THE PHYSIOLOGY OF FLOWERING IN PISUM SATIVUM

(THE GARDEN PEA)

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SUMMARY

THE PHYSIOLOGY OF FLOWERING IN PISUM SATIVUM

(THE GARDEN PEA)

Late varieties of the garden pea are quantitatively long day photoperiodically sensitive and can be vernalised (Barber, 1959). This was verified and the increased response to day length and vernalisation in the tall late variety Telephone over the dwarf late variety Greenfeast was noted.

Late varieties of garden pea grown in the field flowered at a lower average node than was found with any of the plants grown in growth environmental chambers and attempts were made to simulate under controlled conditions the variations between plants grown in the field and plants grown in growth cabinets. Lowering the intensity of light incident on plants reduces plant height and dry weight but does not affect flowering. High intensity blue light and various ratios of red to far red supplementary light were tested but these did not affect plant growth or flowering.

Different growth media (soil, peralite with nutrient solution) and the presence or absence of root nodules were tested and found to have no effect on growth or flowering.

The effects of gibberellic acid on growth and flowering were studied and it was shown that a natural dwarf late variety given the phenotype of a tall variety by applications of gibberellic acid would respond to day-length and vernalisation in a similar manner to a natural tall late variety. A general delay in flowering and fruit set in both natural tall and natural dwarf varieties was noted after applications of gibberellic acid and this was noted to be more pronounced under short day growth conditions.

The effects of the removal of cotyledons on growth, development and flowering of garden pea plants was studied and it was shown that after removal of the cotyledons plant growth rate was reduced, internodes were shorter, leaflets were smaller and plants flowered earlier than control plants which had retained their cotyledons. It was also shown that the reduced vegetative growth rate continued throughout the entire life of the plant provided that the cotyledons were removed before 240 hours after planting, and that plants without cotyledons flowered at a lower average node than controls provided that the cotyledons were removed within 120 hours of planting.

Various compounds (gibberellic acid, sucrose, kinetin, indolyl acetic acid and pea cotyledon extracts) were applied to decotylised plants in attempts to restore vegetative vigour and normal flowering pattern. All compounds failed completely to restore the vegetative vigour and normal flowering pattern of the controls.

Parts of the cotyledon complement were removed and the effects on growth and flowering were recorded. One quarter of the cotyledon complement was shown to be adequate to allow plants to flower normally and one complete cotyledon was sufficient to allow for normal growth.

It was shown that decotylised plants could be vernalised and that the cotyledons are not the site of perception of the cold stimulus of a period of vernalisation.

CONTENTS

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I. INTRODUCTION

The modern study of the flowering process was initiated in 1920 when W.W. Garner and H.A. Allard, working at the U.S. Department of Agriculture Plant Industry Station at Beltsville, Maryland, discovered that flowering in many plants was an environmentally conditioned response. Garner and Allard worked with tobacco and soybean, one variety of each, and in the years prior to 1920 studied the effects of light intensity, temperature and available soil moisture on the flowering behaviour of these species. No definite effects on the flowering pattern could be detected and, as Salisbury (1963) tells us, they finally and almost reluctantly tested the effects of day-length. In the two species being studied it was discovered that flowering occurred when days were shortened and that this was independent of other environmental factors provided that these were not too hot, too dry or too shady for survival.

Other species were examined and days were shortened by placing plants in light proof cabinets or lengthened by the use of artificial light. Species were found which behaved similarly to those initially tested and also other species were found which would flower only under long day conditions.

From this work Garner and Allard classified flowering plants into three main groups.

(1) Those plants which flower after a period of growth in short days the so designated short-day plants;

(2) those plants which flower after a period of growth under long-day conditions the so designated long-day plants, and

(3) those plants whose flowering behaviour is not influenced by day-length and which are termed day-neutral plants.

It is now know that this scheme is not as straightforward as stated above and that many complexities arise in both the long and short-day plants, some of which will be discussed later, but the simplifying beauty of these discoveries still remains as the basis for the classification of plants with regard to their response to the length of day in which the plants are growing.

To this phenomenon Garner and Allard gave the name of 'photoperiodism' which is now a well known and accepted term although later work showed that in many cases the length of the night and not the length of the day was the critical factor (Hamner and Bonner, 1938). Perhaps the most striking feature of the phenomenon of photoperiodism lies in the fact that it is a system in which plants measure time. This is all the more striking when it is considered that, in most cases, the same critical light or dark period is required over a wide range of temperatures. A critical day or night period can easily be thought of as the time required for the completion of a chemical reaction but that this should be independent of temperature is not easy to understand. The site of perception of a particular photoperiod is accepted as being the leaf

of the plant. Exposure of a single leaf of the very sensitive qualitative long day plant 'Dill' to a series of inductive photoperiods will cause the plant to flower even although the rest of the plant is kept under short days which prevent flowering (Hamner and Naylor, 1939).

In the late 19th and early 20th centuries farmers in America noticed that winter wheats which usually flower in the spring, having been planted the previous autumn, will flower, if the seeds are planted in the spring, provided that the moist seeds have been exposed to low temperatures for a few weeks. This flowering response was extensively studied in Europe and Russia in the years following 1920 and a great amount of descriptive information and confusing terminology was amassed. The one term generally accepted is that of 'vernalisation'. Amongst the early pioneers in the study of vernalisation were Gassner, who clearly stated his discoveries in 1910 and again in 1918 in connection with flowering and cold treatment but whose results, unfortunately remained incomplete due to his failure to detect the additional long-day requirement in his species, and Lysenko whose first paper appeared in 1928.

A plant is said to have responded to vernalisation if the cold treatment elicits a positive response from the plant. Chouard (1960) defined vernalisation as follows. "The acquisition or acceleration of the ability to flower by a chilling treatment."

The two main environmental factors which affect flowering in many

plant species have now been briefly discussed and it is, perhaps, relevant at this time to consider some of aforementioned complexities which are encountered. As this introduction progresses more and more of the difficulties which face the plant physiologist working on flowering will be mentioned and a number of references will be made to conflicting reports in the literature but it may suffice, at the moment, to introduce some of the problems and to introduce the plant species used in this work.

Although accepting the basic classification of flowering plants into the three main groups it may be true to say that no two species will be found to operate exactly alike. For that matter, it is a common thing to find differences in flowering response between varieties within a species or even between individuals within a variety.

In each of the two main groups, namely the short-day (long night) plants and the long day (short night) plants a further division is immediately necessary into those plants which show a qualitative response to the day length and those plants which show a quantitative response. In the case of the 'qualitative' plants the requirement for a specific day length is absolute. This means that if these plants are denied a certain critical day length they will remain purely vegetative. On the other hand the 'quantitative' group of plants do not have a specific day length requirement but will, according to the species or to the variety as the case may be, flower 'earlier' if grown under short-day conditions or long day conditions. The plants in this group will flower eventually

and entirely independently of the day length but will flower 'earlier' under increasing longer or shorter days according to whether the plants are quantitative long day or short day plants. Data on flowering collected from such plants will not be a measure of flowering versus no flowering but will be a comparative measurement of the time in the life of the plant at which flowering commences under different environmental conditions. At this juncture it is relevant to make one point clear and that is that when a reference is made to 'earlier' flowering it refers not to earlier flowering with regard to the chronological age of the plant but rather to its physiological age. That is the stage of development reached, in the plant's life, when flowering first occurs and in this case the measurement taken is the node of appearance of the first flower.

The requirement for a period of cold treatment or vernalisation can also be quantitative or qualitative and can be the only environmental condition affecting the flowering behaviour or can be a factor controlling flowering in conjunction with a day length requirement.

A number of plant species, as already mentioned, show highly complex reactions in flowering pattern and amongst these are such plants as Morning Glory (Ipomea purpurea) which is a short-day plant at high growth temperature and a long-day plant at low growth temperature and Winter rye (Secale cereale) which is a quantitative short-long-day plant; short-day effect replaced by low temperature and with no direct temperature effect (Purvis, 1940).

In recent years a number of review articles and books have appeared which cover extensively the study into the flowering process and for further historical background to the subject and general information the undernoted publications can be consulted.

Allsopp (1964); Chouard (1960); Doorenbos and Wellensiek (1959); Murneek and Whyte (1948); Salisbury (1961) and (1963); Searle (1965) and others.

Frequent reference will be made to work on flowering in peas and other species but this will be restricted to work which is pertinent to the present study or is in some way related to the aspects of flowering being considered in the present work. The volume of literature, in general, on the subject is such that space will not permit of more in the way of general discussion.

The many varieties of the garden pea can firstly be divided into two main groups based on the plant's flowering response to day length and temperature and each of these groups can be further divided on the basis of plant height into tall varieties and dwarf varieties. One of the main groups of peas are the 'early' varieties which, as mentioned above, can be divided into 'talls' or 'dwarfs'. These varieties, flowering at the 9th or 10th node, with virtually no exception, fall into the class of 'day neutral plants'. Day length has no effect on the time at which these varieties flower and a period of cold treatment does not result in earlier flowering.

The other main group of varieties into which P. sativum can be divided are all quantitative long day plants and are termed the 'late varieties'. These varieties flower over a wide range of nodes with the first flower appearing between the 14th and 30th nodes, or even higher, according to the environmental conditions under which the plants have been grown. These varieties also show a quantitative response to vernalisation and none of this group have been found which do not show a positive response in flowering behaviour to both long days and to vernalisation (Barber, 1959).

A great amount of research into flowering has been carried out using Pisum sativum and the selection of the garden pea as experimental material is probably, almost entirely, due to the fact that the pea is a self pollinating species and the establishment of pure lines is therefore easily achieved; the result of which is a usually highly uniform population under any particular set of environmental conditions (Went, 1957). This uniformity is all the more important since the data, recording flowering in these late varieties, is quantitative and not qualitative and in many cases varies little between treatments.

Frequent mention is made of the early varieties of pea but the main interest throughout has been centered round the late varieties where changes in the environment or other treatments can bring about considerable variations in the plants flowering behaviour.

The late varieties of the garden pea are, therefore, quantitatively vernalisable and also quantitatively photoperiodically sensitive.

Plant physiologists working with material which shows these responses have postulated a system of 'phasic development'. Klebs was the first to consider phasic development. It was later expressed in its crudest form by Lysenko (Whyte, 1948) and has been developed particularly by Gregory (1948), working on cereals, and by Melchers and Lang (1948), using Hyoscyamus. The concept is that a period of growth at low temperature (vernalisation), at the seedling stage or later, is necessary to provide the substrate, vernalin, from which the flowering hormone, florigen, is formed under the correct photoperiod. Barber (1959) postulates that flowering is not brought about by the production of stimulatory compounds but rather by the destruction of compounds which are inhibitory to flowering. Barber suggests that the proposed sites of production of floral initiating substances, the leaves or plant apex, are in fact sinks for the destruction of floral inhibitors. To these compounds Barber gives the name of 'colysanthins'.

A number of workers do not support this theory of phasic development and propose a form of competitive system. Aitken (1955) working with subterranean clover and Thompson (1953) working with radishes, cabbages and other horticultural crops support this hypothesis. These workers recognise a competition between cold requirement and day length, with prior vernalisation reducing the photoperiodic response.

There is insufficient evidence to unequivocally support either hypothesis and it may well be that both competitive and phasic systems can and do operate in different species.

As already mentioned, the later varieties of the garden pea will flower without having received either a cold treatment or long days but one or other of these treatments will, amongst other things, lower the node of appearance of the first flower. If both long days and a cold treatment are given the node of appearance of the first flower is lower than when only one treatment is given although the effects are not additive (Barber, 1959).

Moore and Bonde (1962) working with the variety Dwarf Telephone have described the effects of vernalisation. They found that after a cold treatment flowering commenced at (a) a lower node, (b) after fewer days, and (c) at a reduced plant height.

Moore and Bonde also found that embryo extracts of 28 day old pea plants when applied to other plants of the Dwarf Telephone variety would significantly lower the average node to the first flower. On the other hand extracts produced from the cotyledons delayed flowering by a significant number of nodes. Earlier work by Highkin (1955) showed similar results and he postulated that the diffusate, obtained from soaking pea seeds in water, at low temperature, contained an active fraction capable of replacing vernalisation. Seeds, to be grown, were soaked for 10 hours in the diffusate and were then grown at 20°C during the day and 17°C during the night. The reduction in the node to the appearance of the first flower is similar to that found after plants have been given a cold treatment. The diffusates were applied to Avena sections but no elongation was recorded and Highkin concluded

that the active fraction was not an auxin but might be a flowering hormone or its precursor.

Removal of the cotyledons at an early stage in the life of a pea plant significantly lowers the average node to the appearance of the first flower (Sprent, 1965). Highkin suggests a flower promoting substance which is destroyed by high temperature. Miss Sprent suggests the presence of an inhibitor of flowering in the cotyledons. Since the effect of removal of the cotyledons is to reduce the number of nodes to the appearance of the first flower by a number similar to that found after pea plants have been vernalised it might be suggested that an inhibitor of flowering is present in the cotyledons which is transported out under high temperature conditions and under low temperature conditions is, in the early stages of growth, slowly destroyed, in situ, in the cotyledons. Conversely it could be that the cotyledons act as a sink for a stimulatory compound or compounds produced in another part of the plant. Similarly, of course, Highkin's results could be explained by the theory that an inhibitory substance is present in the cotyledons which is not mobilised under the low temperature conditions of the period of vernalisation.

In general it is accepted that the site of perception of the cold 'stimulus' is the apex of the plant. However, in the work carried out by Miss Sprent there is a suggestion, without much experimental evidence to support it, that the site of perception of the cold treatment in the garden pea is, in fact, the cotyledons. Wellensiek (1955) reported that the cotyledons of Streptocorpus could be vernalised and later (1961)

found that detached leaves of Lunaria biennis could be vernalised without buds. In subsequent work he was able to show that the leaves had regenerating tissue at their bases and the suggestion was then put forward that, although an actual meristem was not required, dividing cells were necessary. Salisbury reports that the response of seeds below $0^{o}C$ suggests, however, that dividing cells are not essential.

Moore (1964) tested the effect on flowering, in five varieties of pea, of removal of the cotyledons. In the two late varieties Dwarf Telephone and Unica flowering occurred 2-3 nodes lower. In Tall Telephone there was no significant difference and in the early varieties Massey and Alaska flowering was delayed by 1 or 2 nodes.

Moore also noted that the flowering pattern varied with the light intensity. In decotylised and control plants the number of nodes to the first flower is directly proportional to the light intensity. Ryle (1967) working with Lolium perenne and Festuca pratensis found similar effects. He reported that apical growth was slower, and spikelet initiation and inflorescence development were delayed or inhibited in decreased light intensities. This work introduces the possibility of flowering being a nutritionally controlled phenomenon or perhaps at least partially controlled by the nutritional health of the plant. (Klebs was a strong proponent of the nutrition theory). The hypothesis was put forward that, in some species, plants when grown under inductive photoperiods would still only flower provided that their net assimilation rates were above some threshold level.

To return again to the question of vernalisation, it has been reported that de-vernalisation can occur if plants are subjected to high temperature treatments (39°C for 18-24 hours) immediately following the low temperature or vernalisation period. (Gregory and Purvis, 1937).

Leopold and Guernsey (1954) found several compounds active in bringing about earlier flowering. To these compounds they gave the name of 'chemical vernalisation agents'. Amongst the active compounds are auxins, auxin synergists and thiamine. The effect on flowering of these substances can be reversed by high temperature, nitrogen atmosphere and CO_2 free atmosphere. Gregory and Purvis found that these same treatments, following natural vernalisation, can reverse the effect of the cold treatment.

Highkin and Lang (1966) reported that the final height of garden pea plants, the number of pods per plant and the number of seeds per pod in addition to the node to the appearance of the first flower could be affected by a cold treatment. In the same paper, it was also reported that some early varieties react to a cold treatment. Vernalisation of the variety L.5. results in a delay in flowering, i.e. the first flower appearing at a higher node, and vernalisation of the variety Alaskapromotes flowering although Barber (1959) states that vernalisation of this latter variety significantly delays flowering. Experimental work later in the thesis will consider this anomoly.

'Ripeness to flower' (Klebs, 1918) is a concept which should be considered both in relation to vernalisation and to photoperiodism.

Most plants which respond to a cold treatment or to a particular day length require to have reached a certain stage of maturity before these environmental conditions will elicit their response. In some species, however, a cold treatment or a particular day-length elicits a response at a very early stage in the life of the plant although, in some cases, the effect is not manifest for some considerable time. The vernalisation requiring cereals will respond to a cold treatment even before the embryo has reached maturity in the developing seed on the mother plant (Gregory and Purvis, 1936). Chenopodium rubrum will respond to short days and will flower as a seedling on moist filter paper with only the cotyledons and the infloresence present (Cumming, 1959). In general this response at a very early stage is the exception and more typical is, for example, biennial Hyoscyamus niger which must be 10-30 days old before it becomes sensitive to vernalisation (Melchers and Lang, 1948) or as may be the case with some bamboos which are 30-50 years old before they flower, flower once and then die. In this latter case, presumably, the environmental stimulus to flower, if one is required, is received each year, but the 'machinery' to respond to it is not developed until the plant has reached 'ripeness to flower'.

Late varieties of peas can be vernalised at the seedling stage (Highkin 1959; Moore and Bonde 1962; Sprent 1965) and will also respond to a period of cold treatment by initiating flowers earlier, at later stages in the life of the plant. The temperature range which most workers have used is $8^{o}C$ - $10^{o}C$ although the actual total spread of temperatures which can be regarded as vernalising temperatures is about

$0^{o}C$ - $14^{o}C$ or in some cases below $0^{o}C$ (Purvis and Hansell, 1961). Over, at least, part of the range of vernalising temperatures the maximum effect is obtained by a higher vernalising temperature for a shorter time or a lower vernalising temperature for a longer period (Evans, 1962).

Usually treatment durations longer than the 'optimum' (a particular temperature for a particular time according to the species) have the same effect as the optimum but in a few cases a slight reduction in the response has been found. This has been called 'oververnalisation'.

In many cases there is a marked interaction between cold treatments and day length. Short days will essentially replace the effects of cold in rye and in rye the response even then is not absolute since the rye will ultimately flower without any treatment. In Campanula medium the response is absolute with short days and low temperatures being completely interchangeable. If Campanula plants are grown in their early stages under short days and are later exposed to long days the plants shoot and flower without ever having received a cold treatment. This property may be called "Wellensiek's phenomenon" (Wellensiek, 1953). Either one or the other is required for flowers to be initiated. In the late varieties of the garden pea, a cold treatment and in this case long days are not interchangeable nor is the response absolute. A cold treatment, to a degree, can substitute for long days but does not on its own bring about such early flowering as do long days. A combination of both treatments, as already mentioned, further reduces the node to the appearance of the first flower. Barber in his work in the

years up to 1959 showed this for a number of varieties. In the tall late variety Telephone, under a photoperiod of 8 hours out of the 24 hours, a non vernalised plant will commence flowering, on average, at the 29th node against a vernalised plant, under the same photoperiod which will flower on average at the 21st node. Under a photoperiod of 24 hours, continuous light, the non vernalised plant will flower on average at the 18th node against a vernalised plant which will flower at the 16th node.

The concept of a phasic development system, from the data available, would not appear to operate in the late varieties of Pisum sativum. Granted, the garden pea will flower eventually without having received any cold treatment and without having received any long days and this may mean that it cannot be rightly assigned to a group of plants in which 'phasic development' could operate but, even considering this, it would appear that a type of competitive system better explains the situation. A type of system where prior vernalisation reduces the requirement for long days. The hypothesis could be put forward that rather than two different systems operating within the plant, one in response to a cold treatment and the other in response to day length and both hypothetically acting on the flowering process, that both of these environmental factors act on the same system, albeit, perhaps, at different points in the system, the end product of which pathway is a flowering hormone, the 'florigen' of Melchers and Lang (1948) or the precursor or substrate from which the flowering hormone is produced.

Many workers have studied the responses of the garden pea to treatments with auxins, gibberellins and other substances. Brian and Hemming (1955) studied the effects of gibberellic acid on the shoot development of several dwarf varieties of pea. They found that dwarf varieties, after treatment with gibberellic acid had elongated internodes in subsequent growth, i.e. a variety with a dwarf genotype being given the phenotype of a tall variety.

Lockhart and Gottschall (1961) found that by applying gibberellic acid to P. sativum var. Alaska, apical senescence could be delayed. Lockhart also reported that the removal of maturing fruits in this variety and others delayed the senescence of the plants, the application of gibberellic acid prevented or at least delayed considerably the formation of fruits and Lockhart and Gottschall proposed that the delay in senescence following treatment with gibberellic acid was due to the gibberellin interfering with fruit production. Phillips, Vlitos and Cutler (1960) reported gibberellin like activity in substances extracted from Alaska peas.

Brian and Hemming (1958) reported that gibberellic acid does not elicit any internode extension in peas if an auxin is not also present.

Kuraishi, Susumu and Muir (1963) put forward the hypothesis that the accelerated growth noted in peas after treatment with gibberellic acid is due to the gibberellic acid stimulating the production of auxin in the plant. The variety Little Marvel was used in this work. Arney and Mancinelli (1966), working with Meteor peas, studied the

histological action of the gibberellins and were able to report that the elongation of stems treated with gibberellic acid is mainly due to enhanced mitotic divisions in both apical and other meristems and also in internodal regions where cells have already become vacuolated. The increases in length of stems due to the elongation of individual cells is not a direct action of the gibberellic acid but is due to increased levels of auxin following the gibberellic acid treatment. Highkin's flower promoting diffusate, as mentioned earlier in this introduction, was ineffective in an Avena section growth test suggesting that the active substance or substances, in this case, were not auxins. Aqueous extracts from the variety Telephone were found to have either a promotive or inhibitory effect on flowering according to from which part of the plant the extracts were obtained. (Moore and Bonde, 1962). As previously stated, a 28-day-old embryo extract caused treated plants to flower at a lower average node than controls whereas a 28-day-old cotyledon extract significantly delayed flowering.

Kohler and Lang (1963) were able to extract from immature limabean seeds substances which would reduce the effects of gibberellic acid in dwarf peas but which had no effect on the plants in the absence of gibberellin. Similar substances were later found in pea seedlings and in hemp plants and in both of these plant species an inverse correlation was found between growth rate and the amount of inhibitor present. The effect of the inhibitor can be markedly reduced by increasing the amount of gibberellic acid in the plant.

Tall varieties of peas in both light and dark conditions have low

levels of inhibitor, as is also found in dark grown dwarf varieties where in fact the growth rate is very similar to that found in the tall varieties. In the tall varieties the growth rate is much the same in either light or dark. Dwarf varieties in the light have high levels of inhibitor. The suggestion is that in the various varieties of pea in the light, an inhibitor is formed which can be 'neutralised' by applied or endogenous gibberellic acid. Kohler and Lang suggest that a fine balance may exist between gibberellic acid and inhibitor.

Bonner (1949) and Bonner and Thurlow (1949) working with the short-day plant Xanthium noted that auxins (IAA and NAA) would suppress flowering in plants being given optimum photoinductive periods. They also reported that this suppression by these auxins could be reversed by treating the Xanthium plants with an auxin antagonist (2,4-Dichloranisole) and that the auxin antagonist on its own would hasten flowering provided that the plants were being grown in inductive environmental conditions. These chemical treatments could, therefore, be said to modify the environmental response of the plants but per se were not responsible for the initiation or suppression of the formation of flower primordia.

Fisher and Loomis (1954) following the research of Bonner and Thurlow noted also the antagonistic effect of auxins on flowering of soybean. Fisher and Loomis quote work by Dostal and Hosek (1937) and Leopold and Thiman (1949) in which the delaying effects on flowering of the application of auxins was reported. Fisher and Loomis

mention two auxin antagonists or 'anti-auxins' (nicotine sulphate or 2,3,5 tri-iodobenzoic acid). They postulate that the flowering hormone may be an anti-auxin. To support this hypothesis, work by Borthwick and Parker (1938) should be considered where it was found that older, larger soybean plants flowered in photoperiods which were too long to allow younger plants to flower. Fisher and Loomis propose a scheme in which the flower promoting substance is produced in older leaves as opposed to auxin which is produced in the meristem and young expanding leaves. Naturally as the plant grows older the ratio of old foliage to young foliage will alter and the stage will finally be reached where the amount of 'florigen' to auxin will reach a level which will allow flowers to be initiated provided that the correct photoperiod is given. Later treatment with low concentrations of auxin promote flowering or increase the number of flowers and Fisher and Loomis accept this as a reasonable phenomenon on the assumption that the growth hormone is an anti-flowering hormone but, after the initiation of flowers, is again required in the flowering process, although only in low concentration, to support the essentially vegetative growth of the flower parts.

Two month old soybean plants flowered and produced apparently normal flowers under non-photoinductive periods after the plants had been sprayed with a 2,000 ppm. solution of nicotine sulphate (Leopold and Thimann; 1949). The hypothesis put forward in this case was that the correct photoperiod is required to allow the older leaves to

produce the flower promoting hormone and that this daylength requirement can be overcome by spraying the plants with an auxin antagonist.

Morphogenesis of the shoot, of which flowering is one aspect, has been reviewed by Allsopp (1964) and by Sachs (1965) and cell differentiation has been reviewed by Stange (1965).

Oota (1964) has discussed the role of RNA in florally differentiating cells. Bonner and Salisbury (1960), Bonner and Zeevart (1962) and Evans (1964) have established the requirement for active nucleic acid synthesis in the flowering process. The blocking of flowering in Pharbitis nil occurs simultaneously with the arrest of DNA synthesis and active cell division in the bud (Zeevart, 1962). A sharp rise in the mitotic index of the apex is associated with floral induction in Xanthium (Thomas, 1963). Gifford and Tepper (1962) have shown that the RNA concentration of cells in the apex of Chenopodium album increases rapidly soon after photoperiodic induction. Ross (1962) attempted, using Xanthium buds, to find differences in RNA fractions in vegetative and floriferous buds. These investigations failed to give any results supporting the hypothesis but if the transition from a vegetative to a floriferous apex is the result of changes in the relative proportions of the same enzyme systems rather than the synthesis of totally new and different 'floral' proteins then such changes as do occur will not be detectable and no nucleic acid will be associated uniquely with flowering.

From the preceding discussion it has become evident that the flowering process is highly complex and from the conflicting reports in the

literature, of the varying effects of the environment and/or chemical or hormonal treatments on this process and on vegetative growth and also the possible link between these two discrete stages in the life of the plants, it becomes clear that perhaps there does not exist one answer to this problem which will cover the vast range of flowering plant species.

Flowering is an expression of differentiation processes, the initiation of which in many plants marks the transition from vegetative to reproductive growth. Within the range of plants which flower under no particular environmental stimulus, the change from a vegetative to a floriferous meristem is a process which is governed from within the plant. In other plant species, however, the process is regulated by transmissible stimuli generated by the environment acting on parts of the plant, sometimes distant from the flowering locus. "In either case a regulatory mechanism is implicit since all cellular differentiation must be programmed in the plants ontogenetic development (Searle, 1965)."

Meristematic cells in the apex of the plant are continuously embryonic in nature and possess the potential capabilities of any of the plants specialised cells. Specific changes in gene function, either stimulation of passive genes or selective inactivation of gene repressors, results in the dramatic modification of the vegetative apex that results in the production of the plants reproductive structures. The ultimate flower promoting substance, the elusive 'florigen', may be the same or basically the same for all flowering plants but its

ultimate production may result from widely varying processes in different species or varieties.

II. CULTURAL TECHNIQUES AND GROWTH CONDITIONS

As standard practice throughout, excepting where otherwise stated, all plants were grown in coarse Peralite in either 3" green polythene plant bags or in 5 x 3 Vacapots, i.e. 15 pots per tray with each pot having the dimensions 2.1/2" x 2.1/2" x 2.1/2".

Plants were supplied with a modified Hoagland nutrient solution, details of which are given on page 24, twice weekly and with water only, if required, at other times.

Seeds were either planted directly into moist Peralite or if a higher degree of standardisation of germination rate was required, the seeds were soaked in distilled water at 20°C for 6 hours prior to planting.

During all experiments employing varying daylength regimes the very sensitive short-day plant Chenopodium amaranticolor was used as a biological indicator of light failures and also of any leakage of light into the growing area. C. amaranticolor is a qualitative short day plant which will respond to a single photoinductive period by initiating an infloresence. Two or three pots each containing some 6-10 plants were grown along with the experimental plant material.

In each natural growing season, during the course of this work, seeds of the four test varieties, listed below, were sown in the research laboratory garden for the purpose of supplying stock seed for experimental work. The experimental plants, therefore, at all times, were

Nutrient Solution

49%	$MgSO_4 7H_2O$	1 ml. per litre
20%	KNO_3	2.5 ml. per litre
14%	K_2HPO_4	1 ml. per litre
82%	$Ca(NO_3)_2 4H_2O$	1 ml. per litre
Iron Solution		1 ml. per litre
Trace Element Solution		2.5 ml. per litre

Iron Solution

23.5g. Sequestrene 138 Fe. per litre of solution.

Trace Element Solution

	Grams per litre in stock solution
$CuSO_4 \cdot 5H_2O$	0.0354
$MnSO_4$	0.609
$ZnSO_4$	0.0974
H_3BO_3	1.269
$H_2MoO_4 \cdot 4H_2O$	0.0398

grown from seed which was, at a maximum, twelve months old. The percentage germination remained constantly high and was never lower than 98%.

The following is a list of the four test varieties with their respective specifications, as detailed in the introduction, for the various varieties of the garden pea.

Variety	Growth Habit	Flowering Pattern
Massey	Dwarf	Early
Alaska	Tall	Early
Greenfeast	Dwarf	Late
Telephone	Tall	Late

With few exceptions all experiments were carried out in controlled environments using artificial light sources. The growth cabinets were of various designs, details of which are given later. Unless otherwise stated the lighting in the cabinets was from fluorescent tubes, alternating one white or daylight tube and one warm white tube. The lamps were connected through a timeswitch which could allow the selection of any desired daylength. Some experiments, not detailed in the body of the thesis, were conducted to find the best light source which the available facilities could supply. All white fluorescent tubes or all warm white fluorescent tubes did not result in such vigorous growth or generally healthy plants as did the above mentioned pattern although the flowering behaviour of the plants was not affected. Further considerations of light quantity and quality are to be found in Part IV. of this work.

In most cabinets the light intensity and in all cabinets the temperature remained constant throughout the whole chamber. In some cabinets, however, minor variations in light intensity could be detected. In order to avoid the risk of measurable differences between plants being wholely or partially attributable to these fluctuations in light intensity, experimental material was initially randomly distributed in the cabinet and regular changes of position, thereafter, were carried out. This procedure was adopted as standard practice during all experiments in all of the cabinets.

A total of seven growth environmental cabinets were used. Four of these were divided into two separate light-proof compartments where alterations in day length and light quality between the separate halves could be achieved whilst maintaining the same temperature conditions throughout the whole cabinet. The remaining three cabinets consisted of a single growing compartment and had the advantage over the other cabinets of allowing the selection of different temperatures between day and night.

Cabinet I (A Type)

This growth room consists of two compartments. The light source is as previously described, and consists of 4 ft. fluorescent tubes spaced six inches apart. The heating and cooling systems operate through matched thermostats and a control of temperature of $\pm$ 1°C of the desired value can be maintained.

Plants are grown on trays which can be raised or lowered. This growth room shows the greatest fluctuation in light intensity of all of

the chambers. Light intensities vary slightly in a horizontal plane with the lower intensities near the walls of the room and vary markedly in a vertical plane with much lower intensities near floor level. To minimise the effects of these fluctuations the previously mentioned precautions were taken during all experiments and also the levels of the trays were regularly altered such that all plants, whether tall or dwarf or of different age, had their apices at a common level about 15" below the lamps. Supplementary illumination in one or other of the compartments is supplied from a single 2 ft. daylight fluorescent tube wired through a separate timeclock. Details concerning supplementary illumination are given in Part III.

Cabinet II (B Type)

This cabinet again consists of two separate light proof compartments. The heating and cooling systems are wired through matched thermostats and a high degree of temperature control can be maintained. No fluctuations in temperature of more than 0.5°C on either side of the desired value were ever recorded excepting when attempts were made to run the cabinet at temperatures much below ambient, i.e. in the range 8°C - 12°C.

The complete interior walls of this cabinet are lined with Metalised Melinex and no fluctuations in light intensity can be recorded in a horizontal plane and very little variation in a vertical plane. Plant trays in this cabinet are at a fixed level of 4'6" below the lamps which are 5 ft. 65W fluorescent tubes placed 3" apart. Supplementary illumination

is from a single 5 ft. 65W fluorescent tube. In some experiments the supplementary illumination in these cabinets was from five 40W incandescent light bulbs but these are specific cases and will be detailed in the text at the relevant points in Part IV.

Cabinets III and IV (C Type)

These cabinets are of the same basic design as Cabinet II and vary only in the details which are listed below.

The plant trays are lying 5 ft. below the lamp bank.

The fluorescent tubes are spaced 2"-3" apart and supplementary illumination is from two 5 ft. daylight fluorescent tubes.

The degree of temperature control is 0.5°C on either side of the desired value.

In addition to the heating and cooling systems previously described a spray humidifier is incorporated in the design. No high degree of control of humidity is possible but the atmosphere is not as drying as that found in the cabinets so far described and results in a reduction in the number of waterings which plants require each week.

Cabinets I - IV were built in Glasgow and are situated in the Botany Department and at the Garscube Research Laboratories.

Cabinets V, VI and VII (D Type)

These cabinets built to the specifications of the National Institute of Agricultural Engineering by R.K. Saxton (Sax-Air) Ltd are the most sophisticated in design and the least reliable in performance

of any of the cabinets used. A double bank of fluorescent tubes gives high intensities of illumination in these cabinets.

The estimated degree of control of temperature is ± 0.1°C of the desired value and of relative humidity ± 1%. In practice it was found that little control of relative humidity could be maintained and the control of temperature was seldom better that 2°C on either side of the selected value. Furthermore, the daily cycles of temperature were not consistent and in many cases fluctuations of 5°C between the mean temperature value for one day and the next were not uncommon.

Better degrees of control of temperature in these cabinets could only be achieved if the selected temperature was much above or much below ambient.

These cabinets do not have a built in means of supplying supplementary illumination for extending the day length with low intensity light and although the walls are lined with Metalised Melinex fluctuations in light intensity can be recorded on a horizontal plane with the highest intensities in the centre of the cabinets.

In addition to the experiments carried out in the growth environmental chambers some experiments were conducted under glass, using natural light and were subject to daily temperature fluctuations. These experiments and details of the growing conditions will be found in the text dealing with the particular experiments.

The duration of experiments varied but on average was 3-5 weeks

for the 'early' varieties and 10-12 weeks for the 'late' varieties.

In the garden pea, flowers are borne in the axils of the leaves. From the very earliest stages of development floriferous and vegetative axillary shoots are distinctly different and even if a flower abhorts the pedicel which carried the inflorescence does not senesce and can easily be used as an indicator that a flower was present at that node.

In all experiments the node number given as "the node of appearance of the first flower" is the node at which the very first flower occurred although this flower may have been incomplete and possibly consisted of a calyx only.

In a number of cases the first flower was ineffective in setting fruit and some experiments in addition to recording the node of appearance of the first flower have recorded the node of appearance of the first perfect flower, which is the first flower to be seen to be morphologically complete, and also the node at which the first fruit was produced.

The node at which the cotyledons are attached is always taken as being node (0) zero. In all the varieties tested nodes one and two support only scale leaves with node one usually just below and node two usually just above 'ground level'. The first true leaf appears at the third node and consists of a single pair of leaflets with or without a single terminal tendril. Leaves formed later can consist of up to three pairs of leaflets with one or two pairs of tendrils and a terminal tendril. In some cases a single leaflet of a pair may be replaced by a tendril.

Table I. Light Intensities and Temperature Control

Light Intensity (cal./cm.^{2}/min.)

Cabinet Type	Maximum	Minimum	Temperature Control
A	8.10×10^{-4}	7.72×10^{-4}	$\pm 1^{\circ}C$
B	1.17×10^{-3}	1.07×10^{-3}	$\pm 0.5^{\circ}C$
C	2.36×10^{-3}	2.08×10^{-3}	$\pm 0.5^{\circ}C$
D	3.15×10^{-3}	2.79×10^{-3}	$\pm 2^{\circ}C$ *

* Also subject to daily fluctuations as described in paragraph on D type cabinets.

III. RESPONSES TO PHOTOPERIOD AND VERNALISATION

Most of the work on flowering and the effects which the growing conditions have on the flowering process have been carried out using, as test species, those plants which show qualitative responses to changes in their environment. Relatively little, in comparison, has ever been done with plants where the pattern of growth and physiological behaviour, in response to alterations in the growing conditions, has had to be measured solely on a quantitative basis.

When experiments are designed to test the effects of various environments on the growth and development of plants, it becomes immediately apparent that strict environmental control must be established and maintained and that these conditions must be clearly defined if experimental results are to be accepted and the work is to assist in the understanding of the mechanisms involved in the changes which are being measured. These controls are of importance when considering qualitative changes but are of even greater importance when quantitative changes comprise the only measurable factors.

Much of the work on flowering, in response, particularly, to day-length, has been carried out under conditions which are highly unsatisfactory. Many workers when studying the effects of various daylengths on plant growth and development have subjected their plants at all times to high intensities of illumination.

When a true study of day-length effects is the subject under

investigation, plants should be grown in such a way that all plants, in a 24 hour period, receive the same or as near as possible the same total amount of incident energy. This is achieved by growing all plants under the same number of hours of high intensity illumination and extending this, where required, by light of low intensity to give a longer day. The low intensity light should be below that which will allow for active photosynthesis. As already mentioned in Part II, in the experiments, presently to be described, the supplementary illumination for extending the day length was supplied from one or two fluorescent tubes only. From the measures taken of dry weights of plants it will be seen that the total assimilation rates for plants grown under short day and long day conditions is the same.

Barber (1959) reported on the effects of different daylengths on the flowering behaviour of several varieties of garden pea. Plants of the variety Telephone were grown such that different groups received 8, 12, 16 and 24 hours illumination each day. The experiment was conducted in a glass house and the natural day length was extended using high intensities of light from both fluorescent and incandescent lamps. Plants grown under continuous light could have produced three times as much photosynthate per day as plants grown under 8 hours of illumination. No dry weights were given and so it is not possible to say whether or not the above hypothesis is correct, but it does leave a doubt concerning the interpretation of the results. Barber presented the results of his experiments as being the effect of daylength on flowering but did not consider the possibility that the effects measured

might be due to differing nutritional states in the various groups of plants or perhaps a combination of both.

It was thought essential at the beginning of this work to design and carry out some experiments in which the possibility of various interpretations of the results would be eliminated.

Experiment I. To test the effects of daylength on flowering in the garden pea varieties Massey and Greenfeast.

This experiment was conducted in cabinet I. All plants were grown in Peralite in green polythene plant bags and were given nutrient as described and also the routine maintenance as previously mentioned for all plants grown in this cabinet.

Seeds were planted directly into the moist Peralite. The planting date was 3/11/64, hereafter referred to as day zero (0). Three seeds were planted per pot, later to be thinned to one plant per pot. (In subsequent experiments this procedure was abandoned as it was found that a sufficiently high percentage of seeds always germinated and that the above method was, therefore, wasteful of time and materials. In later experiments a few extra pots were included in all sets as replacements).

The selected daylengths for this experiment were an 8 hour photoperiod and a 16 hour photoperiod, later referred as SD (short day) and LD (long day) respectively. The eight hours of supplementary illumination to give a long day immediately followed the eight hour period of high intensity light.

After planting all pots were placed in their respective day length conditions and for the duration of the experiment a constant temperature of 21°C was maintained.

Table II and Fig. I shows the growth of plants measured in centimetres from the third node to the apex. Measurements of height were taken at weekly intervals throughout the duration of the experiment commencing 14 days after planting.

Due to twisting in the stems of the plants it was not possible to take height measurements after 49 days.

Throughout this section of the work comparison of the means was carried out by the 't' test method. A significant difference in height was found between plants grown under long days and short days. Measurements taken 35 days after planting are shown in Table II.

Table III shows the dry weights of the plants at the close of the experiment, 65 days after planting.

Where a result was missing from the table this was given the value of the mean and the degrees of freedom were reduced by one. This procedure was adopted as standard practice throughout excepting in a few cases which are individually marked.

Although marked differences in dry weights within groups were noted, there was no significant difference between long day and short day groups of either of the two varieties under test.

Table IV gives information concerning the node of appearance of the first flower, the node of appearance of the first fruit and the total number of nodes formed at the close of the experiment.

Table II. Height of plants in cms. 35 days after planting

	Massey		Greenfeast	
Pot No.	LD	SD	LD	SD
1	20.0	14.0	20.5	17.2
2	18.5	14.3	20.0	16.5
3	23.2	14.5	25.3	16.4
4	20.9	13.3	19.2	15.8
5	19.9	15.8	21.0	16.2
6	13.5	17.5	23.0	17.4
7	13.0	15.9	21.2	14.0
8	14.9	16.3	21.1	17.5
9	16.2	14.5	19.3	16.5
10	15.8	16.0	13.8	16.5
11	15.8	15.1	20.0	18.0
12	18.2	15.5	20.3	15.1
Mean	17.49	15.22	20.39	16.43
S.E. of Mean	±0.905	±0.336	±0.773	±0.34

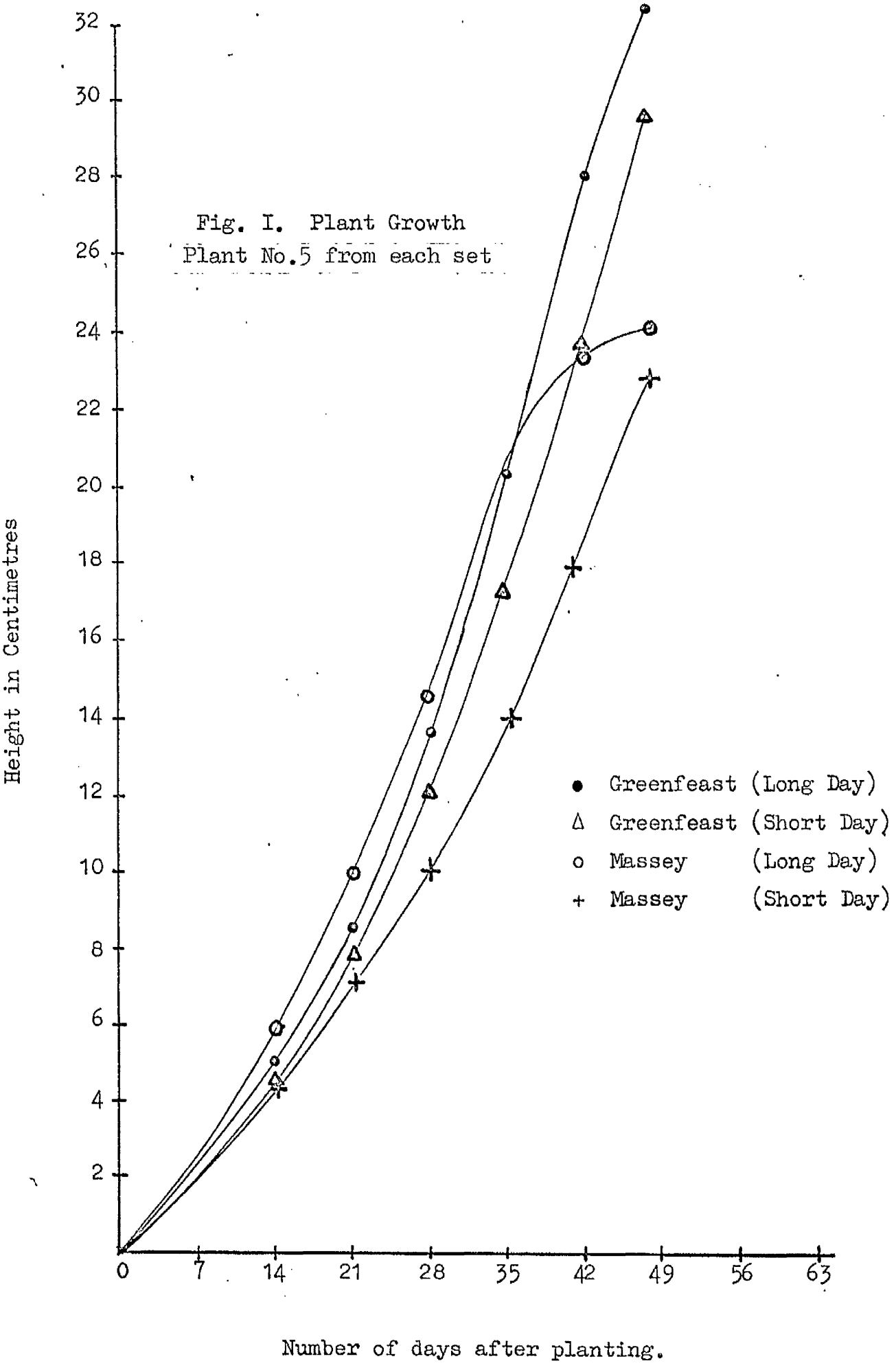


Fig. I. Plant Growth
Plant No.5 from each set
Height in Centimetres
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
7
14
21
28
35
42
49
56
63
Greenfeast (Long Day)
Greenfeast (Short Day)
Massey (Long Day)
Massey (Short Day)
Number of days after planting.

Table III. Dry weight of plants harvested at 65 days

grams/plant

	Massey		Greenfeast	
Pot No.	SD	LD	SD	LD
1	0.36	0.29	0.90	0.95
2	0.41	0.46	0.81	0.41
3	0.31	1.20	0.65	1.00
4	0.38	0.70	0.71	0.70
5	0.30	0.35	0.70	0.85
6	0.67	0.30	0.55	0.88
7	0.65	0.24	0.51	0.75
8	0.61	0.34	0.99	0.85
9	0.19	0.38	0.52	0.61
10	0.44	0.43	1.15	0.75
11	0.50	0.28	0.69	0.66
12	0.55	0.59	0.25	0.65
Mean	0.45	0.46	0.70	0.75
S.E. of Mean	±0.14	±0.24	±0.22	±0.15

Table IV. Node to first flower, node to first fruit and total nodes formed

Pot No.	Massey						Greenfeast					
	LD			SD			LD			SD		
	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes
1	10	15	17	10	15	18	22	22	25	23	No Fruit	28
2	10	15.4	17.9	10	12	15	21	21	24	25	"	27
3	10	14	18	10	16	18	21	21	25	22	"	26
4	10	16	18	10	16	18	22	22	25	23	"	27
5	10	15.4	18	10	14	17	22	22	25	24	"	28
6	10	16	20	10	15	17	22	22	25	24	"	27
7	10	17	18	10	15	18	22	22	25	22	"	25
8	10	15	17	10	15	18	21	21	25	22	22	28
9	10	14	18	10	14.9	19	22	22	25	23	No Fruit	23
10	10	16	18	10	16	18	21.3	21.3	24.6	23	23	29
11	10	15	17	10	15	18	20	20	24	24	No Fruit	28
12	9	16	18	10	15	17	19	19	23	22	"	22
Mean	9.9	15.4	17.9	10	14.9	17.58	21.27	21.27	24.63	23.08	22.5	26.50
S.E. of Mean	± 0.09	± 0.08	± 0.07	± 0	± 0.09	± 0.29	± 0.28	± 0.28	± 0.18	± 0.29	-	± 0.62

The growth rate of plants rapidly decreases after the start of fruit production and accounts for the variation in total number of nodes formed between the LD and SD Greenfeast plants.

As in previous tables, excepting for table IV in the case of column 2 of the short day Greenfeast, where a result was missing in the figures obtained the mean figure for the particular set was given and the degrees of freedom were reduced by one. The two results of fruit set in the short day grown Greenfeast are the exception rather than the rule and therefore cannot be taken as reflecting the performance of the population.

From table IV it can be seen that daylength has no effect on the first flowering node in the variety Massey and has only a slight although significant effect in the late variety Greenfeast with the plants grown under long days flowering on average approximately 0.9 nodes earlier. Short days in the variety Greenfeast have, however, a marked delaying effect on the node at which the first fruit is produced.

Table V shows the results of this experiment alongside the values obtained by Barber. In Barber's experiments the plants were grown at approximately 21°C during the day and approximately 17°C during the night and were subject to slight temperature fluctuations during the day and the normal fluctuations experienced from day to day. In this respect and also with respect to the previously mentioned light quantities and qualities, the growing conditions used by Barber varied from those used in this experiment and may account for the variation

in the results. The fact that the named variety Greenfeast might comprise two or more races with varying physiological behaviour cannot be discounted.

Table V. Experiment I:- Average of 11 or 12 plants

Barber:- Average of 6 plants

Node to first flower

	Exp. 1 SD	Barber SD	Exp. 1 LD	Barber LD
Massey	10 ±0	9.1 ±0.11	9.9 ±0.09	9.1 ±0.11
Greenfeast	23.08 ±0.29	29.4 ±0.32	21.27 ±0.28	22.6 ±0.32

The marked discrepancy appears in the late variety Greenfeast under short day conditions where a difference of over seven nodes between this work and the previous work can be seen.

Experiment II. To test the effects of daylength on flowering in the tall garden pea varieties Alaska and Telephone

This experiment was also carried out in cabinet I and the conditions were exactly the same as for the first experiment. Planting date (day zero) was 15.1.65. Due to the height attained by tall varieties and the difficulties involved in handling the material without causing damage no measurements of height were made in this experiment.

Table VI gives the measurements taken of the node of appearance of the first flower, the node of appearance of the first fruit and and the total number of nodes formed on the main axis.

The figures given in table VI for the total number of nodes formed are recordings taken of the total number of nodes formed at the time of senescence of the main axis. In the variety Alaska senescence occurred at about the 35th day after planting and in the variety Telephone at about the 90th day after planting. In both varieties senescence of the growing region began shortly after the formation of the first fruit.

In a number of plants, growth was continued by the outgrowth of hitherto dormant axillary buds. These axillary shoots supported two scale leaves before the formation of a true leaf at the third node. In most cases the first flower on the axillary shoot occurred in the axil of the first true leaf. This pattern of growth of axillary shoots with a flower appearing in the axil of the first true leaf

Table VI. Node to first flower, node to first fruit and total nodes formed

	Alaska						Telephone					
	SD			LD			SD			LD		
Pot No.	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes	First Flower	First Fruit	Total Nodes
1	9	14	17	10	15	18	27	28	29	19	20	24
2	10	13	18	10	13.7	18	30	30	31	21	21	25
3	10	13	18	9	13	18	31	31	33	20	21	24
4	10	13	19	9	11	15	33	33	33	23	23	26
5	10	12	17	10	15	17	29	30.3	35	20	23	25
6	10	14	17	10	16	19	28	29	32	22	22	25
7	10	12	15	10	15	18	33	33	34	23	23	25
8	10	14	17	9	12	16	33	33	35	21	22	26
9	10	13	18	10	12	17	28	29	31	22	22	27
10	10	13	17	10	15	19	30	30	32	19	19	22
11	10	16	21	9	15	20	31	31	34	22	23	26
12	9	13	16	9	12	17	29	29	31	21	23	26
Mean	9.83	13.33	17.5	9.58	13.72	17.66	30.16	30.52	32.5	21.08	21.83	25.08
S.E. of Mean	± 0.11	± 0.30	± 0.43	± 0.14	± 0.48	± 0.39	± 0.60	± 0.49	± 0.53	± 0.39	± 0.38	± 0.47

only occurred if the main axis had commenced flowering. If the main axis senesced before flowering commenced the first flower on the axillary shoot appeared at a higher node.

Table VII Average results of experiment II and results obtained by Barber (1959)

Node to first flower

	Expt. II SD	Barber SD	Exp. II LD	Barber LD
Alaska	9.83 ±0.11	10.1 ±0.12	9.58 ±0.14	10.0 ±0.12
Telephone	30.16 ±0.60	29 ±0.33	21.08 ±0.39	21.9 ±0.33

Experiment II:- Average of 12 plants

Barber 1959:- Average of 6 plants or less

The values found in this experiment are not different from Barber's results and from experiment II it can be seen that plants of the late variety Telephone flower and set fruit significantly earlier under long day growth conditions than under short day conditions. The significant variation in the total number of nodes formed between the LD and SD grown Telephone plants will be due to the senescence of the main axis commencing 2 - 4 nodes after the formation of the first fruit and the decreased growth rate occurring at the time of fruit set.

Experiment III. To test the effects of daylength and vernalisation on the garden pea varieties Massey and Greenfeast

This experiment was conducted in cabinet I and the cultural techniques and general procedures were the same as, previously described, for experiments I and II.

In addition to the study of the effects of daylength on flowering, plants, during the course of this experiment, were given periods of cold treatment for either 10 days or 20 days at 10°C.

All seeds were planted on the same day (25.4.65), hereafter referred to as day zero. All seeds were soaked for six hours. After this time any seeds which had not shown signs of having started to imbibe were discarded. The 'viable' seeds were planted and the experiment was divided into the groups which were to receive the various treatments. Plants which were to receive no vernalisation treatment were placed in their respective daylength conditions of either 8 hour or 16 hour photoperiods. Plants to receive a cold treatment were placed in 10°C incubators into which had been introduced small fluorescent lights, operated through timeclocks and therefore able to supply the same photoperiods as the plants would later receive in the growth cabinet.

The intensity of light in the incubators was, of necessity, of a low intensity.

After the period of 10 or 20 days vernalisation the plants were

removed from the incubators and placed in their respective daylength conditions in cabinet I.

The chronological age of all plants throughout the experiment was the same but their physiological age, as measured by their degree of development, varied markedly, with the plants which had received vernalisation treatments being physiologically younger than their non-vernalised counterparts.

Chronological time in this experiment is therefore of little significance.

Figure II shows the typical growth pattern of vernalised and non-vernalised plants. No significant differences could be detected between plants grown in long days and those grown in short days, with regard to plant heights, over the period during which these measurements were taken.

Table VIII gives the results of this experiment on node to first flower and node to first fruit in the two varieties tested.

Table IX gives the average results of this experiment on node to first flower compared with that found by Barber. In Barber's experiments the vernalisation treatment was for a three week period at $4^{o}C$ immediately following the soaking of the seed.

From the results shown in table VIII it can be seen that the late variety Greenfeast shows a positive response to a period of cold treatment by initiating flowers at a slightly lower average node. Also from these results it can be seen that a period of cold treatment may slightly delay flowering in the early variety Massey, and may also

Fig. II. Growth of plants of the variety Greenfeast.

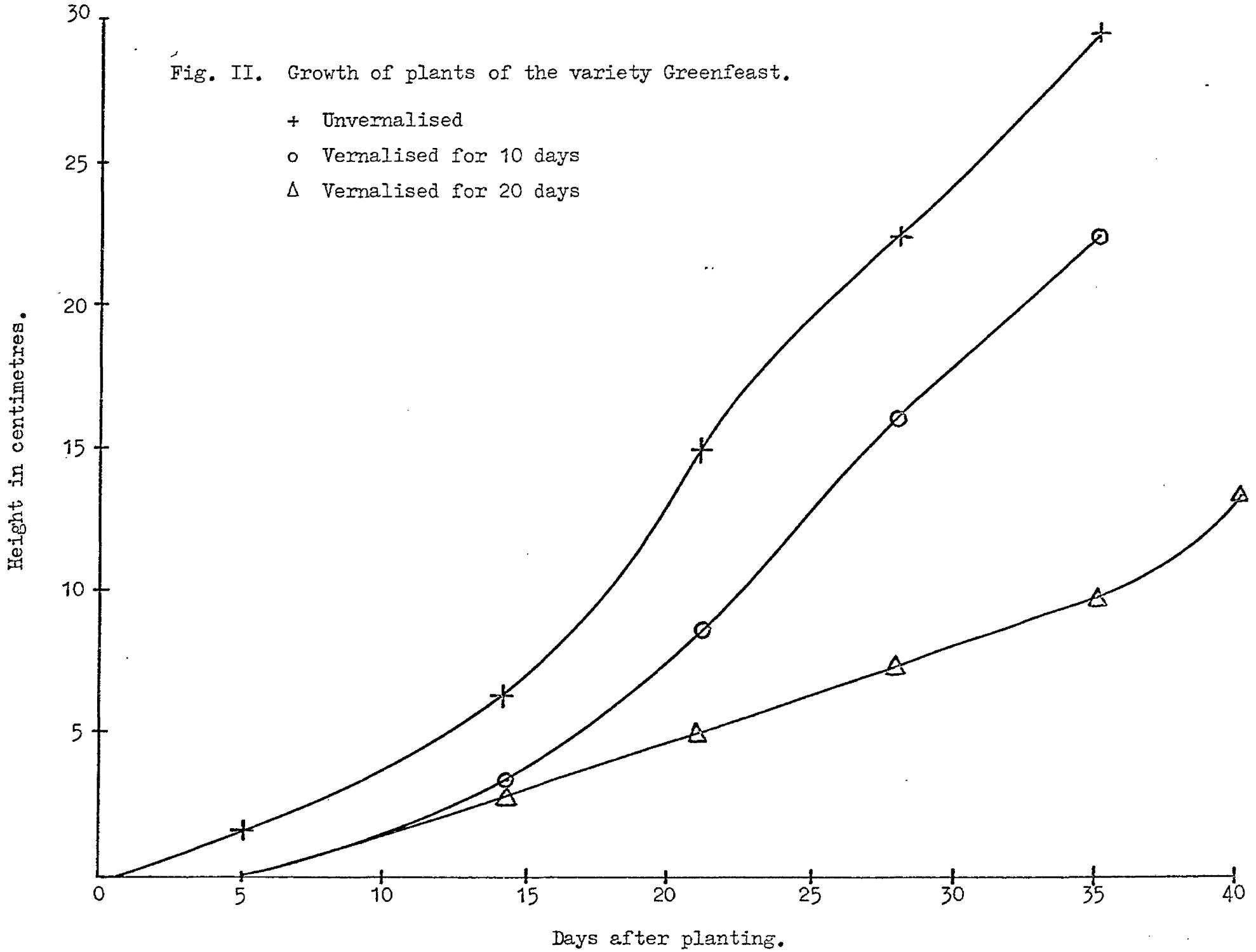


Table VIII. Node to appearance of first flower and node of appearance of first fruit

	Massey						Greenfeast					
	Long Day			Short Day			Long Day			Short Day		
Pot No.	UV	10V	20V	UV	10V	20V	UV	10V	20V	UV	10V	20V
1	10	10	10	9	10	11	21	19	20	25	23	22
2	10	10	12	10	10	13	22	18	19	25	23	22
3	10	9	13	10	10	13	21	20	20	25	23	21
4	10	11	11	10	10	11	20	21	19	26	22	23
5	10	11	10	10	11	10	20	21	19	24	21	22
6	9	10	13	10	10	10	21	19	20	23	24	22
7	10	10	13	10	10	11	19	20	20	26	21	24
8	9	10	12	10	11	12	22	20	21	25	21	21
9	10	10	10	10	10	10	22	21	18	25	22	22
10	10	10	11	10	10	10	20	18	20	23	23	20
Mean	9.8	10.1	11.5	9.9	10.2	11.1	20.8	19.7	19.6	24.7	22.3	21.9
S.E. of Mean	±0.13	±0.18	±0.40	±0.09	±0.13	±0.38	±0.33	±0.37	±0.26	±0.33	±0.33	±0.35
1	13	15	15	13	16	18	21	19	20	25	23	22
2	13	14	15	17	16	19	22	18	19	27	23	24
3	12	13	16	15	16	19	21	20	20	26	24	23
4	12	13	16	15	17	17	20	21	19	26	22	23
5	11	15	14	16	15	17	20	21	20	25	22	22
6	12	14	14	15	18	19	21	20	20	24	24	22
7	13	14	15	17	17	18	20	20	20	26	21	24
8	13	13	13	14	17	16	22	20	21	25	22	22
9	13	15	15	14	19	17	22	21	19	25	22	22
10	12	13	14	13	18	15	20	19	20	24	23	20
Mean	12.4	13.9	14.7	14.9	16.9	17.5	20.9	19.9	19.8	25.3	22.6	22.4
S.E. of Mean	±0.22	±0.27	±0.30	±0.46	±0.18	±0.43	±0.28	±0.46	±0.19	±0.30	±0.30	±0.37

First Flower

First Fruit

Where UV = non vernalised; 10V = vernalised 10 days; 20V = vernalised 20 days

Table IX. Average results of Experiment III on node to first flower and results obtained by Barber 1959

	Massey					Greenfeast				
	Experiment III			Barber		Experiment III			Barber	
	UV	10V	20V	UV	21V	UV	10V	20V	UV	21V
Long Day	9.8 ±0.13	10.1 ±0.18	11.5 ±0.40	9.1 ±0.11	9.8 ±0.11	20.8 ±0.33	19.7 ±0.37	19.6 ±0.26	22.6 ±0.32	18 ±0.32
Short Day	9.9 ±0.9	10.2 ±0.13	11.1 ±0.38	9.1 ±0.11	10 ±0.11	24.7 ±0.33	22.3 ±0.33	21.9 ±0.35	29.4 ±0.32	22 ±0.32

Experiment III:- Average of 10 plants

Barber 1959:- Average of 6 plants

Where UV = non vernalised

10V = vernalised for 10 days

20V = vernalised for 20 days

21V = vernalised for 21 days

delay the node at which the first fruit is produced.

The non-vernalised control plants show approximately the same performance as the plants in Experiment I and show the same trend as the results from Barber's experiment excepting in the case of the short day Greenfeast where the plants in this experiment again flowered markedly earlier than was found by Barber.

Except for the case mentioned above the results of this experiment do not significantly vary from the previous work.

Experiment IV. To test the effects of day-length and vernalisation on the garden pea varieties Alaska and Telephone

This experiment was exactly the same as experiment III but using two tall varieties of pea.

The experiment commenced on 17.6.65 but, as in experiment III, the chronological age of the plants is of little significance due to the difference in growth rate between plants at low temperature and plants at high temperature in the early weeks of the experiment.

Table X shows the results obtained from this experiment on the node of appearance of the first flower and node of appearance of the first fruit.

Table XI compares the results of this experiment with the results obtained by Barber in 1959.

From the results of this experiment it can be seen that a period of cold treatment elicits a positive response in the variety Telephone with vernalised plants grown under long day conditions flowering on average 2 - 3 nodes earlier than non-vernalised plants and vernalised plants grown under short days flowering some 5 - 8 nodes earlier than non-vernalised plants.

In the early variety Alaska there is a slight but significant delay in flowering following a period of vernalisation.

The results of this experiment, as can be seen from Table XI, are very close to those obtained by Barber and the control plants in this

Table X. Node of appearance of first flower and node of appearance of first fruit

	Alaska						Telephone					
	Long Day			Short Day			Long Day			Short Day		
Pot No.	UV	10V	20V	UV	10V	20V	UV	10V	20V	UV	10V	20V
1	10	10	11	9	10	11	19	18	18	30	33	26
2	10	10	11	10	10	11	23	18	17	31	23	19
3	10	10	11	10	10	11	22	19	18	31	27	24
4	10	10	10	10	10	11	19	18.8	17	28	19	21
5	10	10	11	10	10	11	21	20	19	29	23	22
6	10	10	11	10	9	12	22	19	20	33	22	21
7	9	10	11	10	10	11	20	19	17	31	27	20
8	10	10	9	10	10	10	21	18	17	30	26	23
9	10	10	11	9	10	10	19	18	17	29	23	22
10	10	10	11	10	10	11	20	20	18	29	22	21
Mean	9.9	10.0	10.7	9.8	9.9	10.9	20.6	18.8	17.8	30.1	24.5	21.9
S.E. of Mean	±0.09	±0	±0.21	±0.13	±0.09	±0.18	±0.45	±0.25	±0.47	±0.46	±1.22	±0.64
1	13	14	16	15	14	14	20	18	18	30	34	27
2	12	14	14	15	14	13	23	18	18	31	25	22
3	13.2	14	16	16	14	14	22	20	20	31	27	24
4	13	13	13	14	13	15	19	19	17	29	19	21
5	14	14	15	16	12	15	22	20	19	29	23	22
6	12	12	16	16	13	13	22	20	21	33	22	21
7	13	15	14	17	15	12	20	19	18	31	27	21
8	13	14	14	12	14	16	21	18	19	30	26	23
9	15	14	17	12	14	13	20	18	17	29	23	22
10	14	15	13	13	14	13	21	20	18	30	23	21
Mean	13.2	13.9	14.8	14.6	13.7	13.8	21.0	19.0	18.5	30.3	24.9	22.4
S.E. of Mean	±0.28	±0.27	±0.44	±0.56	±0.26	±0.38	±0.39	±0.45	±0.40	±0.39	±1.28	±0.60

First Flower

First Fruit

Where UV = non vernalised; 10V = vernalised 10 days; 20V = vernalised 20 days

Table XI. Average results of Experiment IV on node to first flower and results obtained by Barber 1959

	Alaska					Telephone				
	Experiment IV			Barber		Experiment IV			Barber	
	UV	10V	20V	UV	21V	UV	10V	20V	UV	21V
Long Day	9.9 ±0.09	10.0 ±0	10.7 ±0.21	10.1 ±0.12	10.3 ±0.12	20.6 ±0.45	18.8 ±0.25	17.8 ±0.47	21.9 ±0.33	18.7 ±0.33
Short Day	9.8 ±0.13	9.9 ±0.09	10.9 ±0.18	10.2 ±0.12	10.1 ±0.12	30.1 ±0.46	24.5 ±1.22	21.9 ±0.64	29.0 ±0.33	21.4 ±0.33

Experiment III:- Average of 10 plants

Barber 1959:- Average of 6 plants

Where UV = non vernalised

10V = vernalised for 10 days

20V = vernalised for 20 days

21V = vernalised for 21 days

experiment give results which are not significantly different from those obtained in Experiment II.

The high degree of variability amongst plants of the variety Telephone given the shorter vernalisation period and subsequently grown in short days may be due to the period of vernalisation being on some threshold level with individuals varying in their response to the treatment.

The four experiments described in this section of the thesis were the foundation for future experimentation and formed the basis of the controls to be used in future work.

From the results obtained, it is possible to say, with certainty, that, for the four varieties tested, late varieties of garden pea are -

(1) quantitatively long day photoperiodically sensitive;

(2) respond to a period of vernalisation by initiating flowers at a lower average node;

(3) under short day conditions, respond more markedly to a period of vernalisation (from an analysis of variance carried out on the data obtained and shown in Tables VII and X);

(4) the tall variety responds more markedly to long days than does the dwarf variety; and

(5) the tall variety responds more markedly to a period of vernalisation, particularly under short day conditions, than does the dwarf variety;

and early varieties of garden pea are -

(1) non-photoperiodically sensitive; and

(2) respond negatively to a period of vernalisation by initiating flowers at a slightly higher average node.

A consideration of the node at which the first fruit is produced indicates that this phenomenon is a varietal characteristic. Generally speaking, in the early varieties the first fruit is produced from the fourth or fifth flower irrespective of temperature or daylength, and in the late varieties the first fruit is produced from the first flower or the flower directly above. In the case of the variety Greenfeast it would appear that fruit production is day-length sensitive with short days considerably delaying the node at which the first seed pod is produced. In the variety Telephone day-length has no effect on fruit production relative to the point at which this occurs after flowering has commenced.

In most respects the results obtained from these experiments do not vary significantly from those obtained by previous workers and the one marked variance, in the case of the dwarf, late variety, Greenfeast may be simply that Barber failed to notice or record the small abhortive inflorescences formed when these plants commence flowering under short-day conditions.

All experiments were conducted under controlled conditions with the environment clearly defined and easily reproducible. The true effects of daylength and of vernalisation can be the only interpretation of the results.

IV. EFFECTS OF LIGHT QUANTITY AND QUALITY ON FLOWERING

In the course of casual observations made on the growth, development and ultimate flowering of plants being grown in greenhouses or in the open for the purposes of obtaining seed, it was noted that the general vigour of the plants was better and in a number of cases the node at which the first flower was produced was lower than was observed in plants grown under approximately the same day-length conditions in the growth chambers.

A number of different interpretations of these observations are possible and it was decided to list the possibilities and attempt to test these singly or in desired combinations.

The first obvious factor is the intensity of light under natural conditions compared with the light intensity found in the growth cabinets. The highest intensities of artificial light were to be found in the cabinets of the D type. The light intensity in these cabinets was approximately a quarter of that of an average sunny day and in the other cabinets was less. Although many plants are photosynthesising at their maximum rate when the sunlight is only about one fifth of the maximum intensity (Salisbury, 1963), it is necessary to consider the effect of the intensity of light on flowering and the implication that a nutritional factor is involved in this phenomenon.

The second consideration must be the spectral composition of daylight compared with artificial light and possibly also the variation in

the spectral composition of daylight at different times during the day. The light from fluorescent tubes gives little in the red and far red end of the spectrum and by far the most of the light is in the blue wavelengths.

The third factor to be considered is the natural daily fluctuations in temperature. In the growth cabinets a constant temperature is maintained throughout both day (lights on) and night (lights off) whereas, as a general rule, under natural conditions, higher temperatures are found during the day and lower temperatures during the night.

The fourth and final difference between the plants grown for seed and those used in the experiments described in Part III lies in the fact that experimental plants were grown in Peralite and were given nutrient solution while seed plants were grown in soil. The plants grown in soil became nodulated but the plants grown in Peralite produced no nodules.

Experiment V. To study the effects of light intensity on the growth and flowering of the garden pea varieties Massey, Alaska, Greenfeast and Telephone

This experiment was carried out in a cool greenhouse during the summer of 1965. Ten plants of each variety were given each treatment and the total experiment was repeated. No variations were detectable between the two experiments and the tables of results are set down showing the mean value of twenty plants per treatment.

Vernalisation treatments were also included in this experiment and all seeds were initially planted in seed trays in Peralite after soaking in distilled water for six hours. Plants receiving cold treatments were placed in cooled incubators at 10°C for three weeks. The natural day length was approximately 18 hours and fluorescent lights were placed in the cooled incubators, as previously described, to give an 18 hour photoperiod. At the close of the vernalisation period five or six nodes had fully expanded. Plants receiving no cold treatment were grown in the seed trays in Peralite in the greenhouse until five nodes had fully expanded. At this stage these seedlings were planted out into 10" whalehide pots, in soil, in the greenhouse with seven seedlings per pot later to be thinned to five plants per pot. The same planting out procedure was carried out with the other plants when the vernalisation treatment had been completed.

The various light intensities were achieved by surrounding the pots with layers of white muslin. From measurements taken it was found that

the muslin decreased the total amount of light reaching the plants but did not alter the composition of the light.

Varying numbers of layers of muslin were placed round and over the groups of plants to give the following proportional light intensities. Control plants with no covering received 100% available illumination, other groups received 50% and 25% of the total available light and heavily shaded plants received only 10% of the total available light.

Temperature measurements inside the covers showed no variations between the various treatments.

All plants became nodulated, and plants with no covering grew vigorously. Heavily shaded plants grew more slowly and almost all deaths, amongst plants before flowering commenced, were in these groups.

A total of 640 plants were incorporated in this experiment. No significant differences were detectable within groups for node to flowering. The Tables XII (a), (b), (c) and (d) give the average results of the particular treatments.

A number of interesting observations can be made on these results. The most striking observation is the reduction in dry weights of plants under shade. Between 10% and 100% illumination a factor of approximately X 4 is measured excepting in the case of vernalised plants of the two early varieties where the difference is less. In relation to their overall final size, early varieties have large reserves of carbohydrate in the cotyledons and this may account for results noted in vernalised Massey and Alaska. One hypothesis might be that after or during

Table XII. Node of appearance of first flower, node of appearance of first fruit and total dry weight per plant of four varieties of garden pea grown under four intensities of illumination, with or without vernalisation

XII(a) Variety Massey

	UV 10%	UV 25%	UV 50%	UV 100%	V 10%	V 25%	V 50%	V 100%
First Flower	10 ±0	9.9 ±0.09	9.8 ±0.24	9.9 ±0.09	10.88 ±0.31	11.25 ±0.33	11.5 ±0.27	11.1 ±0.41
First Fruit	- ±	18 ±1.16	16.8 ±0.83	14.2 ±0.70	12.5 ±0.33	11.63 ±0.41	11.5 ±0.63	11.5 ±0.56
Dry Weight	0.86 ±0.22	1.94 ±0.21	3.25 ±0.38	3.85 ±0.34	1.15 ±0.19	0.715 ±0.27	1.47 ±0.44	1.86 ±0.11

Where V = vernalised

UV = non vernalised

and percentages indicate amount of total available light incident on the plants.

XII(b) Variety Alaska

	UV 10%	UV 25%	UV 50%	UV 100%	V 10%	V 25%	V 50%	V 100%
First Flower	9.33 ±0.33	9.7 ±0.29	9.5 ±0.34	9.86 ±0.52	10.63 ±0.26	10.38 ±0.18	9.63 ±0.26	10.75 ±0.25
First Fruit	9.33 ±0.33	9.7 ±0.29	9.5 ±0.34	10.14 ±0.57	10.63 ±0.26	10.38 ±0.18	9.63 ±0.26	10.75 ±0.25
Dry Weight	0.83 ±0.20	1.00 ±0.31	1.21 ±0.33	3.35 ±0.19	0.69 ±0.09	0.82 ±0.38	1.28 ±0.71	1.92 ±0.44

Table XII (contd)

XII(c) Variety Greenfeast

	UV 10%	UV 25%	UV 50%	UV 100%	V 10%	V 25%	V 50%	V 100%
First Flower	18.49 ±0.77	17.3 ±0.83	17.5 ±1.5	17.61 ±1.11	16.5 ±0.19	16.83 ±0.13	16.25 ±0.31	15.83 ±0.29
First Fruit	20.2 ±1.21	17.8 ±0.74	18.5 ±0.71	17.61 ±1.11	16.63 ±0.64	20 ±0.46	17.36 ±0.82	15.83 ±0.29
Dry Weight	2.15 ±0.40	2.77 ±0.25	3.76 ±0.61	7.97 ±0.47	2.24 ±0.72	2.68 ±0.50	4.01 ±0.93	8.23 ±0.74

XII(d) Variety Telephone

	UV 10%	UV 25%	UV 50%	UV 100%	V 10%	V 25%	V 50%	V 100%
First Flower	15.75 ±0.25	17 ±0.37	15 ±0.36	14.75 ±0.25	15.8 ±0.22	15 ±0.42	15.8 ±0.29	15.25 ±0.25
First Fruit	18.5 ±0.29	17.8 ±0.70	18.6 ±1.32	15.6 ±0.5	16.63 ±0.58	15.6 ±0.53	18.45 ±1.13	15.25 ±0.25
Dry Weight	4.69 ±0.18	4.401 ±0.24	5.92 ±0.22	15.59 ±0.48	3.2 ±0.27	4.09 ±0.30	6.83 ±0.54	12.34 ±0.25

vernalisation food reserves in the cotyledons are mobilised and are available to the growing plant. The same may be true for the late varieties but due to their considerably longer period of growth all food reserves in the cotyledons are finally released to the plants either with or without enhanced mobilisation due to vernalisation.

The node to the appearance of the first flower is unaffected by shading or at best is very slightly delayed. The node of appearance of the first fruit is slightly delayed in some varieties under shade conditions. Significant differences in node to first flower and first fruit could only be detected in a very few cases and was in all these cases only slight. Considering the treatments overall it can be said that shading does not affect the node at which the first flower appears but may slightly delay fruit production and that vernalisation, in the late varieties, under long photoperiods has little or no effect.

Vernalisation elicits a slight response only in the late variety Greenfeast and has no significant effect in the variety Telephone but, since long days and cold treatment are to a degree interchangeable, under the long day conditions of growth during this experiment only a slight response to vernalisation would be expected.

A period of vernalisation slightly delays flowering in the two early varieties, although in the variety Massey it would appear later to enhance fruit production.

It is possible that the slight, but in this experiment not significant, delay in flowering, due to shading, might be greater under shorter photoperiods. The two late varieties Greenfeast and Telephone were

used in a similar experiment, but without the incorporation of a vernalisation treatment, during the winter months of 1965-66. Few plants survived to the flowering stage due to the low growth temperatures and shading but from the results obtained it was apparent that by lowering the light intensities during shorter photoperiods the node to the appearance of the first flower was not affected. Table XIII shows the results of this experiment. Average results only are shown and in most cases only four or five plants per treatment survived to the flowering stage.

Generally it can be accepted that shading does not affect the node at which the first flower appears at least over the range of intensities of illumination tested. The experimental plants in the long photoperiods flowered early and the node of appearance of the first flower in almost all cases was approximately the same as Barber had found under a photoperiod of 24 hours. The one exception is in the case of the non-vernalised plants of the variety Telephone where flowering commenced 2-3 nodes earlier than was found by Barber.

It has now been shown that light quantity does not affect flowering. The second factor to be examined is the quality differences between daylight and the artificial light used in the growth cabinets. As mentioned, the main differences lie in the increased red and far red light in daylight compared with fluorescent light and in the high proportion of light at the blue end of the spectrum in fluorescent light compared with daylight. The two suggestions that could be put forward

Table XIII. Node to the appearance of the first flower in two late varieties of garden pea under four different intensities of illumination

	10%	25%	50%	100%
Greenfeast	21.1 ±0.87	22.0 ±0.75	21.7 ±1.67	22.3 ±1.09
Telephone	23.5 ±1.22	23.2 ±1.17	24.7 ±0.74	24.5 ±1.33

The photoperiod was approximately nine hours.

are that red and far red light or both promote flowering or that blue light delays flowering. Experiments carried out in the B and C type cabinets were designed to test these hypotheses.

Experiment VI. To study the effects of red and far red light on flowering in garden peas.

The two late varieties Greenfeast and Telephone were used in this experiment. All plants were grown in green polythene plant bags, in Peralite, and were given the previously described routine maintenance. The experiment was carried out in Cabinet II. Groups of plants were also given the usual vernalisation treatment at 10°C for three weeks, immediately following six hours soaking in distilled water. The temperature in the cabinet throughout this experiment was maintained at 20.5°C. A repeat of this experiment also incorporated the application of gibberellic acid to groups of plants from each of the other treatments but this experiment will be described in detail in Part V.

Planting dates for plants to receive vernalisation and plants not to receive vernalisation were staggered such that all plants were at the same physiological age when the different light treatments were commenced in the growth cabinet. During the period of vernalisation plants were given an 8 hour photoperiod of low intensity light as previously described.

In the growth cabinet all plants were given an 8 hour period daily of high intensity illumination and a further 8 hours of supplementary illumination, in which the ratio of red/far red varied. Three supplementary light sources were used designated white, red and far red. For each light source the ratio of red/far red light is given with Table XIV. The results of the experiment are presented in a single

table although the experiment comprised two sections, i.e. white and red and red and far-red supplementary illumination. This system had to be adopted since the cabinet consists of only two compartments.

The starting dates for the two sections of the experiment were as follows.

White and red - 12th September, 1965.

Red and far-red - 6th December, 1965.

No significant differences could be detected within groups and the plants grown in red supplementary light behaved the same in both sections of the experiment.

The supplementary illumination for white and red light was supplied from a single fluorescent tube of the appropriate colour and for far-red light was supplied from five tungsten bulbs. The tungsten bulbs were wired through a variable resistance which allowed for the intensity of illumination to be adjusted to the same total energy as from the single fluorescent tube.

Table XIV shows the results of this experiment on node to first flower, node to first fruit and dry weights. The figures given are the average results of ten recordings. The ratios of red to far-red light are also shown with Table XIV.

From the results shown in Table XIV it is evident that supplementary light of the three types tested does not affect the node of appearance of the first flower. Node of appearance of the first fruit and the dry weights of plants also remain unaltered.

The possibility that high intensity light of these wavelengths may

Table XIV. Node to the appearance of the first flower, first fruit and the dry weights of two varieties of garden pea under three light wavelength regimes with and without vernalisation

	Greenfeast						Telephone					
	Vernalised			Non Vernalised			Vernalised			Non Vernalised		
	W	R	FR	W	R	FR	W	R	FR	W	R	FR
Node to First Flower	18.2 ±0.54	17.4 ±0.27	17.6 ±0.27	19.7 ±0.33	20.4 ±0.28	20.5 ±0.71	16.7 ±0.42	18.3 ±0.77	17.1 ±0.23	19.2 ±0.34	20.4 ±0.40	19.6 ±0.52
Node to First Fruit	18.2 ±0.54	17.8 ±0.41	17.8 ±0.28	19.8 ±0.34	20.5 ±0.50	20.5 ±0.71	18.3 ±1.47	20.0 ±0.92	17.7 ±0.29	20.7 ±0.56	22.7 ±0.73	21.6 ±0.73
Dry Weight	0.85 ±0.38	0.65 ±0.18	0.78 ±0.27	0.97 ±0.21	0.92 ±0.43	0.97 ±0.33	1.90 ±0.29	1.62 ±0.17	1.81 ±0.28	2.49 ±0.41	2.40 ±0.39	2.34 ±0.27

Where W = white supplementary illumination

R = red supplementary illumination

FR = far red supplementary illumination

Ratios of red/far red light:-

In white supplementary illumination	1.5/1
In red supplementary illumination	4/1
In far red supplementary illumination	0.7/1

affect the flowering node cannot be overlooked but it was not possible to test this experimentally.

On the assumption that increased levels of red and far red supplementary illumination does not promote flowering in garden peas the hypothesis could be put forward that the high proportion of blue light from fluorescent tubes has a delaying effect on flowering. An experiment was designed in an attempt to test this hypothesis.

Experiment VII. To study the effects of increased levels of high intensity blue light on the node of appearance of the first flower in four varieties of garden pea.

This experiment was carried out in cabinets of the C type and plants were grown in 5 x 3 Vacapots, as described in Part II. The temperature in the cabinets was maintained at 20°C. All plants were grown in a photoperiod of 8 hours of high intensity illumination. In one compartment the fluorescent tubes were standard alternating white and warm white and in the other compartment were alternating white and blue tubes. The starting date of this experiment was 14th March, 1967.

Table XV shows the results of this experiment on the node to first flower and node to first fruit in the four test varieties of Pisum. Average results of 20-25 plants are given.

From the results obtained it can be seen that there are no significant differences between treatments. The results are, however, peculiar in that they are indicative of long day conditions. Although the photoperiod in the growth chamber was set at 8 hours it was noted that the Chenopodium amaranticolor indicator plants did not flower. Careful examination of the cabinets showed that there was a slight light leak which could only just be detected with the human eye after a considerable time of exposure in the cabinet with the lights off. This observation indicates that both Chenopodium amaranticolor and the two late varieties of Pisum sativum are sensitive to very low intensities of illumination. The day-length in the cabinets, during this experiment, must, therefore, be regarded as a long day. These observations

Table XV. Node to the appearance of the first flower and first fruit in four varieties of garden pea grown under two different light regimes

	Massey		Alaska		Greenfeast		Telephone	
	White	Blue	White	Blue	White	Blue	White	Blue
Node to First Flower	9.72 ±0.13	9.54 ±0.14	9.87 ±0.14	9.78 ±0.097	22.16 ±0.24	22.55 ±0.33	22.14 ±0.39	22.87 ±0.40
Node to First Fruit	9.72 ±0.13	9.96 ±0.16	9.87 ±0.14	9.78 ±0.097	24.41 ±0.51	23.55 ±0.94	22.78 ±0.46	23.75 ±0.49

may indicate that only the low energy, phytochrome system influences the flowering pattern in peas and that the high energy system has no effect. Mohr (1962) reported that for the high energy system to operate relatively high intensities of illumination were necessary for relatively long periods of time. Experiments to test the validity of the above statement using varying daylengths of high intensity light were not carried out.

Attempts were made to regulate the Saxil cabinets to give temperature regimes in which day and night temperatures varied. The following regimes were fixed.

1. 20°C constant
2. 20°C day : 15°C night
3. 15°C day : 20°C night

The object was to study the effects of fluctuating temperature on flowering in the four test varieties of pea. The experiment was run twice but repeated failures in the lighting, heating and cooling systems of the cabinets invalidated all the results.

Experiment VIII. To study the effects on flowering of growing garden pea plants of the four test varieties in soil and in the standard Peralite with nutrient and to study the effect on flowering of the presence and absence of root nodules

This experiment was conducted under glass in a cool greenhouse during the summer months of 1966. Plants of the four test varieties were grown in 5" plastic pots in either soil, in which case all plants became nodulated, or in Peralite, where normally no root nodules are formed but where the formation of root nodules can be induced if the plants are given an aqueous blend of pea root nodules.

All plants which produced nodules did so at the same time. Table XVI gives the average results of ten plants on the node to first flower and first fruit. No significant differences within sets of plants could be detected.

No significant differences could be detected between treatments and it was concluded that the growing medium in all cases was adequate for healthy growth. In the two late varieties the node of appearance of the first flower was not different from the results obtained by Barber (1959) when growing the same varieties under a photoperiod of 24 hours. The node of appearance of the first flower in the late varieties was not as low as was noted in plants grown for seed purposes in the open. A possible explanation may be that in the greenhouse the temperature between day and night did not fluctuate as widely as in the field.

Table XVI. Node to appearance of first flower and first fruit in four varieties of garden pea grown in Peralite, with or without root nodules, and in soil

	Massey		Alaska		Greenfeast		Telephone	
	First Flower	First Fruit	First Flower	First Fruit	First Flower	First Fruit	First Flower	First Fruit
Soil	9.9 ±0.09	10.2 ±0.57	10 ±0	10 ±0	16.5 ±0.19	16.8 ±0.21	18.0 ±0.71	18.2 ±0.68
Peralite with Nodules	10 ±0	10 ±0	9.9 ±0.09	10 ±0	16.8 ±0.13	16.8 ±0.13	17.7 ±1.11	18.0 ±0.82
Peralite without Nodules	9.9 ±0.09	9.9 ±0.09	10 ±0	10 ±0	16.9 ±0.50	17.1 ±0.82	18.2 ±0.74	18.2 ±0.74

The experiments included in this section were attempts to study the effects of different environments on flowering in peas and to simulate the conditions which the plants experience in the field and under which conditions flower earlier than so far noted in controlled environments.

The results have failed to show any effects of growth medium, intensity of light and quality of light within the limits tested but have perhaps, to some extent, pointed towards the hypothesis put forward earlier that fluctuating temperatures between day and night may influence the flowering pattern in peas.

V. GIBBERELLIC ACID AND FLOWERING

During the course of the preliminary experiments described in detail in Part III of the present work it was repeatedly noted that when considering the two late varieties of pea the tall variety Telephone responded more markedly to variations in day length and to vernalisation under short days than did the dwarf variety Greenfeast. Such variations as were noted between the varieties might be varietal characteristics and it may be that other late varieties whether tall or dwarf might show similar performances to one or other of our test varieties. The readily discernable morphological difference between our two late varieties is that Greenfeast is a 'dwarf' and Telephone is a 'tall'. As discussed in the introduction to this work varieties of pea with a dwarf genotype can be given the phenotype of a tall variety with the application of gibberellic acid. A series of experiments was planned to test the effects of gibberellic acid on both dwarf and tall varieties of pea with regard to their performance in different day length regimes, in different light wavelength conditions and with and without cold treatments. The hypothesis put forward is that dwarf varieties made tall with the application of gibberellic acid will show a similar performance in growth pattern and flowering behaviour to natural tall varieties.

The waxy cuticle on pea plants makes the application of micro drops on the surface or in the axils of leaves very difficult. The rate of

absorption through the leaf surface is slow and the air currents and vibrations in the growth cabinets dislodge droplets. A simple and effective technique of applying the gibberellin was devised. The technique involved the insertion into the stems of pea plants of drawn glass capillaries. After insertion the required amount of gibberellin could be put into the capillary using a Hamilton micro synringe. GA_3 in aqueous solution was used in all experiments. The rate of uptake from the capillary was rapid, some 10 µl. in 6-8 hours, during which time there was little or no evaporation within the capillary and therefore all the applied gibberellin entered the plant. This method of application allowed for exact amounts to be administered and for exact amounts to be taken up by the plants. If repeated applications were required over a number of weeks it was found necessary to insert new capillaries about every two weeks due to callus tissue blocking the end of the tube.

Experiment IX. To study the effects on growth and flowering in Greenfeast and Telephone varieties of garden pea of applications of gibberellic acid under long day and short day growth conditions

The complete experiment was run twice. No significant difference between experiments was detected and the results shown are the combined results of both experiments and represent the means of twenty recordings for each treatment. The two planting dates were 1st November, 1965 and 2nd February, 1966. Both experiments were carried out in cabinet 'A'.

Seeds were planted, after six hours soaking in distilled water, into moist Peralite in 3" green polythene plant bags. The plant pots were then placed in their respective day length conditions of either 8 hour or 16 hour photoperiods. When the first true leaf had fully expanded glass capillaries were inserted into the stem of the seedling in the axil of the uppermost scale leaf.

Control plants also had capillaries inserted and were later given applications of distilled water. Insertion of glass capillaries has no effect on plants of either of the two varieties.

Applications of gibberellin were commenced one day after insertion of the capillary. Three gibberellin treatments were given, i.e. (1) No GA; (2) 1 μg. GA/week and (3) 10 μg. GA/week. This range was chosen as effectively covering, according to Lockhart and Gottscholl (1961),

the optimum gibberellic acid concentration for pea stem elongation of 3 µg./plant/week. i.e. 3 µg. of gibberellic acid applied at weekly intervals was sufficient to bring about the maximum possible internode elongation. Higher amounts of applied gibberellin were ineffective in bringing about further elongation.

No significant differences were detectable within the various groups in this experiment.

Table XVII and Fig. III gives the mean height of plants 21 days after planting. Height measurements could not be continued after 28 days due to twisting in the stems of some of the very tall plants. Final height of gibberellin treated plants at the close of the experiment was 7-9 feet.

In the dwarf variety Greenfeast the effects of gibberellin application were visible within 36 hours. Leaves formed after the application were lighter green, similar to the colour of the tall variety Telephone, and internodes were much extended.

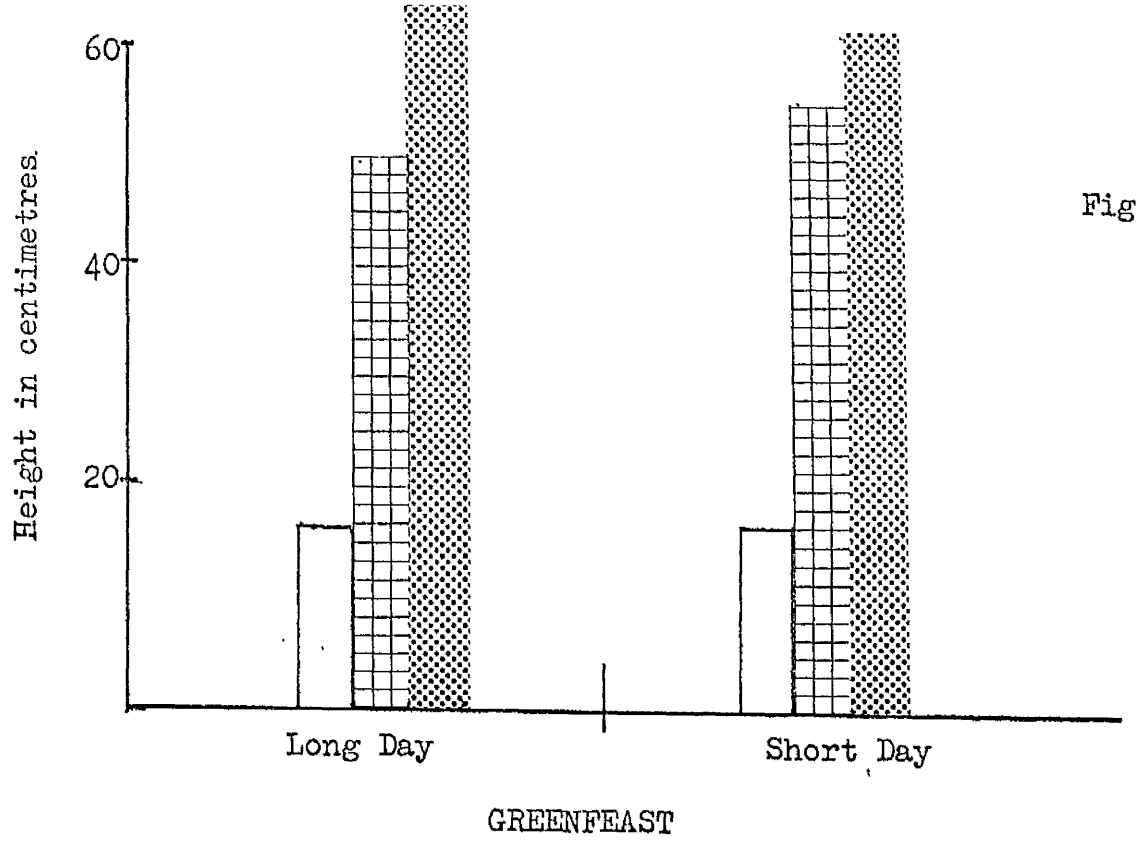
There were no significant differences between long day and short day grown plants in plant height at 21 days and no significant differences in the variety Telephone between the two levels of gibberellin treatment although plants of the variety Telephone after gibberellin treatment differed significantly from plants without applied gibberellic acid. In the dwarf variety Greenfeast there were significant differences between 10 µg. and 1 µg. and 1 µg. and no gibberellic acid.

Growth rate as measured by the number of nodes formed showed no significant differences between day length regimes, gibberellin treat-

Table XVII. Height of plants in centimeters from first true leaf to apex 21 days after planting

Mean of twenty plants

Var.	Day Length	G.A.	Height in cms.	S.E. of Mean
Greenfeast	Short Day	0	16.78	± 2.86
		1 µg.	47.95	± 4.80
		10 µg.	60.83	± 4.15
	Long Day	0	16.88	± 2.71
		1 µg.	44.03	± 3.92
		10 µg.	63.11	± 4.76
Telephone	Short Day	0	60.03	± 2.32
		1 µg.	72.22	± 4.72
		10 µg.	75.6	± 4.26
	Long Day	0	60.22	± 2.65
		1 µg.	75.58	± 2.73
		10 µg.	80.9	± 4.82



GREENFEAST

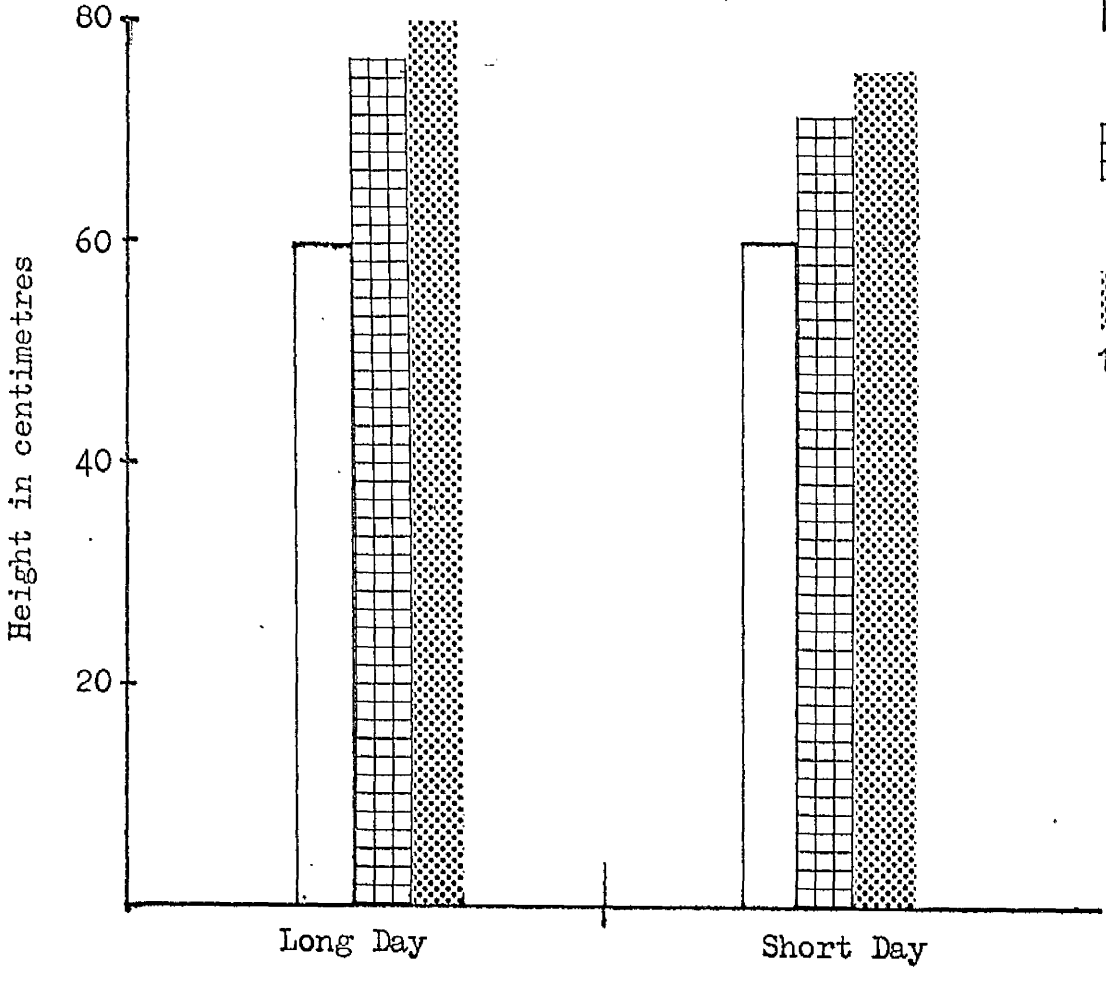
Fig. III

G.A. per week

0 µg.

1 µg.

10 µg.



TELEPHONE

ments or varieties when measured at any time before the start of flowering. Table XVIII gives the mean values obtained on node to first flower and first fruit in the two test varieties. Analysis of the results given in table XVIII shows the following:

(1) A significant delay in flowering and fruit set in short day grown plants of both varieties after treatment with gibberellic acid.

(2) A significant delay in fruit set in long day grown plants of both varieties after treatment with gibberellic acid.

(3) No significant differences between gibberellin treated and non-gibberellin treated plants of either variety in node to first flower after growth in long days.

(4) A significant difference in both node to first flower and node to first fruit in the variety Telephone grown under short days between each of the treatments with gibberellic acid.

(5) No significant difference on node to first flower in short day grown Greenfeast plants between the two levels of gibberellic acid treatment but, as stated in (1), a significant difference between gibberellin treated and non-gibberellin treated plants.

(6) A significant difference between gibberellin treatments in the node to first fruit of short day grown plants of the variety Greenfeast.

Table XVIII. Node to first flower and first fruit in Greenfeast and Telephone varieties of pea under long days and short days with three gibberellin treatments

Var.	Day Length	G.A.	Node to first flower	Node to first fruit
Greenfeast	Short Day	0	26.0 ± 0.19	27.16 ± 0.92
		1 µg.	35.0 ± 1.13	36.5 ± 1.44
		10 µg.	33.3 ± 1.41	39.0 ± 1.28
	Long Day	0	20.83 ± 0.16	21.3 ± 0.28
		1 µg.	21.83 ± 0.81	27.3 ± 0.61
		10 µg.	22.15 ± 0.77	27.3 ± 0.42
Telephone	Short Day	0	30.83 ± 0.58	33.6 ± 0.44
		1 µg.	33.00 ± 0.54	38.66 ± 0.33
		10 µg.	38.33 ± 0.48	41.5 ± 1.84
	Long Day	0	23.66 ± 0.39	25.13 ± 0.38
		1 µg.	24.66 ± 0.50	28.83 ± 0.70
		10 µg.	23.33 ± 0.71	29.83 ± 0.62

The effect of applications of gibberellic acid on flowering and fruit set in the two late varieties Greenfeast and Telephone are very striking. An experiment was designed to measure the effects of gibberellic acid on flowering in the two early varieties Massey and Alaska.

Experiment X. To study the effects of applications of gibberellic acid on growth and flowering in two early varieties of garden pea grown under long day and short day conditions

This experiment was a repeat in all respects of experiment IX excepting for the substitution of the two early varieties of pea Massey and Alaska for the Greenfeast and Telephone used in the previous experiment.

Seed was sown 24th June, 1966.

Each treatment was given to ten plants; Table XIX gives the mean value of plant heights 21 days after planting, and Table XX gives the mean value of node to first flower and node to first fruit in the two varieties.

When considering plant height it was found that the early varieties of pea behaved similarly to the late varieties after application of gibberellic acid. The natural tall variety Alaska became a 'super-tall' but showed no significant difference between the two levels of gibberellin application. The natural dwarf variety Massey showed significant differences between all three treatments as did the natural dwarf variety Greenfeast.

The node at which the first flower occurs is not affected by gibberellic acid at the concentrations tested but a significant delay in fruit production results after the application of the higher concentration of gibberellic acid.

As was found in the previous experiment the first formed flowers

Table XIX. Height of plants in centimetres from first true leaf to apex 21 days after planting

Mean of ten plants

Var.	Day Length	G.A.	Height in cms.	S.E. of Mean
Massey	Short Day	0	13.16	± 1.74
		1 µg.	39.98	± 3.70
		10 µg.	57.31	± 4.15
	Long Day	0	13.64	± 2.11
		1 µg.	39.44	± 4.08
		10 µg.	59.77	± 4.21
Alaska	Short Day	0	49.02	± 3.27
		1 µg.	60.08	± 4.22
		10 µg.	64.39	± 5.16
	Long Day	0	48.86	± 2.24
		1 µg.	63.17	± 4.29
		10 µg.	66.14	± 3.84

Table XX. Node to first flower and first fruit in Massey and Alaska varieties of garden pea grown under long days and short days with three gibberellin treatments

Mean of ten plants

Var.	Day Length	G.A.	Node to first flower	Node to first fruit
Massey	Short Day	0	9.6 ± 0.09	14 ± 0.30
		1 µg.	10 ± 0	17.5 ± 0.61
		10 µg.	10 ± 0	18.5 ± 0.54
	Long Day	0	10 ± 0	13 ± 0.27
		1 µg.	10 ± 0	15 ± 0.44
		10 µg.	9.8 ± 0.11	20 ± 0.61
Alaska	Short Day	0	9 ± 0.12	10.6 ± 0.14
		1 µg.	10 ± 0	11.8 ± 0.17
		10 µg.	9.6 ± 0.14	14.3 ± 0.29
	Long Day	0	9.6 ± 0.09	11.3 ± 0.22
		1 µg.	9.6 ± 0.10	13.6 ± 0.58
		10 µg.	9.6 ± 0.08	15 ± 1.01

following treatment with gibberellic acid were small, incomplete and abhortive. Usually the inflorescence consisted of a calyx only; in a few cases the corolla was also present but in no cases, in the early flowers, were stamens and carples formed.

In all four test varieties after applications of gibberellin the seed pod when formed usually contained only one fully developed seed and in some cases no sizeable seeds were formed. Plants which had received no gibberellic acid treatment usually produced seed pods containing 3-5 fully developed seeds.

The effects of gibberellic acid applications and day length were studied in experiments IX and X; experiments XI and XII were designed to test the effects of gibberellic acid and vernalisation. These experiments were carried out during the period when cabinet II was set to give varying ratios of red to far red supplementary illumination and this was incorporated into experiment XI.

Experiment XI. To study the effects of gibberellic acid on growth and flowering in Greenfeast and Telephone varieties of garden pea with and without vernalisation treatments in three different supplementary light regimes

Details of the setting of the cabinet, sowing of the seed and vernalisation were the same as for Experiment VI and are detailed on page 52.

The insertion of the glass capillaries and the applications of gibberellic acid were the same as for Experiment IX and are detailed on page 61.

Only one level of gibberellin was applied, i.e. 10 µg./plant/week.

As in the case of experiment VI the experiment composed two sections and also as in experiment VI the plants grown under red supplementary light behaved the same in both sections. The results of the two experiments are, therefore, presented in a single table. The dates of commencement of the two sections were 22nd March, 1966 and 4th July, 1966. The period of vernalisation was for three weeks at 10°C.

Three hundred and twenty plants were used in the complete experiment and the results shown are the mean values of ten plants per treatment. All plants were of the same physiological age when gibberellic acid was first applied and when the various supplementary light treatments were commenced.

Table XXI is a combined table giving the data obtained on dry weight and plant height.

An analysis of the data obtained on dry weights showed that there were no significant differences between the various supplementary light

Table XXI. (1) Dry weight of plants at close of experiment

(2) Height of plants from first true leaf to the twelfth node

Mean of ten plants

Var.		Light	G.A.	Dry Weight in Gms.	Height in cms.
Greenfeast	Non Vernalised	White	0	0.98 ± 0.21	20.7 ± 3.98
			10 µg.	2.44 ± 0.52	92.7 ± 7.23
		Red	0	1.22 ± 0.43	23.7 ± 4.19
			10 µg.	2.30 ± 0.19	98.4 ± 5.84
		Far Red	0	0.95 ± 0.33	23.3 ± 5.15
			10 µg.	2.15 ± 0.24	96.7 ± 8.35
	Vernalised	White	0	0.85 ± 0.38	17.9 ± 2.77
			10 µg.	2.30 ± 0.48	72.5 ± 4.63
		Red	0	0.65 ± 0.18	22.4 ± 4.63
			10 µg.	2.04 ± 0.22	79.5 ± 5.18
		Far Red	0	0.91 ± 0.27	19.6 ± 4.30
			10 µg.	2.32 ± 0.31	76.1 ± 3.92
Telephone	Non Vernalised	White	0	2.49 ± 0.41	92.1 ± 7.18
			10 µg.	2.84 ± 0.66	103.8 ± 9.33
		Red	0	2.40 ± 0.39	95.2 ± 6.89
			10 µg.	2.50 ± 0.23	116.4 ± 10.27
		Far Red	0	2.17 ± 0.28	96.6 ± 8.14
			10 µg.	2.77 ± 0.14	109.5 ± 8.70
	Vernalised	White	0	1.90 ± 0.29	85.7 ± 6.33
			10 µg.	2.60 ± 0.28	98.7 ± 7.72
		Red	0	1.60 ± 0.17	89.3 ± 4.46
			10 µg.	2.40 ± 0.37	101.2 ± 7.26
		Far Red	0	2.10 ± 0.23	89.9 ± 5.80
			10 µg.	2.73 ± 0.26	97.3 ± 7.21

regimes either with or without gibberellic acid but that there was a significant difference in the variety Greenfeast between plants which had received gibberellic acid and those which had not. Gibberellin treated plants had increased dry weights over their non-treated counterparts. In the variety Telephone the difference between gibberellin treated and non-gibberellin treated plants, in most cases, was not significant. An experiment employing larger numbers of individuals might show significant differences. There were no significant differences between vernalised and non vernalised plants.

Differences in height between gibberellin treated and non-gibberellin treated plants showed similar trends to the measurements obtained in experiment IX (see Table XVII). There were no significant differences between white, red and far red grown plants of either of the varieties under any of the other treatments. There was, however, the previously noted differences between gibberellin and non-gibberellin treated plants and also a significant difference between vernalised and non vernalised plants.

Table XXII shows the data obtained on node to first flower and first fruit during this experiment.

An analysis of the data obtained on flowering and fruit set showed a number of features already shown for other experiments.

(1) Significant differences were detectable between vernalised and non vernalised plants in first flowering node and consequently first fruiting node with vernalised plants flowering earlier.

Table XXII. Node to first flower and node to first fruit in vernalised and unvernalised plants of the varieties Greenfeast and Telephone with and without applied gibberellic acid and grown under three supplementary light regimes

Var.		Light	G.A.	Node to First Flower	Node to First Fruit
Greenfeast	Non Vernalised	White	0	19.3 ± 0.33	19.5 ± 0.36
			10 µg.	20.6 ± 0.41	27.5 ± 0.72
		Red	0	19.8 ± 0.28	19.9 ± 0.28
			10 µg.	21.3 ± 0.63	27.6 ± 0.81
		Far Red	0	20.5 ± 0.71	20.5 ± 0.71
			10 µg.	21.2 ± 0.52	27.3 ± 0.84
	Vernalised	White	0	18.2 ± 0.54	18.2 ± 0.54
			10 µg.	19.0 ± 0.61	24.7 ± 0.79
		Red	0	18.3 ± 0.27	18.3 ± 0.27
			10 µg.	20.5 ± 0.77	25.0 ± 0.56
		Far Red	0	18.6 ± 0.27	18.8 ± 0.29
			10 µg.	19.4 ± 0.35	25.0 ± 0.67
Telephone	Non Vernalised	White	0	19.7 ± 0.34	20.7 ± 0.41
			10 µg.	21.2 ± 0.51	25.5 ± 0.50
		Red	0	20.7 ± 0.40	22.7 ± 0.82
			10 µg.	22.6 ± 0.55	27.3 ± 0.70
		Far Red	0	19.6 ± 0.52	19.8 ± 0.52
			10 µg.	21.5 ± 0.78	22.1 ± 0.64
	Vernalised	White	0	16.7 ± 0.42	18.3 ± 0.35
			10 µg.	18.0 ± 0.39	24.9 ± 0.82
		Red	0	18.2 ± 0.77	20.0 ± 0.81
			10 µg.	19.8 ± 0.38	27.0 ± 0.76
		Far Red	0	17.1 ± 0.23	18.4 ± 0.16
			10 µg.	19.8 ± 0.31	26.2 ± 0.57

(2) Gibberellin treated plants set fruit significantly later than plants which had not received gibberellic acid applications.

(3) The three supplementary light regimes did not affect the node to first flower or first fruit in the four test varieties.

In this experiment although all plants were grown under long day conditions a slight but significant difference in node to first flower could be found between gibberellin and non-gibberellin treated plants. Plants which had received applications of gibberellic acid flowered slightly later than plants which received no gibberellin treatment. In experiment IX gibberellin and non-gibberellin treated plants, after growth in long days did not flower at significantly different nodes. The difference found in experiment XI is slight and it may be that the total of 120 individuals in experiment IX is too low to give a true indication of the performance of the whole population. At this stage it must, therefore, be said that gibberellic acid coupled with short days causes a marked delay in flowering in garden peas but with long days causes only a slight delay which may or may not be significant.

To summarise the features recorded in pea plants after treatments with gibberellic acid, it has been noted that applications of gibberellic acid cause a delay in flowering and fruit set, cause very marked increases in plant height, particularly in the case of the natural dwarf varieties and bring about increases in dry weight over non-treated plants.

The initial purpose of the study of gibberellic acid and its effect on flowering was to investigate the possibility that if a natural dwarf variety of pea, in this case the variety Greenfeast, were given the

phenotype of a tall variety it would show the same flowering pattern in response to day length and to vernalisation under short days of the natural tall variety, in this case the variety Telephone.

From the results so far obtained the natural dwarf variety when given applications of gibberellic acid assumes the phenotype of the tall variety and with regard to day length shows a similar trend to the natural tall variety in its flowering pattern.

A small experiment was designed to investigate the effect of a period of growth at low temperature coupled with short days on the flowering behaviour of Greenfeast plants given applications of gibberellic acid.

Experiment XII. To study the effects on flowering of gibberellic acid applications in the variety Greenfeast grown under long days and short days with and without vernalisation

The experiment was carried out in growth room 'A' and the experiment commenced on 23rd August, 1966. The temperature was maintained at 21°C and the photoperiods, as previously described, of 8 hours and 16 hours were used. Seeds were given the standard soaking for 6 hours in distilled water and were planted in 3" green polythene plant bags. The previously described arrangements for vernalisation and gibberellic acid application were carried out. The vernalisation period was for three weeks at 10°C. The vernalisation treatment was carried out in cabinet VI. Throughout the vernalisation period the plants were grown under short day conditions. One level of gibberellic acid application was used, that of 10 µg./plant/week.

Only the node to first flower and first fruit were recorded and the results shown in Table XXIII represent the mean values of ten recordings. As before no significant differences within groups could be detected.

The results obtained from this experiment and presented in Table XXIII indicate that there is a greater response to vernalisation in the induced tall plants than in the non-treated natural dwarf plants.

From the data collected from the experiments described in this section it can be said that with the variety Greenfeast induced tallness, brought about by applications of gibberellic acid, will cause the plants to behave in their flowering pattern similarly to the natural tall variety

Table XXIII. Node to first flower and first fruit in Greenfeast peas grown under long days and short days with and without gibberellic acid applications and vernalisation

Day Length		G.A.	Node to First Flower	Node to First Fruit
Long Day	Non Vernalised	0	20.8 ± 0.21	20.9 ± 0.21
		10 µg.	21.7 ± 0.34	29.3 ± 0.46
	Vernalised	0	18.6 ± 0.16	18.6 ± 0.37
		10 µg.	19.5 ± 0.18	23.2 ± 0.29
Short day	Non Vernalised	0	25.8 ± 0.32	27.4 ± 0.44
		10 µg.	32.9 ± 0.48	39.4 ± 0.40
	Vernalised	0	21.8 ± 0.29	22.8 ± 0.11
		10 µg.	24.7 ± 0.33	34.2 ± 0.53

Telephone. Careful application of varying total amounts or weekly applications of gibberellic acid may be able to produce Greenfeast plants of the same height, with the same dry weights and showing the same behaviour in response to day length and vernalisation as the natural tall variety Telephone.

VI. THE PEA COTYLEDONS AND FLOWERING

A paper by Sprent entitled the 'Role of the Leaf in Flowering of Late Pea Varieties' which appeared in the issue of Nature for 5th March, 1966 drew attention to the possible influence of the cotyledons on the flowering of late pea varieties. During the course of this work Miss Sprent showed that severe defoliation caused reduced vegetative vigour but did not affect flowering. If, however, the cotyledons were removed at an early stage a similar reduction in vegetative vigour was noted but also the node to the appearance of the first flower was reduced.

Barber and Paton (1952) proposed that the cotyledons of pea contain a flower inhibitor which is quantitatively transported to the apex during the first two weeks of growth.

Foliage leaves are obviously involved in the photoperiodic response but, the suggestion made by Miss Sprent is that, they affect flowering in a qualitative rather than in a quantitative way; possibly by supplying both phytochrome and a substrate for its action (Borthwick and Downs 1964).

In the work carried out by Miss Sprent the reduction in the node to the appearance of the first flower was by a similar number to that found after plants had been vernalised and, as mentioned in the introduction, the possibility does exist that the site of perception of a cold stimulus

might not be the plant apex but may be the cotyledons. If, in fact, the cotyledons were the plant parts receptive to vernalisation then it would be reasonable to assume that removal of these cotyledons would result in the plant being unable to respond to a period of growth at low temperature by initiating a flower at a lower node.

The work described in this section considers at length the effects on flowering of the removal of the pea cotyledons either as a single treatment or in conjunction with environmental and/or chemical treatments.

Experiment XIII. To investigate the effects on the node to appearance of the first flower of the removal of the cotyledons in four varieties of pea.

Plants of the four varieties, Massey, Greenfeast, Alaska and Telephone were sown in green polythene plant bags in Peralite on 17th June, 1966, after six hours soaking in distilled water.

The plants were grown in a 16 hour day, i.e. 8 hours of high intensity light followed by 8 hours of supplementary illumination, at 21°C in cabinet V. Exactly ten days after sowing, i.e. 240 hours, the Peralite was carefully cleared from around the cotyledons which were then removed by cutting with a scalpel as close to the main axis as possible, taking great care not to damage the main axis. The cotyledons were removed from ten plants of each of the varieties. The Peralite was then replaced and the plants were returned to the cabinet. Control plants, ten of each variety, were allowed to continue growing with their cotyledons attached.

After removal of the cotyledons the growth rate of decotylised plants, as measured by increments in height, was reduced compared with the controls but the plants appeared normal in all other respects. Table XXIV shows the data recorded on node to first flower and also node to first perfect flower i.e. the first flower observed to be morphologically complete. Information on final height and dry weights of plants is not recorded due to the fact that comparisons between decotylised plants and plants with cotyledons could

Table. XXIVA. Node to first flower in four varieties of pea grown under long days with and without cotyledons.

	Massey		Alaska		Greenfeast		Telephone	
	C	DC	C	DC	C	DC	C	DC
1	10	10	10	10	20	22	22	23
2	10	10	10	10	22	22	23	20
3	10	10	10	10	22	23	23	20
4	10	10	11	9	21	20	21	21
5	10	10	10	9	22	20	20	22
6	9	10	9	9	21	21	22	20
7	10	10	9	10	20	22	22	21
8	9	10	10	10	22	19	24	22
9	10	9	10	10	22	22	20	22
10	10	10	9	10	21	19	20	21
Mean	9.8	9.9	9.8	9.7	21.3	21.0	21.7	21.2
S.E. of Mean	±0.12	±0.09	±0.18	±0.14	±0.23	±0.41	±0.41	±0.43

Where C = with cotyledons

and DC = decotylised, i.e. without cotyledons

Table XXIVB. Node to first perfect flower in four varieties of pea grown under long days with and without cotyledons

Mean values of ten recordings

	Massey		Alaska		Greenfeast		Telephone	
	C	DC	C	DC	C	DC	C	DC
Mean	10	9.9	10.1	9.8	26.6	26.1	22.8	21.9
S.E. of Mean	±0	±0.09	±0.11	±0.13	±0.61	±0.58	±0.27	±0.14

not be reasonably made since decotylised plants of the two late varieties were some 3 - 5 weeks later in flowering due to reduced growth rate and control plants by this time had produced fruit and the main axis had senesced. Node to first fruit is not recorded in this experiment.

Table XXV shows the height measurements taken when plants were 20 days old i.e. 10 days after the removal of cotyledons in the decotylised group.

The data obtained indicates that while removal of the cotyledons at ten days markedly affects the vegetative vigour of the plants in that plants without cotyledons are much smaller the node to the appearance of the first flower and first perfect flower are not significantly different between plants with and those without their cotyledons. The rate of production of new nodes is also slowed down after removal of the cotyledons and although no measurements were taken of this it was noted that to reach the flowering node decotylised plants had to be grown for between three and five weeks longer than their respective controls with cotyledons still attached.

Considering the hypothetical flower inhibitor present in the cotyledons and which Barber and Paton (1962) claim is quantitatively transported to the apex during the first two weeks of growth it was decided to remove cotyledons at varying times after imbibition of the seed and to follow the effects on growth and flowering.

Table XXV. Height of plants in centimetres twenty days after planting

Mean values of ten recordings

	Massey		Alaska		Greenfeast		Telephone	
	C	DC	C	DC	C	DC	C	DC
Mean	14.17	9.13	46.31	31.62	16.42	10.66	57.14	41.33
S.E. of Mean	±1.98	±2.14	±2.32	±3.76	±0.77	±1.92	±2.68	±4.88

Experiment XIV. To study the effects on growth and flowering in the variety Greenfeast of removal of cotyledons at 24 hour intervals commencing 18 hours after the pea seeds have been given germinating conditions

The experiment commenced 22nd December, 1966.

Seeds of the variety Greenfeast were soaked in distilled water for six hours and were then planted in moist Peralite in the standard polythene plant bags. The experiment was conducted in cabinet II and all plants were grown in a 16 hour photoperiod i.e. 8 hours of high intensity illumination followed by 8 hours of supplementary illumination at 21°C constant temperature.

At 18 hours after the start of the soaking period a group of ten seeds had the cotyledons removed and the embryo replaced in the Peralite.

An earlier pilot experiment involving the removal of cotyledons at very early stages resulted in very low survival rates due to the rapid dehydration of the embryo or small plant. As standard practice, after the removal of cotyledons, a small beaker was placed over the embryo or young plant, and was removed later when the plant had hardened off.

At 24 hour intervals after the 18 hour period groups of ten plants had cotyledons removed. The total experiment comprised 140 plants.

All the plants which had cotyledons removed prior to 90 hours did not survive and were obviously dead within 48 hours of the removal of the cotyledons. At 90 hours there was 50% survival and all groups which had cotyledons removed after this time showed 100% survival.

Ten weeks after the initial soak period when marked differences in plant height and number of nodes formed could be easily seen a randomly selected plant from each group was photographed and all plants in each set were measured for height and total number of nodes. Plate 1. shows the plants with the upper figure indicating the time after soaking at which the cotyledons were removed and the lower figure showing the total number of nodes formed at ten weeks.

Table XXVI gives the data obtained on plant height and total nodes formed at ten weeks. No significant differences within groups could be detected and the results shown are the mean of ten recordings for each group excepting the group at 90 hours where only five plants survived. At the ten week period many of the plants whose cotyledons had been removed at 215 hours or later had commenced to flower. Other plants required anything up to another two to five weeks of growth before flowering commenced.

In addition to the dwarfing of decotylised plants with respect to height and number of nodes formed the leaflets were also greatly reduced in size. These features persisted throughout the entire life of the plants although in the case of plants where the cotyledons had been removed at later times some of the vegetative vigour of the controls was regained. No actual measurements of this were made and the statement made above is based solely on visual observations of the plants during the growing period.

In Table XXVII the data recorded on node to first flower and first

Plate 1. Plants of the variety Greenfeast ten weeks after planting.

Upper card number indicates number of hours after planting at which cotyledons were removed.

Lower card number shows number of nodes formed in each of the plants.

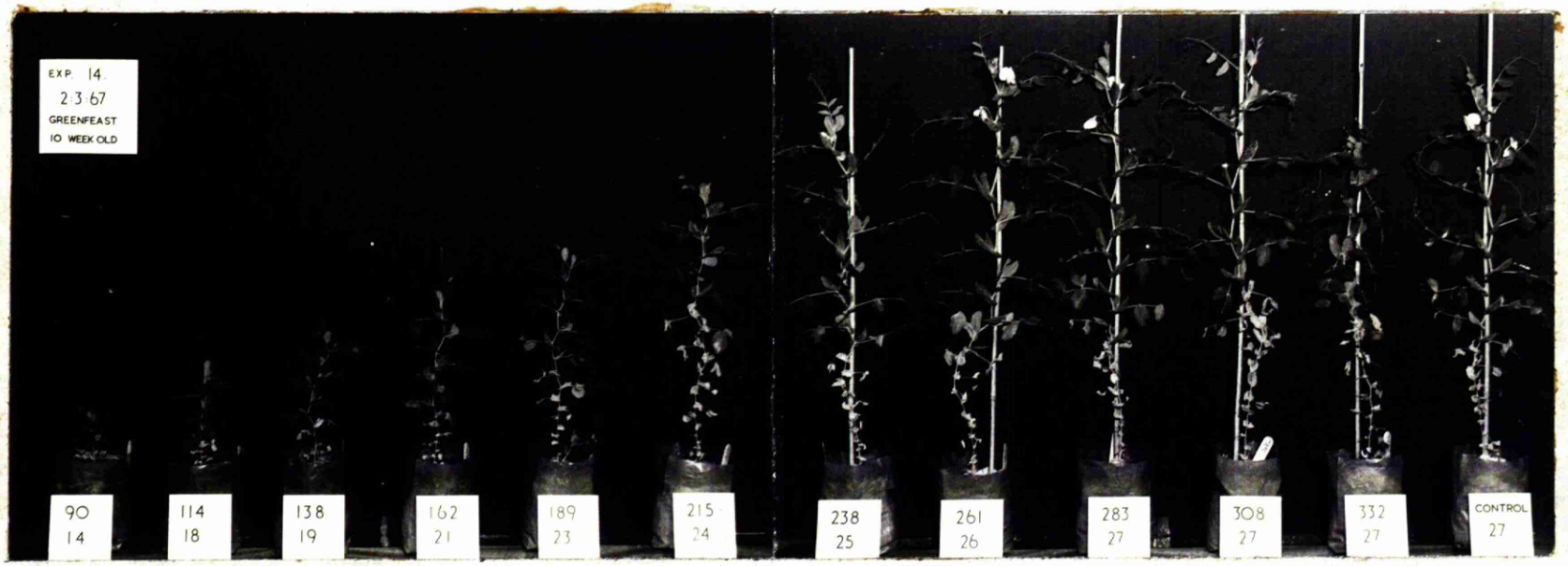


Table XXVI. Total number of nodes formed and height of plants ten weeks after planting

Time after soaking of removal of cotyledons	Nodes formed	Plant heights in centimetres
90 hours	13.8 ± 0.46	6.87 ± 1.27
114 hours	18.2 ± 0.38	9.23 ± 1.11
138 hours	18.8 ± 1.11	13.44 ± 2.08
162 hours	22.1 ± 0.70	17.88 ± 1.94
189 hours	23.4 ± 1.21	22.24 ± 2.64
215 hours	24.1 ± 1.36	31.30 ± 3.19
238 hours	25.6 ± 0.82	39.16 ± 4.26
261 hours	26.9 ± 1.77	42.00 ± 3.83
283 hours	27.8 ± 2.16	43.25 ± 5.16
308 hours	28.2 ± 1.73	45.61 ± 5.21
332 hours	27.3 ± 2.45	35.48 ± 4.98
Control	27.7 ± 2.42	44.70 ± 3.64

Table XXVII. Node to the appearance of the first flower and first fruit against the time of removal of the cotyledons

Time after soaking of removal of cotyledons	Node to First Flower	Node to First Fruit
90 hours	18.3 ± 0.23	21.0 ± 0.56
114 hours	20.6 ± 0.18	23.2 ± 0.61
138 hours	21.3 ± 0.31	24.6 ± 0.28
162 hours	21.8 ± 0.20	24.5 ± 0.46
189 hours	22.8 ± 0.43	26.7 ± 0.51
215 hours	22.5 ± 0.30	25.0 ± 0.44
238 hours	22.2 ± 0.27	26.2 ± 0.37
261 hours	22.2 ± 0.27	24.4 ± 0.62
283 hours	22.2 ± 0.15	24.2 ± 0.38
308 hours	22.4 ± 0.24	24.6 ± 0.55
332 hours	22.6 ± 0.33	25.0 ± 0.40
Control	22.0 ± 0.21	23.33± 0.43

fruit is expressed as the mean of ten recordings except as in the previously specified case of the 90 hour cotyledons removal group where only five plants survived.

A comparison of the means shows a significant difference in node to first flower between the groups which had cotyledons removed at 90 hours or 114 hours and the rest. Early removal of the cotyledons appears to hasten flowering. The node to appearance of the first fruit is similarly affected. When considering the number of nodes formed at ten weeks after planting there are significant differences between each group up to the group which had cotyledons removed at 238 hours. There are also, naturally, significant differences between any of these groups and the rest including the control group.

The same is true for plant heights excepting when considering the group where the cotyledons were removed at 332 hours. In respect of flowering node and nodes formed this group does not vary from the pattern described. No significant differences within the group could be detected and the reason why this single group should appear different is not known.

This experiment indicates the presence in the cotyledons of a flower inhibiting substance transported out to the embryo or a flower promoting substance initially present in the embryo being destroyed in the cotyledons during the first five days of growth. The presence of a growth promoting factor in the cotyledons which is transported out over some ten days is also indicated or conversely the destruction of some growth inhibiting substance in the cotyledons during this period.

The effects noted on growth and flowering may be brought about by the same substance or substances being transported out of or into the cotyledons or may be brought about by different substances being destroyed in or transported out of the cotyledons. The movement may be two way and one or other of the hypothetical substances may be destroyed in the cotyledons while the other is being moved out. Salisbury (1963) discusses the antagonism between vegetative and reproductive growth and certainly in this experiment the plants which flower earliest are those which show the poorest vegetative growth.

The reduction in vegetative growth after the removal of the cotyledons may be the result of the lack of carbohydrate for the developing embryo but the fact that plants, particularly when the cotyledons are removed at a very early stage, never recover the vegetative vigour of the controls suggests that this is not the case. The fact that plants which have the cotyledons removed prior to 90 hours die within a few hours might suggest the presence of an inhibitor or the absence of a promotor of growth but whatever it may be, either presence of or absence of, at least a specific growth regulating substance or substances is suggested.

The reduction in the number of nodes formed when the cotyledons have been removed is similar to that found when plants have been vernalised. Barber and Paton (1962) suggest the presence of a floral inhibitor in the cotyledons. It has been suggested in the introduction, page 8, that this may be true and the possibility that it is not mobilised during a cold treatment at the seedling stage has also been considered.

The position now might indicate that, when considering flowering alone, vernalisation and removal of cotyledons achieve the same end and that being the prevention of a floral inhibitor reaching the apex. If this were the case then it would be reasonable to presume that plants which had their cotyledons removed would not be able to respond to a period of growth at low temperature by initiating flowers at lower nodes.

Since vernalised plants with cotyledons show no retardation in their vegetative vigour, if the above hypothesis is correct, then more than one substance or group of substances is implicit, one controlling vegetative and the other reproductive growth. An experiment was designed in an attempt to clarify this point.

Experiment XV. To study the effects of the removal of cotyledons followed by a period of growth at low temperature on flowering in the garden pea variety Greenfeast.

This experiment was conducted in cabinet V commencing 25th April, 1967. The vernalisation period for the various groups was for a period of three weeks at 10°C in cooled incubators. The day length in the cabinet and incubators was 16 hours and the temperature in the cabinet was maintained at 20°C.

The figures given in the tables are the means of ten recordings for each group. It was anticipated that considerable numbers of deaths would occur in the decotylised plants during the vernalisation period. Three times the number of plants required had cotyledons removed and were given the vernalisation treatment. At the time of recording ten plants were randomly selected from the survivors. During the period of cold treatment approximately 50% of the decotylised plants died. For each of the other groups, as was usual practice, a few extra pots were included in the experiment as replacements.

Seeds were soaked for six hours in distilled water and were then planted in moist Peralite in polythene plant bags. All pots were then placed in the growth cabinet for a further five days. At this time, i.e. 126 hours after plants were first given germinating conditions, the cotyledons were removed from the required number of plants and the vernalisation treatment was commenced.

Small beakers were again placed over decotylised plants to cut down dehydration.

Due to the time involved in growing decotylised plants only the node to first flower was recorded although when flowers could be recorded on the decotylised plants the plants with cotyledons had already set fruit.

Table XXVIII gives the results of this experiment on node to first flower. The results shown for non vernalised plants are very similar to those found in experiment XIV and the general trend is similar to that found in experiment III and shown in Table VIII. The differences between experiments III and XV in the node to appearance of the first flower could be due to the differences in the cabinets or to the age of the seed.

However, from the results obtained in this experiment it becomes clear that plants without cotyledons respond to a period of vernalisation by initiating flowers at a slightly lower average node. The hypothesis put forward that plants without cotyledons would be unable to respond to a period of vernalisation is, therefore, wrong. The suggestion put forward in the introduction, page 10, that the cotyledons are the site of perception of the cold stimulus is also in some doubt. The findings of this experiment do not rule out the possibility that the substance or substances involved are initially to be found in the cotyledons but that shortly after germination they are to be found in the axis of the young plant is evident. A repeat of this experiment removing cotyledons at 90 hours completely failed due to the fact that no decotylised plants survived the period of cold treatment.

The fact that the decotylised plants in this experiment showed all

Table XXVIII. Node to the appearance of the first flower in the garden pea variety Greenfeast with and without cotyledons and with and without a vernalisation treatment

		Node to First Flower
With Cotyledons	Vernalised	19.2 ± 0.31
	Non Vernalised	22.2 ± 0.26
Without Cotyledons	Vernalised	18.3 ± 0.30
	Non Vernalised	20.7 ± 0.42

the visible signs of reduced vegetative vigour as described in Experiment XIV and flowered earlier probably suggest that the substances involved are not the same as those involved in vernalisation responses.

In conjunction with experiment XV it was decided to attempt to apply cotyledon extracts to decotylised plants and to measure the flowering response and vegetative growth of the plants.

Experiment XVI. To study the effects on growth and flowering of the application of aqueous extracts of cotyledons to plants of the garden pea variety Greenfeast with and without cotyledons

This experiment was conducted in a 16 hour photoperiod in cabinet VI with a constant temperature of 20°C being maintained. The experiment commenced 28th April, 1967.

Seeds were soaked for the usual six hours in distilled water and were then planted in moist Peralite. At 126 hours half the plants had their cotyledons removed. After ten days glass capillaries were inserted into the small plants, as previously described, and daily applications of 10 µg. of cotyledon extract were applied.

The extract was prepared by soaking 200 cotyledons, i.e. 100 seeds, for 12 hours; removing the testa and embryo; blending the cotyledons and squeezing the liquid from the mash. The exudate was then filtered and stored at 5°C.

Table XXIX shows the measurements taken after ten weeks of number of nodes formed and plant heights. Table XXX shows the data recorded on node to appearance of the first flower.

From the data recorded in Tables XXIX and XXX it is evident that the aqueous extract applied was ineffective in restoring the vegetative vigour of the controls or any part thereof to decotylised plants. It may be that the substance or substances are not soluble in water or it may be that they are produced during germination and are then moved out

Table XXIX. Total number of nodes formed and plant heights ten weeks after planting in the variety Greenfeast having received ten daily applications of aqueous cotyledon extract

Mean of ten recordings

	Extract	Nodes formed	Plant height in centimetres
With Cotyledons	+	26.9 ± 1.87	41.27 ± 4.83
	-	28.1 ± 2.12	43.38 ± 5.47
Without Cotyledons	+	18.6 ± 2.08	11.27 ± 3.22
	-	18.4 ± 1.73	10.69 ± 4.46

Table XXX. Node to first flower in the variety Greenfeast with and without cotyledons and with and without applications cotyledon extract

Mean of ten recordings

	Extract	Node to First Flower
With Cotyledons	+	22.4 ± 0.21
	-	22.2 ± 0.26
Without Cotyledons	+	20.6 ± 0.39
	-	20.8 ± 0.32

of the cotyledons; conversely the cotyledons may be acting as a sink for substances already present in the embryo. Aqueous extracts of dry seeds and of cotyledons removed from young plants were also ineffective.

In an attempt to discover more about the substances involved it was decided to remove parts of cotyledons and to study the effect of this on growth and flowering.

Experiment XVII(a). To study the effects on growth and flowering of the removal of parts of cotyledons in the garden pea varieties Greenfeast and Telephone

This experiment, starting on 3rd May, 1967, was carried out in cabinet VI under a constant temperature of 20°C and a photoperiod of 16 hours. Seeds were soaked for six hours in distilled water and were then planted in moist Peralite in 5 x 3 Vacapots. After five days different sets had 1/4, 1/2 and 3/4 of the total cotyledon complement removed. One set had the complete cotyledons removed and one set was left with cotyledons attached. In all cases where part of the cotyledon complement was removed great care was taken to avoid damaging the point of attachment of the remaining cotyledons or part of the plant.

One set had the cotyledons removed and then replaced using a paste of lanolin and water.

Table XXXI shows the data recorded on plant height and total nodes formed ten weeks after planting and Table XXXII shows the node to appearance of the first flower.

Considering first the data on node to first flower it is seen from the results obtained that in the variety Telephone there are no significant differences between any of the treatments. The variety Telephone may not be influenced in its flowering pattern by removal of cotyledons or the removal of the cotyledons in this experiment may have been done at some time after the critical period. The time chosen for removal of cotyledons of five days after planting was based solely on the performance of the variety Greenfeast and there is no reason to suppose that

Table XXXI. Total nodes formed and plant heights in Greenfeast and Telephone peas after removal of parts of cotyledons, removal of whole cotyledons and reattachment of cotyledons after removal

Recordings made ten weeks after planting

Mean of fifteen recordings

Var.	Parts removed	Total nodes formed	Height in Centimetres
Greenfeast	1/4	28.6 ± 0.90	44.81 ± 5.49
	1/2	29.5 ± 1.14	45.33 ± 4.73
	3/4	23.7 ± 1.06	20.70 ± 2.98
	1	19.2 ± 1.22	11.72 ± 2.77
	1 + reattachment	18.8 ± 1.11	12.60 ± 3.16
	0	29.3 ± 1.30	47.14 ± 5.28
Telephone	1/4	32.8 ± 2.21	109.82 ± 9.36
	1/2	29.8 ± 2.64	102.66 ± 8.14
	3/4	26.9 ± 1.47	68.47 ± 4.60
	1	20.1 ± 2.30	31.72 ± 3.92
	1 + reattachment	22.3 ± 1.42	29.64 ± 3.94
	0	33.2 ± 0.98	116.30 ± 10.71

Table XXXII. Node to first flower in Greenfeast and Telephone peas after removal of parts of cotyledons, removal of whole cotyledons and reattachment of cotyledons after removal

Mean of fifteen recordings

Var.	Parts removed	Node to first flower
Greenfeast	1/4	22.38 ± 0.41
	1/2	22.46 ± 0.36
	3/4	21.60 ± 0.27
	1	20.10 ± 0.40
	1 + reattachment	19.87 ± 0.39
	0	22.15 ± 0.18
Telephone	1/4	20.23 ± 1.43
	1/2	23.2 ± 0.87
	3/4	21.26 ± 1.17
	1	22.85 ± 1.35
	1 + reattachment	22.24 ± 0.64
	0	22.00 ± 0.83

other varieties will behave similarly.

In this experiment the variety Greenfeast showed some interesting results. Plants which had had the cotyledons removed completely or completely removed and then returned showed the same performance as previously noted with regard to the node at which the first flower occurred. These plants flowered slightly but significantly earlier than the controls. Plants which had retained only parts of the cotyledons, however, also behaved the same as the control plants and this would appear to indicate that, with regard to flowering, a part of the cotyledon is as effective as the whole cotyledon complement.

Total nodes formed and plant heights ten weeks after sowing showed similar trends for both the varieties. The variety Greenfeast showed the same performance as previously noted between plants with and plants without cotyledons. Plants which had the cotyledons removed and then reattached behaved as decotylised plants.

Where only a part of the cotyledon complement had been removed the situation is more complex. Removal of 1/4 or 1/2 of the cotyledon complement does not significantly affect the vegetative growth of the plants. Removal of 1/2 the cotyledon complement involved the removal of one complete cotyledon. An appendix to this experiment in which 1/2 the cotyledon complement was removed by cutting across both cotyledons did show some significant differences from the controls.

Where 3/4 of the cotyledon complement was removed the plants were significantly different from the controls. There was a reduction in the rate of node production and a decrease in plant height. The leaves

formed immediately following the removal of cotyledon parts were smaller than controls, visual observation only, but later formed leaves showed some indication of regaining some of the vegetative vigour noted in plants where the cotyledons had not been removed.

Experiment XVII(b). To study the effects on growth of plants of the variety Greenfeast after removal of half the cotyledon complement

Removal of half the cotyledon complement to be done in various planes.

In the previous experiment it was noted that if one entire cotyledon remained attached to the plant the plant grew normally. This was the case in the plants with 1/4 and 1/2 the total cotyledon complement removed. The plants retaining only 1/4 of the total cotyledon complement were small. Two possibilities seemed to exist (1) that a 1/4 of the cotyledon complement could not supply a sufficient amount of the hypothetical growth substance or substances for completely normal growth or (2) a damaged cotyledon could not supply a sufficient amount of this substance.

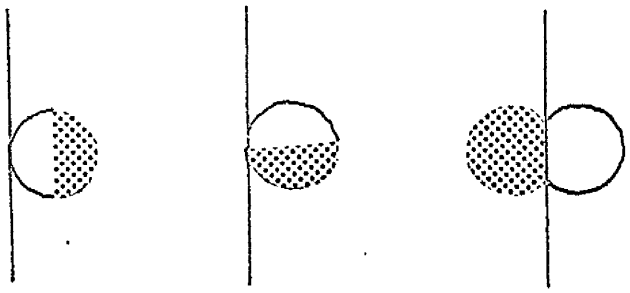
A small experiment with the variety Greenfeast was set up to investigate this problem.

The experiment commenced on 23rd June, 1967 and was carried out in cabinet VII under a constant temperature of 20°C and a photoperiod of 16 hours.

Seeds were handled as in experiment XVII(a) and the plants were grown in 5 x 3 Vacapots.

After five days the experiment was divided into five groups. One group of control plants retained the whole cotyledon complement. One group was completely decotylised and the remaining three groups had 1/2

the cotyledon complement removed in each of the three ways diagrammatically represented below.



(a) (b) (c)

Fig. IV.

Plant height and total nodes formed were recorded five weeks later and the results are shown in table XXXIII as the means of ten recordings.

From the data given it can be seen that removal of half the cotyledon complement results in a marked decrease in vegetative vigour over control plants but that this debilitation is less marked if one entire cotyledon is left attached. The information obtained from the previous experiment would indicate that plants with one cotyledon attached entire do recover vegetative vigour throughout their life and by ten weeks after sowing are not discernably different from controls. This is, perhaps, not surprising since at ten weeks after planting control plants have already been flowering for some time with the attendant reduction in vegetative growth rate.

The work described in this section points clearly to the cotyledons influencing growth and flowering in garden peas and possibly points to two separate processes one controlling or influencing vegetative growth and the other influencing flowering. There is, however, no indication as to how the cotyledons are acting or as to what the nature of the

Table XXXIII. Total nodes formed and height of plants five weeks after planting after several cotyledon removal treatments

Mean of ten recordings

Parts * removed	Total Nodes	Height in Centimetres
0	16.5 ± 0.84	20.7 ± 2.49
1/2 (a)	11.2 ± 1.16	7.11± 1.17
1/2 (b)	12.4 ± 1.14	6.84± 1.20
1/2 (c)	14.3 ± 0.88	13.14± 1.21
1	10.8 ± 1.30	4.85± 1.37

* See Figure IV.

compound or compounds produced by or destroyed in the cotyledons are. Screening of a wide range of compounds might give some indication as to the chemicals involved. The fact that flowering is not affected if even a small part of cotyledonary material remains attached might point to a hormonal type of compound. The reduced vegetative vigour resulting from removal of the cotyledons might point to a gross nutritional factor in the cotyledons without which the plants will never grow vigorously. The reduced vegetative vigour resulting from removal of half the cotyledonary complement but leaving one functional cotyledon entire may support this hypothesis since plants given this treatment appear to recover slowly.

An experiment conducted earlier was designed to test a few possible compounds and to test these in all possible combinations.

Experiment XVIII. To study the effects on growth and flowering of applications of sucrose, gibberellic acid, indolyl acetic acid and kinetin to decotylised peas of the variety Greenfeast.

This experiment was carried out in cabinet VII under a photoperiod of 16 hours and with a constant temperature of 20°C. The experiment commenced on 1st May, 1967.

The standard procedure was adopted for soaking and planting of seeds. After five days half the total number of plants had cotyledons removed, and small beakers were placed over the small plants.

Five days after the removal of cotyledons all plants had glass capillaries inserted into the stem and the various treatments were begun. 10 µg. of each of the test substances were applied in a 10% alcoholic solution and the treatment was repeated at weekly intervals for six weeks.

In an experiment of this type only positive results can be considered as valid. The fact that plants might show no response to certain substances is not a measure of the ineffectiveness of that substance in eliciting a response; also if one or more substances elicit a response this is no indication of the ineffectiveness of other substances which elicit no response. The relative ease of transport of one substance within a plant is no measure of the ease of transport of any other substance.

Sucrose was chosen as one of the test compounds as a potential supplier of a carbohydrate source which might be lacking after removal of the cotyledons.

Dwarfed internodes were noted in all plants after removal of the cotyledons. Gibberellic acid has been noted to bring about internode elongation and was therefore selected as a second test substance.

Kinetin has been found to bring about accelerated division in tobacco pith cultures provided that IAA is also present (Miller et al, 1956 and Skoog and Miller, 1957). Kinetin was also found to bring about expansion in leaf discs of Phaseolus vulgaris (Scott and Liverman, 1956 and Miller, 1956) and this total expansion was found to be brought about by enlargement of the individual cells. (Miller, 1956 and Powell and Griffith, 1960). The effects of kinetin on plant growth have been reviewed by Miller (1961). The very much reduced leaflet size noted in pea plants after the removal of the cotyledons and the possibility that the absence of kinetin and IAA may be responsible for this suggested the possibility of applying these substances as compounds which could overcome the retardation of growth noted in decotylised pea plants.

Due to the size of the experiment and the shortage of space there were only five replicates per treatment. However, for analysis purposes eighty plants received each of the treatments. Including controls the experiment, therefore, comprised 160 plants.

Table XXXIV shows the data recorded on total plant height and total nodes formed six weeks after planting and Table XXXV shows the data recorded on node to appearance of the first flower and first fruit.

An analysis of the results shows clearly that, apart from the pre-

Table XXXIV. Total nodes formed and total plant heights of Greenfeast peas with and without cotyledons and given applications of four compounds in all possible combinations

Recordings made six weeks after planting

	Nodes Formed		Height in Cms.	
	10% Alc.	S	10% Alc.	S
10% Alc.	17.6 11.6	-	26.6 9.6	-
K	17.4 11.4	18.2 10.0	22.6 6.6	24.8 9.4
IAA	17.0 11.8	16.8 12.2	27.8 6.8	20.8 8.4
GA	17.2 9.8	18.0 11.0	77.2 26.4	67.4 21.6
S	17.2 12.0	-	29.0 6.8	-
IAA, K	16.8 10.8	17.2 11.0	22.8 7.4	29.0 9.4
GA, IAA	17.0 11.0	17.8 11.0	78.8 24.4	65.8 28.6
GA, K	17.2 12.0	17.8 11.0	71.2 23.6	73.0 22.4
GA, IAA, K	18.6 11.8	17.4 11.8	74.0 21.8	67.6 23.2

Underlined figures are the data from decotylised plants.

Table XXXV. Node to first flower and first fruit in Greenfeast peas with and without cotyledons and given applications of four compounds in all possible combinations

	First Flower		First Fruit	
	10% Alc.	S	10% Alc.	S
10% Alc.	21.0 19.4	-	21.4 20.2	-
K	21.0 19.4	21.0 20.0	21.6 20.4	21.4 20.8
IAA	20.6 19.4	20.2 18.8	21.4 20.0	21.2 19.4
GA	24.8 22.6	23.4 22.4	27.4 24.6	26.8 25.6
S	21.2 19.6	-	21.4 20.2	-
IAA, K	20.2 19.0	20.4 19.4	20.8 19.0	21.0 19.8
GA, IAA	24.4 22.6	25.4 22.6	27.0 24.8	27.0 25.8
GA, K	24.4 22.6	24.8 24.0	25.8 25.4	26.2 25.8
GA, IAA, K	25.4 21.4	24.4 21.6	27.0 26.4	26.4 25.4

Underlined figures are the data from decotylised plants.

viously discovered features such as the effect on node production, plant height and flowering node of the removal of cotyledons and the effects of applications of gibberellic acid on plant height and first flowering node, little difference can be noted with any of the other compounds tested. Decotylised plants flowered earlier, were smaller in total height and showed a reduced rate of node production over plants with cotyledons. Gibberellin treated plants grew taller and flowered later than non-gibberellin treated plants.

From visual observations only, none of the treatments appeared to increase the leaf area of decotylised plants. The fact that decotylised plants show a response to gibberellic acid is about the only feature of this experiment which gives any additional information to that already known.

In the experiment kinetin and sucrose do not appear to affect the growth of whole pea plants but from an experiment of this type it is not possible to say that either of these substances is completely ineffective. Apparent ineffectiveness may be due to failure in getting the substance to an area of the plant where it could act.

When considering the node to appearance of the first flower IAA and the interaction of GA x cotyledons is just significant at the 5% level (see appendix 2(a)) and further experimentation would be necessary before any conclusions could be drawn as to the true effect of IAA on flowering or of the apparent different effect of GA in plants with cotyledons and those without cotyledons.

Experiments using labelled samples of the same materials, following their movement in the plants and if necessary making applications

to plant parts where the substance is absent would allow positive statements to be made regarding the effectiveness or ineffectiveness of the compound.

Many compounds could be screened in attempts to find the active fraction present in cotyledons and which has such a profound effect on flowering and particularly growth of pea plants. Extracts of cotyledons could be made and tested but at present there is still no indication whether or not the cotyledons are the source of an inhibitor of flowering or a promotor of growth or are acting as 'sinks' for the destruction of already present inhibitors of growth in the embryo. There is no evidence for supposing several compounds, some affecting flowering and other affecting vegetative growth. A reduction in the vegetative growth rate may in itself be the prerequisite for the induction of flower primordia.

From this experiment it is clear that none of the compounds tested can overcome the effects of removal of cotyledons, by restoring to the plants a completely normal vegetative and floriferous growth pattern.

GENERAL DISCUSSION

Late varieties of Pisum sativum are quantitative long day plants and can be vernalised (Barber, 1959).

During the course of the work presented in this thesis the following results were obtained on the growth, development and flowering of four varieties of garden pea.

(1) From experiments conducted under true photoperiodic long day conditions verification that late varieties of garden pea are quantitatively long day photoperiodically sensitive was obtained. Plants of the late varieties grown under long day conditions flower significantly earlier than plants grown under short day conditions.

(2) Late varieties respond to a period of vernalisation by initiating flowers at a significantly lower average node.

(3) The flowering pattern in early varieties is not affected by the length of day in which the plants are growing.

(4) Early varieties respond negatively to a period of vernalisation by initiating flowers at a slightly higher average node.

(5) The tall late variety responds more markedly to photoperiod and to vernalisation than does the dwarf late variety.

(6) Various intensities of illumination incident on plants of all four varieties greatly affected the growth rate, resulted in a marked decrease in dry weight as measured at the time when flowering commenced but did not affect the node at which the first flower occurred.

(7) Different ratios of red/far red supplementary illumination

do not affect flowering in the two late varieties tested.

(8) High intensity blue light does not affect flowering in the two late varieties tested.

(9) Presence or absence of root nodules and various growth media were tested but these did not affect the node at which the first flower occurred in any of the four varieties tested.

(10) Applications of gibberellic acid (GA3) cause marked increases in length of internodes of all four varieties. There are no significant differences in internode lengths between short day and long day grown plants.

(11) In the early varieties with or without gibberellic acid there are no significant differences in the node of appearance of the first flowers in either short day or long day growth conditions.

(12) There is a slight but significant delay in fruit set in the early varieties after applications of gibberellic acid.

(13) Late varieties of pea given applications of gibberellic acid show a significant delay in first flower formation particularly under short days and this is particularly noticeable in the natural dwarf late variety.

(14) In both late varieties either under short days or long days there is a delay in the node at which the first fruit is produced after applications of gibberellic acid.

(15) Plants of the two late varieties grown in long days show marked increases in dry weights over non-treated plants. The increased dry weights between gibberellin and non gibberellin treated plants is greater in the natural dwarf variety than in the natural tall variety.

(16) Plants of both the late varieties with or without gibberellin applications respond to a period of vernalisation by initiating flowers at a lower average node.

(17) Plants of the dwarf late variety given applications of gibberellic acid and thereby given the phenotype of a tall variety respond to photoperiod and to vernalisation in a manner similar to that found in the natural tall variety.

(18) Removal of the cotyledons in all four varieties results in a decrease in plant height and leaf size.

(19) In the dwarf late variety sequential removal of the cotyledons at 24 hour intervals commencing 18 hours after planting showed:-

(a) that plants which had their cotyledons removed prior to 90 hours failed to survive and at 90 hours 50% of all plants survived;

(b) that plants which had their cotyledons removed prior to 240 hours were smaller in height and leaf area and showed reduced vegetative growth rate over plants which retained cotyledons or had them removed after 240 hours;

(c) that plants which had their cotyledons removed prior to 140 hours flowered significantly earlier than plants which retained their cotyledons or had them removed after 140 hours;

(d) that with respect to vegetative growth and flowering the earlier the removal of the cotyledons the more marked the promotion of flowering and the decrease in vegetative growth rate.

(20) In the dwarf late variety the cotyledons are not the site of perception of the cold stimulus of a period of vernalisation. Decotylised plants can be effectively vernalised.

(21) Aqueous extracts of pea cotyledons when applied to decotylised plants of the dwarf late variety appear to be ineffective in restoring to the plants the normal vegetative vigour or flowering pattern of plants with cotyledons.

(22) In the dwarf late variety removal of parts of cotyledons showed the following results:

(a) That as little as one quarter of the cotyledon complement remaining attached allowed the plants to flower normally.

(b) That one complete and undamaged cotyledon remaining attached is sufficient to allow for normal or near normal vegetative growth.

(23) In the tall late variety removal of cotyledons prior to 140 hours affects the vegetative vigour of the plants in a similar way to that already described but does not appear to affect flowering.

(24) Applications of sucrose, gibberellic acid, indolyl acetic acid and kinetin to decotylised plants of the dwarf late variety indicated the following -

(a) that sucrose, kinetin and indolyl acetic acid do not appear to be effective in restoring normal growth and flowering pattern to decotylised plants, but have some effect.

(b) that gibberellic acid causes increases in internode lengths, does not affect the vegetative growth rate and causes a similar delay in flowering to that previously found. The effect of the gibberellic acid is the same when applied singly or in combination with any or all of the other test substances.

(24)(c) that there is an interaction between gibberellic acid and cotyledons with gibberellic acid eliciting a more marked response when applied to decotylised plants than when applied to plants retaining their cotyledons. (See appendix 2b).

The early work presented in this thesis and summarised in (1) - (4) of this discussion formed the basis for further experimentation. The early experiments were, for the most part, verification of previous work by other workers but unlike the previous work gave results which were open only to a single interpretation and as such allowed for the expected performance of any variety under any particular set of conditions to be laid down. This information was valuable in the future work since control plants in most experiments could be compared with the results obtained during the first experiments on day length and temperature.

Little need be said about the second section of the study where attempts were made, under controlled environmental conditions, to simulate selected differences between the normal growth cabinet conditions and the conditions experienced by plants in the field. All the tested conditions of varying light quality and quantity plus different growth media and presence or absence of root nodules failed to affect the flowering pattern. Downs et al. (1958) recorded marked differences in the flowering behaviour of a number of long day species between plants given fluorescent and plants given incandescent supplementary light to give the inductive long day. Pirenger and Cathey (1960) reported the earlier flowering in Petunia, a quantitative long

day plant, when the supplementary light was from an incandescent source and the marked delay over these plants when the supplementary light was from a fluorescent source, and Paleg and Aspinall (1964) noted the earlier flowering in barley when at least some of the supplementary light was from an incandescent source.

No effects could, however, be detected in the experiments with pea plants grown under various ratios of red to far red supplementary light.

Went (1953), Kramer (1957) and Highkin (1956 and 1960) studied the effects of constant temperature on growth of various species and all came to the conclusion that this abnormal environment was detrimental to growth. Fluctuations in temperature improved growth in the test species and Hillman (1956) showed that the injury in tomato plants resulting from growth in continuous light could be overcome by alternating the temperature. This work supports the theory put forward that the fluctuating temperature in the field enhances the growth of pea plants and is perhaps responsible for the noted promotion of flowering.

A considerable review of the literature on the effects on growth of applications of gibberellic acid was undertaken in the introduction to this thesis and it will now suffice to almost exclusively limit the discussion to the effects which gibberellic acid has on flowering. Sachs et al. (1967) reported on the effects of gibberellic acid on flowering in Fuchsia hybrida cultivar 'Lord Byron' and noted the complete inhibition of flowering in this qualitative long day plant after applications of gibberellic acid. The gibberellic acid was applied to

the terminal bud and repeated applications could completely inhibit flowering even under highly inductive photoperiods. Harder and Bünsow (1958) found that gibberellic acid would inhibit flower initiation in Kalanchoë.

In plants which show a qualitative response to the environment a particular environment must be encountered before flowering or some other feature will be manifest. A quantitative response to the environment, on the other hand, shows as a promotion or delay in some feature of development. In the experiments of sections V and VI the delay in flowering following applications of gibberellic acid was repeatedly noted and it could be postulated that the delay noted in the pea (a quantitative long day plant) is analogous with the inhibition which Sachs found in Fuschia (a qualitative long day plant). Repeated applications of gibberellic acid to pea plants will not indefinitely delay the onset of reproductive growth but even the shortest of photoperiods will not inhibit flowering.

After application of gibberellic acid the natural tall variety Telephone, used in the present study, was not as markedly delayed in commencing to flower as was the dwarf late variety Greenfeast, and the suggestion could be put forward that the mechanisms involved in the flowering of Telephone, which has endogenous GA or GA like substances, are more easily able to cope with increased concentrations of gibberellic acid. In both the late varieties under short day conditions the delay in flowering due to gibberellic acid applications is greater than under long day conditions. Since there is a natural delay under short days

it could be proposed that the slower rate of production of a floral stimulator or destruction of a floral inhibitor to the critical level which would allow flowering to commence might be more adversely affected by the introduction into the plant of inhibitors or delay causing compounds when the plants are growing under short photoperiods than when the plants are growing under longer photoperiods.

Guttridge and Thompson (1964) postulated that the native hormone in strawberries acts simultaneously as a vegetative growth promotor and inhibitor of flower initiation. Sachs et al. (1967) discussed the promotion in flowering which results from using growth retarding chemicals which probably lower the level of naturally occurring gibberellins.

In the present work the relatively greater response to day length and temperature in the variety Telephone over the variety Greenfeast and the imposition on the variety Greenfeast of a pattern similar to that found in Telephone after Greenfeast plants have received gibberellic acid would fit nicely into the theory of an antagonism between vegetative growth and reproductive growth. The increase in dry weight of gibberellin treated plants over non-gibberellin treated plants would give further support to this hypothesis.

The much decreased dry weights recorded in the shaded plants of Experiment V over non-shaded plants but this ineffectiveness of shading in altering the node to the appearance of the first flower would, however, tend to suggest that there is not a simple relationship involved and that it is not possible to say that by reducing the vegetative growth rate flowering will be promoted in every case. Nevertheless it does appear

that under certain conditions by reducing the vegetative growth rate flowering can be accelerated. The effect of growth retarding chemicals in bringing about earlier flowering can be reversed by simultaneous applications of gibberellic acid (Stuart 1961; Batjer, Williams and Martin, 1964; Cathey, 1964 and Sachs, 1966).

The cotyledons of the garden pea have been shown to have a profound effect on the growth and flowering of the plants. Several suggestions have already been put forward concerning the mode of action of the cotyledons in either supplying a promotor of growth or acting as a 'sink' for the destruction of an inhibitor. Activity in the cotyledons commences during the early hours of germination and in response to a signal from the axis tissue (Varner, Balce and Huang, 1963). These workers noted that very early removal of the cotyledons resulted in the very rapid senescence of the cotyledonary mass. If the cotyledons were removed at a slightly later stage, after receiving the stimulus from the axis, normal amylase and phosphatase activity would continue in the excised cotyledons.

Vyvyan (1924) and Went (1938a. and 1938b.) showed that the growth of mesophyll of leaves was dependent on materials stored in the cotyledons of pea. Bonner (1939) tested cotyledon extracts. Pure substances which included a number of substances in the vitamin B complex were applied to decotylised pea plants. In a few cases a return of some of the vegetative vigour of control plants was achieved but in no case even with applications of all the known cotyledon substances could completely normal plants be produced (Bonner, 1942).

Considering only vegetative growth the inference is of a two way system. The production and/or mobilisation of materials stored in the cotyledons and necessary for growth of the embryo being triggered off by a stimulus to the cotyledons coming from the axis. The work described, however, indicates an undiscovered factor or factors which would allow for completely normal growth.

The complete failure to survive amongst plants which had their cotyledons removed prior to 90 hours might be explained by proposing that at 90 hours none of the necessary growth factor had passed from the cotyledons into the axis.

In the present work the normal flowering pattern was observed if plants retained their cotyledons for up to 140 hours but for normal vegetative growth the cotyledons had to remain attached for at least 240 hours. This might indicate two systems, one controlling vegetative growth and the other flowering or might be due to a different balance of the same substances in a single system. The hypothesis could be put forward that for meristematic activity to commence in the apical meristem of the embryo a certain concentration of some growth factor must be reached in the axis. Thereafter, higher concentrations which would be gained from leaving the cotyledons attached for longer periods would progressively improve the vegetative growth to the stage at which the optimum concentration would be present and which would allow for normal growth. This stage being reached at about 240 hours after planting at $20^{\circ}C$. The earlier flowering, as suggested, might be due to the absence of an inhibitor or to the removal of the cotyledons resulting in a flower

promoting substance not being destroyed. On the other hand it again might be proposed that the severe reduction in vegetative growth noted when cotyledons are removed prior to 140 hours is in itself sufficient to promote earlier flowering. Wheeler (1966) showed that the removal of cotyledons at an early stage in the Dwarf French Bean (Phaseolus vulgaris) resulted in a marked reduction in the final area of the primary leaves due to a decrease in cell size and number. A marked reduction in leaf size was noted in the experiments described in this thesis and was carried a stage further by the observation that the leaf area in all leaves, not only the primary leaves, was reduced.

Several growth substances were tested and shown to be ineffective in restoring normal growth pattern or flowering behaviour to decotylised plants. The profound effects of the cotyledons on growth and flowering remain at this stage unanswered. Later research may show the cotyledons of pea to be the site of production of the 'colysanthin' of Barber (1959) or the site of destruction of the 'florigen' of Melchers and Lang (1948). Equally possible, however, is the suggestion that no compound will be found to be uniquely associated with flowering and that the change from vegetative growth to reproductive growth may result from a change, and possibly only a slight change, in the balance of already existing growth regulators (Ross, 1962). The conspicuous failure to find a compound or compounds which universally promote or delay, induce or inhibit flowering throughout the range of flowering plants fits slightly better the suggestion of a fine balance of compounds rather than the production of totally new compounds.

The role of the pea cotyledons may be that of a regulator of relative amounts of different plant hormones and may not be the production site of a new compound itself capable of promoting flowering or by regulating the vegetative growth allowing for floral induction to commence.

Appendix 1. Analysis of variance of results of Experiments III and IV, Tables VIII and X on flowering in Greenfeast and Telephone varieties of garden pea.

Source	df.	Sum of Squares	Mean Square	Variance Ratio	
Replicates	9	34.67	3.85	1.766	N.S.
Daylength	1	662.67	662.67	303.97	* *
Temperature	2	301.67	150.84	69.19	* *
Daylength x Temperature	2	256.43	128.22	58.81	* *
Residual	105	250.93	2.18	-	
Total	119	1,506.37	-	-	

Appendix 2(a) Analysis of variance of results of Experiment XVIII, Table XXXV on node to first flower in the Greenfeast variety of garden pea.

Source	df.	Sum of Squares	Mean Square	Variance Ratio	
Replicates	4	2.975	0.744	0.458	N.S.
Cotyledons	1	119.6	119.6	73.69	* *
GA.	1	490.0	490.0	301.91	* *
K	1	0.0	0.0	0.0	N.S.
S	1	0.025	0.025	0.0015	N.S.
IAA	1	6.4	6.4	3.943	*
GA x cotyledons	1	6.4	6.4	3.943	*
GA x IAA	1	2.5	2.5	1.54	N.S.
IAA x cotyledons	1	3.65	3.65	2.24	N.S.
IAA x K	1	3.6	3.6	2.22	N.S.
K x cotyledons	1	0.0	0.0	0.0	N.S.
S x cotyledons	1	0.325	0.325	0.2	N.S.
GA x K	1	0.1	0.1	0.06	N.S.
GA x S	1	0.025	0.025	0.0015	N.S.
K x S	1	4.525	4.525	2.78	N.S.
S x IAA	1	0.215	0.215	0.132	N.S.
Residual	140	227.26	1.62328	-	
Total	159	867.6	-	-	

Appendix 2(b) Analysis of variance of results of Experiment XVIII, Table XXXV on plant height in the Greenfeast variety of garden pea.

Source	df.	Sum of Squares	Mean Square	Variance Ratio	
Replicates	4	44.187	11.047	0.414	N.S.
Cotyledons	1	42,315.025	42,315.025	1,586.25	* *
GA	1	39,000.025	39,000.025	1,461.98	* *
GA x cotyledons$^{\phi}$	1	9,394.225	9,394.225	352.16	* *
IAA	1	0.025	0.025	0.009	N.S.
K	1	40.00	40.00	1.499	N.S.
S	1	60.025	60.025	2.25	N.S.
Residual	149	4,074.913	26.676	-	
Total	159	94,828.375	-	-	

ϕ All other first order interactions were tested but none were significant.

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