

THE ROLE OF THE COTYLEDON ON THE FLOWERING BEHAVIOUR OF PISUM SATIVUM

A PHYSIOLOGICAL STUDY

by

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B.Sc.(Melb.), B.Sc.(Hons.)(Tas.)

submitted in fulfilment of the requirements for the degree of

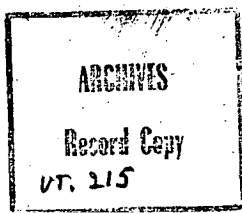
Doctor of Philosophy

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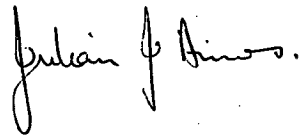
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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

A handwritten signature in black ink, appearing to read 'Julian J. Amos'.

(Julian J. Amos)

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Chapter 8SUMMARY

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ABBREVIATIONS

| | |
|------------------|--|
| S.D. | short day photoperiod |
| L.D. | long day photoperiod |
| U | unvernalized |
| V | vernalized |
| NF | node out of which the first flower initiates |
| FT | flowering time - in days |
| NE | number of expanded nodes |
| ND | node out of which the first flower develops |
| NT | number of nodes (total) |
| T | time |
| $S_2 \equiv S_n$ | symbols used for flowering genes |
| $E \equiv E$ | |
| $S_1 \equiv Lf$ | |
| ED | phenotypic classifications |
| EI | |
| L | |
| | (early developing |
| | (early initiating |
| | (late |
| P | probability |
| n | number |
| kgm | kilogram |
| mgm | milligram |
| mm | millimetre |
| mM | millimolar |
| μ M | micromolar |
| ml | millilitre |

INTRODUCTION

a GENETICS

The present knowledge concerning the genetic control of flowering in peas has recently been reviewed by Murfet (1971a). Rowlands (1964), who used as his criterion FT (the number of days between sowing of the seed and opening of the first flower) and Barber (1959), who recorded the flowering response in terms of NF (the node at which the first flower is initiated), both concluded that flowering was fundamentally under the control of one major gene, dominant for late flowering. Barber used the symbol Sn, first proposed by Tedin and Tedin (1923), to designate this gene. The effect of Sn is increased under S.D. (short day) conditions, and subject to modification by a system of polygenes. Barber also suggested a second polygene system working outside the orbit of the Sn gene. More recently, Wellensiek (1969) has suggested the presence of a multiple allelic gene system controlling flowering behaviour in peas, with alleles for late, intermediate and early flowering such that each later gene is incompletely dominant over the earlier one.

Little work has been carried out in which both FT and NF have been recorded. Paton and Barber (1955) have presented data for some commercial lines, as has Marx (1969). Rowlands (1964), who scored plants for both FT and NF for two successive generations, reported a high correlation between the two. Rowlands also stated that any data found not to be correlated may result from a difference in the rate of internode production for those particular varieties.

However, by graphing FT against NF, Murfet (1971a) observed three distinct phenotypic classes in the F₂ generation of a cross between a "late-flowering" cultivar and an "early-flowering" one. Further, as a

result of a detailed crossing programme (Murfet, 1971b), he was able to demonstrate quite convincingly that flowering in peas was predominantly under the control of a 3-gene system (c.f. the multiple allelic system described by Wellensiek). These three genes he called S2, E and S1. He found that gene S2 was responsible for conferring lateness, gene E was epistatic to S2, and gene S1 was epistatic to E. Murfet (1971b) suggested that the historic symbols Lf and Sn replace the symbols S1 and S2 respectively and take on the meaning attached to S1 and S2 as defined in his paper. This has been done in the remainder of this thesis. The role of these three genes - Sn, E and Lf - is dealt with in greater detail in Chapter 6.

In reviewing the literature, Haupt (1969) commented that NF was the more reliable of the two criteria from a physiological standpoint, since minor environmental fluctuations could cause a change in FT without altering either the physiological status of the plant with respect to flowering or NF. However, he suggested that both factors should be taken into account so that information regarding aborted flowers and vegetative reversion was not lost, as these facts are important when discussing the physiological aspects of flowering.

b PHYSIOLOGY

TECHNIQUES AND ENVIRONMENTAL VARIABLES

With regard to the physiological approach towards flowering in peas, the two most common variables used have been photoperiod and vernalization. In addition, the two techniques of cotyledon removal and grafting have been commonly employed. These environmental variables and techniques are explained in greater detail in the following chapter.

VARIETIES

Most workers have made use of two types of varieties categorized by their flowering habit.

Late varieties typically flower above node 12, are capable of being vernalized and respond to photoperiod behaving as quantitative L.D. (long day) plants. Vernalization advances flowering by approximately two nodes without affecting the rate of node formation. The effect of vernalization increases with the progressive delay in NF. Cotyledon removal soon after germination will also promote flowering by about two nodes, and this effect is additive to both photoperiod and vernalization. Cotyledon removal causes a reduction in the growth rate. Grafting has an effect similar to cotyledon removal under L.D. conditions but under S.D. conditions the prolonged period of vegetative growth appears to nullify this effect, irrespective of the vernalization status of the stock (see Amos and Crowden, 1969).

Early varieties typically flower between nodes 8 and 12, behave as day-neutral plants and respond only slightly - and in a negative way - to vernalization. Cotyledon removal soon after germination can cause a delay in NF of up to two nodes and the plants can then show a response to photoperiod (Haupt, 1969). However, this photoperiod response is not always observed, (e.g. Johnston and Crowden, 1967). This lack of response has been attributed to either the physical fact that flower initiation occurs in these varieties soon after germination has commenced, thereby allowing only a brief period of time when the plants are above ground for them to be able to respond to photoperiod (Haupt, 1969), or to a difference in the genetic constitution of the early varieties involved (Murfet, 1971a). As just mentioned, Murfet (1971a) has observed a number of phenotypically different classes, which will be dealt with more fully in Chapters 2 and 6.

INTERACTIONS

Barber (1959) and Haupt (1969) have both stated that photoperiod and vernalization act in a competitive manner, each reaction vying for a common substrate. However, both Paton (1969) and Amos and Crowden (1969) have presented evidence to support the concept that these two reactions are independent of each other and in fact work in a complementary manner rather than a competitive one.

It is of interest to note that up until this time no data has been brought forward comparing the effect of the growth rate on flowering behaviour, although mention has been made by both Haupt (1969) and Murfet (1973) that a causal relationship may exist. In particular the ratio total nodes : expanded nodes has not been discussed before, and it will be seen in Chapter 7 that there is a strong correlation between this ratio (called the growth pattern) and flowering behaviour.

THEORIES

Although transmission of a floral stimulus from the leaves to the apex is an accepted partial process of photoperiodic induction (Zeevart, 1962), the actual hormonal regulation of floral induction in Pisum has been interpreted differently by different workers, and the controversy has polarised into two opposing schools of thought.

The first interpretation as expounded by Haupt (1958, 1969) and Köhler (1965) is that flowering in Pisum is mediated by the positive action of a floral stimulus present in the cotyledons of early varieties. Haupt has carried out a number of experiments involving cotyledon removal of early varieties - which causes a delay in NF - and grafting of early scions (e.g. Haupt, 1952, 1954, 1957). Most of his work has been carried out under only one photoperiod (L.D.) regime. Köhler, using a cross-grafting technique

between early and late varieties, supports the idea of a promoter being present in the cotyledons of early varieties. In his view, late varieties normally initiate flowers autonomously, as an effect of ageing, but can be induced to flower at a lower node if grafted to a stock of an early variety. Early varieties normally are induced to flower at a very early age by their own cotyledons, but autonomous determination can occur if the cotyledons are removed as early as possible. This theory has been supported by experiments using pea-seed diffusates (Highkin, 1955), although flowering has been found to be delayed as well as promoted by this technique (Moore and Bonde, 1962).

The second interpretation involves the presence of a floral inhibitor which is present in the cotyledons of late varieties of peas. Paton and Barber (1955) found that cotyledon removal had no effect on the flowering behaviour of early varieties but led to an advancement of NF in late varieties. Grafting experiments between varieties (i.e. cross-grafting) confirmed this idea. Barber (1959) employed an approach combining both physiology and genetics, and suggested that the gene *Sn* - which delays NF and induces a response to photoperiod and vernalization - produces a flower-delaying substance which he named colysanthin. This substance must be destroyed before flowering can occur, and is preferentially destroyed by low temperatures (vernalization) and a long-day photoperiod regime. In conjunction with Sprent (Sprent and Barber, 1957) he discovered that cuttings of a late variety would flower out of a lower node once they had been leached, and further postulated that the cotyledonary inhibitor was both mobile and available up to fourteen days after germination. Experiments involving removal of the cotyledons of a late cultivar at various stages after germination have supported this interpretation (Johnston and Crowden, 1967), and other workers (e.g. Moore, 1964, 1965) have also obtained results consistent with the hypothesis of a cotyledonary inhibitor in late varieties of peas.

It has also been observed that removal of cotyledons in early varieties will only delay flowering if carried out at a very early stage

of germination (up to four days), and perhaps this behaviour is also the result of a cotyledonary inhibitor which is rapidly mobilized, but subsequently deactivated or destroyed by the shoot as germination progresses if the cotyledons remain intact (Johnston and Crowden, 1967).

A third proposal has been brought forward by Murfet (1971c). In discussing his experiments on the genetic control of flowering behaviour in peas, he has proposed a balance model controlling flower initiation, where NF is determined more by a balance between promoter and inhibitor than by an absolute amount of either. He suggested that all varieties can produce a promoter in their leaves and shoots, but that varieties containing the dominant gene Sn also produces a floral inhibitor in their cotyledons and shoots by virtue of gene Sn. To explain some of his results, he found it necessary to postulate that the mutant form, sn, is possibly a leaky mutant causing some inhibitor to be present in early varieties. Short days favour the production of inhibitor in the shoot until the normal process of ageing reduces its synthesis or its effectiveness. Gene E lowers the level of inhibitor in the cotyledons, and Lf increases the sensitivity of the apex to inhibitor or alternatively reduces the sensitivity of the apex to the stimulus.

Finally, it has been found that many environmental variables such as nutrient levels, light intensity and growing temperature can alter NF and/or the growth rate to some degree (e.g. Fries, 1954; Sprent, 1966b, 1967; Stanfield et al, 1966). This aspect will be dealt with to a greater extent in Chapters 3 and 4.

The work to be described in this present programme is concerned primarily with a consideration of the role of the cotyledon on the flowering behaviour in Pisum sativum, with special emphasis on the late pea cultivar Line 24 - derived from the commercial line "Greenfeast" - and in its relationship with the environmental conditions of photoperiod and vernalization.

MATERIALS AND METHODS

a GROWTH HABIT

The growth habit of the various cultivars of Pisum sativum used in this programme is monoaxial, with one main stem on which the leaf axils bear either dormant vegetative buds or flower primordia. If these vegetative buds grew into lateral branches, which is a common condition for a number of cultivars under S.D. conditions, they were pinched off ^{at an} early ^{stage} to maintain the monoaxial condition.

Flowers develop singly or as small inflorescences from the reproductive nodes and once a node has undergone transition from the vegetative to the reproductive state, all succeeding nodes are usually reproductive until the plant finally senesces. However under special circumstances reversion to the vegetative state can occur (Barber, 1959; Köhler, 1965; Murfet, 1971a). Fully developed flowers normally arise from these reproductive nodes, but with certain genotypes and under certain conditions, the initiated flower may abort before the development of the flower primordium into a flower bud has proceeded to any great extent.

Aborted flowers can easily be identified as a withered stalked structure in the leaf axil. In very rare instances a smooth axil was observed, and these were regarded as the flower primordia having aborted at a very early age.

b CHARACTERS RECORDED

Three main characters were recorded, although in some experiments only one may have been noted. The first was growth rate. In a number of experiments, the rate of leaf expansion was recorded on the decimal system as proposed by Maurer et al (1966). To determine the rate of node formation, the apices of a sample of plants in each treatment were dissected at various intervals and both the number of nodes laid down and the size of the apical meristem - again on a decimal system as proposed by Maurer et al (1966) - were recorded.

The second was the node of initiation of the first flower (NF). This node is taken to represent the physiological stage at which the plant changes from the vegetative to the reproductive condition, irrespective of any vegetative reversion that may subsequently occur, and also irrespective of the degree of subsequent development of the flower primordium into a flower bud or open flower. In a number of experiments both vegetative reversion and flower abortion were recorded. The cotyledonary node is taken as zero.

The third character was flowering time (FT). This is taken to be the number of days from the sowing of the seed to the appearance of the first open flower. Obviously this character would not be related to any flower initial which did not proceed with subsequent development into an open flower.

With many of the experiments that were carried out, the only character recorded was that of NF, as it was assumed that this factor would be more representative of the true physiological state of the plant than FT, which could be affected by a change in the growth rate that was not specific for floral initiation. However in some of the experiments with "Greenfeast" both FT and the growth rate were noted, and an attempt made to correlate these characters and NF

c CULTIVARS

All the cultivars used were dwarf varieties, since this facilitated easy handling, and have previously been described by Murfet (1971 a,b). The majority of the work has centred around a late-flowering cultivar originating from a single seed selection of the commercial cultivar "Greenfeast", and designated "Line 24". This pure line is late for both NF and FT and has the genetic constitution for flowering $Sn\ e\ Lf$ (Murfet, 1971b). Some of the early work mentioned previously has involved the commercial cultivar "Greenfeast" (Amos and Crowden, 1969). Murfet (1971a) has classified the various Pisum cultivars into three major phenotypic classes, depending on their flowering habit. Class ED (early developing) is a plant which flowers early in both time and node and is unaffected by photoperiod. The first initiated flower primordium develops through to a mature flower in both photoperiods. Class EI (early initiating) is a plant whose NF is unaffected by photoperiod but whose FT is delayed under S.D. conditions. This delay in FT is due mainly to the first initiated flower primordia having aborted. Class L (late flowering) plants behave as quantitative L.D. plants in both node and time. In making a comparison between Line 24 and the other genetic cultivars, five other lines were employed, and these are explained in detail in Chapter 6.

d GROWING CONDITIONS

The growing conditions have been described previously (Murfet, 1971a). Plants were grown in 2.7 kgm. tin cans and plastic boxes in a 50/50 by volume mixture of 6.4 mm. dolerite chips and vermiculite and watered each day. Nutrient in the form of a modified Hoagland's solution was supplied twice weekly. The

controlled environment facilities provided good control over the length of the light period but only limited control over temperature. Plants were grown on trucks which automatically moved in and out of the dark compartments at prescribed times, and a system of heaters and fans maintained the same temperature in the L.D. and S.D. compartments. The heaters were able to maintain the night temperature above the ambient and lessened the possibility of vernalization on frosty nights. No cooling was provided apart from glasshouse vents which opened automatically at a predetermined temperature to allow a cross circulation of outside air. Long days were supplied by supplementing natural photoperiod with banks of incandescent and fluorescent lights which were adjustable in height.

Seeds were selected so that their testae were free from cracks or obvious infections, surface-sterilized with Thiram-80, and grown in fresh vermiculite/gravel growth medium. It was found necessary with Lines 2 and 60 to nick the testa with a razor blade so that full and regular germination would follow.

e EMBRYO CULTURE

Seeds whose embryos were to be excised were imbibed for 8 hours in sterile moist vermiculite, after which time the embryo was aseptically removed and placed on 10 mls. of growth medium. The medium, sterilized in an autoclave, contained a range of salts that are detailed in Chapter 3, together with 2% dextrose and 0.75% agar to give it a gel-like consistency.

Once embryos had been placed on the medium, they were left to grow until both a healthy root system had developed and the shoot had reached a stage of development where the 5th internode

was expanding, counting as zero the cotyledonary node. Once this stage had been reached (see Figure 2.1) plants were transplanted out onto a vermiculite/gravel mix identical with that for intact plants.

f VERNALIZATION

Vernalized plants were sown as for unvernallized plants, and the cans placed in a cool room that was kept at a temperature of $3 \pm 1^{\circ}\text{C}$. The normal period of vernalization was four weeks. However, experiments were conducted where the vernalization period was varied.

Embryo vernalization was performed by placing the embryos onto a sloped nutrient agar growth medium to allow for illumination during the vernalization period. This illumination was provided by artificial light only, given by banks of incandescent and fluorescent lights such that the light intensity at plant level was 50 lumens, unless otherwise stated.

Experiments were staggered such that at the end of the vernalization treatment, plants had reached a similar level of development as unvernallized plants (see p. 4.2).

g GRAFTING

The grafting procedure has been detailed previously (Paton and Barber, 1955; Amos and Crowden, 1969; Murfet, 1971c). Seeds

were germinated at a depth of approximately 15 cms. in moist vermiculite/gravel mix for four days to encourage extension of the epicotyl. For the stock, the shoot was decapitated below the first scale leaf, a small rubber band made from bicycle-valve rubber was slipped over the cut top and the epicotyl slit down the middle by a sharp scalpel. For the scion, the epicotyl was cut off above the cotyledons, cut into a wedge shape with a sharp razor-blade, and wedged into the stock (see Figure 2.2). (For grafts involving cotyledon removal, cotyledons also were removed at this time.)

A firm union was usually achieved within two to three days and vigorous growth commenced within a week. The cotyledonary axils were checked at regular intervals and any lateral shoots removed.

h EXPERIMENTAL DESIGN

To allow for statistical treatment of the data, all experiments were planned as randomized block experiments with four and sometimes five replications in each treatment. A minimum of 20 plants were involved in each treatment although with some treatments, the survival rate was extremely low. The values for means, standard errors and numbers of plants scored for the various treatments are quoted in the tables, where appropriate.

FIGURE 2.1CONDITIONS OF EMBRYO CULTURE

Pictured are Line 24 seedlings that are ready for transplanting. These embryos were excised from the seed after 8 hours' imbibition, and grown under S.D. conditions. (From Johnston, M.J. (1966), B.Sc.(Hons.) thesis - University of Tasmania.)

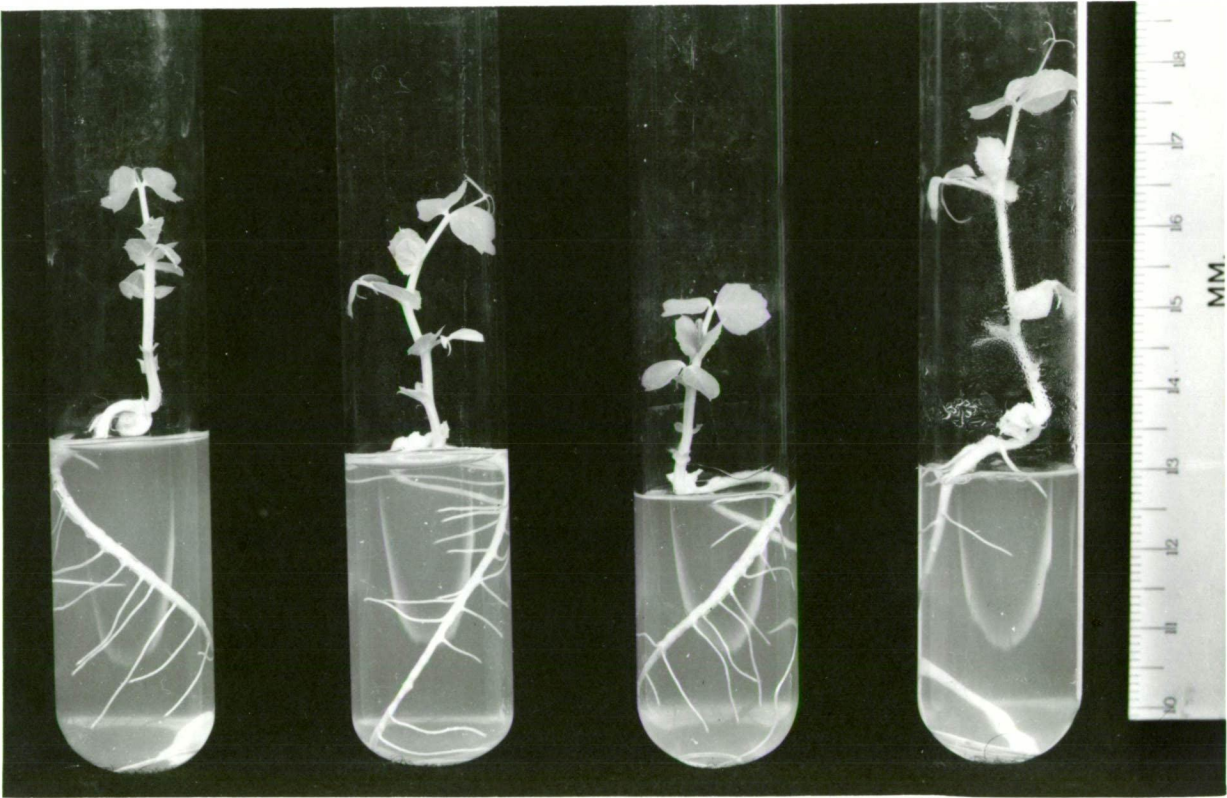
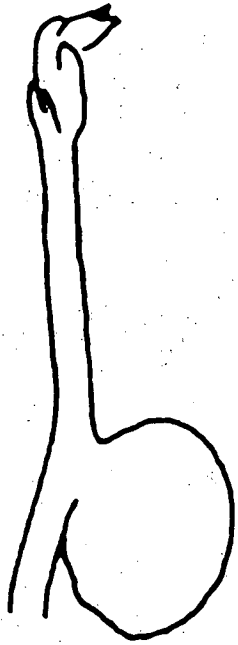
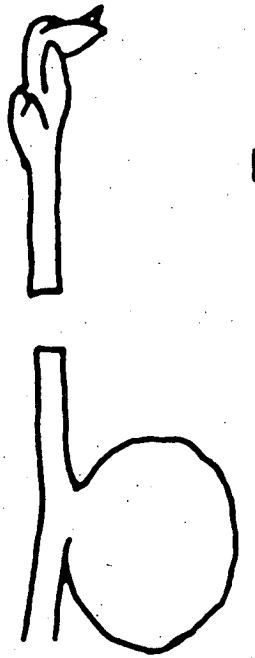


FIGURE 2.2THE GRAFTING TECHNIQUE

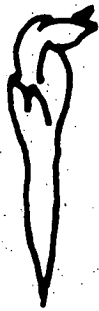
- A - intact seedling.
- B - decapitation between the cotyledonary node and leaf node 1.
- C - wedge-shaped scion, and stock with *affixed* rubber ring and longitudinal slit.
- D - the completed graft.



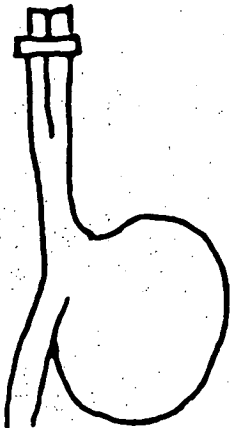
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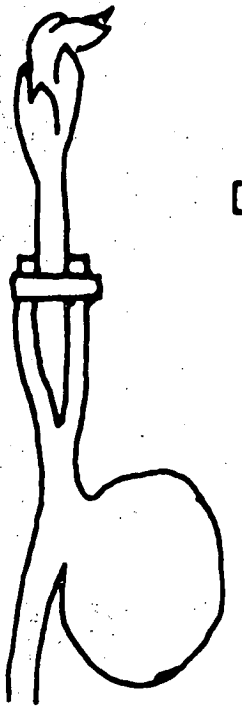
B.



C.



D.



COTYLEDON REMOVAL AND EMBRYO CULTURE

a INTRODUCTION

Many workers have commented that the cotyledons of Pisum sativum play a direct and incisive role in controlling flower behaviour, either by the production in late varieties of a flower inhibitor (e.g. Paton and Barber, 1955; Sprent and Barber, 1957; Barber, 1959; Johnston and Crowden, 1967; Chailakhyan and Podol'nyi, 1968; Paton, 1969) or by the production in early varieties of a flowering stimulus (e.g. Köhler, 1965; Haupt, 1969). The results have been inconclusive either way. In the present investigation, a series of experiments has been designed involving the removal of cotyledons of a late variety in an attempt to determine more precisely how the cotyledons affect the flowering process.

Previous experiments in the laboratories at Hobart have shown that plants which have had their cotyledons removed within four days of germination were unable to survive by themselves, and so a technique of aseptic embryo culture was adopted similar to that described previously (Fries, 1954; Johnston and Crowden, 1967; Amos and Crowden, 1969). The nutrient agar medium used is described in detail in Section b, sub-section I of this chapter (p. 3.2). The method of embryo culture is also described in the previous chapter.

The presentation of material in this chapter requires some explanation. In Section 3b, a number of experiments are described where nutrient and light levels have been varied, and their effect on growth and flowering observed. Although the work presented in this section was not pursued to any great extent, it did point out the need to exercise a rigid control over these levels.

At the commencement of this experimental programme, two distinct approaches were made as to the effect of cotyledon removal on flowering.

The first approach was to remove cotyledons at various stages of development, as described by Johnston and Crowden (1967); the second was to remove cotyledons once imbibition had been completed. Although the second approach could be regarded as being but a small part of the first, it was decided to present the results in two different sections, as the two approaches each contributed different information as to the role of the cotyledons in the flowering process. Further, although the experimental programme began with the "sequential cotyledon removal" approach, the majority of the work that is described in later chapters was involved with the removal of cotyledons at the early stage of development. The purpose behind these two approaches will be appreciated when the vernalization response is discussed in the following chapter.

The two approaches were separated into two distinct sections: the first of these, Section 3c, records the results of experiments designed as "sequential cotyledon removal" experiments, and the second, Section 3d, records the results of "early removal" experiments. Section 3d also contains a general discussion which links the two sections together. It is acknowledged, however, that this method of presentation necessitates some repetition of material.

b SOME ENVIRONMENTAL FACTORS AFFECTING EMBRYO GROWTH AND FLOWERING

In this section, experiments are described in which both nutrient application and light intensity have been varied, and the effect on the vegetative growth and NF of embryos in particular are briefly discussed.

I. MEDIUM

The first experiment concerns the nutrient levels in the medium on which the embryos were initially grown. An experiment was conducted

under L.D. conditions where the nutrient levels in the agar medium were altered. This experiment was in part inspired by the results that other workers had obtained in altering the nutrient balance and observing the effect on excised tissue (e.g. Fries, 1954; de Fossard, 1967).

Experiment 3.1:- Ten different media were prepared as shown in Table 3.1 and 30 embryos were placed onto each medium. Plants were transplanted at the stage of "5th internode expanding" (with the exception of one treatment where it was found necessary to transplant plants at the stage of "4th leaf open"), and their stage of development recorded, both for roots and shoots (Table 3.2). Plants were then grown to anthesis and their NF recorded, together with an approximate FT for each group (Table 3.3).

Results:- Table 3.2 shows the effect of the different media on the rate of growth of embryos. The No. 8 medium especially had such a profound effect on root development that it was felt necessary to transplant the embryos at an earlier stage of stem development than normal. Media 6, 7 and 8 all produced strong root growth. The presence of glycine and the two vitamins thiamine and pyridoxine had a deleterious effect on root growth (c.f. media 2 with 1, or 8 with 10), as also did a lowering of the sugar level (c.f. media 3 with 1, or 9 with 8).

The NF for each different category - based on the rate of shoot development as shown in Table 3.2 - is given in Table 3.3, together with an overall average for each medium. From this Table, it would appear that the rate of node expansion (shoot development) experienced by the embryos while in test-tubes is reflected in the final NF. This appears to be the case both within a specific treatment and between treatments. For example, the results for NF of medium 6 show a significant trend for more rapid growth to cause a delay in NF (a - c, $P = 0.01$). (In fact this phenomenon was observed throughout the entire investigation, although it was not always as significant as shown here.)

Also the more rapidly growing plants on medium 8 flowered out of a higher node (14.00) than e.g. their medium 4 counterparts (13.30) ($P < 0.001$).

It is of interest to note that the survival rate in this experiment was in the order of 85%. This figure was found to be normal for all cotyledon removal work involving Line 24 plants.

Discussion:- These results show that the embryo growth medium can influence NF to some extent. The medium may well exert this effect by altering the growth rate, since plants that exhibited a more rapid rate of growth tended to flower at a higher node. The more rapid growth rate exhibited by plants on media 6 - 10 may well be linked to the higher phosphate levels in these media, especially when it occurs in conjunction with increased $MgSO_4$ levels. The fact that plants on media 8, 9 and 10 all tended to flower at a higher node than those grown on media 1 - 7 suggests that the growth rate and subsequent NF may be affected by the levels of potassium, nitrate and phosphate in the medium. FT shows no such effect.

This effect was not pursued further, but the results from this experiment emphasized the need to exercise a rigid control over the consistency of the medium. Although this particular experiment was conducted after the major experimental programme had begun, it was decided to change from medium 1 to medium 8 as the latter medium gave a more rapid growth rate. All future experiments reported therefore state which medium was used as there is a minor variation apparent in both the growth rate and NF.

II. NUTRIENT

As a result of the findings reported in the previous sub-section, it was thought advisable to test whether the amount of liquid nutrient given to both intact plants and embryos after transplant had any effect

on vegetative growth and flowering. Two experiments were conducted at different times in the programme to determine this effect. Although the first was essentially a pilot experiment to the second, both are reported since there is a subtle difference in design between the two.

Experiment 3.2:- Intact and decotyledonized plants were grown under L.D. conditions to anthesis. Plants were given nutrient either once or twice weekly, using one of two nutrient stocks (Table 3.4). The results for NF are given in Table 3.5.

Experiment 3.3:- Intact plants were grown under either S.D. or L.D. conditions and were given nutrient once or twice weekly from Nutrient Stock a. After 32 days of growth the stage of node expansion was observed, and the plants then grown through to anthesis. The results are given in Table 3.6.

Results:- It can be seen from Table 3.5 that an increase in the level of applied nutrient will cause a delay in NF for both intact and excised plants. The dosage rate had a greater effect on NF (P significant - see Table 3.5) than a change in the nutrient stock (P not significant). It was with this fact in mind that Experiment 3.3 was designed. Table 3.6 shows that an increase in the dosage rate delayed NF for intact plants under either photoperiod regime, as well as increasing the rate of node expansion. However, FT was not affected by nutrient treatment. Plants had a more rapid rate of node expansion under L.D. conditions than under S.D. conditions (c.f. Table 3.11a).

Discussion:- Although obvious photoperiod and cotyledon removal effects can be observed, these will be discussed in greater detail at a later stage. The reason for discussing these two experiments here is to point out that the level of nutrient supplied will affect both NF and the growth rate, for both intact and excised plants, and under either photoperiod. Sprent (1966b, 1967), who obtained similar results to those presented here, also found that whereas the nutrient level can affect both NF and growth rate,

a change in the water level will only affect the growth rate - not NF.

As also shown in Experiment 3.1, it seems that there is a definite link between the growth rate and NF, perhaps by allowing plants to lay down more nodes in a certain period of time. Thus, although plants will initiate flowers after receiving a certain quantity of light, their NF will depend on the number of nodes that can be laid down within that period. This matter will be discussed in detail in Chapter 7.

Again, although this matter was not pursued further, the results emphasized the need for consistency in the nutrient treatment, and as a standard practice, it was decided to apply nutrient to plants twice each week from nutrient solution a, the solution that was normally used in the Hobart glasshouse.

III. LIGHT LEVELS

The third factor to be investigated was the level of light intensity under which embryos were grown prior to transplanting. This experiment was performed primarily to determine whether the amount of light reaching the plant at an early stage of development would have an effect on NF, as the seasonal variations in the light levels are quite marked in Hobart.

Experiment 3.4:- Decotyledonized plants, 24 per treatment, were grown under L.D. conditions at light levels of 1400, 700, 350 or 175 lumens until their 5th leaf had opened. The period of time taken to reach this stage was recorded and an average time calculated (Table 3.7). At this stage, embryos were transplanted and grown under normal daylight conditions to anthesis. FT after transplanting and NF were recorded, both for each light treatment and for the embryo growth rate irrespective of the light treatment (Table 3.7).

Results:- It can be seen from Table 3.7 that an increase in the light intensity will cause an increase in the rate of node expansion. The different light intensities under which plants were grown while in the test-tubes had no significant effect on either NF or FT after transplanting. The initial growth rate had a negligible effect both on the subsequent NF and on FT after transplanting.

Discussion:- Since both NF and FT after transplanting are not affected by the initial light intensity, and since all plants were transplanted at a similar stage of development, it is apparent that the growth rate of all plants is the same after transplanting. Although the initial light intensity had an effect on the initial growth rate, this effect was not expressed in the resultant NF. It would therefore seem likely that the growth behaviour of the plant has been affected in a different manner from that exerted by the nutrient levels as noted in the previous sub-section. This matter is also discussed further in Chapter 7.

At this stage, the more important observation is that the range of light intensities used did not affect the resultant NF, and it was concluded that embryos could be grown in test-tubes under normal lighting conditions on the floor of the glasshouse, irrespective of season.

c SEQUENTIAL COTYLEDON REMOVAL

As stated in Section 3a, this section deals with two experiments involving the removal of cotyledons of Line 24 plants at various stages of development. Although the first experiment was in effect a trial experiment for the second, the conditions under which the experiments

were conducted varied slightly, and different types of data were recorded. Experiment 3.5 involved the old medium (medium 1) and was carried out in winter, while Experiment 3.6 involved the new medium (medium 8) and was carried out in summer. Experiment 3.5 was concerned primarily with the number of nodes laid down at the time of cotyledon removal; Experiment 3.6 recorded the number of expanded nodes after 33 days' growth to determine whether photoperiod had an effect on the growth rate.

Experiment 3.5:- Cotyledons were removed at intervals, after 8 hours imbibition and then at 1, 2, 4, 6, 9 and 12 days after germination had commenced. For each treatment, 35 plants were involved. The apices of 5 plants in each treatment were dissected at the time of cotyledon removal to determine the total number of nodes laid down; the other 30 were grown to anthesis under L.D. conditions. Plants that were excised at 8 hours, 1, 2 and 4 days were grown initially on a nutrient agar medium (medium 1) until the root growth had become established, whereupon they were transplanted. Plants were then grown to anthesis 5 to a tin. The results are shown in Table 3.8 and Figure 3.1.

Experiment 3.6:- Cotyledons were removed at the same stages of development as in Experiment 3.5. However, two photoperiod regimes were used, and each treatment contained 20 plants. Plants were again grown 5 to a tin. The rate of development was recorded as the number of nodes expanded after 33 days of growth (NE), using a decimal system first proposed by Maurer et al (1966). The time taken for the first bud to reach anthesis - FT - was also recorded. The results are shown in Table 3.9 and NF is diagrammatically shown in Figure 3.1.

Results:- Table 3.8 shows that the rate of node formation for intact plant during the first 12 days of germination was approximately 0.5 nodes/day. The effect of cotyledon removal on the rate of node expansion (NE) and FT was quite marked (Table 3.9). Plants that were decotyledonized and placed in test-tubes grew much less rapidly and had a much longer FT than intact

plants which were placed directly into tins. NF showed a different response. In fact NF was advanced for plants that had their cotyledons removed, and the results shown here agree very well with those presented by Johnston and Crowden (1967).

Under L.D. conditions a progressive delay in the time until cotyledon removal led to a gradual increase in NF until at 12 days there was no further effect. This delay in NF does not linearly correspond with the more rapid rate of node expansion experienced. There appears to be a seasonal difference in that the winter crop flowered 1.2 - 1.3 nodes earlier than the summer crop, irrespective of the time of cotyledon removal. This difference is greater than that observed for the effect of the medium in Experiment 3.1 (approximately 0.5 nodes).

Plants grown under S.D. conditions flowered approximately 6 nodes later than their L.D. counterparts, irrespective of the cotyledon status. However, plants that were excised 2 days and 4 days after germination showed an extra response, delaying NF by a further 3 nodes. Photoperiod did not appear to have a significant effect on the rate of node expansion.

One other observation that should be noted is that symptoms of overcrowding appeared in the 9-day, 12-day and control plants.

Discussion:- Plants that were excised after 8 hours germination needed to put down another 6 - 7 vegetative nodes before flowering could occur - from node 6.0 (NT) to node 12.75 (NF), whereas plants that were germinated for 12 days before excision occurred needed to lay down an extra 3 - 4 vegetative nodes before transferring from the vegetative to the flowering state - from node 12.2 (NT) to node 15.40 (NF). It is interesting that plants excised after 8 hours germination flowered at the same node (node 12 - 13) as had been laid down by plants prior to excision at 12 days.

At the 12-day stage of germination, the cotyledons are beginning to disintegrate, suggesting that all the available material has been mobilized and moved into the axis by this time. This is supported by the fact that the difference between 12-day excised plants and the intact controls is minimal, for both NF and FT.

Since the retention of the cotyledons causes a delay in NF of some 3 - 4 nodes which is not specifically related to the level of growth achieved prior to cotyledon removal, it is thought that the cotyledons are playing a direct role in the flowering process by producing a substance intimately associated with the delay in NF.

The delay in NF experienced by plants excised at the 2-day and 4-day stage of germination under S.D. conditions is not fully understood. This phenomenon was not observed in all experiments, and is thought to be caused by seasonal factors, allowing for either a build-up of inhibitor (see pp.3.13-14) or an alteration in the growth pattern (see Chapter 7).

point is discussed more fully in Chapter 7.

The growth response of embryos is similar to that reported by Fries (1954) and Killeen and Larson (1968). The seasonal difference as shown in Figure 3.1 existed throughout this experimental programme. This difference may be the result of a mild vernalization response (see Chapter 4), a combination of minor seasonal variations (e.g. temperature, light intensity) or a change in the growth pattern (see Chapter 7). Whatever the cause, it did not alter the overall qualitative response to cotyledon removal. Theoretical considerations of this qualitative response are discussed in the next section.

Plants that had their cotyledons removed during the first 4 days of germination experienced a similar reduction in their growth rate - as measured by NE - together with a concomitant delay in FT. Plants that were excised at the 9-day and 12-day stage of growth exhibited a similar NE and FT to that of the control plants. NF did not show a similar response to cotyledon treatment

It therefore appears that there is a connection between FT and the growth rate, and it was felt that NF would be a much more reliable indicator of a direct cotyledon effect on flowering. There was however, a lingering feeling that the reduced rate of growth itself may have had some direct effect on flowering. This point is discussed more fully in Chapter 7.

The growth response of embryos is similar to that reported by Fries (1954) and Killeen and Larson (1968). The seasonal difference as shown in Figure 3.1 existed throughout this experimental programme. This difference may be the result of a mild vernalization response (see Chapter 4), a combination of minor seasonal variations (e.g. temperature, light intensity) or a change in the growth pattern (see Chapter 7). Whatever the cause, it did not alter the overall qualitative response to cotyledon removal. Theoretical considerations of this qualitative response are discussed in the next section.

d EARLY EXCISION

In the last section, it was observed that cotyledon removal had its greatest effect on NF if performed as soon as imbibition is complete. To further study this cotyledon effect, a number of experiments were performed with the cotyledons removed at this early stage. In fact the remainder of the experiments to be described in this thesis involving cotyledon removal were designed on the basis of obtaining a maximum cotyledon effect.

Three experiments are reported in this section. The first two deal solely with the effect of cotyledon removal on the growth rate, and were designed as pilot experiments. Experiment 3.7 was performed on medium 1, was limited to one photoperiod, and measured the growth rate by the rate of node formation. Experiment 3.8 was performed on medium 8, involved both photoperiods, and measured the growth rate by the rate of node expansion. The results of two experiments are given in Experiment 3.8 in order to draw attention to one aspect of the photoperiod response. (Although in retrospect it seems obvious, at the time there was no thought given to measure both parameters in the same experiment.) Experiment 3.9 is a fully factorial experiment showing the effect of cotyledon removal and photoperiod on NF and FT. It has the same basic design as Experiment 3.8 but was performed at a different time of the year.

Experiment 3.7:- Intact and decotyledonized plants were grown under L.D. conditions, and the apices of 8 plants were dissected at various time intervals up to 30 days after germination to determine the rate of node formation. The results are shown in Table 3.10 and Figure 3.2.

Experiment 3.8:- Intact and decotyledonized plants were grown under S.D. and L.D. photoperiod regimes, and their stage of development after 33 days' growth was recorded as the number of expanded nodes. The results for two such experiments, conducted one year apart, are given in Table 3.11.


Experiment 3.9:- Intact and decotyledonized plants were grown under S.D. and L.D. photoperiod regimes as in Experiment 3.8, and both NF and FT recorded. The results are given in Table 3.12.

Results:- From Table 3.10 and Figure 3.2 it can be seen that cotyledon removal drastically reduced the rate of node formation. Under the conditions of this experiment the average rate of node formation for intact plants was 0.42 nodes/day, and for decotyledonized plants 0.16 nodes/day. From Table 3.11, the rate of node expansion was also much reduced by cotyledon removal (see also Table 3.9). Although photoperiod may have an effect on the rate of node expansion (e.g. Table 3.11b) this did not always occur (Tables 3.9, 3.11a). When it did, the rate of node expansion was always greater in plants grown under L.D. conditions.

The effect of cotyledon removal on NF can be observed in Table 3.12 (see also Table 3.9). Cotyledon removal reduced NF by approximately 3 nodes under either photoperiod, and plants in S.D. conditions flowered approximately 6 nodes later than those under L.D. conditions.

Discussion:- The results presented here further demonstrate that cotyledon removal at imbibition causes a reduction in both the rate of node formation and the rate of node expansion, and causes a reduction in NF of approximately 3 nodes. These results are in general agreement with those obtained by Moore (1964, 1965). However, it must be emphasized that not all experiments showed such a clear-cut response of 3 nodes as did the one reported here (c.f. Table 3.9).

Many workers have suggested that the cotyledons of late varieties of Pisum sativum contain a floral inhibitor in their cotyledons which passes to the shoot during germination and delays flowering (e.g. Paton and Barber, 1955; Barber, 1959; Sprent, 1966a; Paton, 1969); removal of the cotyledons during germination will remove the supply of inhibitor and cause plants to flower out of

a lower node. These present experiments did in fact show that NF can be advanced  if the cotyledons are removed during the first 12 days of germination. (By the 12-day stage of growth, the cotyledons have begun to rot away, so it would not be expected that they would exercise any effect after this time.)

On this assumption, the movement of inhibitor begins soon after imbibition is complete and continues during the first 12 days of germination. This fact agrees with the results of leaching experiments on cuttings of a late pea variety (Sprent and Barber, 1957). The inhibitor would set a threshold level which must be surpassed by an opposing inductive stimulus before flowering can occur. Further, if production of the stimulus is dependent upon the amount of light reaching the plant, the effect of short days would be to delay the attainment of this threshold level by reducing the rate of production of the inductive stimulus. Therefore, S.D. conditions would enable an extended period of vegetative growth to occur - approximately 6 nodes - before this threshold is surpassed.

Both Sprent (1966a) and Paton (1967) have conducted experiments involving leaf removal in efforts to determine the role of the leaves in the inductive process. Whereas Sprent suggests that the role of the leaf is more of a qualitative nature than a quantitative one, Paton disagrees and later experiments involving masking of leaves support this view (Paton, 1971). However, one of the greatest problems involving leaf removal was its effect on the rate of node formation, and it was thought that the primary effect on NF may have been a result of a change in the growth rate (Sprent, 1966a; Paton, 1967).

The apparent anomaly existing with the 2-day and 4-day treatments requires further discussion (Table 3.9). Murfet (1971b) has suggested that the inhibitory substance is produced in the shoot as well as in the cotyledons. If this is in fact the case, the longer the period of time that plants are subjected to S.D. conditions, the more inhibitor would be produced in the shoot.

From Table 3.9, 2-day and 4-day plants take approximately 40 more days to reach a flowering condition than plants which have been excised at 9 and 12 days. This extra period of time would presumably be sufficient for an amount of inhibitor to be manufactured to raise the threshold level sufficient to produce an additional delay of approximately 3 nodes as observed in Experiment 3.6.

Other workers have interpreted their results differently. Both Köhler (1965) and Haupt (1969) have suggested that cotyledons of late varieties do not contain any inhibitor at all, and plants will flower autonomously once they have reached a certain stage of development. The results presented here do not appear to support this theory. Although Haupt (1969) comments that cotyledon removal may advance NF by temporarily arresting growth, the difference in the number of expanded nodes between intact and decotyledonized plants does not correspond with the difference in NF.

From Table 3.9, it is apparent that plants excised at 1-day, 2-days and 4-days can be grouped together on the basis of growth rate (NE), as can plants that were excised at 9-days, 12-days, or had their cotyledons left intact. Although FT shows a similar response, the effect of cotyledon removal on NF is seen to be a gradual one, especially under L.D. conditions (Tables 3.8, 3.9). Although there may be a connection between the effect of cotyledon removal on both the growth rate and NF, it does not explain fully the results presented here. This "connection" is discussed further in Chapter 7.

It was with these conclusions in mind that experiments involving various vernalization treatments were conducted in an attempt to further understand the cotyledon response.

TABLE 3.1

CHEMICAL COMPOSITION OF 10 DIFFERENT AGAR MEDIA

Medium 1 was used at the beginning of the experimental programme, but ~~medium 8 was substituted~~ on the completion of this experiment. Figures underlined show where the various media differ from the two standards.

| Unit | Ingredients | Medium | | | | | | | | | |
|---------------|-----------------------------------|--------|-----|------------|-----------|----------|-------------|-------------|------|------------|------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| mM | Ca(NO ₃) ₂ | .6 | .6 | .6 | .6 | .6 | .6 | .6 | 2 | 2 | 2 |
| | KNO ₃ | .25 | .25 | .25 | .25 | .25 | .25 | .25 | 1 | 1 | 1 |
| | MgSO ₄ | .2 | .2 | .2 | .2 | <u>5</u> | .2 | <u>5</u> | 5 | 5 | 5 |
| | KH ₂ PO ₄ | .25 | .25 | .25 | .25 | .25 | <u>12.5</u> | <u>12.5</u> | 12.5 | 12.5 | 12.5 |
| | FeSO ₄ | .05 | .05 | .05 | <u>.5</u> | .05 | .05 | .05 | .05 | .05 | .05 |
| | E.D.T.A. | .05 | .05 | .05 | <u>.5</u> | .05 | .05 | .05 | .05 | .05 | .05 |
| μM | CuSO ₄ | - | - | - | <u>.1</u> | - | - | - | .1 | .1 | .1 |
| | MnSO ₄ | - | - | - | <u>10</u> | - | - | - | 10 | 10 | 10 |
| | ZnSO ₄ | - | - | - | <u>2</u> | - | - | - | 2 | 2 | 2 |
| | H ₃ BO ₃ | - | - | - | <u>10</u> | - | - | - | 10 | 10 | 10 |
| mgm/ litre | + glycine | 3 | - | 3 | 3 | 3 | 3 | 3 | - | - | <u>3</u> |
| | + thiamine | 0.1 | - | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - | - | <u>0.1</u> |
| | + pyridoxine | 0.1 | - | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - | - | <u>0.1</u> |
| % | sugar | 2 | 2 | <u>0.2</u> | 2 | 2 | 2 | 2 | 2 | <u>0.2</u> | 2 |
| | agar | 0.75 | .75 | .75 | .75 | .75 | .75 | .75 | .75 | .75 | .75 |

TABLE 3.2
EFFECT OF THE MEDIUM ON TRANSPLANT TIME (T), ROOT DEVELOPMENT AND SHOOT DEVELOPMENT
IN EXCISED EMBRYOS OF LINE 24 (SPRING CROP)

L.D. photoperiod only was used. n, number of plants scored.

| Characters Recorded | Medium | | | | | | | | | |
|--------------------------------|---------|--------|------|--------------------------|--------------------------|--------|--------|----------|---------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| T (days) | 25 | 19 | 25 | 20 | 20 | 18 | 18 | 13 | 18 | 18 |
| Root development | average | strong | weak | average slow start | average slow start | strong | strong | v.strong | average | weak |
| Shoot development * | | | | | | | | | | |
| (a) 5th leaf open | 12 | 10 | 10 | | 6 | 7 | | | 12 | |
| (b) 5th internode expanding | 16 | 12 | 11 | 6 | 8 | 6 | 6 | 10 | 10 | 6 |
| (c) 4th leaf open | | 4 | 6 | 16 | 12 | 17 | 12 | 10 | 5 | 12 |
| (d) 4th internode expanding | | | 3 | 5 | | | 10 | 5 | | 7 |
| n | 28 | 26 | 30 | 27 | 26 | 30 | 28 | 25 | 27 | 25 |

* The figures in the columns score the number of plants which have reached the stage of development as shown at the time of transplant.

The NF data for plants transplanted at different stages of development is given in Table 3.3.

TABLE 3.3

EFFECT OF THE MEDIUM ON NF AND FT IN EXCISED EMBRYOS OF LINE 24 (SPRING CROP)

The results for NF are ~~separated~~ in the first instance to relate with Table 3.2. L.D. photoperiod only was used. n, number of plants scored. The figures for NF and n of intact controls are also given.

| Characters Recorded | Medium | | | | | | | | | |
|----------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| NF (a) | 13.5 | 13.9 | 14.0 | | 14.0 | 14.3 | | | 14.3 | |
| (b) | 13.6 | 13.7 | 13.4 | 13.8 | 13.5 | 14.0 | 14.0 | 14.0 | 13.7 | 14.3 |
| (c) | | 13.0 | 13.8 | 13.1 | 13.8 | 13.3 | 13.8 | 14.4 | 14.0 | 13.9 |
| (d) | | | 13.5 | 13.2 | | | 13.9 | 13.2 | | 13.9 |
| NF (average and) | 13.56 | 13.64 | 13.69 | 13.30 | 13.77 | 13.69 | 13.75 | 14.00 | 14.04 | 14.00 |
| ± SE | .11 | .11 | .12 | .09 | .12 | .15 | .12 | .14 | .10 | .12 |
| n (surviving plants) | 26 | 25 | 26 | 27 | 26 | 26 | 28 | 25 | 23 | 24 |
| FT (approx.) | 63 | 61 | 63 | 62 | 62 | 62 | 62 | 59 | 63 | 64 |

Control NF 15.70 ± .16
(Intact Plants) n 20

Experiment 3.1

TABLE 3.4

CHEMICAL COMPOSITION OF TWO NUTRIENT STOCKS

| Ingredients | Nutrient Solution a | Nutrient Solution b |
|----------------------------|---------------------|---------------------|
| $\text{Ca}(\text{NO}_3)_2$ | 10 | 20 |
| KNO_3 | 10 | 10 |
| MgSO_4 | 4 | 8 |
| KH_2PO_4 | 2 | 4 |
| FeSO_4 | .2 | .5 |
| E.D.T.A. | .2 | .5 |
| | | mM |
| H_3BO_3 | 90 | 100 |
| ZnSO_4 | 2 | 20 |
| CuSO_4 | 0.6 | 1.0 |
| MnSO_4 | 18 | 100 |
| NaMoO_4 | 0.2 | 1.0 |
| | | μM |

Nutrient solution a: The solution normally used in the Hobart glasshouse.

Nutrient solution b: A solution based on de Fossard (1967).

TABLE 3.5

EFFECT OF TWO NUTRIENT STOCKS ON NF IN INTACT AND DECOTYLEDONIZED
PLANTS OF LINE 24 (SUMMER CROP)

Both nutrient stocks were administered once or twice weekly.
L.D. photoperiod only was used. n, number of plants scored.
Nutrient agar medium 8.

| Stock | Dose/ Week | Cotyledon Status | | | | | |
|-------|---------------|--------------------|-------|-----|-------------------|-------|-----|
| | | Intact | | | Excised | | |
| | | n | NF | SE | n | NF | SE |
| a | 1 | 20 | 16.35 | .15 | 14 | 13.57 | .17 |
| | 2 | 20 | 16.95 | .14 | 12 | 14.33 | .26 |
| | | (P = 0.01) | | | (P = 0.02) | | |
| b | 1 | 20 | 16.50 | .14 | 8 | 14.00 | .33 |
| | 2 | 20 | 17.05 | .15 | 10 | 14.90 | .23 |
| | | (0.001 < P < 0.01) | | | (0.02 < P < 0.05) | | |

TABLE 3.6

EFFECT OF NUTRIENT DOSE ON NF, FT AND NE (NUMBER OF EXPANDED
NODES AFTER 32 DAYS' GROWTH) IN INTACT PLANTS OF LINE 24
(SPRING CROP)

Both S.D. and L.D. photoperiods were used.

| Dosage Rate/ Week | Characters Recorded | S.D. | | | L.D. | | |
|-------------------------|------------------------|------|-----------|------|-----------------|-----------|-----|
| | | n | \bar{x} | SE | n | \bar{x} | SE |
| 1. | NE | 16 | 9.14 | .10 | 15 | 10.37 | .09 |
| | NF | 16 | 20.94 | .35 | 15 | 15.38 | .24 |
| | FT | 16 | 63.44 | .88 | 15 | 46.08 | .56 |
| 2. | NE | 15 | 9.97 | .10 | 15 | 10.75 | .12 |
| | NF | 15 | 22.13 | .50 | 15 | 16.07 | .21 |
| | FT | 15 | 63.67 | 1.04 | 15 | 46.03 | .44 |
| P (for NF) | | 0.05 | | | 0.02 < P < 0.05 | | |

TABLE 3.7

EFFECT OF LIGHT INTENSITY AND GROWTH RATE ON NF AND FT IN EXCISED PLANTS OF LINE 24 (SPRING CROP)

L.D. photoperiod only was used. n, number of plants scored at flowering. Nutrient agar medium 8. The results for NF and FT of intact control plants are also given.

| Time to Reach 5th Leaf Open (Days) | Light Intensity (Lumens) | | | | FT - From Transplant (Days) | n | NF |
|--|--------------------------|-----------------|-----------------|-----------------|-----------------------------------|----|-----------------|
| | 1400 | 700 | 350 | 175 | | | |
| | n | n | n | n | | | |
| 17 | 15 | 12 | 3 | - | 44.20 \pm .31 | 30 | 14.47 \pm .10 |
| 19 | 8 | 4 | 6 | 3 | 43.47 \pm .56 | 20 | 14.55 \pm .17 |
| 21 | | 8 | 9 | - | 43.94 \pm .60 | 17 | 14.24 \pm .14 |
| 23 | | | 6 | 11 | 43.71 \pm .63 | 17 | 14.35 \pm .23 |
| 25 | | | | 8 | 45.50 \pm .10 | 8 | 14.13 \pm .04 |
| 28 | | | | 2 | - | - | - |
| Average Time to Transplant (Days) | 17.70 \pm .20 | 18.67 \pm .37 | 20.50 \pm .40 | 23.58 \pm .47 | | | |
| FT - From Transplant (Days) | 43.95 \pm .44 | 43.71 \pm .35 | 43.75 \pm .65 | 44.73 \pm .61 | | | |
| n | 22 | 24 | 24 | 24 | | | |
| NF | 14.55 \pm .14 | 14.25 \pm .35 | 14.38 \pm .16 | 14.41 \pm .17 | | | |

CONTROL

FT

n

NF

42.58 \pm .62

12

17.08 \pm .36

TABLE 3.8

EFFECT OF COTYLEDON REMOVAL AT VARIOUS STAGES OF GERMINATION ON NF IN PLANTS OF LINE 24 (WINTER CROP)

L.D. photoperiod only was used. Data is also given for the number of nodes laid down at the time of excision (NT). n, number of plants scored. Nutrient agar medium 1 (see Chapter 3.b.I).

| Characters Recorded | Time of Cotyledon Removal | | | | | | | |
|---------------------|---------------------------|-------|-------|-------|-------|-------|--------|---------|
| | 8 hrs. | 1 day | 2 day | 4 day | 6 day | 9 day | 12 day | Control |
| NT | 6.0 | 6.2 | 7.0 | 8.0 | 8.8 | 10.5 | 12.2 | - |
| NF | 12.75 | 12.88 | 13.04 | 13.24 | 14.00 | 14.54 | 15.40 | 15.43 |
| ±SE | .14 | .19 | .14 | .10 | .11 | .15 | .14 | .12 |
| n | 20 | 17 | 25 | 21 | 19 | 28 | 30 | 30 |

Experiment 3.5

TABLE 3.9

EFFECT OF COTYLEDON REMOVAL AT VARIOUS STAGES OF GERMINATION ON NF IN PLANTS OF LINE 24 (SUMMER CROP)

Both S.D. and L.D. photoperiods were used. Data is also given for the number of expanded nodes after 33 days' growth from germination (NE), and the flowering time (FT). n, number of plants scored. Nutrient agar medium 8.

| Photo-period | Characters Recorded | Time of Cotyledon Removal | | | | | | |
|--------------|---------------------|---------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | 1 day | 2 day | 4 day | 6 day | 9 day | 12 day | Control |
| S.D. | NE | 6.6 | 6.8 | 7.8 | 9.2 | 11.4 | 11.8 | 11.8 |
| | NF \pm SE | 21.60 .58 | 23.69 .44 | 23.63 .52 | 20.84 .43 | 22.11 .34 | 21.76 .41 | 22.11 .31 |
| | n | 15 | 16 | 16 | 19 | 18 | 17 | 19 |
| | FT (days) | 103 | 106 | 98 | 69 | 62 | 58 | 58 |
| L.D. | NE | 7.2 | 7.2 | 7.6 | 9.2 | 11.2 | 11.8 | 11.8 |
| | NF \pm SE | 14.21 .14 | 14.41 .17 | 14.63 .20 | 15.00 .19 | 15.70 .13 | 16.70 .25 | 16.65 .18 |
| | n | 19 | 17 | 19 | 19 | 20 | 20 | 20 |
| | FT (days) | 54 | 54 | 51 | 43 | 39 | 39 | 39 |

TABLE 3.10

EFFECT OF COTYLEDON REMOVAL ON THE RATE OF NODE
FORMATION IN PLANTS OF LINE 24 (SUMMER CROP)

L.D. photoperiod only was used. The average rate of node formation is also given. Nutrient agar medium 1.

| Time (Days) | Cotyledon Status | |
|--------------------------------|------------------|-----------------|
| | Intact | Excised |
| 0 | 6.00 \pm .00 | 6.00 \pm .00 |
| 4 | 7.00 \pm .00 | 7.00 \pm .10 |
| 6 | 8.00 \pm .00 | 7.50 \pm .15 |
| 9 | 9.70 \pm .15 | 7.80 \pm .10 |
| 12 | 11.20 \pm .13 | 8.30 \pm .13 |
| 16 | 13.00 \pm .00 | 8.50 \pm .17 |
| 20 | 14.90 \pm .10 | 9.00 \pm .12 |
| 26 | 17.00 * | 10.40 \pm .14 |
| 30 | | 11.20 \pm .13 |
| Average Rate of Node Formation | 0.46 nodes/day | 0.15 nodes/day |

* flowering

TABLE 3.11

EFFECT OF COTYLEDON REMOVAL ON NE (THE NUMBER OF
EXPANDED NODES AFTER 33 DAYS' GROWTH) IN PLANTS OF
LINE 24 (SPRING CROP)

Both S.D. and L.D. photoperiods were used. n, number of plants scored. Nutrient agar medium 8.

(a)

| Cotyledon Status | S.D. | | | | L.D. | | | |
|------------------|------|-----|-----|----|------|------|-----|----|
| | n | NE | ± | SE | n | NE | ± | SE |
| Intact | 10 | 9.9 | .10 | | 10 | 10.2 | .12 | |
| Excised | 10 | 5.4 | .17 | | 10 | 5.6 | .17 | |

(b)

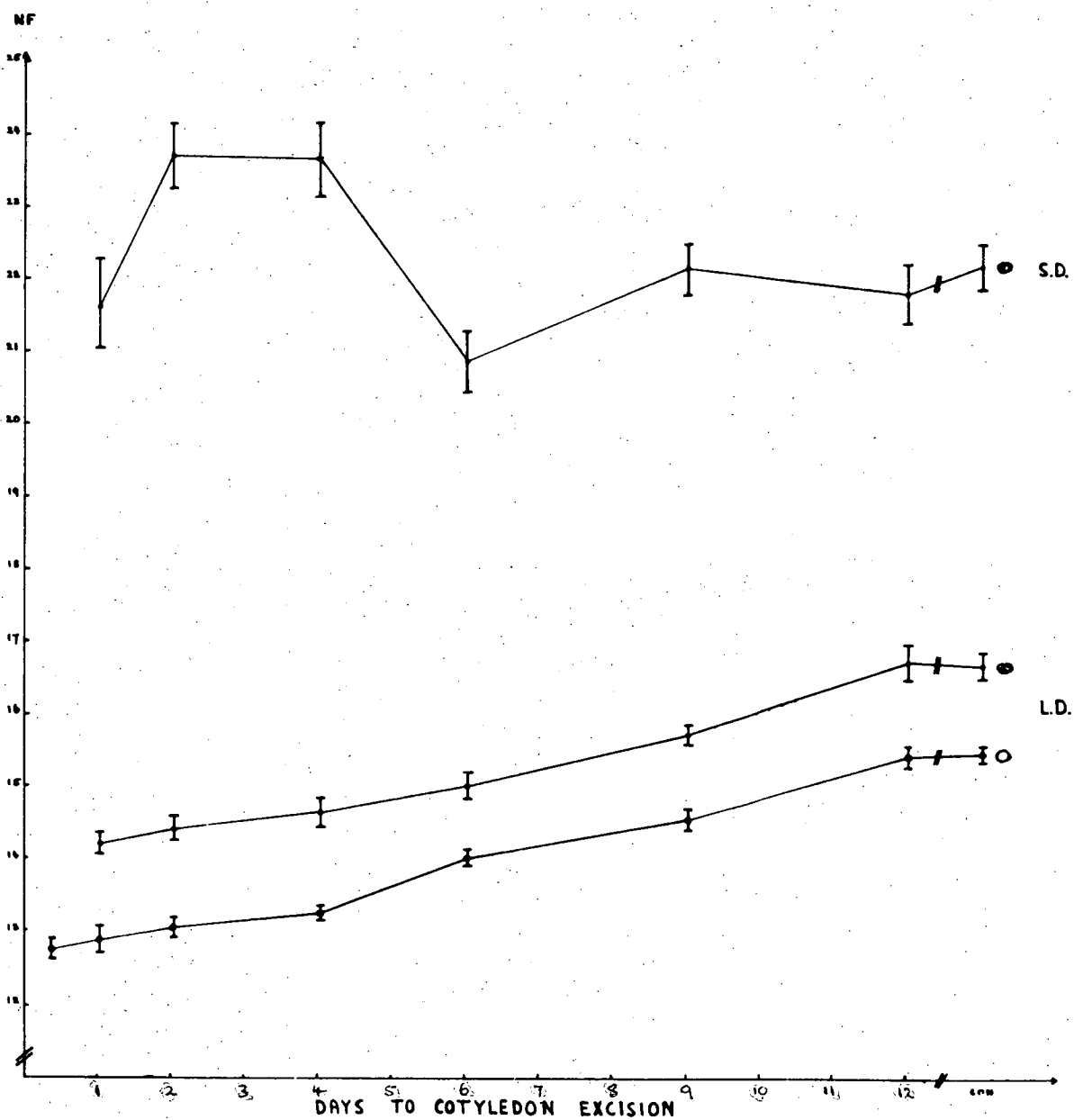
| Cotyledon Status | S.D. | | | | L.D. | | | |
|------------------|------|-----|----|----|------|------|----|----|
| | n | NE | ± | SE | n | NE | ± | SE |
| Intact | 28 | 9.4 | .1 | | 29 | 10.4 | .1 | |
| Excised | 39 | 5.4 | .1 | | 35 | 5.8 | .1 | |

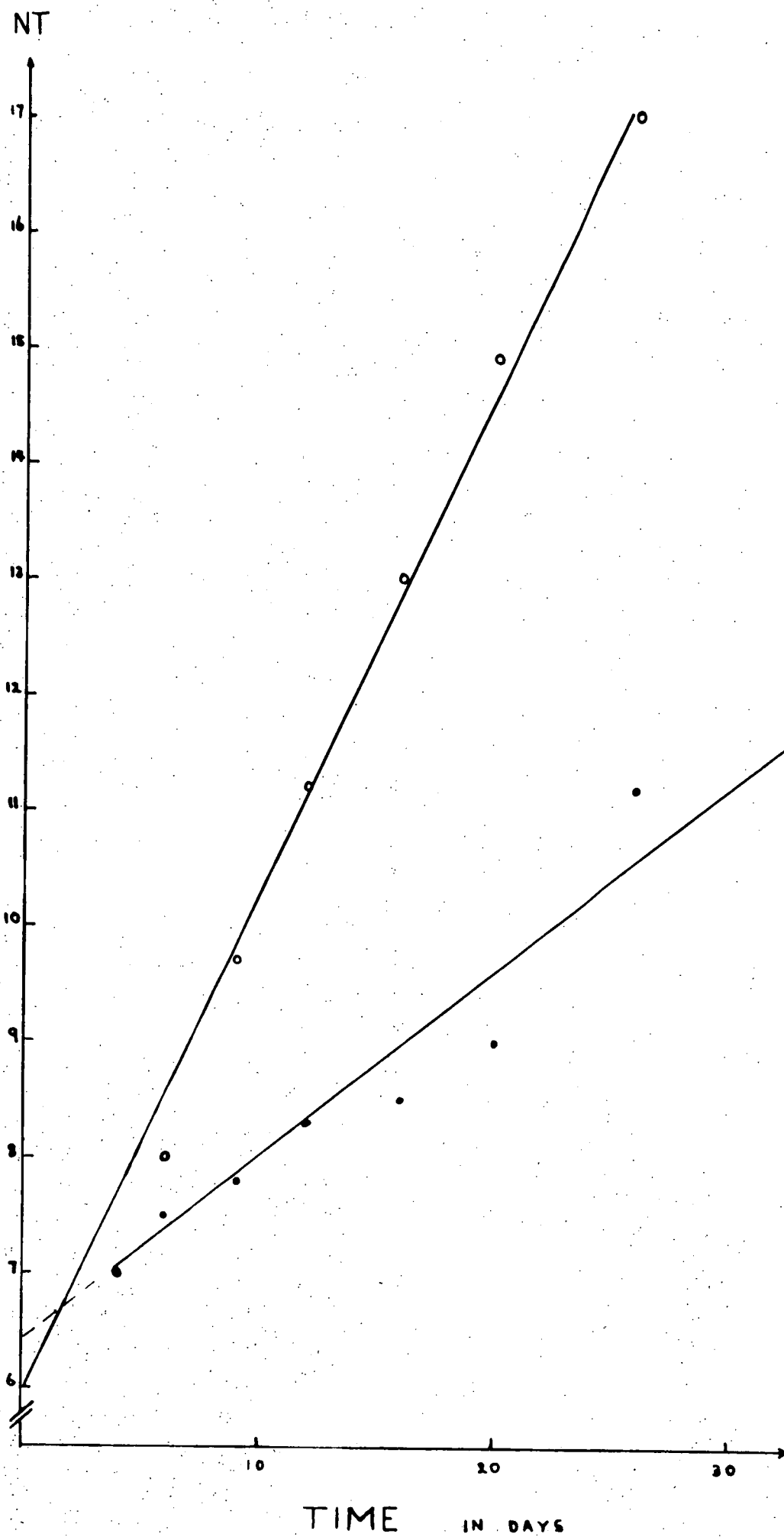
TABLE 3.12

EFFECT OF COTYLEDON REMOVAL ON NF AND FT
IN PLANTS OF LINE 24 (WINTER CROP)

Both S.D. and L.D. photoperiods were used. n, number of plants scored. Nutrient agar medium 8.

| Cotyledon Status | S.D. | | | | L.D. | | | |
|---------------------|------|-------------|-----|----|-------------|----|--|--|
| | n | NF \pm SE | FT | n | NF \pm SE | FT | | |
| Intact | 16 | 22.06 .36 | 75 | 13 | 16.00 .23 | 54 | | |
| Excised | 23 | 19.00 .50 | 109 | 22 | 13.00 .13 | 78 | | |





VERNALIZATION

a INTRODUCTION

It is a well documented fact that late varieties of Pisum sativum will respond quite markedly to a period of vernalization treatment given during germination (e.g. Barber, 1959; Highkin, 1956; Moore and Bonde, 1958, 1962; Stanfield et al, 1966; Paton, 1969). This response to vernalization consists of advancing both NF and FT, and occurs in both intact and decotyledonized plants under both S.D. and L.D. photoperiod regimes (Amos and Crowden, 1969).

Although Highkin (1956) observed that the effect of temperature is most easily detected during the early stages of development, Barber (1959) has stated that vernalization in late varieties of peas is most likely a continuous process and not restricted to the early stages of growth. He further observed that prior vernalization reduces the photoperiodic response and suggested that the two reactions compete with each other. Paton (1969), who demonstrated a separation of those events related to the leaf requirement (induction) and those between induction and initiation of the first flower (evocation - Knox and Evans, 1968), surmised that the effect of seed vernalization is probably additive to that of photoperiod. He suggested that whereas photoperiod influences induction, vernalization influences evocation by repressing the synthesis of a graft-transmissible floral inhibitor.

From the results of experiments reported in this chapter, Amos and Crowden (1969) showed that in late cultivars vernalization appears to have two separate effects, both of which promote flower initiation to a lower node. Although the smaller effect is manifest on the cotyledonary inhibitor system, the major effect occurs in the young embryo and is effective before photoperiodic induction has occurred.

They further suggested that this effect may be partially obscured by the cotyledonary inhibitor (colysanthin), unless the cotyledons are removed soon after vernalization is completed.

In preliminary experiments leading up to this present investigation, it was observed that a vernalization period of 1 week will result in a number of new nodes formed approximately equivalent to 1 day's growth under normal temperatures, 2 weeks equivalent to 2 days, and so on. This fact has been taken into account in the experimental design such that unvernallized plants were planted e.g. 4 days prior to vernalized plants completing a vernalization period of 4 weeks. It has also been taken into account when calculating FT and growth rate.

In this chapter, the vernalization response is dealt with in some detail in an attempt to determine both its site of action and the stage of development at which the response to vernalization is manifest. The presentation of material is in four sections. The first of these, Section 3b, deals with a pilot experiment where embryos were vernalized under different light intensities to determine any effect on NF. Section 3c deals with the effect of vernalization on plant growth. The third section, Section 3d, is concerned with the effect of vernalization on flowering; Section 3e is a general discussion.

b VERNALIZATION AND LIGHT INTENSITY

This section is concerned with the light intensity under which embryos were vernalized. In most experiments, embryos were vernalized under L.D. conditions with a light intensity of approximately 50 lumens. However, in the first experiment where embryos were vernalized under both S.D. and L.D. conditions, a relatively high light intensity (approximately 1400 lumens) was used and most of the embryos died (c.f. Experiment 3.4). The purpose of this experiment was both to find a satisfactory level of light intensity which would allow embryos to

survive through to the post-vernalization stage, and to observe any effect of light intensity on the subsequent NF.

Experiment 4.1:- Decotyledonized plants, 20 plants per treatment, were subjected to a vernalization period of 4 weeks under L.D. conditions at light intensities ranging from 50 - 800 lumens. At the completion of the vernalization treatment, plants were grown to anthesis under normal L.D. conditions and scored for NF. The results are given in Table 4.1.

Results:- Table 4.1 shows that no embryos survived if vernalized at a light intensity of 800 lumens, and only 3 of the 20 survived if vernalized at 600 lumens. There was no significant effect of light intensity on the subsequent NF (P - not significant), while a normal vernalization effect was observed, although not as large as normal. Although not recorded in the Table, it was also noted that high light intensities promoted shoot growth, while low light intensities favoured root growth.

Discussion:- The low values for NF of the control plants are attributed to the winter conditions, and it is thought that plants may have been affected by the low growing temperature during the post-vernalization period of growth. It seems that the maximum light intensity at which embryos can be vernalized under L.D. conditions is between 400 and 600 lumens. Since high light intensities encourage shoot growth, it is thought that plants which are exposed to high light intensities during vernalization die from exhaustion, as the temperature would not allow for growth to be as rapid as is required by the light intensity.

The light intensities used here did not have any significant effect on NF, and future ^{vernalization} experiments were conducted at the low light intensity of 50 lumens to promote a vigorous root growth.

c VERNALIZATION AND GROWTH RATE

In a previous experiment (Experiment 3.7) it was observed that cotyledon removal had a marked effect on the growth rate of excised plants, and the subsequent advancement of NF was discussed - at least in part - in relation to this effect. Since vernalization also advances NF, it was felt necessary to determine whether prior vernalization also affected the growth rate of plants, and in this Section, an experiment is described where the effect of vernalization on the growth rate of both intact and excised plants was examined.

Experiment 4.2:- Intact and decotyledonized plants were either left unvernallized or vernalized for periods of from 1 to 4 weeks and then grown to anthesis under L.D. conditions. The apices of 10 plants were dissected at various time intervals after completion of the vernalization treatment until flowers had been initiated to determine the total number of nodes laid down. The results are given in Table 4.2 and Figure 4.1.

Results:- Under the conditions of this experiment the rate of node formation for intact plants was 0.50 nodes/day, and for decotyledonized plants 0.24 nodes/day (c.f. Experiment 3.7). Prior vernalization had no effect on the rate of node formation (see Figure 4.1).

Discussion:- The results are in general agreement with those presented for cotyledon removal in Experiment 3.7, and in Paton (1969). The effect of vernalization on flowering behaviour is in no way related to a change in the growth rate - as may be the case with cotyledon removal - since the growth rate is not affected by prior vernalization treatment.

d VERNALIZATION AND FLOWERING

In this section, the effect of vernalization on flowering is examined in some detail. The section is divided into three sub-sections. The first is concerned with the effect of vernalization on excised plants under L.D. conditions, the second deals with an experiment which was designed to determine whether immature seeds have the capacity to respond to vernalization while still in the pod, and the third is concerned with the question of an interaction between photoperiod and vernalization.

I. VERNALIZATION OF EXCISED PLANTS

Four experiments are described which were designed to determine the likely site of action of the vernalization response. All of them were conducted under a L.D. photoperiod regime and all embryos were grown on medium 1.

The first of these experiments deals with both the period of vernalization necessary to elicit a maximum response and the effect of cotyledon removal on that response.

Experiment 4.3:- Six different vernalization treatments were employed. Within each treatment, 50 intact and 30 decotyledonized plants were exposed to a temperature of 3°C for periods ranging from 0 - 6 weeks. At the completion of the vernalization treatment, cotyledons were removed from 30 of the intact plants, and then all plants were grown to anthesis under L.D. conditions. The results are shown in Table 4.3 and Figure 4.2.

Results:- For intact plants, there was a gradual advancement in NF with increasing exposure to vernalization up to 4 weeks, at which stage NF again began to be delayed. For decotyledonized plants, the advancement of NF was more abrupt, and it was obvious that some plants responded maximally to vernalization with only a 1-week treatment. However, the average NF fell to a minimum

after a 3-week treatment. After this time, the NF for plants that were decotyledonized after vernalization was again delayed (to NF 13.33 at 4 weeks and NF 13.66 at 6 weeks), whereas the NF for plants that were decotyledonized prior to vernalization remained around 12.

It is obvious that not only do excised plants respond to vernalization in a different fashion than intact plants, but also the time of cotyledon removal is important, since plants that were excised prior to a 4-week or 6-week vernalization treatment showed a different response to those that were excised after similar periods of vernalization treatment. This fact was explored further in the second experiment, where plants were excised during the vernalization treatment.

Experiment 4.4:- Embryos were dissected from imbibed seeds which had been vernalized for varying periods up to 4 weeks. Some of these embryos were then given extended vernalization treatments up to a total of 4 weeks, in isolation from the cotyledon influence. The rate of node formation from the conclusion of the various vernalization treatments was determined for both intact and excised plants by dissection of the apices of a number of plants. Plants were grown to anthesis under L.D. conditions and their NF recorded. The results are shown in Table 4.4.

Results:- Again it can be seen that intact plants showed a progressive advancement in NF as the vernalization period was extended to 4 weeks. However, if the cotyledons were removed before post-vernalization growth commenced, then a vernalization period of 1 week only was sufficient to obtain an almost maximum advancement of NF.

Cotyledon removal during a vernalization treatment of greater than 1 week had little effect in further advancing NF. Plants that were decotyledonized after vernalization flowered out of a higher node than those that were decotyledonized prior to vernalization, although in this case the difference was not as marked as in Experiment 4.3.

The growth rate of intact plants was in the order of 0.50 nodes/day, and for excised plants about 0.25 nodes/day. Prior vernalization had no effect on the growth rate (see also Experiment 4.3).

It would seem from the previous two experiments that the cotyledons do in fact play a fairly substantial role in the flowering behaviour of Line 24 plants. In the third experiment, this role was examined further by removing only one cotyledon and subjecting these plants to a vernalization treatment.

Experiment 4.5:- Intact, semi-decotyledonized and fully-decotyledonized plants were either left unvernallized or given a vernalization treatment of 4 weeks. On completion of vernalization, plants were grown under L.D. conditions to anthesis and scored for NF. The results are given in Table 4.5 and Figure 4.3.

Results:- Table 4.5 shows that the vernalization effect was approximately 2 nodes, irrespective of cotyledon status, and that further, the cotyledon effect was about 2 nodes, irrespective of vernalization treatment. Removing one of the cotyledons caused plants to flower at an intermediate NF.

The previous three experiments have shown that vernalization has a marked effect on the flowering behaviour of both intact and excised plants if given once germination has commenced. A further experiment was designed to see whether the vernalization effect was modified in any way if given to plants at different stages after germination had commenced.

Experiment 4.6:- Intact plants were germinated for varying intervals to 14 days before vernalization treatments of 4 weeks were begun, and the cotyledons were removed from different groups of plants immediately before or after vernalization. The entire experiment was conducted under L.D. conditions. Plants were

grown to anthesis, and the results for NF, the number of nodes laid down prior to and at the conclusion of the vernalization treatment are given in Table 4.6.

Results:- It can be seen from Table 4.6 that, irrespective of the cotyledon status, the response to vernalization decreased as germination and growth of the plants progressed. In fact, once plants had reached the node 12 - 13 stage of development (between 10 and 14 days after germination), vernalization had no significant effect on NF. Cotyledon removal at 14 days was without effect on unvernallized plants, but an effect of marginal significance ($P = 0.05$) was still apparent with vernalized plants. As shown previously (Tables 4.3 and 4.4), cotyledon removal before vernalization resulted in a greater advancement of NF than did post-vernalization excision.

The decreasing effect of vernalization on NF is not correlated with a change in the rate of node formation during the vernalization period. For intact plants, the rates of node formation during the vernalization period given after 6, 10 and 14 days' germination were approximately the same in all cases (approximately 0.5 nodes/week). If the cotyledons were removed prior to vernalization, the number of nodes formed per week in the embryos during vernalization increased sharply from 0.05 at 6 days to 0.15 at 10 days to 0.5 at 14 days, whereas the effect of vernalization in these groups of plants showed an almost linear decline.

Discussion:- The nature of the vernalization response is clarified by the results of the series of experiments presented here. From the outset, it must be emphasised that the vernalization response differs markedly from that of cotyledon removal in that vernalization does not affect the rate of subsequent node formation to any great extent (see Tables 4.2, 4.4; Figure 4.1).

Tables 4.3 and 4.4 show that a major effect of vernalization is manifest directly on the embryo, and that this response to vernalization requires only a comparatively short-term exposure

to low temperature in order to yield a maximum response. Since under normal growing conditions the NF of vernalized plants does not regain the original value for unvernallized controls, it would appear that the effect of vernalization on the embryo is partially obscured if the cotyledons are left attached to the growing plant after vernalization.

It is this series of experiments, dealing with the vernalization response, that has provided the best evidence for the presence of a floral inhibitor in the cotyledons of late varieties.

Both Barber (1959) and Paton (1969) have implied that the response to vernalization can be interpreted in terms of a direct effect of vernalization on the cotyledon inhibitor, either by destruction or by reduced synthesis. However, it appears from these experiments that any effect of vernalization on the cotyledon system is significantly less than the maximum response that can be realised. This maximum response to vernalization is achieved only if cotyledons are removed before post-vernalization growth at normal temperatures is allowed to occur; the presence of the cotyledons appears to reduce this maximum response.

Nor is there evidence in these experiments to endorse the proposal brought forward by Barber (1959) that vernalization leads to the destruction of colysanthin at the plant apex. In most experiments involving both vernalization and cotyledon removal, vernalization is more effective in advancing NF than cotyledon removal alone. If colysanthin is indeed the substrate for the vernalization response, then it must follow from Barber's hypothesis that the embryo of the imbibed seed already contains a significant quantity of the inhibitor - or alternatively, as Murfet (1971b,c; 1973) suggests, it has the ability to manufacture colysanthin during the post-vernalization phase of growth. However, experiments involving sequential cotyledon removal (see Johnston and Crowden, 1967; also Chapter 3) and leaching (Sprent and Barber, 1957) show that the movement of colysanthin from the cotyledons does not begin until about day 4 after germination.

Nevertheless, it would seem that a flower inhibitory substance is manufactured in the cotyledons, and is transported into the shoot during the first 14 days of growth. This period corresponds to the decreasing sensitivity of the shoot to vernalization treatment (Table 4.6), and it may be argued that it is the presence of colysanthin that has arrived at the apex prior to vernalization which decreases or masks the effect of vernalization in young plants.

It can also be observed in Tables 4.3, 4.4 and 4.6 that cotyledon removal is more effective in advancing NF if carried out prior to vernalization, irrespective of how long after germination the vernalization period is commenced. Whilst it can be expected that some colysanthin has already entered the shoot during the period of germination preceding the vernalization treatment (at 6, 10 and 14 days), thus providing for the progressive delay of NF in both groups of decotyledonized plants, it is apparent from this theory that in plants involving post-vernalization excision, movement of inhibitor from the cotyledons continues throughout the vernalization treatment, in company with the limited growth which takes place during this period. In each of these cases, it is implied that colysanthin present at the apex survives the vernalization treatment and effectively reduces the vernalization response.

There is evidence that vernalization has a direct effect on the cotyledon inhibitor system. Although it can be seen from Table 4.5 that there is no interaction between vernalization and cotyledon removal, both effects being additive to each other, a strong interaction between these two factors does appear to exist in experiments where both intact and excised plants were subjected to varying lengths of vernalization treatment (see Tables 4.3, 4.4). This apparent interaction is explained by the fact that excised plants show a rapid response to vernalization, achieving maximum effect after 2 weeks, whereas intact plants show only a gradual response to vernalization up to 4 weeks.

It is this progressive increase in the effect of vernalization on intact plants which suggests an effect on the cotyledon system. It may reflect either a steady, low-temperature destruction of colysanthin or more probably a progressive repression of inhibitor synthesis.

In either case, the net effect appears to be that before the inhibitor level can be restored to its normal effective threshold, a lesser level of growth is achieved before the plant becomes photo-induced, and flower initiation is evoked. Unlike the apical vernalization response, vernalization of the cotyledon system requires a longer period of treatment in order to register the full effect.

One result which cannot readily be explained at this stage is the response of intact plants to a vernalization treatment of greater than 4 weeks. It may well be that the extended period of vernalization has either delayed the production or reduced the capacity of the plant to synthesize the inductive stimulus. Alternatively, the growth pattern of the plant may have been altered by the long period of cold treatment (see Chapter 7).

II. PARENT VERNALIZATION

Throughout the investigation, it was thought that vernalization may have had an effect on seed which was still developing in the pod, and as such an experiment was designed to see whether such an effect did exist.

Experiment 4.7:- Intact plants were vernalized for 4 weeks and, together with unvernallized controls, grown under L.D. conditions to anthesis. Once the pods appeared, whole plants were subjected to one of four treatments, involving vernalization temperatures for 8 hours each night. These were respectively:

- (i) no vernalization treatment at all.

- (ii) night vernalization from the time the pod first appeared through the flower petals until it had swollen to maximum size.
- (iii) night vernalization from the time the pod had swollen to maximum size until it had desiccated.
- (iv) night vernalization from the time the pod first appeared through the flower petals until it had desiccated.

Seed was collected from the pods of nodes 15 and 16 for unvernallized plants, and from node 14 pods for vernalized plants. Half of the progeny were then subjected to a vernalization treatment of 4 weeks, and the other half grown as unvernallized controls. All plants were grown under L.D. conditions to anthesis as before and scored for NF. The results are given in Table 4.7.

Results:- In all progeny, irrespective of the different pod treatment conditions, vernalizing the seed for 4 weeks during germination had the effect of advancing NF by 2 - 3 nodes. Vernalization had less effect on the spring crop (approximately 1.9 nodes) than on the summer crop (approximately 2.4 nodes).

Seed from vernalized plants had a tendency to flower out of a higher node than those from unvernallized plants. Although the difference was not significant for seed which had not been vernalized (NF 17.54 - NF 17.69, $P = 0.1$) vernalized seed did show a significant difference (NF 15.01 - NF 15.40, $P = 0.001$). Pods of vernalized plants which were themselves subject to vernalization temperatures produced progeny that flowered out of a slightly lower node (e.g. NF 15.00 - NF 15.67, $P = 0.01$). However, the same was not true for pods of unvernallized plants.

Discussion:- Overall, the effect of vernalization does not seem to be transmitted between generations. Although there is a tendency for a vernalized plant to produce progeny that are less responsive to vernalization, the scale of response in no way

approaches the regular 2 - 3 node response observed for vernalization. Further, unvernallized progeny show no significant effect. Seeds which are exposed to vernalization temperatures while still developing in the pod show little sign of being affected.

In the previous sub-section, the various sites of the vernalization response were discussed. From this experiment, it would seem that the apex is incapable of perceiving a vernalization response until it has both become fully developed and undergone a period of dormancy. The same applies to the cotyledon system.

III. THE INTERACTION BETWEEN PHOTOPERIOD AND VERNALIZATION

The next step was to compare the effects of vernalization on intact and decotyledonized plants under different photoperiods.

Experiment 4.8:- Many experiments were conducted involving the effect of all three factors - namely cotyledon removal, vernalization and photoperiod - on NF in Line 24. The results for a typical experiment are shown in Table 4.8 and Figure 4.4.

Results:- The results shown in Table 4.8 are in agreement with those shown previously for plants grown under L.D. conditions (Tables 4.3 and 4.4). Intact plants showed a gradual advancement in NF as the vernalization period was extended to 4 weeks, whereas in decotyledonized plants, maximum advancement was obtained after a 2-week vernalization period. Photoperiod did not alter the qualitative nature of the vernalization and cotyledon removal responses, but simply superimposed its own response over those of the other two effects. In other words, the photoperiod response was an additive one.

Discussion:- Paton (1967, 1968, 1969) has proposed that in "Greenfeast" peas (equivalent to Line 24), photoperiod has a quantitative effect which is directly concerned with the attainment of a minimum leaf requirement for flowering (i.e. induction) and

the production in the leaves of an inductive stimulus. This stimulus, he suggests, passes from the leaves to the stem apex where flower initiation takes place.

Under S.D. conditions the minimum node at which flower initiation can occur is approximately node 18. In other words, the supply of inductive stimulus necessary to induce the apex to flower does not reach its effective threshold level until node 18 has been laid down (c.f. node 12 under L.D. conditions). This difference of 6 nodes is regarded as an expression of the quantitative difference in photoperiodic induction between the two photoperiod regimes for this cultivar. The production of inductive stimulus would be dependent on the quantity of light received by the plant.

In Barber's hypothesis (1959), vernalization and long photoperiod both act in a competitive fashion to destroy colysanthin. However, Johnston and Crowden (1967) reported that photoperiod and cotyledon removal appeared to be additive in effect, and now it has been shown that the photoperiod effect is additive to both cotyledon removal and vernalization. Paton (1969) has also demonstrated a physiological separation of the photoperiod and vernalization effects (c.f. Chapter 7). It would therefore seem that photoperiod is relatively independent of the other two treatments in its effect.

Although there is no evidence as to the precise nature of the apical vernalization reaction it appears fairly certain that it does not involve colysanthin. Rather it seems more logical to interpret the embryo response in terms of the inductive stimulus. Since under L.D. conditions photoperiodic induction in Line 24 can be completed by about node 12, it is apparent that the effect of seed vernalization is stable for at least 5 - 6 plastochron (e.g. Lyndon, 1968) intervals. Under S.D. conditions this period is even longer.

It can be observed from Table 4.6 that vernalization has no significant effect on apices which have reached the node 12 stage

of development. This would imply that vernalization only has an effect on the embryo when given to plants prior to the time of photoperiodic induction. It is therefore possible that vernalization acts in some manner to predispose the young plant to the photoinductive processes, rather than be implicated at a later stage in the evocation events, as Paton (1969) has suggested. On the other hand, colysanthin appears to be more concerned with the post-inductive events, and may partially obscure the vernalization response.

e GENERAL DISCUSSION

Pisum sativum, like many plants, undergoes a period of vegetative growth before reaching the ripeness-to-flower condition, a condition which is probably an absolute expression of the genetic constitution of the plant (see Chapter 6). However, the realization of this genetic potential may require an appropriate combination of environmental conditions. There is good evidence in this present experimental programme to show that the minimum node out of which Line 24 plants may flower is at node 12. Since there are 6 nodes already present in the embryo at the time of imbibition, the attainment of the minimum node number for flower initiation involves the formation of a further 6 vegetative nodes after the commencement of germination. Normally the observed NF for Line 24 under L.D. conditions is delayed beyond node 12, but quantitative reduction of this delay may be brought about by treatments such as vernalization and cotyledon removal given independently or in combination.

In these present experiments, it can be seen that vernalization and cotyledon removal, when given together, allow flowering to occur as early as node 12 under L.D. conditions. Under these conditions, at least, the inductive stimulus has reached its effective threshold by about the node 11 - 12 stage of development. Therefore, provided seed

vernalization has been performed, the induced apex can proceed immediately to floral initiation, and this will occur in the absence of cotyledons. However, should cotyledons remain attached to the growing plant after vernalization then flower initiation is delayed, and if seed vernalization is not given, flower initiation is delayed still further.

Other workers have proposed that this delay in floral initiation can be largely attributed to the presence of an inhibitor produced both in the cotyledons (Paton and Barber, 1955; Barber, 1959; Paton, 1969) and in the shoot (Murfet, 1971b,c; 1973) of Line 24 plants. The data in these present experiments is consistent with the view that flowering in Line 24 is at least in part regulated by an inhibitory effect of the cotyledons. This inhibitory effect of the cotyledons is not only stable to vernalization, but can obscure the vernalization response. Whether the cotyledon effect is due to the presence of an inhibitor (i.e. colysanthin) or to the absence or retarded formation of a florigenic substance is not unequivocally determined, but on the evidence available the former view is favoured. Further comment on the cotyledon effect can be found in Chapters 3 and 7.

It has been shown that vernalization does not influence the subsequent growth rate of plants. However, vernalization does have two other direct and separate effects, both of which promote flower initiation to an earlier node. The smaller effect is manifest on the cotyledon system, requires a long period of exposure and probably results from a reduction in the effective level of the cotyledonary inhibitor. The major effect is manifest on the young embryo, requires only a short-term period of exposure, and is effective before the completion of photo-periodic induction. In fact it is probably related to the photo-inductive process in some way. Further, this effect can be partially obscured by the cotyledons, unless they are removed soon after vernalization is completed.

TABLE 4.1
EFFECT OF LIGHT INTENSITY ON NF AND FT OF EXCISED
LINE 24 PLANTS UNDER VERNALIZING CONDITIONS (WINTER CROP)

L.D. conditions only were used. n, number of plants scored.
 Nutrient agar medium 8.

| Vernalization Status | Light Intensity (Lumens) | n | NF ± SE | FT (approx.) |
|----------------------|--------------------------|----|-----------|--------------|
| U | | 20 | 13.20 .19 | 49 |
| V | 800 | - | | |
| | 600 | 3 | 11.67 .33 | 50 |
| | 400 | 17 | 12.18 .21 | 47 |
| | 200 | 15 | 11.87 .16 | 48 |
| | 50 | 18 | 11.72 .14 | 48 |

TABLE 4.2

EFFECT OF DURATION OF VERNALIZATION TREATMENT ON THE GROWTH RATE OF INTACT AND EXCISED "GREENFEAST" PLANTS (SPRING CROP)

Figures are for total number of nodes (NT). L.D. photoperiod only was used. Nutrient agar medium 1.

| Days After Vernaliz- ation | Intact Plants | | | | | Decotyledonized Plants | | | | |
|----------------------------------|---|------|-------|-------|-------|--|------|-------|-------|-------|
| | Weeks Vernalized | | | | | Weeks Vernalized | | | | |
| | 0Wks. | 1Wk. | 2Wks. | 3Wks. | 4Wks. | 0Wks. | 1Wk. | 2Wks. | 3Wks. | 4Wks. |
| 0 | 5.9 | 5.9 | 6.1 | 6.5 | 7.3 | 5.9 | 6.0 | 6.2 | 6.7 | 7.0 |
| 4 | 7.0 | 8.1 | 8.6 | 8.8 | 10.0 | 6.5 | 7.1 | 7.5 | 7.7 | 8.2 |
| 8 | 9.3 | 10.1 | 10.7 | 10.8 | 11.5 | 8.1 | 8.2 | 9.0 | 8.9 | 9.7 |
| 12 | 11.5 | 12.2 | 12.8 | 12.9 | 13.6 | 9.3 | 9.5 | 10.2 | 10.1 | 10.7 |
| 16 | 13.5 | 13.9 | 14.7 | 14.7 | 15.6 | 10.0 | 10.5 | 11.3 | 10.8 | 11.5 |
| 20 | 15.5 | 15.6 | | | | 10.7 | 11.0 | 12.1 | 11.6 | 12.7 |
| 24 | | | | | | 11.4 | 12.0 | 12.6 | 12.4 | |
| 28 | | | | | | 12.2 | 12.7 | 13.5 | 13.5 | |
| 32 | | | | | | 13.8 | | | | |
| Average growth rate | Slope = 0.50 (nodes/day) Correlation coefficient = 1.0 | | | | | Slope = 0.24 (nodes/day) Correlation coefficient = 0.98 | | | | |

TABLE 4.3
EFFECT OF DURATION OF VERNALIZATION TREATMENT ON NF OF INTACT
AND DECOTYLEDONIZED "GREENFEAST" PLANTS (WINTER CROP)

Two classes of decotyledonized plants are shown — pre- and post-vernalization excision. L.D. conditions only were used. n, number of plants scored. Nutrient agar medium 1.

| Weeks Vernaliz- ation | Cotyledon Status | | | | | | | | |
|-----------------------------|------------------|-------|------|---|-------|------|--|-------|------|
| | Intact Plants | | | Cotyledons Removed After Vernalization | | | Cotyledons Removed Before Vernalization | | |
| | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE |
| 0 | 20 | 16.60 | .22 | 24 | 13.92 | .08 | | | |
| 1 | 20 | 15.80 | .14 | 24 | 12.57 | .23 | 26 | 12.73 | .10 |
| 2 | 20 | 15.35 | .18 | 27 | 12.93 | .07 | 22 | 12.55 | .13 |
| 3 | 20 | 14.70 | .16 | 22 | 11.86 | .10 | 22 | 12.00 | .13 |
| 4 | 20 | 14.79 | .09 | 29 | 13.33 | .09 | 29 | 12.14 | .14 |
| 6 | 20 | 15.25 | .14 | 29 | 13.66 | .10 | 27 | 12.11 | .12 |

TABLE 4.4

EFFECT OF VERNALIZATION ON NF OF EXCISED EMBRYOS IN "GREENFEAST" PLANTS (WINTER CROP)

L.D. photoperiod only was used. n, number of plants scored. N/D, rate of node formation (nodes/day) from end of vernalization treatment to time of initiation of first visible flower primordium. Nutrient agar medium 1.

| Length of Vernalization Treatment* (Weeks) | Cotyledon Status | Length of Vernalization Treatment After Cotyledon Removal | | | | | | | | | | | | | | | |
|--|------------------|---|-------|------|------|--------|-------|------|---------|-------|------|---------|-------|------|---------|-------|------|
| | | 0 Weeks | | | | 1 Week | | | 2 Weeks | | | 3 Weeks | | | 4 Weeks | | |
| | | n | NF | ± SE | N/D | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE |
| 0 | Intact | 20 | 15.40 | .15 | 0.46 | | | | | | | | | | | | |
| | Excised | 23 | 13.48 | .14 | 0.24 | 24 | 12.67 | .12 | 27 | 11.76 | .09 | 7 | 12.14 | .14 | 16 | 12.06 | .11 |
| 1 | Intact | 19 | 15.50 | .12 | 0.45 | | | | | | | | | | | | |
| | Excised | 29 | 12.48 | .15 | 0.23 | 30 | 12.33 | .12 | 27 | 12.22 | .11 | 27 | 12.44 | .12 | | | |
| 2 | Intact | 20 | 14.60 | .15 | 0.51 | | | | | | | | | | | | |
| | Excised | 24 | 12.17 | .10 | 0.27 | 17 | 11.53 | .24 | 26 | 11.92 | .13 | | | | | | |
| 3 | Intact | 20 | 13.50 | .11 | 0.51 | | | | | | | | | | | | |
| | Excised | 24 | 12.33 | .12 | 0.25 | 26 | 12.58 | .11 | | | | | | | | | |
| 4 | Intact | 19 | 13.00 | .09 | 0.50 | | | | | | | | | | | | |
| | Excised | 24 | 12.33 | .10 | 0.26 | | | | | | | | | | | | |

*Preceding cotyledon removal.

Experiment 4.4

TABLE 4.5
EFFECT OF VERNALIZATION AND COTYLEDON STATUS
ON NF IN "GREENFEAST" PLANTS (SUMMER CROP)

L.D. photoperiod only was used. n, number of plants scored.
 Nutrient agar medium 1.

| Cotyledon Status | Vernalization Status | | | | | | |
|------------------|----------------------|-------|-----|----|------------|-------|------|
| | Unvernalized | | | | Vernalized | | |
| | n | NF | ± | SE | n | NF | ± SE |
| Intact | 29 | 15.97 | .14 | | 26 | 13.73 | .12 |
| Semi Excised | 53 | 14.83 | .09 | | 55 | 13.00 | .06 |
| Fully Excised | 10 | 13.80 | .17 | | 10 | 11.90 | .10 |

TABLE 4.6

EFFECT OF TIME OF COTYLEDON REMOVAL AND VERNALIZATION ON NF IN "GREENFEAST" PLANTS (SUMMER CROP)

18-hr. photoperiod only was used. n, number of plants scored. NF_1 , number of nodes at commencement of vernalization treatment; NF_2 , number of nodes at conclusion of vernalization treatment. Nutrient agar medium 1.

| Days from Sowing to Commencement of Vernalization | Length of Vernalization Treatment (Weeks) | Cotyledons Intact | | | | | Cotyledons Removed Before Vernalization | | | | | Cotyledons Removed After Vernalization | | | | |
|---|---|-------------------|-------|------|-----------------|-----------------|---|-------|------|-----------------|-----------------|--|-------|------|-----------------|-----------------|
| | | n | NF | ± SE | NT ₁ | NT ₂ | n | NF | ± SE | NT ₁ | NT ₂ | n | NF | ± SE | NT ₁ | NT ₂ |
| 0 | 0 | 18 | 16.78 | .21 | 6.0 | - | 23 | 14.84 | .14 | 6.0 | - | 10 | 12.80 | .20 | 6.0 | 7.0 |
| | 4 | 18 | 14.39 | .12 | 6.0 | 7.0 | 20 | 12.28 | .12 | 6.0 | - | | | | | |
| 6 | 0 | 20 | 15.60 | .15 | 8.8 | 11.0 | 17 | 15.76 | .25 | 8.7 | - | 18 | 14.39 | .12 | 9.5 | 11.4 |
| | 4 | | | | | | 13 | 13.92 | .08 | 8.8 | 9.0 | | | | | |
| 10 | 0 | 19 | 15.47 | .16 | 11.2 | 12.5 | 19 | 15.37 | .14 | 11.2 | - | 18 | 15.44 | .16 | 11.6 | 14.5 |
| | 4 | | | | | | 18 | 14.22 | .10 | 11.2 | 11.8 | | | | | |
| 14 | 0 | 20 | 16.50 | .15 | 13.1 | 15.4 | 19 | 16.37 | .19 | 13.2 | - | 18 | 15.94 | .19 | 13.2 | 15.5 |
| | 4 | | | | | | 20 | 15.90 | .16 | 13.1 | 14.7 | | | | | |

TABLE 4.7

EFFECT OF VERNALIZATION OF THE PARENT PLANT ON NF IN LINE 24 PLANTS

The four pod treatments (i, ii, iii, iv) are as described in the text. (Parent treatment spring crop. Progeny treatment summer crop.) L.D. conditions only were used. n, number of plants scored.

| Parent Seed Treatment | | U | | | | | | V | | | | | | | | | |
|------------------------|------|----|-------|-------|----|----|-------|-----|----|-------|-------|-----|-----|----|-------|-----|----|
| | | n | | NF | | ± | SE | n | | NF | | ± | SE | | | | |
| | | 93 | | 15.87 | | | .08 | 97 | | 13.96 | | | .05 | | | | |
| Progeny Seed Treatment | | U | | | | V | | | | U | | | | V | | | |
| | | n | NF | ± | SE | n | NF | ± | SE | n | NF | ± | SE | n | NF | ± | SE |
| Pod Treat-ment | i. | 18 | 17.22 | .24 | | 19 | 15.00 | .13 | | 18 | 17.78 | .17 | | 18 | 15.67 | .14 | |
| | ii. | 25 | 17.72 | .16 | | 24 | 15.08 | .15 | | 18 | 17.56 | .18 | | 19 | 15.00 | .19 | |
| | iii. | 25 | 17.40 | .12 | | 25 | 14.92 | .08 | | 20 | 17.70 | .16 | | 22 | 15.41 | .14 | |
| | iv. | 25 | 17.72 | .11 | | 25 | 15.04 | .11 | | 18 | 17.72 | .14 | | 18 | 15.56 | .12 | |
| Totals | | 93 | 17.54 | .08 | | 93 | 15.01 | .06 | | 74 | 17.69 | .08 | | 77 | 15.40 | .08 | |

TABLE 4.8
EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD ON NF IN LINE 24 PLANTS (AUTUMN CROP)

n, number of plants scored. Nutrient agar medium 8.

| No. Weeks Vernal- ization | S.D. | | | | | | L.D. | | | | | |
|------------------------------------|--------|-------|-----|-----------------|-------|-----|--------|-------|-----|-----------------|-------|-----|
| | Intact | | | Decotyledonized | | | Intact | | | Decotyledonized | | |
| | n | NF ± | SE | n | NF ± | SE | n | NF ± | SE | n | NF ± | SE |
| 0 | 19 | 24.05 | .32 | 21 | 20.95 | .56 | 20 | 15.80 | .21 | 22 | 13.32 | .14 |
| 2 | 19 | 22.42 | .32 | 22 | 18.32 | .41 | 19 | 14.95 | .22 | 19 | 12.58 | .16 |
| 4 | 20 | 20.65 | .23 | 21 | 18.72 | .27 | 18 | 13.83 | .15 | 22 | 12.23 | .11 |

FIGURE 4.1

EFFECT OF DURATION OF VERNALIZATION TREATMENT
ON THE GROWTH RATE OF INTACT AND EXCISED "GREENFEAST" PLANTS
(SPRING CROP)

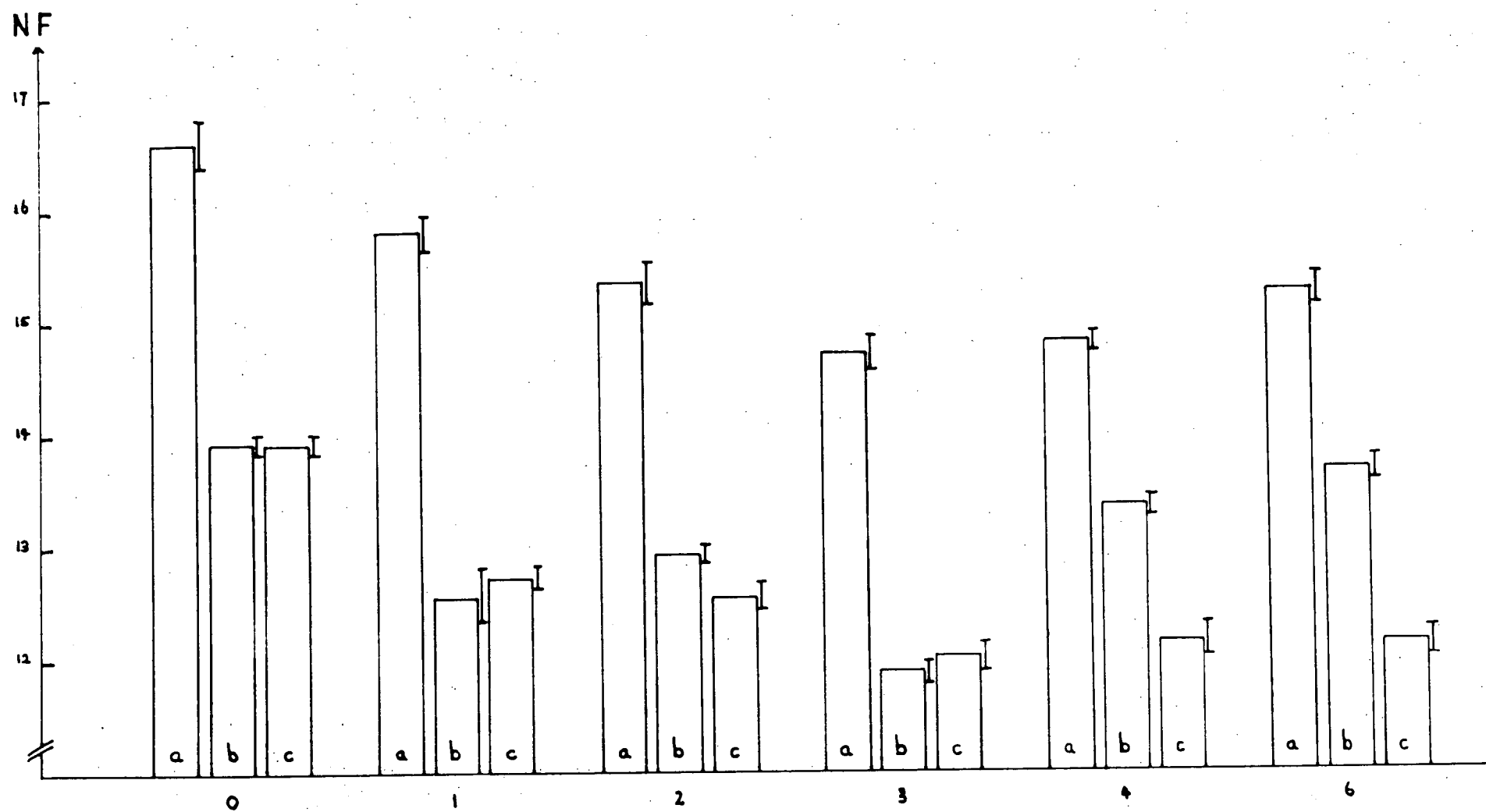
| | | |
|-------|---|------------------------|
| _____ | | Intact plants |
| ----- | | Excised plants |
| o | - | 0 weeks (unvernalized) |
| + | - | 1 week |
| x | - | 2 weeks |
| △ | - | 3 weeks |
| ▽ | - | 4 weeks |

FIGURE 4.2

EFFECT OF DURATION OF VERNALIZATION TREATMENT
ON NF OF INTACT AND DECOTYLEDONIZED "GREENFEAST" PLANTS
(WINTER CROP)

- a - Intact plants.
- b - Cotyledons removed after completion of the vernalization treatment.
- c - Cotyledons removed prior to the vernalization treatment (i.e. at imbibition).

(The standard errors are shown at the top of each histogram.)



VERNALIZATION TREATMENT IN WEEKS

FIGURE 4.3EFFECT OF VERNALIZATION AND COTYLEDON
STATUS ON NF IN "GREENFEAST" PLANTS (SUMMER CROP)

- a - Intact plants
- b - Plants with one cotyledon removed
- c - Plants with two cotyledons removed

(The standard errors are shown at the top of each histogram.)

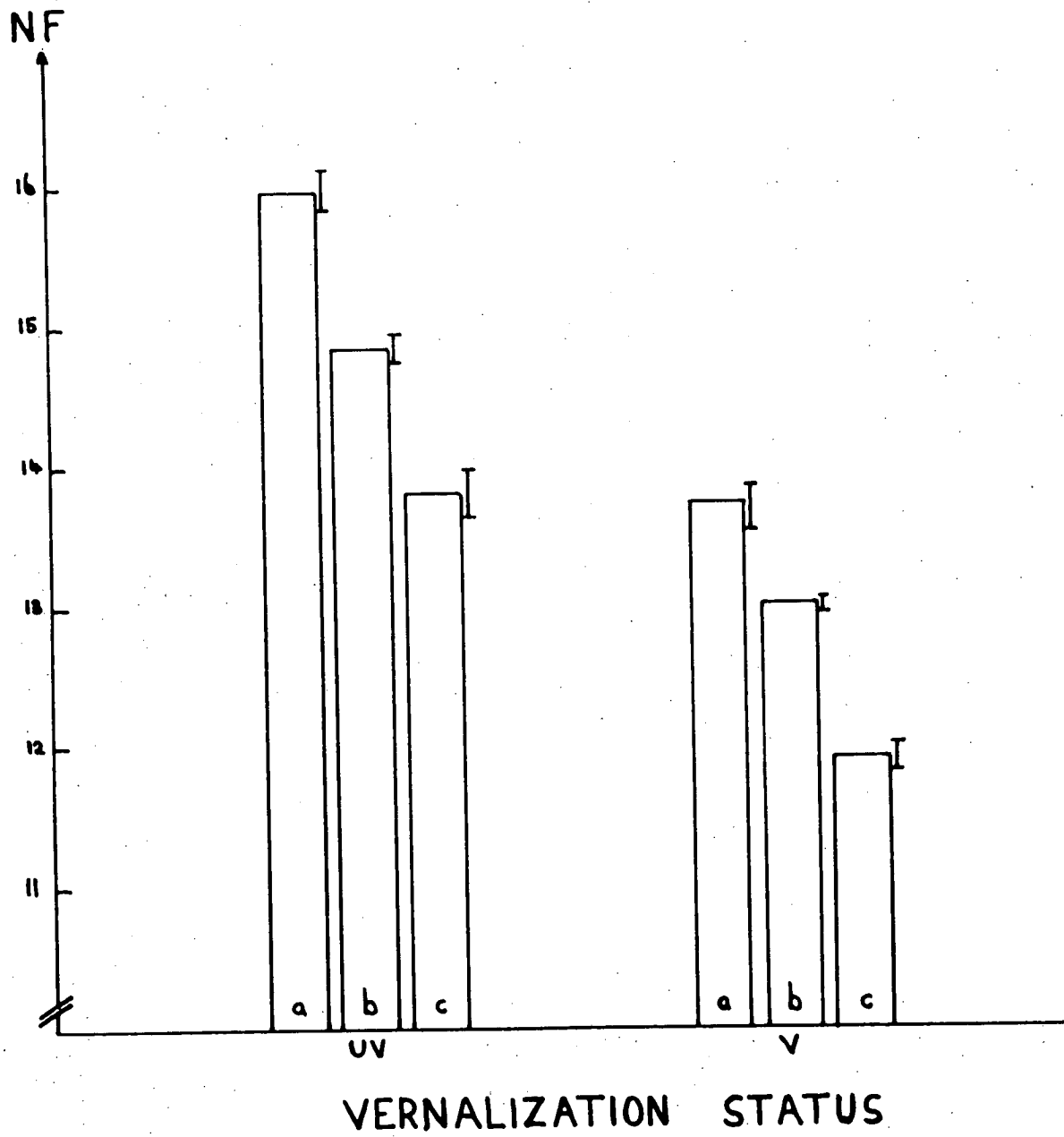
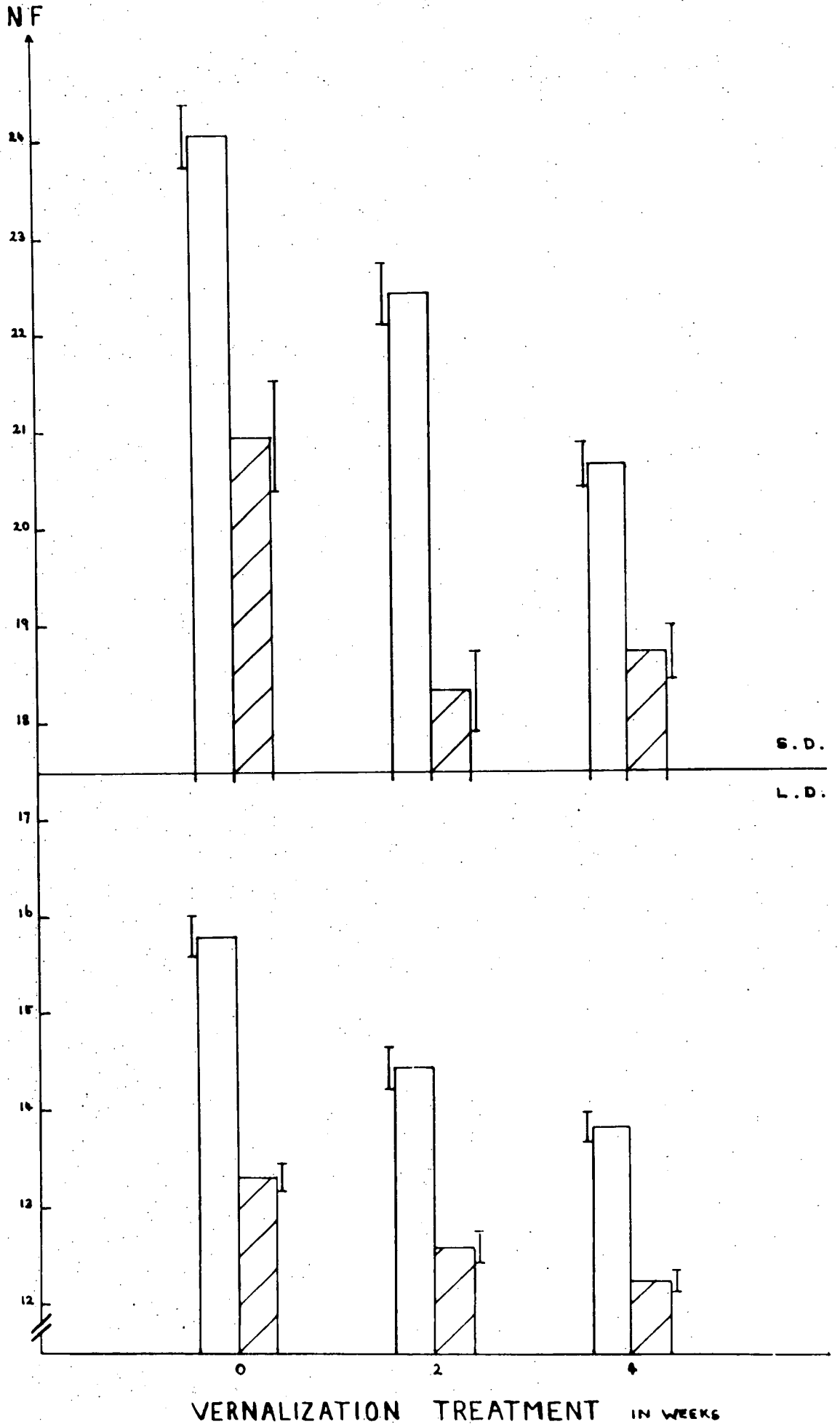


FIGURE 4.4EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF IN LINE 24 PLANTS
(AUTUMN CROP)

Plain bars - Intact plants

Cross hatched bars - Excised plants

(The standard errors are shown at the top of each histogram.)



GRAFTING

Much work has appeared in the literature concerning the effect of grafting on flowering in "Greenfeast" peas (e.g. Paton and Barber, 1955; Amos and Crowden, 1969; Murfet, 1971c). It has in fact been stated that one of the most convincing pieces of evidence concerning the presence of a floral inhibitor in this variety is based on the results of grafting experiments (Paton and Barber, 1955).

Although a grafting experiment had been conducted previously (Amos and Crowden, 1969), it was felt desirable to expand this experiment to involve the three factors mentioned in previous chapters - namely vernalization, photoperiod, and cotyledon removal - to see whether any further information could be gained concerning the cotyledon system and the vernalization response.

Experiment 5.1:- Unvernalized and vernalized plants were grown under both S.D. and L.D. photoperiods for a period equivalent to 4 days, after which a full factorial experiment was performed involving grafting, vernalization and cotyledon removal. Plants were then grown to anthesis under S.D. or L.D. conditions as before and their NF recorded. The results are given in Table 5.1.

Results:- As can be seen from the control plants, each of the effects of vernalization, cotyledon removal and photoperiod was additive. Cotyledon removal advanced NF by some 3 nodes, irrespective of any other treatment, vernalization of the scion advanced NF by some 3 nodes, and the photoperiod effect was of the order of some 6 nodes. These results agree with those reported in previous chapters.

First taking stocks with intact cotyledons under L.D. conditions, it can be seen that vernalized scions, either V/U or V/V (scion/stock), flowered at an earlier node than the vernalized controls.

The NF of these vernalized scions was approximately 1 node higher than those resulting from the dual treatments of seed vernalization plus cotyledon removal. This is in sharp contrast to the performance of vernalized scions in short days, where no effect of grafting was evident. Thus, under S.D. conditions plants from each of the treatments V, V/U and V/V had almost identical NF values (e.g. V - V/U, $P = 0.05$). Moreover, this value of NF (approximately 20.7) was some 2 - 3 nodes higher than that for vernalized, decotyledonized plants grown under short days.

Similarly, with unvernallized scions (U, U/U and U/V), a significant effect of grafting was evident only when plants were grown under L.D. conditions. The state of the stock had little significance on the resultant NF (e.g. for U/U - U/V, $P = 0.1$ under both photoperiods). However, the state of the scion had a large effect on the resultant NF (U/U - V/U, $P < 0.001$ under both photoperiods).

For decotyledonized plants, not much can be said for those grafts grown under S.D. conditions as the survival rate was so low. Under L.D. conditions, decotyledonized plants behaved in much the same manner as their intact counterparts: the main effect on NF was the vernalized condition of the scion, whereas the condition of the stock had no effect at all. Unvernallized scions flowered out of a lower node than did the excised controls.

In all cases bar one (intact V/U; V/V plants grown under L.D. conditions), there was a tendency for a vernalized stock to cause a scion to flower out of a lower node than one grafted onto an unvernallized stock. However, in no case was this difference significant.

Discussion:- These results are consistent with the theory that flowering in Line 24 is controlled to some degree by a cotyledon-based inhibitor. The presence of this inhibitor at the apex determines a threshold level which the floral inducer, produced

in the leaves, must attain before it can induce the plant to flower. It is significant that a discrete effect of grafting can only be observed under L.D. conditions and not under S.D. conditions. This difference in NF between comparable graft treatments under different photoperiods may simply reflect the length of time that is required to establish a functional graft union and permit transfer of inhibitor from the cotyledons of the stock to the scion. At the time of grafting, plants have already laid down 8 or 9 nodes, so that some inhibitor has presumably already moved into the shoot, irrespective of whether the stock is kept intact or is subsequently decotyledonized. Also vernalized scions only need to form 4 more nodes before flower initiation occurs.

Thus under L.D. conditions, events in the vernalized scion leading to flower initiation may well be completed before the graft union is adequate for regular transport of the cotyledonary inhibitor. This would cause a lowering of the normal threshold set by the inhibitor, thereby allowing flowering to occur at a lower node. Similarly with unvernallized scions under L.D. conditions, NF is always below that for the ungrafted control plants, suggesting that the graft union is still not fully functional in time for the quantity of inhibitor reaching the apex to delay initiation to the normal extent as in the ungrafted controls.

In short days however, 12 nodes at least of vegetative growth have been laid down from the time of grafting before flower initiation occurs. By this time, it is most likely that the graft union is fully established, and normal transport of the inhibitor is restored.

Alternatively, this effect of photoperiod on NF between comparable graft treatments could be the result of an alteration or at least a temporary halt in the growth of the plant. Under L.D. conditions, flower initiation may occur before normal growth can be restored, thus causing an advancement in NF. Under S.D. conditions, however,

full restoration of normal growth may precede flower initiation, and thus no effect of grafting would be evident. This explanation is expanded in Chapter 7.

The results also support the view that the major site of vernalization is in the apex of the plant. Vernalization of the scion has the effect of advancing NF by approximately 3 nodes under S.D. conditions and from 1 - 2 nodes under L.D. conditions. The reduced effect under L.D. conditions is to be expected since the threshold level set by the inhibitor is quickly surpassed by the inductive floral stimulus. Under S.D. conditions however, full production and transport of the inhibitor is restored before the inductive stimulus can reach the reduced threshold.

Vernalization of the stock has no significant effect in advancing NF, although an obvious tendency is observed. Vernalization does not seem to have any lasting effect on the supply of inhibitor from the cotyledons to the shoot, although it may cause a slight delay in its rate of supply, at least in the initial stages of growth.

As stated in the previous chapter, the major effect of vernalization would appear to be in the apex, and is most likely involved with preconditioning the apex to the inducer, thereby reducing the effective threshold level set by the cotyledonary inhibitor.

Many workers have carried out a number of experiments involving cross-grafting between different varieties, in an attempt to clarify the position with regard to the presence of a cotyledonary promoter in early varieties (Haupt, 1954, 1957; Köhler, 1965) or to that of a cotyledonary inhibitor in late varieties (Paton and Barber, 1955; Amos and Crowden, 1969; Murfet and Reid, 1973). Further discussion on this aspect will be left until the end of Chapter 6, once the response of a number of genetically different cultivars to conditions previously described in Chapters 3 and 4 have been dealt with.

TABLE 5.1
EFFECT OF VERNALIZATION, GRAFTING, COTYLEDON REMOVAL
AND PHOTOPERIOD ON NF IN PLANTS OF LINE 24 (SUMMER CROP)

n, number of plants scored; U/V, Unvernalized scion grafted to a vernalized stock.
 Nutrient agar medium 8.

| Cotyledon Status of Stock | Vernaliza- tion Status | S.D. | | | | L.D. | | | |
|---------------------------------|---------------------------|-----------------|-------|-----|----|------|-------|-----|----|
| | | n | NF | ± | SE | n | NF | ± | SE |
| Intact | U(Control) | 19 | 23.63 | .44 | | 20 | 17.05 | .14 | |
| | V(control) | 20 | 20.15 | .32 | | 20 | 14.05 | .15 | |
| | U/U | 11 | 25.09 | .59 | | 14 | 15.29 | .29 | |
| | U/V | 16 | 23.63 | .53 | | 16 | 14.63 | .26 | |
| | V/U | 9 | 21.33 | .58 | | 16 | 13.00 | .22 | |
| | V/V | 12 | 20.67 | .43 | | 16 | 13.19 | .23 | |
| Excised | U | 12 | 20.17 | .69 | | 27 | 14.11 | .17 | |
| | V | 28 | 17.04 | .21 | | 28 | 12.00 | .07 | |
| | U/U | 5 only survived | | | | 19 | 13.58 | .21 | |
| | U/V | 2 only survived | | | | 16 | 13.31 | .22 | |
| | V/U | 13 | 16.38 | .45 | | 20 | 12.00 | .15 | |
| | V/V | 3 only survived | | | | 16 | 11.94 | .14 | |

THE RESPONSE OF OTHER LINES OF KNOWN GENOTYPE TO
COTYLEDON REMOVAL, PHOTOPERIOD AND VERNALIZATION

a INTRODUCTION

In the past, experiments have been conducted by different workers using various commercial varieties of Pisum sativum and the results reported. One of the major problems in comparing these results is that the genetics of these commercial varieties are not known, which puts an element of doubt into comparing not only results between the different varieties but also those which have been reported for the same variety. Both Rowlands (1964) and Murfet (1971b) have either suspected or demonstrated genetic heterogeneity in commercial varieties, and Murfet (1971a) has suggested that a pool of standard genotyped varieties should be established to remove any doubts as to the equivalence of the varieties used by different workers.

In 1971, Murfet published three papers which demonstrated that flowering in a number of lines of Pisum sativum is under the major control of a 3-gene system (Murfet 1971 a,b,c). He first separated the varieties into three distinct phenotypic classes, depending on their flowering behaviour. These were:-

1. ED (early developing) varieties, which have a flowering response unaffected by photoperiod, both FT (flowering time) and NF (node of first flower) being early under S.D. conditions.
2. EI (early initiating) varieties, in which NF is unaffected by photoperiod but FT is delayed under S.D. conditions. These varieties flower out of an early node under S.D. conditions, but the flowers on the first few nodes abort.

3. L (late) varieties, which show a response to photoperiod, both NF and FT being delayed under S.D. conditions.

He then demonstrated by a series of genetic crosses that the class differences were controlled by three dominant major-genes, which he designated Sn, E and Lf. The triple recessive is ED. Addition of Sn creates an L-type. E is epistatic to Sn in terms of flowering node and Sn E lf is EI. Lf is epistatic to E and Sn E Lf is again L. Sn e Lf is also L. Lf and E have little or no effect by themselves and sn e Lf, sn E Lf and sn E lf are essentially ED.

Marx (1968, 1969) has also developed a system of phenotypic classification based on 4 photo-dependent response classes. In his scheme, I and G2 types both flower out of a low node and are not affected by photoperiod. However, the reproductive phase of G2 plants is greatly prolonged under S.D. conditions. Both K and G types are late and similar under L.D. conditions, but whereas K plants show a limited quantitative response to photoperiod, G plants show almost a qualitative response and may develop up to 70 vegetative nodes under S.D. conditions.

Murfet (1971a) has suggested that his phenotypic classes ED, EI and L may correspond to the classes I, G2 and K of Marx (1968, 1969). Although not relevant to this work, Murfet (1971a) also described a class LHR (late high response), which corresponds very closely to the G class of Marx.

In this present programme, two different physiological experiments were carried out on six of the available lines in an attempt to further clarify the role of these three genes in the flowering behaviour of Pisum sativum. The six lines used, their phenotypic classification and their genetic constitution are given in the table below.

| | | | | | | |
|-----------|---------|---------|---------|---------|---------|---------|
| Line | 58 | 59 | 60 | 53 | 24 | 2 |
| Phenotype | ED | ED | EI | L | L | L |
| Genotype | sn e lf | sn E lf | Sn E lf | Sn e lf | Sn e Lf | Sn E Lf |

At the time of writing, the other two possible ED genotypes - $sn\ e\ Lf$ and $sn\ E\ Lf$ - had not been isolated and were therefore not available for use in this experiment. Further information on the genetics and history of these lines may be found in Murfet (1971 a,b,c).

b THE RESPONSES OF SOME OTHER CULTIVARS

Experiment 6.1:- This experiment involved sequential cotyledon removal and photoperiod, and is similar to Experiment 3.6, except that the six genetic lines were involved. The results are given in Table 6.1 and Figure 6.1.

Results:- For ED plants (Lines 58 and 59), photoperiod had a small but significant effect if the cotyledons were removed up to 6 days after germination had commenced. Cotyledon removal caused a progressive delay in NF under both photoperiod regimes, which reached a maximum at the 4-day excision stage. Line 59 plants always flowered out of a lower node than did their Line 58 counterparts, irrespective of the conditions.

L plants behaved in a manner similar to that described previously (Experiment 3.6). Under S.D. conditions there was a different behaviour pattern for Line 53 on the one hand, which showed a progressive delay in NF with an increasing time-delay to cotyledon removal, and Lines 2 and 24, which showed only a slight delay - if any. However, a general pattern can be observed. It is also worth noting that, given the same conditions, Line 53 plants flowered out of a lower node than Line 2 and especially Line 24, which must relate to the intrinsic genetic difference between these late varieties. These differences occur at the E and Lf loci. Under L.D. conditions, Line 53 behaved similarly to Line 58 up to the 6-day excision stage, whereupon there was a progressive delay in NF with increasing time to cotyledon removal. Also under L.D.

conditions, Line 2 plants flowered out of a lower node than did their Line 24 counterparts.

EI plants (Line 60) exhibited a marked response to photoperiod, reaching a maximum at the 4-day excision stage and then decreasing to zero for intact plants. Under L.D. conditions, Line 60 behaved like an ED plant (Line 59) prior to the 4-day excision stage, but after this stage like an L-type. The difference in NF between Line 60 and Line 53 under L.D. conditions was remarkably similar to that between Line 59 and Line 58. Under S.D. conditions, Line 60 behaved as an L-type until the 4-day excision stage and in fact flowered out of the same node as Line 53. After this time however, NF fell dramatically until, for intact plants, it was the same as for plants grown under L.D. conditions.

Unfortunately in this experiment no record was kept of the node at which Line 53 and Line 60 developed (c.f. initiated) their first flower (i.e. ND). However, it was observed that under S.D. conditions there was a marked delay between NF and ND for both varieties. In other words, although flower buds were initiated, they did not develop into flowers but aborted.

Experiment 6.2:- This experiment parallels that of Experiment 4.8, in that the three factors of cotyledon removal, photoperiod and vernalization were employed. Again all six varieties were used. The results are given in Table 6.2 and Figures 6.2 - 5).

Results:- ED varieties (i.e. Lines 58 and 59) showed little response to photoperiod, irrespective of the other conditions imposed. Cotyledon removal caused a delay in NF, which was reduced by vernalization. Vernalization caused a slight delay in NF of intact plants, so that after a 4-week vernalization treatment, intact and excised plants flowered out of the same node. Line 58 plants flowered out of a slightly higher node than did Line 59 plants, irrespective of the treatment given.

Two of the three L varieties, Lines 2 and 24, behaved in a similar fashion to that described previously in Chapter 4. The other L variety, Line 53, initiated its first flower in a similar fashion to Lines 2 and 24, but at a much earlier node ($P < .001$). What is of interest is that ND was greatly delayed under S.D. conditions, and much more so for excised plants than for intact plants. Vernalization caused an advancement in the ND of Line 53 plants grown under S.D. conditions. Under L.D. conditions, NF and ND were the same in all cases.

The EI variety, Line 60, is of special interest. The NF of intact plants showed no response to vernalization under S.D. conditions. However, under L.D. conditions intact plants showed a slight response to vernalization (approximately 0.4 nodes). Unvernalized plants showed no response to photoperiod. Under S.D. conditions, cotyledon removal caused a large delay in NF ($P < .001$) which also was unaffected by vernalization. However, under L.D. conditions, NF was slightly advanced by cotyledon removal ($P < .001$) but this was negated by vernalization. Line 60 behaved in a similar manner to Line 53 in relation to the difference between NF and ND.

Discussion:- The results are in general agreement with the thesis propounded by Murfet (1971 a,b,c). He put forward a theory in which he proposed that:-

1. Sn produces a substance in the cotyledons and shoot which opposes floral initiation.
2. E lowers the level of floral inhibitor in the cotyledons.
3. Lf increases the sensitivity of the apex to inhibitor.
4. short days favour the production of inhibitor in the shoot.
5. the level of Sn product falls inevitably with ageing either through diminution of Sn activity or destruction of its product.

Further he suggests that all cultivars "are able to produce a flower promoter in their cotyledons and shoots, that they each have fairly similar capacities in this respect, and that a low level of inhibitor is available in recessive 'sn' plants" (Murfet, 1971c).

Consideration of this proposal in relation to these present results leads to the following conclusions being drawn.

I. ED Varieties (Line 58 - sn e lf; Line 59 - sn E lf)

The results obtained here for Lines 58 and 59 are in general agreement with those obtained by other workers working with the early varieties "Massey" and "Alaska". These varieties have been found to respond in a negative fashion to vernalization (Barber, 1959; Highkin and Lang, 1966) and to be insensitive to photoperiod (Leopold and Guernsey, 1954; Moore, 1965), although it has been suggested that flower initiation proceeds too soon after germination for it to be influenced by photoperiod (Haupt, 1969).

Cotyledon removal has delayed NF in the early varieties "Kleine Rheinländerin" (Haupt, 1952; Haupt and Nakamura, 1970), "Massey" and "Alaska" (Moore, 1964; Johnston and Crowden, 1967), causing at least one cultivar, "Kleine Rheinländerin", to behave as a quantitative long-day plant (Haupt, 1954; Köhler, 1965) and to respond positively to vernalization (Haupt and Nakamura, 1970). In fact, as a result of this level of response, Murfet (1971c) has suggested that "Kleine Rheinländerin" may well be a class EI type, and not ED.

As stated in Chapter 1, the transmission of a floral stimulus from the leaves to the apex is an accepted partial process of photoperiodic induction. According to Murfet's theory, ED varieties contain no inhibitor, although he has suggested that the recessive "sn" may in fact be a "leaky" mutant. The apex in these varieties will transfer from the vegetative to the reproductive state once

the floral stimulus arrives at the apex from the leaves. If no inhibitor is present at the apex at the time the stimulus arrives, then NF would not be affected by the rate of arrival of the stimulus at the apex. Since it has already been suggested that the effect of photoperiod is a quantitative one, concerned with the rate of stimulus production (pp. 3.13, 4.14, 5.4), then an effect of photoperiod on NF in ED plants would not be expected.

Cotyledon removal delayed NF in ED plants. By the time the first true leaf at node 3 has opened, node 9 has been laid down in the apex. Therefore any difference in NF between treatments would be the result of the varying time-of-arrival of the stimulus at the apex. A delay in the commencement of production of stimulus could conceivably be caused by a drastically reduced growth rate, as the first formed leaves may not be able to produce the stimulus when they first open, due perhaps to some form of metabolic imbalance. A delay in the production of stimulus would cause an increase in NF.

The photoperiod effect observed in excised plants may well be the result of a more rapid recovery from this metabolic imbalance under L.D. conditions, and an earlier production of the flowering stimulus in the leaves. Depending on the degree of "leakiness" of the mutant "sn" gene, the rate of production of stimulus affected by the photoperiod may also have some bearing under these conditions. Whatever the explanation, excised plants demonstrated a greatly retarded growth rate, a delay in NF, and a photoperiod effect.

Alternatively photoperiod may affect the growth rate of excised plants slightly (e.g. excised plants under L.D. conditions may expand their leaves more rapidly than those under S.D. conditions, and therefore may affect the time of initial production of the stimulus). This phenomenon was observed in Line 24 embryos (see Table 3.8).

If "sn" was indeed a leaky mutant, then the growth rate of the plant may also affect the amount of inhibitor present at the apex at the time the stimulus arrives. A slower growth rate could cause

a greater build-up in the level of inhibitor present. Once inhibitor was present, then it would be expected that both a delay in NF and a photoperiod effect would be observed, and this was observed with excised plants.

The small delay in NF caused by vernalization of intact ED plants could be the result of a slight delay in the time of initial production of the stimulus. It must be remembered that this difference between unvernallized and vernalized plants is always less than one node. For decotyledonized plants, the positive effect of vernalization on NF may result from a slight increase in the growth rate. This effect of vernalization on the growth rate of excised plants has already been observed in excised plants of Line 24 (see Figure 4.1). Again it could be argued that if "sn" was a "leaky" mutant which was able to build up a small supply of inhibitor in excised ED plants, then a positive vernalization effect would be expected by either increasing the rate of production of stimulus or preconditioning the apex to be more sensitive to the stimulus.

It can also be seen that Line 59 plants always flower out of a lower node than do their Line 58 counterparts. As the only difference between these two lines occurs at the E locus, then gene E must be active in the shoot as well as in the cotyledons, since there is a constant difference in NF between these two lines, both in the intact and decotyledonized state. If this is so, then it would support the theory that "sn" was a leaky mutant as the effect of E is (according to theory) on Sn. Gene E may also be responsible for the production of a floral stimulus. It would also seem to suggest that the effect of vernalization is to delay initially the production of floral stimulus (affecting intact plants) but to increase its effectiveness (affecting excised plants).

II. L Varieties with Gene Lf (Line 24 - Sn e Lf; Line 2 - Sn E Lf)

The results are in accord with the view that gene Sn causes the production of a floral inhibitor whose effects have been discussed

previously (Chapters 3, 4 and 5). Since Sn has an effect on NF in both intact and decotyledonized plants, the inhibitor must be produced in the shoot at least. The presence of inhibitor at the apex would most likely set a threshold level which must be overcome by the floral stimulus before flower initiation can occur.

Excised plants flower out of a lower node than their intact counterparts. The difference in the growth rates could play a significant part in the advancement of NF, and this point is discussed in greater detail in the next chapter. Cotyledon removal could also remove the supply of Sn-inhibitor from the cotyledons, which would also explain the lowering of NF.

The photoperiod effect in both intact and excised plants is probably the result of a differential rate of production of floral stimulus. As mentioned previously, a photoperiod effect would not under normal situations be observed in "sn" (i.e. ED) plants. Although there would still be a differential rate of supply of floral stimulus, the threshold level which it would need to overcome would be zero, or nearly so, and so flowering would occur at the same time under both photoperiod regimes.

Both intact and decotyledonized plants respond to vernalization, although the response is much more rapid in decotyledonized plants (see also Chapter 4), and is less under L.D. than it is under S.D. conditions (see also Barber, 1959). In fact for Line 2 the response is not significant at all. Vernalization obviously has its main effect in the shoot. It could on the one hand lower the rate of production of Sn-inhibitor (or cause the apex to be less sensitive to it) or on the other hand enhance the production of floral stimulus (or cause the apex to be more sensitive to it). The results do not really distinguish which mechanism is operating, since each would give the same result. However, the fact that excised ED plants can respond to vernalization would suggest that the response to vernalization is more likely to be associated with the floral stimulus than with the Sn-inhibitor.

The effect of vernalization in embryos under L.D. conditions may be obscured by the tendency for these plants to have a slightly more rapid growth rate than their unvernallized counterparts. However, no measurements were taken of this phenomenon. It could also be affected by the fact that the threshold level set by the Sn-inhibitor production in the shoot is fairly rapidly reached by the stimulus under L.D. conditions, before Sn has been able to realize its full potential. Under S.D. conditions Sn-inhibitor production in the shoot is well advanced before the level of stimulus required for transition from the vegetative to the reproductive state can be produced. Either Sn-inhibitor production is favoured by S.D. conditions or, more likely, S.D. conditions cause a reduction in the rate of production of floral stimulus (c.f. Murfet, 1973).

Finally, Line 2 differs in its flowering genetics from Line 24 by the presence of the dominant gene at the E locus. Under L.D. conditions, Line 2 flowers at a lower node than does Line 24 whilst under S.D. conditions, the reverse is true (Figure 6.1). If E affects the Sn-inhibitor supply from the cotyledons, rather than from the shoot, then it would be expected that plants containing E would flower out of a lower node under L.D. conditions, as the production of stimulus would soon reach the reduced threshold set by the cotyledonary inhibitor before Sn-inhibitor production reached maximum capacity in the shoot. Under S.D. conditions however, with its resultant decrease in the rate of stimulus production, the supply of Sn-inhibitor from the shoot is more than adequate to compensate for the effect of E on the cotyledonary inhibitor. E can still be effective in the shoot, but presumably has its maximum effect either at an early stage of growth or on the inhibitor arriving from the cotyledons.

III. L Varieties - Without Lf (Line 53 - Sn e lf)

In many respects Line 53 behaves in a similar fashion to Lines 2 and 24. It is an L-variety which responds positively to both photo-period and vernalization. The ability to respond to these two

treatments must therefore be the result of the presence of Sn, and not Lf. Photoperiod and vernalization would therefore be connected in some way with the threshold level set by the Sn-inhibitor, although not necessarily directly connected with the substance itself.

Line 53 flowers out of a lower node than do Lines 2 and 24. In fact under L.D. conditions, cotyledon removal prior to the 4-day growth stage causes the plant to behave like an ED-type.

These results show that gene Lf has a strong boosting effect to the expression of gene Sn, in both the presence and the absence of gene E. This could be achieved on the one hand by increasing the rate of production of Sn-inhibitor in the shoot (or cause the apex to be more sensitive to it) or on the other hand inhibit the rate of production of floral stimulus (or cause the apex to be less sensitive to it).

The effect of removing cotyledons prior to the 4-day growth stage under L.D. conditions is to remove Sn-inhibitor supply from the cotyledons. Before inhibitor production in the shoot can be fully established, the production of stimulus has caused the plant to initiate flowers. If gene Lf was present (i.e. Line 24 genotype), plants would initiate flowers 2 - 3 nodes later (see Figure 6.1). Since inhibitor production in the stem has not yet become fully established, it would seem more likely that gene Lf has its effect by either encouraging the rate of inhibitor production in the shoot or causing the apex to be more receptive to it.

Under S.D. conditions the rate of supply of stimulus is reduced and Sn-inhibitor production in the shoot has become fully established before the lowered threshold level can be reached by the stimulus. However, the photoperiod effect (in the vicinity of 4 to 5 nodes) is not as large as for Lines 2 or 24. Obviously the rate of production of the floral stimulus is enough to reach the slowly increasing threshold set by the inhibitor for flower initiation

to occur. However, the rate of stimulus production is not high enough to allow the flowers to develop, and so they abort. (This assumes of course that both flower initiation and flower development are controlled by the balance between flower inhibitor and flower promoter.)

Intact plants show a huge response to vernalization under S.D. conditions, although flowers, once initiated, abort. In fact, intact vernalized plants show only a minor photoperiod response. Decotyledonized plants on the other hand show a negligible response to vernalization. It would seem therefore that vernalization causes an increase in the rate of supply of stimulus from the leaves (or an increased sensitivity by the apex to the stimulus). Under L.D. conditions, the threshold level set by the Sn-inhibitor is still very low by the time the floral stimulus reaches the apex, and so vernalization would not be expected to have any great effect.

IV. EI Varieties (Line 60 - Sn E 1f)

Under S.D. conditions, Line 60 plants behave in a similar manner to Line 53 plants until the 4-day excision stage after which NF falls dramatically (see Figure 6.1). Decotyledonized plants behave in a similar fashion to their Line 53 counterparts. However, intact plants initiate flowers out of an early node and show a negligible response to photoperiod and vernalization. Obviously the presence of E has almost completely counteracted Sn-activity and further, the presence of E is manifest either in the cotyledons themselves or on the Sn-product from the cotyledons since the removal of the cotyledons at an early stage removes the effectiveness of E. However, it would appear that the lack of a photoperiod response may be the effect of E-activity in the shoot.

It is perhaps appropriate at this stage to examine the effects of cross-grafting and other experiments reported by other workers on different commercial varieties and to examine their findings in the light of the discussion just presented. Johnston and Crowden (1967) carried out an

experiment similar to that described in Experiment 3.6 where cotyledons were removed from "Massey" plants at varying intervals of time after germination. They found that there was a progressive delay in NF up to 4 days after germination, after which time NF reverted to that of the control plants. This effect is called the "hump effect". Similar results were obtained in the present programme. It is obvious therefore that the effect of cotyledon removal in early varieties will depend to a large extent on the stage at which cotyledons are removed, and comparisons of cotyledon removal with grafting will be dependent on this fact. Johnston and Crowden (1967) advanced the hypothesis that this effect may be the result of "Massey" cotyledons containing a flower-inhibitory substance which is "rapidly mobilized after germination commences, and which is subsequently deactivated...as germination and development of the plumule progresses". This may be the result of "sn" being a "leaky" mutant, as Murfet (1971 a,b,c) suggests, especially considering both the degree of metabolic imbalance which is probably present at that time, and the reduced growth rate.

Haupt (1952, 1957) and Köhler (1965) have shown that "early" scions grafted to "late" stocks flowered at the same node as an excised "early" plant. However, both Paton and Barber (1955) and Murfet and Reid (1973) found that "early" grafts flowered later than excised plants. The latter authors also found that an "early" scion grafted to a "late" stock was sensitive to photoperiod (as did Paton (1969)), whereas the self-graft was not. Both grafted and excised plants flowered at a higher node than the control plants.

Haupt (1969) argues that it is not the "late" cotyledons which contain an inhibitor, but rather that it is the "early" cotyledons which contain a promoter. He found that "late" scions grafted onto an "early" stock had the same NF as if it was grafted onto an "old late" stock, both of which were less than the "late" self-graft NF. However, the supply of inhibitor - or any substance from the cotyledons for that matter - would presumably be minimal by the time that the "old late" graft was made, and thus it can be argued just as convincingly that the cotyledons of late varieties contain an inhibitor.

He further found that "late" scions plus cotyledons grafted onto an "early" stock flowered out of an earlier node than did the "late" controls, but later than scions without cotyledons. Again the evidence is not convincing either way and much could depend on the relative growth rates of the three groups involved.

Köhler (1965) carried out a number of grafting experiments to come to the conclusion reported previously (see pp.1.4-5, 3.14). However, most of his work was carried out under sub-optimal conditions (Haupt, 1969), which could alter the situation drastically by affecting the growth pattern (see Chapter 7) and certainly the inductive process.

On the other hand, Paton and Barber (1955), Amos and Crowden (1969) and Murfet and Reid (1973) have suggested that late varieties contain an inhibitor in their cotyledons. In particular, Murfet and Reid (1973) have presented strong evidence for this by showing that an "early" scion grafted to a "late" stock will flower out of a similar node to the self-graft under L.D. conditions, but at a much higher one under S.D. conditions. They further suggest that gene Sn is suppressed under L.D. conditions (also Murfet, 1973). However, this seems an unnecessary proposal as they have not taken into account the quantitative nature of the photoperiodic stimulus, which can quite adequately explain the photoperiodic response. Further, if Sn was inoperative under L.D. conditions, then the following facts still require explanation:

1. that under L.D. conditions, "Sn" plants flower out of higher node than do "sn" plants.
2. that Line 58 plants always flower out of a higher node than Line 59 plants. (This has been explained in terms of the leaky "sn" gene.)
3. that excised "Sn" and "sn" plants can respond to vernalization treatment under L.D. conditions.

Summary

From these results and discussion, the following can be inferred (c.f. Murfet, 1971 a,b,c):-

1. Gene "Sn" confers the ability of a plant to respond to photoperiod and vernalization by the production of a floral inhibitor in the shoot and cotyledons. The inhibitor establishes a threshold level at the apex which must be overcome by the floral stimulus before flower initiation and development can occur. There was no need to postulate the decline in "Sn" activity as a result of ageing. The recessive "sn" most likely behaves as a leaky mutant.
2. Gene "E" reduces the effectiveness of the cotyledonary inhibitor, perhaps by the production of a cotyledonary flower promoter, and further may delay the initial production of inhibitor in the stem.
3. Gene "Lf" both represses the activity of "E" and encourages the activity of gene "Sn" in the shoot.
4. Photoperiod causes a differential rate of production of stimulus in green tissue.
5. Vernalization directly encourages the activity of the inductive stimulus. It also has a minor effect on the cotyledonary inhibitor system.

TABLE 6.1
EFFECT OF COTYLEDON REMOVAL AT VARIOUS STAGES OF GERMINATION
ON NF IN LINES 2, 24, 53, 60, 59 and 58 (SUMMER CROP)

Both S.D. and L.D. photoperiods were used. n, number of plants scored. Nutrient agar medium 8.

| Photo-period | Lines | 1 Day | | | 2 Days | | | 4 Days | | | 6 Days | | | 9 Days | | | Control | | |
|--------------|-------|-------|-------|-----|--------|-------|------|--------|-------|------|--------|-------|------|--------|-------|------|---------|-------|------|
| | | n | NF | ± | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE |
| S.D. | 2 | 15 | 21.21 | .58 | 17 | 21.53 | .45 | 13 | 22.41 | .43 | 13 | 22.92 | .66 | 15 | 22.33 | .43 | 18 | 23.61 | .20 |
| | 24 | 15 | 21.60 | .58 | 16 | 23.69 | .44 | 19 | 24.05 | .75 | 19 | 20.84 | .43 | 18 | 22.11 | .34 | 19 | 22.11 | .31 |
| | 53 | 12 | 16.25 | .48 | 18 | 16.72 | .52 | 4 | 19.00 | 1.58 | 12 | 19.08 | .42 | 17 | 20.47 | .43 | 14 | 21.64 | .49 |
| | 60 | 19 | 17.21 | .42 | 15 | 17.27 | .57 | 8 | 18.00 | .42 | 17 | 17.76 | .29 | 18 | 17.00 | .29 | 19 | 12.68 | .27 |
| | 59 | 11 | 11.27 | .36 | 14 | 11.14 | .27 | 2 | 12.00 | .50 | 18 | 9.05 | .05 | 18 | 9.00 | - | 20 | 9.00 | - |
| | 58 | 15 | 12.20 | .24 | 9 | 12.33 | .29 | 3 | 13.67 | .34 | 19 | 10.00 | .11 | 19 | 10.05 | .08 | 20 | 9.80 | .09 |
| L.D. | 2 | 17 | 13.35 | .26 | 17 | 13.47 | .17 | 16 | 13.63 | .15 | 20 | 13.40 | .13 | 20 | 14.40 | .11 | 20 | 15.65 | .13 |
| | 24 | 19 | 14.21 | .15 | 17 | 14.41 | .17 | 19 | 14.63 | .21 | 19 | 15.00 | .20 | 20 | 15.70 | .13 | 20 | 16.65 | .17 |
| | 53 | 16 | 11.19 | .16 | 18 | 11.67 | .16 | 13 | 12.08 | .21 | 17 | 11.41 | .15 | 18 | 13.11 | .08 | 13 | 14.31 | .17 |
| | 60 | 15 | 10.53 | .22 | 19 | 9.79 | .18 | 18 | 10.44 | .18 | 16 | 10.63 | .12 | 19 | 11.42 | .21 | 18 | 12.00 | .21 |
| | 59 | 19 | 9.79 | .20 | 18 | 10.17 | .27 | 9 | 10.78 | .47 | 18 | 8.94 | .06 | 18 | 9.00 | .08 | 20 | 8.90 | .07 |
| | 58 | 20 | 11.25 | .10 | 20 | 11.11 | .12 | 14 | 12.00 | .23 | 20 | 9.20 | .09 | 20 | 9.50 | .11 | 20 | 9.45 | .11 |

Experiment 6.1

TABLE 6.2

EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD
ON NF IN LINES 2, 24, 53, 60, 59 and 58 (AUTUMN CROP)

n, number of plants scored. Nutrient agar medium 8. ND for Lines 53 and 60 under S.D. conditions are also given in brackets beside figures for NF.

| Line | Vernal- ization (Weeks) | S.D. | | | | | | | | L.D. | | | | | | | |
|------|-------------------------------|--------|--------------|---|----------|-----------------|--------------|---|----------|--------|-------|-----|----|-----------------|-------|-----|----|
| | | Intact | | | | Decotyledonized | | | | Intact | | | | Decotyledonized | | | |
| | | n | NF | ± | SE | n | NF | ± | SE | n | NF | ± | SE | n | NF | ± | SE |
| 2 | 0 | 19 | 26.79 | | .22 | 20 | 20.25 | | .48 | 20 | 15.45 | .17 | | 20 | 13.00 | .14 | |
| | 2 | 14 | 25.21 | | .50 | 21 | 18.52 | | .25 | 20 | 15.40 | .11 | | 16 | 12.81 | .16 | |
| | 4 | 18 | 23.83 | | .76 | 21 | 18.71 | | .28 | 20 | 15.00 | .16 | | 19 | 12.95 | .12 | |
| 24 | 0 | 19 | 24.05 | | .32 | 21 | 20.95 | | .56 | 20 | 15.80 | .21 | | 22 | 13.32 | .14 | |
| | 2 | 19 | 22.42 | | .32 | 22 | 18.32 | | .41 | 19 | 14.95 | .22 | | 19 | 12.58 | .16 | |
| | 4 | 20 | 20.65 | | .23 | 21 | 18.72 | | .27 | 18 | 13.83 | .15 | | 22 | 12.23 | .11 | |
| 53 | 0 | 15 | 21.47(23.12) | | .36(.51) | 19 | 14.21(23.67) | | .29(.35) | 19 | 12.84 | .30 | | 20 | 10.35 | .22 | |
| | 2 | 10 | 18.30(20.85) | | .68(.64) | 21 | 13.78(22.38) | | .23(.50) | 20 | 12.40 | .31 | | 17 | 10.24 | .18 | |
| | 4 | 20 | 11.40(17.53) | | .27(.34) | 22 | 13.45(22.48) | | .14(.34) | 20 | 11.15 | .13 | | 23 | 10.26 | .09 | |
| 60 | 0 | 21 | 10.57(19.79) | | .12(.59) | 18 | 12.56(22.71) | | .37(.47) | 20 | 10.40 | .11 | | 23 | 9.52 | .11 | |
| | 2 | 20 | 10.00(17.00) | | .07(.36) | 23 | 13.57(22.17) | | .27(.47) | 20 | 10.15 | .15 | | 20 | 9.35 | .11 | |
| | 4 | 23 | 10.09(16.75) | | .06(.42) | 20 | 13.40(23.22) | | .32(.28) | 20 | 10.00 | - | | 21 | 9.95 | .05 | |
| 59 | 0 | 19 | 8.89 | | .11 | 16 | 10.94 | | .17 | 19 | 8.79 | .10 | | 19 | 10.11 | .26 | |
| | 2 | 19 | 9.05 | | .12 | 18 | 9.83 | | .15 | 20 | 9.00 | .15 | | 22 | 9.32 | .12 | |
| | 4 | 19 | 9.16 | | .09 | 19 | 9.37 | | .11 | 20 | 9.40 | .11 | | 23 | 9.43 | .11 | |
| 58 | 0 | 21 | 9.00 | | .07 | 17 | 11.24 | | .16 | 18 | 9.28 | .14 | | 19 | 11.00 | .20 | |
| | 2 | 19 | 9.63 | | .19 | 17 | 10.47 | | .17 | 17 | 9.18 | .09 | | 17 | 9.82 | .26 | |
| | 4 | 19 | 9.84 | | .09 | 20 | 9.90 | | .10 | 19 | 10.00 | .08 | | 20 | 10.00 | .07 | |

FIGURE 6.1
EFFECT OF COTYLEDON REMOVAL AT VARIOUS STAGES
OF GERMINATION ON NF IN LINES 2, 24, 53, 60, 59 AND 58
(SUMMER CROP)

_____ L.D. conditions
----- S.D. conditions

(The standard errors are shown where appropriate.)

Although it would be more accurate to depict these results in histogram form, the line drawing was chosen so that the figure could be more easily understood.

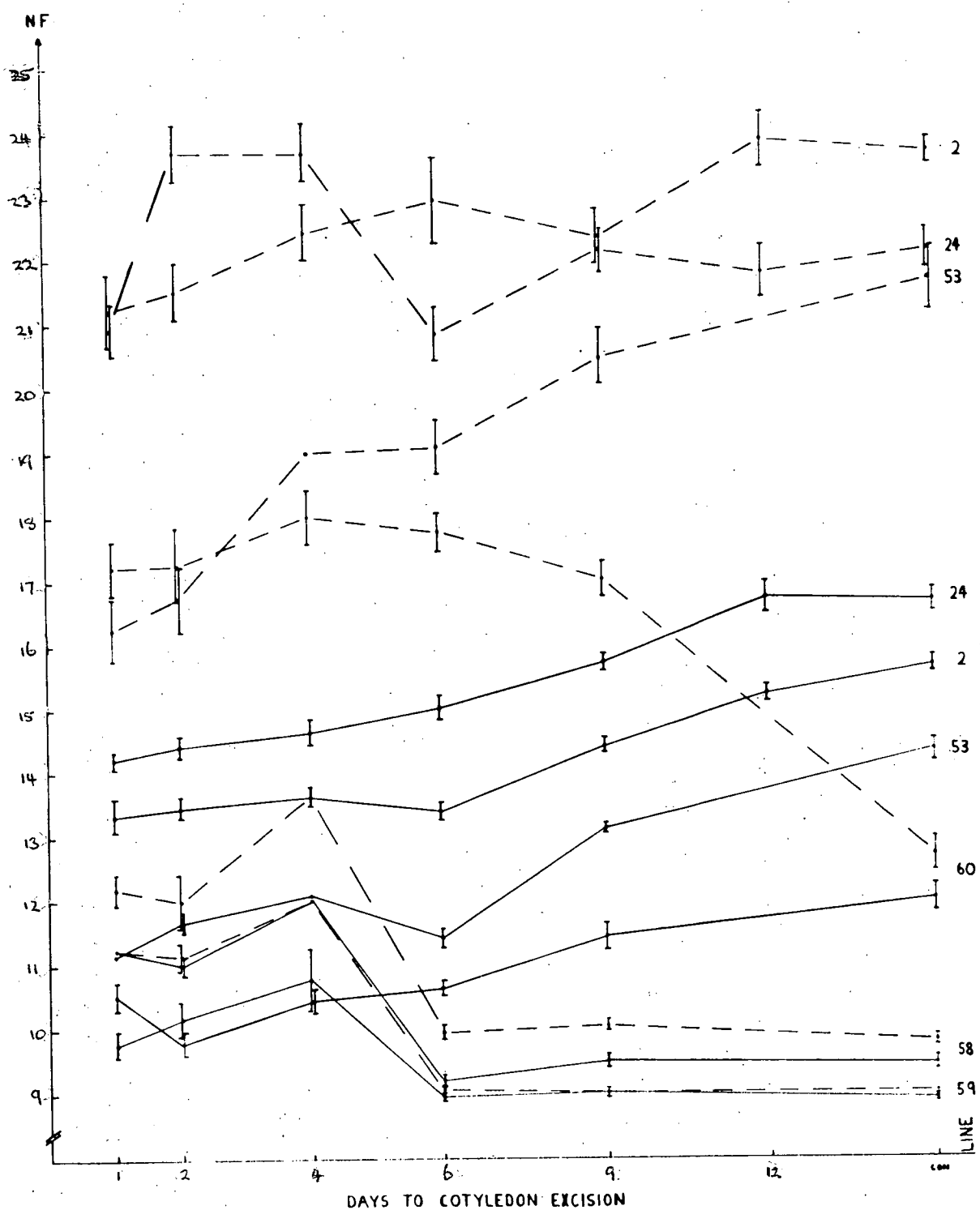


FIGURE 6.2EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF IN LINES 59 AND 58
(AUTUMN CROP)

Plain bar - Intact plants

Hatched bar - Excised plants

(The standard errors are shown at the top of each histogram.)

The data summarized is based on the number of plants indicated at the base of each bar.

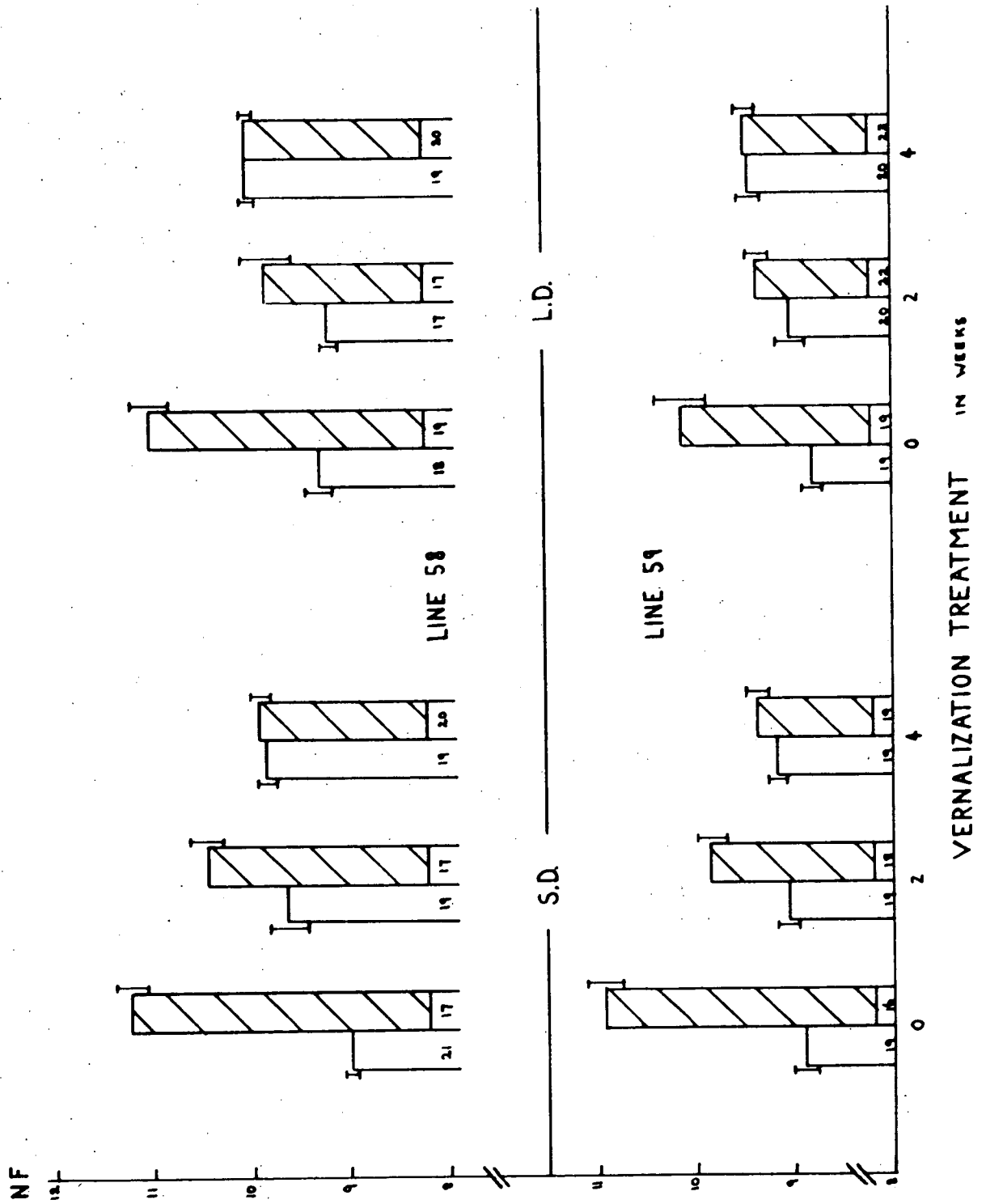


FIGURE 6.3EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF IN LINES 2 AND 24
(AUTUMN CROP)

Plain bar - Intact plants
Cross hatched bar - Excised plants

The data summarized is based on the number of plants indicated at the base of each bar.

(The standard errors are shown at the top of each bar.)

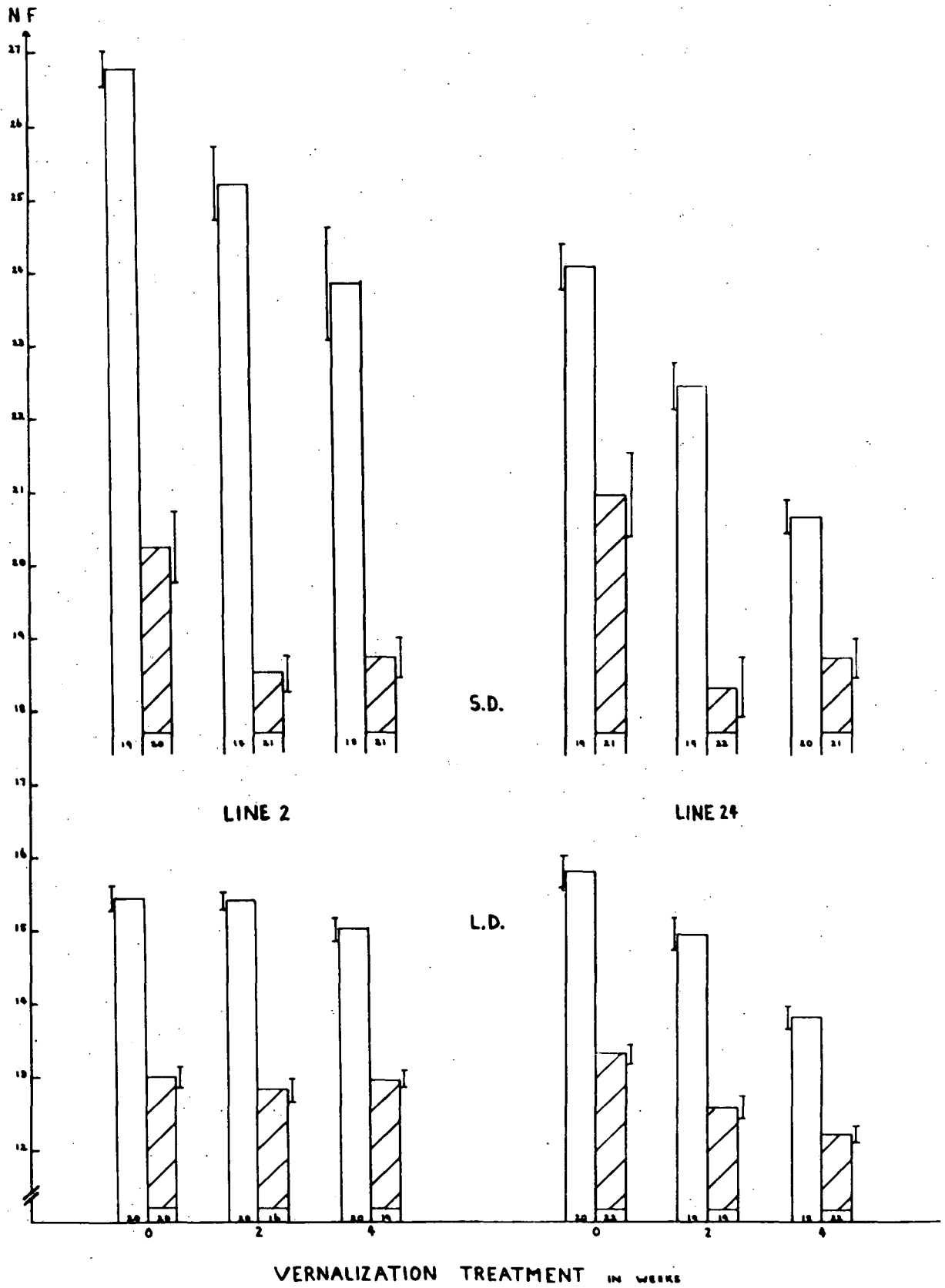


FIGURE 6.4EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF AND ND IN LINE 53
(AUTUMN CROP)

| | | |
|-------------------|---|----------------|
| Plain bar | - | Intact plants |
| Cross hatched bar | - | Excised plants |
| Full line | - | NF |
| Broken line | - | ND |

The data summarized is based on the number of plants indicated at the base of each bar.

(The standard errors are shown at the top of each bar.)

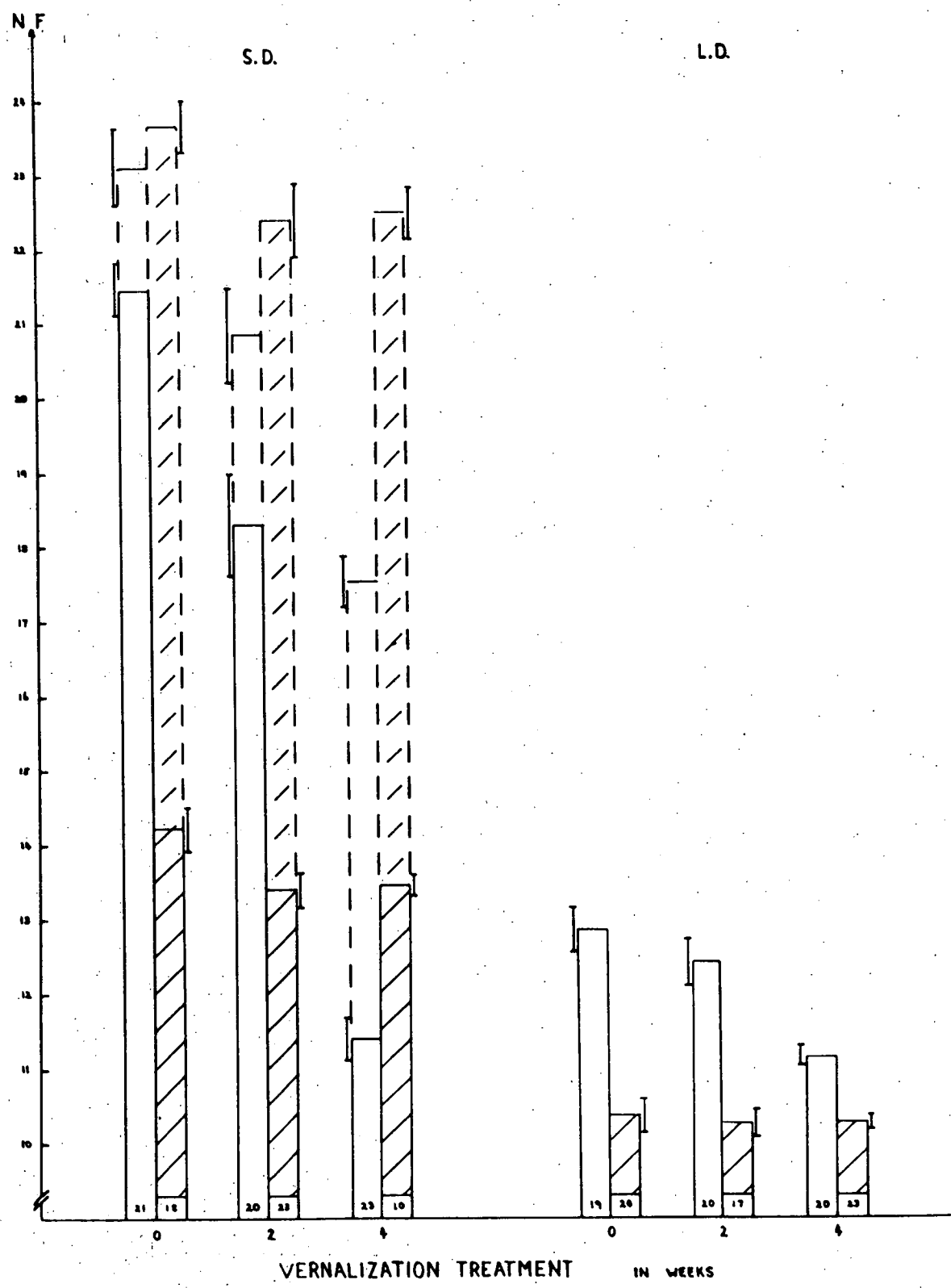
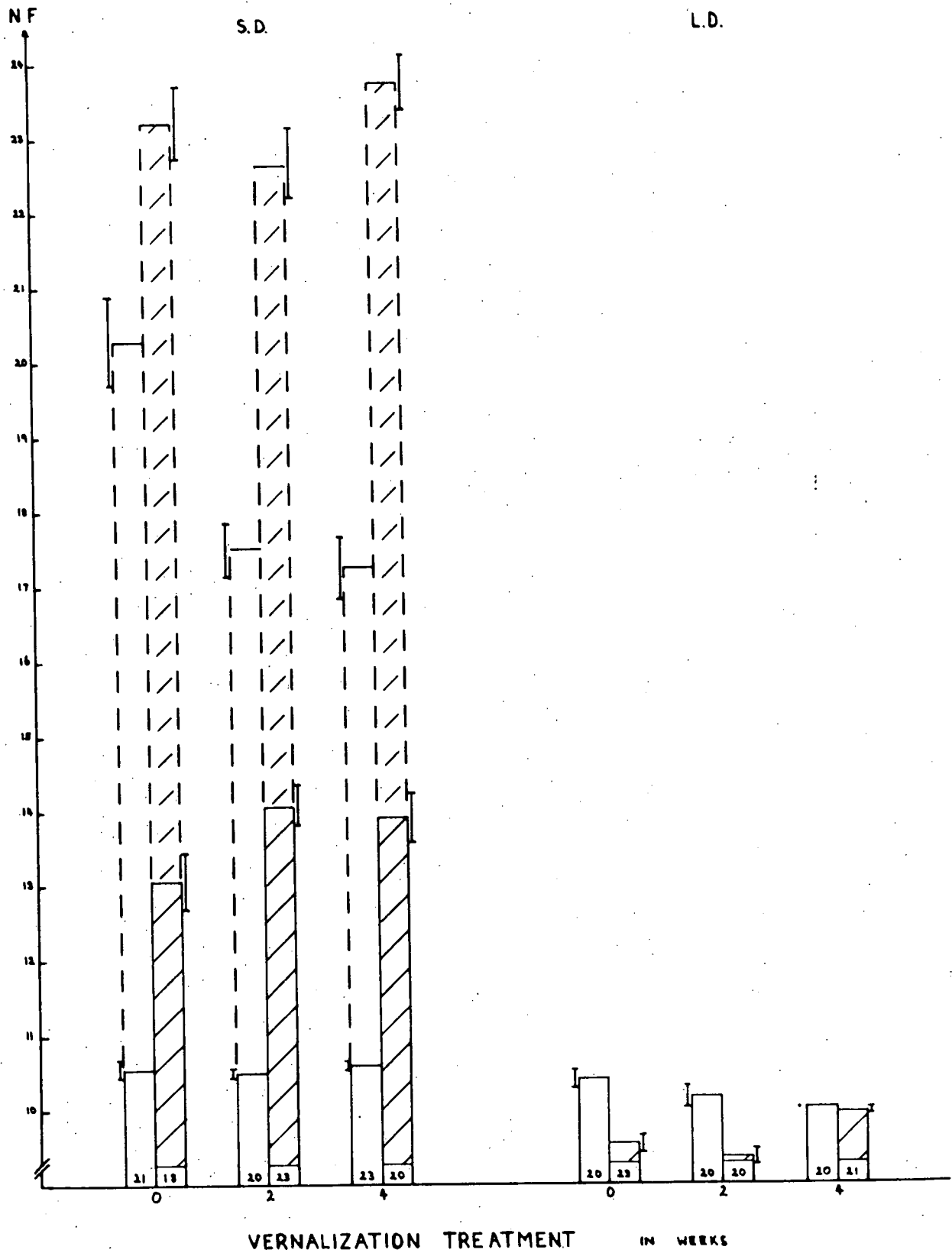


FIGURE 6.5EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF AND ND IN LINE 60
(AUTUMN CROP)

- Plain bar - Intact plants
- Cross hatched bar - Excised plants
- Full line - NF
- Broken line - ND

The data summarized is based on the number of plants indicated at the base of each bar.

(The standard errors are shown at the top of each bar.)



THE GROWTH PATTERN

a INTRODUCTION

Many workers have made reference to the idea that an alteration to the growth of a plant may have a direct effect on the resultant NF, without affecting the flowering process as such. Haupt (1969) certainly suggests that the effect of temperature on flowering behaviour may be the result of such a phenomenon, and both Sprent (1966a) and Paton (1967) mentioned this as being a possibility with respect to leaf removal experiments. Further, two recent papers have contained the words "growth pattern" without definition when discussing the effect of cotyledon removal on NF (Murfet, 1973; Murfet and Reid, 1973). This phrase has been mentioned in previous chapters, and in the context of this thesis "growth pattern" is defined as the ratio total nodes : expanded nodes.

In the introductory chapter, reference was made to a possible relationship between the growth pattern and flowering behaviour. Throughout this thesis, it has been suggested that cotyledon removal may have a direct effect on NF in Line 24 plants by somehow affecting the physical development of excised plants. Certainly, attributing the effect of cotyledon removal on NF solely to the removal of a cotyledonary inhibitor has only been done with some discomfort since the growth rate has obviously been reduced by cotyledon removal (see Chapters 3 and 4), and it was felt that NF may also have been affected directly by this slower development.

It was not until the final stages of the work programme however, that a method was devised whereby an effect of growth rate on NF could be separated from other factors (e.g. vernalization, photoperiod), and a clue was obtained from an experiment reported by Paton (1969) (see also Amos and Crowden, 1969).

Previously, it had been suggested that the events in the leaf and those in the ~~apex~~ leading to floral initiation were likely to be so different in space and time that different terms should be used to distinguish them (Evans, 1969). Evans suggested "induction" and "evocation" respectively.

In his paper, Paton demonstrated what appeared to be a physiological separation of the vernalization and photoperiod response by transferring plants from L.D. to S.D. conditions at various stages of development. He presented results which suggested that the inductive process was affected by photoperiod and the evocation process by vernalization. He further suggested that the inductive process involved the production of a stimulus, and that the evocation process was affected by the presence of an inhibitor. Vernalization reduced the effectiveness of the inhibitor, thus causing rapid evocation.

The purpose of the experiment to be described was threefold. In the first instance it was designed so that the effect of cotyledon removal on the rate of vegetative development (as measured by the growth pattern) could be measured. Secondly, it was thought desirable to expand Paton's experiment to include excised plants, to see whether cotyledon removal influenced the effect reported by Paton. And thirdly, it was thought that if the conditions were reversed (i.e. plants transferred from S.D. to L.D. conditions) the results obtained by Paton with respect to the induction and evocation processes should still be observed.

b THE GROWTH PATTERN

Experiment 7.1:- A full factorial experiment involving vernalization, cotyledon removal and photoperiod was performed as previously described in Chapter 4 (Experiment 4.8), with the

addition that plants were either left intact or had one or both cotyledons removed, cotyledon removal occurred prior to any vernalization treatment, and the vernalization treatment lasted for 4 weeks.

The rate of growth was measured in terms of two parameters, these being the stage of node expansion (NE) and the total number of nodes initiated (NT). The decimal system of defining NE and NT as described by Maurer *et al* (1966) was used in quantifying these measurements.

For intact plants, progressive recording of growth data was made at 3-day intervals by scoring 4 plants taken at random from each treatment. These readings gave a fair spread during the entire vegetative phase, and because of the quantity of data involved, are presented in purely statistical form in Table 7.1. At the same time as the growth data was recorded, 20 plants per treatment were transferred from one photoperiod to the alternate one, wherein they were allowed to grow to anthesis.

Because of the limitations of time and space in the facilities available, the experiment needed to be limited in size, and for the remainder of the treatments, only 3 plants were transferred between photoperiod regimes at any one time. For semidecotyledonized plants, readings were taken of 3 plants at 3-day intervals, and for fully excised plants, readings were made of 3 plants at 6-day intervals.

After plants had been transferred from one photoperiod to the other, periodic checks on the growth rate were made to determine whether any change had occurred. Flowering was measured in terms of NF. The progress of each individual plant was followed, and for plants transferred between photoperiods, their NF was related back to their NE at the time of transfer.

addition that plants were either left intact or had one or both cotyledons removed, cotyledon removal occurred prior to any vernalization treatment, and the vernalization treatment lasted for 4 weeks.

The rate of growth was measured in terms of two parameters, these being the stage of node expansion (NE) and the total number of nodes initiated (NT). The decimal system of defining NE and NT as described by Maurer et al (1966) was used in quantifying these measurements.

For intact plants, progressive recording of growth data was made at 3-day intervals by scoring 4 plants taken at random from each treatment. These readings gave a fair spread

All treatments were carried out concurrently during the summer season, and to allow for statistical treatment of the data, they were each planned as randomized block experiments.

The results are given in Tables 7.1 - 2 and Figures 7.1 - 7.11.

Results:- The results are best considered in two parts. The first part is concerned simply with the measurement of the growth rate using the two criteria NE and NT. When NE was plotted against time (Figure 7.1) it was found that:-

- (a) the within-treatment variances were unacceptably high, especially in the case of fully excised plants)
- (b) the rate of node expansion for fully excised plants was dependent on the photoperiod and vernalization treatment, whereas intact or semi-decotyledonized plants displayed no such effect.
- (c) although semi-decotyledonized plants showed a similar rate of node expansion to intact plants, a closer examination of the NT data showed that they were in fact at a different stage of physiological development (see Figure 7.2).
- (d) a non-linear relationship held for fully excised plants (see Figure 7.1)

It was found to be both more convenient and more accurate to determine the actual stage of plant development by measuring NT as a function of NE (Table 7.1, Figure 7.2). The ratio NT : NE is hereafter referred to as the growth pattern. The measurement of growth by the growth pattern had advantages over the time rates of measurement in that:-

- (a) high within-treatment variances were largely eliminated.
- (b) linear relationships existed for all treatments.
- (c) all treatments had a growth pattern of the same or similar slope.

Examination of Figure 7.2 shows that the growth pattern was not affected to any great extent by the vernalization and photoperiod treatments. The displacement in the growth pattern was caused primarily by the cotyledon status. It is thus apparent that cotyledon removal caused a reduction in the number of unexpanded nodes in the apex at any given stage of node expansion. However, one anomalous result does need mentioning.

Fully excised plants which had been vernalized and grown under L.D. conditions (VL) displayed a growth pattern which was displaced such that they contained a greater number of unexpanded nodes in the apex than did the other excised plants at similar stages of node expansion.

The second part of the experiment concerned the effect of vernalization, photoperiod and partial or complete cotyledon removal on NF. These results are given in Table 7.2 and Figure 7.3, and are completely in accord with data given previously, with one exception. Fully excised plants which had been vernalized and grown under L.D. conditions flowered out of a higher node ($NF = 12.39$) than their unvernallized counterparts ($NF = 12.11$). Although this difference was not significant, this phenomenon had never before been observed in our laboratories.

The third part of the experiment concerned the transfer of plants from one photoperiod to the other, and especially the comparison of the final NF with the growth pattern. The results for plants that were transferred from S.D. to L.D. conditions are shown diagrammatically in Figures 7.4 - 7.6, and Figures 7.7 - 7.9 show the results of plants that were transferred from L.D. to S.D. conditions.

The time at which the transfer of plants was carried out from one photoperiod regime to the other is specified by NE. The standard errors for partially or fully excised plants are not shown as the actual number of plants transferred at any particular stage of NE was small for these treatments.

Plants that were transferred from one photoperiod regime to the other behaved in a similar fashion, irrespective of the vernalization or cotyledon treatment. The effect of the pre-transfer photoperiod treatment was proportional to the duration of exposure, and resulted in either a progressive delay (Figures 7.4 - 7.6) or

a progressive advancement (Figures 7.7 - 7.9) in NF, depending on the direction of the transfer. Vernalization and cotyledon removal had effects that were additive to that of photoperiod transfer.

Figures 7.4 - 7.9 are concerned with the relationship between NF of plants transferred between photoperiod treatments and the growth pattern curve for each treatment. The respective curves are seen to converge and finally intersect at the point corresponding to the NF for the stated treatment without transfer. There is no evidence to support the observation by Paton (1969) of "evocation plateaus".

In Figures 7.10 and 7.11, the NF for non-transferred plants is shown against NE for all treatments, together with the relevant section of the growth pattern curves. NF occurred at a particular point of NE which had been predetermined by photoperiod and vernalization but was independent of the cotyledon status. Thus, unvernallized plants flowered under L.D. conditions once NE reached 6, and under S.D. conditions once NE reached 10. Vernalization lowered the necessary NE to 5 under L.D. conditions and 8 under S.D. conditions.

Discussion:- It has been stated previously that the Line 24 cultivar of Pisum sativum undergoes a period of vegetative development before reaching the ripeness-to-flower stage; the minimum node at which flowering can occur appears to be node 12.

In the light of the results obtained from this experiment, this statement needs to be modified.

This experiment shows that NF is highly correlated with NE and that NE at flower initiation is determined by photoperiod and vernalization treatments. The cotyledon status has no significant effect on NE at flower initiation (see Figures 7.10, 7.11) although it does affect NT for a particular NE by lowering the number of unexpanded nodes in the apex. Cotyledon removal is the only

treatment of the three which affects the growth pattern in this manner.

From the transfer experiments it is obvious that from the time node 3 has opened plants are able to respond to a change in the photoperiod regime (see also Sprent, 1966a; Paton, 1967). The period of vegetative development before flower initiation can occur is in fact a continual and gradual build-up of potential to transfer from the vegetative to the flowering state. This potential is realized at the ripeness-to-flower stage, which occurs once a particular stage of leaf expansion is reached. This minimum leaf requirement has been shown to be affected by photoperiod and vernalization, the earliest that a plant can flower being once node 5 has expanded (see Figure 7.11). The earliest NF will thus be dependent on the growth pattern of the particular plant, and would be reduced below the present minimum of node 12 if the growth pattern could be displaced further to the right (i.e. the number of unexpanded nodes in the apex be further reduced).

The photoperiod response:- It would seem that photoperiod is involved with the quantitative production of an inductive stimulus in the leaves. There is no reason to suspect that this process does not occur in all green tissue, including the stipules and the stem itself (Haupt, 1969). From the transfer experiments, the progressive and continuing response to photoperiod exposure from the time node 3 has expanded indicates that all true leaves are involved in the inductive process, and will respond to any change in photoperiod until such time as flower initiation has occurred.

Obviously, an increase in the photoperiod will cause an increase in the production of the inductive stimulus with the result that the threshold level will be surpassed at an earlier stage and flowering will occur.

The vernalization response:- Vernalization seems to be involved in the flowering process by reducing the effective threshold level which

the inductive stimulus must reach before flowering can occur. It is independent of but additive to the photoperiod response as can be seen from the results of the transfer experiments (Figures 7.4 - 7.9). As it is independent of the photoperiod response, and since it occurs in fully excised plants, the most likely site of action for the vernalization response would appear to be in the apex, where the inductive stimulus causes the changeover from the vegetative to the reproductive state.

Both vernalization and photoperiod have a direct effect on flowering in that neither treatment has any effect on the growth pattern. The one anomalous result where vernalization and photoperiod did appear to have an effect on the growth pattern (i.e. for fully excised plants which had been vernalized and grown under L.D. conditions) explains the other anomalous result where vernalized plants flowered out of a higher node than did their unvernallized counterparts. It can be seen quite clearly from Figure 7.9 that a greater displacement of the growth pattern to the right would have allowed these plants to flower out of a lower node, and in fact some plants did show the normal response.

The delay in the evocation events of intact unvernallized plants as reported by Paton (1969) was not reproduced here. Although Paton used as his co-ordinates NT and time, the effect should still be apparent if growth is measured by the growth pattern, since NE and time show a linear relationship - at least for intact plants (see Figure 7.1). It may be that the conditions under which the experiment was conducted played some part in the obtaining of such different results. The quality control over such factors as light intensity, light quality and daily temperature levels were not as strict in this present experiment as they were in his experiment.

The cotyledon response:- The effect of cotyledon removal appears to be an indirect one and its main effect is in determining the number of unexpanded nodes in the apex at the completion of photo-

periodic induction. Thus the role of the cotyledon in producing the flower inhibitor, colysanthin - as reported previously by Barber (1959), Paton (1969), Johnston and Crowden (1967), and Amos and Crowden (1969) - needs to be revised.

It seems likely that a flower inhibitor is produced in Line 24 plants as a result of activity of the Sn gene, which sets a threshold level that the photoperiodic stimulus must attain before flowering can occur. However, as reported by Murfet (1971,c), the site of activity of the Sn gene appears to be in the shoot as much as it is in the cotyledons.

It is necessary now to return to some of the points raised earlier in this thesis and to discuss them in relation to the growth pattern effect.

In Chapter 3, it was shown that the level of available nutrient had an effect on NF in both intact and decotyledonized plants. Haupt (1952, 1955) has shown that an increase in the level of nitrogen will delay NF in an excised early cultivar, and Sprent (1966b, 1967) has also shown an increase in NF with an increase in the nutrient level for intact "Greenfeast" plants. It may be that if the nutrient level becomes a limiting factor to growth, then the growth pattern is displaced such that the number of unopened nodes in the apex is reduced.

Similarly, it has been shown that an increase in the growing temperature will also delay NF in late varieties of peas (e.g. Barber, 1959; Stanfield *et al*, 1966; Ormrod *et al*, 1970). This effect could also be caused by a displacement of the growth pattern, allowing for a greater number of nodes to be laid down in the apex relative to the number of open leaves. Although a similar situation has not been observed in early varieties (Haupt, 1952; Highkin and Lang, 1966), the period of time available before flower initiation occurs may not be sufficient to allow for a change in the growth pattern in these varieties.

The light intensity under which plants are grown has also been shown to have had an effect on NF in late varieties. Moore (1964) has shown that a reduction in the light intensity causes late varieties to flower at an earlier node whereas early varieties are delayed in their flowering (also Leopold and Guernsey, 1954; Haupt, 1957). This latter point is important when considering the results of Köhler (1965) who carried out a number of experiments at sub-optimal conditions. Although no effect of light intensity on NF was observed in these present experiments, plants were only grown under differing light regimes for a short while, and not through to anthesis. Yet again this effect may well be the result of an alteration to the growth pattern. The reduction in light intensity could cause fewer nodes to be produced at the apex of late varieties relative to the number of open leaves.

It is of interest to note that both Sprent (1967) and Maurer et al (1968) found that the level of water supply would affect the rate of growth of the plants but not NF. Thus it would appear that the growth pattern is not disturbed by the water level, and that the rate of node production is affected to the same extent as the rate of node expansion.

All of these minor variations - nutrient supply, growing temperature and light intensity - could be involved in the seasonal variations that were observed throughout the programme and commented upon in Chapter 3.

A more important point, however, was the suggestion (Chapter 3, p. 3.12) that the effect of cotyledon removal was a result of the removal of the cotyledonary inhibitor, which was supported by the leaching experiments of Sprent and Barber (1957). It would seem more feasible to interpret the results of cotyledon removal not so much as one of removing the supply of inhibitor but more as a displacement of the growth pattern. Although more work will be required to support this comment, especially with reference to Experiments 3.5 and 3.6, it seems the more logical interpretation.

This does not mean, however, that cotyledons of late varieties do not contain an inhibitory substance. In fact, it would appear from the results of Murfet and Reid (1973) and from the vernalization studies presented here (comparing the effect of an increasing vernalization treatment between intact and excised plants) that there is still a need to postulate the presence of a floral inhibitor in the cotyledons of late varieties. However, the effect of cotyledon removal on NF may not be primarily the result of the removal of the cotyledonary inhibitor.

It is tempting to suggest that the effect of the time of cotyledon removal (e.g. prior to or at the completion of a vernalization treatment) is also an effect of growth pattern displacement. However, more work will be required before this can be stated unequivocally.

One important piece of evidence which can fairly safely be presumed to be an effect of growth pattern displacement is the effect of grafting. It was found that the effect of grafting was to be found mainly under L.D. conditions, but not under S.D. conditions. It appears likely that under S.D. conditions the growth pattern is fully normalized before flower initiation occurs, but that under L.D. conditions, the scion is still at a stage of development approximating that of the excised plant.

TABLE 7.1

THE EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD ON THE GROWTH PATTERN (SUMMER CROP)

Readings were taken for the duration of each treatment. Nutrient agar medium 8.

| Treatment | | | Number of Readings | Slope \pm SE | | Correlation Coefficient |
|-------------|------------------|----------------------|--------------------|----------------|-----|-------------------------|
| Photoperiod | Cotyledon Status | Vernalization Status | | | | |
| S.D. | Intact | U | 63 | 1.59 | .02 | 0.99 |
| | | V | 53 | 1.72 | .07 | 0.99 |
| | Semi Excised | U | 14 | 1.44 | .05 | 1.00 |
| | | V | 11 | 1.60 | .07 | 0.99 |
| | Fully Excised | U | 18 | 1.36 | .04 | 0.98 |
| | | V | 15 | 1.31 | .07 | 0.98 |
| L.D. | Intact | U | 40 | 1.72 | .04 | 0.99 |
| | | V | 25 | 1.96 | .08 | 0.98 |
| | Semi Excised | U | 11 | 1.80 | .10 | 0.99 |
| | | V | 10 | 2.04 | .06 | 1.00 |
| | Fully Excised | U | 10 | 1.54 | .13 | 0.97 |
| | | V | 9 | 1.35 | .12 | 0.97 |

TABLE 7.2

EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD ON NF OF LINE 24 PLANTS (SUMMER CROP)

n, number of plants scored. Nutrient agar medium 8.

| Cotyledon Status | S.D. | | | | | | L.D. | | | | | |
|------------------|--------------|-------|------|------------|-------|------|--------------|-------|------|------------|-------|------|
| | Unvernalized | | | Vernalized | | | Unvernalized | | | Vernalized | | |
| | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE | n | NF | ± SE |
| Intact | 63 | 21.02 | .17 | 35 | 18.97 | .11 | 60 | 14.98 | .09 | 29 | 13.86 | .14 |
| Semi Excised | 35 | 19.97 | .19 | 25 | 18.28 | .15 | 27 | 14.26 | .11 | 26 | 13.77 | .10 |
| Fully Excised | 24 | 17.38 | .41 | 22 | 15.23 | .11 | 18 | 12.11 | .16 | 23 | 12.39 | .17 |

FIGURE 7.1

EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD
ON THE RATE OF NODE EXPANSION IN LINE 24 PLANTS
(SUMMER CROP)

As there was no difference in the rate of node expansion for intact and semi-decotyledonized plants, these results have been combined.

_____ - Intact and semi-decotyledonized

----- - Fully excised

U - Unvernalized

V - Vernalized

S - Short day photoperiod

L - Long day photoperiod

EXPLANATION OF FIGURES

The growth patterns for the 6 different treatments are shown in Figure 7.2. These curves were obtained by recording NT and NE at various stages of vegetative growth and calculating the slopes and correlation coefficients (see Table 7.1).

Figures 7.4 - 7.9 show the effect on NF when unvernallized and vernalized plants are transferred between the two photo-period regimes at various stages of vegetative growth.

1. The appropriate growth pattern curves are represented by the straight lines

—— NT at transfer for unvernallized plants
----- NT at transfer for vernalized plants

2. The labelled lines show NF data for plants having undergone transfer treatment

—— (US-UL) NF for unvernallized plants
----- (VS-VL) NF for vernalized plants

Additional details for specific figures are given in the respective legends.

NODES
EXPANDED

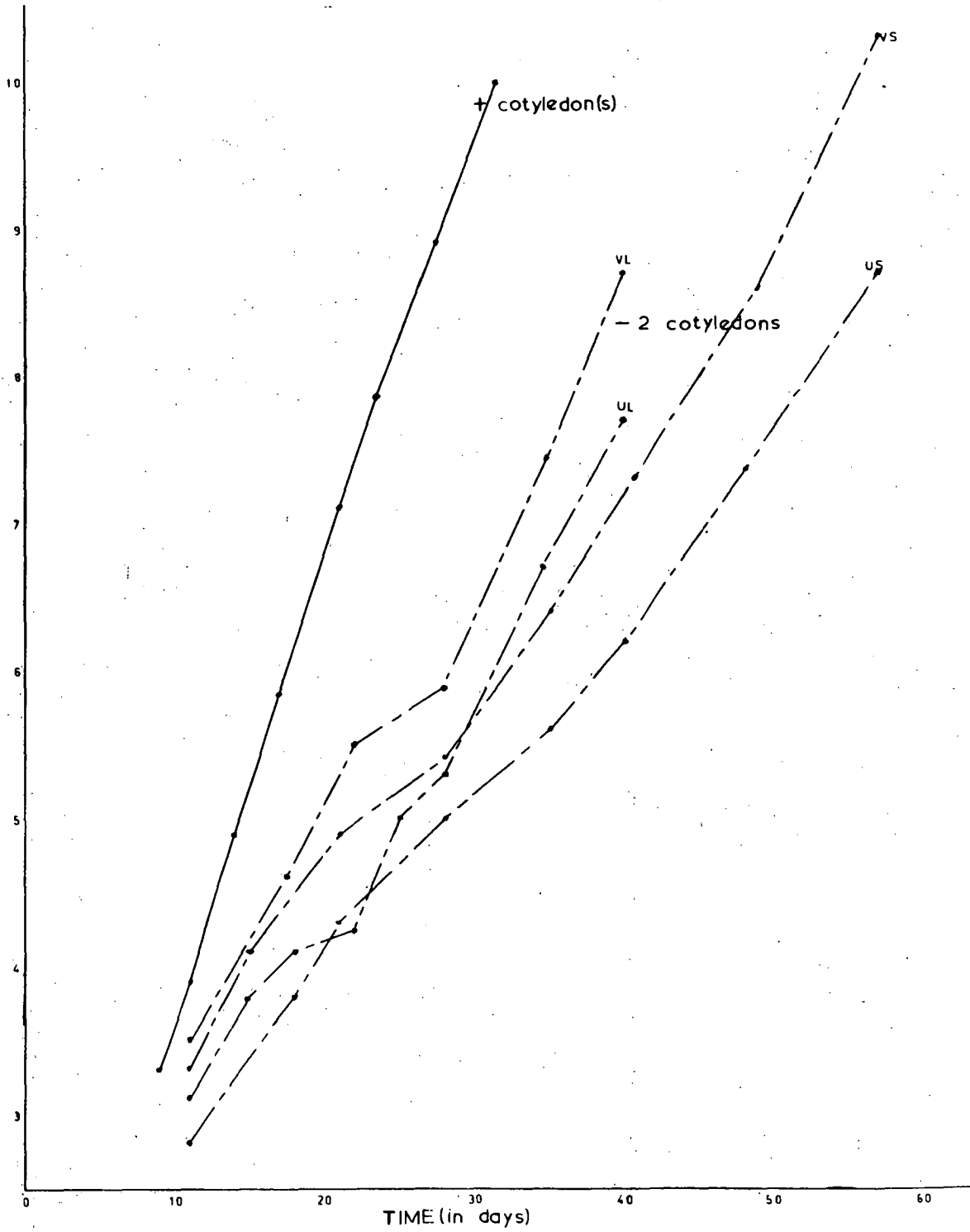


FIGURE 7.2

THE EFFECT OF COTYLEDON REMOVAL, VERNALIZATION AND PHOTOPERIOD
ON THE GROWTH RATE OF LINE 24 PLANTS, AS MEASURED BY
THE GROWTH PATTERN
(SUMMER CROP)

U - Unvernalized

V - Vernalized

S - Short day conditions

L - Long day conditions

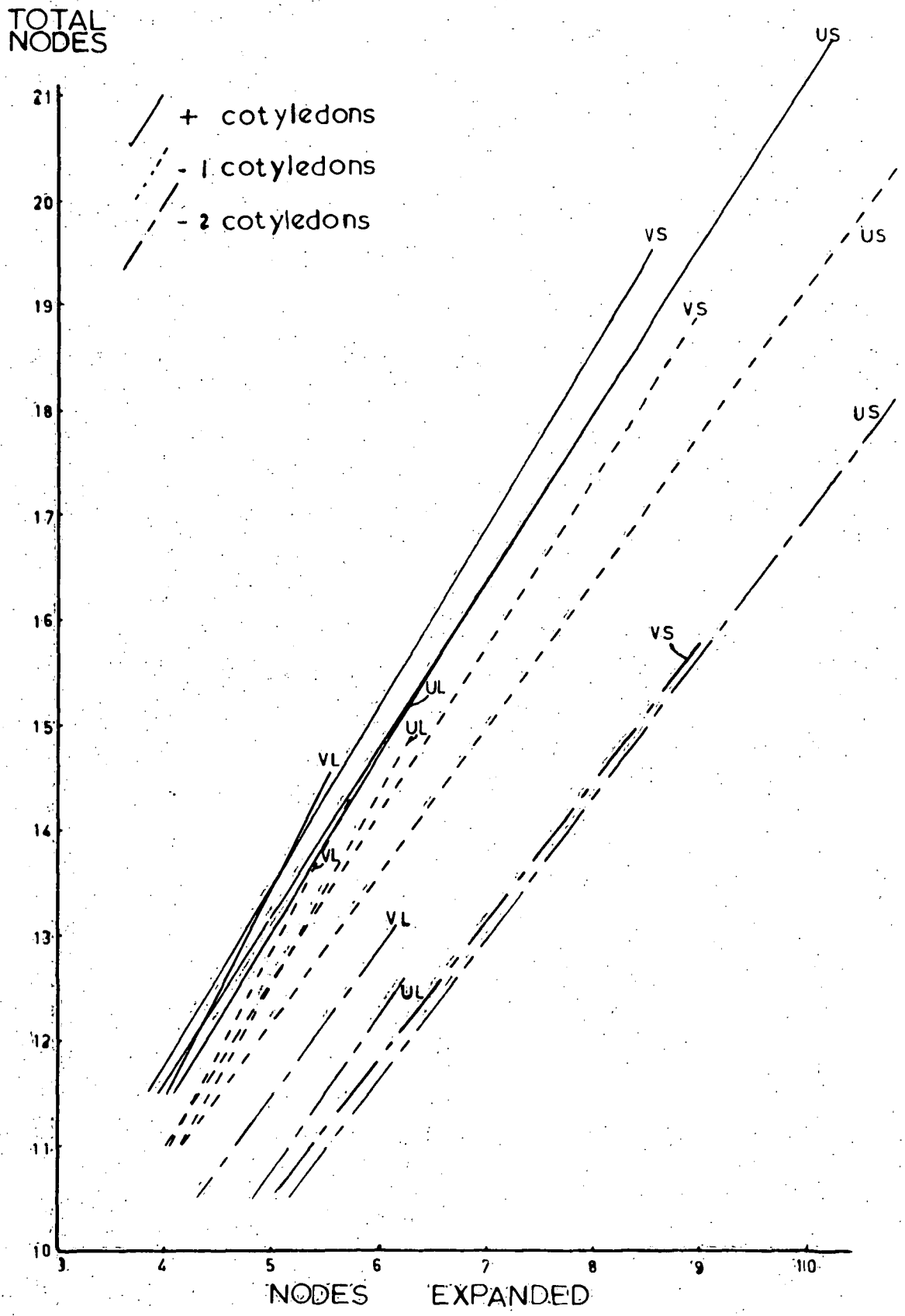


FIGURE 7.3EFFECT OF COTYLEDON REMOVAL, VERNALIZATION
AND PHOTOPERIOD ON NF IN LINE 24 PLANTS
(SUMMER CROP)

The data summarized is based on the number of plants indicated at the base of each bar.

The standard errors are shown at the top of each bar.

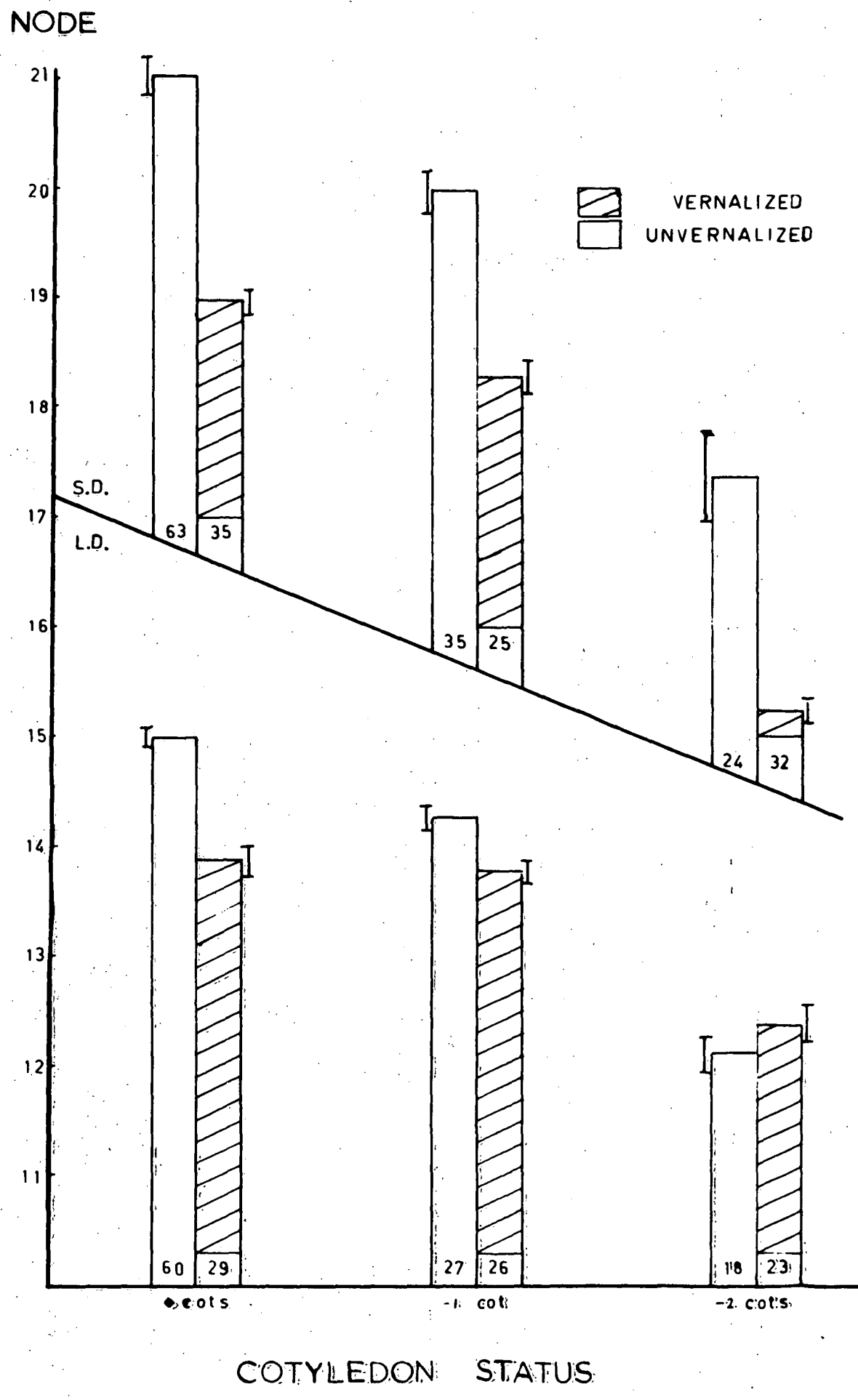


FIGURE 7.4
THE EFFECT OF S.D. → L.D. TRANSFER AND VERNALIZATION
ON NF IN INTACT LINE 24 PLANTS
(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for intact unvernallized and vernalized plants under S.D. conditions is also shown.

- _____ - Unvernallized
- - Vernalized
- U - Unvernallized
- V - Vernalized
- S - Short day conditions
- L - Long day conditions

(The standard errors are shown as vertical lines.)

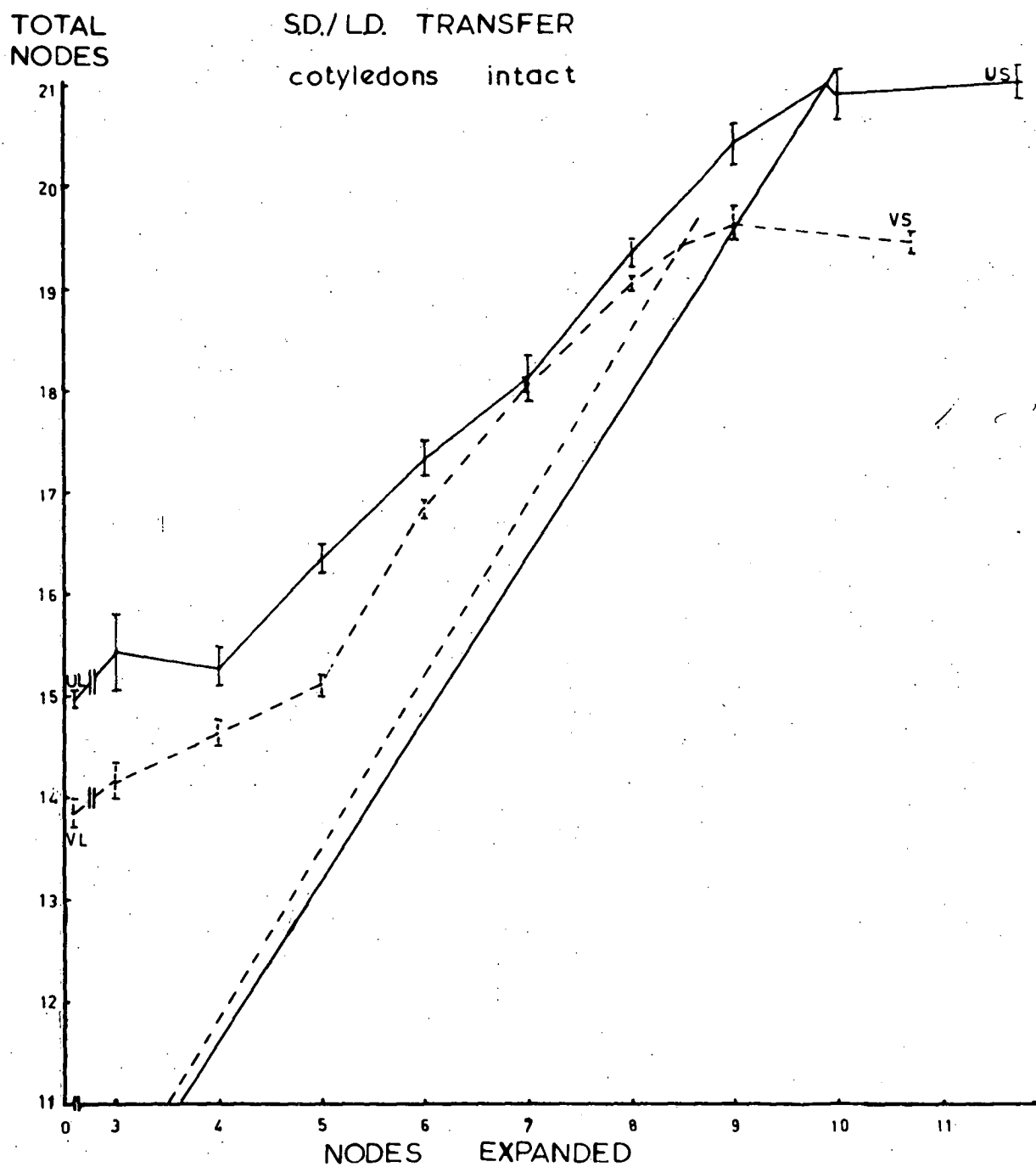


FIGURE 7.5
THE EFFECT OF S.D. → L.D. TRANSFER AND VERNALIZATION
ON NF IN SEMI-DECOTYLEDONIZED LINE 24 PLANTS
(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for semi-decotyledonized unvernallized and vernalized plants under S.D. conditions is also shown.

- Unvernallized
- - Vernalized
- U - Unvernallized
- V - Vernalized
- S - Short day conditions
- L - Long day conditions

(The standard errors are not shown since the sample sizes were small.)

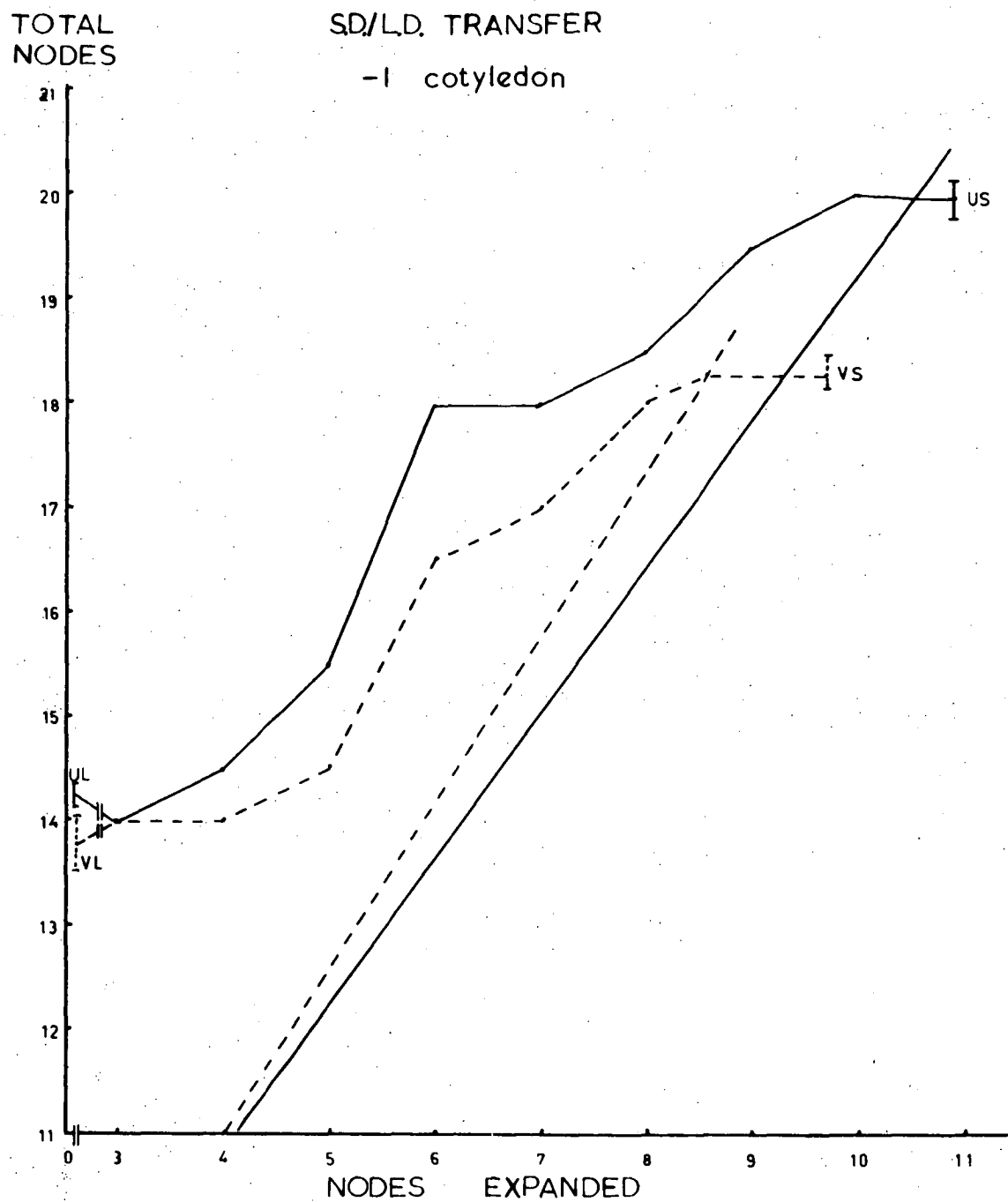


FIGURE 7.6

THE EFFECT OF S.D. → L.D. TRANSFER AND VERNALIZATION
ON NF IN FULLY EXCISED LINE 24 PLANTS

(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for fully excised unvernallized and vernalized plants under S.D. conditions is also shown.

- _____ - Unvernallized
- - Vernalized
- U - Unvernallized
- V - Vernalized
- S - Short day conditions
- L - Long day conditions

(The standard errors are not shown since the sample sizes were small.)

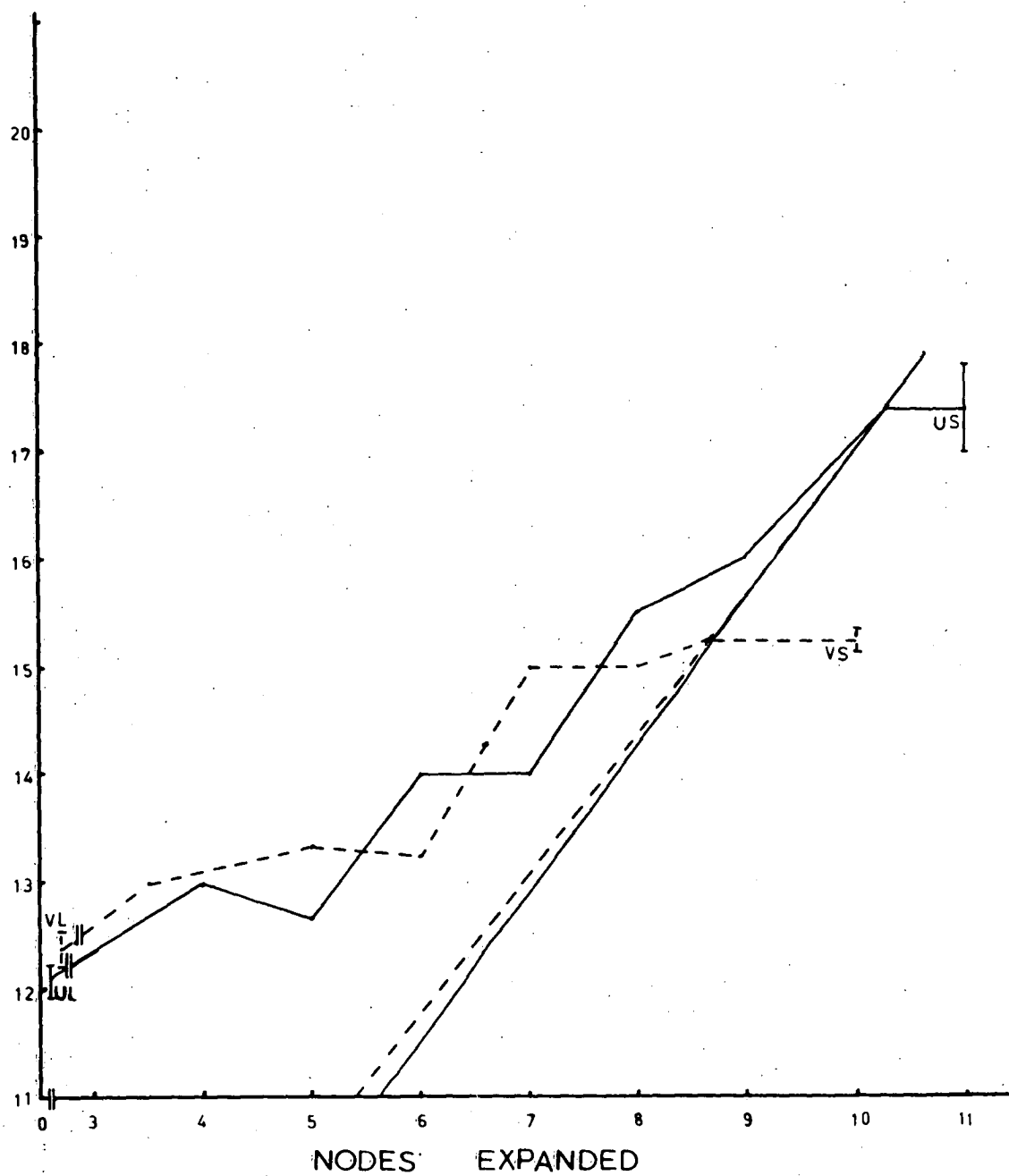
TOTAL
NODESS.D./L.D. TRANSFER
- 2 cotyledons

FIGURE 7.7

THE EFFECT OF L.D. → S.D. TRANSFER AND VERNALIZATION
ON NF IN INTACT LINE 24 PLANTS
(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for intact unvernallized and vernalized plants under L.D. conditions is also shown.

| | | |
|-------|---|----------------------|
| _____ | - | Unvernallized |
| ----- | - | Vernalized |
| U | - | Unvernallized |
| V | - | Vernalized |
| S | - | Short day conditions |
| L | - | Long day conditions |

(The standard errors are shown as vertical lines.)

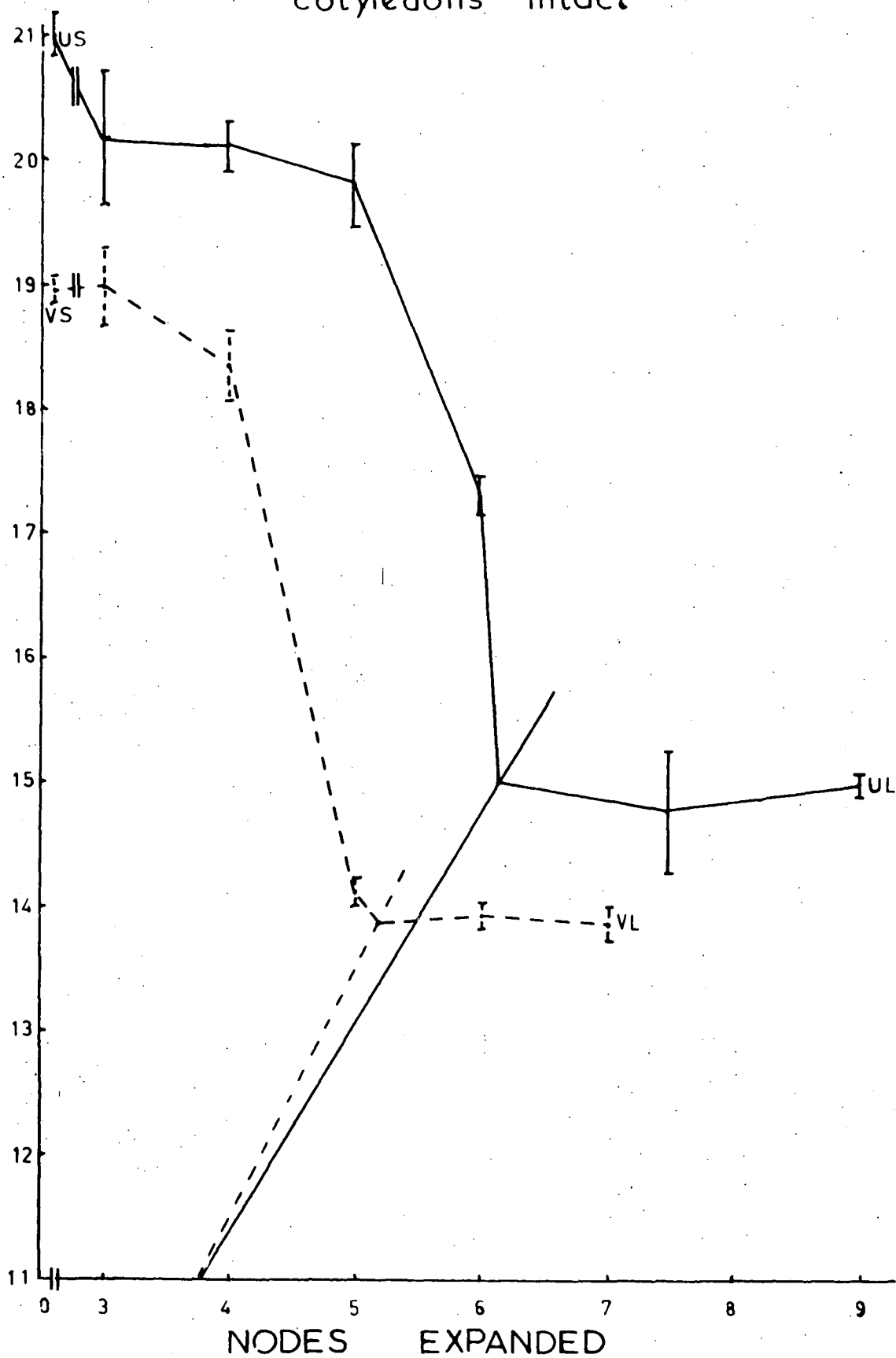
TOTAL
NODESL.D./S.D. TRANSFER
cotyledons intact

FIGURE 7.8

THE EFFECT OF L.D. → S.D. TRANSFER AND VERNALIZATION
ON NF IN SEMI-DECOTYLEDONIZED LINE 24 PLANTS
(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for semi-decotyledonized unvernallized and vernalized plants under L.D. conditions is also shown.

| | | |
|-------|---|----------------------|
| _____ | - | Unvernallized |
| ----- | - | Vernalized |
| U | - | Unvernallized |
| V | - | Vernalized |
| S | - | Short day conditions |
| L | - | Long day conditions |

(The standard errors are not shown since the sample sizes were small.)

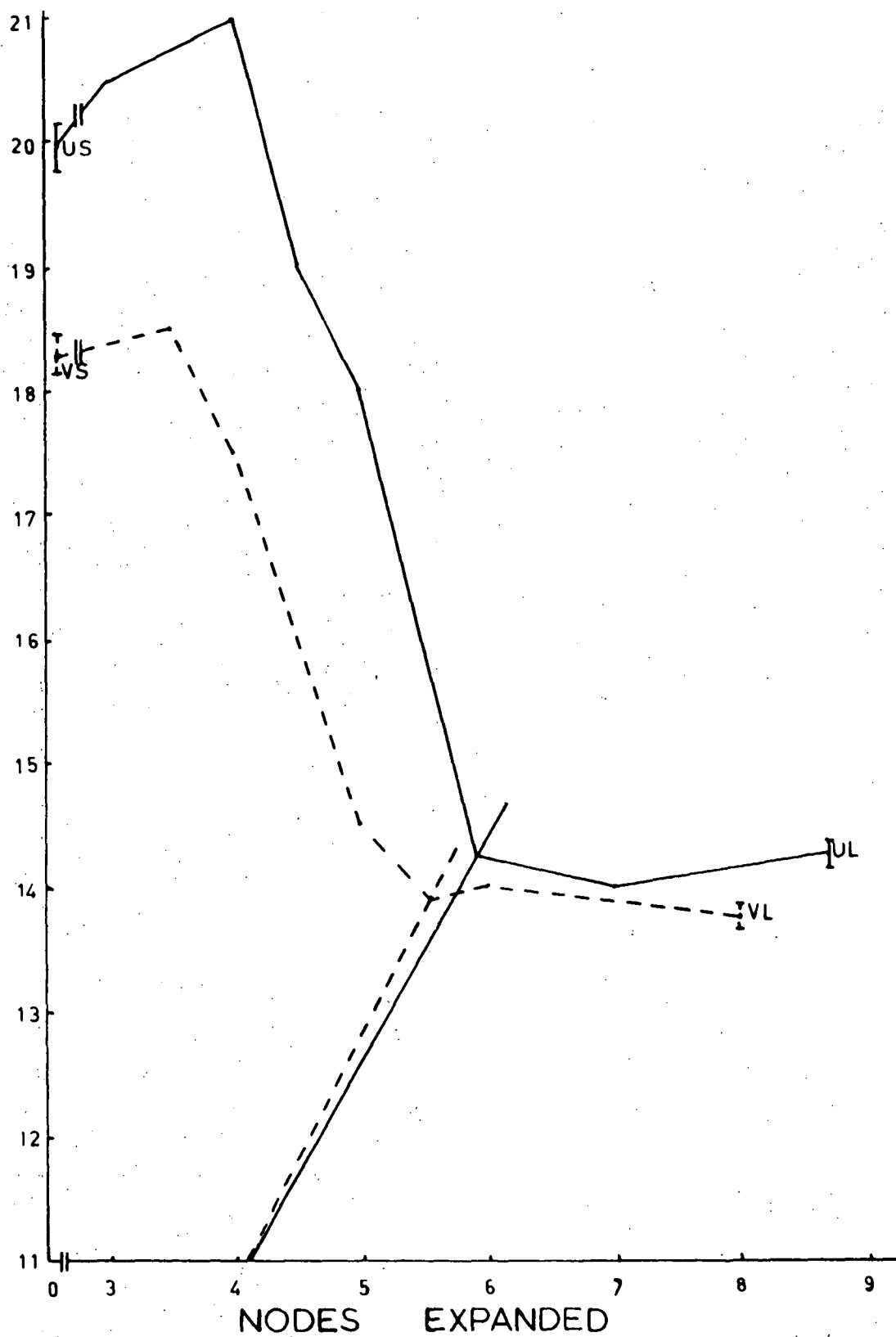
TOTAL
NODESL.D./S.D. TRANSFER
-1 cotyledon

FIGURE 7.9
THE EFFECT OF L.D. → S.D. TRANSFER AND VERNALIZATION
ON NF IN FULLY EXCISED LINE 24 PLANTS
(SUMMER CROP)

The stage of development at which plants were transferred is shown by NE.

The growth pattern for fully excised unvernmalized and vernalized plants under L.D. conditions is also shown.

| | | |
|-------|---|----------------------|
| _____ | - | Unvernmalized |
| ----- | - | Vernalized |
| U | - | Unvernmalized |
| V | - | Vernalized |
| S | - | Short day conditions |
| L | - | Long day conditions |

(The standard errors are not shown since the sample sizes were small.)

TOTAL
NODES

L.D./S.D. TRANSFER
- 2 cotyledons

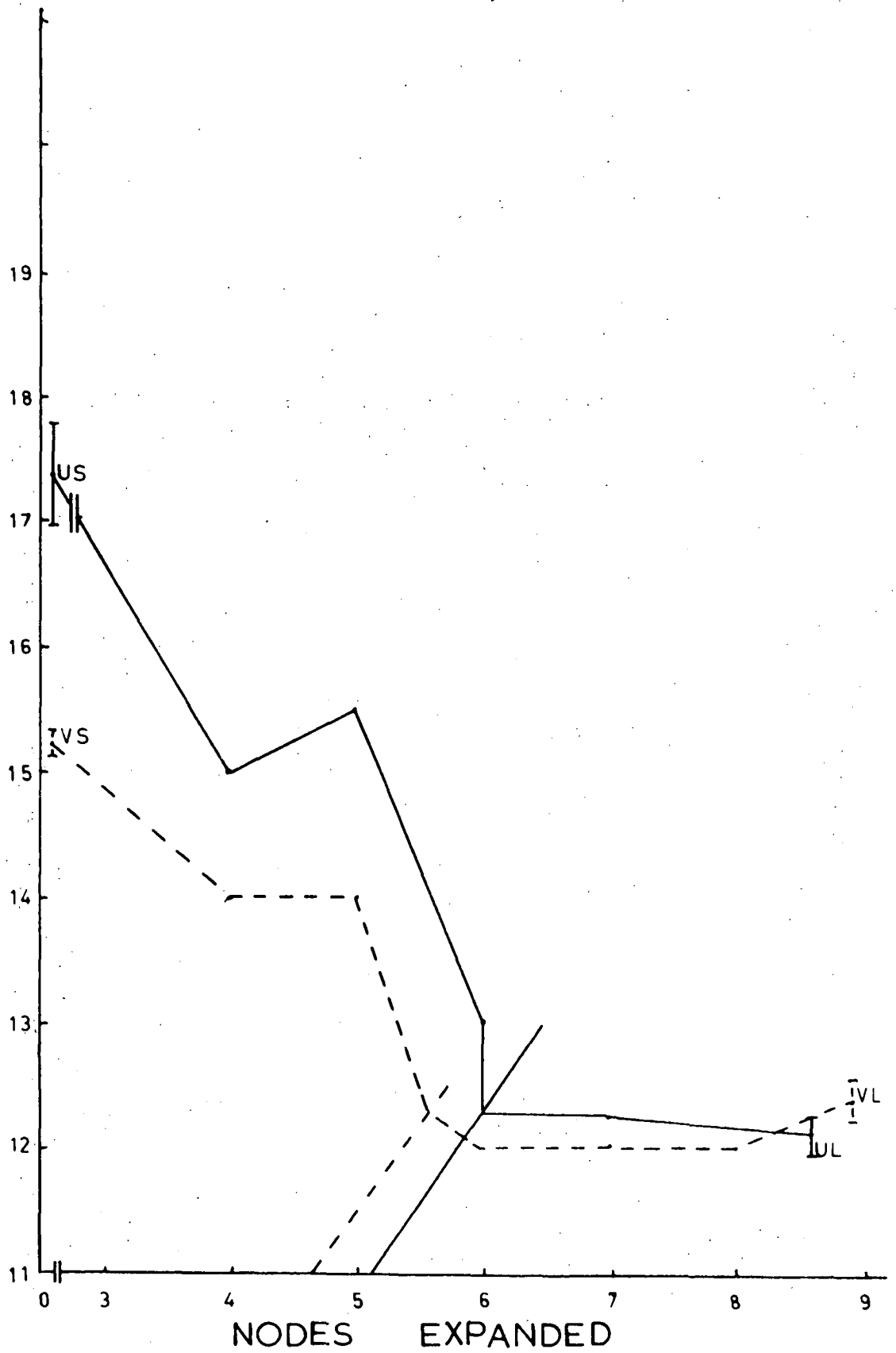


FIGURE 7.10

THE EFFECT OF COTYLEDON REMOVAL AND PHOTOPERIOD
ON NF IN UNVERNALIZED LINE 24 PLANTS
(SUMMER CROP)

The relevant section of the appropriate growth pattern curve is also shown.

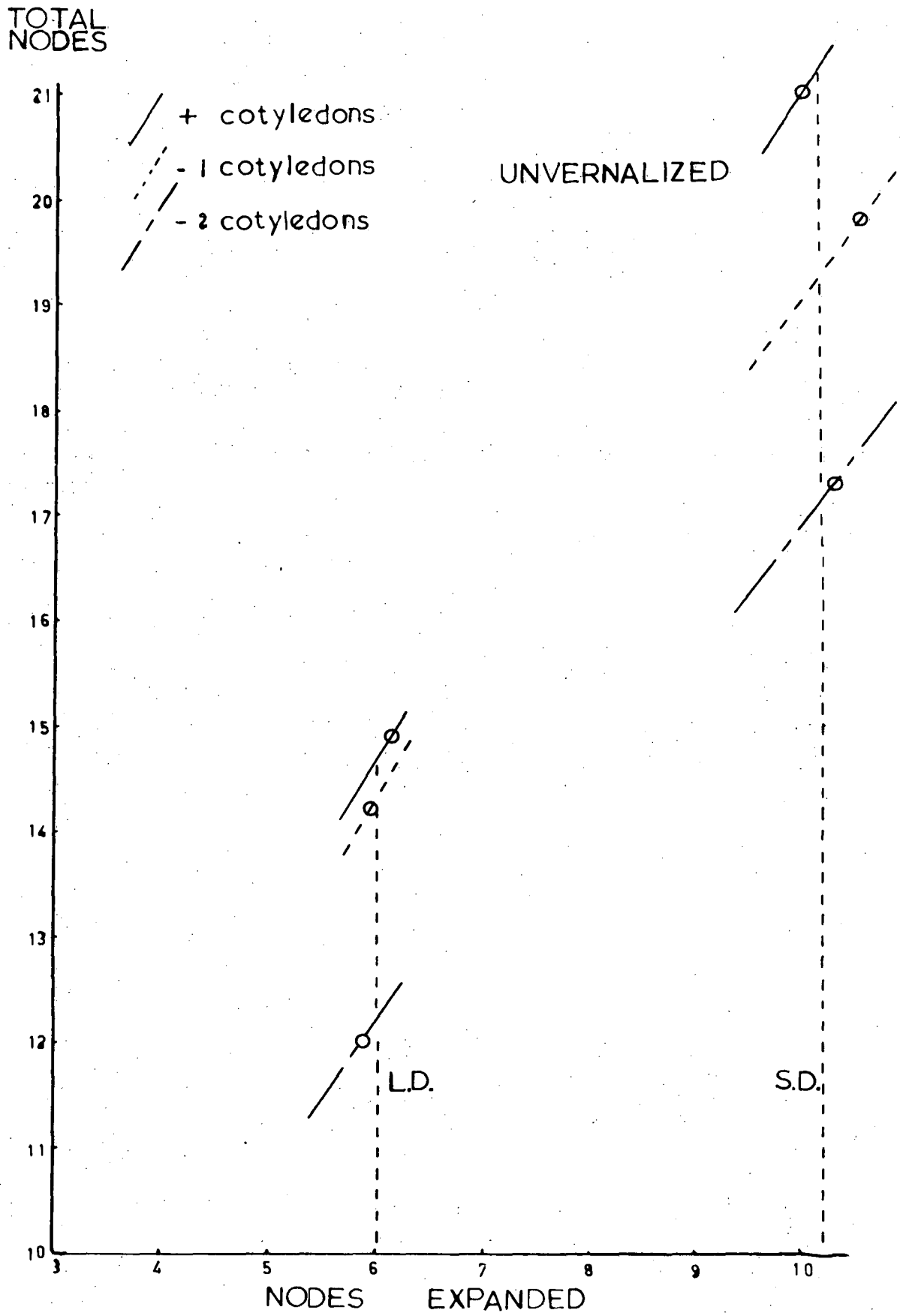
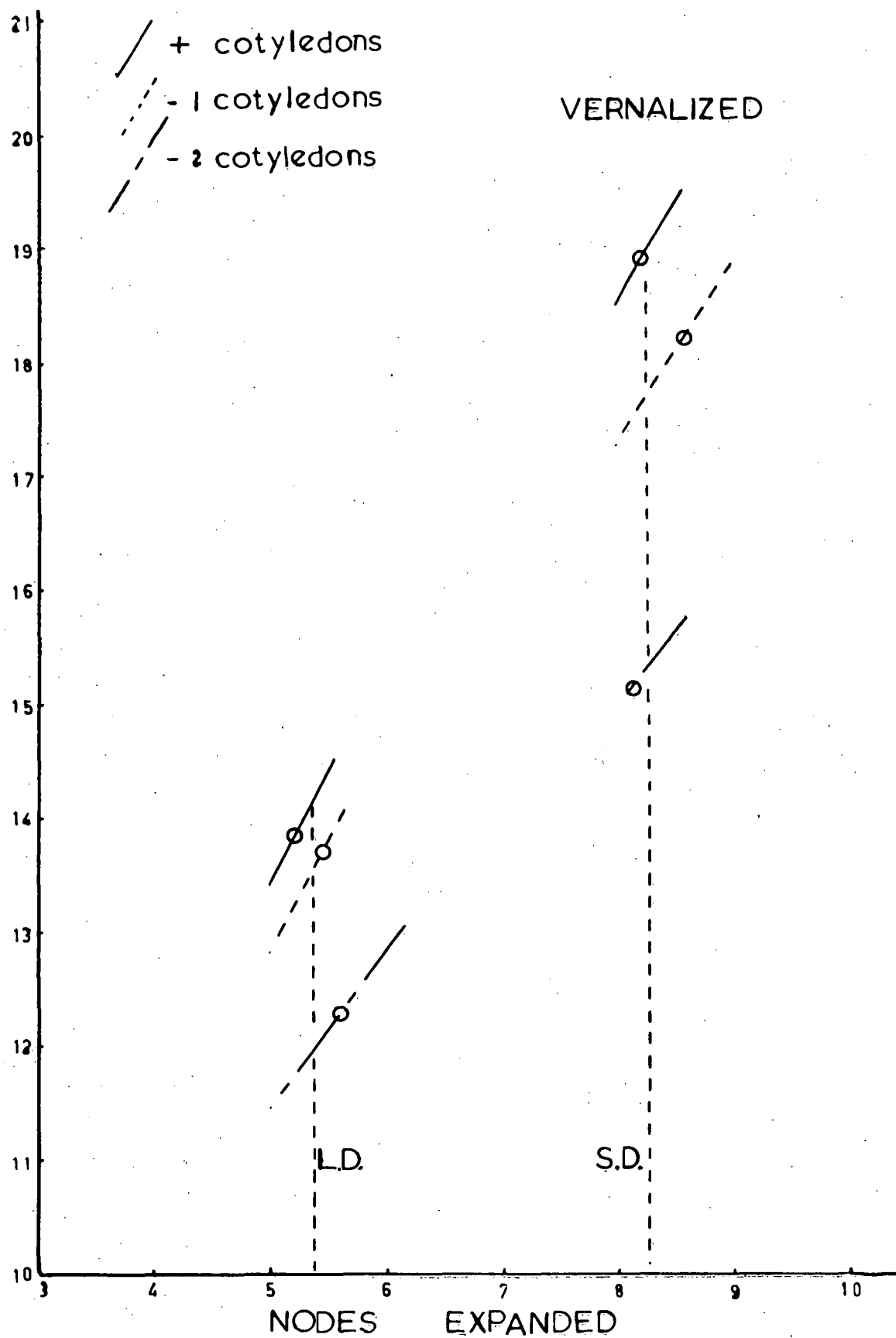


FIGURE 7.11THE EFFECT OF COTYLEDON REMOVAL AND PHOTOPERIOD
ON NF IN VERNALIZED LINE 24 PLANTS
(SUMMER CROP)

The relevant section of the appropriate growth pattern curve is also shown.

TOTAL
NODES

SUMMARY

1. In this thesis, the role of a number of variables affecting the flowering behaviour in Pisum sativum have been examined. In particular, discussion has centred around the role of the cotyledons, vernalization and photoperiod on the flowering of a late variety - Line 24, derived from the commercial variety "Greenfeast" - and a comparison of that line with other lines of known genotype.
2. It is suggested that an accurate method of determining the true physiological age of a plant is to measure its growth pattern. The growth pattern is defined as the ratio total nodes : expanded nodes, and has been measured for Line 24 plants. It was found that the growth pattern has a direct effect on the flowering behaviour of Line 24 plants, as measured by NF, and examination of this effect has shown that flower initiation is dependent on a minimum leaf requirement.
3. All cultivars of Pisum sativum have the ability to produce a floral stimulus in the green tissue of the plant.
4. Photoperiod has a direct effect on the flowering process by causing a differential rate of production of stimulus in the green tissue. It is a continuing process which commences at the time of opening of the first true leaf. Photoperiod does not affect the growth pattern. Line 24 plants will flower under S.D. conditions once the leaf at node 10 has expanded, and under L.D. conditions once the leaf at node 6 has expanded.
5. The major effect of vernalization is to directly encourage the activity of the photoperiod stimulus. It reduces the minimum leaf requirement under either photoperiod. It is a direct effect on

the flowering process, it requires only a short period of time to register a full effect, and does not affect the growth pattern. Vernalization reduces the minimum leaf requirement to 8 under S.D. conditions and 5 under L.D. conditions.

Vernalization also reduces the effectiveness of the cotyledonary inhibitor system, but requires a longer period of exposure to register a maximum effect.

6. Cotyledon removal only affects the flowering process indirectly. Its main effect is to displace the growth pattern such that the number of unopen nodes at the apex is reduced.
7. It is suggested that the grafting effect may be similar to that of cotyledon removal, especially under L.D. conditions.
8. The effects of nutrient supply, light intensity and growing temperature may produce a small displacement in the growth pattern.
9. Gene "Sn", which confers the ability of a plant to respond to photoperiod and vernalization, produces a floral inhibitor in both the shoot and the cotyledons. The recessive "sn" gene behaves as a leaky mutant in the shoot. The inhibitor establishes a threshold level at the apex which must be overcome by the floral stimulus before flower initiation and development can occur.
10. Gene "E" greatly reduces the effectiveness of the cotyledonary inhibitor, perhaps by the production of a cotyledonary flower promoter, and further may delay initial production of the inhibitor in the stem.
11. Gene "Lf" both represses the activity of gene "E" and encourages the activity of gene "Sn" in the shoot.

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EFFECTS OF VERNALIZATION, PHOTOPERIOD, AND THE COTYLEDON ON FLOWER INITIATION IN GREENFEAST PEAS

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Summary

Estimates have been made of the quantitative contribution of each of the determinant factors, photoperiod, vernalization, and colysanthin (a presumed inhibitor of flower initiation formed in the cotyledon), in regulating flower initiation in the late-flowering pea cultivar Greenfeast.

Photoperiod appears to be quantitatively related to the production of an inductive stimulus. This stimulus reaches its threshold level at about node 12 under 18-hr photoperiods, but not until node 18 under an 8-hr photoperiod.

Colysanthin delays events between photoperiodic induction and flower initiation (evocation), and causes a slightly greater delay to flower initiation in short than in long days (3 and 2 nodes respectively).

Vernalization appears to have two separate effects, both of which promote flower initiation at an earlier node. The smaller effect is manifest on the cotyledonary inhibitor system, and probably results from a reduction of the effective level of colysanthin. The major effect does not appear to involve colysanthin, but is manifest on the young embryo and is effective before photoperiodic induction is completed. The embryo response to vernalization results in advanced flower initiation of some 4 nodes in long days and nearly 6 nodes in short days. This effect may be partially obscured by colysanthin, unless the cotyledons are excised soon after vernalization is completed.

The evidence favours the view that the three determinant factors act in a complementary manner, rather than competitively, to regulate flower initiation in Greenfeast.

I. INTRODUCTION

Greenfeast, a late-flowering cultivar of *Pisum sativum* L., behaves as a quantitative long-day plant [node to first flower (NF) = 17 under an 18-hr photoperiod (P₁₈); NF = 24 under an 8-hr photoperiod (P₈)]. Significant advancement of NF can be brought about by grafting Greenfeast scions onto stocks of early-flowering varieties [e.g. Massey (Paton 1956)], by vernalization (Barber *et al.* 1958), or by cotyledon excision during early stages of germination (Johnston and Crowden 1967).

Paton and Barber (1955) proposed a mechanism based on a mobile inhibitor produced in the cotyledons of late-flowering varieties, to account for the grafting behaviour, and this idea is well supported by cotyledon-removal experiments. Barber (1959) introduced the name "colysanthin" for this inhibitor, and suggested that flowering in late varieties occurred when colysanthin was destroyed. Moore (1964) has proposed that vernalization and cotyledon excision in peas may have a common basis, and Paton (1956) concluded from grafting experiments that vernalized stocks of Greenfeast contained less inhibitor than unvernallized stocks.

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An alternative proposal is due to Haupt (1952), who suggested that cotyledons of at least one early-flowering variety, *Kleine-Rhinelanderin*, produce a flower-stimulatory substance which is graft transmissible. This florigenic substance is thought to be absent from late-flowering varieties, or alternatively its formation is blocked by an inhibitor (possibly a colysanthin) which is produced in the cotyledons of these plants (Haupt 1969).

To date unambiguous experimental verification of either hypothesis has not been made, and all attempts at definitive isolation of a florigenic substance or of a colysanthin have been unsuccessful.

In these present experiments, an attempt has been made to determine more precisely the relationship between the cotyledon system, photoperiod, and vernalization in regulating NF in *Greenfeast* peas, and in particular to show possible independent or interacting effects.

II. MATERIALS AND METHODS

Seeds used in these experiments were obtained commercially in Hobart, in a single batch. Before any treatments were commenced, seeds were selected so that their testae were free from cracks or obvious infections, and were surface-sterilized by dusting with Thiram-80. Seeds were planted in a mixture of moist vermiculite-small dolerite chips (1:1), contained in 5-lb fruit pulp tins, five seeds per tin. The plants were grown in a glasshouse, under controlled photoperiods of either 8 or 18 hr. Illumination in both photoperiods was provided by natural daylight, supplemented and extended as required by mixed banks of fluorescent and incandescent lamps. Plants were supplied twice weekly with Hoagland's complete nutrient solution (one-quarter strength) and watered as required.

Seeds to be vernalized were planted in tins as above, and placed in a room at 3°C for periods of up to 4 weeks. Excision and culture of embryos was carried out as described by Johnston and Crowden (1967). When embryos were to be vernalized, they were planted onto sloped agar in tubes to afford good illumination during the vernalization period. In these cases photoperiod was provided by artificial light only.

The technique used for grafting was as described previously by Paton and Barber (1955). For grafting vernalized plants, seeds were planted 3 in. deep in moist vermiculite and given 4 weeks vernalization at 3°C. This deep planting encouraged extension of the epicotyl and facilitated the grafting procedure. When mixed grafts were performed, i.e. vernalized with unvernallized partners, seeds for the unvernallized material were planted 4 days before the due completion of the vernalization treatment. This ensured that both graft partners were at a comparable stage of development as determined by apical dissection. The grafts were made at the stage of opening of the plumular hook, when the epicotyl was approximately 1 in. long (about 6-8 days for unvernallized plants).

To allow for statistical treatment of the data, the experiments were planned as randomized-block experiments with four replications in each treatment. A minimum of 20 plants was involved in each treatment. For the scoring of NF, all plants were grown to anthesis, and the node at which the first flower (or aborted rudiment) appeared was recorded, taking the cotyledonary node as zero. Values for means, standard errors, and numbers of plants scored for the various treatments are quoted in the tables. Average rates of node formation for plants in various treatments were determined by dissection of groups of 10 plants at intervals throughout the growing period. Experiments were conducted throughout the year under controlled photoperiod conditions, yet there is evidence of variations in NF due to seasonal (but not photoperiodic) differences. These variations are possibly related to seasonal variations of the night temperatures but this point has not been investigated thoroughly. Control of temperature in our glasshouse is not absolute, and whereas reasonably uniform day temperatures can be maintained, it is not uncommon for the night temperatures, particularly in winter, to fall to about 12°C. Since all plants in any one experiment were grown under comparable conditions with adequate randomization, it is assumed

that there is no significant effect of this phenomenon within individual experiments. All results are recorded showing the season in which the plants were grown.

III. RESULTS

Factors affecting the determination of NF in Greenfeast were investigated in a series of experiments involving cotyledon removal, grafting, vernalization, and photoperiod in various combinations of treatments.

Table 1 shows the effects of cotyledon removal and vernalization treatments on flower initiation under 18-hr and 8-hr photoperiods. It can be seen that vernalization and cotyledon excision led to advancement of NF in both photoperiods, and that the two treatments supplemented one another in effect, the maximum advancement of

TABLE 1
EFFECT OF COTYLEDON EXCISION, VERNALIZATION, AND PHOTOPERIOD ON NODE TO FIRST FLOWER IN GREENFEAST (WINTER CROP)

| Photo-period | Treatment | Node to First Flower (no vernalization) | No. of Plants | Node to First Flower (4 weeks vernalization) | No. of Plants |
|--------------|-------------------------|--|------------------|---|------------------|
| 18 hr | Cotyledons intact | 16.90 ± 0.21 | 20 | 14.42 ± 0.16 | 19 |
| | Cotyledons removed | 14.94 ± 0.15 | 18 | 12.50 ± 0.22 | 6 |
| 8 hr | Cotyledons intact | 24.35 ± 0.21 | 20 | 20.11 ± 0.21 | 18 |
| | Cotyledons removed | 21.43 ± 0.25 | 14 | 18.57 ± 0.20 | 7 |
| 18 hr* | Both cotyledons intact | 15.97 ± 0.14 | 29 | 13.73 ± 0.12 | 26 |
| | Right cotyledon removed | 14.85 ± 0.13 | 26 | 13.04 ± 0.04 | 26 |
| | Left cotyledon removed | 14.81 ± 0.12 | 27 | 12.97 ± 0.12 | 29 |
| | Both cotyledons removed | 13.80 ± 0.17 | 10 | 11.90 ± 0.10 | 10 |
| | | | | | |

* Summer crop.

NF being achieved when both treatments were given. Under long days, removal of both cotyledons advanced flowering by 2 nodes for vernalized as well as unvernallized plants, whilst the vernalization effect was to advance NF by 2.5 nodes for both intact and decotyledonized plants. In contrast, when plants were grown in short days cotyledon removal had a much greater effect in unvernallized than in vernalized plants (2.9 and 1.6 nodes respectively), and the vernalization treatment was more effective in intact than in decotyledonized plants (4.2 and 2.9 nodes respectively). Removal of one cotyledon gave an intermediate level of effect in both vernalized and unvernallized plants.

Rates of node formation for control, vernalized, and decotyledonized plants under long photoperiod are shown in Figure 1. For unvernallized plants the average rates of node formation to the time of flower initiation were 0.47 nodes/day (cotyledons intact), and 0.26 nodes/day (cotyledons removed). For vernalized plants the average rates of node formation in the post-vernalization interval were 0.51 and 0.27 nodes/day respectively. The apparently slower rate for non-vernalized plants reflects the lag of 2-3 days following imbibition before any new node formation

becomes evident. In contrast, vernalized plants show no such lag in the immediate post-vernalization period, and, in fact, have laid down one additional node during the 4-week period of the vernalization treatment. If the average rate for control plants is estimated from day 2 onwards, a rate equivalent to that for vernalized plants is obtained (0.51 nodes/day). Similar results to those shown in Figure 1, obtained in a separate experiment, are summarized in Table 3.

An equivalent rate of node formation for both vernalized and unvernallized plants is also reported by Paton (1969). The slightly higher rate in Paton's experiments (0.67 nodes/day) is probably related to a higher and constant ambient temperature during the main growing period. That the plastochron interval should also be similar for vernalized and unvernallized plants after cotyledon removal indicates that the effect of seed vernalization on the flowering response of this plant is not manifest

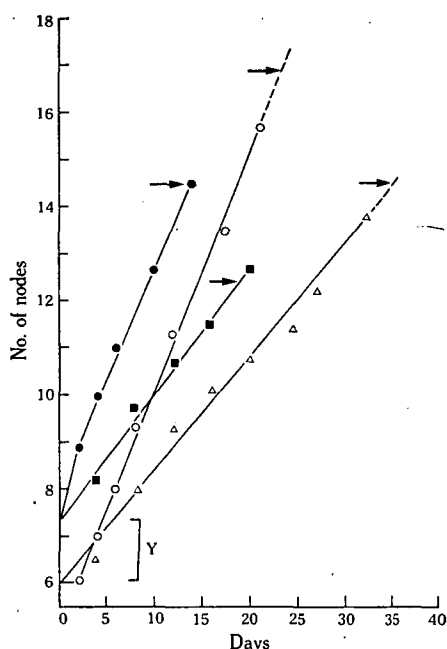


Fig. 1.—Rates of node formation in control, vernalized, and decotyledonized Greenfeast plants under an 18-hr photoperiod (summer crop). The data were obtained in an experiment parallel to that recorded in Table 1. Arrows indicate the node of initiation of the first flower. Y = nodes formed during the vernalization treatment.
 ○ Cotyledons intact, no vernalization.
 △ Cotyledons removed, no vernalization.
 ● Cotyledons intact, 4 weeks vernalization.
 ■ Cotyledons removed, 4 weeks vernalization.

through any alteration in the rate of leaf formation. On the other hand, there is a clear correlation between the retarded rate of node formation in decotyledonized plants and the initiation of flower primordia at an earlier node.

To investigate the effect of the time of vernalization and cotyledon-removal treatments on NF, plants were germinated for varying intervals before vernalization treatments were begun, and cotyledons were excised from different groups of plants immediately before or after vernalization. Treatments were staggered to allow all plants to commence post-vernalization growth concurrently. Because of limited facilities in the vernalization room, this experiment was conducted under long-day conditions only. The results are given in Table 2. It can be seen that, irrespective of whether the cotyledons were present or not, the response to vernalization decreased as germination and growth of the plants progressed. In fact vernalization had no

TABLE 2

EFFECT OF TIME OF COTYLEDON-REMOVAL TREATMENTS AND VERNALIZATION ON NODE TO FIRST FLOWER IN GREENFEAST (WINTER CROP)
 18-hr photoperiod only was used. n , number of plants scored; N_1 , number of nodes at commencement of vernalization treatment; N_2 , number of nodes at conclusion of vernalization treatment

| Days from Sowing to Commencement of Vernalization | Length of Vernalization Treatment (weeks) | Cotyledons Intact | | | | Cotyledons Removed before Vernalization | | | | Cotyledons Removed after Vernalization | | | |
|--|--|-------------------|------------------|-------|-------|--|------------------|-------|-------|---|------------------|-------|-------|
| | | n | NF | N_1 | N_2 | n | NF | N_1 | N_2 | n | NF | N_1 | N_2 |
| 0 | 0 | 18 | 16.78 ± 0.21 | 6.0 | — | 23 | 14.84 ± 0.14 | 6.0 | — | 10 | 12.80 ± 0.20 | 6.0 | 7.0 |
| | 4 | 18 | 14.39 ± 0.12 | 6.0 | 7.0 | 20 | 12.28 ± 0.12 | 6.0 | — | | | | |
| 6 | 0 | 20 | 15.60 ± 0.15 | 8.8 | 11.0 | 17 | 15.76 ± 0.25 | 8.7 | — | 18 | 14.39 ± 0.12 | 9.5 | 11.4 |
| | 4 | | | | | 13 | 13.92 ± 0.08 | 8.8 | 9.0 | | | | |
| 10 | 0 | 19 | 15.47 ± 0.16 | 11.2 | 12.5 | 19 | 15.37 ± 0.14 | 11.2 | — | 18 | 15.44 ± 0.16 | 11.6 | 14.5 |
| | 4 | | | | | 18 | 14.22 ± 0.10 | 11.2 | 11.8 | | | | |
| 14 | 0 | 20 | 16.50 ± 0.15 | 13.1 | 15.4 | 19 | 16.37 ± 0.19 | 13.2 | — | 18 | 15.94 ± 0.19 | 13.2 | 15.5 |
| | 4 | | | | | 20 | 15.90 ± 0.16 | 13.1 | 14.7 | | | | |

TABLE 3

EFFECTS OF VERNALIZATION OF EXCISED EMBRYOS ON NODE TO FIRST FLOWER OF GREENFEAST (SUMMER CROP)

18-hr photoperiod only was used. *n*, number of plants scored; N/D, rate of node formation (nodes/day) from end of vernalization treatment to time of initiation of first visible flower primordium

| Length of Vernalization Treatment* (weeks) | Cotyledon Status | Length of Vernalization Treatments after Cotyledons Removed | | | | | | | | | | |
|--|------------------|---|------------|------|----------|------------|----------|------------|----------|------------|----------|------------|
| | | 0 Weeks | | | 1 Week | | 2 Weeks | | 3 Weeks | | 4 Weeks | |
| | | <i>n</i> | NF | N/D | <i>n</i> | NF | <i>n</i> | NF | <i>n</i> | NF | <i>n</i> | NF |
| 0 | Intact | 20 | 15.40±0.15 | 0.46 | | | | | | | | |
| | Excised | 23 | 13.48±0.14 | 0.24 | 24 | 12.67±0.12 | 27 | 11.76±0.09 | 7 | 12.14±0.14 | 16 | 12.06±0.11 |
| 1 | Intact | 19 | 15.50±0.12 | 0.45 | | | | | | | | |
| | Excised | 29 | 12.48±0.15 | 0.23 | 30 | 12.33±0.12 | 27 | 12.22±0.11 | 27 | 12.44±0.12 | | |
| 2 | Intact | 20 | 14.60±0.15 | 0.51 | | | | | | | | |
| | Excised | 24 | 12.17±0.10 | 0.27 | 17 | 11.53±0.24 | 26 | 11.92±0.13 | | | | |
| 3 | Intact | 20 | 13.50±0.11 | 0.51 | | | | | | | | |
| | Excised | 24 | 12.33±0.12 | 0.25 | 26 | 12.58±0.11 | | | | | | |
| 4 | Intact | 19 | 13.00±0.09 | 0.50 | | | | | | | | |
| | Excised | 24 | 12.33±0.10 | 0.26 | | | | | | | | |

* Preceding cotyledon excision.

significant effect on the NF of plants which had already reached the node-12-13 stage of development (between 10 and 14 days after germination). Cotyledon removal at 14 days was without effect on unvernallized plants, but an effect of marginal significance ($P = 0.05$) was still apparent with vernalized plants. Removal of cotyledons from growing plants before giving the vernalization treatment resulted in greater advancement of NF than did post-vernalization excision.

The decreasing effect of vernalization on NF does not appear to be correlated to the change in rate of node formation of the plants during the vernalization period. For intact plants, the rates of node formation during vernalization at 6, 10, and 14 days after germination were nearly the same in all cases (approximately 0.5 nodes/week). When cotyledons were removed before vernalization, the number of nodes formed per week in the embryos during vernalization increased sharply from 0.05 at 6 days to 0.15 at 10 days and 0.5 at 14 days, whereas the effect of vernalization on NF in these groups of plants showed an almost linear decline.

In a further experiment, embryos were dissected from imbibed seeds which had been vernalized for varying periods up to 4 weeks. Some of these embryos were then given extended vernalization treatments, up to a total of 4 weeks, in isolation from the cotyledon influence. This experiment also was conducted under long days only. The results are shown in Table 3. The data show that 1 week of vernalization was sufficient to obtain nearly maximum advancement of NF, provided the cotyledons were excised before the plants were allowed to grow under normal temperatures. It did not matter whether cotyledons were present or not during the vernalization interval. On the other hand, when cotyledons remained intact during the post-vernalization period of growth, there was only progressive advancement of NF as the vernalization treatment was extended to the full 4 weeks.

Two grafting experiments were conducted. In the first experiment, vernalized and unvernallized scions of Greenfeast (GV and GU respectively) were grafted to both vernalized and unvernallized Greenfeast stocks. The results are shown in Table 4. For plants grown in a long photoperiod it is seen that grafting vernalized scions, either GV/GU or GV/GV (scion/stock), promoted flowering at an earlier node than did GV controls. The NF of these vernalized scions is in fact comparable with that resulting from the dual treatments of cotyledon removal plus seed vernalization shown in Tables 1, 2, and 3. This is in sharp contrast to the performance of vernalized scions in short days, where no effect of grafting was evident. Thus plants from each of the treatments GV, GV/GU, and GV/GV have almost identical NF values. Moreover, this value of NF (approximately 20.5) is some 2 nodes higher than that for vernalized, decotyledonized plants grown under short days (Table 1). Similarly, with unvernallized scions (GU, GU/GU, and GU/GV), a significant effect of grafting is evident only when plants are grown under long-day conditions. Thus it would seem that the effect of grafting in Greenfeast is nullified during the prolonged interval of vegetative growth which precedes flower initiation in short days.

In the second grafting experiment, an early flowering variety, Massey, was used as stock. Grafts on Massey stocks grown under short days were not always successful, and scion mortality was high. However, survival was satisfactory under long-day conditions. In all cases grafting to Massey stocks promoted flowering at an earlier node than did comparable grafts to Greenfeast stocks. More significant

perhaps is the observation that vernalized Greenfeast scions grafted to Massey stocks flowered out of the same nodes as vernalized, decotyledonized plants (Table 1) in both photoperiods, in marked contrast to the behaviour of GV/GU and GV/GV grafts.

TABLE 4
EFFECT OF VERNALIZATION, GRAFTING, AND PHOTOPERIOD ON NODE TO
FIRST FLOWER IN GREENFEAST (WINTER CROP)

| Graft Type (scion/stock)* | 8-hr Photoperiod | | 18-hr Photoperiod | |
|------------------------------|------------------|----|-------------------|----|
| | NF | n† | NF | n† |
| GU (control) | 23.35±0.30 | 20 | 16.90±0.18 | 20 |
| GV (control) | 20.50±0.21 | 20 | 14.53±0.12 | 19 |
| GU/GU | 23.13±0.23 | 8 | 15.60±0.21 | 18 |
| GU/GV | 22.71±0.29 | 14 | 14.17±0.19 | 18 |
| GV/GU | 20.67±0.20 | 18 | 12.79±0.14 | 19 |
| GV/GV | 20.42±0.20 | 24 | 12.90±0.17 | 20 |
| GU/MU | 19.86±0.54 | 7 | 13.88±0.09 | 8 |
| GU/MV | 20.71±0.47 | 7 | 13.37±0.22 | 19 |
| GV/MU | 18.10±0.52 | 10 | 12.67±0.14 | 12 |
| GV/MV | 19.00±0.68 | 7 | 12.67±0.14 | 18 |

* G = Greenfeast; M = Massey; U = unvernallized; V = vernalized.

† Number of plants scored.

IV. DISCUSSION

Most plants undergo a period of vegetative development before reaching the ripeness-to-flower condition, whereupon they may produce reproductive structures. The ripeness-to-flower condition is probably an absolute expression of a plant's genetic constitution, but the subsequent realization of this genetic potential may require an appropriate combination of environmental conditions. There is good evidence in these present experiments that in Greenfeast peas the minimum node at which the initiation of flower primordia may occur is about node 12. (We have scored only a very small number of NF-11 plants, less than 5% of the total, following treatments which promote the maximum advancement of NF.) Since there are usually 6 nodes already present in the dormant embryo, then the attainment of the minimum node number for flower initiation (which may well coincide with ripeness-to-flower for this plant) involves vegetative development of a further 6 nodes after the commencement of germination. However, under normal growing conditions the observed NF for this plant is delayed beyond node 12. Quantitative reduction of this delay may be brought about by treatments such as cotyledon removal, vernalization, or long photoperiod given independently or in combination.

Other workers (Paton and Barber 1955; Barber 1959; Paton 1969) have proposed that this delay to flower initiation can be largely explained in terms of a graft-transmissible inhibitor produced in the cotyledons of Greenfeast (and probably other late-flowering varieties as well). The data in these present experiments is consistent with this view that flowering in Greenfeast is regulated, at least in part,

by an inhibitory effect of the cotyledon. Whether the cotyledon effect is due to the presence of an inhibitor (i.e. colysanthin; Barber 1959), or to the absence or retarded formation of a florigenic substance (Haupt 1969) is not unequivocally determined, but on the evidence available we favour the former view.

Perhaps the best evidence that a colysanthin is directly involved comes from the results of grafting experiments, summarized in Table 4. Thus, considering the Greenfeast on Greenfeast grafts, it is significant that a discrete effect of grafting is seen only under a long photoperiod, when the NF of grafted scions is comparable to that of decotyledonized plants. In contrast, the NF of scions in a short photoperiod is the same as for intact plants. This difference in NF between comparable graft treatments in different photoperiods may simply reflect the length of time that is required to establish a functional graft union (presumably a phloem connection), and permit transfer of colysanthin from the cotyledons of the stock to the scion. Plants at grafting already contain 8 or 9 nodes, so that vernalized scions only need to form 4 more before flower initiation occurs. Thus, in long photoperiods events in the vernalized scion leading to flower initiation may well be completed before the graft union is adequate for regular colysanthin transport, and hence colysanthin does not attain its normal inhibitory threshold. Similarly, with unvernallized scions under long days, NF is always below that for the ungrafted control plants (first reported by Paton and Barber 1955), suggesting that the graft union is still not fully functional after about 6 or 7 nodes of growth, and the quantity of colysanthin reaching the apex is insufficient to delay initiation to the normal extent as in the ungrafted controls. In short days, 12 or more nodes of vegetative growth from the time of grafting precede the formation of the first flower primordium. By this time it is most likely that the graft union is fully established, and normal colysanthin transport has been restored.

In contrast, by using stocks of the early flowering variety, Massey, a grafting effect was apparent in both photoperiods, and the Greenfeast scions behaved in all treatments simply as decotyledonized plants. The Massey stocks contributed no effective inhibitor to the graft partner.

Apart from the physiological property of causing delayed flower initiation there is little additional evidence available concerning the nature of colysanthin. Paton (1969) has commented that it has some properties characteristic of abscissic acid, with possibly a variety of physiological effects. In these present experiments we have shown a significant correlation between removal of colysanthin (by cotyledon excision) and the effects of this treatment on flower initiation and rate of node formation (Fig. 1), but the mechanism of this relationship is not at all clear.

In Barber's hypothesis (1959), vernalization and long photoperiod both act in a competitive fashion to destroy colysanthin. However, Johnston and Crowden (1967) reported that photoperiod and cotyledon removal appeared to be additive in their effect, and Paton (1969) has recently shown physiological separation of the photoperiod and vernalization effects. The degree of interaction of these three factors in regulating NF in Greenfeast is shown in the variance analysis of the data in Table 1. Thus the interaction between cotyledon removal and vernalization is highly significant ($P < 0.001$). However, the interaction of photoperiod with both cotyledon removal and vernalization is comparatively weak ($0.02 < P < 0.05$ in each case), indicating that photoperiod is relatively independent of these other treatments in its effects.

Paton (1969) has proposed that in Greenfeast photoperiod has a quantitative effect, which is directly concerned with the attainment of the minimum leaf requirement for flowering (i.e. induction), and the production within the leaves of an inductive stimulus. This stimulus passes from the leaves to the stem apex where flower initiation takes place. Vernalization, on the other hand, influences those reactions at the stem apex which follow induction and culminate in the initiation of flower primordia (i.e. evocation, Knox and Evans 1968). From Paton's data (1969), the processes of evocation occupy about 3 plastochron intervals, or less following seed vernalization treatment. It can be estimated also that photoinduction is completed in Greenfeast (under continuous light) by about node 13 or 14.

In these present experiments, it can be seen that treatments which promote maximum advancement of NF, i.e. both vernalization and cotyledon removal, allow flower initiation to occur as early as node 12 (mean 12.5) under an 18-hr photoperiod. Thus, under these conditions at least, the inductive stimulus has reached its effective threshold by about the node-11-12 stage of development. In short photoperiods (8 hr), this threshold is not reached until about 18 nodes are produced ($NF = 18.57$ for vernalized, decotyledonized plants; Table 1). This difference of 6 nodes is regarded as an expression of the quantitative difference in photoperiodic induction between 18- and 8-hr photoperiods for this variety. If it may be assumed that photoperiodic induction occurs at the same minimum leaf number in intact as in decotyledonized plants, then the duration of the evocation processes in this plant is extended from 3 plastochron intervals, as suggested by Paton's data (1969), to about 5 (4.40 in P_{18} and 5.78 in P_8).

Since flowering at node 12 has been observed in these present experiments, it appears that, provided seed vernalization has been performed, the induced apex can proceed immediately to floral initiation, in both photoperiods, and this will occur in the absence of cotyledons. However, should cotyledons remain attached to the growing plant after vernalization then flower initiation is delayed. The magnitude of the delay is about 2 nodes in both photoperiods (2 nodes in P_{18} , and slightly less, 1.6 nodes, in P_8). If seed vernalization was not given, flower initiation is delayed still further, by some 2.5 nodes in P_{18} and about 4 nodes in P_8 .

Both Barber (1959) and Paton (1969) have implied that the response to vernalization can be interpreted in terms of a direct effect of vernalization on the cotyledon inhibitor, either by destruction (Barber 1959) or by reduced synthesis (Paton 1969). In contrast, the present experiments show that any effect of vernalization on the cotyledon system is significantly less than the maximum vernalization response that can be realized. Thus removal of cotyledons from vernalized plants, e.g. Table 1, advanced NF by 4.5 nodes in P_{18} and nearly 6 nodes in P_8 compared with the unvernallized controls. However, when the cotyledons were left attached to vernalized plants, apparent vernalization responses of 2.5 and 4 nodes in P_{18} and P_8 respectively were obtained. These differences in NF between intact and decotyledonized plants after vernalization could result from colysanthin which had moved into the shoot of the intact plant during the post-vernalization period of growth. The quantitative nature of the colysanthin effect is shown (Table 1) by removal of single cotyledons, when values of NF intermediate between intact and fully decotyledonized plants were obtained. In Table 3, it is seen that the maximum

vernalization effect was achieved only if cotyledons were removed before post-vernalization growth at normal temperature was allowed to take place.

These results suggest that a major effect of vernalization is manifest directly on the embryo itself. Since under normal growing conditions the NF of vernalized plants with intact cotyledons does not regain the original value for unvernallized controls, it seems that the embryo vernalization effect is not readily reversible, but that it may be partially obscured when cotyledons are left attached to the growing plant after vernalization. Table 3 also shows that this effect of vernalization on the embryo requires only a comparatively short-term exposure to low temperature in order to yield maximum response.

There is no evidence in these present experiments to endorse Barber's proposal (1959) that vernalization leads to destruction of colysanthin at the plant apex. In all experiments where both vernalization and cotyledon treatments have been investigated simultaneously, it is evident that vernalization treatment was more effective in advancing NF than cotyledon removal alone (e.g. Table 1: 0.5 nodes in P_{18} , $0.02 < P < 0.05$; 1.5 nodes in P_8 , $P < 0.001$). Thus if colysanthin is indeed the substrate for the vernalization reaction, then it follows from Barber's hypothesis that the embryo of the imbibed seed already contains a significant quantity of the inhibitor. However, experiments involving sequential cotyledon removal (Johnston and Crowden 1967) and leaching (Sprent and Barber 1957) show that the removal of colysanthin from the cotyledons does not start until about day 4 or 5 after germination.

Further, it is clear that the period of active movement of colysanthin into the shoot (up to about day 14–15) corresponds to the period of decreasing sensitivity of the shoot to vernalization treatment (Table 2), and it may be argued that it is the presence of colysanthin at the apex which decreases or masks the effect of vernalization in young plants. In Table 2 it is also seen that cotyledon excision from growing plants at 6, 10, and 14 days after germination, prior to a vernalization treatment, was more effective in advancing NF than post-vernalization excision. Whilst it can be expected that some colysanthin had already entered the shoot during the period of germination preceding the vernalization treatment, thus providing for the progressive delay to NF in both groups of decotyledonized plants, it is apparent that, in the latter group, inhibitor movement from the cotyledons continued throughout the vernalization treatment, in company with the limited growth which took place during this period. In each of the above cases, it is implied that colysanthin present at the apex survives vernalization treatment and effectively reduces the vernalization response.

Although there is no evidence as to the precise nature of the apical vernalization reaction it appears fairly certain that it does not involve colysanthin. Rather it seems more logical to interpret the embryo response in terms of production of a positive flowering stimulus. Since photoperiodic induction in Greenfeast is completed by about node 12 (under long days), it is apparent that following seed vernalization treatment this stimulus is stable for at least 5–6 plastochron intervals and even longer (12 or more plastochron intervals) under an 8-hr photoperiod. It is apparent from the data in Table 2 that vernalization does not have a significant effect on apices which have passed the node-12 stage of development (between 10 and 14 days after germination). This implies that vernalization has an effect on the embryo only when given to plants before the time of photoperiodic induction. Thus it is possible

that apical vernalization acts in some manner to predispose the young plant to photoinductive processes, rather than be implicated at a later stage in the evocation events, as Paton (1969) has suggested. On the other hand, colysanthin appears to be more concerned with the post-inductive events, and may partially obscure the vernalization response.

There are two lines of evidence which suggest that vernalization has a direct effect on the cotyledon inhibitor system. The magnitude of this effect on the cotyledon is appreciably less than that on the embryo. Firstly, comparison of the values of NF obtained by grafting unvernallized scions to both vernalized and unvernallized stocks (14.17 and 15.60 respectively in P_{18}) shows a significant difference of 1.43 nodes ($P < 0.001$). However, NF values for the corresponding grafts under short days, although showing the same trend, are not significantly different. Secondly, there is the observation of a progressive increase in the effect which vernalization has on intact plants with lengthening exposure to cold treatment (Table 3). This effect of vernalization on the cotyledon system may reflect either a steady, low-temperature destruction of colysanthin (cf. Barber 1959), or more probably that there may be a progressive repression of the capacity to synthesize the inhibitor (cf. Paton 1969).

In either case, the net result appears to be that before the inhibitor level can be restored to the effective threshold, the minimum level of growth is achieved by the shoot for it to become photoinduced, and for flower initiation to be evoked. Unlike the effect of vernalization in dissected embryos, vernalization of the cotyledon system requires a long period of treatment (at least 4 weeks) in order to register the full effect. An apparent reversal of vernalization in peas (devernallization) at high growing temperatures has been reported (Highkin 1956; Barber 1959; Moore and Bonde 1962). The mechanism of this effect is not known but it may well be explained in terms of higher colysanthin synthesis at elevated temperatures.

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