Title

ASPECTS OF THE REGULATION OF INFLORESCENCE INITIATION IN WHITE CLOVER (*Trifolium repens* L).

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ABSTRACT

Thomas (1962), found that after a pretreatment of warm short days, one genotype of 'Grasslands Huia' white clover, clone C, flowered in long days. Another 'Grassland s Huia' genotype did not, (clone B).

Experiments with clone C revealed the following:

(a) Production of a translocatable floral stimulus occurred in long days and continuous light.

(b) Production of either translocatable inhibitory or promotive factors did not occur in short days.

Although flowering was caused by long days, flowering eventually stopped. Experiments designed to test the hypothesis that this was caused by the build up of translocatable inhibitors were inconclusive. There was no evidence in the same experiments that translocatable products produced in short days stopped the cessation of flowering.

The effect of the short day light intensity on flowering in long days was also examined. Results indicated that in long day conditions when the photoperiod was near the critical daylength, the light intensity of the short day pretreatment limited flowering. At higher daylengths and in higher long day light intensities, the short day light intensity had no influence on flowering. This supports the idea of Thomas (1981), that a balance between two factors, one inhibitory one promotive controls flowering.

Further support for this concept came from studies with clone B in which it was found that a cool pretreatment would

enable clone B to flower in continuous light but not in 16h photoperiods.

Other experiments with clone B, showed that it produced a translocatable floral stimulus in continuous light. There was no evidence that clone B produced translocatable inhibitors in vegetative conditions although there were indication that warm conditions could inhibit the response of the apex to the floral stimulus.

Grafts of clone C on clone C, clone B on clone C, Kalinin A on clone C, were used to test the hypothesis that apical factors limited apical responses to the floral stimulus. Given that clone B had the weakest response to the floral stimulus from clone C, and Kalinin A had a stronger response than clone C it would seem that the hypothesis is correct.

Grafts were also used to test the hypotheses that

(a) Clone B produced translocatable inhibitors which blocked flowering.

(b) Clone B produced a translocatable floral stimulus which it was inhibited from responding to.

Neither hypothesis was supported by the results.

In conclusion it appeared that a balance between two factors controlled the amount of the floral stimulus translocated from the leaves. It also seemed likely that a interaction between the floral stimulus and the apex had a regulatory role.

Differences between white clover genotypes are probably due to differences in apical and leaf processes.

The limitations of the experimental methods and futive experiments were also discussed.

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INTRODUCTION

ENVIRONMENTAL CONTROL OF FLOWERING

Flower and inflorescence production is often regulated by environmental factors acting individually, or together. Garner and Allard (1920), and earlier workers such as L.H. Bailey, J. Tournois and G. Klebs (reviewed in Garner and Allard 1920, Murneek and Whyte 1948), found that in some plants, flowering only occurred or occurred sooner in certain daylengths broadly classified as short (under 12 hours) and long (over 12 hours). For example, Garner and Allard (1920) found that Nicotiana tabacum cultivar Maryland Mammoth growing in warm conditions flowered earlier in short days than in long days. Flowering in a five hour daylength took from 55 to 61 days, and from 152 days to 160 days in a 12 to 15 hour daylength. In other examples a long day treatment was required. For example Raphanus sativus and Spinacea oleracea were found by Garner and Allard (1920), to require long days. This was demonstrated by artificially extending the daylength, when only plants exposed to the lengthened day flowered.

In other cases flowering is faster in one daylength than another but eventually occurs irrespective of the daylength. For example Halse and Weir (1970), found that some spring wheat cultivars flowered earlier in long days than in short. The difference in days to inflorescence initiation between the long day (14 hour) and the short day (10 hour) treatments varied from three to 14 days. In another example Gott, Gregory and Purvis (1955), found that double ridge

formation in spring rye Secale cereale took three weeks in continuous light, but in short days (10 hours) about seven weeks were required.

In some examples flower initiation is enhanced by dual daylength treatments. Bryophyllum daigremontanum (see Lang 1965) and Cestrum nocturum (Sachs 1969) require long days followed by short days. Echevevia harmsii (Runger 1962 in Lang 1965) and Campanula medium (Wellensiek 1960) flower in response to a short day pretreatment followed by exposure to long days.

Another environmental variable known to cause flower or inflorescence initiation is temperature. Cool temperatures cause flower and inflorescence initiation in: Allium cepa (Holdsworth and Heath 1950), Citrus sinensis (Moss 1969), Nerine flexuosa alba (Fortanier, Van Break and Wellensiek 1979), Trifolium subterraneum (Aitkin 1955, Evans 1959, Morley and Evans 1959) and Brassica oleracea (Stokes and Verkerk 1951).

Warm or high temperature effects on inflorescence or flower initiation are a little more difficult to evaluate, as an apparent induction of flowering may be due to the speeding up of growth and other processes which may eventually occur irrespective of daylength and other environmental factors. However as Cosmos sulphureus cultivar Orange Flame, Delphinium cultorum, Rudbeckia bicolor and Silene armeria which have strict photoperiodic requirements for flowering at moderate temperatures (about 20°C), become daylength neutral at high temperatures (Vince-Prue 1975), there are indications that high temperatures cause flowering. In other examples such as Beta vulgaris and Brassica pekinensis flowering eventually

occurrs in non inductive photoperiods (short days) at high temperatures (Vince-Prue 1975), which also suggests that in some cases high temperatures promote flowering by means other than effects on growth and other continuing processes.

There is some evidence that temperature-and-photoperiod-caused flower or inflorescence initiation, are related. Cathey (1955), found that the critical daylength for flowering of some Chrysanthemum cultivars decreased with decreasing temperatures. For example "White Wonder" only flowered in daylengths greater than 16 hours at 15.6°C and 26.7°C, but at 10°C flowering occurred in 1375 hours of light. Another cultivar "Encore", has critical daylengths of 13.75 hours at 10°C, 14.5 at 15.6°C and 15.25 at 26.7°C. Melchers and Lang (Chouard, 1960), found a similar effect of cool temperatures on the critical daylength at 20°C being 11.5 hours and at 11.5°C, 8.5 hours.

Rudbeckia bicolor, normally a long day plant at high temperatures (37.8°C to 32.2°C), flowers in seven hour days (Murneck 1940). While this can be taken to be an example of daylength neutrality induced by high temperatures, or direct high temperature induction of flowering. It is also possible that high temperatures have affected the photoperiodic mechanism so that is responds to short days. This interpretation implies either that high temperature induction is secondary, as it acts through the photoperiodic mechanism, or that both temperature and photoperiod act in the same way.

In some situations, an exposure to cool temperatures or warm temperatures (30°C or more), will either induce flowering or cause earlier flowering in the conditions following exposure to them. Because some other treatment besides the exposure to warm or cold temperatures is required, the temperature

effect by itself does not directly cause flowering. (When a cool temperature is required as a pretreatment the process is called vernalisation.) This can be illustrated by a quote from McKinney and Sando (1933), who wrote that "The early cessation of the formation of leaf primordia in "Harvest Queen" (a winter wheat Triticum aestivum) does not take place during the exposure to the low growing temperatures (1.7°C to -1.1°C) but during the subsequent exposure to high temperatures and long days". In this plant the effect of a cool temperature pretreatment is to reduce the number of days to stigma receptivity and anther extrusion from 128 days to 66.

While in some plants cool temperatures reduce the number of days to flowering, as in winter wheat and winter rye (Gott, Gregory and Purvis, 1955), in other plants such as Hyoscyamus niger a cool treatment is necessary if flowering is to occur at all in subsequent conditions. Digitalis purpurea is another plant that will remain vegetative without a cool treatment. It differs from Hyoscyamus niger in that it will flower after a cool treatment in either long or short days (Chouard, 1960). Biennial strains of Hyoscyamus niger require a long day treatment to flower (Chouard, 1960). Other examples of plants that require both long days and cool temperatures (within the range -2.0°C to 15°C) are Silene armeria (S.J. Wellensiek, 1969), some cultivars of carrots (Lang, 1957) and Beta vulgaris. Owen and Stout (1940), and other workers, found that although sugar beet flower rapidly only in long days given after cool temperatures, flowering will eventually occur in cool short days, (as it does in Silene armeria). In Beta vulgaris the cool treatment was also effective when given concurrently with long days. The situation in Chrysanthemum morifolium Ramat cultivar Sunbeam is slightly different

(Schwabe, 1951). This plant flowers on transfer from cold short days to warm short days, it may also flower if left for four weeks in cool temperatures (Schwabe, 1954). It is possible that in some cases the transfer from cool to warm conditions simply sped up processes which were already occurring thereby leading to earlier flowering. For example, in plants like Beta vulgaris and Digitalis purpurea where inflorescence or flower initiation occurs in long days after a cool pretreatment and in cool conditions irrespective of the daylength if left for long enough (five weeks) (Owen and Stout, 1940). Flowering may have occurred earlier in the long day or short day treatments because of the superior growing conditions. This cannot be the sole answer as some plants such as Hyoscyamus niger require a definite post-cool treatment daylength which a simple improvement in growing conditions would not explain.

In at least two plants Silene armeria and Chrysanthemum morifolium high temperatures have the same effect as cool temperatures ($32^{o}C$ optimum in Silene, and and $32/27^{o}C$ in Chrysanthemum). S.J. Wellensiek (1966), found in Silene that flowering in long days was 20 days earlier following four weeks of short day pretreatment at $35^{o}C$ than after short day pretreatment at $20^{o}C$. Francois Blondon (1976), discovered that Chrysanthemum cultivar "Shuokan" flowered in warm short days if pretreatment with continuous light for 24 days at $32/27^{o}C$ or 16 days at $2^{o}C$. High temperatures given in 9 hour days ($27^{o}C$), also caused flowering in 'Shuokan'.

Flowering is not always caused by specific environmental conditions, in Buckwheat (Garner and Allard

1920) and in glasshouse roses (Horridge and Cockshull, 1974) flowering occurs irrespective of environmental factors such as daylength, which suggests that an internal system of control of flowering exists independently of the environment in some cases.

GENERAL FLOWERING PHYSIOLOGY

In some plants flowering is caused by an interaction between the plant and its environment and many experiments have shown that this interaction results in the production in the leaves of factors which cause flower or inflorescence formation in stem apices or buds. Hamner and Bonner (1938), exposed *Xanthium pennsylvanicum* leaves to short days leaving some leaves and the tip of the plant in long days (*Xanthium pennsyvanicum* does not flower in long days). As flower initiation occurred, they concluded that the leaves in short days produced some factor which caused flowering. Heinze, Parker and Borthwick(1942), grafted an "Agate" soyabean leaf onto a "Biloxi" soyabean plant which was growing in conditions which would not cause flowering (long days). "Agate" flowers independently of day length so that the evocation of flowers in the "Biloxi" plant was concluded to have occurred as a result of some factor produced by the "Agate" leaf. In other experiments Moskov (1937), induced foliated stems of the short day plant *Nicotiana tabacum* cultivar Maryland Mammoth to produce flowers in long days, by grafting them onto a daylength neutral tobacco cultivar. As Maryland Mammoth does not usually flower in long days, it was concluded that the daylength neutral plant probably produced some factor which caused evocation of flowers in Maryland

Mammoth. The work of Melders and Lang also supports the previous experiments. Hyoscyamus niger plants do not usually flower in short days but by grafting them on to plants which are flowering in long days, stems growing in short days can be made to flower (Lang, 1965).

Not only do some daylength neutral plants, and plants that respond to long or short days, produce factors which cause evocation of flowers and inflorescences, plants that respond to cool temperatures also produce a translocatable floral stimulus. Wellensiek (1969) found that Silene armeria plants exposed to 5°C produced translocatable factors. Deronne and Blonden (1973) discovered that Perilla leaves exposed to 5°C in continuous light, and then granfted on to plants growing in warm conditions caused the plants not exposed to "inductive" conditions to flower.

There is also some evidence that leaves in conditions in which flowering does not occur, produce factors which block flowering. When the leaves of the long day plant Hyoscyamus niger were removed in short days the plants flowered, suggesting that the leaves suppressed flowering in short days. Usually dodder only flowers when its host does. However, when a "Biloxi" soyabean was used as a host, and was defoliated, the parasitic dodder flowered although the "Biloxi" host remained vegetative. This suggests that the "Biloxi" leaves in long days produced factors which suppressed flowering (Fratiane, 1965). Fratiane (1965), also found that a "Biloxi" plant growing in long days and connected to a "Biloxi" plant in short days through a dodder bridge, inhibited the flowering of the plant in short days. The implication being that the long day plant produced a translocatable inhibitor. Interestingly Heinze et al

(1942), were unable to demonstrate that "Biloxi" soyabean leaves could produce translocatable factors which caused flowering. Guttridge (1956), found that strawberry mother plants in non flowering conditions inhibited the flowering of daughter plants in conditions in which flowering usually occurs. Guttridge was also unable to demonstrate a translocatable promoter in strawberries (Guttridge, 1969). Based upon his work Guttridge suggested that in some plants flowering is controlled by translocatable inhibitors rather than a "flowering hormone". The absence of a translocatable promoter and the presence of translocatable inhibitors in "Biloxi" soyabean and strawberry supports this idea. Further support for this hypothesis comes from grafting. In many plants such as in Perilla (Zeevaart, 1958) the scion or stock in vegetative photoperiods must be defoliated. The same applies to studies using ungrafted plants, for example where one branch is exposed to flowering photoperiods and another to vegetative photoperiods. Chailakhyan defoliated Chrysanthemum tips in vegetative conditions (Cholodny, 1939). Garner and Allard (1925) had to keep Cosmos stem tips in continuous dark before they would flower. Thus as there were no mature leaves in vegetative photoperiods in these examples flowering can be explained on the basis of an absence of some factor which repressed flowering. However, the work of Hamner and Bonner (1938), Heinze et al (1942), Melchers and Lang (Lang, 1965) and Sachs (1969), in which scions, stocks or branches in vegetative photoperiods, flowered without defoliations when they were attached to plants in flowering photoperiods shows that regulation of flowering by control of inhibitor levels is not a general phenomenom. Were it so, then the leaves of the stems in vegetative photoperiods would have blocked the flowering of the apices.

Therefore in some situations neither the hypothesis that leaves produce translocatable flowering promotors in conditions in which flowering occurs, nor the hypothesis that leaves in vegetative conditions produce a factor which blocks flowering, can be eliminated. The work of Chailakhyan (1936, 1937), with Chrysanthemum from which the term "Florigen" (flowering hormone or hormone complex) was developed, the work of Garner and Allard (1925) with Cosmos and Knott's work with spinach (1934) can be interpreted as by the authors concerned, in terms of a positive stimulus, or as the result of a lack of a factor inhibitory to flowering (the interpretation of van Denffer and Lona (Evans, 1969)).

Several authors have found that both promotive and inhibitory translocatable factors are produced in the same plant depending upon the daylength. Lang, Chailakhyan and Frolova (1977), found that Nicotiania silvestris, a long day plant, produced inhibitory substances in short days and promotive substances in long days. "Trapezoid" tobacco, a daylength neutral plant, did not flower when grafted onto Nicotiania silvestris and grown in short days. However, when "Trapezoid" was grafted on to Nicotiania silvestris and grown in long days, flowering was promoted. Phatak and Wittmer (1965), showed that late flowering tomato stocks delayed flowering and growth in early tomato scions. Late tomato scions flowered earlier and grew faster on early flowering stocks. The inhibitory or promotive effect depended on the presence of leaves which suggests that tomato leaves produce inhibitory or promotive compounds. Though the primary effect of these factors may be to do with growth and not with flowering. The work by Lang and Melchers on Hyoscyamus niger (Lang, 1965) also indicates that dual

control mechanisms may operate in other plants. For example, they demonstrated that leaves in long days produced promotive factors and leaves in short days inhibitory ones (Lang 1952, 1965).

Peas (Pisum sativum) may be another example of an inhibitor: promoter system. Late lines inhibit flowering in early scions, while early lines promote flowering in late scions (Murfet, 1971). In pea the cotyledons appear to be inhibitory as cotyledon removal in late lines resulted in earlier flowering (Murfet, 1974). Cotyledons also appear to be a source of promoter as cotyledon removal in early lines delayed flowering. However, the evidence for a promoter is not as strong as the evidence for an inhibitor, as delayed flowering in early lines as a result of cotyledon removal, may be due to other causes such as the removal of food reserves. The other evidence for a promoter in pea, the earlier flowering of late scions on early stocks may be due to the removal of inhibitory cotyledons. Haupt's evidence (1958) for a promoter in pea is also open to other interpretations. Murfet (1977) and Haupt (1958), interpreted the stronger flowering in a late cultivar (Alderman) when it was grafted on to a leafy stock of Klein Rhein Landerin, than when it was grafted on to a defoliated Klein Rhein Landerin stock, as showing the presence of a promoter. However, poorer flowering in scions on defoliated stocks may have been due to a lack of food reserves and enhanced translocation of inhibitors from the cotyledons.

Pea shoot tips also appear both inhibitory and promotory as flowering in scions is to some extent unaffected by the nature of the stock. For example, late scions on

early stocks did not flower as early as early scions on early stocks (Murfet, 1971).

While many experiments suggest that the leaves are possible sources of inhibitory or promotive flowering factors, Miginiac (1978), has proposed that the roots also produce inhibitory compounds. As Chenopodium polyspermum and Scrofularia arguta flowered in response to root removal, in daylengths in which flowering did not usually occur. Van Der Pol (1972), found that flowering was enhanced in long days, and occurred in warm short days when the roots of the long day plant Silene armeria were removed. Roots are also inhibitory to flowering in Ribes nigrum. While there could be many reasons for root inhibition of flowering, such as root production of inhibitory compounds or root activity as sinks for promotive factors, roots probably have a major role flowering in conjunction with leaf processes.

In some plants flower initiation is accompanied by a decrease in apical dominance as in Trifolium repens (Thomas, 1962), Silene coeli rosa (Lyndon, 1978) and in Chenopodium rubrum (Seidlova, 1980). The removal of apical dominance in Rosa canina (Cockshull and Horridge, 1974, 1977, Aranda cv. "Deborah" and Vanilla planifolia (Goh and Seetoh, 1973) was associated with promotion of flowering. Lateral buds of Mangifera indica were blocked from flowering by the terminal bud until it had produced fruit, so that they only flowered in the following flowering season. When the terminal bud was removed laterals became capable of responding to the flowering stimulus (Reece, Furr and Cooper, 1946). Cockshull (1972), suggested that apical dominance, duration and intensity of the short day stimulus all control the

pattern of flowering in Chrysanthemum morifolium (Ramat). Presented differently, the lateral buds will not flower if apical dominance is strong, and when it is removed will only flower if a stimulus is present. The suppression of axillary bud response to the floral stimulus probably accounts for many inflorescence structures, and patterns of flowering especially in determinate cymose inflorescences.

Hussey (1963), found that the removal of leaf primordia in tomatoes decreased the time and number of nodes to flowering and Seidlova (1980), found that leaf primordia decreased in size during evocation. In another example Lyndon (1978) found that by examining the position of leaf and sepal primordia initiation on the apical dome before and during evocation that the youngest leaf primordia had little effect on the position of sites of sepal initiation, which may indicate that leaf primordia cease to be inhibitory to sites of primordium initiation during evocation. As leaf primordia are thought to control apical dominance, the weakening of apical dominance which seems to accompany flowering may be related to effect of factors causing evocation. This may be an important step in inflorescence or flower initiation. But decreases in leaf primordium size, the weakening of apical dominance and other associated phenomena, may be more related to internal changes in apical structure and to the diversion of nutrients away from the sites controlling apical dominance than to flowering. Further more it does not necessarily follow from examples where the removal of leaf primordia, or apices has caused flowering, that flowering is directly caused by the removal of apical

inhibitors, (as Charles-Edwards (1979) and Thornly and Cockshull (1980), have suggested) because only daylength neutral plants (tomato) and lateral apices of flowering plants, flowered in response to leaf removal or decapitation. In both of these situations floral stimulus is likely to be present. However, it is likely that the apex apart from being a site of flower or inflorescence initiation is a regulatory site of flowering, probably in conjunction with others.

Further evidence of this role is shown by the fact that in many cases vernalisation only occurs when the tip is cooled. Curtis and Chang (1930) found that celery flowered only if the crown was cooled but not if the mature expanded leaves were. Schwabe (1954), vernalised Chrysanthemum morifolium (Ramat) cultivar Sunbean, if the tips were cooled, and Purvis (1940), found that rye fragments required growing points for the regenerated plants resulting from chilled fragments to be vernalised. Another line of evidence (see Lang (1965)) is that unvernalised shoot apices requiring vernalisation do not flower when grafted on to vernalised leafy shoots. The implications thus are, that mature leaves do not produce factors which can cause a shoot tip to be vernalised, and that mature leaves cannot be vernalised.

Melchers and Lang, Gregory and Purvis, and Napp-Zinn (Purvis, 1962) have proposed that vernalisation produces factors which in certain conditions result in the production of factors which cause evocation. Melchers and Lang (Lang, 1951, Melchers, 1939) found that vernalised biennial Hyoscyamus niger which requires vernalisation, when grafted on to the short day plant Maryland Mammoth tobacco

flowered in long days without a cool treatment. While Maryland Mammoth eventually flowers in long days, and so a build up of the factors causing flowering could occur, no flowering occurred in Maryland Mammoth in these experiments. Melchers and Lang concluded that some factor from Maryland Mammoth replaced the need for vernalisation in Hyoscyamus niger. When scions of an annual form of Hyoscyamus niger (flowers in long days without vernalisation) were maintained in short days after being grafted onto a biennial form (requires vernalisation to flower in long days) 50% of the biennial stocks (kept in long days) flowered without vernalisation. This suggests that the annual Hyoscyamus niger produced some factor in short days which enabled the unvernalised biennial Hyoscyamus niger to respond to the long days. Melchers (1939), called this factor vernalin, which because it did not cause flowering in either the annual Hyoscyamus niger or Maryland Mammoth was not thought to be the translocatable factor causing evocation. However, there is another interpretation, annual Hyoscyamus flowers in short days if defoliated this indicates that a low level of floral stimulus is present in short days, Maryland Mammoth eventually flowers in long days which also suggests the presence of floral stimulus, if biennual Hyoscyamus niger is more sensitive to floral stimulus then either annual Hyoscyamus in short days or Maryland Mammoth in long days it is possible that the biennual Hyoscyamus niger responded to floral stimulus, and that vernalin is a floral stimulus. Other attempts to show translocation of vernalisation products produced during the cool treatment have not shown the translocation of such products. For

example, Schwabe (1951), did not find "vernalin" in Chrysanthemum. A possible exception is winter rye, where unvernalised shoots or tillers were found to flower on a vernalised shoot system (Purvis, 1962). Many unvernalised plants which require vernalisation will flower when grafted onto a plant which is flowering, for example Hyoscyamus niger (Lang, 1965). Therefore the rye shoots in Purvis' example may have responded to floral stimulus produced by the vernalised shoots. While the evidence for a translocatable "vernalin" can be interpreted in other ways, there is no evidence against a non-translocatable "vernalin".

In Pisum sativum several workers, for example Reid and Murfet (1975), and Paton (1969), have found that vernalisation reduced the effectiveness of the gene Sn which produces inhibitors. As the gene Sn also operates in the shoot tip (Murfet, 1971) it is possible that inhibitor levels are lowered in the tip in response to cool temperatures. Indeed the hypothesis that vernalisation removes inhibitors from the tip has been recognised as an alternative to precursor or co-factor production models of vernalisation. Decreases in inhibitor levels in the tip could increase the sensitivity of the tip to promoters or result in the removal of blocks to promoter production in juvenile leaves, which stops mature leaves from responding to photoperiod.

Given the evidence that translocatable factors inhibit or promote flower initiation one would expect that known plant hormones such as auxins gibberellins and cytokinnins regulate flowering. To some extent this is so, for example gibberellin treatment will sometimes replace the need for a cool treatment (Lang, 1957), or cause flowering

in long days (Lang, 1965, Evans, 1971, Zeevaart, 1976). Other examples are sometimes inhibitory, for example cytokinnins (Migniac, 1976), auxins (Lang, 1965). However given the variability of response to these hormones and the fact that timing of application can be important (Seidlova, 1980) it is difficult to regulate known plant hormones to a general theory of regulation of flowering.

In conclusion to this section, it would appear that the leaves, roots and stem apex have regulatory roles in flowering, and that this regulation may or may not be due to auxins, gibberellin, natural plant phenolics etc.

FLOWERING PHYSIOLOGY IN WHITE CLOVER

Environmental Control of Flowering in White Clover

Roberts and Struckmeyer (1938), published the first studies on environmental control of flowering in white clover. Their studies revealed that flowering was initiated by growing plants in 13°C long days but not in 13°C short days, or 21°C long or short days. This suggested that white clover was a long day plant, in which warm temperatures were inhibitory to flowering. Later studies by Laude, Stanford and Enloe (1958), in glasshouse conditions probably as warm as 21°C also showed a long day requirement. They found that increasing the daylength reduced the number of days to flowering (the unfolding of the first petal on the first inflorescence). They also found that overwintering the plants in the field reduced the number of days to "flowering" from 70 to 51 days, which indicates that cool temperature may have some effect on flowering at least in Pilgrim Ladino white clover.

Beatty and Gardner (1961) also studies photoperiod and temperature effects on the flowering of Ladino white clover. In a range of photoperiods varying from 11-18 hours, at a temperature of 26°C, two types of response were noted. In some genotypes flowering increased with increasing daylength to a range of optima varying from 14 to 15½ hour daylength, and in other flowering was weak to not occurring at all, irrespective of the daylength. To test the effect of temperatures, plants were exposed to either cold (winter) or 26°C 18 hour or 9 hour days for three weeks, they were then transferred to either a 26°C 9 or 18 hour day.

5 weeks of 26°C
10 hour days
→ 3 weeks
9 hours cold, 9 hours warm → 9 hours warm
18 hours cold, 18 hours warm → 18 hours warm

In three genotypes which failed to flower in response to warm long days, cool temperatures (around freezing point at night) caused flowering in both short and long days. Cool temperatures increased the level of inflorescence production in one genotype from 3.6 to 6.6 inflorescences per plant. Pretreatments of plants with varying lengths of temperature treatments at 10°C and 16°C, increased the level of inflorescence production in 18 hour treatments. Thus they found that cool temperatures could directly stimulate inflorescence production, and promote flowering in situations such as long days which also caused flowering.

A direct cool temperature effect on flowering was also found by Britten (1960, 1961). Plants that failed to flower in response to short photoperiods (13 hours) at 22-28°C, flowered in response to a day temperature of 28°C and a night temperature of 8°C.

Another study of photoperiod and temperature effects was undertaken by Haggar (1961). Kent wild white clover flowered sooner and produced more flower heads with increasing daylengths. Exposure to cool temperatures (3°C) of either seeds or mature plants increased the flowering in subsequent long days. As plants or seeds were only exposed for one or two weeks to cold continuous dark, Haggar's results seem to show that vernalisation

occurs in white clover. Zaleski (1964), also found an apparent vernalisation effect of cool short day treatment: natural winter daylengths at 4°C to 13°C were better than warm winter daylengths in promoting flowering in subsequent 17 hour days.

Cool treatments are not alone in promoting flowering in following long photoperiods. Thomas (1961), found that seedlings and selected genotypes of a Grasslands Huia white clover population; growing in warm conditions required a short day pretreatment if flowering was to occur in following long day treatments. The experiments of Britten (1960, 1961), Beatty and Gardiner (1961), Laude, Enloe and Stanford (1958), do not contradict this result, as in all cases experimental plants were exposed to short days prior to the photoperiodic treatments. However, as Haggar (1961), recorded flowering in seedlings grown exclusively in long days, the short day requirement can not be a general necessity for all seedling phenotypes.

Thomas (1962, 1979) also found that flowering could be caused by cool temperatures. For example a 15.5°C day and 4.7°C night temperature caused flowering in Clone B of "Grasslands Huia". Temperature treatments of 10°C or lower caused all three clones tested (C, B and A) to flower in short days.

While all clones selected by Thomas (1961, 1962) flowered in response to cool temperatures irrespective of the daylength, one, Clone B, did not respond to warm short days followed by either 16 hour photoperiods, or continuous light. Britten (1960, 1961), and Gardner and Beatty (1961), found the same thing. Some lines of white clover were induced to

flower by long photoperiods and cool temperatures, but others, for example Beatty and Gardner's "Minnesota" only flowered in response to cool temperatures. Red clover, Trifolium pratense (Stoddart, 1962) also has phenotypes which flower only in response to cool temperature, while others respond to both cool temperatures and long photoperiods.

In conclusion, it would appear that daylength, short days followed by long days, or long days alone, and cool temperatures alone, or in combination with daylength (long days) regulate inflorescence initiation and production. Some plants responding to both cool temperatures, and photoperiods, others to only cool temperatures.

Internal Control of Flowering in White Clover

Cool temperatures and warm long days are known to cause inflorescence initiation in white clover. However, some genotypes produce flower heads only in cool conditions, while others flower in both cool conditions and in response to photoperiod treatments. This indicates that there are phenotypic differences in the regulation of inflorescence production.

As shown in a previous section; in some species cool temperatures and inductive photoperiods cause the production of translocatable floral stimuli which cause flowering at stem apices. Other plants also produce translocatable inhibitors which block flowering, while in others root and apical factors also influence flowering. Thus the regulation of flowering involves a complex of factors, associated with the leaves, roots and stem apices.

If the regulation of inflorescence production in white clover is similar to regulation of flowering in other

species, the situation is likely to be complex. Some studies for example those of Cohen and Dovrat (1976), and Thomas (1979, 1981) indicate that flowering in white clover is controlled by a balance between inhibitors and promoters, and that the level of known inhibitors of some plant processes such as stomatal opening, are higher in some cultivars than in others (Cohen and Dovrat, 1976). However there is little direct evidence of flowering promoters or inhibitors, or of stolon tip or root activity in the regulation of inflorescence production in clover. The aim of this thesis then, is to examine the role of some components of the plants such as the leaves and stolon tip in flowering, and to develop a model of the regulatory system which would explain the difference between white clover phenotypes such as "Grasslands Huia" Clone B which do not flower in warm photoperiods and those like "Grasslands Huia" Clone C which do.

MATERIAL AND METHODS

Plant Materials

The general morphology of white clover (Trifolium repens) has been described by Erith (1924).

Description of Genotypes

Three white clover genotypes were used in experiments: two genotypes of 'Grasslands Huia', clones B and C, and a Kalinin genotype Kalinin A. Both clones B and C were selected by Thomas (1962, 1979). Kalinin A was obtained from material grown at Grasslands Division DSIR Palmerston North from seed sent from Leningrad U.S.S.R /from N.I. Vavilov All Union Institute for Plant Research, Leningrad (collection number WTR 34587)7.

The genotypes differ from each other in colour and size. Kalinin A has smaller leaves, shorter stolon internodes and smaller stolon diameters, than either clones B and C. The leaves of Kalinin A are also more bluish green than the leaves of clones B and C, and has fainter leaf marks.

In comparison to Kalinin A and clone C, clone B is characterised by the redness of the pulvini, stipules, stolon internodes and florets. Clone B also has slightly larger stolons than clone C.

Environmental conditions influence the morplology of clones B and C and Kalinin A. Under high light intensities the internodes, stipules pulvini and florets of clone C become red though this is duller than the red in clone B. Kalinin A also develops a red colouring this is mainly in

the stipules and is slightly purplish.

Light intensity also affects leaf characteristics under low intensities in the glasshouse Kalinin A leaves sometimes become as large as clone C leaves.

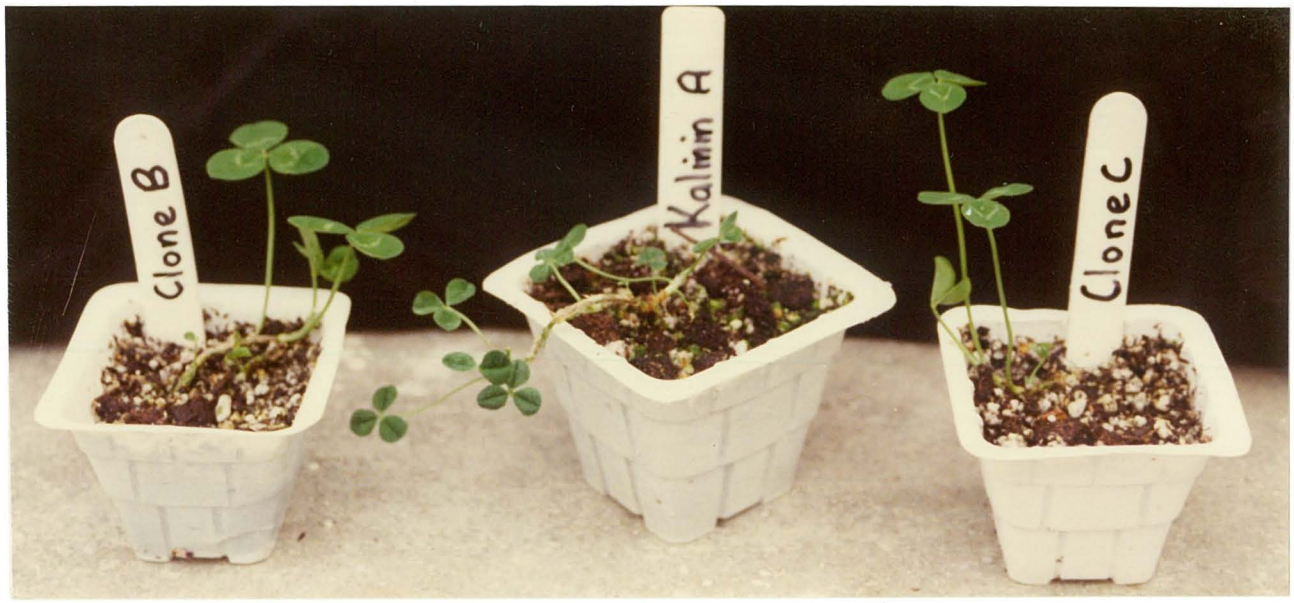


Plate 1

Young plants of clone B, Kalinin A and clone C.

Propagation and Plant Maintenence

Plants were usually multiplied by taking tip cuttings from stock plants. The best stolons to use as cuttings seemed to be young and elongating, with at least two visible nodes, that of the youngest and next to youngest unfolded leaves. Longer stolons, despite their potential for greater production of roots, tended to rot, and root only near the tip. Shorter stolons, for example young stolons in which elongation had yet to occur in internodes, or stolon with stunted internodes, took longer to root.

If it was possible the youngest unfolded leaf, and the next youngest unfolded leaf, were kept on the cutting. This depended upon whether or not it was possible to maintain the cuttings in a humid environment which prevented wilting. In such cases the plants were left in a shady humid environment for up to two weeks, then transferred to a higher light intensity, where they were frequently watered.

Cuttings were grown in a perlite - pumice - peat mix supplemented with 'Osmocote' short term fertiliser. Occasionally liquid fertiliser was given, to speed up rooting.

When the root system had sufficiently developed to survive less humid conditions, the cuttings were transferred to 10cm pots.

Another system of multiplication was that plants were broken up and potted into new potting mix. This was used in experiment 16.

The potting mix was as follows:

two bushels of basic mix, 70cm^3 of super phosphate 100cm^3 of short term osmocote, and 500cm^3 of lime. In experiment 16 50cm^3 of long term osmocote was also given. The basic mix consisted of one part peat, one part pumice and one part perlite.

The potting mix used in Climate Laboratory (experiment B) was a North Carolina mix, (Brooking 1976) supplemented daily with Hoagland's solution.

Two systems of watering used. In one the plants were subirrigated standing by pots in a tray of water. This system was probably best for grafts because splashing water onto grafts often resulted in graft death. One problem was that the level of water had to be closely watched to make sure that the plants were not standing permanently in a pool of water. The distribution of water in the trays also had to be watched to make sure that all plants received adequate water. The other system of watering was daily or twice daily application of water to the plants by pouring water onto the pot and allowing it to soak down.

Plants were potted on the root system completely filled the pot. This was usually done when it had been necessary to grow plants for some time before the experiment, as for example in grafting experiments and experiments concerned with long day cessation and the rate of the short days.

Liquid fertilisers eg Lush (Arthur Yates Co) were given to plants, usually on a weekly basis.

Aphids especially pea and blue green lucerne aphids were sometimes a problem, as were mealy bugs, white flies and red spider mites. To cope with these pests, especially in long term experiments in the growth room, insectide sprays were

used. Aphids were usually controlled with Madison (Coopers); and a synthetic pyrethrum Permethrin (Ambush ICI Tasman). White flies seemed to be resistant to Madison but were easily controlled with Ambush. Mealy bugs were generally no problem, but red spider mites ruined several experiments. Madison had no effect, and the mites were uncontrollable until a supply of Kumatox, (obtained from Climate Laboratory, no longer available) and Phosdrin (Mevinphos, Shell Oil) became available. Mites were also effectively controlled by Plictran. Oil was added to most sprays and was sometimes used by itself. However, clone B was sometimes badly 'burnt' when this was used. Malathion (Madison) also 'burnt' clone B.

Insecticides were applied by watering or spraying. Kumatox was applied by watering. The other chemocals were sprayed on. The best time being late in the evening, when the leaflets were beginning to fold upwards exposing the undersides of the leaflets In hot weather short term systemics such as Phosdrin, and non-systemics like Malathion (Madison) had to be used frequently to overcome rapid increases in pest population sizes.

Grafting

When it became apparent that clone C produced a translocatable floral stimulus, grafting procedures were tried on white clover. Various types of grafts were tried, approach grafts, which had been tried in the past, cleft grafts and bud grafts. Of these the most successful and fastest was the cleft graft (see figure 1). In this method the base of a stolon segment with a stolon (scion) tip was shaped into a wedge. Another stolon (the stock), was decapitated just above a node, and slit longitudinally. The first stolon (scion) was then inserted into the cut. One problem that arose was that one side of the wedge of the scion was sometimes longer that the other. This was overcome by slitting the stock with the razor blade at an angle.

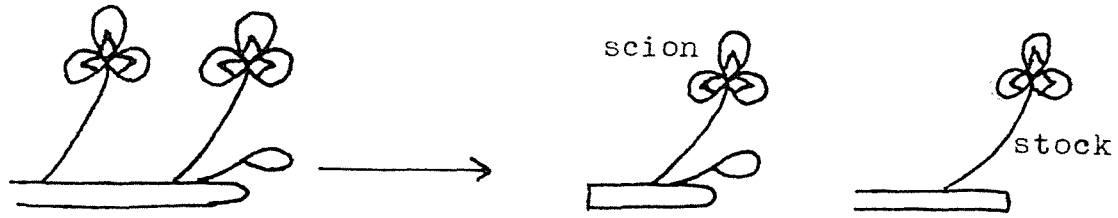
Grafts were generally held together with surgical rubber rings two to three mm in diameter.

The length of the scion and stock was determined by the diameter of the scion, as it seemed important that the scion and stock diameter matched. Long (over 4cm) scions were better than short (4cm or less) scions, as the wedge in short scions was shaped at low angles which resulted in some scions 'popping out'. The internodes used for the wedge or slit was determined, also by length and age. Young internodes especially those above the youngest unfolded leaf tended to be too short, in old internodes the pith degenerates.

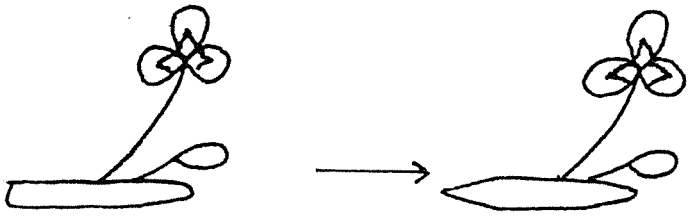
Best results occurred when grafting was done on cloudy or rainy conditions, or where the humidy was high. Late

Figure 1 Diagrams of Grafting Procedures

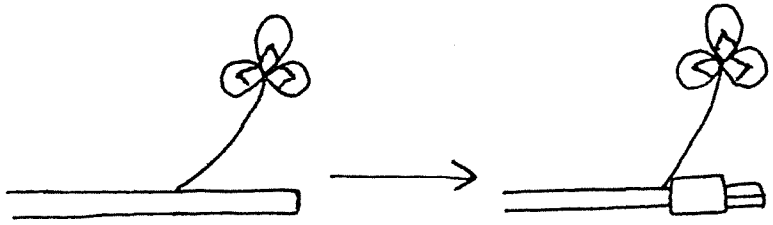
1. Stolons decapitated to provide scions (stolon tips) and stocks.



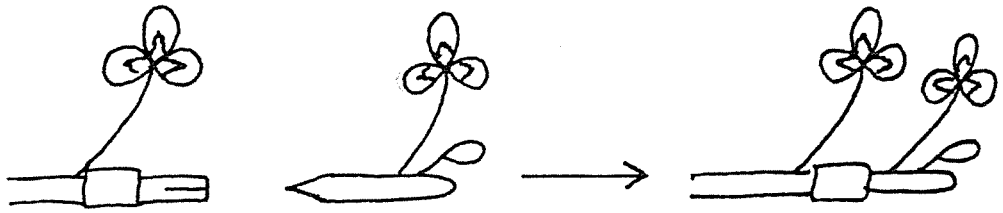
2. Lower end of scion shaped into a wedge by razor blade.



3. Rubber ring pulled down stock stolon and the upper end of the stock stolon slit along the middle.



4. Scion wedge inserted into cut in stock stolon. Rubber ring pulled up over the junction of scion and stock.



evening or early morning was also a good time. Speed was also important as slowness increased the possibility of desiccation.

In general grafts which were going to take, had stuck together by one day. However, good vascular unions, and scion elongation took at least five weeks (as shown by internode elongation and node production).

Once the grafts had been made, plants were transferred to humid shady areas. For the growth room experiment, grafts were transferred to a "plastic film cabinet", made by dropping plastic film over the frame of a bench. To keep the humidity high the concrete floor was daily hosed with water. In other experiments (14, 15, 16) plants were transferred to a large standard box in which there was a tray filled with water. After two weeks, or when the scions had produced at least one unfolded leaf, the plants were transferred to a higher light intensity. Light intensity was gradually increased as determined by the ability of the scions to remain turgid. Too much low intensity light, coupled with high temperatures caused leaf and root death, particularly in Kalinin A. Determining when to transfer plants for one light intensity to another was one of the more difficult aspects of this method of grafting.

Plastic bags were sometimes used but high losses of grafts occurred. One other system that was successful, in that the stock did not need to be exposed to low light intensity; was that holes were cut near the rim of small plastic pots. The pots were then filled with water, and the scions pushed through the holes, so that they were just above the water level. Unfortunately this method was space consuming; and the number

Plate 2

Kalinin A grafted onto clone C. The Kalinin A scion later flowered despite the one sided graft union.

Plate 3

Kalinin A - clone C graft union.

of grafts per pot limited.

Plants were found to be best watered by hand, as automatic systems did not take account of the low evapouration that occurred in the shade. Care was also taken to make sure that water was not spilt on the grafts, as this caused them to rot. It also seemed to be a good idea to remove the rubber rings by putting them down the stock stolon. (generally after two to three weeks), as there was a tendency for more grafts to rot when the rings were left on. Initially the plan was to replace the main stolon apex with a scion. This was changed when it became apparent that because the graft union took so long, most of the stock leaves were dead by the time they were wanted. Another problem was that to get good grafts it was sometimes necessary to remove the youngest unfolded leaf, on a stock, a leaf which has a good response to photoperiod. Also the number of sites (leaves) producing regulatory factors was limited by removing the source of the leaves, the apex.

For these reasons scions were grafted onto lateral branches, though only after it had been checked that the floral stimulus could move between major stolons, with the same root system. This also meant that the stock was producing potential sites of regulator production, that could influence the scion.

Replacement of the main stolon apex by a scion although poor for study of the effect of a stock on a scion is better for studying the effect of a scion on a stock. For example in clone C on clone B grafts the scions can be used to try to induce inflorescence production in more than one clone B lateral stolon, which incidently are usually growing well. The problem is that stock laterals sometimes restricted the

growth of the scions, but this may have been a sign of poor graft unions, as it is my impression that in good grafts apical dominance is not seriously diminished by replacement of the apex with a scion.

Many people defoliate their stocks, but as plant death was a serious problem when this was done to white clover it was discontinued (Apart from removing some old leaves to make grafting easier).

Pots with many main stolons which in turn had large numbers of lateral stolons were used for grafting, as this increased the number of sites available for grafts.

When two or more main stolons produced good grafts the plants were sometimes broken up. Most scions and stocks survived this procedure well. This method was used in exp 16.

Experiments were not started until most grafts were elongating and producing new leaves. If this was not possible, because, for instance, poor elongating was occurring, the plants had to be able to survive high glasshouse temperatures (30°C or more). Growth but little elongation occurred in the first batch of clone C on clone B grafts, this may have been a result of the small clone C scion size with respect to both diameter and length

Analysis of Flowering

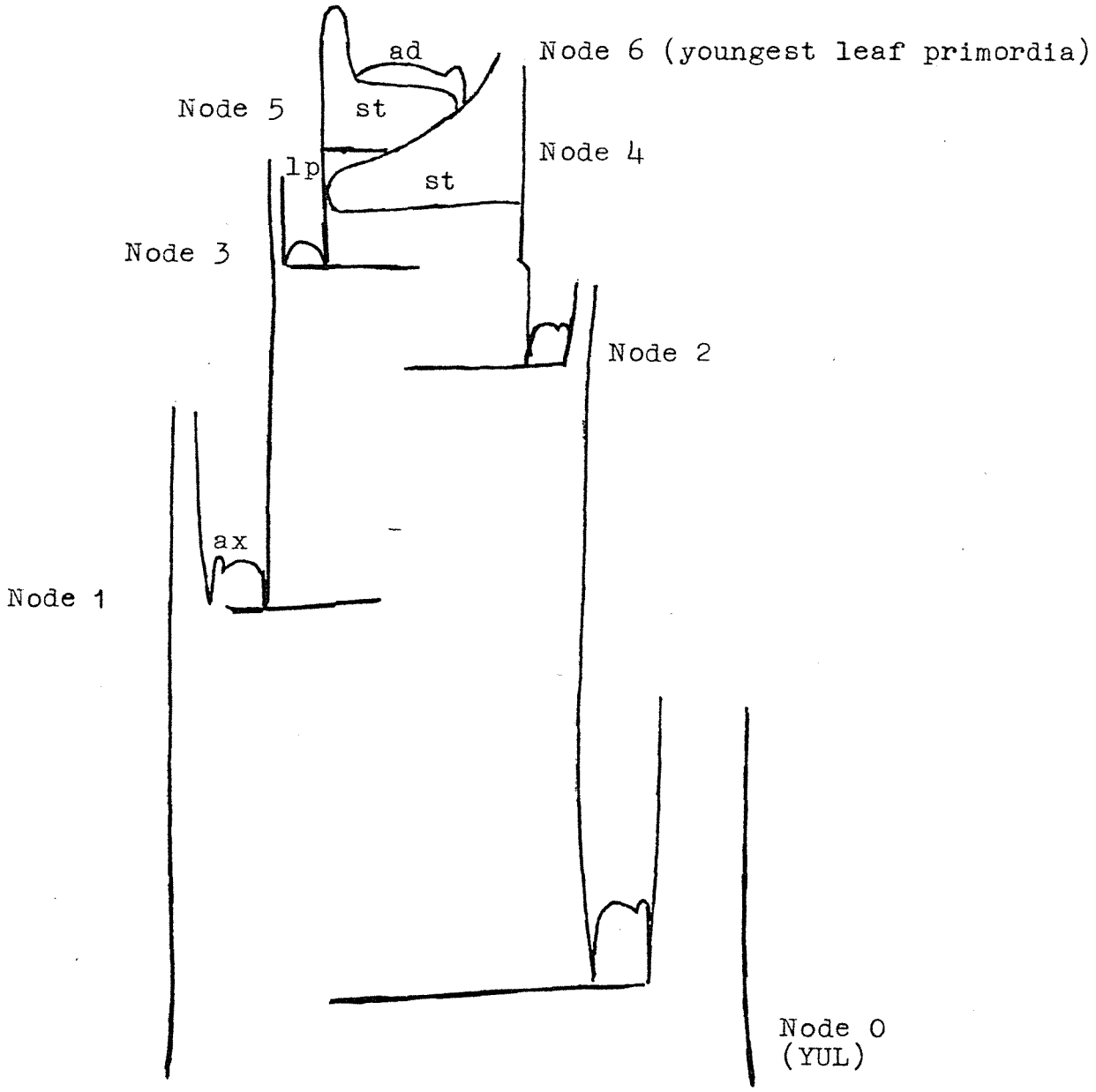
Flowering was measured by counting the number of inflorescences produced by a stolon during a particular treatment. To determine whether or not flowering had occurred before the treatment, and also to determine the length of time to inflorescence initiation, stolons were tagged at the youngest unfolded leaf, or defoliated to the youngest unfolded leaf.

In healthy stolons the number of leaves or nodes, younger than the youngest unfolded leaf (YUL) ranged from five to seven. Most stolons had six, for example in one sample, eight out of nine stolons had six and one had seven. Poor growing conditions increased the number of stolons which had five or less leaves after YUL, as in experiment 8 where most had five and some, four.

If it is assumed healthy stolons have six nodes from the youngest unfolded leaf (YUL) see figure 2, an inflorescence which forms seven or more nodes distal to YUL must have been initiated after tagging or defoliating. Inflorescences which form six nodes from the youngest unfolded leaf may have formed before the experiment or during it. Short day controls can be used to determine which.

Another point of interest besides whether or not a plant had iniated flowers was the time to inflorescence initiation. One way in which to do this, is to derive a value for the plastochron (time internal between formation of two nodes)

Figure 2 Diagram of Stolon tip showing the number of Nodes from Yul (Node 0) to the apical dome



ax = axillary bud
ad = apical dome
Yul = Youngest unfolded leaf
lp = leaf primordium
st = stipule

and to multiply the plastochron time by the number of nodes to an inflorescence (Time for inflorescence initiation = time for a node to be produced x nodes to an inflorescence). This method assumes that the plastochron is constant .

By tagging YUL it is possible to determine the plastochron values and the number of nodes to an inflorescence. The production of nodes during a time interval (plastochron value) can be determined by subtracting six from the total number nodes from YUL, and the number of nodes produced to the first inflorescence, by subtracting six from the total number of nodes to the first inflorescence (see paragraph 2).

This method actually measures the time taken before an inflorescence - bearing node is produced, and as inflorescences were rarely seen at the youngest node on a stolon (usually the second) there may be delay (at the most a plastochron) between the time a node was produced and when an inflorescence was microscopically visible. (This may be an erroneous observation earlier dissection may show inflorescence initiation at the youngest node).

Another problem is that the apex may not be sensitive to the floral stimulus during the whole of the plastochron, so that flowering may be delayed to the next plastochron, relative to another stolon whose apex is sensitive to the floral stimulus.

Plastochron values may also vary, and as some stolons have five or seven nodes instead of six nodes from YUL, flowering could appear to be earlier or later when it is infact not.

Large sample sizes would probably have overcome some of these problems. However this was not always possible because space and the number of viable grafts was limited.

Another problem was identifying the 'oldest ' YUL at the end of an experiment. This was usualy done by tagging it at the beginning of an experiment or by defoliating stolons up to YUL. On several occasions, especially in long term experiments, the cotton string on the tags rotted off, or the leaves died and the petioles snapped. The best method is probably to tag the first internode infront of YUL with plastic or nylon thread. Alternatively the inter - node could be marked with ink.

Analysis of Results

Where applicable, experimental results were expressed in terms of the treatment mean plus or minus the standard error. Statisical significance tests ie Student's t test, and analysis of variance were not used to test differences between treatments. Either because it was apparent from the means and variation within treatments that there were or were not differences between treatments or because the result that was required was whether or not flowering occurred or was stopped. Another factor taken into consideration was that given the availability of space and material the number of replicates within a treatment was often less than 10, such small sample sizes can give high standard deviations and variances which may affect the accuracy of significance tests.

Light Intensity and Temperature Measurements

Temperature

Growth and flowering in white clover is affected by temperature; for example, cool temperatures (eg $10^{o}C$) will cause white clover to flower in short days. For this reason maximum and minimum temperatures were measured by a maximum and minimum thermometer, or by a thermo-graph (September 1980 onwards).

Light Intensity

Light intensities were measured with a Lambda L 1-185 Quantum/Radiometer/Photometer, using a Quantum Probe Serial Number Q 316-7309. This measures quantum flux ($uE/m^2/sec$) at photosynthetically active wavelengths (380-700nm). Measurements were taken at the same height as leaf laminae.

Departmental Glasshouse

The departmental glasshouse was used for propagating plants for all experiments except for experiment 13. Pre-treatments and some experiments were also carried out within the glasshouse.

Average minimum and maximum temperatures, and the lowest and highest temperatures are shown in figure 3. From August 20th 1980 the 'cut in' temperatures for turning on the heaters was lowered from 18.3°C to 15.6°C.

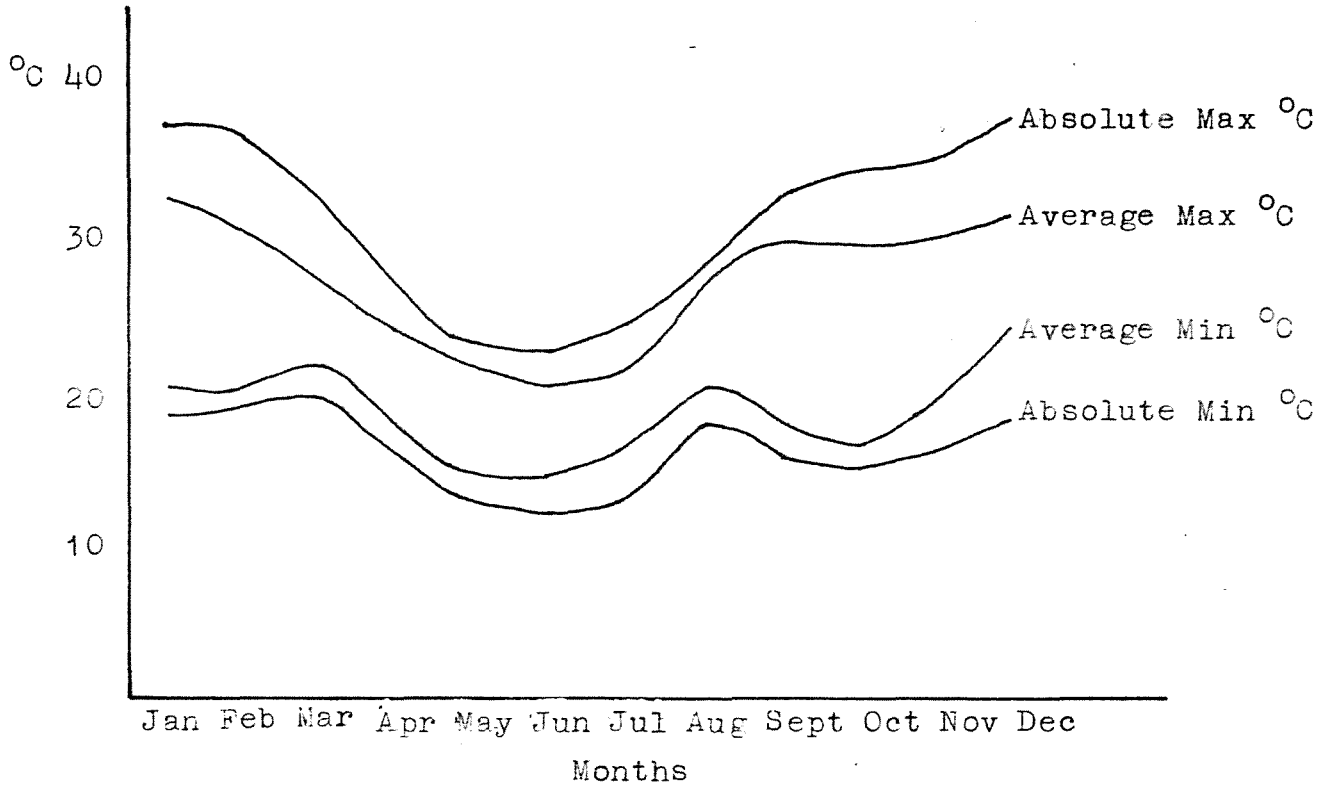
Temperatures within the glasshouse were sufficiently warm to stop either clone B or clone C from flowering in short days. However, clone B plants overwintered in the glasshouse responded to continuous light which is an effect of cool temperature pretreatment.

Light intensity within the glasshouse was measured on one occasion on a cloudless day at 11.00 in mid January, to see how light intensity varied with position inside the glass-house. It ranged from 33 to 1250 uE/m^2/sec (average 640 uE/m^2/sec. Outside the glasshouse the light intensity in fall sunlight was 1500 uE/m^2/sec.

Figure 3 Glasshouse Temperatures (Bot - Zoo)

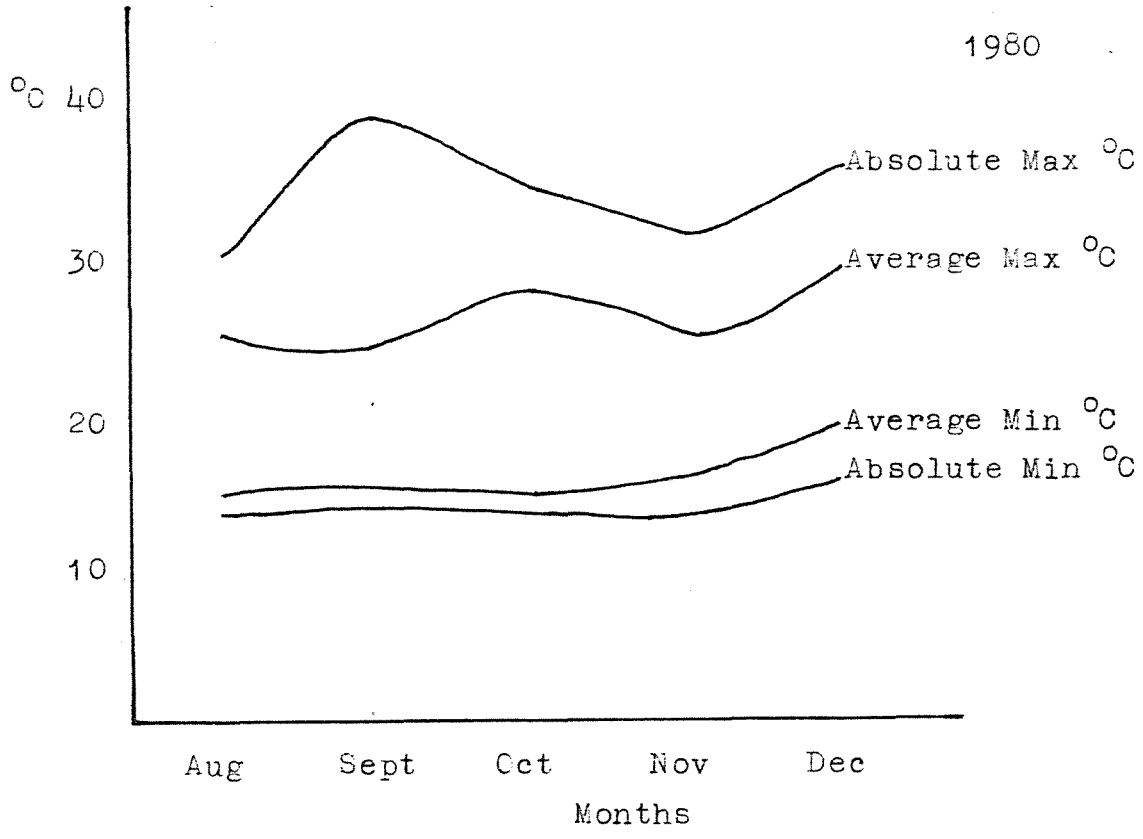
1978 to August 1980

Thermostat 18.3°C



Thermostat 15.6°C

1980



Departmental Growth Cabinets and Continuous Light Banks

Lighting

In both the growth cabinets and the continuous light banks, cool white fluorescent light tubes (Thorn, Philips, Osman, cool white TLA 33 watt) and incandescent lights were used (Thorn 15 watt). The use of incandescent lights was discontinued after Experiment 3 because they were too short lived. Growth cabinets contained 10 cool white fluorescent tubes, and the continuous light banks nine. The light intensity of photosynthetically active radiation (P.A.R) under the lights varied from 300 u $E/m^2/sec$ to 200 $uE/m^2/sec$. The incandescent light produced 5 $uE/m^2/sec$.

Temperature

Temperature under the continuous light banks and in the growth cabinets were constant ie there was little day to day or hourly variation in temperature. Temperatures under the lights was measured at 25°C. During Experiment 2 the cooling system broke down with the result that the temperature rose into the high 30's for a maximum of up to eight hours.

Dimensions of Growth Cabinet and Continuous Light Banks

Growth Cabinets: 1.24 x 0.48 x 0.5 metres.

Continuous Light Banks a) 1.26 x 0.50 x 0.36 metres.

b) 1.28 x 0.50 x 0.36 metres.

Plant Growth Unit Growth Cabinets and Growth Room (Climate Lab)

Light intensity, temperature and daylength of experiments using these facilities are described with each experiment. The lighting system of each is based upon a mixture of Quartz Halogen, Metal Halide and incandescent lamps. (Warrington, Dixon, Robotham, and Rook, 1978)

Growth Room	4 x 1000 W Metalarc metal halide lamps 4 x 1000 W Quartz halogen lamps 6 x 150 W Incandescent lamps
Growth Cabinet	6 x 375 W Mercury halide lamps
P.G.U	2 x 1000 W Quartz halogen lamps
Temper zone	3 x 100 W Incandescent lamps

The spectral energy distrubation for each facility is given by Warrington et al (1978).

It is possible that high light intensities (700 to 800 $uE\ m^{-2}\ sec^{-1}$) given for long photoperiods (18h or more) injure white clover. This is based upon the observation that stolons in experiment 13 exposed to 18h photoperiods were stunted and reddish, while lateral stolons of the same plant given 8h photoperiods were healthy.

Plant Physiology Division (P.P.D) Glasshouse.

Plants for experiment 14 were kept in a P.P.D glasshouse (No 2) for propagation, grafting and short day pretreatment.

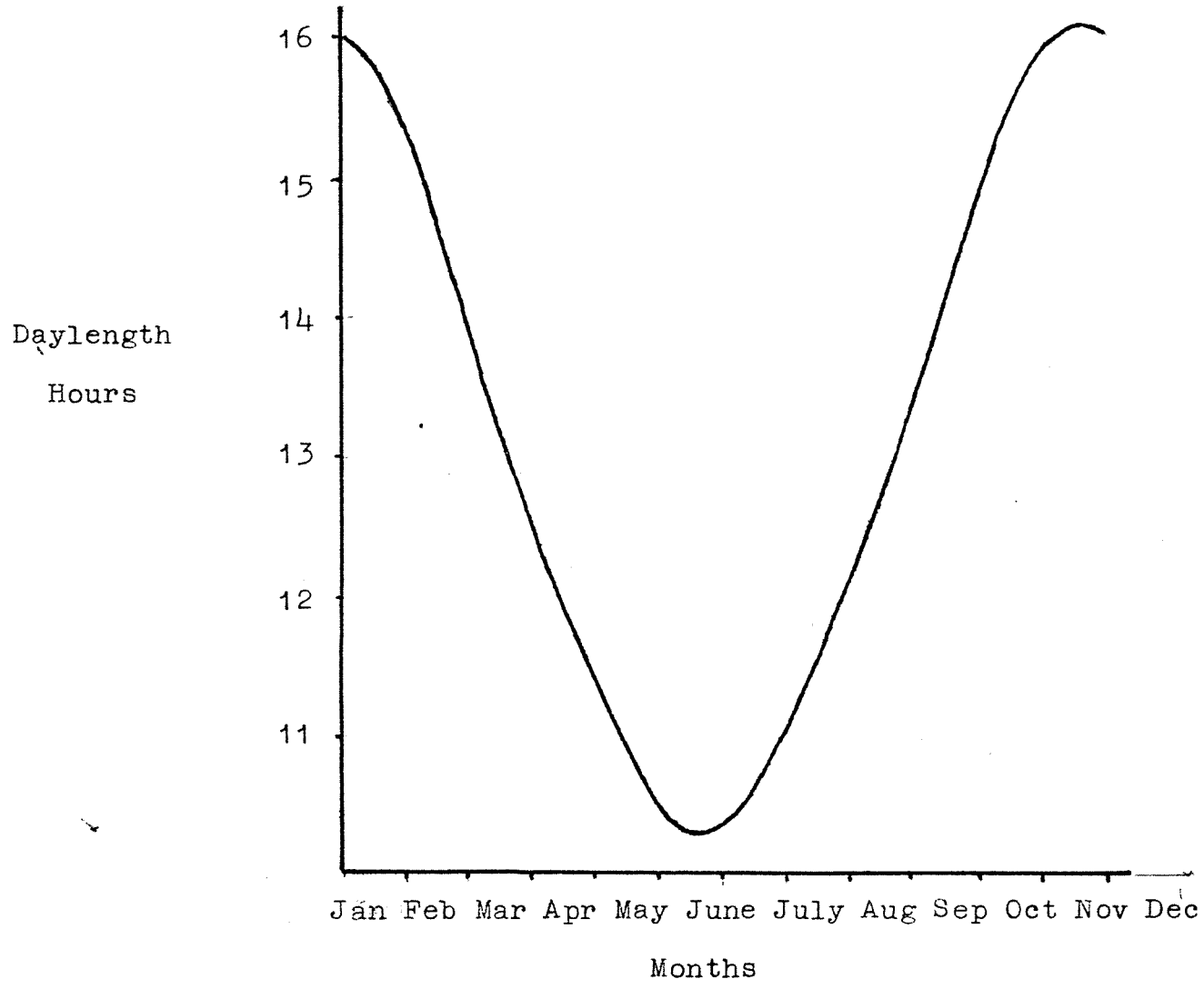
The minimum glasshouse temperature varied from 11 to 19°C (mostly between 15 and 16°C). On serval occasions when the heating broke down, minimum temperatures dropped to between 11 and 13°C. Maximum temperatures varied from 20 to 30°C.

Temperatures were not sufficently cool to cause flowering in short days as clones B and C and Kalinin A did not flower in short days. But as some (3) clone B plants initiated inflorescences when transferred to 18h photoperiod, temperatures were sufficiently cool enough to promote flowering marginally in clone B in long days.

Daylength

Daylength, or rather night length, is known to affect flowering in white clover. For example, in warm conditions inflorescence initiation is caused by daylengths greater than $14\frac{1}{2}$ hours, (Thomas, personal communication). In figure 4, to show how daylength varied throughout the year and to show when long day conditions were suitable for inflorescence initiation, daylength was plotted against the time of year. Daylength was calculated as being the length of time from the beginning of civil twilight in the morning to the end of civil twilight in the evening. This probably over estimates the daylength as perceived by white clover. The condition of the plant and the temperature probably influence the plants perception of daylength. By how much the daylength calculated by the method used here over estimates (or under estimates) the daylength preceived by white clover is unknown. In plants such as Japanese Morning Glory (Pharbitis nil) daylength as perceived by the plant is determined by astronomical sunset and the beginning of morning civil twilight. While in other plants sunrise and sunset determine the length of day/night, (Salisbury, 1963).

Figure 4 Graph of Daylength verses Month



Daylength was determined by subtracting the time of morning civil twilight (time of sunrise minus 27 to 33 minutes), from the time day of evening civil twilight (time of sunset plus 27 to 33 minutes). Sunrise, sunset, and civil twilight correction factors obtained from Civil aviation figures for Palmerston North Airport.

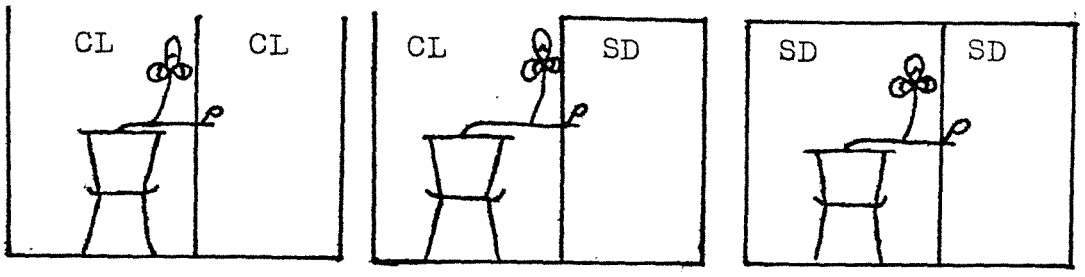
Transmission of Regulatory Factors

To test the hypothesis that inflorescence initiation is controlled by translocatable regulators, parts of the same plant were exposed to different photoperiods or temperatures. Whole plants were also exposed to different conditions to determine or check the effect of a photoperiod or temperature on flowering. The method used for each experiment is discussed under the following experimental headings.

Experiments 1 and 2

Cardboard boxes (26.5cm x 21.0cm x 26cm) were divided into two compartments are 11cm and the other 15cm.

Figure 5 Diagram of Boxes



Stolon tips (1cm or less) were pushed through holes in the dividing walls (a). Alternatively slits were made in the dividing wall (a), at the end of which were holes the size of stolons. The stolons were then pulled through the slits and down into the holes. To increase the light intensity given to plants within the box, a tray was placed on two pots (height 6cm) and the plants placed on top of the tray.

Each box contained three plants (pot size 7cm x 6.5cm). All plants within the same box received the same treatment.

Two boxes were used per treatment.

Plants were placed inside the boxes rather than outside to make it easier to move the experimental treatments around, and because more plants and treatments could be placed under the fluorescent lights.

Between 17.00 and 17.30 hours lids were placed on the short day compartments. These were then covered with aluminium foil to reflect heat and light. To ensure that the compartments were light 'tight', light intensity was measured inside the compartments with the lids on, at the beginning of the experiment. The light intensity within the boxes was also checked by looking through peep holes which were later covered (daily). The lids and aluminium foil were removed between 9.00 and 9.30 hours to give 7 to 8 hour days inside the compartments.

Experiment 3

To expose one stolon on a plant to long days, and another to short days, plants were placed within cardboard boxes as in figure 18. Slits were cut in the side walls of three boxes through which either main stolons or lateral stolons were pulled. To ensure that constriction of the stolons did not occur the base of the slit was widened to the diameter of a stolon. Black plastic tape was used to cover up the slits and gaps between stolons and the walls of the cardboard box. The tops of the cardboard boxes were also edged with tape to stop light entering the box between the lids and walls of the boxes.

One box was used per treatment, with each box containing nine plants (pot size 7.5cm x 7.5cm x 6cm). Boxes for

treatments 3.1, 3.3 and 3.4 were covered for seven to eight hours daily. The plants used in each treatment consisted of two stolons (one main stolon and one lateral stolon).

Some stolons were damaged during dissection, so that it was not always possible to obtain flowering data for every stolon.

Experiments 4 and 5

In two treatments, 4.1 and 5.1, plants were kept in a seven to eight hour photoperiod for at least five weeks. To do this six plants were kept in a cardboard box (55 x 47 x 24cm). At the end of five weeks plants for treatment 5.1 were taken out and placed on a glasshouse bench to receive a long day treatment.

Treatment 4.3 in which the stolon tip (initially YUL and the apical bud) were kept in long days was given by keeping the rest of the stolon and associated lateral stolons inside a cardboard for eight weeks. Plants for treatment 5.3 were kept in the same box (48.0 x 49 x 20cm) except that at the end of the five week period they were removed or placed on a glasshouse bench.

Plants in treatments 4.2 and 5.2 were given the reverse of treatments 4.3 and 5.3, the stolon tips being given short days and the rest of plant long days. In treatment 4.2 the stolon tip which consisted initially of the apical tissue beyond YUL, was put inside a cardboard box while YUL, the older leaves and the associated lateral stolon were kept outside. (YUL was left onto maximise floral stimulus production). Treatment 5.2 was similar to treatment 4.2 with exception that the short day treatment was only given for five weeks,

and that different boxes were used for exposing the stolon to short days.

To give the short day pretreatments, lids were placed on short day compartments at night (17.00 - 17.30 hours) and removed in the morning (9.00 hours).

Experiment 10

In experiment 10 stolon tips (apical tissue beyond YUL) were pushed through holes in a cardboard box (26.5cm x 26 x 21cm). A lid was placed on the box so that the stolon tips were kept in continuous dark, while the leaves etc were exposed to continuous light.

Experiment 11

The aim of experiment 11 was to test for translocatable inhibitors. This was done by exposing an apical bud and YUL of a lateral stolon to continuous light, while keeping the rest of the plant in warm short days. One lateral stolon per plant was pulled through a hole in a cardboard box while the rest of the plant, and the short day controls were kept inside the box. The boxes were covered at night and uncovered in the morning to give seven to eight hour days inside the box. Two boxes were used (box size 45 x 45 x 20cm pot size 14cm x 14cm x 11cm).

Experiment 13

This experiment was set up inside a climate laboratory growth room. Plants were put inside one of six boxes (45 x 45 x 20cm) which had been covered with aluminium foil, and for which lids had been made. Inside each box there were nine plants (pot size 14cm diameter x 12cm deep). Five boxes contained the following:

1 to 2 clone C short day controls;

2 grafts of clone B on clone C;

2 grafts of clone C on clone C;

2to 3 clone C plants where the main stolon kept in 18 hours

and the laterals in short days (8 - 7½ hours).

Grafts of clone B on clone B, clone C on clone B were also present in some boxes.

One box contained three clone C short day controls, and two grafts of clone B on clone C also short day controls. Numbers were altered by scion death (mostly clone B) and by removal of plants. Kalinin A scions on clone C stocks were also transferred into some of the boxes at a later date.

Main stock scions were exposed to 18hr photoperiods by putting them through slits cut through the cardboard walls. Black tape was used to make the boxes light tight. (Growth room conditions weakened the adhensive, so that tape was constantly replaced).

Lids were removed at 10.00 hours ± 15 minutes and replaced at 18.00 hours ± 15 minutes.

Experiment 15

In some treatments in experiment 15 clone B stocks were kept in a 8hr photoperiod and the clone C scion in an 18hr photoperiod. This was done by keeping the stocks inside a cardboard box (45 x 45 x 20cm) and the scions outside the box.

Experiment 16

In, the clone C scions in experiment 16 were kept in 7 - 8hr photoperiods for three weeks by keeping the base of the stock and the scion inside a cardboard box. Clone B stocks were pulled through slits out of the box. The slits and the gaps between the clone B stocks and the cardboard walls, were covered with black plastic tape. Short day controls were kept inside each box (45 x 45 x 20cm). Two boxes were

used to accommodate both the short day controls and the experimental treatments. At the end of three weeks the lids were taken off each box. (Pot size 14cm x 14cm x 11cm)

CHAPTER ONE

THE ROLE OF TRANSLOCATABLE REGULATORY FACTORS IN INFLORESCENCE INITIATION, AS A RESPONSE TO LONG DAYS, IN CLONE C

Thomas (1962) found that some genotypes of Grasslands Huia white clover such as Clone C would initiate inflorescence on exposure to a treatment of short days followed by long days. However some genotypes such as Clone B did not flower in these conditions. This would suggest that the regulatory system of the two genotypes (Clone C and Clone B) differ in some way in their physiological responses to either short or long days.

To determine the nature of the differences in the physiology of the control of flowering between Clones B and C it is necessary to understand the control of flowering in each Clone. Therefore one of the first steps in determining the differences between Clones B and C is to develop a model of the control of flowering for each clone. The aim of this chapter is to develop an understanding of the regulation of inflorescence initiation and production in Clone C, in particular the role of the long day reaction which directly causes inflorescence initiation and to use this understanding to determine the causes of the inability of Clone B to flower in a short-long day treatment.

In developing a model of inflorescence initiation in Clone C, two things need to be taken into consideration: firstly, in warm conditions inflorescence initiation only takes place in long days, and secondly, inflorescence are produced in the axils of young leaf primordia near the apical summit. Several models can explain this, and are listed below:

LIST OF MODELS

1/ Tissue exposed to long days produces a factor which causes inflorescence initiation in the tip.

2/ On exposure to long days, light-sensitive tissue stops producing factors which block inflorescence formation at the tip. (Inhibitor production occurs in short days).

3/ The apex controls flowering through the production of inhibitors, promoters or both.

4/ The roots produce an inhibitor to flowering, until production is stopped by a leaf factor produced in long days.

5/ An inhibitor produced in the roots blocks flowering. Inhibitor production is controlled by the production of leaf factors in short days.

This list could be extended by models in which balances of inhibitors and promoters control flowering. For example, in short days the leaves could produce inhibitors, while in long days the leaves could produce promoters. Equally possible is a model in which the leaves and the apex control flowering.

TRANSLOCATABLE REGULATORY FACTORS AND INFLORESCENCE INITIATION IN CLONE C - EXPERIMENT ONE -

The purpose of this experiment was to test the hypothesis that either a translocatable floral stimulus or floral inhibitor controlled inflorescence initiation. There are three ways in which this could occur, 1/ translocatable factors produced in the leaves, in long days, stimulate inflorescence initiation, 2/ translocatable factors produced in short days block flowering and 3/ a balance between the floral stimulus and the inhibitors produced in short days control flowering. These hypotheses can be tested by exposing plants to treatment 3 in table 2 as the prediction of each hypothesis with respect to the level of flowering is different.

This experiment also tested the possibility that control of inflorescence initiation is localised in the stolon tip. This hypothesis predicts that flowering will only occur in stolon apices directly exposed to short then subsequent long days. Based on this prediction the hypothesis was tested by comparing the flowering of stolon apices in short days and associated leaves in reproductive conditions to the flowering of apices directly exposed to reproductive treatment.

TABLE 2

TABLE OF PREDICTIONS FROM HYPOTHESES

Hypothesis	Treatments		
	1. Whole plant in short days	2. Whole plant SD- LD	3. Leaves of plant SD- LD, apex in SD
	(FS)(I)(F)	(PS)(I)(F)	(FS)(I)(F)
1. Floral stimulus in long days. No floral stimulus SD. No inhibitor LD. or SD	- - -	+ - +	+ - +
2. Inhibitor short days. No inhibitor long days. No floral stimulus in SD or LD.	- + -	- - +	- + -
3. Floral stimulus in long days. Inhibitor in short days	- + -	+ - +	+ + +

F = flowering FS = floral stimulus
SD = short days LD = long days
I = inhibitor
SD- LD (plants transferred from short to long days)

- predicted absence of flowering, inhibitor or floral stimulus

+ predicted presence of flowering, inhibitor, floral stimulus

MATERIALS AND METHODS

A population of clone C plants was grown in the glasshouse during the winter. In September, when the stolons were long enough to handle (5cm or more), the plants were trimmed to single stolons, which were d defoliated to the youngest unfolded leaf. The plants were defoliated because an inverse relationship between leaf area and inflorescense production was found to occur after an intialy promotive effect of leaf area on flowering The youngest unfolded leaf was left on stolons because it was known to be the most responsive to long days,, (Thomas, personal communication).

Five treatments were devised :a short day control to check that the short day conditions were non inductive, and that inflorescence initiation had occurred prior to the experiment (1-1);two treatments where the tips were exposed either to short days (1-2.) or continuous dark (1-3), and the rest of the plant to continuous llight ; a long control to use as a stanard to the other treatments (1-4); and one treatment where the tips alone were given continuous light (1-5) .

TABLE 3 LIST OF TREATMENTS

Treatment Number	Tip	Leaf and Rest of Plant	No. of Plants	No. of Stolons
1-1	Short days (8 hour)	Short days (8 hour)	6	6
1-2	Short days	Continuous light	6	6
1-3	Dark	Continuous light	6	6
1-4	Continuous light	Continuous light	6	6
1-5	Continuous light	Short days	6	6

Plants were transferred from a 12 hour daylength to their various treatments. The experiment was terminated after ten days, and the plants dissected.

To evaluate the various treatments, the youngest unfolded leaf at the time of transfer of the plants from short days to long days was tagged. From this the number of leaves produced, and the node at which inflorescences first form was determined. This relies on the assumption that at tagging six nodes are present after the youngest unfolded leaf (see main Material and Methods). Therefore any leaves or nodes produced after the sixth leaf or node from the tag can be assumed to be produced during the experiment. The last leaf or node produced before the experimental treatment (sixth node or leaf from the tag) can serve as a reference point to determine the number of vegetative nodes produced before an inflorescence bearing node, and that inflorescence initiation did not take place before the transfer to long days.

At the end of the experiment the number of inflorescences per stolon was counted. The number of leaves produced after the tag (Yul) was also determined. This was done, by dissecting the stolons to the apical dome

RESULTS

In treatments where the youngest unfolded leaf, and the lower stolon tissue were exposed to continuous light (treatments 1-2, 1-3, 1-4), the number of leaves and inflorescences produced per stolon was similar, irrespective of the conditions in which the stolon tip (apical summit and leaf primordia) was growing. For example, the number of leaves and inflorescence produced in tips grown in continuous dark (5.00±0.07 leaves and 1.0±0 inflorescences per stolon) was similar to that of the continuous light control (5.00±0.07 leaves and 1.3±0.2 inflorescences per stolon) even though the tips were completely etiolated.

When the tip alone (leaf primordia and apical summit) was exposed to reproductive conditions (1-2), growth was more characteristic of the short day control (1-1), and the production of inflorescences per stolon lower than the control (treatment 1-5).

TABLE 4 TABLE OF RESULTS

Treatment Number	Treatment Tip-Base	Leaf Production	Node Number of 1st Inflorescence	Inflorescence Per Stolon	Vegetative Nodes Produced Before Inflorescenc
1-1	SD-SD	4.00±00	0	0	-
1-2	SD-CL	4.8±0.3	8.6±0.2	1.2±0.2	1.6±0.3
1-3	D-CL	5.00±0.0	8.8±0.3	1.0±0	1.8±0.3
1-4	CL-CL	5.00±0.0	8.3±0.8	1.3±0.2	1.3±0.3
1-5	CL-SD	3.8±0.5	8.7±0.9	0.5±0.2	2.0±0.6

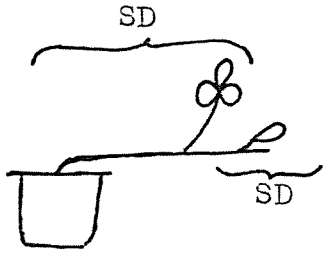
= 8 hours
D = dark
CL = 24 hours
error = ± standard error of the mean the number of plants and stolons per treatment equals 6
Node number of inflorescence = position from tag
Leaf production = Total leaves present from tag minus number of leaves previously produced.

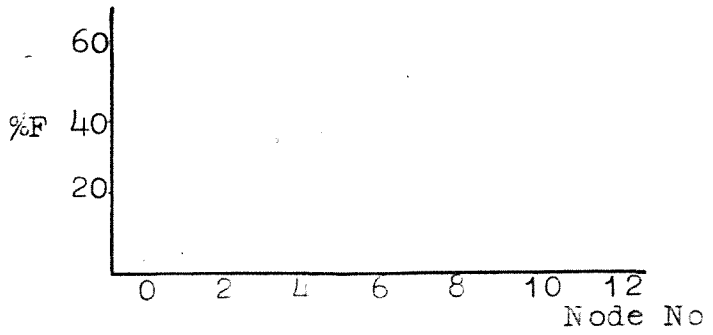
Figure 6 Patterns of Inflorescence Production (Experiment 1)

Diagrams of Treatments

Histogram of Results

1.1





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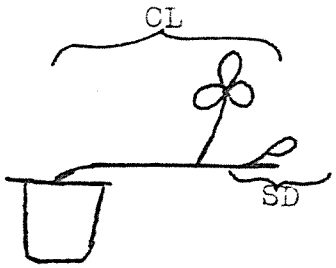

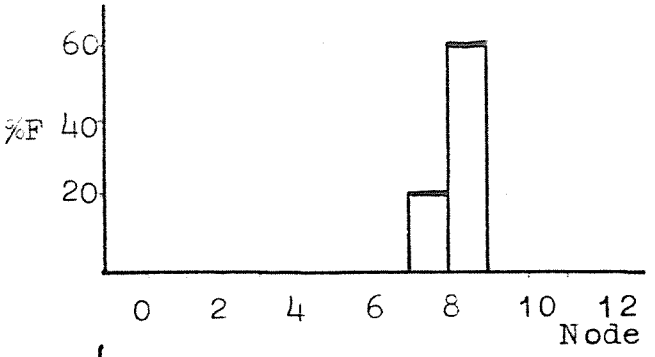

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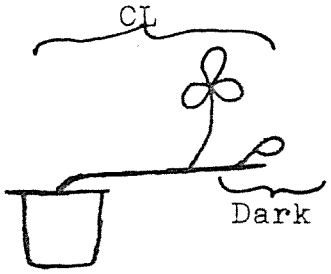

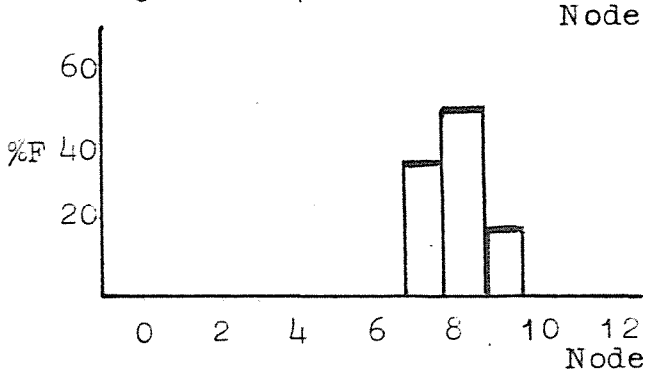

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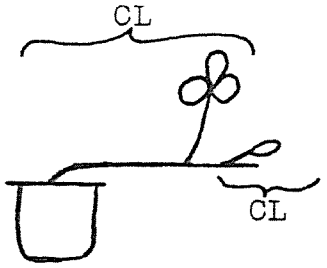

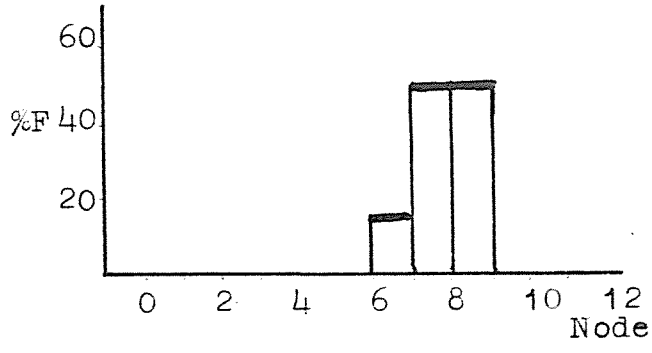

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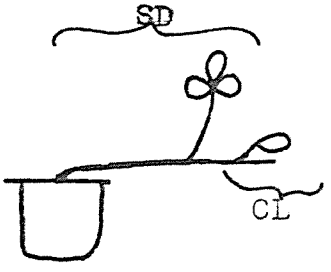


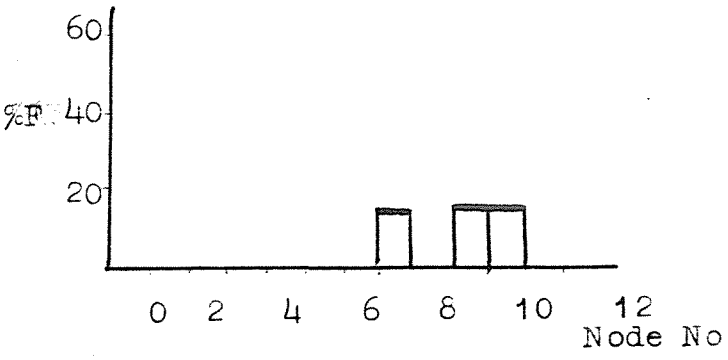


Figure continued

SD = short day.

CL = continuous light.

%F = % of stolons with an inflorescence at specific node within a treatment.

Node No = order of nodes along the stolon (0 = post of Yul) at the beginning of the experiment.

DISCUSSION OF RESULTS

The results of this experiment are consistent with the idea that inflorescence production is controlled by a transmissible promoter, as inflorescence initiation occurred in stolon apices in vegetative conditions that were connected to tissue in "inductive" photoperiods but not otherwise. There was no evidence of a transmissible inhibitor; if one existed, as flowering in treatment 1-5, where the stolon apex was exposed to continuous light in the presence of a mature leaf exposed to warm short days, would not have been expected. This is because if inflorescence initiation was controlled solely by levels of inhibitor produced by mature leaves in short days the presence of a source of inhibitor should block flowering unless the inhibitor was deactivated by continuous light. Deactivation would seem to be unlikely as some stolon apices in continuous light did not flower. The reduced level of flowering in treatment 1-5 supports the hypothesis that inhibitors may be produced by mature leaves in short days, so that an inhibitor-promoter balance might control flowering. However, the same result (reduced flowering compared to other flowering treatments) could have been due to the fact that in treatment 1-5 mature leaves were not exposed to continuous light.

Another hypothesis which was examined was that in short days an apical inhibitor to flowering existed, but as the photoperiod of the tip had little effect on flowering, this hypothesis would appear to be incorrect. Because these treatments were carried out in continuous

light, which causes the strongest flowering is Clone C (Thomas, 1981), the levels of promoter translocated to the tip may have overcome apical inhibitors, so that the hypothesis is not completely invalidated, as inhibitor may have been present

Thus one can conclude from this experiment that light sensitive tissue in continuous light, produces a transmissible promoter, but that apical inhibitors and transmissible inhibitors produced in short days could also control flowering.

THE INFLUENCE OF TISSUE EXPOSED TO SHORT DAYS, ON THE FLOWERING OF TISSUE EXPOSED TO LONG DAYS - EXPERIMENT 2

The results of experiment 1 indicated that a translocatable factor which stimulated inflorescence production was produced in long days. There were also indications that translocatable inhibitors produced in short days by either the roots or leaves could influence the flowering of tissue in long days, though this evidence was subject to other interpretations such as decreased promoter production. To examine the hypothesis that mature leaves in short days could inhibit the flowering of stolons exposed to long days, treatments in which stolons were exposed to both long days and short days were set up.

case of products from leaves exposed to continuous light it was possible that the donor activity of the leaf in short days could interrupt the flow of long day products, such as the promoter or floral stimulus, resulting in weaker flowering.

TABLE 8 TABLE OF TREATMENTS

Treatment Number	Treatment	Plants and stolons per treatment
2—1	Youngest unfolded leaf CL 4 leaves SD.	6
2—2	Youngest unfolded leaf CL. 0 leaves SD.	6
2—3	Tip plus Yul exposed to short days. Two leaves exposed to long days.	6
2—4	Tip minus Yul exposed to short days. Two leaves exposed to long days.	6

Plants were exposed to these conditions for 11 days and dissected. The results were obtained by determining the number of inflorescences per stolon, the number of nodes from the youngest unfolded leaf to the first inflorescence, and the number of nodes produced to theal and before the first inflorescence.

RESULTS

The presence of leaves exposed to short days distal to leaves in long days had little effect on the numbers of inflorescences initially formed, or on the node of first inflorescence initiation (Treatment 2-1), compared to the control (Treatment 2-2). However, the total number of inflorescences produced per stolon was lower in Treatment 2-1 than in Treatment 2-2 which lacked a distal short day leaf. From the histogram inflorescence initiation was reduced in the later node positions in Treatment 2-1. Growth was also slower in comparison to the control (Treatment 2-2).

When some mature leaves were exposed to short days and the youngest unfolded leaf and the stolon tip to long days (Treatment 2-3), inflorescence production and growth were slightly increased relative to the control treatment (2-4) which did not contain as many mature leaves, or any leaves in short days.

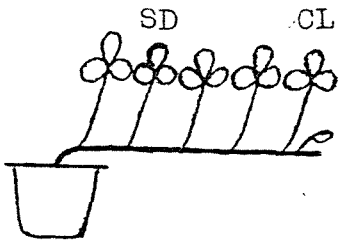
TABLE 6 TABLE OF RESULTS

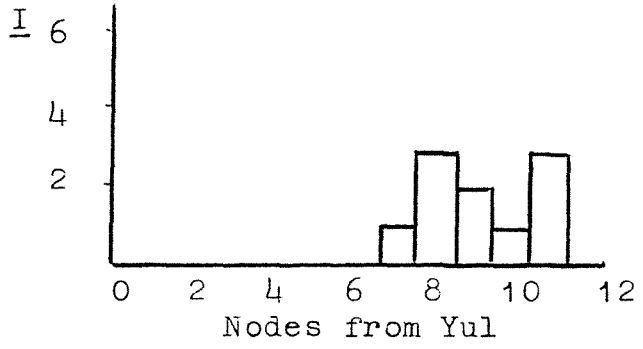
	Treatment Numbers			
	2.1	2.2	2.3	2.4
Nodes produced ± SEM	5.5±0.2	5.2±0.2	4.0±0	4.7±0.2
Inflorescence per stolon	2.0±0.3	1.5±0.2	1.5±0.2	2.3±0.2
Position of first inflorescence	8.0±0.3	8.0±0.4	7±0	7±0
Nodes before first inflorescence	1.0±0.4	1.0±0.3	0±0	0±0
Number of stolons	6	6	6	6

Figure 7 Histogram of inflorescences per treatment per node versus nodes from YUL

Diagrams of Treatments Histograms of Results

2.1





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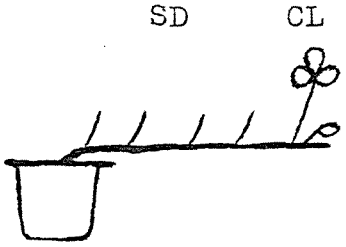

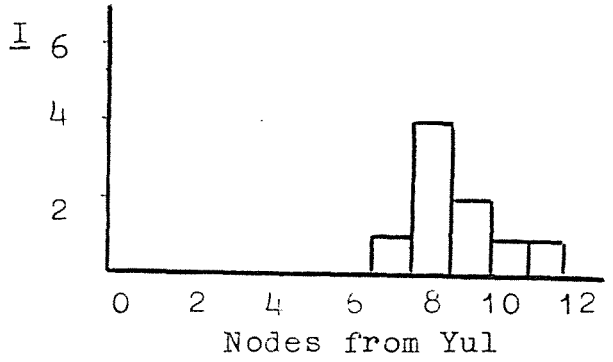

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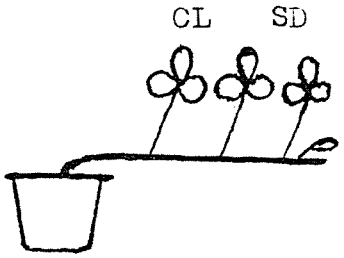

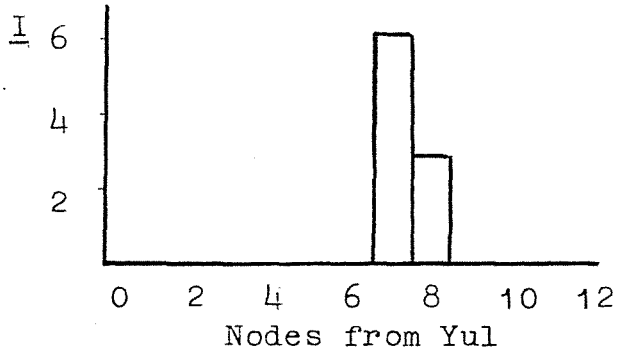

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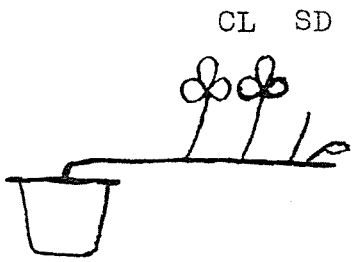
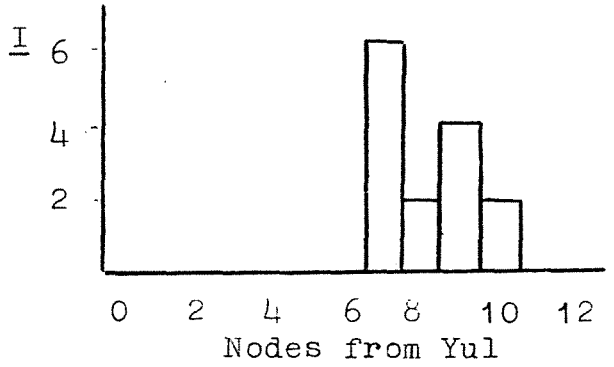




SD = short day treatment

CL = continuous light treatment

Node 7 = is the first node produced in the experiment

I = number of inflorescences

DISCUSSION OF RESULTS

The aim of this experiment was to test the hypothesis that factors produced by the leaves in short days inhibited flowering. Some evidence for this hypothesis is found by comparing treatment 2-3, in which the youngest unfolded leaf was kept in short days, and two older leaves in continuous light, to the control treatment 2-4. A mature leaf in short days apparently inhibited flowering. However, this inibition only seemed to occur after inflorescence initiation had occurred, when it would be expected that high levels of inhibitor would have delayed flowering relative to a control where a source of inhibitor was lacking (higher levels of promoter being needed to overcome inhibitor levels). If mature leaves in short days produce inhibitors it would have been expected that the inflorescence production in treatment 2-1 relative to that in 2-2 would have been decreased by the presence of leaves in short days. In fact the opposite occurred, slightly more inflorescences being produced, which indicates that the inhibitor hypothesis is incorrect, as even if the leaves in long days inhibited translocation of the inhibitor a promotive effect would have not been expected.

Miginiac (1976) postulated that the roots produced an inhibitor factor whose production was controlled by leaf factors. This system of control is unlikely to occur in white clover. For example, from this hypothesis the prediction would be made that a long day factor stops inhibitor production in the roots, yet in treatment 2-3 where leaves exposed to continuous light were the closest

leaves to the root system, inflorescence production was inhibited. Alternatively, in treatment 2-1 where leaves in short days would be expected to inhibit translocation of the long day factor, flowering was enhanced relative to the control.

Another hypothesis is that inhibitor production in the roots is stimulated by the presence of leaves in short days. In treatment 2-3, the translocation of the short day factor is likely to be inhibited by leaves in continuous light, therefore diminished translocation could explain why some but not total inhibition of flowering occurred in 2-3 relative to the control 2-4. However, in treatments 2-1 where short day factors would arrive in the roots, inhibition rather than the promotion which occurred would be expected.

These results would suggest that alternative explanations of the result of treatments 2-3 and 2-4 are required. One explanation is that the presence of leaves in short days reduces the translocation of promoting factors from the leaves in continuous light as the leaves in short days were interposed between the source of the promoter and the site of action. The diminished production of inflorescences with time can be explained on the basis that as the leaves in continuous light aged, promoter production declined. However, at the same time, the leaf in short days would have become more important as a source of carbohydrates for the shoot apex. Thus, promoter production would have declined and interference with promoter translocation increased as the experiment continued. The effect of this would have been to lower the levels of promoter in the apex near the end of the experiment.

TRANSLOCATION OF THE FLORAL STIMULUS BETWEEN STOLONS - EXPERIMENT 3

Both the previous experiments were concerned with the translocation of the floral stimulus from leaves to the stolon apex. In this experiment the movement of the floral stimulus or promoters between stolons was studied.

MATERIAL AND METHODS

Forty Clone C plants which had overwintered in the glasshouse were transferred to continuous light in the growth room in late October. Thus, before the experiment they received short day conditions from 8 to 12 hour long, followed by a daylength (14 hours), close to the critical daylength ($14\frac{1}{2}$ hours). Temperatures varied from 18^{o} to over 30^{o}C. The plants were trimmed to a main stolon and a major lateral stolon (usually the oldest), which were similar in length (20-30cm long). Four treatments were used, as listed below.

LIST OF TREATMENTS

Treatment Number	Main Stolon	Lateral Stolon	Number Plants
3-1	SD	SD	10
3-2	CL	CL	10
3-3	CL	SD	10
3-4	SD	CL	10

Treatment 3-1 was the short day control. Whole plants were exposed to eight hours, in order to make sure that inflorescence initiation did not occur before the experiment or in short days. The long day control treatment 3-2, was set up to make sure that both main stolons and laterals were capable of flowering. To test for translocation of the floral stimulus, in treatment 3-3 the main stolon was given continuous light and the lateral stolon an eight hour photoperiod. Treatment 3-4 the reverse of treatment 3-3. The lateral stolon was given continuous light and the main stolon eight hours.

Stolons in short days were defoliated except for the youngest unfolded leaf, and leaf primordia. Those in long day (continuous light) were defoliated except for two mature leaves, the youngest and next to youngest unfolded leaves.

Defoliation of stolons was continued throughout the treatment period but defoliation of stolons in continuous light ceased when newly formed leaves were smaller than usual.

The experiment was terminated after 13 days and the plants dissected.

Details on the propogation methods and photoperiodic treatments are given in the main "Material and Methods" section.

RESULTS

Two out of 18 stolons in short days produced one inflorescence each, but both inflorescences were too close to the youngest unfolded leaf to have been produced during the experimental treatments and so were excluded from the table.

Inflorescence production in stolons exposed to continuous light was extremely high, an inflorescence being produced at most new nodes (Table 8).

Both main and lateral stolons exposed to continuous light caused inflorescence initiation in stolons connected to them, that were growing in short days (treatment 3-3, 3-4). Main stolons in continuous light caused more laterals to flower than lateral stolons in continuous light caused main stolons to do so. However, main stolons in short days on average produced nodes more slowly than lateral stolons in short days. This may have affected the ability of the main stolon to respond to the floral stimulus, and limited the number of sites available for inflorescence production. Because none of the short day control stolons (treatment 3-1) produced inflorescences in the course of the experiment, and because inflorescence initiation occurred at nodes produced after the start of the experiment (the nodes at which inflorescences formed were more than five or six nodes away from the tag (see Main Material and Methods)), the inflorescences formed on stolons growing in short days but connected to stolons exposed to continuous light must have formed in the course of experimental treatment.

TABLE OF RESULTS

Treatment Number	Treatment(n)	Nodes Produced	Position of first Inflorescences	Inflorescences per Stolon	Production Inflores-cences over node production
3-2	Main stolon continuous light (7)	4.8± 3	6.7 0.2	3.3 0.3	0.69 0.6
	Lateral stolon (9) short days	3.9 0.3	8.2 0.3	1.0	0.26 0.01
3-1	Main stolon continuous light (7)	4.4 0.2	6.0 0.2	3.1 0.1	0.71 0.05
	Lateral stolon (8) continuous light	4.3 0.2	5.6 0.2	2.9 0.2	0.67 0.05
3-4	Lateral stolon short days (9)	2.9 0.3	-	-	-
	Main stolon (9) short days	3.3 0.3	-	-	-
3-3	Main stolon short days (8)	2.8 0.1	8.3 0.3 (3)	0.4 0.1	0.17 0.06
	Lateral stolon (8) continuous light	5.0 0	6.6 0.4	2.7 0.1	0.56 0.03

DISCUSSION OF RESULTS

The results of this experiment indicate that the transfer of the floral stimulus between lateral and main stolons occurs, as would have been predicted from results of experiments on other genera, and on studies using a simple stolon system.

One unusual feature of this experiment, compared to other experiments in continuous light (e.g. experiment 2), was the high number of inflorescences formed per stolon (up to four) and the lack of vegetative nodes between inflorescences. One possible cause of this may have been the greater number of leaves exposed to continuous light than in experiments 1 and 2.

GENERAL DISCUSSION

From the results of these three experiments it seems very likely that a factor produced in the leaves in long days causes evocation of inflorescences in the stolon tip (Experiments 1,2 and 3). Thus there are at least two possible areas of control, one located in tissue responsive to light (manufacture of the floral stimulus), and another located in the stolon tip. The response of Clone B to continuous light or long days (Thomas, 1962) may be related to processes either concerned with the production of the floral stimulus or to processes related to the response of the stolon tip to the floral stimulus or promoter. Clone B could differ from Clone C in both ways.

If the difference between Clone B and C is related to the production of a floral stimulus, or to the activity of the floral stimulus, inhibitory factors could block synthesis or activity of the floral stimulus. In Clone C there was no evidence of translocatable inhibitory factors produced by leaves in short days. In Clone B such factors could exist.

Clone C requires a pre-exposure of short days to flower in long days (Thomas, 1962). Thomas (1981), has suggested that the role of short days is to remove inhibitors which block the long day response. If this is so, then the lack of evidence for a translocatable inhibitor produced in short days is not surprising. The failure of Clone B to respond to continuous light by flowering may be due to the inactivity of the short day reactions. If these fail to stop inhibitor production, inhibitor levels in Clone B may be

high enough to inhibit the response to continuous light or long days. These inhibitors need not be translocatable, as they could be localised in the site of production and activity of the floral stimulus.

The actions of responses to short days need not be solely concerned with the removal of chemical inhibitors. Production of essential components for production of the floral stimulus and its activity may also occur.

CHAPTER 2

SHORT DAY PRETREATMENT AND THE CESSATION OF INFLORESCENCE PRODUCTION IN LONG DAYS

Laude, Stanford and Enloe (1958), and Thomas (1979), found that inflorescence initiation stopped in conditions initially favourable to flowering. In a 16h daylength in the growth cabinets, clone C stopped producing inflorescences (personal observation), yet plants given short days and transferred to this daylength produced inflorescences.

Short day pretreatments in certain conditions overcome the inability of clone C to produce inflorescences in long days. Thus the short day mechanism and the cessation of flowering in long days maybe related. For example depletion of cofactors or precursors could occur in long days and replenishment in short days.

Long days	→	Long days	→	Short days	→	Long days
flowering		essential components for flowering depleted		essential components produced		flowering

Alternatively a build up of inhibitors which block production or activity of the floral stimulus could occur in long days, followed by removal of the inhibitors in short days and flowering in later long day treatments.

Long days	→	Long days	→	Short days	→	Long days
flowering		inhibitor present flowering stops		no inhibitor no floral stimulus		floral stimulus flowering

The experimentsin this chapter were designed to find out the cause of the cessation of flowering in long days, and the action of short day pretreatments, with the intention of relating the results of the experiments to flowering in clone B. For example, if one action of short day treatments results in the production of precursor, in clone B the lack of precursor production could block floral stimulus production and flowering in long days.

Alternatively should short day treatment result in inhibitor depletion, short day treatments in clone B might be less effective so that higher levels of inhibitor might exist in clone B than in clone C.

Localisation of the Effect of Short Days and its Relationship to Long Day Processes

Experiment 4

Short days may affect one of two processes:

(1) Production of the floral stimulus

(2) The apical response to the floral stimulus

If short says affect flowering by enabling the production of the floral stimulus to occur in long days, they would be expected to act on the leaves, as this is the most probable site of production of the floral stimulus. However, if exposure to short days affects the sensitivity of the apex to the floral stimulus, the site of action of the short day pre-treatment is more likely to be the apex, although sensitivity of the apex could be affected by translocatable factors (precursor or inhibitor) produced in the leaves.

It is possible to localise and determine the effect of short days by exposing different parts of a plant (leaves and stolon tip) to either short days or long days, after flowering has ceased in long days and to compare the results with those predicted by various hypotheses, and with plants kept solely in long days. For example, if the action of short days is to cause the removal of inhibitory influences (inhibitors or the absence of precursors) from the stolon tip, which block the response of the apex to the floral stimulus, exposure of the stolon tip to short days as in treatment 4.3 Figure should enable it to respond to the floral stimulus from leaves in long days, and to flower. If short days act by influencing the production of the floral stimulus, exposure of the stolon tip alone to short days will have no effect on flowering.

Should the action of short days be to remove translocatable inhibitors, or to produce translocatable components essential for the activity or production of the floral stimulus, flowering should be enhanced by treatment 4.4, in Figure 8 , as inhibitor levels should be lower, or precursor - cofactor levels higher, relative to plants where leaves have not been transferred to short days.

Material and Methods

The aim of this experiment was to see how various treatments would affect flowering in plants which had ceased flowering in long days. Therefore plants which had been exposed to daylengths increasing from 10 to 16h, and on which two macroscopically visible inflorescences had emerged per stolon (near the maximum of three inflorescences for inflorescence production in natural 16h photoperiods) were selected. To ensure that inflorescence production would not cease because of poor root conditions, the plants were planted into larger pots (16×16×11cm). The temperature during inflorescence initiation (November) varied from 18°C at night to over 30°C at midday.

To examine the localisation of the effect of short days on flowering, the following treatments were set up (see Figure 8). There were three potsper treatment and from three to four stolons per pot.

(4.1) Short day control. Whole plants transferred from 16h to 8h for $8\frac{1}{2}$ weeks, then dissected.

(4.2) Long day control. Whole plants kept in natural decreasing long days for $8\frac{1}{2}$ weeks, to determine level of inhibition of flowering.

Pretreatment
SD → 16h

(4.3) Stolon tips and some mature leaves kept in natural long days. The rest of the plant transferred to 8h days. Treatment designed to test for translocatable factor whose production is controlled by short days.

(4.4) Stolon tip plus some leaves in 8h days. The rest of the plant kept in decreasing natural long days. Treatment designed to test the hypothesis that the effect of short days is localised in the stolon tip.

These treatments began on 16th December 1979 and ended on 15th February 1980. During the later stages of the experiment natural long days decreased from 15h to 14h.

One of the problems with these treatments was that the stolon tips produced new leaves and internodes, pushing the stolon tip further away from sources of inhibitory or promotive factors, and potentially decreasing transfer of inhibitors etc. To reduce the effect of leaves on the translocation of

regulators from lateral stolons and other leaves, it was necessary to defoliate the stolon tips partially. While it may have been better to defoliate the plants as the mature leaves unfolded, the stolon tips were not defoliated until three or four leaves had accumulated because constant defoliation had been observed to reduce tip elongation and leaf growth in other experiments, (e.g experiment 3)

Table 9

Table of Hypothesis Concerning the Relationship Between the Decline of Flowering in Long Days and Short Day Pretreatments.

List of Hypotheses

Hypothesis A Floral stimulus activity declines in long days. Short day pretreatments given to stolon apices recondition apices to respond to the floral stimulus.

Hypothesis B Inflorescence initiation declines in long days because the levels of translocatable cofactors or precursor produced in short days, are no longer sufficient for continued production or activity of the floral stimulus to occur in long days. Thus further short day conditions are required.

Hypothesis C Cessation of inflorescence initiation in long days is caused by an increase in the level of translocatable inhibitor, which follows increased floral stimulus production. Inhibitor levels decline in short days so a transitory flowering response occurs in following long day treatments.

Hypothesis D Short day pretreatments enable leaves which have stopped producing floral stimulus in long days to produce floral stimulus on re-exposure to long days by causing within the leaf, the removal of inhibitors or the production of precursors.

Note (1) A short day pretreatment is usually required before flowering will occur in long day conditions.

(2) Flowering starts and stops in long days.

Table 10 Table of Predicted Results with Respect to Treatment and Hypothesis

Hypothesis	Tip Leaves	4.1 SD→SD SD→SD	4.2 LD→LD LD→LD	4.3 LD→LD LD→SD	4.4 LD→SD LD→LD
Hypothesis A		0	0	0	+
Hypothesis B		0	0	+	+
Hypothesis C		0	0	+	0/+ *
Hypothesis D		0	0	0	0

SD = short days

LD = long days

tip = one mature leaf and apical bud

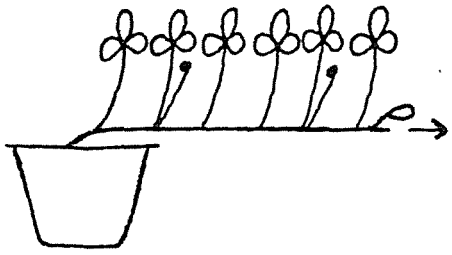
\+ = flowering continues/enhanced

0 = flowering stops

* If short days deactivate inhibitor and production of floral stimulus is still occurring flowering should continue.

Figure 8 Diagram of Experimental Treatments

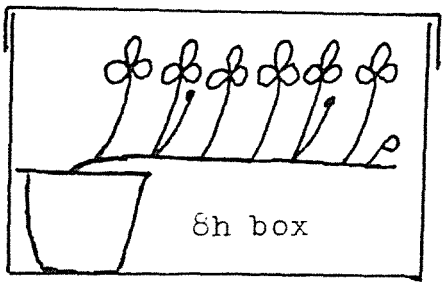
Pretreatments



Natural SD-LD's pretreatment stopped when two inflorescences visible

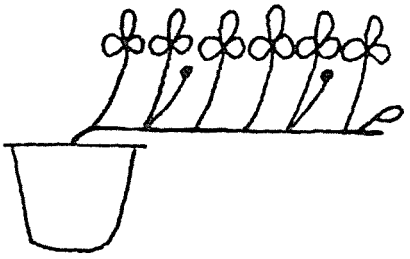
Treatment

4.1



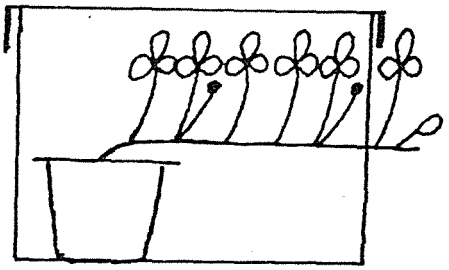
Short day control - whole plant transferred to 8h.

4.2



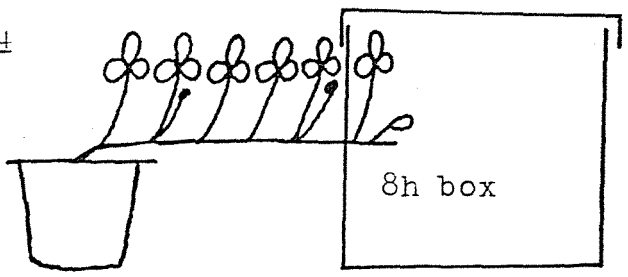
Long day control - whole plant kept in nature LD's.

4.3



Lateral stolons and most of the mature leaves 8h.
Stolon tip and young mature leaves natural LD's.

4.4



Lateral stolons and most of the mature leaves in natural long days.
Stolon tip and young mature leaves 8h.

Table 11 Table of Experimental Results

		Treatment Number			
		4.1	4.2	4.3	4.4
	Treatment	LD→SD LD→SD	LD→LD LD→LD	LD→SD LD→LD	LD→LD Ld→SD
1	Inflorescences/stolon during long day pretreatment	3.0±0.2	3.3±0.2	3.0±0 n=6	3.1±0.1 n=7
2	Inflorescences/stolon during treatment	0	1.1±0.4	1.0±0.2	1.5±0.3
3	Number of vegetative nodes formed after initiative of last inflorescence	13.0±0.6	8.4±0.7	7.7±0.3	9.7±0.8
4	Number of nodes produced between the inflorescences produced in the first long day treatment (node no=1), and the fourth inflorescence (inflorescence produced in the second long day treatment)	9±0 n=1	11±1 n=6	12.0±0.9 n=6	12.0±0.6 n=7
5	Number of plants	3	3	3	3
6	Number of stolons per plant used in experiment	8	10	9	10

Results

Inflorescence production had ceased in all stolons by the time of dissection, as shown by the third line in the table of results (table 41) where the number of vegetative nodes from the youngest node bearing an inflorescence to the youngest node produced by the stolon varied from seven to 13.

Stolons produced inflorescences during the long day pretreatment (line 1). Inflorescence production also occurred in experimental treatments where leaves exposed to long days were present irrespective of whether or not the apex was in short or long days. (Treatments 4.2, 4.3, 4.4 compared to treatment 4.1)

On average two to three vegetative nodes occurred between the beginning of inflorescence production in the pretreatment phase of the experiment and the treatment stage.

Two to three vegetative nodes also occurred between inflorescences produced during the long day pretreatment.

The number of inflorescences produced by stolons was unaffected within the range of experimental error by exposing the stolon tip (Treatment 4.4) or all the plant except the tip to short days (Treatment 4.3). The higher inflorescence production during the experiment stolon tips exposed to short days (1.5±0.3) was not significantly different from that in tips in long days (4.1±0.4 and 1.0±0.2).

Discussion of Results

The aim of this experiment was to test various hypotheses concerning the nature of the physiology of the cessation of flowering in reproductive photoperiods, and its relationship to the short day pretreatment. The hypotheses are listed in table 9 . Predictions based on these hypotheses with respect to the experimental treatments, are given in table 9 . Each hypothesis and its prediction as to the outcome of the experimental treatments are also discussed in the following paragraphs.

One hypothesis under test (Hypothesis A table 9) was that the apex eventually became unable to respond to the floral stimulus, and that this inhibition was removed by a short day pretreatment. If this hypothesis is correct then an apex transferred from long to short days should become capable of response to the floral stimulus, i.e flowering should restart continue of the floral stimulus is present. However in comparison to the control treatment 4.1, flowering was not enhanced when the stolon tip was transferred from long to short days (Treatment 4.4). It is possible that the short day pretreatment was too short to be effective. However, inflorescence initiation occurred within one to three nodes of the last node produced in the pretreatment. As inflorescence initiation would not have occurred if the apex was blocked from responding to the floral stimulus, it would seem likely that at the beginning of the experimental treatment a block to the activity of the floral stimulus was not present. If it later occurred, it was not stopped or weakened by keeping the apex in short days. This would suggest that hypothesis A is incorrect.

In hypothesis B (table 9), it was suggested that a translocatable precursor or cofactor produced in short days, declined in long days, which caused flowering to stop. If this is so, in any treatment in which the plants have leaves to produce (a) the floral stimulus (leaves in long days), and (b) precursor or cofactors (leaves in short days), stolons should continue to flower. Treatments 4.3 and 4.4, in which plants had leaves in both short or long days, did not result in significantly more inflorescences than the long day control (Treatment 4.2). Therefore the prediction of hypothesis does not agree with the experimental results.

It is possible that flowering ceases in long days because a increase in the level of translocatable inhibitors blocks the activity of the floral stimulus. As production of both the floral stimulus and the inhibitor occur in long days this would imply that a balance between the two controls flowering. Plants would intially flower in long days because the short

day pretreatment would cause a decline in the level of inhibitor. (Hypothesis C table 9).

To test this hypothesis, older leaves were transferred to short days (Treatment 4.3). If the action of short days is slow, high but declining levels of inhibitor could have occurred in the leaves transferred to short days. As a result it could appear that the transfer of leaves to short days is ineffective and that a translocatable inhibitor removed by short days does not exist. However, as the plants were exposed to short days for over five weeks, and as plants flower in long days in response to five week long, short day treatments, inhibitor levels in treatment 4.3 would be expected to drop causing flowering to restart. Because flowering was not significantly higher in treatment 4.3 than in the control treatment 4.4, the hypothesis that a balance between a translocatable inhibitor, (which acts at the apex, and which increases in long days and declines in short days), and the floral stimulus would appear to be incorrect.

The only hypothesis listed in table whose predictions are similar to the experimental results (no significant difference between treatments exposed to long days) would appear to be hypothesis D. If the results of long and short day actions are localised in the leaf, and are solely concerned with the production of the floral stimulus, short day treatments will have no effect unless they are given prior to a long day treatment. For example, a short day treatment given to the stolon tip, will not stop the build up of inhibitors or the depletion of precursor etc, in leaves treated with long days because in this case the exposure to short days is localised to the stolon tip, and not at the site of the long day effect.

The Cessation of Flowering in Long Days, and the Production of Translocatable Inhibitors

In experiment 4, localised simultaneous short and long day treatments were used to study the decline of flowering in long day conditions. A different approach was used in experiment 5, the effects of localised and simultaneous long day and reproductive treatments (SD-LD) being used to test various hypotheses. Similar experimental treatments were used in both experiments but those in experiment 4 which involves a short day treatment were extended by the addition of a following long day treatment in experiment 5.

Material and Methods

As this experiment was undertaken to test the hypothesis that clone C produced a translocatable inhibitor in long days, the treatments set out in figure 9 were performed. Before the experiment, plants were given the same treatments as those in experiment 4 (see experiment 4 for details).

The aim of each experimental treatment is outlined below.

Aims of Treatments

5.1 Treatment 5.1 was set up to make sure that the short day and long day treatments were effective when (a) the plant was given one photoperiod at a time and (b) the plants had previously flowered in long day treatments.

5.2 The aim of this treatment was to test for the transmission of long day inhibitors into the stolon apex and leaf tissue exposed to short then long day photoperiods.

5.3 There were two purposes to this treatment; (1) to see if an apex kept in long days would respond to the floral stimulus from leaves given short to long day treatments; (2) to examine the possibility that transfer of some leaves to short days from long days would increase flowering.

Because this experiment used plants taken from the same population as experiment 4, and because both experiments 4 and 5 were run at the same time and place, treatments 4.1 and 4.2 in experiment 4 were used as the short day and long day control respectively. The only difference between similar treatments in experiments 4 and 5 was that plants in experiment 5 were transferred to a $14\frac{1}{2}$ - $14\frac{3}{2}$h photoperiod on the 26th of January, whereas those in experiment 4 were left unaltered till dissection.

Diagram of Experimental Treatments

Figure 9

Experiment 5

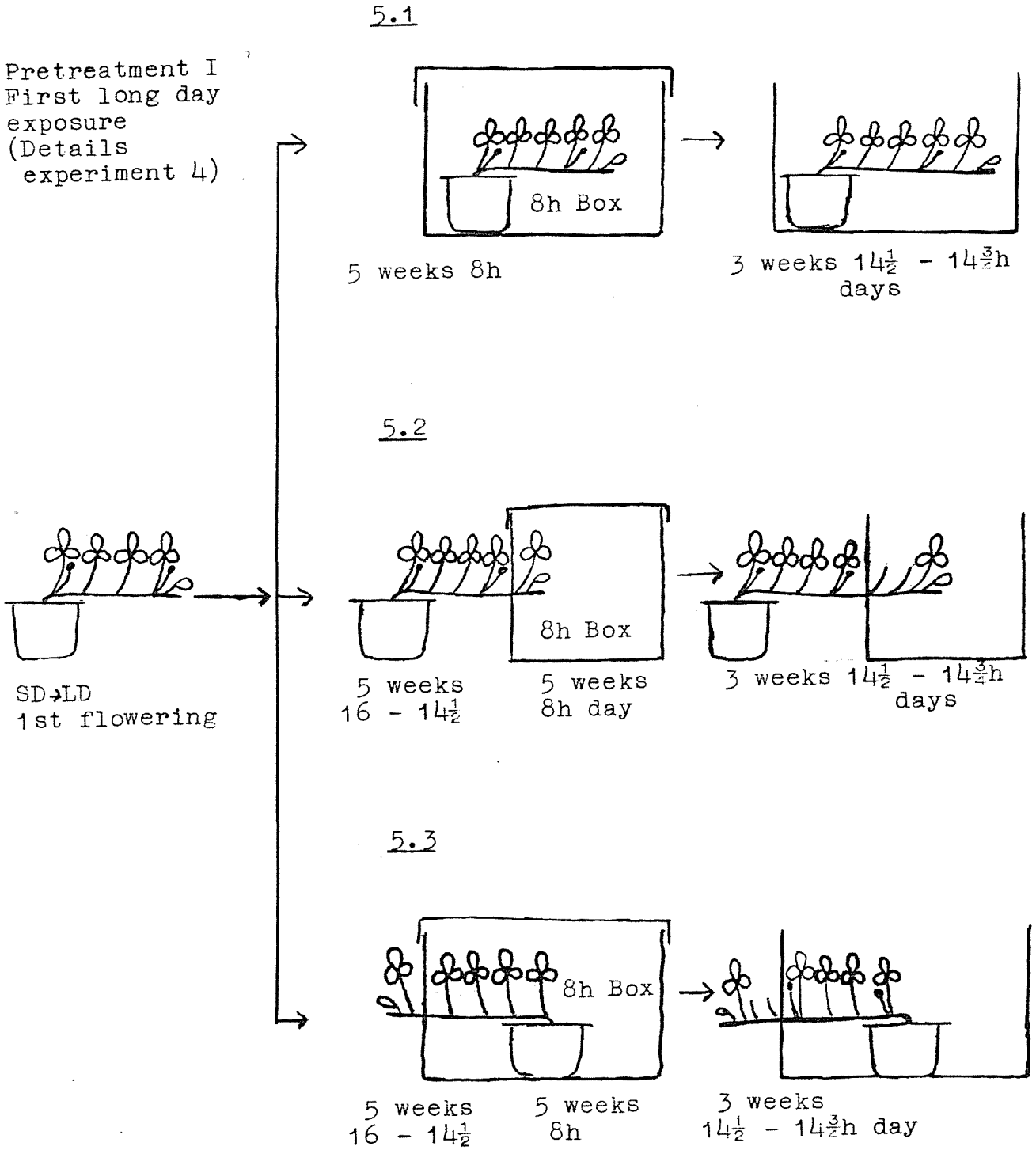


Table 12 Table of Results - Experiment 5

Treatment Number

Treatment Base / Tip	LD→LD→LD / LD→LD→LD	LD→SD→LD / LD→SD→LD	LD→LD→LD / LD→SD→LD	LD→LD→LD / LD→LD→LD
Inflorescences per stolon pretreatment I	3.0±0.2	3.0±0.3	3.0±0.3	3.0±0.1
Inflorescences per stolon pretreatment II	1.1±0.4	0	0.7±0.2	1.2±0.1
Mean position 4th inflorescence pretreatment II	11±1	0	12.1±0.4	12.4±0.2
Inflorescence per stolon treatment	0	1.2±0.2	0	0
Vegetative nodes to last leaf primordic	8.4±0.7	3.2±0.4	11.1±0.5	8.4±0.5
Node production during treatment	-	5.6±0.3	5.3±0.3	4.8±0.1
Number of plants	3	3	3	3
Number of stolons used per pot	-	4, 3, 4	4, 3, 4	4, 4, 3

Error = ± standard error of the mean

Results

Node production in stolons during the second long day exposure was similar in all experimental treatments. It was marginally slower in treatment 5.3 than in the others (5.1, 5.2).

Inflorescence initiation occurred in pretreatment I (first LD exposure). It also occurred in pretreatment II if some leaves were kept in long days (Treatments 5.2, 5.3) irrespective of whether or not the stolon apex was in short or long days, within the production of one to three nodes. However, inflorescence initiation eventually stopped, as can be seen from the table of results, as the number of vegetative nodes from the last inflorescence to the last node varied from 11.1±0.5 to 8.4±0.5.

After five weeks of short days, plants in treatment 5.1, lateral stolons in treatment 5.3, and the stolon tip plus a mature leaves, were exposed to long days ($14\frac{3}{4}$ - $14\frac{1}{2}$h). Inflorescence initiation occurred in the long day treatment in two cases.

(1) In main stolons of plants given the short day pretreatment, (Lateral stolons not dissected). Treatment 5.1.

(2) In lateral stolons which were pretreated with short days prior to the long day treatment, while the main stolons tip and mature leaves were kept in long days. (I/S = 0.7)

In the latter example the lateral stolons were not tagged. Thus it was impossible to determine growth, and the position of the first inflorescence with respect to the last node produced in the short day pretreatment. At the time of dissection three of the four inflorescences were present at the second to youngest node, and one at the sixth youngest node In comparison, the first inflorescences in treatments 5.1, which formed during the long day treatment, occurred at the sixth to fourth youngest internode (mean=5). So that it is possible that inflorescence initiation was delayed in the lateral stolons. This maybe due to the slower growth of plants in treatment 5.3 (node production of main stolons in comparison to plants in other treatments). Lateral stolon varied in length from 10 to 15 nodes. Therefore at the time of inflorescence initiation at least three fully expanded leaves would have been present on most of the dissected lateral stolons.

Inflorescence initiation did not occur during the long day treatment in the main stolons of plants in treatments 5.2 and 5.3. Despite the fact that in treatment 5.2 the main stolon tips and mature leaves were given the short day

pretreatment prior to the long day treatment.

Discussion of Results - Experiment 5

The inability of treatments which caused inflorescence production in treatment 5.1 (SD-LD) to do so in the presence of leaves continually exposed to long days (Treatment 5.2) indicates that some factor associated with long term exposure to long days (five weeks or more) has prevented the response of the plant to either short or long day processes causing flowering.

Thomas (1979), has suggested that in short days production of the inhibitor produced in long days ceases. If this is so, the effect of short days might have been negated by the transfer of inhibitor from other sites of production into the main stolon region exposed to short and long days (Treatment 5.2).

Inflorescence production in treatment 5.3 was not increased relative to the control treatment 4.2 (Table 12), by exposing most of the leaves to short days. Theoretically this would lower the levels of inhibitor and promote flowering. Furthermore some lateral stolon in treatment 5.3 flowered even though some leaves continuously exposed to long days were present on the main stolon to which they were attached, and even though the main stolons stopped flowering. This does not support the translocatable inhibitor hypothesis. Perhaps the inhibitory effect of long day leaves in treatment 5.2 was due to processes other than the production of inhibitors.

In treatment 5.3 both lateral stolons and mature leaves were exposed to a reproductive treatment (SD-LD), which should have caused the production of the floral stimulus. Inflorescence initiation in some laterals indicates that this occurred. In clone C the floral stimulus is translocatable (see experiments 1, 2 and 3). Yet the main stolon apices to which these laterals were attached did not flower. Two things could have happened to stop the main stolons from flowering:

(a) The apex was blocked from responding to the floral stimulus by inhibitors in the apex.

(b) Leaves in long days on the main stolon tip inhibited the translocation of the floral stimulus to the stolon apex.

However, given the fact that short days did not seem to affect the sensitivity of the main stolon tip to the floral stimulus, and that flowering was weak in the lateral stolons, it seems more likely that (b) is the correct interpretation.

The results of this experiment are difficult to interpret. Given this, confirmation of these results using longer daylengths, more plants, and lateral stolons instead of main stolons is needed.

General Discussion

From the results of experiment 4 it was concluded that the effect of short days was to remove an inhibitory effect such as the absence or presence of some factor from the leaves, enabling the production of the floral stimulus to occur. If this is related to the ability of short days to 'restart' flowering it could be concluded that short days result in the removal of an inhibition localised in the leaf which build up in long days after the production of the floral stimulus. However, the results of treatment 5.2 support the hypothesis that long days produce a translocatable inhibitor.

Treatments 5.3 and 4.4, in which all leaves except for one were moved from inhibitory long day conditions to short days, did not have higher levels of inflorescence production than similar plants kept in long days (4.1). Such treatments would be expected to promote flowering on the basis that short day treatments cause the depletion of translocatable inhibitors. As this did not occur, the results of treatments 5.3 and 4.4 do not support the hypothesis that short days remove translocatable inhibitors. Neither does the result that, while main stolon kept in long days remained vegetative, the lateral stolons given the reproductive treatment (SD→LD) flowered (Treatment 5.3). Therefore some contradiction exists; treatment 5.2 supporting the translocatable inhibitor hypothesis while others such as 5.3 and 4.4 conflict with the hypothesis.

To explain this contradiction, either new hypotheses are required, or factors such as sink effects have confounded the results. These are discussed in the following paragraphs.

One confounding effect may have been interference with the translocation of regulatory components by the activity of roots and leaves. If consideration is given to this effect, some hypotheses are no longer testable by the methods used in this experiment. For example, in treatments 4.4 and 5.3 where some leaves and stolons were transferred from long days to short days, the parts of the plants kept in short days could have acted as sinks for either root factors or long day factors associated with flowering. This could have negated the effect of transferring the sites of inhibitor production to conditions in which inhibitor production did not occur.

Alternatively treatment 5.2, which indicated a translocatable inhibitor, could also have been misleading. The inhibitory effect of basal leaves in long days on the flowering of stolon tips exposed to short days followed by long days, could have been due to sink activity of basal

leaves in long days. These leaves could have blocked translocation of essential root components or could have been sinks for the floral stimulus.

In both these examples, the results can be interpreted in terms of either a translocatable inhibitor or a localised one, depending upon whether or not the potential for impaired translocation or some other factor is considered.

Another hypothesis under consideration was that short days caused the production of translocatable factors such as 'vernalin' or 'anthesin', (Treatments 4.4 and 5.3). While there was no evidence of such factors, sink effects of leaves in long days may have confounded the result of these treatments too. For example, if the short day factors act in the stolon tip, leaves in long days in treatments 4.4 and 5.3 could have blocked translocation to the stolon tip. However, in treatments 4.3 and 5.2, where the stolon tip and a few mature leaves were kept in short days, and leaves capable of producing the floral stimulus wre present, translocation of the precursor or cofactor should have resulted in greater flowering. Here leaves in short days could have blocked transfer of the floral stimulus so that the presence of precursor etc had no effect. This can be dismissed, as inflorescence initiation occurred in stolon tips kept in short days while connected to basal leaves in long days, even in the presence of mature short day leaves. So that promotion should still have occurred if translocatable products such as precursor or cofactors of the floral stimulus were produced in short days. Thus, there is no evidence of translocatable precursors or cofactors, or sink affects on translocation which could alter the interpretations.

The most likely explanation of the need for short days and the cessation of flowering in photoperiods which initially cause flowering, is that long days have one of two inhibitory effects. Either:

(1) a leaf-localised effect such as depletion of non translocatable 'vernalin' or the build up of inhibitors;

(2) the production by leaves in long days of translocatable inhibitors follows the production of floral stimulus has occurred.

As has been shown, experiments designed to test these hypotheses gave contradictory results, and were subject to multiple interpretations because of the unknown effect of leaves on translocation. However, if the 'demonstrated' translocation of the floral stimulus in experiments 1, 2, 3 and 4 is a general phenomenon, despite the presence of mature

leaves between the sites of production and activity of the floral stimulus (4.3, 5.2), it is possible that the sink activity of mature leaves in minimal. Therefore other explanations are required to resolve the conflicts in interpretations.

One possibility is that in the near critical daylength ($14\frac{3}{4}$ to $14\frac{1}{2}$), of the experiment 5, early morning and late evening shade may have been sufficient to reduce the daylength in some glasshouse areas below $14\frac{1}{2}$h. So that stolons in treatment 5.2 may have failed to respond to the long day treatment because the long daylength in that part of the glasshouse was too low. Perhaps experiment 5 would be best repeated in a growth room where conditions are less variable.

Another possibility is that the production of, or the prescence of, very young inflorescence primordia inhibits further inflorescence production. This is based upon the observation that in treatments where inflorescences were produced at the beginning of the experiments either in response to direct long day exposure such as stolon tips in long days, or as a result of the translocation of the floral stimulus (stolon tips in short days, leaves in long days), further inflorescence initiation did not occur. This was irrespective of the experimental conditions. For example, five weeks of short days followed by three weeks of long days or the stolon in short days.

If this hypothesis is correct the reason for the lack of flowering in treatment 5.2 may have been that the short day treatment was too short for the time the last inflorescence was initiated to the beginning of the short day pretreatment. Treatment 4.1 would not be a adequate control because inflorescence initiation stopped sooner in treatment 4.1 than in 5.2.

There are some problems with the hypothesis that young inflorescences inhibit following inflorescence production. One is that inflorescence production would be expected to recommence once the inflorescences had aged, unless the process once started by inflorescences became independent of them. Another is that short days were required in plants which had not flowered, for example 'Grasslands Huia' white clover (Thomas 1961). Possibly some residual factor was still present from the parent plants which was still inhibitory making a short day pretreatment necessary. Inflorescences also form directly after each other in some conditions and cultivars which suggests that if inflorescences are inhibitory it is not merely a matter of inflorescence formation. Thomas, (1979), found that warm long days inhibited flowering if flowering had been initiated prior to the treatment but not if flowering had not been initiated, which supports the hypothesis that some processes associated with inflorescence initiation are inhibitory to flowering.

While in some ways the results of these experiments are inconclusive, they have shown that translocation of the floral stimulus occurs under natural conditions, and that the presence of translocatable 'vernalin' or anthesin type components is unlikely. Results tend to support the hypothesis that the inhibitory effect of long days and the effect of short days are localised in the leaf. However, to clarify the mechanisms of both the long day inhibitor and short day pretreatment it would be useful to examine the influence of leaves on translocation of regulatory factors, and the effect of young inflorescences on flowering. More plants, lateral stolons instead of main stolons and a more constant and uniform environment would improve the experiment.

CHAPTER 3

THE INFLUENCE OF THE LIGHT INTENSITY OF SHORT DAY PRETREATMENTS ON INFLORESCENCE PRODUCTION IN LONG DAYS

Earlier work with white clover (Experiments 4 chapter 2) indicated that the primary site of the effect of short days might be the leaf.

Zaleski (1964), has shown that light intensity affects the inductive strength of photoperiods, so that it was wondered if light intensity also influenced the effectiveness of the short day pretreatment.

This would also help to distinguish between the hypothesis either that short days remove inhibitory factors, or that short days produce essential components (e.g. precursor or cofactor) of the regulatory system. Breakdown of inhibitor might be expected to be a passive process, whereas the other might be more influenced by the level of irradiation.

Three experiments were performed. In the first (experiment 6) 8h short days of four different light intensities were followed by natural long days also of various light intensities, so that there were 16 different treatments, each differing in the combination of short day and long day light intensities.

The second experiment (experiment 7) used the same plants and directly followed on from the first, treatments with 8h short days of different light intensities (outside) being followed by exposure to a 23h photoperiod (200-300uEm^{-2} sec^{-1} PAR), in the growth room.

In the third experiment (experiment 8) growth cabinets were used so that short days of different light intensity and temperature, and long days of different duration, could be given to the plants.

The Effect of Varying Light Intensity Under Natural Conditions, on the Effectiveness of the Long and Short Day Treatments.

Experiment 6

This experiment was designed to test the hypothesis that the light intensity of both the short day pretreatment and long day treatment affected inflorescence production.

If the light intensity of the short day pretreatment influences the effectiveness of the long day treatment, one would expect a trend of either decreasing or increasing effectiveness of long days with light intensities given during the short day pretreatment.

In the same way, should the intensity of the long day treatment affect inflorescence initiation, a trend of greater or lesser effect on flowering should occur with decreasing or increasing light intensity.

Materials and Methods

Experiment 6

Clone C cuttings were prepared in the standard way and grown to small rooted plants. As this was done during late November and the first three weeks of December, and because the plants were kept in artificial short days (8h, glasshouse sunlight), the plants were exposed to high intensity, warm short days before the experiment.

On December the 24th, 48 plants were transferred to outside conditions, and 12 placed in each of four boxes (1.15 x 0.71 x 0.29 m). Each box was shaded differently to give a different light intensity.

At night the boxes were covered with opaque lids for 16h for the first five weeks of the experiment. Pots inside each box were shifted around weekly to minimize light intensity variations between plants.

At the end of January, all the plants were transferred to natural long days at four different light intensities, by leaving the lids off the boxes. From each short day intensity three plants were exposed to each of four different long day light intensities. The daylength of the long day treatment varied from nearly 15h at the beginning to nearly 14h at the end of the treatment. The experiment ended after three weeks, and the main stolons dissected. (3 to 6 stolons per plant).

Table 13 — Lists of Treatments - Experiment 6

Short day Treatment 8 hours	Long day Treatment (15 - 14 hours)	Treatment Number
Intensity 1 (1500 uE/m^2/sec PAR) * →	Intensity 1	(1 - 1)
	Intensity 2	(1 - 2)
	Intensity 3	(1 - 3)
	Intensity 4	(1 - 4)
$Intensity_2$ (810 uE/m^2/sec PAR) * →	Intensity 1	(2 - 1)
	Intensity 2	(2 - 2)
	Intensity 3	(2 - 3)
	Intensity 4	(2 - 4)
$Intensity_3$ (360 uE/m^2/sec PAR) * →	Intensity 1	(3 - 1)
	Intensity 2	(3 - 2)
	Intensity 3	(3 - 3)
	Intensity 4	(3 - 4)
$Intensity_4$ (129 uE/m^2/sec PAR) * →	Intensity 1	(4 - 1)
	Intensity 2	(4 - 2)
	Intensity 3	(4 - 3)
	Intensity 4	(4 - 4)

* Intensities of photosynthetically active radiation (P.A.R) 400 - 700nm were measured at midday on a clear day December 24th. (See Main Materials and Methods for further details)

Table 6 Tables of Results

Table 6a Inflorescences per stolon ± standard error of the mean

Short day light intensity	Long Day Light Intensity 1	2	3	4
1 (high P.A.R)	0.4 ± 0.1	0.9 ± 0.1	0.1 ± 0.1	0
2	0.4 ± 0.1	0.5 ± 0.2	0	*
3	0	0	0	*
4 (low P.A.R)	0	0	0	*

Table 6b Position of Inflorescences with Respect to First Node Produced in Long Days.

Short day light intensity	Long Day Light Intensity 1	2	3	4
1 (high P.A.R)	3.5 ± 0.5	3.7 ± 0.1	3.0 ± 0	0
2	3.1 ± 0.2	3.7 ± 0.3	0	*
3	0	0	0	*
4 (low P.A.R)	0	0	0	*

Table 6c Nodes Produced Per Stolon in Long Days ± Standard Error of Mean

Short day light intensity	Long Day Light Intensity 1	2	3	4
1 (high P.A.R)	6.7 ± 0.2	5.6 ± 0.2	5.4 ± 0.2	5.9 ± 0.7
2	5.1 ± 0.2	5.2 ± 0.2	5.7 ± 0.2	*
3	4.8 ± 0.2	5.2 ± 0.2	5.4 ± 0.2	*
4 (low P.A.R)	4.7 ± 0.2	5.6 ± 0.2	5.0 ± 0	*

Table 6d Number of Stolons Dissected.

Short day light intensity	Long Day Light Intensity 1	2	3	4
1 (high P.A.R)	14	17	7	6
2	17	6	9	
3	7	5	5	*
4 (low P.A.R)	6	7	4	*

* Plants not dissected.

Results of Experiment 6

Despite the fact that there were no major differences in growth during the long day treatments in the plants examined, plants given low intensity short days foiled to produce inflorescences in conditions where unshaded or lightly shaded plants did so. For example in treatments 4.1, 4.2 inflorescence initiationdid not occur, but in treatments 2.1, 2.2, where the long day conditions were the same, it did, while there are no data as to the rate of leaf production with respect short day light intensity, plants from intensities three and four were poorer in conditions (poor internode elongation and a high rate of leaf death apparent), than plants from the other treatments.

It was also apparent that the long day light intensity was important to flowering as not all the light intensities of long day treatment were effective: for example, plants given natural short days did not produce inflorescences in the lowest and next to lowest long day light intensities (Treatments 1.4, 1.3).

Conditions for inflorescence initiation were poor, as despite the number or sites potentially available for inflorescence initiation one, once two, (generally none) sites per stolon, were used for inflorescence production in even the best of treatments.

Discussion of Results

These results show that in conditions where the natural photoperiod is just above the criticial daylength ($14\frac{1}{2}$ hrs), the intensities of the short day pretreatment can affect inflorescence initiation in long days. This would suggest that some factor produced in short days is necessary for flowering to occur in long days. This factor need not be a precursor. It could be a reserve of energy or a product which deactivates inhibitors.

There is some evidence that light intensities given during the long day treatment affect the effect of the short day light intensities. For example, although the level of inflorescence initiation in treatment 1.3 was very low (one untagged stolon out of five produced on inflorescence) no inflorescence initiation occurred in treatment 2.3. Give the variation in stolon response this needs confirmation.

Inflorescence Production in Growth Room Conditions After Various Short Day Intensities in Natural Conditions.

Experiment 7

Introduction

Because of the low number of inflorescences per stolon produced in even the best of the short day and long day light intensity combinations in Experiment 6, (Treatments 2.1, 1.1), plants from this experiment were reused in a further experiment to test the hypothesis that the light intensity of the short day pretreatment would affect the production of inflorescences in long days. However, in this experiment the photoperiod of the long day Treatment (23hrs) was not limiting for inflorescence initiation, as it possibly was in the previous one.

Material and Methods

In this experiment the plants used in experiment 6 were reused. This meant that individual sets of plants received short and long day intensities of different strength prior to re-exposure to short day pretreatments in experiment 7. Fourteen treatments were generated.

Table of Treatments

In the table below the first number refers to the light intensity during the first short day pretreatment (experiment 6). The second number indicates the light intensity of the long day treatment in experiment 6 and the third number shows the final short day pretreatment. (Intensities expressed as an approximate percentage of the unshaded value because natural light intensities vary during the day).

100	→	100	→	100	50	→	100	→	50	24	→	100	→	9	9	→	100	→	24
100	→	50	→	100	50	→	50	→	50	24	→	50	→	9	9	→	50	→	24
100	→	24	→	100	50	→	24	→	50	24	→	24	→	9	9	→	9	→	24
					50	→	9	→	50	24	→	9	→	24					

To allow for differences in light intensity at different positions within boxes: intensities were measured at various positions and an average obtained for each box.

Light Intensity Within Boxes ($uE/m^2/sec^{-1}$ P.A.R)

	(a)		(b)	
Box 1	670 ± 40	(100%)	93 ± 7	(100%)
2	310 ± 15	(46%)	50 ± 2	(54%)
3	147 ± 2	(22%)	24 ± 2	(26%)
4	57 ± 7	(8%)	9 ± 1	(9.4%)

Reading Taken When:

(a) Sky overcast and (b) Boxes in shadow of glasshouse

The third period of light intensity treatment consisted of an eight hour day given outside for five weeks from the beginning of March. Before this experimental period plants received a daylength of 14 to 14½ hours which was probably effectively shorter in the more heavily shaded boxes.

The maximum temperature during the final short day pretreatment varied from 26.8 to 14.7°C, while the air or grass minimum temperature varied from 18.5°C to 11°C.

(Meteorological Records Massey University Library).

To overcome the possibility of death or very poor growth, plants which had been given the lowest light intensity in the first short day pretreatment were returned to the next lowest light (intensity three). Plants given intensity three (24%) were transferred to intensity four.

As there may have been some leaf 'memory' of the previous light intensity, the plants were defoliated up to and including the youngest unfolded leaf. This also facilitated determination of leaf production in the short day treatment by counting the number of leaves appearing during the treatment.

After five weeks of short days all plants were transferred to a cabinet in the growthrooms and exposed to 23h photoperiods at 200 to 250uE/m^2/sec P.A.R at 25^{o}C for three weeks.

The youngest unfolded leaf at the beginning of the long day treatment was tagged and the position of the first inflorescence was determined relative to this. Plants were defoliated to the youngest unfolded leaf at the beginning of the long day treatment.

Table 16 Table of Results Experiment 7

Final short day light intensity *	100%	50%	24%	9%
Inflorescence per stolon * (Treatments bulked).	2.3 ± 0.2 n ± 132	2.4 ± 0.1 n ± 131	2.4 ± 0.1 n ± 19	3.0 ± 0.2 n ± 17

* Intensities expressed as a percentage of the light intensity in the unshaded treatments. Peak light intensities in boxes exposed to full sunlight varied from 1200 to 1500 $uE/m^2/sec^{-1}$, light intensities in other conditions are shown in table in the Material and Methods.

* Results bulked as flowering was similar in treatments irrespective of pretreatment, for example in treatment 100 - 50 - 100 there were 2.7 ± 0.2 inflorescences per stolon, and in treatment 100 - 24 - 100 2.3 ± 0.2.

Results

The intensity of the short day pretreatment appeared to have little or no effect on the number of inflorescences produced per stolon. Even plants that had only been exposed to light intensities 9% or 24% of full sunlight strongly initiated inflorescences.

Discussion of Results - Experiment 7

In this experiment the light intensity of the short day reaction did not limit the production of inflorescences in long days. This would suggest that the hypothesis that light intensity of short day pretreatments can limit inflorescence production caused by long days, is not always correct.

How the hypothesis could be supported in one set of conditions (experiment 6), and not in another (experiment 7) is a matter of conjecture, as the conditions were not strictly comparable. For example, the minimum temperatures in experiment 6 were slightly lower than those in experiment 7 (the range of minimum temperatures 18.5°C to 11°C compared to 19.1 to 11.7°C) though the average temperatures for both treatments were similar (16.8 January 17.1 March). Also, different sources of light were used in the long day treatments: natural light was used in one to fluorescent light in the other. If neither of these differences mattered, the most likely cause is the difference in the long day photoperiod.

Growth Room Studies of the Effectiveness of Short Days with Respect to Light Intensity, Temperature and Daylength of the Long Photoperiod

Experiment 8.

To clarify the role of light intensity in the short day reaction, the effect of different light intensities on short day activity was re-examined using growth chambers for both the long day and short day treatments.

The hypothesis under examination was that the light intensity of the short day pretreatment linked inflorescence production in long days, but that cool temperatures during the short day reaction, or longer photoperiods in the long day treatment could overcome this effect.

This hypothesis predicts that in some situations the light intensity of the short day reaction will limit inflorescence production, but that this can be overcome by using cool short days or large photoperiods (e.g. 23h compared to 16h).

Material and Method

Plants were grown from cuttings in the usual way, during September and October receiving high day temperatures (maxima from 25 to 33°C), warm night temperatures (19 to 15°C), and 13 to 14h days. Cuttings were exposed to shorter days and cooler temperatures while still attached to parent plants which had over wintered in the glasshouse.

In the last week of October (14h day) the youngest unfolded leaves were tagged and transferred to growth chambers in the plant growth unit. There sets of plants were given one of three treatments; a low intensity 111 ± 3 uE/m^2/sec 20°C 10h day, a high intensity(338 ± 14 uE/m^2/sec 20°C 10h day, or a low intensity 100 ± uE/m^2/sec 10°C 10h day). These treatments were given for five weeks. The youngest unfolded leaf on a stolon was then tagged. After three weeks of long day treatments the experiment was terminated and the plants dissected.

Treatments (Nine plants were used per treatment)

Short day treatment (10hrs, five weeks)	Long day treatment
10°C 101 ± 1 (n=10) uE/m^2/sec P.A.R (low intensity)	16hrs, 20°C 338 ± 14 uE/m^2/sec (n=10)
20°C 111 ± 3 (n=23) uE/m^2/sec^{-1}	16hrs etc 23hrs
20°C 338 ± 14 (n=10) uE/m^2/sec^{-1}	16hrs etc

All light intensities P.A.R

Table of Results - Experiment 8

Treatment Number	Treatment	No. of Inflorescences per stolon	Position of first inflorescence (no. of nodes after tagged leaf)
8.1	High intensity warm short days to 16 hours	3.0 ± 0	7.0 ± 0.2
8.2	Low intensity warm short days to 16 hours	3.4 ± 0.2	6.6 ± 0.2
8.3	Low intensity cold short days to 16 hours	3.13 ± 0.08	7.3 ± 0.2
8.4	Low intensity warm short days to 23 hours	5.0 ± 0.2	6.8 ± 0.3

Results

While the short day pretreatments 8.1, and 8.3 did not impair the condition of the plants the low intensity warm short day pretreatment, (treatment 8.2) resulted in sickly looking plants, in which little elongation of internodes or production of lateral stolons occurred. The apparently high death rate of leaves which occurred apart from affecting growth also meant that tags on youngest unfolded leaves at the beginning of the experiment became lost and detached. The adverse conditions did not stop inflorescence production which was similar to that of the control treatment (8.1). The cool temperature, low intensity short day pretreatment (8.3) did not produce a significantly different number of inflorescences per stolon to either treatment 8.2, (a warm low intensity short day pretreatment) or to treatment 8.1 (high intensity short day pretreatment).

Discussion of Results

As inflorescence production was unaffected by the light intensity of the short day pretreatment, it would appear that in the conditions of this experiment light intensity did not limit the activity of the short day reaction, nor did cool temperature enhance it. This suggests that the hypothesis that the light intensity of the short day pretreatment limits flowering is incorrect.

However, one possible reason for this, is that the plants used in this experiment received short days before experimental treatments began and this may have been sufficient to allow flowering to occur even after long periods of low intensity light treatments and regardless of the conditions received during the short day pretreatments. Because of this it could be advisable to repeat this experiment using plants that have flowered in long days, and to transfer them directly from long days to the various combination of short day light intensities, temperatures and photoperiods, and then back to long days.

Plants in experiment 6 were also exposed to a period of short day treatment before the main short day treatment. As this did not enable plants to respond to long day treatments the thoughts expressed in the previous paragraph may not be completely valid, and the intepretation that the hypothesis that short day intensity limits flowering in long days is invalid may still be correct.

General Discussion

It is difficult to know from experiment 6 whether or not the effect of shading during the short day pretreatment was due to the actual light intensity, to decreased daylength, or to a combination of both decreased daylength and light intensity. Whatever the cause, in near critical daylengths for the production of inflorescences by long days, some aspects of short day treatment can limit inflorescence initiation.

If the short day reaction involves a passive breakdown of inhibitors which either block production or activity of the floral stimulus, light intensity would not be expected to be a limiting factor or to have any effect, as the process would occur anyway. Infact if inhibitor production results from light requiring reactions, lower light intensities would be expected to be better than higher light intensities in promoting inflorescence initiation in long days.

Should the short day reaction result in the production of factors which either decrease inhibitor activity or are essential components of the system causing inflorescence production (e.g. precursors), low light intensity or daylength would be expected to be limiting in treatments such as low intensity very short days.

Experiments 7 and 8 showed, however, that low light intensity (111 ± 3 $uE/m^2/sec$) did not limit flowering in 16h photoperiods (300 - 350 $uE/m^2/sec$) or in 23h photoperiods (200 - 300 $uE/m^2/sec$). This was so even in situations in which, judging by the appearance of plants conditions were marginal for plant survival. Thus it seems that the long day conditions, viz photoperiod and light intensity determine whether or not the short day reaction is limited by light intensity. The hypothesis that short days result in the production of some factor which either removes inhibitors or cuts as a precursor etc for the floral stimulus does not fully explain this result. Neither does the alternative hypothesis that during short days (probably in the dark) an inhibitor passively breakdown. The reason being that in either hypothesis if a short day treatment has been such that either inhibitor levels drop or precursor levels rise and are sufficient to allow flowering in one long day treatment, they should also be sufficient for other long day conditions. (The block on long day processes has been removed).

One hypothesis which explains the results that a short day pretreatment maybe effective for one long day treatment (16h/23h) but not for another (natural long day etc) is the hypothesis that a balance between promoters and inhibitors controls inflorescence initiation (Thomas 1979).

In a situation where a balance controls flowering the effect of one component on flowering e.g. an inhibitor or a promoter will depend on the relative 'pool' size of the other component. The same applies to processes which control the levels of the balance components. For example, as it seems likely that daylength controls promoter production, the effect of a long day treatment on flowering will be determined by the relative amount of promoter produced in that daylength to the amount of inhibitor present. In a similar manner the 'apparent' effectiveness of a short day pretreatment is also related to the ratio of promoter to inhibitor as it is thought that the promotive effect of a short day pretreatment is due to a decrease in inhibitor levels (an active process). Accordingly, the relative amount of promoter produced in a long day treatment to the amount of inhibitor remaining after a short day pretreatment will determine whether or not a plant will flower.

In terms of the inhibitor - promoter hypothesis the difference between the low intensity treatments of experiment 6 and experiments 7 and 8 where inhibitor levels were probably similar might have been due to the higher levels of promoter relative to inhibitor in the latter experiments.

If a shorter LD photoperiod had been used in experiments 7 and 8, results similar to experiment 6 may have been obtained. For this reason, and because there is evidence that cool temperatures and short days both influence the level of inhibitor (Thomas 1981 and chapter 4), it maybe profitable to repeat experiment 8 using a wider range of light intensity, daylength and temperature combinations.

CHAPTER 4

THE EFFECT OF COOL TEMPERATURE ON THE PHYSIOLOGY OF FLOWERING IN CLONE B

Thomas (1962), and others such as Britten (1960), have found that cool temperatures (8 to 12°C) will cause white clover to flower indepently of daylength. (Direct Cool Temperature Pronotion of Flowering /Lang 1965/).

Therefore cool temperatures ultimately cause the same thing as photoperiodic treatments, (flowering), and also enhance the photoperiodic mechanism. This suggests that the photoperiodic mechanism (SD → LD) and the effect of cool temperatures are related.

The aim of this chapter is to develop understanding of some of the mechanisms involved in the effect of cool temperature, and to relate this to the inability of clone B to flower regularly after warm photoperiodic treatments such as transfer from short to long days.

This involved a study of inflorescence initiation and production caused by cool temperature clone, or in combination with continuous light. In particular the possibility of translocatable inhibitors or promoters of flowering was studied.

Indirect Promotion of Flowering in Clone B in Long Days by Cool Temperatures.

Introduction

Experiment 9

While clone B does not usually flower in long days (16h), on several occasions plants have initiated one or two inflorescences on transfer from the glasshouse to long days, (18h or continuous light). In other cases clone B plants which over wintered in the glasshouse sometimes produced inflorescences while still in the glasshouse, (12 - 14h daylength). This was especially noficeable when the minimum temperature of the glasshouse was lowered from 18 to 20°C to 15°C.

It was suspected from this that in some cases of inflorescence production a cool temperature pretreatment enabled clone B to produce inflorescences in response to long days.

To test this hypothesis clone B plants were given warm or cool pretreatments, and transferred to long days.

Material and Methods

Lateral stolons with root systems were detached from parebt plants that had overwintered in the glasshouse and immediately potted up. This was done in November. As the plants were kept in the glasshouse to December 14th, they were exposed to warm temperatures varying from 18°C to 30°C, and daylengths form 14 to 15½hrs. On previous occasions plants which had been exposed to cool short days followed natural warm long days in November did not flower on transfer to continuous light. Thus it seemed likely in these cases the effect of any cool pretreatment that the plants received as cuttings etc was negated by the temperature and daylengths the plants received before the experimental treatments.

Prior to the experiment, plants were defoliated to the youngest unfolded leaf. They were then transferred to growth cabinets in the Plant Growth Unit. This occurred on December 14th 1979.

One growth chamber was set at 10°C, the other at 20°C. The daylength and light intensity in both cabinets were 10hrs and 300 uE/m^2/sec respectively. After two weeks of these treatments, plants from the chambers were transferred to the deportmental growth room for exposure to long day treatments of 16 or 23hrs. Plants from the glasshouse were also transferred from natural long day to either a 16 or 24h photoperiod.

		2 Weeks	
		10° SD	16 hours
			23 hours
Glasshouse long days and warm temperatures	→	20° SD	16 hours
			23 hours
		Glasshouse long days up to 16 hours	16 hours
			23 hours

The experiment was terminated when inflorescences were visible, which was approximately three weeks after transfer.

Table 18 Table of Results Experiment 9

Treatments	Mean Number of Nodes produced in short days	Mean Number of Nodes produced in long days	Mean Inflorescences per stolon
Warm short days to 23h	3.0 ± 0.2	9.9 ± 0.3	0.3 ± 0.1
Cool short days to 23h	2.0 ± 0	10.2 ± 0.4	0.9 ± 0.2
Warm short days to 16h	3.0 ± 0.2	9.1 ± 0.6	0
Cold short days to 16h	2.0 ± 0	6.9 ± 0.5	0
Long days to 16h	-	-	0
Long days to 23h	-	-	0

	% Reproductive stolons	Inflorescence/flowering stolon
Warm short days to 23h	30%	1.0 ± 0
Cold short days to 23h	58%	1.4 ± 0.1

Experimental means expressed as ± standard error of the mean.

Results of Experiment 9

A cool short day pretreatment followed by 23h photoperiod was the most effective treatment, as more inflorescences, up to two per stolon were produced, and more stolons produced inflorescences. Warm short days followed by 23h resulted in some inflorescence initiation. The other treatments, warm long days preceding either 16 or 23h photoperiods, or cold or warm short days preceding a 16h photoperiod did not result in inflorescence initiation.

Cold short days ($10^{o}C$, 10hrs) eventually resulted directly inflorescence formation: some stolons of plants set aside for another experiment at the same as plants in this experiment, and dissected after six weeks had just initiated inflorescences. After seven weeks well-developed inflorescence primordia (inflorescences with floret primordia) were present on most stolons. No inflorescence initiation occurred during the two weeks pretreatment at $10^{o}C$ in this experiment, however.

Discussion of Results

It is apparent from these results that clone B can behave as a short-long day plant but that daylength required to initiate inflorescences in long days (critical daylength) is long, as plants flowered in 23hrs but not in 16, regardless of the temperature of the pretreatment.

The marginal response of clone B to near continuous light after warm short days indicates that the differences between clones B and C maybe only quantitative in either the short day or long day effect.

Cool temperature pretreatment clearly enhances the sensitivity of the regulatory system to photoperiod as more inflorescences are produced in long days after a cool treatment.

There are several eays in which cool temperatures could affect flower initiation in white clover.

Cool temperatures could cause the production of the floral stimulus through the production of precursors or cofactors

(i) $A \xrightarrow[CT/SD]{\text{Reaction 1}} B \xrightarrow[LD]{\text{Reaction 2}} f.s \xrightarrow{\text{Reaction 3}} Fl$

Cool temperatures could result in inhibitor breakdown which allows production or activity of the floral stimulus.

(ii) Reaction one $I \xrightarrow[CT/SD]{} x$ (inhibitor levels drop)

Reaction two $B \xrightarrow[\substack{CT/SD \\ \text{No inhibitor}}]{} f.s \xrightarrow[CT/LD]{} Fl$

Ratio of Pr to I or x (Pr/I (x)) controls flowering by affecting (a) Production of floral stimulus (?)
(b) Composition of floral stimulus (?)
(c) Response of apex to f.s (?)

(iii) Reaction one $I \xrightarrow[CT/SD]{} x$

Reaction two $B \xrightarrow[CT/LD]{} Pr$

f.s = floral stimulus SD = short days LD = long days
Pr = promoter I = inhibitor CT = cool temperatures
A and B precursor of f.s
x = decomposition product of inhibitor

The flowering of clone B in 23h photoperiods after a pretreatment of 10°C 10h days shows that the cool short day pretreatment was effective. Thus in terms of hypothesis where inhibitor levels are increased and/or precursor levels of the floral stimulus increased, the reactions proceeded, enabling flowering to occur.

However, despite the effectiveness of the short day pretreatment, flowering did not occur in the 16h photoperiod, which suggests that the 16h photoperiod limited flowering. Perhaps reaction two in hypothesis i and ii only occurs in long days greater than 23h. This is unsupported by the observation that clone B sometimes flowers in the glasshouse (daylength maximum 16h) and in both high intensity (300-700 $uE/m^2/sec$) and low intensity (200 $uE/m^2/sec^{-1}$) 18h photoperiods. The fact that clone B also flowers in short days if exposed to four to six week periods of cool temperatures suggests that with longer periods of cool temperature clone B will flower in progressively shorter daylengths. However this is in need of testing, thus it seems unlikely that flowering did not occur in the 16h photoperiods simply because reaction failed to occur.

The balance hypothesis put forward by Thomas (1981/1979), could also be applied to the effect of cool temperatures on flowering in white clover, (Hypothesis iii). If 16h daylengths do not produce pr as effectively as 23h, the proportion of inhibitor to promoter may still be high enough to block flowering. However with longer periods of cool treatment the proportion of inhibitor to promoter produced by 16h daylengths maybe lowered, enabling flowering to occur.

Further studies of daylength - temperature interaction and the effect of duration of temperature are needed to provide more inflormation.

Translocation of Promoters in Clone B

Experiment 10

Introduction

There is some evidence that on transfer from short days to continuous light or natural long days a translocatable factor causes inflorescence initiation in clone C. To cause inflorescence initiation in clone B after short periods of cool temperature (10°C), very long photoperiods are required (previous experiment). Thus it is possible that clone B produces a translocatable stimulus in continuous light like clone C.

To examine this possibility clone B plants which had come from a population known to respond to continuous light (exposed to a nightly glasshouse minimum temperature of 15°C), were transferred to continuous light. Some clone B stolon tips were exposed to continuous dark and their leaves to continuous light. Should the hypothesis that clone B in continuous light produce a translocatable floral stimulus be correct, then such plants would be expected to produce inflorescences.

Material and Methods

Plants of clone B which had been growing in the glasshouse during the autumn were transferred to continuous light on May 16th 1980. Plants would have been exposed to daylengths as low as $10\frac{1}{2}$h and temperatures as low as 15°C.

The plants were tagged and each stolon tip, which consisted of tissue beyond the youngest unfolded leaf, of a main stolon was poked through a hole in a cardboard box (see plate 4). The cardboard box was then closed up and the stolon tips exposed to continuous dark for three weeks, lateral stolons on main stolons whose tips were in continuous dark (treatment 10.2), and whole plants (treatment 10.1) were given continuous light to determine whether the plants would respond to continuous light. When inflorescence were macroscopically visible on lateral stolons and plants in continuous light and inflorescence initiation had apparently stopped, the experiment was terminated. The stolon tips growing in continuous dark were only examined at the beginning and end of the experiment. Between the leaves exposed to continuous light and the stolon tip on average three mature leaves developed.

Results

Three out of the six stolon tips growing in continuous dark, whose leaves were in continuous light, produced inflorescences. The other stolon tips which did not were not noticeably different. For example they produced as many nodes (in one case more), and as many basal lateral stolons flowered. However as can be seen from table the proportion of laterals which produced inflorescences was very variable ranging from 0.33 to 1.

ble 19 Table of Results (Main Stolons)

eatment No.	Treatment	% Stolons Flowering	Inflorescence no. per Flowering Stolon	Nodes to first Inflorescence
0.1	Whole plant continuous light (n=6)	85%	1	9
0.2	Tips continuous dark Leaves continuous light (n=6)	50%	1	9

ble 20 Table of Inflorescence per Stolon - Laterals and Main Stolons

Treatment 10.2

	Main Stolon (tip dark)	Lateral Stolons (in CL)
; 1	1	1 (n=5)
; 2	0	0.75 (n=4)
; 3	1	1 (n=5)
; 4	0	0.67 (n=3)
; 5	1	0.33 (n=3)
; 6	0	0.33 (n=3)

Plate 4

Photograph of plants in experiment 10 showing that when the leaves of clone B stolons were kept in continuous light the stolon tips kept in continuous dark initiated inflorescences.

Discussion of Results

Continuous dark treatment of clone B plants, at other times, has shown that clone B does not produce inflorescences as a result of a continuous dark treatment. For example, at one time when clone B plants were left in continuous dark for five weeks (by which time only a few stolon tips were still alive), inflorescence initiation did not occur.

Therefore the production of inflorescences in the main stolon tips of treatment 10.2 is probably due to the translocation of a floral stimulus from the main stolon leaves and lateral stolons exposed to continuous light, to main stolon apices in continuous dark.

In comparison to the lateral stolons (74%), and to main stolons exposed to continuous light (85%), not as many stolons produced inflorescences when the tips were kept in continuous dark (50%). While this indicates that continuous dark is inhibitory to inflorescence initiation, the fact that in two pots only one lateral stolon out of the three theoretically capable of responding to continuous light produced an inflorescence, suggests that the difference between the control (10.1), the laterals on plants in treatment (10.2) and the main stolons in 10.2 was due to the variation in response to continuous light.

The Role of Inhibitory Factors in the Regulation of Flowering in Clone B

Experiment 11

Experience has shown that cool temperatures enhance the response of clone B to subsequent continuous light. This was shown by a higher production of inflorescences after a cool temperature exposure (Experiment 9)than after warm temperature .

This may have been caused in many ways. For example if inhibitor levels block the production and activity of translocatable or localised promoting factors, cool temperatures may have decreased the level of the inhibitors. Alternatively as Melchers has suggested (Lang, 1951), cool temperatures may result in the production of precursors (vernalin) of the flowering hormone (florigen). Though the factor produced in cool temperatures need not be a precursor, it may be a cofactor, of the flowering hormone.

While the effect of short days and cool temperatures maybe different, similar hypothesis have been formulated for the activity of both. For example, it has been suggested that short days remove inhibitory factors or provide precursors or cofactors; processes which are also thought to account for the effect of cool temperatures.

The inability of clone B to respond strongly to warm photoperiodic treatment maybe due to the ineffectiveness of the short day pretreatment. For example, inhibitor destruction or breakdown might not occur in clone B with the result that high levels of inhibitor may exist. The effect of cool temperatures maybe to remove or stop the production of inhibitors which have been unaffected by activity of short days.

Another possibility is that in short days, precursors or cofactors of the floral stimulus are produced not in clone B until a cool temperature treatment. However, as work by Thomas (1981), indicates that the effect of short days is tc remove an inhibitor which blocks floral stimulus activity, and as experiment 9 indicates that inhibitor removal is one action of cool treatments, the hypothesis that cool temperatures remove an inhibitor, and that clone B contains an inhibitor in warm short days were examined.

One way in which to test the hypothesis that clone B produces translocatable inhibitors in warm short days is to expose lateral stolons to conditions which should cause

inflorescence initiation, and main stolons to conditions which do not. If production of translocatable inhibitors occurs in warm short days, inflorescence initiation should be blocked in the lateral stolons. Should the main stolons in vegetative conditions have no effect on the flowering of lateral stolons, it is possible that translocatable inhibitors do not affect flowering, or that the cool conditions necessary to promote flowering have deactivated the inhibitor. This means that while a positive result (the inhibition of flowering in lateral stolon exposed to cool short days followed by continuous light), would indicate a translocatable inhibitor, a negative result would neither prove or disprove that hypotheis that a translocatable inhibitor is present. A negative result would not rule out the alternative hypothesis, that an inhibitor localised to the apex, or production of essential components 'induced' by the cold could also be correct.

Material and Methods

Clone B plants which had reverted is the vegetation state after producing inflorescences in continuous light were transferred back to the glasshouse, trimmed, potted on and divided into two groups, (August 13th 1980). In each group there were five plants.

The experimental treatment of each group is outlined in figure 10. One group (I) of plants was transferred to the wintery conditions outside the glasshouse for five weeks, during which time they were exposed to temperature as low as 3°C and as high as 25°C, (Average minimum and maximum temperature 2.1°C and 17.4°C respectively). The daylength from the beginning of the five week period to the end increased from $11\frac{1}{2}$ to 13hrs.

The other group (II) was kept in the glasshouse but one lateral stolon per plant (main stolon) was exposed to cool temperatures. This was done by pushing the lateral stolon between a pair of glass plates which replaced a pane of glass in the glasshouse, (See figure 10). The lateral stolon in this treatment were exposed to the cold for three weeks. Inside the glasshouse during this period the average minimum temperatures were 14.8°C and average maximum temperatures 23.4°C.

At the end of the five week period for the plants in group I and the three week period for the plants in group II the plants were transferred into the growth room. There each plant, except for a lateral stolon on each which had been exposed to cold short days, was placed in a 25°C 8h day. Lateral stolons exposed to cool temperatures in group II and a similar lateral in group I were exposed to continuous light.

Plants were exposed to the experimental conditions inside the growth room for three weeks.

Results

Inflorescence initiation occurred in all clone B lateral stolons pretreated with cool temperatures and then transferred to continuous light, even when the main stolons to which they were attached remained vegetative, (lines 4 and 6 Table 21).

Some lateral stolons treated in the same way as the main stolons produced inflorescences, (line 5 Table 21).

One stolon in treatment 11.2, (CSD - WSD) produced an inflorescence, (line 5 Table 21). This was possibly caused by the length and degree of exposure to cool temperature during the pretreatment.

Figure 10 Clone B plant showing main stolon, lateral stolon, and secondary lateral stolon.

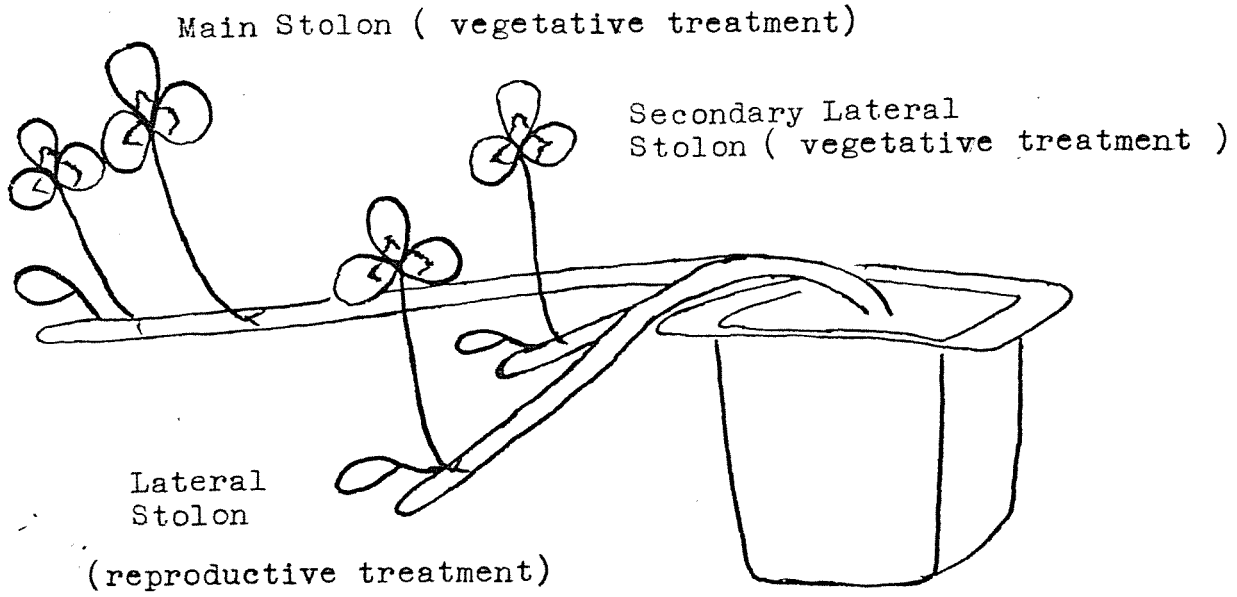


Table 21 Table of Results - Experiment 11

Line No		Treatment Number 11.1	11.2	11.3	11.4
		Treatments			
1	Main Stolon	CSD-WSD	CSD-WSD	GHSD-WSD	GHSD-WSD
2	Lateral Stolon (a)	CSD-WSD	CSD-WSD	GHSD-WSD	GHSD-WSD
3	Lateral Stolon (b)	CSD-CL		CSD -CL	
		Inflorescences per Stolon			
4	Main Stolon	0 (n=5)	-	0 (n=5)	0.25 (n=4)
5	Lateral Stolon (a)	0.11±0.07 (n=19)	0.33±0.33 (n=3)	0.14±0.07 (n=28)	0±0 (n=23)
6	Lateral Stolon (b)	1±0 (n=5)	-	1±0 (n=5)	-
		Nodes from Tag to first Inflorescence			
7	Lateral Stolon (b)	5.4±0.2 *	-	6.1±0.1	-
		Nodes from last Inflorescence			
8	Lateral Stolon (b)	5.0±0	-	5.8±0.1	-

Inflorescence Initiation in Secondary Lateral Stolons

	Lateral Stolons (Parent) Treatment	Inflorescences per stolon	Secondary Lateral Stolon Treatment	Inflorescences per stolon
9	CSD-CL	1±0 (n=5)	CSD-WSD	0.13±0.07 (n=23)
10	GHSD-CL	1±0 (n=5)	GHSD-WSD	0.17±0.11 (n=12)
11	CSD-WSD	0.11±0.07 (n=19)	CSD-WSD	0 (n=5)
12	GHSD-WSD	0.14±0.07 (n=28)	GHSD-WSD	0 (n=5)

* Differences between 11.1 and 11.2 probably due to differences in nodes from Yul at the beginning of the experiment.

GHSD = glasshouse short days WSD = warm short days growth room
CL = continuous light growth room.

Lateral Stolons

Each plant contain one main stolon and a number of lateral stolons. One set of laterals was treated in the same way as their main stolon, either CSD or GHSD - WSD, /Lateral stolon (a)/. All but one of the lateral on plant were given this treatment. The other lateral stolon on a plant (main stolon) was given CSD-CL, /Lateral stolon (b)/.

Secondary Lateral Stolons

Stolons which developed on lateral stolons during the experiment. Treated with either CSD or GHSD and then by WSD.

Discussion of Results

The aim of this experiment was to test the hypothesis that a translocatable inhibitor in clone B stopped inflorescence initiation in warm conditions. To do this, selected lateral stolons were given cool short days followed by to continuous light (a reproductive treatment) while their main stolons were kept in vegetative conditions (warm short days). On the basis of the above hypothesis, the main stolons should have inhibited inflorescence initiation in lateral stolons given a reproductive treatment; yet inflorescence initiation in the lateral stolons was apparently normal. Therefore it would appear that the experimental results do not support the hypothesis that clone B produces a translocatable inhibitor.

It is possible that in reality it does, but that in the conditions of this experiment it had no effect. This may have occurred in four ways:

(a) Cool glasshouse temperatures (14.8°C) could have reduced the levels of inhibitor produced by the main stolon.

(b) Mature leaves between lateral stolons and main stolons could have blocked translocation of the inhibitor.

(c) Translocatable factors produced in cool temperatures could have reduced the levels or activity of the inhibitor.

(d) Cool temperatures could have reduced the activity of the inhibitor in lateral stolon tips, although production of inhibitors was unaltered.

As all of the main stolons and most of the lateral stolons in vegetative conditions, remained vegetative throughout the experiment, continued production of translocatable inhibitors would have been expected to continue. In experiments 2, 3 and 9, mature leaves did not stop the effect of translocatable floral stimulus, so that is unlikely that translocation of inhibitors was blocked by leaves on clone B stolons. With respect to (c) above clone C does not seem to produce translocatable factors in short days with a role in flowering, so it is unlikely that clone B does. The fourth possibility (d) is also unlikely. If inhibitor activity is reduced only by cool temperatures, inhibitor activity should occur again on transfer to warm conditions and the effect of a translocatable inhibitor be apparent. As this did not occur ie flowering was not inhibited it is unlikely that a translocatable inhibitor whose activity is reduced by cool temperatures exists.

There is some evidence that clone B stolons in warm conditions have an impaired response to the floral stimulus. Clone B produces a floral stimulus which is translocated from leaves and stolons in reproductive conditions to stolon tips in vegetative conditions (Experiment 10). In this experiment stolons in reproductive conditions were connected to stolons in vegetative conditions (see figure 10) and theoretically should have caused the latter to flower. However only a few did. which suggests that apical response to the floral stimulus is impaired by vegetative conditions, (refer to inflorescence initiation in secondary laterals lines 8, 9 and 10 Table of Results).

Clone B has only a short 'burst' of inflorescence initiation in continuous light (one inflorescence per stolon) which indicates that production of the floral stimulus declines quickly. This coupled with apical inhibitors may have also resulted in poor transfer of the floral stimulus between stolons.

The Physiology of Inflorescence Production Caused by Direct Exposure to Cool Temperatures

Experiment 12

Introduction

Exposure to cool temperatures will cause direct inflorescence initiation in clones B and C, in both short and long days (Thomas 1979). In some plants this effect of cool temperatures is associated with the production of a floral stimulus which causes flower or inflorescence production in apices not directly exposed to cool temperatures, for example in the short day plant Perilla and the long day plant Silene armeria. In other plants such as in celery and Chrysanthemum (Schwabe 1954 and Curtis and Chang 1930) a translocatable floral stimulus has not been detected. Other plants such as Nicotiana silvestris produce translocatable inhibitors in non flowering conditions (Lang et al 1976), an experiment was therefore designed to see if a translocatable floral stimulus was produced by exposure to cool temperatures, and translocatable inhibitors by exposure to warm conditions.

If cool temperatures cause the production of translocatable factors which cause inflorescence initiation in stolon tips, plants whose tips are in warm conditions, and whose leaves and lateral stolons are in the cold should produce inflorescences, as a result of the transfer of the floral stimulus. Should the effect of cool temperatures be localised in the stolon tip, stolon apices in warm conditions should not flower even if their leaves and lateral stolons are in cool conditions and flowering.

Clone B might also produce inhibitors in warm conditions. If it does, then an apex growing in warm conditions is unlikely to respond to floral stimulus, as is an apex exposed to cool conditions, connected to material growing in warm conditions. Localised inhibitors in the former case, and translocatable inhibitors in the latter are likely to inhibit the activity of the floral stimulus.

Thus there are two treatments which can be used to test the translocatable floral stimulus hypothesis, and the inhibitor hypothesis. One treatment involves exposing a stolon tip to the cold and the rest of the plant to the warm, the other the stolon tip to the warm and the rest of the plant to the cold. The results that would be expected from these treatments with respect to the hypothesis are outlined in table (22).

Table 22 Flowering of Stolons with Respect to Hypothesis and Treatment.

Hypothesis	Treatments			
	Whole plant cold	Whole plant warm	Tip cold rest warm	Tip warm rest cold
Translocatable floral stimulus produced by tissue in cold	+	-	+	+
Translocatable inhibitor produced by warm conditions	+	-	-	-
Localised inhibitor (tip), produced by warm condition	+	-	+	-
Localised tip floral stimulus	+	-	+	-
Translocatable inhibitor-deactivated by the cold	+	-	+	-

From table 22 it is apparent that this approach is limited in its ability to differentiate between the hypothesis. For example, the hypothesis that flowering is controlled by a floral stimulus localised in the tip gives the same expected results as flowering controlled by a localised inhibitor in the tip. If a translocatable inhibitor produced in warm conditions was deactivated by the cold, there would be no evidence of a translocatable inhibitor, as the cool treatment which possibly produces a floral stimulus could also remove translocatable inhibitors.

Material and Methods

On 6th August five treatments using six clone B plants per treatment were set up. These are outlined in Figure .

Treatment Aims

Treatment 12.1 the cold short day control was designed to measure the effectiveness of the cold short day treatment, and to use as a comparison to treatment 12.3. The long day control was treatment 12.2, this treatment was used to determine whether or not the glasshouse conditions were inhibitory to flowering. Translocatable inhibitors were tested for in treatments 12.3 and 12.4 and a translocatable floral stimulus in treatment 12.5 by comparison with the flowering of the short day control.

The glasshouse temperature varied from 12°C to 33°C (average minimum 18.0°C average maximum 28°C). Daylength during the course of the experiment increased from 11½ to 13hrs.

To expose are part of a plant to warm conditions and the rest to cold (or vice versa), glass frames in the glasshouse were removed and replaced with bubble plastic film. Two plastic cloches were placed next to plastic film, and holes were punched through both. Stolons inside the glasshouse were then pulled through. The cloche was heated with a electric heater set by thermostat to switch on when the temperature dropped below 20°C.

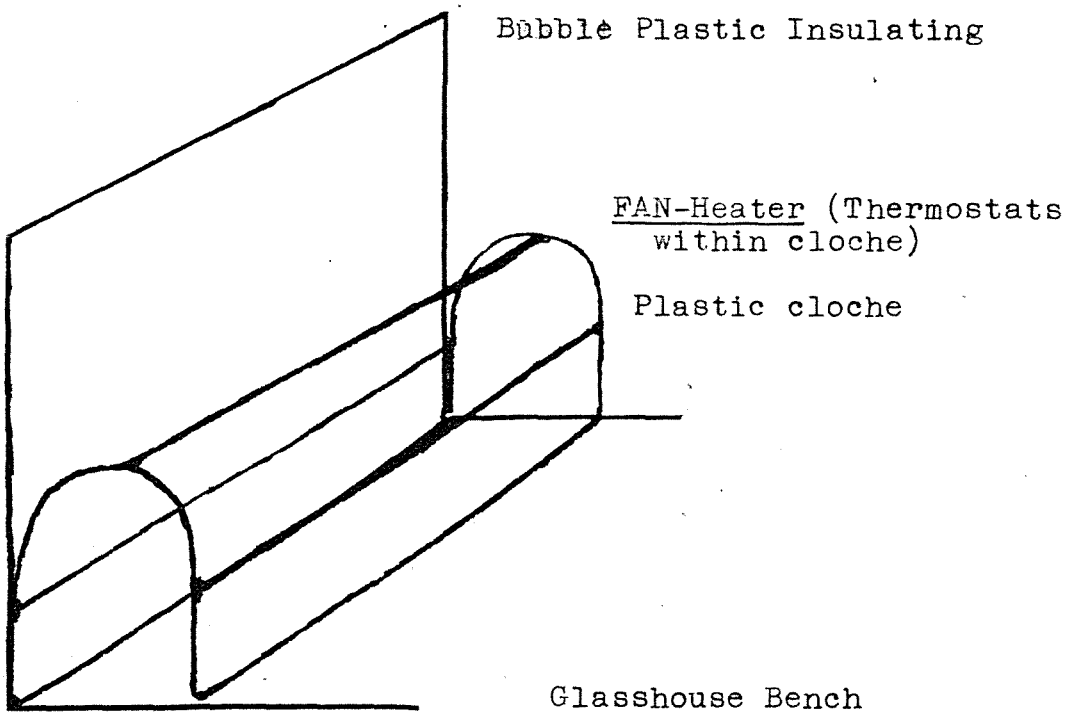
Immediately infront of the heater was an electric fan designed to push heated air through the cloche. As the experiment continued this system of heating became inefficient as leaves interrupted the air flow. It was also difficult to direct heated air along the sides of the cloche. Main stolons were continuously defoliated to one mature leaf. The internodes of main stolons in treatment 12.5 were pulled back through the plastic film to outside the glasshouse when they reached the other side of the cloche.

Over a two week period at the beginning of the experiment maximum and minimum temperatures were recorded.

Distribution of Temperature Across Glasshouse

	Average Minimum	Average Maximum	Range Minimum Temperatures
Outside temperatures	6.0°C	14.5°C	6½ - 8°C
Glasshouse temperatures middle bench	16.3°C	22.5°C	15 - 18°C
Cloche temperatures	15.7°C	24.5°C	13.5 - 17°C

Figure 11 Diagram of Methods



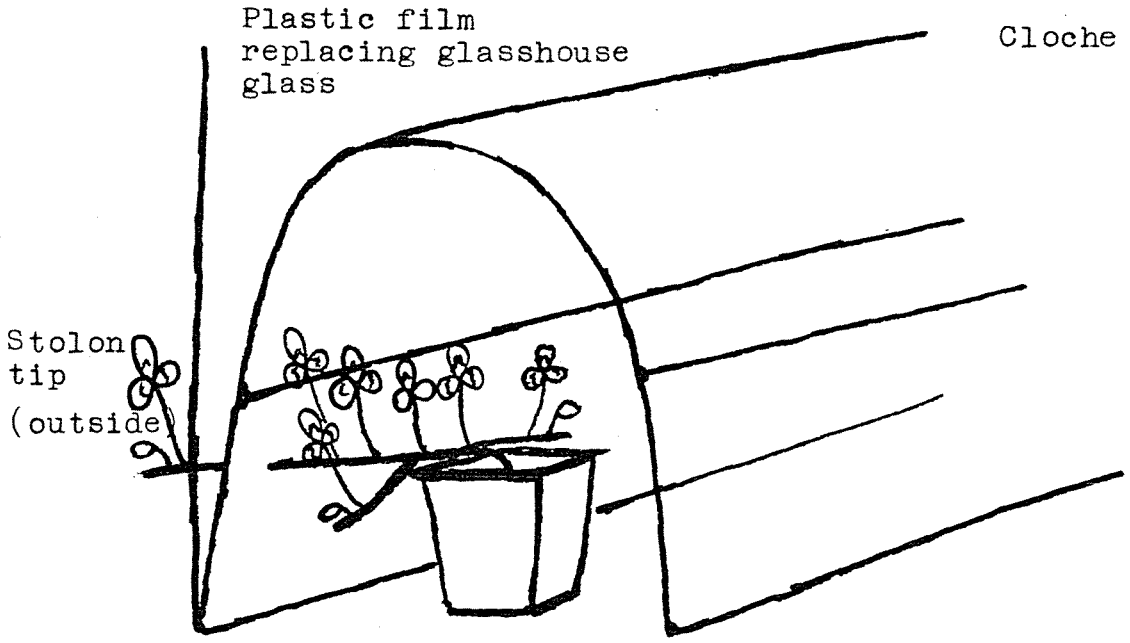
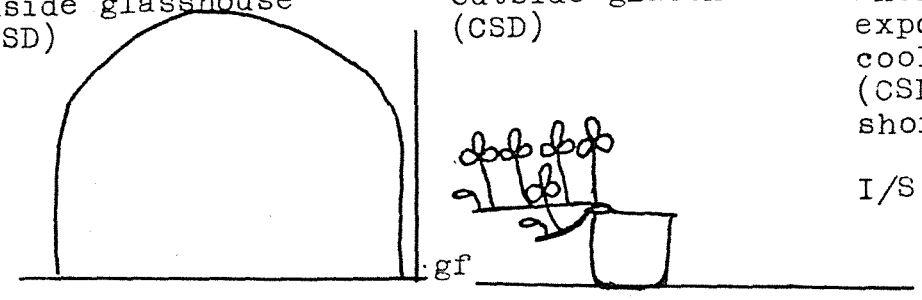


Figure 12 Diagram of Treatments with Respect to Results (I/S)

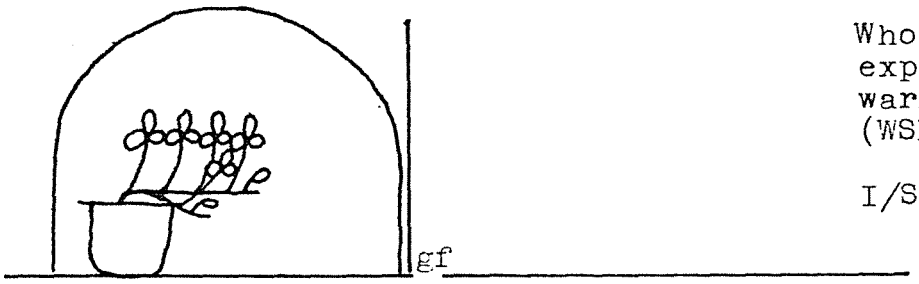
12.1 Inside glasshouse (WSD)



Whole plant exposed to cool conditios (CSD) during short days

I/S = 1.0±0

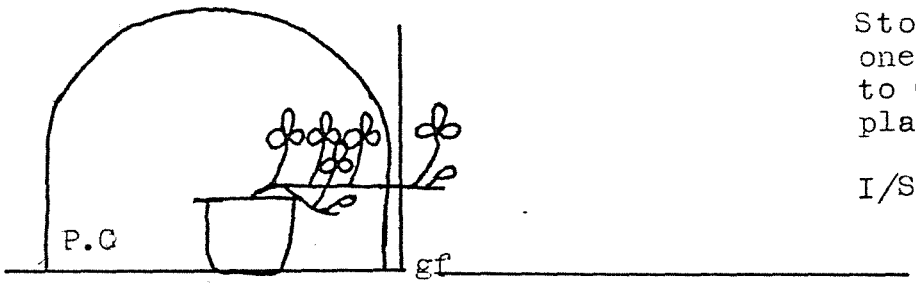
12.2



Whole plant exposed to warm conditions (WSD)

I/S = 0

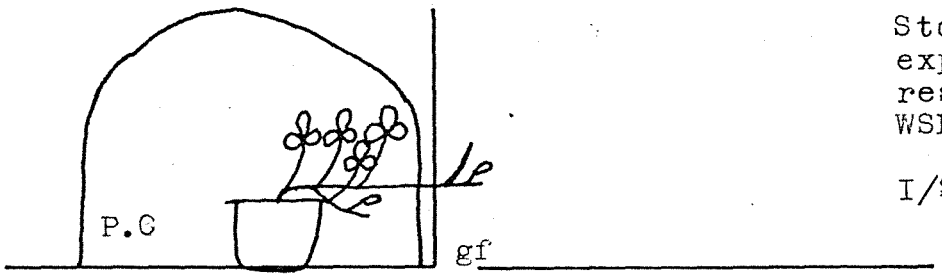
12.3



Stolon tip plus one leaf exposed to CSD rest of plant to WSD

I/S = 1.0±0

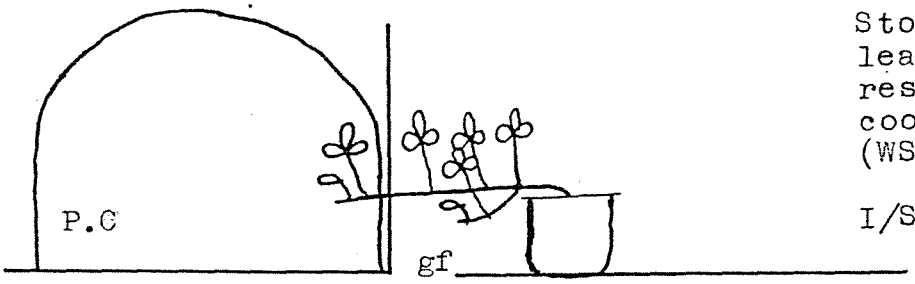
12.4



Stolon tip exposed to CSD rest of plant to WSD

I/S = 1.0±0

12.5



Stolon tip plus leaves (CSD) rest of plant cool short days (WSD)

I/S = 0

P.C = plastic cloche
gf = glasshouse frame
CSD = cold short days
I/S = inflorescences per stolon

Results - Experiment 12

Inflorescence initiation occurred in all stolons directly exposed to cool temperatures. This included defoliated and partially defoliated lateral stolons whose main stolons were inside the glasshouse, (Treatments 12.1, 12.3, 12.5; figure 12).

Stolon tips inside the glasshouse, whether the main stolon of a plant outside the glasshouse, or secondary laterals of lateral stolons outside the glasshouse, did not produce inflorescences. Although connected stolons outside the glasshouse did. (Treatment 12.5 figure 12)

With the exception of one lateral stolon (Treatment 12.2), stolons of plants solely in the glasshouse did not produce inflorescences.

It was observed that stolons the tips of which were in the warm, elongated and grew much faster than stolon tips in the cold, even when the rest of the plant was in the cold, (Treatment 12.5).

Despite an aphid attack and midday temperatures as high as 33°C within the plastic tunnel, plants grew well. They were perhaps a little shaded and petioles seemed longer than usual as a result. As the experiment continued, overcrowding became a problem

Discussion of Results

One hypothesis tested by this experiment was that clone B produced translocatable inhibitors in warm conditions. This hypothesis predicts that inflorescence initiation will be inhibited in stolons exposed to cool conditions when they are connected to stolons or leaves in warm vegetative conditions due to the production of inhibitors by the latter which inhibit inflorescence initiation. However, as stolon tips exposed to the cold, foliated and defoliated, produced inflorescence when connected to stolons growing in the warm (Treatments 12.3, 12.4), this hypothesis seems invalid.

Although one lateral stolon produced an inflorescence, main stolons and other lateral stolons did not produce inflorescences inside the glasshouse (Treatment 12.2). Thus, despite low minimum temperatures near the sides of the cloche (sometimes as low as 12°C), conditions inside the glasshouse were still unfavourable for inflorescence initiation.

The other hypothesis under examination was that cool temperatures caused the production of inflorescences through the production of a floral stimulus, which was translocatable. The non production of inflorescences in (a) warm main stolon tips, the leaves and lateral stolons of which were growing outside in the cold (Treatment 12.5), and in (b) lateral stolons growing in the warm connected to stolon growing in the cold, would suggest that the effect of cool temperatures is localised to the stolon tip. So that inflorescence initiation in response to direct cool temperature exposure may not necessarily involve the production of a translocatable floral stimulus.

If inflorescence initiation is controlled by an interaction between apical inhibitors and a translocatable floral stimulus, and in warm conditions level of inhibitor are high, inflorescence initiation in stolon tips and lateral stolons in response to a translocatable floral stimulus may have been blocked by apical inhibitors. Given that a translocatable floral stimulus has been shown to exist in clone B (experiment 9) this would seem to be a more reasonable explanation of the results of treatment 12. than the proposal that inflorescence initiation in response to cool temperature does not involve the production of a translocatable floral stimulus.

Another possible cause of the results is poor transmission of the floral stimulus, but as translocation of the floral stimulus was unaffected by leaves in experiments 1, 2, 3 and 10 this is unlikely.

Thus it would seem that apical factors, which limit inflorescence initiation and the production of the floral

stimulus, determine inflorescence initiation in clone B. However, to determine whether or not the cause of these results (non transmission of floral stimulus) is due to apical factors or to the levels of the floral stimulus, clone C could be grafted onto clone B, and used as indicator of the presence or absence of the floral stimulus, when clone B is exposed to cool conditions. Alternatively the experimental treatments could be repeated using clone C as well as clone B.

General Discussion

Studies in this chapter revealed that not only could cool temperature cause flowering in short days, but flowering in subsequent 23h photoperiods could also be enhanced by a pre-treatment with cool temperatures. (Experiment 9) In the latter case it was suggested that this was due to the effect of cool temperatures on a balance between inhibitors and promoters of flowering, (cool temperatures resulting in lower inhibitor levels).

At the outset it was hypothesised that flowering in clone B might be blocked by translocatable inhibitors. Cohen and Devrat (1976), have suggested that high levels of inhibitors relative to promoters might block flowering. No evidence of translocatable inhibitors was found in experiments 11 and 12. Thus the balance between inhibitors and promoters indicated in experiment 9 maybe localised in the apex or leaves.

In experiment 10 it was found that clone B produced a translocatable floral stimulus, after a suitable pretreatment, in continuous light. However in experiment 11 there was some evidence that apical factors decreased the activity of the floral stimulus. The same factor may have accounted for the apparent lack of a translocatable floral stimulus in conditions were flowering was caused by cool temperatures (Experiment 12).

From this it could be concluded that inflorescence initiation is controlled by a balance between the floral stimulus and localised apical inhibitors.

Inflorescence initiation could also be regulated by a second process - one which controls the amount of floral stimulus or promoter leaving the sites of production (leaves ?). In this case either a balance between inhibitors and promoters controls the amount of floral stimulus produced

$$[\text{Floral stimulus}] = K\,\frac{[\text{Promoter}]}{[\text{Inhibitor}]}$$

or inhibitor deactivates promoter in proportion to its concentration

$$[\text{Floral stimulus/promoter exported}] = [\text{Amount produced} - \text{amount deactivated}]$$

Therefore two balances might control inflorescences initiation in clone B, are localised at the site of production of the floral stimulus, the other at the sites of inflorescence

production.

Both process maybe same ie the components are the same, or they maybe different but controlled by the same environmental variable (temperature).

CHAPTER 5

GRAFTING STUDIES OF INFLORESCENCE PRODUCTION IN WHITE CLOVER

The results of studies of inflorescence initiation in long days after short day pretreatments, with clone C, have led to the hypothesis that inductive photoperiods cause leaves of clone C to produce translocatable factors which stimulate inflorescence formation in the stolon tip (evocation). If this is correct, the inability of clone B to respond to warm long days after a warm short day pretreatment may be due to

(a) blocked floral stimulus production in the leaf.

(b) blocked floral stimulus activity (due to breakdown etc) in the stolon tip.

(c) a combination of both blocked production and activity of the floral stimulus.

Grafting can be used to test which one of these hypothesis is correct. For example, to determine whether or not stolon apices contain factors which inhibit the activity of the floral stimulus apices of different genotypes or treatments can be grafted onto genotypes which are known to produce floral stimulus, and there by exposed to floral stimulus. In the case of clone B, clone B could be grafted onto clone C, and both the clone B scions and the clone C stocks exposed to long days to produce floral stimulus. If clone C scions flowered when grafted onto clone C stocks, while clone B scions grafted onto clone C did not, the implication would be that the clone B stolon tip was inhibited from responding to floral stimulus. However if the clone B grafts (scions) flowered as a result of exposure to floral stimulus from clone C it could be concluded that the failure of clone B to flower is probably brought about by the non production of floral stimulus in warm long days.

Experiments in this chapter were designed to test the various hypothesis listed below concerning the cause of non flowering of clone B on transfer from warm short days to warm long days.

Hypothesis

(1) Factors localised in the leaves block production of the floral stimulus.

(2) Localised inhibitory factors in the stolon apex of clone B inhibit the response of the apex to floral stimulus.

(3) Clone B leaves produce high levels of translocatable inhibitor which inhibits the production or activity of the floral stimulus.

(4) Clone B produces floral stimulus but is incapable of responding to it.

(5) Clone B is blocked from producing and responding to the floral stimulus.

In experiment 13 another genotype besides clone B and C was used. This was to see whether differences in responses to long days were generally associated with apical or leaf mechanisms.

The Role of the Leaves, and the Stolon apex in the Regulation of Inflorescence Production

Experiment 13

Experiment 13 was designed to test whether differences in response to long days were due to apical factors or to factors associated with the leaves. To do this, a series of grafts was made between clone C and two other genotypes, clone B and Kalinin A.

Grafts of clone C scions on clone C were used to test for the transmission of the floral stimulus across a graft union, and to provide a standard by which to compare the results of the grafts of clone B on clone C, and Kalinin A on clone C. The other grafts, clone B on clone C, and Kalinin on clone C, were made to see whether their response to long days was due to factors located in the leaf or in the apex, (see table 23). Kalinin A produces more inflorescences than clone C in warm long days, while clone B does not usually produce any.

Table of Expected Results with Respect to Scion and Hypothesis

(Table -23)

Hypothesis	Genotype grafted onto Clone C		
	Clone C	Clone B	Kalinin A
Flowering determined by the production of the floral stimulus (leaf characteristic)	Clone C type flowering	Clone C type flowering	Clone C type flowering
Flowering controlled by apical factors which determine the response of the apex to the floral stimulus	Clone C type flowering	Clone B type flowering	Kalinin type flowering
Flowering controlled by apical factors and the floral stimulus produced by the leaves	Clone C type flowering	Clone C type flowering	Kalinin type flowering

Clone C type Flowering - Flowering characteristic of Clone C (2 to 3 inflorescences with vegetative nodes between inflorescence bearing nodes)

Clone B type Flowering - Flowering response characteristic of Clone B. (No flowering or one inflorescence per stolon.)

Kalinin A type-Flowering - Strong flowering inflorescences at most nodes.

Material and Methods

The plants used in this experiment were propagated and maintained in a glasshouse at DSIR Plant Physiology Divison Palmerston North. Grafting was carried out in the same glasshouse, (see main Material and Methods).

In August 1979, scions were grafted in lateral positions onto main stolons. When there were sufficient numbers of scions growing, the plants were transferred to a growth room operating at 23°C with an 18h photoperiod, on the 23rd of October 1979.

Scions were defoliated except for the youngest unfolded leaf, to minimize leaf interference with the transfer of the floral stimulus. While continuous and complete defoliation would have been ideal, preliminary experiments with clone B showed that this resulted in scions which grew poorly and ultimately died. Despite this, scions were defoliated one week after begining of the experiment and at other times following the appearance of inflorecences on some scions growing in an 8h photoperiod.

Because clone C and Kalinin A both flower in long days, exposing scions to long days does not test the response of the stolon tip to imported floral stimulus as the floral stimulus produced by the scions may have caused flowering. For this reason, and because it was possible that clone B produced inhibitors in long days, clone C, clone B and Kalinin A scions were kept in an 8h photoperiod, which does not cause flowering. The main stolon of each plant was kept in 18hrs. This was done by using cardboard boxes in which the base of the plants and the grafts were kept inside the box, and the rest of the plant outside the box. (see figure 13)

Although the cardboard boxes were covered with aluminium foil, light distribution problems occurred. Light intensity ranged from 750 uE/m^2/sec to 350 uE/m^2/sec. In previous experiments (chapter 1) clone C was shown flower in light intensities of as low as 200 uE/m^2/sec so that the light intensity was probably not limiting, (borne out by the fact that all clone C plants exposed to 18hrs flowered). However, to minimize the effects of the variability of light intensity within the room. The boxes were rearranged weekly by shifting the trollies on which the boxes were placed (one box per trolley) to different positions in the room. Rearranging pots inside the boxes would have been costly in terms of damage to main stolons.

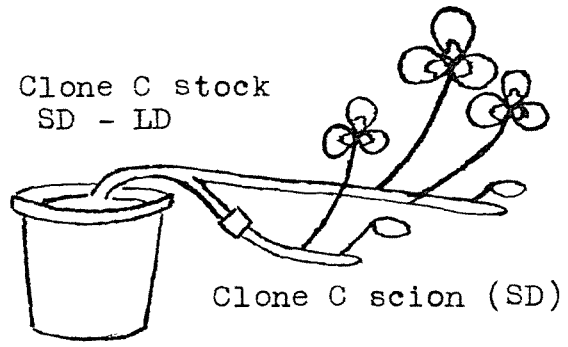
The Kalinin A grafts were transferred from natural photoperiods to the growth room in late November when space became available, which happened when the clone C on clone C,

and clone B on clone C grafts which had produced inflorescences were removed. As a result of the delay, some clone C plants produced one inflorescence in natural long days before transfer into the growth room. However, no Kalinin A stolons either on Kalinin plants or on clone C stocks initiated inflorescences before transfer. This was checked by dissections of Kalinin stolons on stock plants the day Kalinin plants and scions were transferred to the growth room from the youngest unfolded leaf and by the number of nodes which existed prior to the experiment. Shown to be from 6 to 5 nodes by dissections of 7 Kalinin A stolons taken from stock plants which were not flowering at the time the Kalinin on C grafts were transferred into the growth room.

Clone C and clone B scions which flowered during the experiment were given the experimental treatment for five weeks. Non flowering plants and scions of clone C on clone B were kept in the growth room for nine weeks. Kalinin A scions were given four weeks of experimental treatment.

Figure 13 Diagram and List of Treatments using Grafts

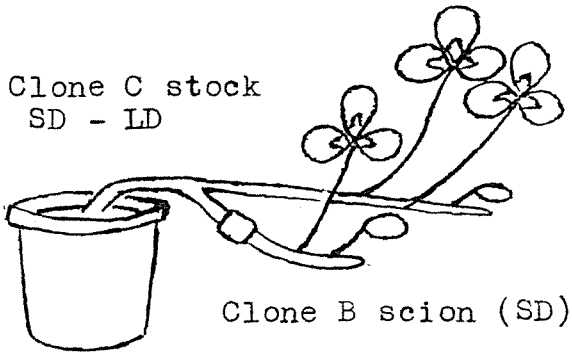
13 1. Clone C grafted onto clone C



Clone C stolon exposed to 8 to 13h daylength then transferred to 18h daylength.

Clone C scion exposed to 8 to 13h daylength then transferred to 8h daylength within cardboard box.

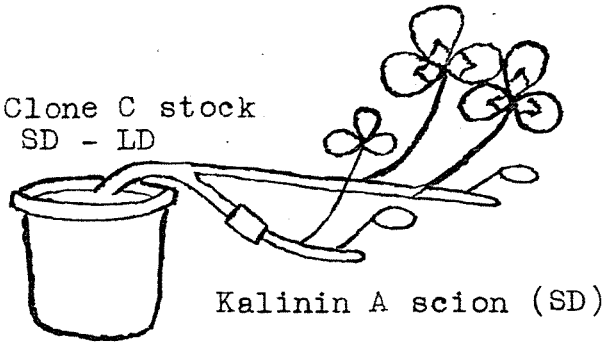
13.2 Clone B grafted onto clone C



Clone C stolon exposed to 13h daylength, then transferred to 18h daylength.

Clone B scion exposed to 8 to 13h daylength then transferred to 8h daylength within cardboard box.

13.3 Kalinin A grafted onto clone C



Clone C stolon exposed to 8 to 14h daylength then transferred to 18h daylength.

Kalinin A scion exposed to 8 to 14h daylength then to 8h day within cardboard box.

Results of Experiment 13

Control plants of clone B, clone C and Kalinin A did not produce inflorescences in short days, even after a one to two month exposure to short days. However, scions of clone C, Kalinin A and some clone B scions, growing in short days and grafted onto clone C main stolons, growing in long days, produced inflorescences, despite the fact that they were growing in non inductive conditions (warm short days).

The percentage of scions that produced inflorescences in response to the stimulus from clone C stocks, varied from 86 and 85 percent for Kalinin A and clone C respectively, to 33 percent for clone B.

Kalinin A scions on clone C stocks produced more inflorescences, and in a different pattern of production, than the clone C stocks. They also produced more inflorescences than clone C scions on clone C stocks in similar conditions (see figure).

The inflorescence production on clone B scions was much less than on Kalinin A and clone C scions, even though it was apparent by the growth and elongation of the scions that a graft union between clone C and clone B had formed.

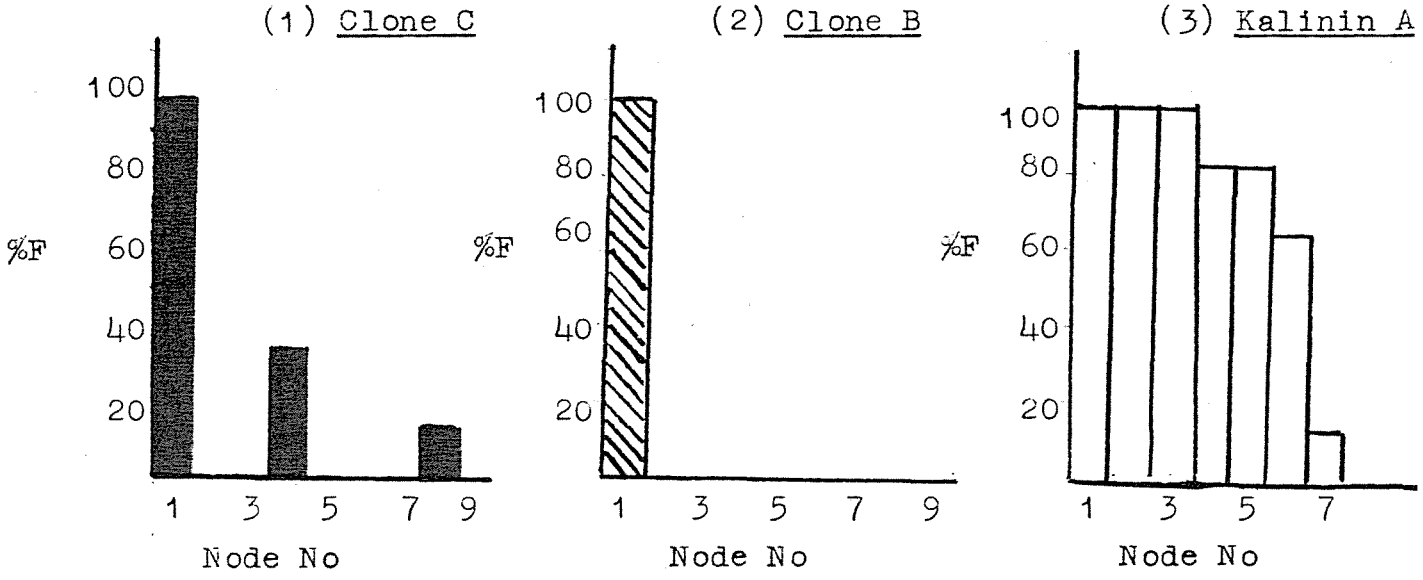
Table 23 Table of Results

Inflorescence production on scions growing in short days, grafted on to clone C stocks growing in long days.

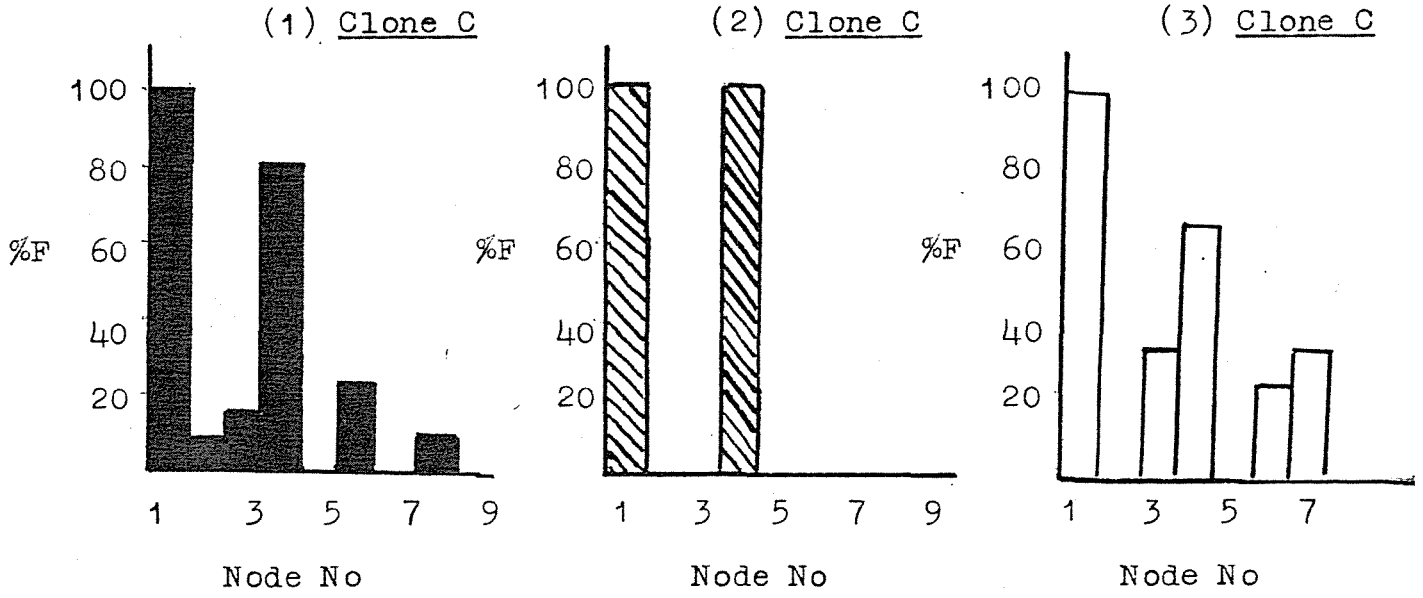
Treatment No	Scion (8 hours)	Numbers of grafts	% in which initiation of inflorescence occurred	Mean Inflorescence no per flowering scion
13.1	Clone C	13	85%	1.4 ± 0.4
13.2	Clone B	9	33%	1.0 ± 0
13.3	Kalinin A	7	86%	4.7 ± 0.3

Figure 14 Patterns of Inflorescence Production in Flowering Scions and Stocks

SCIONS (8h photoperiod)



STOCKS (18h photoperiod)



%F = Percentage of scions or stocks within a treatment with an inflorescence at a specific node number.

Node No = The order of nodes along a stolon where node 1 represents the position of the first inflorescence.

Plate 5

Inflorescence initiation in a clone B scion grafted onto a clone C stock. The clone B scion was kept in short days (vegetative) the clone C stock in long days (reproductive).

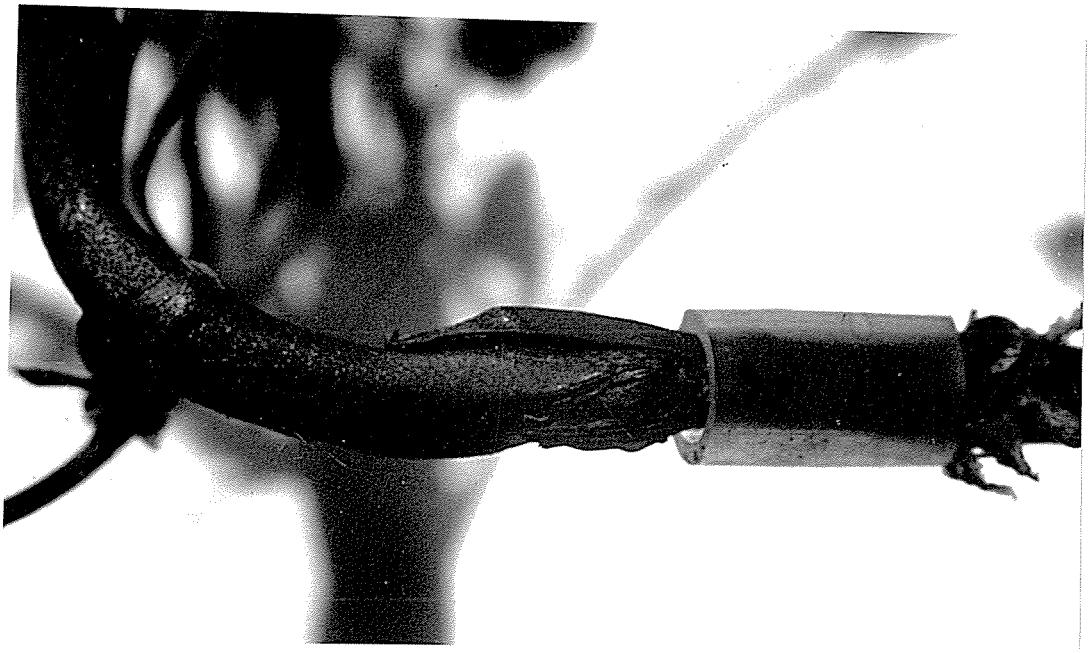


Plate 6

The graft union of the clone B scion and clone C stock.

Discussion of Results from Experiment 13

It is apparent from these results that the stolon tip plays a major role in the regulation of inflorescence production, as, despite occasional irregular defoliations and good graft unions, genotyptic differences in response to the same stimulus emerged. This was well shown in Kalinin A scions grafted onto clone C, where the inflorescence production in Kalinin scions was greater than the production in the clone C stocks and in clone C scions. The amount of inflorescence production was essentially characteristic of the usual response of Kalinin A to conditions causing inflorescence initiation.

The poor response of clone B indicated that inhibitory factors may have blocked the activity of the floral stimulus. The response of Kalinin A and clone C showed that the floral stimulus crossed a graft union, and that clone C, in the conditions of these experiment, produced a floral stimulus, so that it is difficult to explain the low inflorescence production in clone B by means other than decreased activity of the floral stimulus due to either breakdown of the floral stimulus or an inability of clone B to respond to the floral stimulus. Failure of clone B to flower in long days is not necessarily due to a blocked apical responses, though the possible presence of such a block in warm short days supports the hypothesis that a block may exist in long days.

The marginal response of clone B to the unknowns from flowering clone C plants shows that clone B is capable of responding to floral stimuli, even if this response is weak. This indicates that one possible reason for the lack of inflorescence production in warm long days by clone B is failure to produce adequate levels of the floral stimulus.

This experiment showed that white clover genotypes vary in their ability to respond to the floral stimulus. Further more as clone B marginally responded to the floral stimulus from clone C, it is possible that genotypes also vary in their ability to produce floral stimulus, because if in general clone B produced a floral stimulus in long days, the apices would be expected to flower.

The Effect of Long days on the Apical Response of Clone B to the Floral Stimulus from Clone C

Experiment 14

The previous experiment (experiment 13) did not examine the response of clone B scions and long days to the floral stimulus from clone C. It is possible that the effect of long days on the apical response to the floral stimulus is inhibitory which could account for the non-flowering of clone B in warm long day conditions. Long days could also promote the response of the apex to the floral stimulus, for example: by causing production of additional but usually ineffective levels of floral stimulus the total level of floral stimulus in clone B could be increased.

To see if long days would inhibit or promote the response of a clone B scion to floral stimulus from clone C, clone B scions were grafted onto clone C. Both scion and stock were then transferred to continuous light, which causes are of the strongest flowering responses of clone C to long day conditions (Thomas 1981, and personal experience).

Grafts in which clone C was grafted onto clone B were also used to examine the effect of long days on the apical response of clone B to floral stimulus from clone C.

Material and Methods

Experiment 14

Clone B and C plants which had over-wintered in the glasshouse were used as a source of scions and stocks. Two types of grafts were made. In some the main clone B stolon tip was replaced by a clone C scion, in others a clone B stolon was grafted onto a lateral position on a clone C main stolon.

The grafts were made in early November so that scions and stocks would receive a daylength close to 14hrs. The temperatures in the glasshouse during this time (November - December) ranged from 15°C night temperatures to midday temperatures up to 30°C. On January 1st the clone B controls, and 11 clone B and C grafts were transferred to continuous light in the growth room. Prior to this the plants were given two weeks of low intensity light (just after grafting) and four weeks of 8h days in the glasshouse.

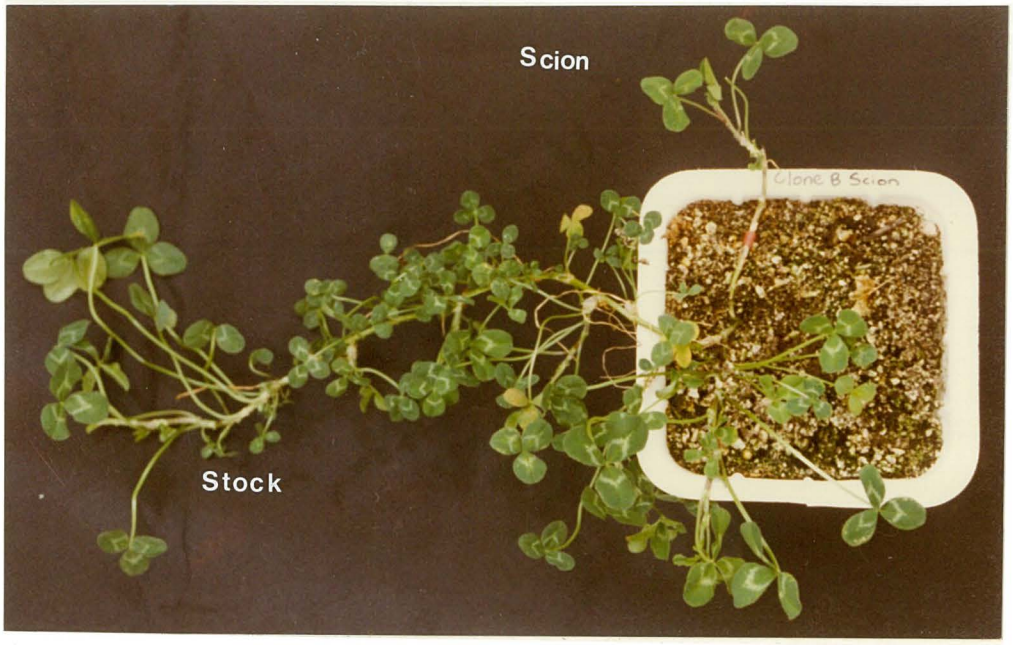


Plate 7

Clone B scion grafted on a clone C stock (Treatment 14.2). The clone C stock flowered while the clone B scion remained vegetative.

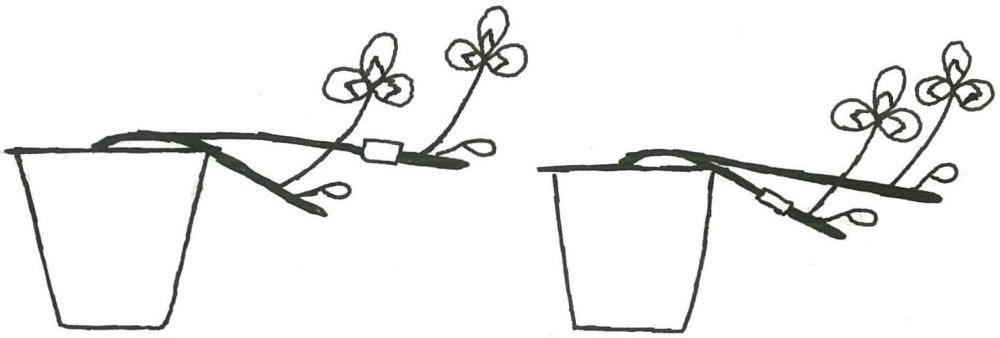


Figure 1

Types of grafts used in experiment 14.

(a) Clone C scion, clone B stock (Treatment 14.1).

(b) Clone C stock, clone B scion (Treatment 14.2).

Results of Experiment 14

Clone B lateral stolons and clone B scions grew more slowly than either clone C scions grafted onto decapitated clone B main stolons, or clone B scions grafted onto clone C, (Table of Results). With respect to the grafts of clone B on clone C, (treatment 14-2) this may indicate poor graft unions. This is supported by the fact that 55% of the clone B on clone C grafts died during the experiment. Some connection between the clone B scions and clone C stocks must have existed, however, because the scions remained turgid throughout the experiment.

The slow growth of the clone B lateral stolons below of clone C scions (treatment 14-1, see figure 15) was possibly due to removal of adventious roots, and because the lateral stolons were less than 5 cm long at the beginning of the experiment.

Defoliation of the clone B scions and laterals on the 5 and 13th of January may have also slowed growth, relative to clone C stolons, (grafted or not).

Neither clone B lateral stolons on main stolons, of which the tips had been replaced by clone C (treatment 14-1), nor clone B scions on clone C stocks (treatment 14-2), produced inflorescences in continuous light, even though the experiment was run for over one month, by which time inflorescences had emerged on the clone C stolons.

Table of Results

Treatment Number		[1] Flowering	[2] Mean Leaf Production
14-1	Clone C scion	+	5.0 ± 0 (n=2)
	Clone B laterals	-	3.0 = 0.5 (n=3)
14-2	Clone C stock	+	5.2 ± 0.2 (n=5)
	Clone B scion	-	2.3 ± 0.4 (n=6)

[1] Flowering

+ indicates flowering occurred (at least 3 inflorescences per stolon)

- stolons did not flower

[2] Production measured by counting the number of leaves which unfolded during the first 12 days of the experiment.

Discussion of Results

The non-production of inflorescences in clone B stolons theoretically exposed to the floral stimulus produced by clone C, could have been caused in two ways:

(i) poor transfer of the stimulus to clone B

(ii) impaired stimulus activity in the stolon tip.

The relatively poor growth of clone B laterals on scions, compared to the clone C scions and main stolons, would indicate the possibity of poor transfer of the floral stimulus. The elongation of the clone C scions, where clone C was grafted on to clone B, indicates that good connection existed between some clone C and B stolons. Young axillary buds of clone C, (first leaves just protruding from the stipules of the adjacent leaf) will flower in continuous light (personal observation based upon dissections), thus despite the slow growth of clone B laterals connected to a clone C scion one would have expected the clone B lateral to have responded to the floral stimulus produced by the clone C scions. This suggests that other factors block the response of the clone B apex to the floral stimulus.

Inhibitors produced by the apex, or by leaves, in long days could have blocked the activity of the clone C floral stimulus in the clone B apex. In other species (Chailakhyan 1936 , and Lang 1965) mature leaves inhibit transfer and activity of the floral stimulus. To avoid inhibition by mature leaves, clone B stolons, as either scions or laterals, were defoliated to one mature leaf throughout the experiment. Inhibitor production by leaves would have been expected to decrease, and flowering to increase. As flowering did not occur with partial defoliation other sources of inhibitory factors may possibly exist for example in the stolon tip.

Though this experiment supports the hypothesis that factors block the activity of the floral stimulus in clone B further evidence is required before one can conclude whether or not the hypothesis is correct for clone B. Better elongating scions of clone B are required. The effect of warm or cool temperatures, long and short photoperiods, and the influence of mature leaves on the response of clone B apices to the floral stimulus should be examined, using a wider range of treatments so that direct comparisons can be made. For example because of the low number of grafts all the grafts were transferred to continuous light, with more grafts some scions could be exposed to short days and others to long days.

The Influence of Clone B Stocks on the Flowering of Clone C Scions

Experiment 15

Clone B does not usually initate inflorescences in long days after a warm short day treatment this possibly occurs because clone B contains high levels of translocatable inhibitors which inhibit production or activity of the floral stimulus. To test this hypothesis, a series of grafts in which clone C was grafted onto clone B was made. The clone C scions were exposed to long days, to make them produce inflorescences, and the clone B stocks to either warm long or short days.

If clone B produces translocatable inhibitors in either one or other, or perhaps in both photoperiods, inflorescence production in clone C (scions) should be inhibited. If clone B does not produce translocatable inhibitors, inflorescence production in clone C should be unaffected by the presence of clone B stocks.

This experiment was performed twice. The first attempt was made in conjuction with studies on the role of the scion in regulating inflorescence production. However, because of the very poor elongation of clone C scions which indicated poor graft unions it was regarded as inconclusive as transfer of the inhibitor could have been blocked.

The second attempt was made in late December 1980 early January 1981.

Materials and Methods

Clone B and C plants maintained in the glasshouse from March onwards were grafted together as scion and stock in October. In November 10 plants in which clone C had been grafted onto clone B and three plants in which clone B had been grafted onto clone C were transferred from shade boxes to an 8h photoperiod. From January 2nd 1981 clone C scions and laterals were exposed to an 18h photoperiod in a growth cabinet in the growth room. Seven clone B stocks were exposed to an 18h photoperiod and three to an 8h photoperiod, (kept in a cardboard box). Three clone B scions and their clone C stocks were given an 18h photoperiod.

At the beginning of the experiment the clone C scions were defoliated to one mature leaf (Yul), the production of the number of leaves can be determined by using this leaf as a tag. The experiment was terminated when inflorescences were visible on all clone C scions or laterals.

Table of Treatments

Treatment Number	Stock	Scion
15.1	Clone B (18h)	Clone C (18h)
15.2	Clone C (18h)	Clone B (18h)
15.3	Clone B (8h)	Clone C (18h)

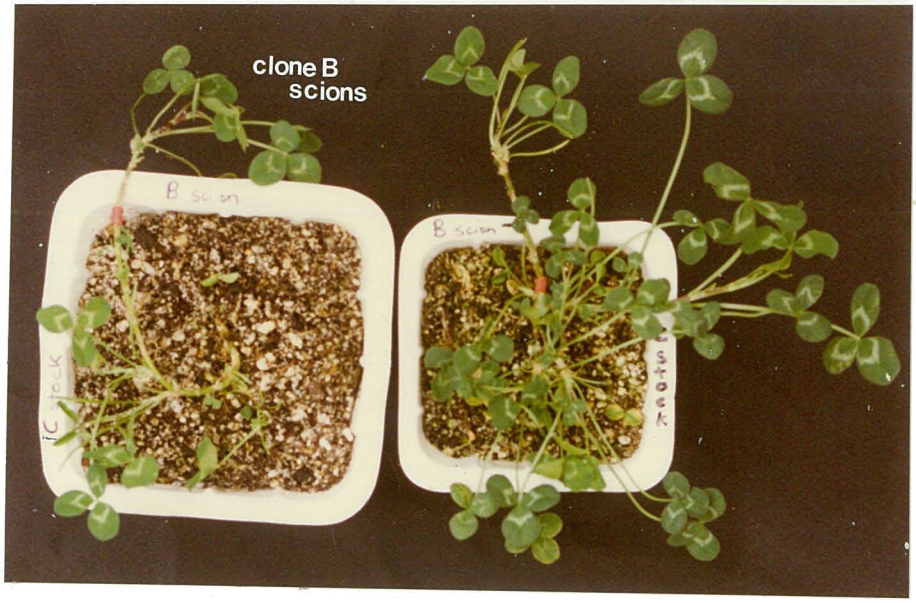


Plate 8

Clone B grafted onto clone C. Stem elongation in the clone B scion indicates that vascular connection exist between scion and stock, (Treatment 15.3)

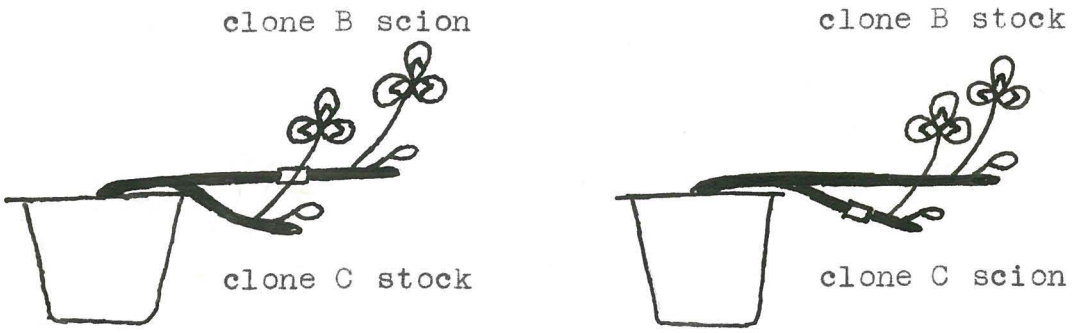


Figure 16

Diagram of scion/stock combinations used in experiment 15.

(a) Clone C grafted onto clone B (Treatments 15.1, 15.2)

(b) Clone B grafted onto clone C (Treatment 15.3).

Results of Experiment 15

With the exception of two clone C scions, which died in the course of the experiment, all clone C scions grafted onto clone B produced inflorescences, as did major lateral stolons on main stolons where the clone C stolon tip was replaced by a clone B stolon.

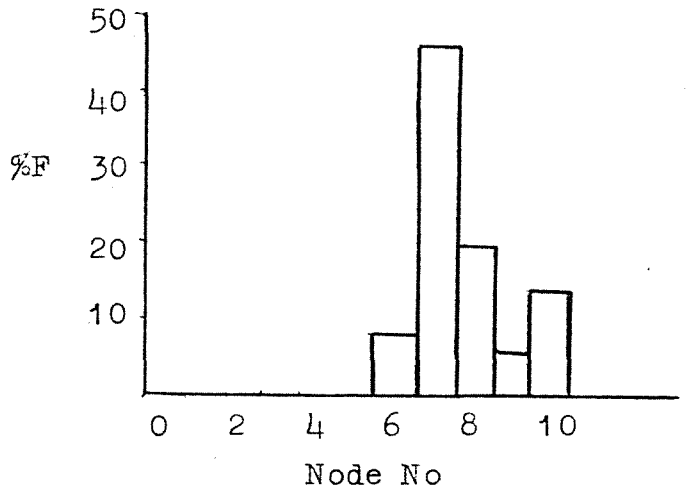
One clone B stolon, unattached to any clone C scion, also produced an inflorescence. Lateral stolons of clone B immediately adjacent to graft unions did not produce inflorescences in either long days or short days.

Table of Results

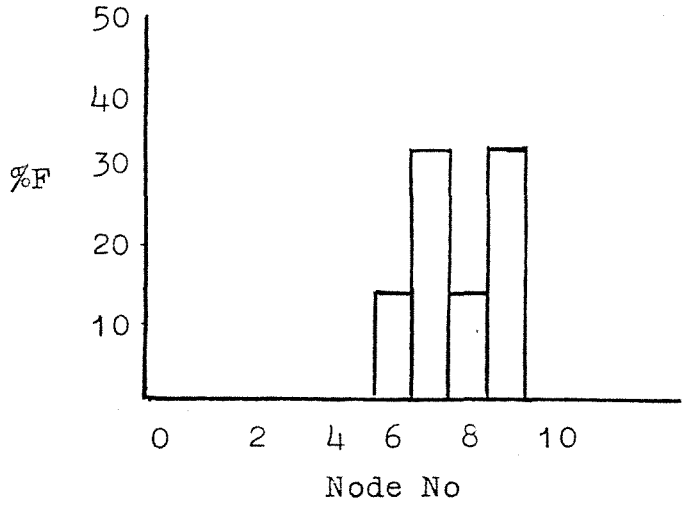
Table 27

Treatment Number	Treatment	Number of stolons	Nodes from Yul to first Inflorescence	Inflorescences per stolon
15.1	Clone C scion (18h) on Clone B stock (18h)	7	6.6 ± 0.2	1.3 ± 0.2
15.2	Clone C scion (18h) on Clone B stock (8h)	3	6.0 ± 0.5	1.3 ± 0.3
15.3	Clone B scion (18h) on Clone C (18h) Clone C laterals	6	7.7 ± 0.5	1.0 ± 0
15.4	Clone C controls (18h)	12	7.3 ± 0.2	1.2 ± 0.2

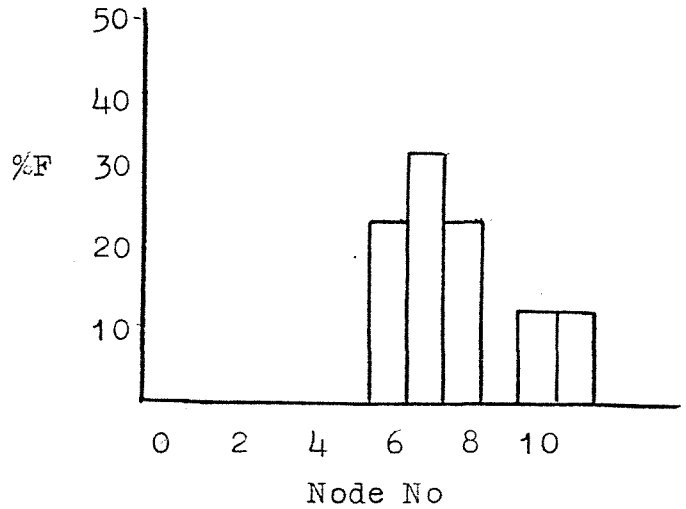
Figure 71 Pattern of Inflorescence Production in Experiment 15



(a) Clone C Control



(b) Clone C laterals below a clone B scions



(c) Clone C scions on a clone B stock

%F = percentage of flowering scions or stocks with an inflorescence at a specific node.

Node No = The order of nodes along the stolon where Node No 0 represents the position of the youngest unfolded leaf at dissection.

Discussion of Results of Experiment 15

Several explanations of the failure of clone B to inhibit inflorescence production in clone C scions and, in clone C lateral stolons below clone B scions are possible.

One is that the experimental system hindered the transfer of inhibitors. For example, the presence of graft unions and / or the presence of an unfolded leaf on clone C scions or lateral stolons below clone B scions might have reduced the amount of inhibitor translocated from clone B to clone C. There is evidence against this interpretation. Clone C scions or clone B stocks received water and other nutrients from clone B as evidenced by the steady growth of the clone C scions. Clone B scions on clone C stocks also grew well. Therefore the vascular connections between scion and stock were probably sufficient to allow transfer of inhibitors. In experiment 13 graft unions and unfolded leaves did not stop the translocation of the floral stimulus from stocks to scions, so it is unlikely that graft unions and mature leaves would stop the translocation of inhibitors.

If the experimental system is capable of allowing the free flow of inhibitors, other explanations are required. For example, the sensitivity of clone's B and C to inhibitory factors may be different, so that while inflorescence production in clone B is blocked, in clone C inflorescence production is unaffected. This could arise if a balance between promotor and inhibitor controls flowering. If clone C produces higher levels of promoter, higher levels of hibitor will be required to block or delay inflorescence production. However because inflorescences production was low compared to other experiments this seems unlikely.

Another interpretation of these results is that clone B does not produce inhibitory levels of translocatable inhibitors, or inhibitory effects in the conditions of this experiment. The low amount of inflorescence production that occurred in this experiment and in the aborted experiment run at the DSIR (see introduction this experiment) when clone B was transferred from short days to long days supports this notion, as flowering would not be expected to occur if inhibitors were present. Thus it would seem that in some situations, for example on transfer from short to long days, clone B does not produce effective levels of translocatable inhibitor even when clone B is vegetative.

Further Studies of the Influence of Clone B on the Flowering of Clone C

Experiment 16

Introduction

As clone B sometimes flowers on transfer from short to long days (refer to experiment 9), it is possible that although inhibitor levels in clone B are high enough to inhibit flowering in most clone B stocks, they are too low to inhibit the flowering of clone C scions, in a short to long day treatment.

Experiment 9 chapter 4, showed that inflorescence production by clone B in response to transfer from cold short days to continuous light was of short duration. This indicates that inhibitor levels may build up in long days. Therefore one way in which to test for translocatable inhibitors in clone B is to maintain the clone B stock in long days before the clone C scions are transferred from short to long days, allowing sufficient time for the build up of inhibitors to occur.

This can be done by grafting clone C onto clone B, keeping the clone C scions in short days, and the clone B stolons (stocks) in long days. When the clone B stocks have been exposed to long days for two weeks or more (further inflorescence initition in clone B does not occur after this length of time in plants given cold short day to continuous light treatments), the clone C scions could be 'induced' to flower by giving them long day treatments. If inhibitors are present flowering in the clone C scions will be inhibited, if not, flowering in clone C scions will be unaffected by the clone B stocks.

This method also makes it possible to test the hypothesis that clone B produces a translocatable floral stimulus whose activity is blocked by inhibitory factors. Clone C scions respond to the floral stimulus in short days (experiment 13) so that if the floral stimulus is produced by clone B leaves on a transfer from short to long days at the beginning of the experiment, the clone C scions should flower. Should the clone C scions flower but not the clone B stocks the conclusion would be that clone B produces floral stimulus but is uncapable of responding to it in warm long day conditions.

Materials and Methods

Clone C scions were grafted onto clone B plants in late September, after they that had grown through the winter. When graft unions had formed, the plants were broken up and potted on, so that 12 'new' plants each consistingly of a main stolon of clone B and one clone C lateral which had been grafted on were produced. On 12th November the plants were transferred from the near inductive daylength (approximately 14½hrs) to a short day box (8hrs). The plants were kept in this box till 10th December to make sure that stem elongation and good graft unions had formed. From the short day box the plants were transferred to a growth cabinet in the growth room, where the clone C scion was exposed to an average daylength of 8hrs and within a cardboard box the clone B main stolons exposed to an 16h photoperiod.

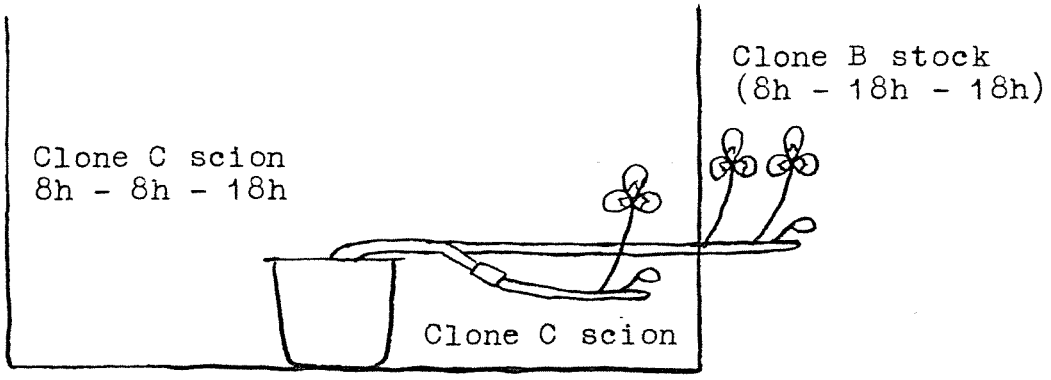
To encourage translocation of either inhibitory or promotive factors to the clone C scions, the clone C scions were defoliated to one mature, and one growing unfolded leaf. At the beginning of the experiment only one unfolded leaf was present. Also, in the first week, the clone C scions were received only 4h photoperiods. On 25th December the plants were unintentionally given one day of continuous dark. The quantity of light received by the clone C scions initially proved to be too harsh. Four of the control scions died and some leaves on other control scions turned yellow. For this reason on 27th December the daylength of the long day cabinet was increased from 16 to 18hrs, and the daylength to which the clone C scions were exposed, to 10 hours.

When on average five leaves had been produced in short days the scions were exposed to the 18h photoperiod. Leaves continued to be removed until the second inflorescence was visible. The experiment was terminated on February 1st 1981.

During the course of the experiment plants were sprayed with Phosdrin and Plictran to control an infestation of red spider mites.

Figure 18 Diagram of Treatments

Treatment 1



Treatment 2

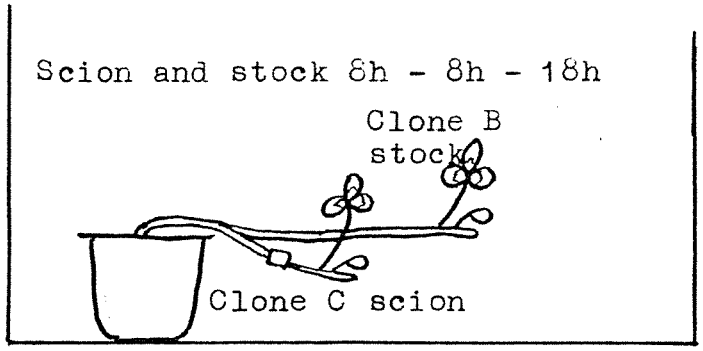


Diagram of arrangement of treatments within boxes

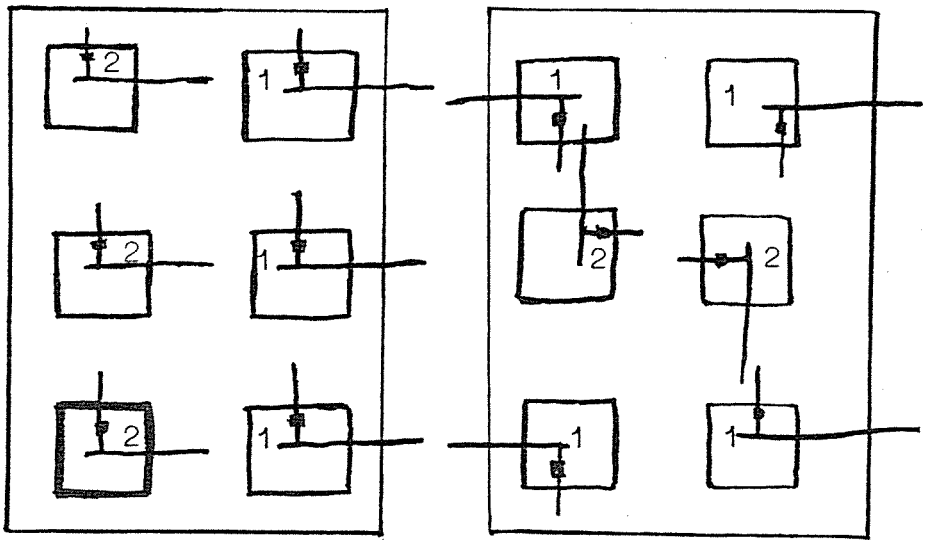


Plate 9

Clone C scions grafted onto clone B stocks.

Results of Experiment 16

The clone B stocks (see figure 18) did not produce inflorescence during this experiment.

Based on the assumption that at the begining of the experiment the clone C scions had six further leaves (nodes) from Yul 1, and produced five leaves during the short day pretreatment, inflorescence initiation in the clone C scions only ocurred on transfer from short to long days (Refer figure 19). One stolon had an inflorescence 10 nodes from Yul 1. This may have been produced during the short day pretreatment, or may have been produced during the long day treatment if the clone C scion initially had five leaves from Yul 1 and produced four during the short day pretreatment. Thus it seems likely that the clone B stolons in long days did not induce inflorescence initiation in clone C scions growing in short days.

Inflorescence initiation in clone C scions on clone B plants was not inhibited by clone B stolons kept in warm long days. In comparisons to other experiments inflorescence initiated the clone C scions was not lower than would be expected. (Refer to experiments 1 to 3, and experiment 8).

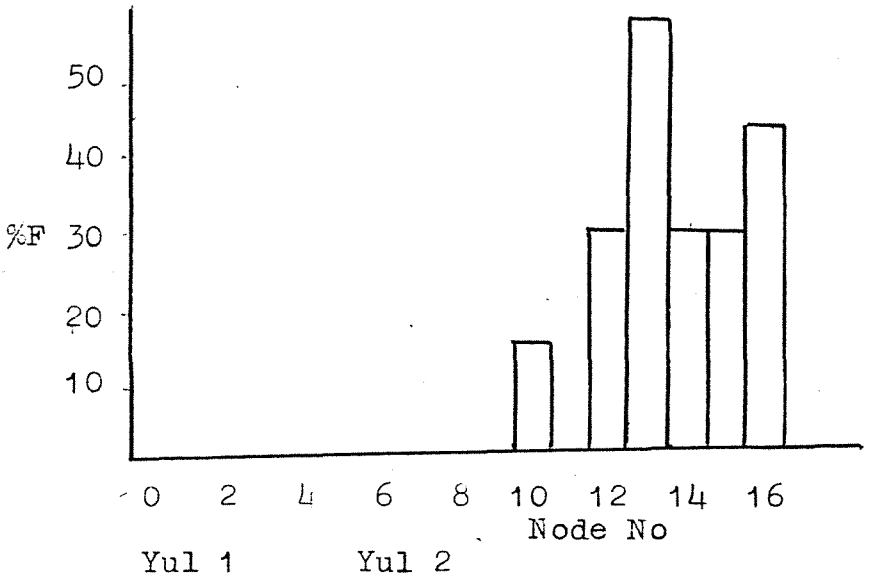
The time taken for inflorescence initiation to occur in the clone C scions varied from six to nine days, in some stolons, and from zero to two and a half in others.

Figure 19

Patterns of Inflorescence Production in Clone C scions (8-18h) Grafted onto Clone B stocks (16-18h)

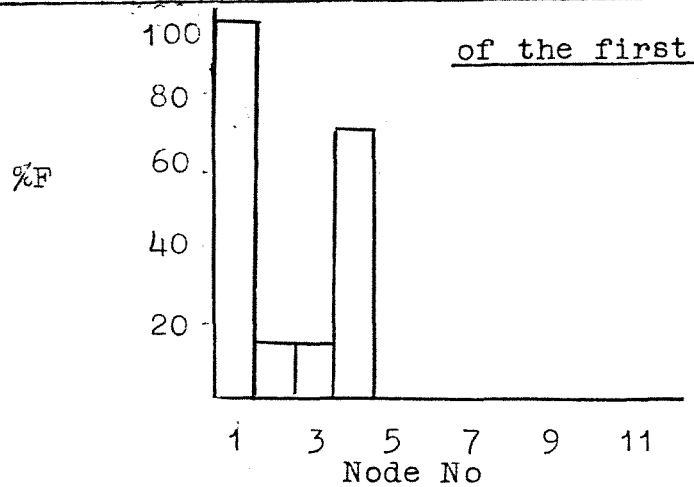
Graph A

Positions of Inflorescences with respect to the Youngest Unfolded leaf at the begining of the experiment



Graph B

Position of Inflorescences where Node 1 represents the position of the first inflorescence



%F = % of scions with an inflorescence at a specific node number.

Node No = order of nodes along a scion.

Yul 1 = Youngest unfolded leaf at the begining of the experiment.

Yul 2 = Youngest unfolded leaf at the start of the 18h treatment.

Table 28 Table of Results Experiment 16

Clone C scion (8 to 18h) Clone B stock (16 to 18h)

Number of grafts = 7

Nodes produced in short days (8h)	Inflorescences produced in short days per stolon
5.0 ± 0.2	0 *

Nodes produced in long days (18h)	Inflorescences produced in long days per stolon	Inflorescences per node
9.3 ± 0.5	2.0 ± 0 *	0.22 ± 0.1

Nodes produced in long days to first inflorescence	Long days to first inflorescence
0.9 ± 0.4	2.2 ± 0.8

* One scion produced on inflorescence in either short days or long days as the node at which it was produced was produced either at the end of the short day pretreatment or at the begining of the long day treatment.

****

Clone C scion (8 to 18h)

Number of grafts = 5
only one survived

Nodes produced in short days	Inflorescences produced in short day per stolon
4.0	0

Nodes produced in long days	Inflorescences produced in long days per stolon	Nodes produced in long days to first inflorescence
9.0	1	6

Discussion of Results

Transmission of promotors from long day donors to short day receptors, across graft unions has been shown to occur in clone C, thus the failure of clone B to evoke inflorescence production in clone C is unlikely to have occurred as a result of either the state of clone C or poor transfer of the floral stimulus from clone B to clone C.

This is supported by the fact, that while most of the short day clone C scions on clone B plants maintained in short days died, the short day clone C scions on clone B stolons which were exposed to long days survived and grew. Further correlation between scion and stock growth was apparent in that the two slowest growing scions were on the weakest clone B stolons. If poor graft unions or poor communications existed between the scions and their stocks, growth would have ceased, in what were poor conditions for white clover survival as shown by the death of 80% of the short day controls.

Thus white clone B may produce translocatable promotors in long days, (see experiment 10) in this experiment the levels of floral stimulus was insufficient to induce either clone C receptor stolons, which are capable of responding to promotor, or clone B stolons, to produce inflorescences.

Another aim of this experiment was to test for translocatable inhibitors which might block or delay inflorescence production in clone C when it was growing in inductive conditions.

Assuming that the transfer of inhibitors from clone B stolons to clone C was possible, the present result, (no inhibition of clone B on the flowering of clone C), would seem to indicate clone B did not produce translocatable inhibitors in the conditions of this experiment.

Another possibility is that clone B produced a translocatable inhibitor which was ineffective because of the high levels of floral stimulus in the clone C scions. The clone C scions in this experiment produced two inflorescences per stolon, in an 18h daylength with in most cases a gap of two vegetative nodes between inflorescence bearing nodes (see figure 19). In comparison to experiment 8 where after five weeks of very low intensity short days (100 $mE/m^2/sec$) some plants produced three and five inflorescences in 16h and 23h photoperiods respectively, inflorescence production was not unusually high in this experiment. So it is likely that high levels of floral stimulus did not exist to overcome translocatable inhibitors from clone B. However, to be sure that this latter interpretation is correct, the experiment should be repeated using clone C scions in shorter photoperiods for example 14.5hrs which are only just inductive.

Until more evidence becomes available, the best hypothesis concerning the inflorescence initiation in clone B, would appear to be that non flowering in warm conditions is due to lack of the floral stimulus, and not to the presence of translocatable inhibitors.

General Discussion

The aim of this chapter was to determine the nature of the physiological differences between clones B and C, by using grafts, (see introduction to chapter 5).

The first approach was to determine whether or not clone B was capable of responding to the floral stimulus. This was done by keeping clone B scions, grafted onto clone C stocks, in short days, while the clone C stock was kept in an 18h photoperiod. Three clone B scions produced inflorescences which showed that clone B scions were able to respond to the floral stimulus. However, in comparison to the other genotypes (Clone C and Kalinin A) which were also grafted onto clone C, the apical response to the floral stimulus was not as productive, nor did as many clone B scions respond to the floral stimulus. This indicates that in conjunction with the regulation of the production of the floral stimulus, another regulatory mechanism located in the stolon tip also controlled inflorescence production, and that this process was more inhibitory in clone B than in clone C, and least inhibitory in Kalinin A.

Photoperiod may affect the tip mechanism in clone B as clone B scions only responded to a floral stimulus from clone C when the scions were in short days. Inflorescence production did not occur in clone B stolons grafted onto clone C in continuous light or in 16h photoperiods (experiments 14 and preliminary experiments). Also clone B lateral stolons below clone C scions in experiment 14 did not produce inflorescences even when the clone C scions produced inflorescences.

In experiment 13 the clone B plants were subject to a minimum temperature usually as low as 15°C but sometimes as low as 11°C. This was sufficient to cause some clone B plants to flower in response to the 18h photoperiod used in the experiment (see experiment 13). At other times clone B plants have flowered in a 23 or 24h photoperiod after an exposure to 10 to 15°C temperature. So that a cool temperature pretreatment rather than photoperiod may have enabled the clone B scions to respond to the floral stimulus for clone C.

To test the hypothesis that clone B produced a floral stimulus in warm long days after a warm short day pretreatment, but was unable to respond to it because of an inhibition localised in the apex clone C scions were grafted onto clone B plants which were then exposed to long days (16h) after a short day pretreatment. Inflorescence initiation did not occur in the clone C scions which indicates that because clone C is able to respond to the floral stimulus, clone B does not produce detectable levels of floral stimulus in warm short to long day conditions.

Another hypothesis which was examined was that clone B produced high level of translocatable inhibitor which blocked it from flowering. As clone C scions or laterals connected to clone B stocks or scions produced inflorescences in response to short to long day conditions it would appear that clone B does not produce translocatable inhibitors.

The non production of inflorescences in clone B after a treatment of warm short days followed by long days probably due to both:

(a) The lack of production of the floral stimulus.

(b) Inhibitory processes localised in the stolon tip.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Flowering in clone C, (Thomas 1961, 1962, 1979, 1981 and chapter 3) and sometimes in clone B (chapter 4 experiment 9) is caused by exposure to long days, following a pretreatment of short days. In clone B the short day pretreatment is more effective under cool conditions ($\leq$ 10°C). Inflorescence initiation in both clones B and C is also caused directly by cool temperatures (c. 10°C) (chapter 4 experiment 12, Thomas 1962).

The effect of the short day and cool temperature pretreatment may relate to the long day processes in two ways:

(a) Removal of inhibitors which block the long day reactions.

(b) Production of co factors and precursors for long day reactions.

In chapter 3, where the effect of different light intensities on the effectiveness of the short day pretreatment was studied, there were indication that the light intensity during the long day treatment determined whether or not the intensity of the short day pretreatment limited flowering. For example in experiment 7 plants which had been given heavily shaded short day treatments during both experiments 6 and 7 and which had failed to flower in experiment 6, flowered in a 23h photoperiod.

Thomas (1981) studied the interaction between the number of short days and the initiation response to various daylengths during the long day treatment. He found that inflorescence initiation occurred in continuous light after a few short days, but not in 16h photoperiods following the same pretreatment (Thomas, 1981).

The effect of both short days and cool temperatures has been described in terms of the two hypothesis mentioned previously. In both the plant is conditioned to respond to the long day treatment by the pretreatment. Both clones B and C initiated inflorescences in continuous light which indicates that the pretreatments (warm short days for clone C, and cold short days for clone B) were effective. Yet inflorescence initiation did not occur after the same pretreatments in shorter 16h photoperiods. Despite the fact that clone C is capable of flowering in response to such daylengths, (clone B may or may not be capable of such a response). The simple removal of some block, be it

the presence of inhibitors or the absence of a co factor/precursor, does not explain these results. Inflorescence initiation in continuous light shows that in terms of hypothesis (a) and (b) that the pretreatments were effective ie inhibitors removed, co factors etc produced. Thus it should follow that the pretreatment should have been effective for a response to a 16h photoperiod. The lack of inflorescence initiation in the 16h photoperiod after the same pretreatments indicates that the pretreatments were infact ineffective.

Thomas (1979, 1981) has proposed that a balance between inhibitors and promoters controls flowering. In this system, inhibitors and promoters are produced in long day conditions and 'removed' in short days. Levels of promoters in long days are initially higher than the levels of inhibitors so as to achieve flowering. This is expressed in the following equations:

$$\underline{\text{Concentration inhibitor}} = [I] = [I]_0 - k_{CT/SD}[I]_0$$

$[I]_0$ = the amount of inhibitor produced in long days where: $k_{CT/SD}$ is a rate constant dependent upon temperature, duration of temperature, and the photoperiod.

CT = cool temperatures.

SD = short days.

$$\underline{\text{Concentration promoter}} = [Pr] = kpp\,[x]$$

kpp is a rate constant dependent upon daylength (photoperiod) and duration of treatment.

$[x]$ = amount of percursor etc.

If flowering is proportional to the ratio of promoter to inhibitor it follows that:

$$\text{Inflorescence initiation} = \frac{K\ kpp\ [x]}{[I]_0\ (1-k_{CT/SD})}$$

K = proportionality constant.

This model can be used to explain the result that inflorescence initiation did not occur in a 16h photoperiod when (a) inflorescence initiation occurred in 23/24h photoperiods after the same pretreatment and (b), inflorescence initiation will occur in a 16h photoperiod after a suitable pretreatment. If the amount of promoter produced is proportional to the photoperiod the ratio of promoter inhibitor in a 16h photoperiod will be lower than that in a 23/24h photoperiod. Thus, in situations where inhibitor levels are high the ratio of

promoter to inhibitor will be insufficient to cause flowering, while in a 23/24h photoperiod the higher production of promoter will be enough to overcome the level of inhibitor. In situations of reduced inhibitor levels, the level of promoter produced in a 16h photoperiod might be sufficient to result in flowering.

Experiments described in chapters 1 to 5, in which apices maintained in vegetative conditions (short days) flowered, if associated leaves and stolons were exposed to reproductive conditions (SD - 18h, 24h, natural long days) showed that a translocatable floral stimulus exists in white clover. This may relate to the balance in three ways:

(i) Both inhibitors and promoters are translocatable. Flowering occurs when a high level of promoter is translocated relative to the levels of inhibitor.

(ii) The balance between promoter and inhibitor controls the production of the floral stimulus.

(iii) The promoter which is translocated to the apex, and an apical inhibitor, are the components of the balance.

Examples of balance systems exist in other plants. Tran Thanh Van (1973), found that flowering in tissue cultures derived from the epidermal cells of peduncles taken from Nicotiana tabacum was influenced by a balance between auxins and cytoKinins. If the level of cytoKinins was increased, shoots instead of flowers were produced; if the level of auxins was increased, roots formed. Lang et al (1977), found the Nicotiana sylvestris produced translocatable inhibitors in vegetative conditions, and a translocatable floral stimulus in reproductive conditions. In peas, Murfet et al (1971), have suggested that a balance between translocatable inhibitors and promoters controls flowering.

Another balance system involved in the regulation of flowering is the photochrome system where in some situations the ratio of one form of photochrome to another determines flowering, (Vine-Prue 1975, 1976). In this situation a balance is controlling production of the translocatable products which cause flowering.

The other possible system of control: an interaction between a translocatable floral stimulus/promoter and apical factors also exists: for example in Chrysanthemum morifolium Ramat (Cockshull, 1972).

To determine which type of balance controlled flowering in clones B and C a process of elimination was used.

Thomas (1979, 1981) has suggested that inhibitor levels decrease in short days and increase in long days after the levels of promoter have risen to cause inflorescence initiation. In chapter 2, experiments were described in which stolon apices of clone C were given a reproductive treatment (SD - LD) in the presence of leaves and lateral stocks in long days. Relative to a control in which leaves and stolon had not been subjected to inhibitory long day conditions, inflorescence initiation was completely inhibited, (experiment 5). However, other experimental results did not support the concept of a translocatable inhibitor. For example, lateral stolons exposed to reproductive conditions (SD - LD) produced inflorescences in the presence of stolons which had stopped flowering in long days. Thus the evidence for a translocatable inhibitor is inconclusive.

It is possible that clone B does not initiate inflorescences in warm conditions (SD - LD) because it produces higher levels of inhibitor than clone C. To test this, clone C scions were grafted onto clone B stocks. The clone C scions were kept in short days for five weeks then transferred to an 18h photoperiod, while the clone B stocks were kept in a 16 to 18h photoperiod, (experiment 16). In such situations clone B does not flower. If the failure to flower results from the presence of a translocatable inhibitor, this inhibitor would be expected to prevent inflorescence initiation in the clone C scions. Inhibitors were not detected as inflorescence production in the clone C scions was apparently unaffected being as strong as in control grafts of C scions on C stocks, (two inflorescence per stolon). This suggests that the non flowering of clone B in warm conditions is not due to the presence of translocatable inhibitor. Translocatable inhibitors were not detected in other experiments, either; e.g. in experiments 11 and 12. If translocatable inhibitors do not stop flowering in clone B, it is unlikely that flowering in white clover is controlled by an interaction between translocatable inhibitors and the floral stimulus/promoter.

Another way in which a balance may regulate inflorescence initiation is that an interaction between a translocatable floral stimulus/promoter and an environmentally controlled apical inhibitor (sensitive to photoperiod and temperature) occurs. There was no evidence of an apical inhibitor, in clone C, whose levels were controlled by short days, (chapter 1 and 2), and as flowering is controlled by a balance between two photoperiodically sensitive processes in clone C, the balance must operate in some other way.

A third option is that a balance between promoters and inhibitors controls the production of the floral stimulus. In this case the terms promoter and floral stimulus are not synonymous, one controlling the production of the other.

Alternatively an interaction between an inhibitor localised in sites of promoter production might control the amount of promoter (floral stimulus) translocated to the sites of activity.

On the basis of elimination, a system of regulation in which either a balance controls floral stimulus production, or an inhibitor controls the amount of promoter (floral stimulus) translocated to the sites of activity, best explains the experimental results.

As flowering starts and stops in long days, it would appear that promoter and inhibitor are both produced in long days. One possible mechanism by which this could occur is shown in the following diagram.

(1) $x \xrightarrow[k_1]{LD} Pr \xrightarrow[k_2]{} I \xrightarrow[k_3]{} Y$

(2) Pr/I control production of the floral stimulus.

In a series of grafts in which clones B and C, and Kalinin A stolons (scions) were grafted onto clone C, and the clone C stolons exposed to long days, (experiment 13) graft transmission of the floral stimulus occurred. However, the response of the scions to the floral stimulus varied. Kalinin A produced more inflorescences than either the C stocks, or the C scions, and only 33% of the clone B scions flowered. Assuming that the amount of floral stimulus transferred from clone C to each scion was similar, some factor (either an inhibitor or the lack of an essential component) would appear to limit inflorescence initiation. Differences in apical response to the floral stimulus may have been caused by a difference in the relative levels of the limiting factor. If the level of inflorescence initiation depends upon the amount of an apical inhibitor, and a certain amount of the floral stimulus removes a certain amount of inhibitor, the amount of inflorescence initiation will decrease with an increase in the level of inhibitors.

Clone B scions only initiated inflorescences when they were kept in short days (experiment 13). In long days (18/CL), despite the fact that the clone C stocks produced inflorescences, initiation did not occur (experiments 14, 15, 16). Thus it is possible that in clone B the apical inhibitor is sensitive to photoperiod. However, because the scions in experiment 13 were grown in a glasshouse where rather cool temperatures (c. 15°C) occurred nightly, cool temperatures rather than short day conditions may have enabled inflorescence initiation in the clone B scions to occur in response to floral stimulus from clone C.

When clone B was exposed to cool temperatures for five weeks, inflorescence initiation occurred in stolons directly exposed to cool temperatures. Lateral or main tips of these stolons, kept in warm conditions did not flower (experiment 12). This indicates that either

(a) clone B does not require or produce a translocatable floral stimulus when inflorescence initiation is caused by cool temperatures

or

(b) that warm conditions inhibit apical responses to the floral stimulus.

Given that clone B is known to produce a translocatable floral stimulus in continuous light after a cool-short day pretreatment, (b) is probably correct. However, the floral stimulus could be a secondary factor in which that it removes a cold-sensitive inhibitor of inflorescence initiation from the apex.

Another method of causing inflorescence initiation in clone B is to expose plants to continuous light after a cool pretreatment. Experiment 10 showed that in such situation a translocatable floral stimulus is produced. Yet in experiment 11, translocation or activity of a floral stimulus was not demonstrated. There are two possible reasons for this:

(a) Lateral stolons were used instead of a main stolon as a 'receptor' for the floral stimulus from the main stolon.

(b) The laterals were kept in short days instead of continuous dark.

If an inhibitor in the apex decreases the response of the apex to the floral stimulus, and if the floral stimulus is not as effectively transferred from the main stolon leaves to lateral stolons as it is to the main stolon apex, the ratio of inhibitor to the floral stimulus will be higher in lateral stolons than in main stolons.

In response to continuous light, clone C produces more inflorescences than clone B (experiment 9), so that it probably produces a higher level of the floral stimulus than clone B. This may account for inflorescence initiation in clone B stolons in warm short days in response to the floral stimulus from clone C, but not in response to a floral stimulus from clone B.

Relevant to apical and leaf factors in flowering, Ridley and Laude (1968), found that by warming the apex the average number of inflorescences produced per node was increased

(more available sites of inflorescence initiation were used). They also found that the leaf-temperature could limit inflorescence initiation. Thus there appeared to be a system of regulation in which both the apex as the site of inflorescence production, and the leaves (site production of the floral stimulus) controlled the number of inflorescences initiated.

Further support of the idea that the difference between clones B and C is due to a combination of leaf and apical factors, comes from grafting studies. If the difference between clones B and C was solely due to apical sensitively to the floral stimulus, clone B in response to long days after a warm short day pretreatment, should produce a translocatable floral stimulus. To test this, clone C (sensitive to the floral stimulus) was grafted onto clone B and the clone B stock exposed to a 16h photoperiod, (experiment 16). If production of a floral stimulus occurred the clone C scions should have initiated inflorescences. Lack of inflorescence initiation in the clone C scions implies an absence of translocatable floral stimulus in clone B. Melchers obtained similar results with Hyoscyamus niger - non flowering biennals did not 'induce' flowering in annual forms, when the former were exposed to long day conditions without a cool pretreatment, (Lang 1965).

While the difference in apical response to the floral stimulus between clones B and C may be due to differences in amounts of one limiting factor, the differences between clones B and C in the process of production of the floral stimulus may be many. There is evidence that short days, cool temperatures, and long days act on a balance between inhibitory and promotive factors. If flowering is proportional to the ratio of promoter to inhibitor, at least two reaction may control intensity of flowering. This is shown in the following formula.

$$\text{Inflorescence initiation} = K.\ \frac{Pr}{I} = \frac{K.\ kpp\ \bar{x}}{\bar{I}_0\ (1-k_{CT/SD})}$$

K is a proportionality constant, the meaning of kpp, $\bar{x}/\bar{I}_0$ $k_{CT/SD}$ has been expressed previously in this chapter.

In warm conditions the rate of inhibitor (I) breakdown, ($k_{CT/SD}$) rate of promoter (pr) production (kpp) may be slower in clone B than in clone C. The rate of production of the inhibitor ($\bar{I}_0$) might also be greater so that clone B plants have higher levels of inhibitor, or inhibitory levels sooner than clone C.

/Results show that the period of inflorescence initiation in continuous light (experiments 9, 10, 11) is shorter in clone B than in clone C, one or two nodes in clone B compared to seven or more in clone C, (personal observation)/

In clone B cool temperatures are apparently required to cause production of the floral stimulus or promoter in continuous light. (Experiments 9, 10, 16). There is also a possibility that cool temperatures enhance the sensitivity of clone B apices to the floral stimulus. (Experiment 13, 14, 15 and 16).

Given that destruction of inhibitors by cool temperatures may account for increased production of the floral stimulus, and increased apical sensitivity one process might control the levels of inhibitor in leaves and apices. If so then one would expect that there should be a trend of increasing critical daylength with decreasing apical sensitivity within a group of genotypes.

In experiment 13 Kalinin A was more sensitive to the floral stimulus than clone C, yet Kalinin A plants used in experiment 13 as a source of scions for experiment 13 initiated inflorescences later than clone C plants. Thus a relatively higher critical daylength, (or longer critical exposure to long photoperiods), was not associated with decreased apical sensitivity, rather the reverse.

This association does not support the hypothesis that apical and leaf processes involved in flowering are regulated by one process ie by the levels of a common inhibitor. Hypothesis which are supported are as follows:

(a) Critical daylength is determined by the rate of promoter/floral stimulus production not by levels of leaf-localised inhibitors.

(b) Levels of cofactors in apices rather than levels of inhibitors control the activity of the floral stimulus.

(c) Inhibitor levels in leaves and apices are controlled by separate processes. (In the case of clone B both processes respond to the same environmental variable).

In clone C warm short days seem to influence leaf processes rather than apical ones (Experiments 4 and 5), which also supports the notion that separate processes control the production and activity of the floral stimulus.

While many of the experimental results do not support the hypothesis of a translocatable inhibitor which could link apical and leaf processes involved in flowering, there is some evidence that translocatable inhibitors may exist. This is:

(a) Leaf removal stimulates flowering (Thomas personal communication).

(b) Detachment of lateral stolons from plants which have stopped flowering in long days enables the lateral stolons to flower (Thomas personal communication).

(c) Repotting plants which have stopped flowering (in long days) causes further inflorescence initiation (experiments 4 and 5, Thomas personal communication and personal experience with experiments which were discontinued).

The results suggest that the roots produce translocatable inhibitors, yet grafts which should show translocatable inhibitors did not (e.g. experiment 16). Perhaps these results are caused by the influence of factors such as ion and energy availability upon the balance proposed by Thomas (1979, 1981) or upon apical processes. Clearly regulation of inflorescence initiation is a complex and dynamic system. Two processes both of which were possibly balances between promotive factors (floral stimulus promoters) and inhibitors, may account for the complexity of inflorescence initiation in white clover.

The major approach used in this thesis to determine the presence or absence of translocatable regulatory factors has been to expose differentially: leaves and stolon tips; main stolons and lateral stolons; scion and stocks, to different conditions (short or long days). For example, in experiment 1, the leaves were kept in continuous light, the stolon tip in short days.

A problem with this approach is that the efficiency by which translocatable regulators are transferred from one organ to another is unknown, and may vary. It is probably affected by such things as: the age and number of leaves between the various components; the distance between them; the energy status of the sink; and, where present, the nature of the graft union. This must mean that where negative results have been obtained some doubt exists as to the validity of the conclusions. For example, in experiment 15 translocation of the floral stimulus from clone C stocks to clone B scions may have been blocked by the leaves and graft union.

Other approachs are necessary to improve the model of flowering in white clover and to determine the differences between clone B and C. One could be to bioassay chemicals extracted from white clover plants for activity as either a floral stimulus or as an inhibitor, and then to measure differences between the various genotypes with respect to content and activity.

This has already been tried in white clover. Cohen and Dovrat (1976) established by bioassay that in cv Tamar, gibberellin levels rose in warm long days, and growth inhibitors (not of flowering) in vegetative conditions (warm SD). They also found that gibberellins could cause flowering, from which it could be concluded that the floral stimulus in white clover might be a gibberellin. Yet gibberellins were not detected in the leaves which produce the floral stimulus, nor do they cause flowering in clones B or C in warm short days (Thomas personal communication). Given this, and the low level of flowering induced by gibberellins in 'Tamar', gibberellins probably have a secondary role in the regulation of flowering, possibly acting as modifiers of inhibitor levels.

Given that translocatable inhibitors were not found in either clones B or C, and that the balance hypothesis (Thomas 1981), proposes that inhibitor levels decrease during short days, the inhibitors found by Cohen and Dovrat (1976), which increased in SD, may be totally unrelated to flowering. However, because there were indications that genotypic responses to the floral stimulus varied, the inhibitors may be related to apical responses to the floral stimulus. This example shows how difficult it is to relate chemical extracts to regulation of flowering. For example it is possible that two balances control flowering in white clover: one localised in the leaf the other in the apex. Chemicals may affect the balances by stimulating inhibitor production or destruction, rather than acting as promoter the floral stimulus, or inhibitors of floral stimulus activity. Despite this, it would probably be useful to use chemical methods, using a range of genotypes, possibly in conjunction with other techniques such as grafting.

Another direction of approach would be to examine leaf processes involved in production of the floral stimulus so as to obtain some idea of the nature of the balance controlling floral stimulus production.

In conclusion the following experimental results were obtained.

(a) Clone B flowered in continuous light if pretreated with cool temperatures for two weeks. Indicating that clone B has a photoperiodic response to long days. (Experiment 9)

(b) Both clones B and C produce a translocatable floral stimulus in continuous light (after a suitable pretreatment). (Experiments 1, 2, 3, and 10)

(c) Kalinin A responded better to floral stimulus translocated across a graft union than either clone C or clone B. Both clone C and Kalinin A responded better than clone B. (Experiment 13)

(d) Clone B stolons in vegetative conditions did not inhibit inflorescence initiation in either clone C scions or in clone B stolons, kept in reproductive conditions. (Experiments 11, 12, 15 and 16)

(e) Clone C leaves in short days neither promoted or inhibited flowering. (Experiments 2 and 13)

(f) Warm short days did not inhibit or promote the response of clone C apices to the floral stimulus. (Experiments 2, 3, and 13). There is some evidence that warm short days inhibit the apical response of clone B to the floral stimulus. (Experiments 11 and 12)

(g) Clone B stocks kept in long days did not cause inflorescence initiation in clone C scions kept in short days.

Experiment 9, and experiments 6, 7 and 8, as well as the work of Thomas (1981), indicates that a balance between promoters and inhibitors controls flowering in clones B and C. Thomas has proposed that inhibitor breakdown occurs in short days, and as experiment 4 indicates that short day activity occurs in leaves, the balance between promoter and inhibitor probably relates to the amount of promoter/floral stimulus leaving the leaf. Based upon this and the other experimental results (a to g) the production of floral stimulus/promoter in clone C when it doesn't occur in clone B could be due to: (i) lower levels of inhibitor in clone C than in clone B and; (ii) higher rates of promoter/floral stimulus production in clone C.

Another difference between clone C and clone B is apical sensitivity to the floral stimulus. Differences in the amounts of apical co factors and inhibitors could be the

cause of the lower amount of inflorescence initiation in clone B apices in response to either continuous light or to floral stimulus from clone C.

Appendix 1	Definition of Term as used in this Thesis
Anthesin	Flower forming substance Cholodny (1937). Component which interacts with gibberellins to cause flowering (Chailakhyan 1976).
Apex	Meristematic dome where inflorescence initiation and leaf initiation occurs.
Critical Daylength	Daylength below or above which flowering on photoperiod response does not occur.
Evocation	Apical activities which result in flowering.
Florigen	Single or multi component factor which is translocated from the leaves to the apex and which causes flowering. Sometimes equivalent to the flowering hormone.
Floral stimulus	Translocatable unknown(s) which cause(s) flowering. In some situations known hormones such as gibberellins and cytokinins can be considered to be a floral stimulus.
Flowering	The initiation and production of flowers on inflorescences. Not simply the visible appearance of flowers.
Hormone	A compound which exists in small amounts and which causes activity in sites other than the site of production. (Translocatable regulator)
Long day plant	A plant which flowers etc in photoperiods above a critical daylength or flowers etc more strongly with increasing daylengths.
Induction	A permanent change of state caused by some external factor. In white clover only the short day treatment can be considered to be induced. Flowering stops in long and short days. Sometimes the verb 'inducted' is used to mean 'caused'.
Inhibitor	A factor which its inhibits flowering by presence.

Initiation	The formation of an inflorescence or flower at the apex or in meristenatic tissue.
Promoter	A factor which causes flowering. It may or may not be translocatable.
Scion	Stolon grafted onto a plant.
Short day plant	A plant which flowers below a critical daylength, or which flowers more strongly with decreasing daylengths.
Short - long day plant	A plant which requires a pretreatment with short days (12hrs or below) to enable it to flower in long days.
Stock	Plant onto which a stolon has been grafted.
Stolon tip	The stolon tissue from but not including Yul. Consists of leaf primordia, axillary buds and the apical dome.
Vernalin	A precursor or cofador produced in cool temperatures which enables flowering to occur later. Considered to be translocatable. May be equivalent to gibberellins, as gibberellin sometimes replaces the need for cool temperatures and ocassionally increases in cool conditions.
Vernalisation	A pretreatment by cool temperatures, needed to make plants flower in following warm short days or long.
Yul	Youngest unfolded leaf present on a stolon. Leaflets no longer touching each other.

Appendix 2 Unusual Inflorescences in Clone C

Sometimes two types of inflorescences developed in continuous light or in the glasshouse, though continuous light was the strongest source of such inflorescences.

In the most common of these, some peduncle internodes and the pedicels of the terminal florets, elongated more than was usual. This may have been caused by gibberellins as these are known to induce peduncle tissue below the first whorl of florets to elongate (Thomas personal communication). Other factors may also have been operating.

The other type of inflorescence was quite unusual in that vegetative buds and modified beacts were produced by the inflorescence, as well as the normal scale - like bract subtending the florets. Initially the development of these structures appeared normal, as the first whorl or first turn of the low angled spiral was the usual array of bracts and axillary florets. However, in the next whorl or spiral turn three modified bracts and inflorescences were produced. These bracts were often small versions of trifoliate leaves, or green unifoliate structures. The inflorescences produced instead of florets were apparently normal. Ocasionally in higher whorls vegetative buds were produced in the axils of modified bracts. This is interesting as it shows that meristenatic tissue in an inflorescence is capable of forming either florets or inflorescences. Modified inflorescences were usually terminated by one or more vegetative stolon tips.

While many of these modified structures were quite complicated, with structures apparently fasciated, and the humbers of whorls and spirals many. Some were quite simple. The simplest was a whorl of florets, one whorl of modified bracts, and then a normal vegetative structure.

Often the second type of modified inflorescence was found at either the first or last node formerly an inflorescence on a given stolon, indicating that perhaps they were formed under marginal conditions. This may have been caused by the time at which inflorescence development was initiated in the plastochron or by a low level of floral stimulus or high levels of inhibitor.

Another possibility is that as continuous light is one of the strongest inductive causes of inflorescence production, high levels of floral stimulus may have induced floret formation in young exillary buds in which development was incomplete. The type of modified inflorescence might then have been dependent upon the stage of development of the exillary bud. However, if this is the case, the first whorl of florets on these structures would be difficult to explain, unless it

Plate 10

Type II inflorescence.

Plate 11

Type II inflorescence with a single whorl of florets and a whorl of bracts with axillary inflorescence. Note the sepaloidy.

occurred before the axillary buds became a dome of cells and a leaf primordiam.

Further studies of these modified inflorescences, particularly the mixed vegetative and reproductive inflorescences, could possibly increase our understanding of the processes involved in inflorescence development. For example, it would seem that meristems on an inflorescence are capable of producing inflorescences, florets, normal bracts, modified bracts and vegetative buds. The fact that they orginate on an inflorescence does not necessarily mean that they will be florets. What then causes or influences the development of a meristem on an inflorescence?

It is also tempting to speculate that bract modification from a scale like structure to a leaf like structure, influenced the development of the axillary bud so that an inflorescence was produced instead of a floret. This would support the hypothesis that leaves are inhibitory to flower or floret development (Refer to Lyndon 1978). However it is equally likely, considering the present state of knowledge, that some process may have modified axillary bud and bract development at the same time. (Inflorescence development is uninfluenced by bract development, both being a function of something else).

In some florets phyllody occurred.

Plate 12

Type I inflorescence (elongated peduncles).

Plate 13

Type II inflorescence on which a normal inflorescences has developed.

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