same number of inflorescences under varying photoperiodic conditions. However, on examination of Table 13.1 it is shown that the number of macroscopically visible inflorescences in each photoperiod treatment ranges from 4.81 to 7.76 per flowering plant. If this V-Factor is broken down in conditions of higher temperature then one would expect that the faster reproductive development occurs in long days the more inflorescences that would form. For example, growth in a 17 hour photoperiod, after low temperature treatment, would be expected to produce more inflorescences than an equivalent but 15 hour photoperiod treated population if this hypothesis was correct. Reference to Table 13.1 shows that in fact the 17 hour photoperiod treated population has fewer macroscopically visible inflorescences per flowering plant, and fewer per stem, than the 15 hour treated population. Thus from these examples one can rule out the hypothesis that a "build up" of a V-Factor occurs during low temperature treatment that is used later for inflorescence development.

The results of Chapters 12 and 13 can, however, be explained by proposing that a senescence factor (S-Factor) causes this reproductive inhibition. It would appear that, in the normal reproductive development of a plant, when an inflorescence in initiated, a S-Factor is soon produced. During the morphological development of the first inflorescence I suggest that a flowering (F) factor (this might be more than one compound) dominates over a S-Factor until approximately 60-80 florets have been produced. At this stage the S-Factor would dominate over the V-Factor thus effectively stopping the further development of that inflorescence. When many inflorescences on a stem are produced this same process would occur for each. This in effect would lead to a gradual build up of the S-Factor level in the stem. This, after reaching a certain critical level, would result in the complete inhibition of further inflorescence development thus explaining why flowering stems nearly always have small aborted inflorescences at the apical end no matter what treatment or pretreatment they received.

It would also be suggested that the production of this S-Factor

even occurs on inflorescences initiated in cool short days. In an environment where growth is slow and the F-Factor responsible for continued inflorescence development is missing (i.e. only produced in long days) the S-Factor level builds up higher than is normal for an equivalent long day produced inflorescence. If this hypothesis is correct then one would expect that plants held in low temperature environments for a longer time period would have fewer blossoming inflorescences as these plants would have initiated earlier. Therefore, it would be expected that these plants would have a higher concentration of this S-Factor in the plant system at the time of transfer. In effect, in long days, the ratio of S-Factor to F-Factor would be higher than normal. Examination of Table 12.5 shows this is exactly what occurred. The fewer the number of days of low temperature pretreatment the more plants that had blossoming inflorescences.

This same hypothesis can explain why the 17 hour warm glasshouse treated/short day low temperature pretreated population had a reduced flowering vigour when compared to the equivalent 15 hour treated population (Figure 13.4). In this case the initial vigorous reproductive growth might produce greater than normal S-Factor, and this coupled with the early production of the S-Factor in short days, could cause an upset in the S-Factor to F-Factor ratio for normal reproductive development.

It is also possible that once plants have flowered they must receive a period of cool temperatures to remove this S-Factor from the plant system as plants from all cultivars that flowered in the glasshouse in the 1975-76 season then held in the glasshouse until the 1976-77 spring/summer season had a much reduced flowering vigour for the second season (Table 12.3).

One now asks the question of why the natural outside treated population did not show this S-Factor inhibitory effect to the same extent. In some growth aspects this S-Factor inhibition does occur early: for example, this would explain why inflorescences abort in cool short days after reaching a developmental stage of 4 or 5 (Table 4.1). Because inflorescence development to blossoming cannot occur due to the lack of long days inflorescences "waiting" for natural long days to come are overcome by this S-Factor. The reason why overall reproductive development is not inhibited early is most probably because the production by the inflorescences of a S-Factor is slow under low temperature conditions so that when long days do arrive there is still

a favourable F-Factor to S-Factor ratio for normal development. In natural conditions when continued inflorescence development does occur temperatures are still relatively cool especially at night.

15.5 Possible endogenous processes controlling flowering in sainfoin

To describe a mechanism for flowering in sainfoin the following must be taken into account.

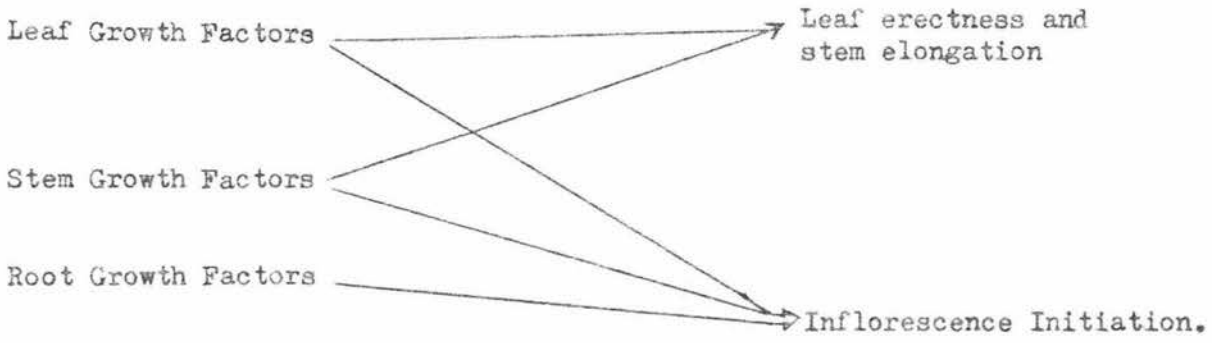
(i) the requirement for long days.
(ii) in some cases pretreatment in short days to be followed by long days.
(iii) direct low temperature treatment.
(iv) low temperature pretreatment.
(v) the need for a "healthy", root system (Chapter 7, 8).
(vi) warm temperatures for continued inflorescence development.

A. Inflorescence Initiation

To explain how inflorescence initiation does occur in sainfoin I suggest a hormonal-balance type mechanism with the active site being at the stem apex. Production of various growth factors making up this balance would occur throughout the plant system with various types of plant tissues (i.e. root, leaf, stem) having differing contributing effects. For example, in response to long days the young leaves might produce a growth factor, possibly GA, that would effect increased leaf angle, and stem elongation. There could also be a synergistic response with increasing daylength, and temperature, in that the plant tissue could become more responsive to growth factors. It is highly possible that this initial response does not directly affect inflorescence initiation but could be just a stage in the reproductive process which allows a more favourable internal situation to occur for later inflorescence development. For example, this leaf angle, stem elongation response might occur in conjunction with inflorescence initiation, but is a seperate process, which allows the later formed inflorescence to have a greater "reproductive fitness" value i.e. a greater chance to reach anthesis.

While this is occurring other reproductive processes would also be taking place. For example, the roots would be synthesizing, or interconverting growth factors that would later be transported to the apex. Stem growth factors could also move to the apex.

For example:



To explain differences in flowering response (e.g. why some plants have stem elongation and no flowering) one could postulate that the processes associated with stem elongation have been activated, although the processes associated with inflorescence initiation have not.

To explain why some genotypes have one flowering control whereas others appear to have different controls it could be suggested that each plant tissue produces varying amounts of growth factors. For example, in a "normal" flowering plant the root might supply factors 6 x, 2y, the stem 2y 2z, and the leaf 6z 2x giving a total at the apex of 8 x 4y 8z. However in some genotypes, or in some environments, the production of say 'root Z' might be slightly inhibited and is only able to produce 4z. With time, however, the leaf might be able to make up this deficit so that in the end the overall total remains at 8x 4y 8z.

From the data obtained with sainfoin it would appear that the growth processes involved in vegetative axillary bud initiation are are very similar to the initial processes associated with inflorescence primordium initiation (Chapter 11). Wetmore et al (1959) showed that vegetative and floral meristems are homologous. These conclusions are in agreement with the view of Goethe (1790) that the flower is a modified shoot and not a structure sui generis. If the view of Goethe is correct then one would expect that the growth processes involved in vegetative bud initiation are very similar to the growth processes involved in inflorescence initiation. For the short day plant Bryophyllum crenatum Dostal (1967) observed that marginal vegetative buds on the leaves start to appear at the same time that flower primordia are formed. Dostal (1967), from his surgical experiments, concluded that the leaf primordium was the first structure to be irreversibly determined on the vegetative tip; and from the beginning buds are dependent on their supporting leaves.

The main factor that determines whether an axillary bud develops into a vegetative bud or reproductive bud might be its time of initiation in relation to its associated leaf. For example, if the bud can be initiated at the same time as, or slightly earlier than, the associated leaf primordium, then it might have sufficient time to be 'genetically determined' towards reproductive growth. With the arrival at the apex of all the plant's growth factors, a sufficient stimulus would be present to effect vigorous axillary bud growth. If the correlative growth effects of the plant do not favour strong bud development then possibly the leaf may have sufficient time to gain dominance over its axillary bud. This might explain why inflorescence initiation can occur while plants are held in low temperature conditions, that is, leaf development at the apex may be very slow thus allowing time for the axillary bud to be 'genetically programmed' as an inflorescence.

B. Inflorescence Development

Under some environmental conditions it was shown that full inflorescence development did not occur after inflorescence initiation (Chapter 12). Thus it would appear that growth processes involved in inflorescence initiation are not the same as those responsible for inflorescence development. However, the growth processes involved in inflorescence development might be similar to those that effect initiation with possibly a change in the ratios of growth substances or even the addition of another growth substance.

The flowering behavior of sainfoin fits the general pattern of mechanisms proposed for other long day pasture legumes.

Evans (1959) and Morley and Evans (1959) noted that flower initiation in Trifolium subterraneum was under the control of three interacting partial processes, one of which was inhibitory to early initiation, while the other two favour it. The three processes noted were:

(a) a long day photoperiod process.

(b) a high temperature - favoured, light-independent process. However, Evans (1959) noted that the evidence for the existence of this process was only indirect in that high temperatures accelerate flowering initiation considerably.

(c) a vernalization process.

These authors explained that the variations in the flowering behaviour of subterraneum clover cultivars could be explained in terms of

an interraction between two synergistic promotive processes (one favoured by high temperatures, and the other by low temperatures) and an inhibitory process evident in long warm nights.

For flower initiation to occur in sainfoin the same partial processes were noted. For example, it was shown in the environment conditions of Chapter 5 that long days were necessary for inflorescence initiation and development to occur, although, in three cultivars (Melrose, Krasnodar, Common) some, or in the case of Common all, plants did not flower under these environmental conditions. Results presented in Section IV showed that low temperature treatment or low temperature pretreatment, with long days allowed all plants of all cultivars to blossom. In addition the high temperature-favoured process indirectly noted in subterraneum clover was also noted in sainfoin. For example, in early spring when plants remained outside growth and development was slow, however, if plants were transferred into the glasshouse at this period development was more rapid. However, this latter process was not shown to affect inflorescence initiation but it certainly had a promotive effect on inflorescence development.

Jones and Stoddart (1973) for Trifolium pratense put forward the hypothesis that young expanding leaves are sites of active gibberellin synthesis and that senescent older tissues contain large amounts of abscisic acid. From this they proposed that leaves of different ages make varying contributions to both stem elongation and inflorescence initiation. Again in sainfoin a similar process might also be occurring. It was observed that when plants began to elongate their stems leaves prior to this became more erect. It was later shown (Chapter 9) that this leaf angle response to long day treatment could be repeated in short days with GA_3 application. With time this initial leaf angle response was followed by an increase in the rate of leaf emergence (Chapter 5, Chapter 7). Thus what might be occurring is that the ratio of young leaf tissue to older leaf tissue was increasing. According to the hypothesis of Jones and Stoddart, (1973) this would result in more promotive growth substances. However, when sainfoin plants that had begaun to elongate their stems received a mite attack as described in Chapter 6 the oldest leaves were the first to senesce. According to hypothesis of Jones and Stoddart (1973) this should have resulted in an increased flowering vigour. In sainfoin mite defoliation severely arrested reproductive development. This suggests that in sainfoin older leaves on the plant do play some promotive role in reproductive

development. Results for sainfoin do however, agree with those for red clover in that young leaf tissue appears to supply an increased amount of growth promotor, possibly GA.

Thomas (1961b) for Trifolium repens noted that some clones were short-long day plants. Bawaloski (1966) for two Polish cultivars of sainfoin also observed this short-long day response. In this present study I also observed this short-long day response in some genotypes of Melrose.

Comparison with results obtained by other authors on flowering of forage and pasture legumes (see section 5.1, 12.1 and this Chapter) shows that sainfoin is very similar in response.

15.6 Future experiments

In a study such as this many interesting observations are made that lead the way for further experimentation. Listed in this section are a number of possible research projects that would provide further information to that already obtained.

1. When plants are grown under natural short day outside environmental conditions at what time of the year/season do inflorescences initiate noting at the same time if there is any difference in initiation time between cultivars that require low temperature pretreatment/treatment (i.e. Common) and those that do not (i.e. Giant).

2. In Chapter 11 it was noted that GA_3 appeared to decrease the critical photoperiod. Further experimentation is required to determine in more detail what effect GA_3 does have on critical photoperiod and to what extent the photoperiod can be lowered. It would also be interesting to determine whether this observed GA_3 effect on critical photoperiod could be correlated with the temperature x photoperiod interaction effect observed in this study in natural outside environmental conditions.

3. A more detailed study that would, or would not, confirm my hypothesis that allelopathic substances produced by the roots inhibit reproductive development. If this hypothesis is correct then a new field of study could be opened on developmental physiology, that is, what effect does one species have on the growth and development, especially flowering, on another species. Could this be another mechanism that controls, or

partly controls, reproductive development in some plants when grown in association with others?

4. A thorough study on aspects of inflorescence abortion noting in detail the effects of environment. From that data future studies, including hormonal, could be undertaken to determine why inflorescences abort. For all plant species a study such as this could have immense economic implications.

5. To examine in more detail my hypothesis for the occurrence of a senescence factor that controls growth and development.

6. Surgical experiments at the shoot apex to determine whether the hypothesis of Dostal (1967) is correct (i.e. the leaf primordium determines at an early stage the future of its axillary bud primordium). Associated with this would be further experimentation on the hypothesis that an inflorescence primordium and an axillary bud primordium are analogous and that the processes involved in the development of each are the same.

7. For future agronomic experimentation the researcher must be aware that some cultivars require low temperature pretreatment before they will flower. Thus cultivars must not be "written off" on the basis of the first growth seasons results.

8. Field trials that would determine if early spring sowing of the low temperature requiring cultivars would enable the low temperature requirement to be satisfied so that flowering would occur in the first season of growth. If this does occur then aspects associated with persistence must also be closely examined as it has been shown overseas that these low temperature requiring cultivars are more persistant than the Giant type cultivars that do not require low temperature pretreatment (Thomson, 1938a).

APPENDIX I

EFFECT OF TEMPERATURE ON LEAF ORIENTATION

Introduction

While testing for a juvenile response in sainfoin one treatment of 30 Melrose plants was held over the main summer period in artificially shortened short days prior to long day treatment in Chapter 6. It was noted that many plants from within this population held in warm short days had erect leaves and swollen buds in a manner similar to plants that receive long day treatment. As plants were held over summer in the 'short day cabinets', as described in Chapter 2, it was thought that some environmental factor other than photoperiod, possibly temperature, might have been causing this leaf angle increase under artificial short days.

Thus the aim of this present investigation was to determine what effect temperature has on early reproductive development in Melrose plants that have previously been held in artificially shortened warm short days.

Materials and Methods

The experiment, which began on December 9 1975 and ended on January 13 1976, involved two treatments, each of four Melrose plants that had previously been held in the short day cabinets in the glasshouse, that had relatively high leaf angle scores. In one treatment plants were placed daily into a 4°C dark cool room at 4.30 pm and returned to the glasshouse at 8.00 am. The second treatment group was left in the short day cabinet in the glasshouse for the period of the experiment.

At intervals of seven days leaf angle scores were measured using the same technique as described in Chapter 5, Plate 5.1. In addition daily maximum and minimum temperatures were recorded and are provided below.

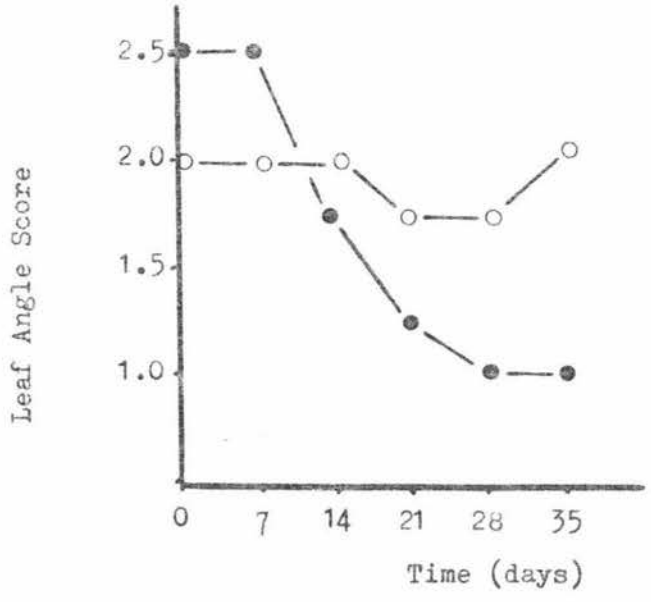
Temperature (°C)	Plants receiving cool (4°C) nights	S.D. cabinet treated plants
Maximum (day)	29.9	29.9
Minimum (night)	4.0 ± 1.0	21.8
Maximum (night)	4.0 ± 1.0	27.1

Results

For those plants that were transferred to the 4°C cool nights the leaf angle score steadily decreased beginning on day 7 and ending at day 28 (Figure 1.1); by day 28 all plants had a leaf angle score of 1. For the short day cabinet treated plants the average leaf angle score did not significantly change.

Figure I.1

The effect of low night temperatures on leaf angle score.
●——● population placed into cool (4°C) dark night temperatures;
○——○ population held in warm (greater than 20°C) night temperatures.



Discussion

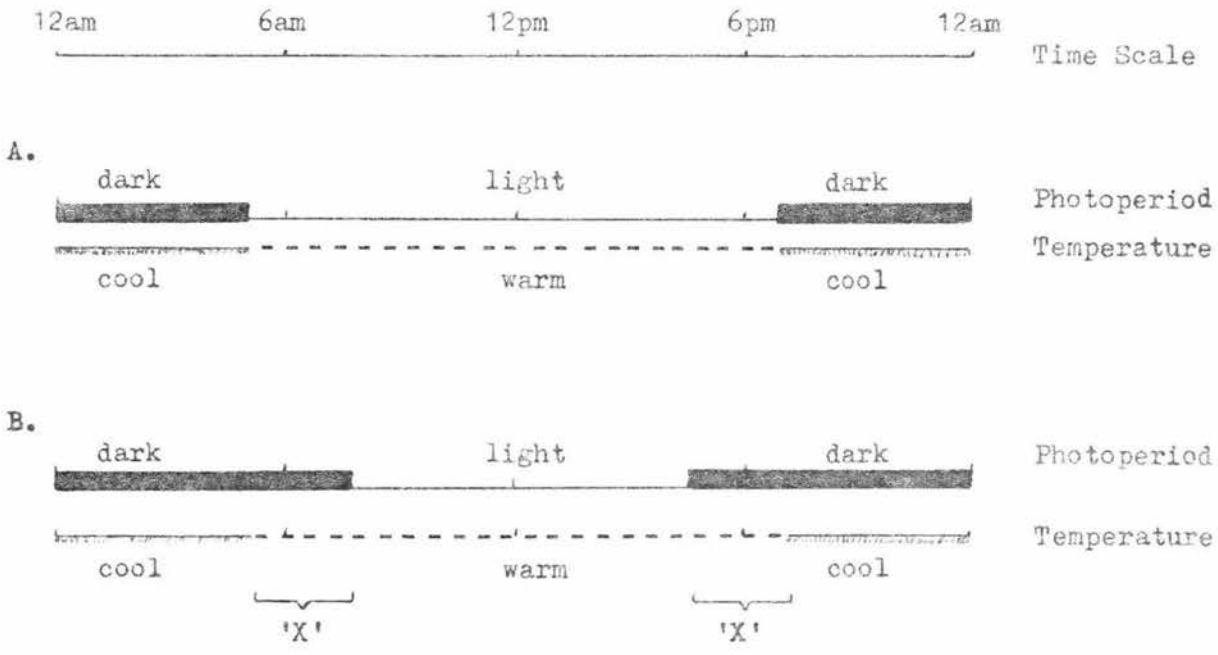
The results of this investigation show that for plants that have been initially held in short days with relatively high average night temperatures, and have increased the angle of their leaves, revert back to the normal natural short day prostrate growth habit when placed into short days with cool nights.

In Table I.1 the small decrease in the leaf angle score between day 21 and 28 for the short day cabinet treated plants might have been

caused by two consecutive coolish nights during this period (minima 17 and 19°C). With later higher night temperatures the leaf angle score again increased after this period of cooler temperature.

The results of this experiment raise an interesting question; that is, does the effect of warm nights in short days (SD) have a similar effect to the normal long photoperiod effect, and are these two processes closely associated with the overall reproductive process.

This is diagramatically represented below.



'X' = Period of "high dark" temperature

In diagram A, which represents the natural long day (LD) environment, both temperature and photoperiod are closely associated together. However, in diagram B, which represents the environmental conditions that were experienced by the SD cabinet treated plants, light conditions resembled a typical SD, however, temperature conditions resembled a LD.

Comparison with Figure 9.1 shows a correlation between leaf angle response in long days. Taking into account the results of this present investigation one could suggest that with an increase in temperature there is an increase in the sensitivity to GA. Under natural environmental conditions this would prove most beneficial as when warmer spring conditions arrive the plant would be able to respond in a more vigorous manner to the initial phases of the reproductive process. It is also possible that with an increase in temperature there might be an increase in gibberellin synthesis.

APPENDIX II

EFFECT OF DAYLENGTH ON FLOWERING IN CV. MELROSE

Introduction

From the results obtained in Chapter 6 it was observed that some genotypes within a Melrose population were perhaps short-long day plants.

The experiment in this present investigation on the effects of various daylength combinations for vernalized and non-vernalized seedling plants also supports this short-long day effect.

Materials and Methods

Vernalization treatment involved placing seeds in a petri-dish moistened with filter paper into a refrigerator held at 4°C for 21 days. Seedlings that had no vernalization treatment were germinated in a dark cupboard (temperature = 20 - 25°C) for 2½ days. At the end of this period seedlings had germinated to the same stage as those that were vernalized.

On September 19 1975, seedlings were planted into 5 centimetre plastic pots containing an osmocote soil/nutrient mix and placed into the growth cabinets (temperature = 21 - 25°C; PAR at plant height = 320 microeinsteins/m^2/sec.). Treatments, each of 15 plants, consisted of eight weeks pretreatment with either short (9 hour) or long (17 hour) photoperiods followed by either short or long photoperiods as shown below.

(a) 8 weeks SD ⟶ LD
(b) 8 weeks SD ⟶ SD
(c) 8 weeks LD ⟶ LD
(d) 8 weeks LD ⟶ SD.

Each treatment (a to d) was replicated with one group grown from vernalized seed and the other group grown from non vernalized seed.

On December 8 1975 all plants were repotted into 10 centimetre pots and transferred into the glasshouse where they were kept in their respective growth cabinet photoperiods by artificially shortening or lengthening the natural photoperiod. This move was necessary as the light intensity within the cabinets appeared to be too low for sainfoin plant growth.

On February 20 1976 data were obtained from a final harvest.

Results

There was no vernalization response apparent in this experiment, however, a definite photoperiodic effect was observed (Table II.1).

Table II.1

The effect of photoperiod treatment on the percentage of plants with macroscopically visible inflorescences, and the percentage of plants with elongated stems. Data from vernalized and non-vernalized groups have been bulked thus increasing the treatment populations to 30 plants each.

Photoperiod Treatment	% Inflorescence initiation	% of plants with elongated stems
SD ⟶ SD	0	0
SD ⟶ LD	18	39
LD ⟶ LD	4	16
LD ⟶ SD	0	0

Discussion

The results presented in Table II.1 demonstrate a definite short-long day response for flowering in Melrose sainfoin. Thomas (1961b) observed a similar short-long day response in Trifolium repens. Sainfoin, a high light intensity requiring plant (Bawolski, 1966), showed no vernalization response in this investigation. However, this latter result must be treated with caution as the plants were initially held for a considerable period of time under environmental conditions of low light intensity. Thus by the time the plants were removed to the glasshouse the normal response associated with seedling vernalization as found in other legumes (Clarkson and Russell, 1975; Medicago spp.) might have by that time dissipated. Cyusski (1957) (quoted by Bawolski, 1966) noted that vernalization of young sainfoin seedlings had no significant effect on flowering.

It must be noted also that the percentages of plants flowering in the long day treatments (i.e. a and c) were low. This might have been due to the initial growth in low intensity light in the growth room. However, it is shown that plants having received prior short days had a higher percentage of plants with visible inflorescences. This could have resulted from a pot size effect, as described in Chapters 7 and 8, as when the experiment was ended, it was noted that the plants' root systems were at a stage which appeared to have affected reproductive development in other experiments. With this in mind one might go so far as to suggest the possibility that the short-long day flowering genotypes might not be affected by pot size to the same extent as the normal long day genotypes.

APPENDIX III

SEM VALUES ASSOCIATED WITH FIGURE'S 8.1, 8.2.

(a) SEM values for Figure 8.1 (Shoot Dry Weights).

Cultivar/Pot size		Stage of Development (days)							
		0	7	14	21	28	35	42	49
Small Pots	Melrose	0.070	0.054	0.089	0.156	0.230	0.222	0.128	0.181
	Giant	0.049	0.135	0.107	0.137	0.173	0.103	-	0.281
	Common	0.057	0.080	0.066	0.114	0.118	0.053	-	0.106
Large Pots	Melrose	0.026	0.072	0.085	0.145	0.209	0.364	0.178	0.224
	Giant	0.033	0.096	0.128	0.135	0.125	0.346	-	0.551
	Common	0.049	0.041	0.069	0.114	0.183	0.108	-	0.156

(b) SEM values for Figure 8.2 (Root Dry Weights).

Cultivar/Pot size		Stage of Development (days)							
		0	7	14	21	28	35	42	49
Small Pots	Melrose	0.019	0.014	0.023	0.046	0.063	0.093	0.045	0.093
	Giant	0.010	0.048	0.038	0.044	0.053	0.076	-	0.129
	Common	0.021	0.030	0.022	0.040	0.048	0.034	-	0.078
Large Pots	Melrose	0.018	0.026	0.025	0.052	0.060	0.051	0.056	0.181
	Giant	0.012	0.030	0.038	0.200	0.049	0.147	-	0.191
	Common	0.017	0.009	0.022	0.025	0.061	0.028	-	0.089

GLOSSARY

Definition of terms as used in this thesis.

1. AN ELONGATED STEM

   A stem which has a visible internode longer than three millimetres.

2. CULTIVAR

   An assemblage of cultivated plants which is clearly distinguished by any characters and which retains its distinguishing characters when reproduced, sexually or asexually.

3. INDUCTIVE PHOTOPERIOD

   A photoperiod that has the potential to bring about inflorescence initiation.

4. INFLORESCENCE

   A flowering shoot bearing more than one flower.

5. INFLORESCENCE INDUCTION

   When the first biochemical events occur at the apex that lead to inflorescence initiation.

6. INFLORESCENCE INITIATION

   Is the transformation of the shoot apical meristem from a vegetative axis to a potentially reproductive axis.

7. MACROSCOPICALLY VISIBLE INFLORESCENCE

   An inflorescence visible to the naked eye (can be aborted).

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