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Title

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

A thesis presented in partial fulfilment
of the requirement for the degree
of Master of Science in Botany
at Massey University.

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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.