THE TRANSITION FROM VEGETATIVE TO FLORAL DEVELOPMENT IN THE SHOOT APEX

by

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A C K N O W L E D G E M E N T S

My sincere thanks are due to Dr. Robert F. Lyndon, my supervisor, for the invaluable help and encouragement he has given me throughout this work and in the writing of this thesis.

I also owe thanks to Professor R. Brown, F.R.S., who made this work possible by accepting me as both an undergraduate and a postgraduate student in his department.

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SUMMARY

During this work a study was made of changes in the shoot apex during floral transition. A plant (Silene coeli-rosa L.) in which the shoot apex transforms directly from leaf production to floral-part production, was used. Silene is a LD plant and will flower if exposed to 4 or more LD.

It has been shown that during induction the growth rate of the apex is the same as in vegetative plants. During early flower morphogenesis however, both the cell cycle of the apical cells lessened and the proportion of dividing cells in the apex increased.

Following the start of induction the first change (apart from perhaps a slight stimulation of growth following exposure to 1LD) recorded in the apex was a rise in the concentration of RNA. If full induction took place this increased RNA concentration was maintained. Following sub-inductive LD treatment on ephemeral rise in RNA concentration took place in the apex. This suggests evocation in Silene is a stepwise phenomenon. The effects of sub-inductive LD treatment remained in Silene for up to 4 days.

The concentration of RNA in the apex rose, in the absence of any change, in growth rate, following GA_3 application suggesting the RNA rise in induced plants may result from the arrival of hormone(s) in the apex.

On transfer to cold conditions both the RNA concentration and the growth rate in the apex fell. Induction was unaffected by cold conditions and it was shown the RNA concentration and the growth rate was similar in the apices of vegetative plants at 20°C and plants producing flowers at 12°C. This suggests the maintenance of high RNA levels may be linked to the increases in growth rate which normally takes place during flower morphogenesis.

Although the overall protein concentration in the apex was shown to increase in Silene during flower morphogenesis no evidence could be found for any change over this period in the electrophoretic pattern following polyacrylamide gel electrophoresis of apical protein samples.

Publication of thesis material

Some of the material published in this thesis has been published in:-

Miller, M. B. and R. F. Lyndon, 1975. The Cell Cycle in Vegetative and floral shoot meristems measured by a double labelling technique. Planta 126, pp 37-43.

A paper entitled "Rates of Growth and Cell Division in the Shoot Apex of Silene during the Transition to Flowering" by M. B. Miller and R. F. Lyndon has been submitted to the Journal of Experimental Botany.

A paper entitled "Changes in RNA Levels in the Shoot Apex of Silene During the Transition to Flowering" by M. B. Miller and R. F. Lyndon is in preparation.

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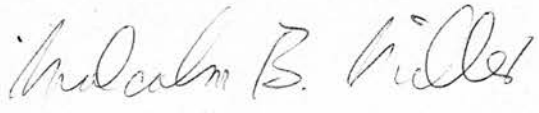
The work recorded in this thesis and the composition of this thesis is my own.



Signed

I N D E X

PART I

INTRODUCTION

Introduction.

Until about 15 years ago research on flowering in higher plants was mainly concerned with the control of induction by environmental factors, especially day-length. This early work led to the description of a bewildering array of conditions which suppress or promote flowering in different species (Lang, 1965).

Since the early 1960 s research has been more directed to the study of changes at the molecular and cellular level in the shoot apex during floral induction, evocation, and morphogenesis* of the flower. The distinction of the processes of evocation and morphogenesis is conceptually important though at the practical experimental level it is difficult to classify observations into two distinct categories. In any meaningful interpretation of changes at the apex associated with these events knowledge of the precise timing of induction and evocation is almost essential. Because of this the majority of recent investigations on flowering have depended upon the use of plants where flowering is under the absolute control of day-length.

Induction.

Bernier (1970) and J. and Y. Heslop-Harrison (1970) pointed out that the majority of flowering plants are, as far as is known, day-length neutral with respect to flowering and suggested that the concentration by investigators on day-length sensitive plants, especially those where only one 24 hour cycle of the appropriate/

* These terms are used as defined by Evans (1969): induction referring to events in the leaf which commit the plant to flower; evocation to events in the shoot apex which commit that apex to floral development; and morphogenesis referring to the process of differentiation of the flower at the apex.

appropriate day-length is required for induction, may lead to an over emphasis of the importance of the switch response. The experimental advantages of using plants where induction can be manipulated precisely, are, however, immense. As argued by Lang (1970) the very fact there is such a variation in the environmental conditions required for flowering in different species (there are, in fact, cases where totally different conditions induce different strains of a single species) suggest that though different external conditions may be required to trigger the process, the internal control at the molecular level is probably similar throughout the higher plants. In photosensitive plants evocation appears to consist of a distinct all or nothing response. A sudden and total alteration in developmental direction from vegetative to floral growth is not clearly exhibited by day-length neutral plants and it has been argued that in these plants, and also in day-length sensitive plants maintained in conditions unfavourable to flowering, there is a gradual advancement towards the reproductive state (Nougarède, 1965; J. and Y. Heslop-Harrison, 1970). However, in spite of this argument, in practically all plants, whether or not flowering can be manipulated by changes in environmental conditions, the actual beginning of floral morphogenesis, and therefore presumably also evocation, is in fact an all or nothing response for each meristem, though the reason for the developmental switch may not be obvious.

Evocation.

A picture of the sequence of events taking place in the apex from the start of inductive treatment to well into the morphogenesis phase of flower production is beginning to emerge for a number of species (Bernier, 1971). Where the timing of the arrival of the floral stimulus at the apex and the beginning of the morphogenesis of the flower is known/

known it is possible to interpret certain events as being concerned with evocation and others with morphogenesis of the flower. The data discussed in this section are those which are considered by both myself and the respective authors as being directly associated with the process of evocation at the apex. The distinction between evocation and morphogenesis becomes less and less clear as more data are considered and because of this difficulty many observations which may be associated with evocation are included in the following section headed "morphogenesis".

Gressel at al. (1967) described an ephemeral increase in the rate of synthesis of total RNA, as measured by the rate of incorporation of tritiated uridine, and also an increase in the measurable ribonuclease activity in the apical tissue of Pharbitis nil following the start of the inductive treatment but at a stage where the flowering process could be prevented by the removal of the cotyledons i.e. prior to complete evocation. This observation led these workers to conclude that the stimulus from the cotyledons was multi-staged. An increase has been recorded in the rate of incorporation of labelled precursors into RNA in the apical tissue of Sinapis alba and Lolium temulentum. In these plants, however, the rate of synthesis of RNA did not increase until the apex was irreversibly committed to flowering, (Bernier at al, 1970; Bronchart at al, 1970; Knox and Evans, 1968). In Lolium temulentum an ephemeral increase in the incorporation of ^{32}P phosphate into nucleic acid and ^{35}S sulphate into protein in the apex takes place around or shortly after the time of the commitment of the apex to flower (Knox and Evans, 1968; Evans et al, 1970). In Sinapis a very similar result was recorded following the application of tritiated uridine to plants undergoing induction. An increase in the incorporation of tritiated uridine into RNA was recorded in the Sinapis apex about/

about 18 hours after the start of inductive treatment which is when the apex becomes committed to floral development (Bernier et al, 1970; Bronchart, 1970). Leaf production does not however cease until 24 hours later (Bernier et al, 1970). It was concluded that these short-lived increases in the rate of synthesis of RNA in Lolium and Sinapis, like that in Pharbitis, were associated with the process of evocation itself rather than the subsequent development of the flower at the apex.

The application of plant growth hormones has resulted in a similar increase in the rate of synthesis of RNA in a variety of plant tissues (Trewavas, 1968). There is, for example, a doubling of RNA concentration 30 minutes after treatment of onion roots with kinetin (Jensen et al, 1964); an increase in total RNA synthesis following IAA treatment of oat coleoptiles (Hamilton et al, 1965) and a stimulation in the rate of ^{32}P incorporation into R N A in the coleoptiles of Avena by auxin (Zimmerer and Hamilton, 1965).

The similarity of the changes in the apex during evocation with established effects of plant hormones was remarked upon by Evans, (1970). There is considerable evidence that inhibitors of R N A synthesis such as actinomycin-D and 8 - azaguanine prevent hormone-mediated responses in plant tissues (Key, 1964; Mitra and Sen, 1965; Penny and Galston, 1966; Nooden and Thimann, 1966). Inhibitors of RNA synthesis have also been shown to be effective in preventing induction when present on shoot apices during inductive treatment (Collins et al, 1963; Bonner and Zeevaart, 1962; Salisbury and Bonner, 1960; Galun et al, 1964; Evans, 1964; Bernier, 1969).

As pointed out by Cherry (1970) and Lang (1970) all of these experiments with inhibitors are open to the criticism that when a growing system is preturbed by some interfering substance, even/

even when the primary effect is known to be specific, it is dangerous to draw too specific a conclusion because of possible secondary effects consequent upon the primary effect of the inhibitor.

It has been shown by Seidlová (1970) and Arzee et al (1970) that there is some ephemeral retardation of growth at the apex following the application to the apex of inhibitors of R N A synthesis. It is likely some degree of inhibition may occur at the apex following the application of all such anti-metabolites, appropriate growth rate measurements are, however, seldom made in such studies.

Morphogenesis

Subsequent to these early effects of inductive treatment upon the rates of synthesis of R N A and protein there are a number of observations on changes in the mitotic cycle. Because of differences in method and detail in different reports it is difficult to generalize on these observations. Certain consistencies are, however, apparent. Early increases in mitotic index following inductive treatment, or in the number of mitotic figures per apex have been described in Sinapis (Bernier et al, 1970), Xanthium (Thomas, 1963; Gifford, 1963), Pharbitis (Wada, 1968), and Chenopodium album (Gifford and Tepper, 1961). Whilst it is appreciated that an increase in mitotic index does not necessarily indicate a shortening of the cell cycle, where appropriate measurements have been reported there is evidence there is a decrease in the mean cell generation time in the shoot apex following evocation. Corson (1969) demonstrated there was an increase in the rate of cell division in the apical tissue of Datura stramonium during the early stages of flower morphogenisis from the rate of accumulation of metaphases following colchicine treatment. Using the same technique Bodson(1975) recorded a similar increase in growth rate in the Sinapis apex following inductive treatment.

Bernier (1972) remarked upon the possibility that the mitotic phase now established to occur in a number of species could be a mechanism, as originally suggested by Stern (1966), whereby the cells of the apex destroy their nuclear history prior to the change in morphogenetic direction from leaf production to flower production.

Where the timing of the start of morphological changes associated with flower production at the apex is known it seems the "mitotic phase" (usually an increase in the mitotic index) takes place before there is any recorded change in the shape of the apex. For example, an increase in mitotic index was recorded in Xanthium 38 hours after the start of inductive treatment (Thomas, 1963), while morphological changes in the apexwere not apparent until 64 hours after the start of inductive treatment (Arzee et al, 1970); in Pharbitis an increase in the number of mitotic figures takes place in the induced apex 40 hours after the start of the inductive long day (Wada, 1968) while the first changes in shape in the apex were recorded 50 hours later (King and Evans, 1969); in Sinapis peaks of mitotic index have been recorded around 30 and 60 hours after the start of the inductive short day (Bernier et al, 1967; Jacqmard and Mische, 1971) and since initiation of flower buds takes place 60 hours after the start of the inductive long day (Bernier, 1969) the first peak is well before the start of flower morphogenesis. There is of course difficulty, presumably experienced by all these authors, in obtaining an objective assessment of exactly when morphogenesis of the flower commences.

Inhibitors of D N A synthesis have been shown to be effective in suppressing flowering under certain conditions. In Xanthium, Sinapis, and Lolium, inhibitors of D N A synthesis only become effective in preventing flowering if applied during or just prior to the beginning of the morphogenesis phase (Kinet et al/

(Kinet et al - see Bernier 1971; Bonner and Zeevaart, 1962; Evans, 1964) and are not effective if applied during induction and evocation. In Pharbitis however, inhibitors of D N A synthesis are effective in preventing flowering when applied during the inductive dark period (Zeevaart, 1962). These effects of D N A synthesis inhibitors, with the exception of that on Pharbitis, are consistent with the hypothesis that active cell division during or just prior to the morphogenesis phase, i.e. around the time when the number of mitotic figures increases, is necessary if floral induction is to be effective.

In Chenopodium album (Gifford and Tepper, 1965), Kalanchoe (Stein and Stein, 1960), and Lobelia (Philipson, 1948) an increase in the length of embryonic internodes at the apex takes place shortly after photo-periodic induction. This effect is even more pronounced in rosette plants, (Philipson, 1947; Rouk and Reynick, 1951; Vaughan, 1955; Sachs, Bertz and Lang, 1959).

All of these data (mitotic activation, D N A inhibitor studies, internode elongation, as well as direct measurements of cell division rates) suggests there may be an overall increase in the growth rate following evocation at the time of the beginning of the morphogenetic transformation of the vegetative apex into a flower or an infloresence.

Several authors (Gifford, 1963; Healey and Jensen, 1965; Bernier, 1969; Knox and Evans, 1966; Gifford and Tepper, 1962; Jacqmard et al, 1972; Lance, 1957) have reported an increase in the concentration of R N A in the cells of the apex following evocation and prior to the start of the morphogenesis of the flower. This increase in R N A concentration commences about the same time as the increase in mitotic activity and is distinct from the ephemeral increase in the rate of synthesis of R N A associated with evocation.

In Sinapis/

In Sinapis an increase in total R N A concentration in the apex was recorded 32 hours after the start of inductive treatment (i.e. about 16 hours after the presumed time of arrival of the stimulus at the apex). The concentration of R N A further increased over at least the following 24 hour period (Jacqmard et al, 1972). The increase in the concentration of R N A in the apex occurs simultaneously with an increase in nucleolar size (Bernier et al, 1967) and in nuclear R N A synthesis (Bronchart et al, 1970) and it was concluded by Bernier (1971) that in Sinapis the increase in R N A concentration was associated with a rise in ribosome concentration and a subsequent increased ability to synthesise protein. In Lolium there is an overall increase in the R N A concentration in the presumptive spikelet sites in the apex 48 hours after the start of inductive treatment (about 24 hours after the presumed time of arrival of the stimulus at the apex) with a further increase in R N A concentration over at least the following 24 hours (Knox and Evans, 1966). In both Sinapis and Lolium these latter increases in overall R N A concentration in the apical tissue are apparently distinct from the ephemeral stimulation in the rate of R N A synthesis associated with the arrival of the morphogenetic stimulus at the shoot tip discussed above. A similar series of events has been described in Pharbitis. There is an ephemeral increase in the rate of synthesis of R N A associated with evocation (Gressel et al, 1967) and later an overall increase in the concentration of R N A in the apex (Healey and Jensen, 1965; Gifford, 1963). Gifford (1963) also described an increase in the concentration of R N A in the induced Xanthium apex around 40 hours after the start of the inductive long day and Gifford and Tepper (1961) recorded an increase in the concentration of R N A in the induced Chenopodium album apex.

As with the initial increase in the rate of synthesis of R N A associated with evocation, there/

there is again evidence from work with growth hormones, that indicates the overall increase in R N A concentration may be an effect of hormonal action at the apex. All of the principal categories of growth hormone are known to cause fairly long term increases in total R N A concentration, in the tissue affected by the hormone. For example 2, 4 - D treatment results in a five-fold increase in R N A concentration in cucumber stems five days after treatment (West et al, 1960), gibberellic acid promotes an increase in the R N A concentration in dwarf pea internodes (Giles et al, 1966; Broughton, 1969), and the R N A concentration is increased in certain roots from 24 hours onwards following kinetin treatment (Olzewska , 1959). There are at least two possible interpretations of this type of hormone - mediated increase in concentration of R N A - presumably principally ribosomal R N A. The increase could be a direct effect of the hormone, or it could be a secondary effect which has resulted from a primary effect of the hormone such as an increase in growth rate. In many hormone reactions in plants activation of a tissue is the characteristic response, and it may be that the rise in R N A concentration results from this activation or increase in growth rate. Other tissues such as isolated carrot root tissue show large increases in R N A concentration when cultured in mineral salt/sucrose medium (Steward et al, 1964) or aged in distilled water (Leaver, 1966), (it may be that in these cases excision itself results in the internal release of hormones). Gibberellin and kinetin have been shown to exercise control over bulk protein synthesis in excised leaf tissue (Fletcher, 1965; Sugiura, 1962) and auxin to increase the rate of synthesis of protein (Fan and MacLachlan, 1966; Key, 1964; Noodén and Thimann, 1965). There have been several reports of increased bulk protein concentration in the plant shoot apex following floral induction (Gifford and Tepper, 1962; Corson and Gifford 1969; Jacqmard et al, 1972). In each of these/

In each of these cases the rise in apical protein concentration, like the increase in total R N A concentration and rise in mitotic activity, began to take place just prior to the start of flower morphogenesis.

It might be expected that during the period from evocation to flowering there is likely to be an increasingly different complement of enzymes present in the apical tissue with, for example, the appearance of enzymes concerned with flower pigment production, pollen walls, etc and the disappearance of those associated with the photosynthetic process. Nitsan (1962), using the technique of free-boundary electrophoresis, was unable to record qualitative differences between the protein complements of vegetative and reproductive buds of Xanthium. On the contrary Marushige and Marushige (1962) were able to demonstrate qualitative differences between proteins from leaves and various flower parts of Pharbitis. In both of these investigations the techniques used were not sufficiently sensitive to demonstrate small qualitative differences in the protein complements and also the tissue samples were taken from plants where flower morphogenesis was complete. The data therefore have little relevance to the study of early changes in the apex consequent upon induction. The major contribution in this field is that of Barber and Steward (1968). Using polyacrylamide gel electrophoresis they were able to demonstrate qualitative differences between the band patterns of proteins from apical tissue of vegetative and temperature-induced tulip bulbs. These differences were apparent both following non-specific amido black staining as well as staining for esterase activity. Qualitative differences appeared several days before the tulip apex showed signs of flower morphogenesis and the differences increased as floral morphogenesis progressed.

This experimental demonstration of differences in the electrophoretic protein band pattern/

pattern from temperature-induced and non-induced tulip apical tissue supports the hypothesis that new proteins are synthesised over the period of transition from vegetative to floral development.

Cherry and Van Huystee (1965) have shown by the fractionation of bud nucleic acid on columns ofmethylated albumin on kieselguhr that the floral stimulus resulted in an increased synthesis of messenger R N A in Xanthium buds. Using the double labelling techniques of Kano-Sueoka and Spiegelmann (1962) data from Xanthium indicated there may be qualitative differences in the messenger R N A fraction following induction suggesting there was de-novo synthesis of protein controlled at the transcriptional level (Cherry and Van Huytsee, 1965; Cherry, 1970). It is, however, unlikely the technique is sufficiently sensitive to differentiate between two comparatively similar m R N A fractions. Watson and Mathews (1966) described a ribonuclease resistant R N A component detected in apical buds of Chenopodium amaranticolor following floral induction. This component was shown by fractionation on columns of methylated albumin on kies elguhr to be associated with D N A probably as a D N A-R N A hybrid. Watson and Mathews went on to speculate this fraction may reflect the production of new floral messenger R N A in the induced bud.

This summary of published data shows that although there are considerable differences in detail in different reports, there are nevertheless several features which have been shown to occur in a number of plants in the apex during the transition from vegetative to floral development. Perhaps the first of these is that around the time of commit ment of the apex to flower (evocation) there are ephemeral increases in the rate of synthesis of R N A and also of protein. The similarity of this observation with the early effects of growth hormones on other plant tissue, suggests evocation takes place in response to the arrival of a hormone or hormone-like substance at the apex. There is/

There is evidence that in Pharbitis and in Sinapis the arrival of the stimulus is multi-staged. Sometime later the concentration of R N A and protein in the tissue of the apex increases. This increase in concentration of R N A and of protein would appear from most accounts to begin just prior to, and to continue into the morphogenesis phase. A difficulty exists in the interpretation of almost all of these results because they are seldom accompanied by systematic reports of the sequence of morphological changes in the apex or by information on the cell division rates in the apex over the period from evocation to flower morphogenesis. Many reports suggest there may, in fact, be an increase in the apical growth rate associated with the transition to flowering. An increase in the concentration of R N A would be expected if there were an increase in the growth rate in the apex since an increase in growth rate is likely to depend on an increased capacity to synthesize protein which in turn would depend upon the concentration of ribosomes and other R N A species.

In the present investigation on the changes in the apex associated with flowering, cell division rates in the apex and the morphological sequence in the apex are systematically recorded from the start of inductive treatment until morphogenesis of the flower is well advanced. The possibility of an increase in growth rate being the causative factor of the higher R N A concentration associated with morphogenesis of the flower is studied by measuring R N A concentration in the apex following the experimental alteration of growth rates.

There is considerable evidence which supports the hypothesis that evocation is brought about by the arrival in the apex of a hormone or hormone-like substance. It has been shown that in plants hormone treatment can result in a comparatively long-lived (several days) increase in total R N A concentration. Though it is not clear why R N A concentration should increase following hormone treatment/

treatment it is nevertheless possible to explain the increase in the concentration of R N A in the apex during flower morphogenesis as resulting directly from the arrival of hormone at the apex. The effects of the external application of giberellic acid on apical growth rates and on the accumulation of R N A in the apex are recorded in the present study.

It has been argued that there is a likelyhood of an increase in the rate of apical growth during the period of flower morphogenesis and that such an increased growth rate is likely to depend upon an increased rate of protein synthesis. It has also been argued that as flower morphogenesis progresses there is likely to be an increasingly different protein complement in the apical tissue of induced plants compared with non-induced plants, the data of Barber and Steward (1968) supports this hypothesis. There is however no information on changes in the protein complement in the apical dome of photosensetive plants during floral transition. In the present work an electrophoretic study of the protein eomplement of the apical dome from the start of inductive treatment until flower morphogenesis is well advanced is described.

Because of experimental convenience a plant where the flowering response is under the absolute control of day-length was selected for these present series of investigations. The great advantage of such a plant is that it is possible to time induction precisely by the manipulation of day-length. Such control is simply not possible with day-length neutral plants. The most favoured plants for work on flowering are plants such as Sinapis alba, Pharbitis nil, and Lolium temulentum, which show an extreme response and flower after exposure to a single 24-hour period of the appropriate day-length. It is probably simply due to coincidence, but with the exception of Pharbitis nil, all of the more popular plants used in recent investigations on the control of flowering/

flowering produce a terminal infloresence rather than a single terminal flower. Although it is unlikely the inductive process and evocation are any different in a plant producing a terminal flower rather than an infloresence it was felt a more direct comparison could be made between the vegetative apex producing leaf primordia and the apex when, following induction, it was producing flowerpart primordia. For this reason a plant which produces a single terminal flower was selected.

One other factor which determined the choice of plant material for this work was the ease with which long itudinal apical sections could be cut through a known and repeatable plane. The small size and relative inaccessibility of the shoot apex dictates that perhaps the most useful experimental techniques rely upon tissue sectioning. The design of several experiments (see methods section) necessitated sections being cut in the precise plane of a particular primordial pair. The simplest method of accomplishing this was to select a plant with a suitable leaf arrangement so that the plane of sectioning could be aligned by reference to an older leaf or leaf pair. Pharbitis nil was rejected as a suitable plant because it has a spiral leaf arrangement which would make this type of manipulation impossible.

These three principal considerations greatly limited the choice of a suitable plant. A horticultural variety of Silene coeli-rosa (L) Godron, known by seedsmen as Viscaria cardinalis was finally selected. This plant has an opposite and decussate leaf arrangement facilitating alignment of the tip tissue during sectioning. Silene has an absolute requirement for long days to flower under the conditions used, and upon induction the vegetative apex transforms into a single terminal flower.

PART II

MATERIALS AND METHODS

Materials and Methods.

This study was conducted using a horticultural variety of *Silene coeli-rosa* (L.) Godron, supplied as *Viscaria cardinalis* by Stewarts Ltd. seedsmen, Edinburgh.

This particular plant was chosen for the following reasons:-

1. It is photoperiodically sensitive, being a qualitative long day plant. Plants have been shown to remain vegetative for at least six months when grown in a twenty-four hour cycle of sixteen hours darkness and eight hours light. Such plants proceed rapidly to flower if transferred to a cycle of at least sixteen hours light and eight hours dark. Figs 1 and 2 show plants of *Silene* grown from seed in these two sets of conditions.
2. The vegetative apex, upon induction, is directly transformed into a single terminal flower making possible a direct comparison between the apex involved in the formation of floral part primordia and that involved in the formation of leaf primordia.
3. *Silene* has an opposite and decussate leaf arrangement. This facilitates alignment of apical tissue during microtomy.
4. The plant grows reasonably quickly under experimental conditions allowing experiments to be completed in about six weeks from sowing. Also since it is a popular garden variety there is a ready and plentiful commercial supply of seed.

Growth Conditions

Plants were grown from seed in 9cm diameter plastic plant pots in a natural peat based compost "Levington's Germinating Compost" prepared by Fisons Research Co. Ltd.

Sowing was carried out by sprinkling approximately seventy seeds per pot onto the surface of compost thoroughly wetted with tap water. The pots were stood in about one centimetre of Hoagland number one solution/

Fig. 1 Unsorted 28-day old vegetative Silene plants grown under SD conditions.

Fig. 2 Silene plants flowering. These plants have been grown under LD conditions.

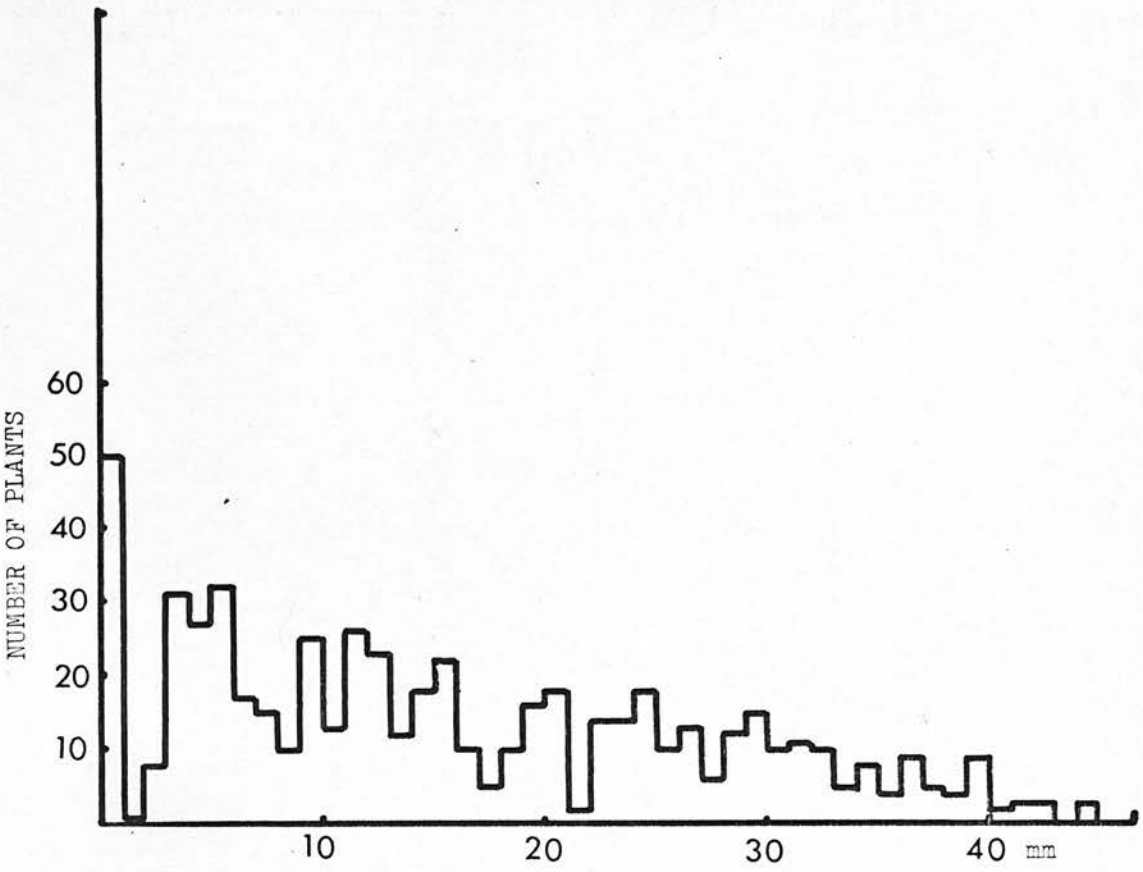


Fig. 3 Variation in an unsorted population of Silene plants grown in SD, as demonstrated by the length of the third leaf pair 28 days from sowing (day 0).

solution (Hoagland and Arnon, 1938) in plastic trays in a specially constructed controlled environment room. Light was supplied at an intensity of 87-105 Watts metre^{-2}, equivalent to 2,500 - 3,000 lumens ft^{-2}, by Phillips 65-80 Watt warm white fluorescent tubes and 40 watt tungsten bulbs.

The plants were grown in a 24 hour cycle consisting of eight hours light and sixteen hours darkness. Temperature was maintained at 20^{o}C.

These growth conditions are short day conditions (SD) and under them all from a sample of 15 plants remained vegetative for at least six months.

Induction was carried out in long days (LD) by substituting a 16 hour period of low intensity tungsten light (2 Watts meter^{-2}, equivalent to 60 lumens foot^{-2}) for the dark period. Conditions were otherwise the same as for SD conditions.

Germination occured in about three days and vegetative growth was allowed to continue for a total of 28 short days before further treatment.

Selection of Plants Growth was highly variable but it was not until the plants were about four weeks old that a sufficient number (about one third) were large enough to permit easy handling. These plants had produced seven or more leaf and primordial pairs and would flower if exposed to five or six LD then returned to SD. Photoperiodic sensitivity increased with age; a larger proportion of plants flowered after exposure to a set number of long days as the plants became older. Four-week old plants required, on average, one more LD to become induced than did six-week old plants. Because of limited growth room space however, it was decided to limit vegetative growth to twenty-eight days. Since all experiments were commenced when the plants were 28 days old, day 28 of growth will subsequently be referred to as day 0; day 29 of growth as day 1, etc. Because of plant to plant variability (fig. 3) the plants were subjected to a selective weeding process in order to obtain a population/

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Selection of Plants Growth was highly variable but it was not until the plants were about four weeks old that a sufficient number (about one third) were large enough to permit easy handling. These plants had produced seven or more leaf and primordial pairs and would flower if exposed to five or six LD then returned to SD. Photoperiodic sensitivity increased with age; a larger proportion of plants flowered after exposure to a set number of long days as the plants became older. Four-week old plants required, on average, one more LD to become induced than did six-week old plants. Because of limited growth room space however, it was decided to limit vegetative growth to twenty-eight days. Since all experiments were commenced when the plants were 28 days old, day 28 of growth will subsequently be referred to as day 0; day 29 of growth as day 1, etc. Because of plant to plant variability (fig. 3) the plants were subjected to a selective weeding process in order to obtain a population/

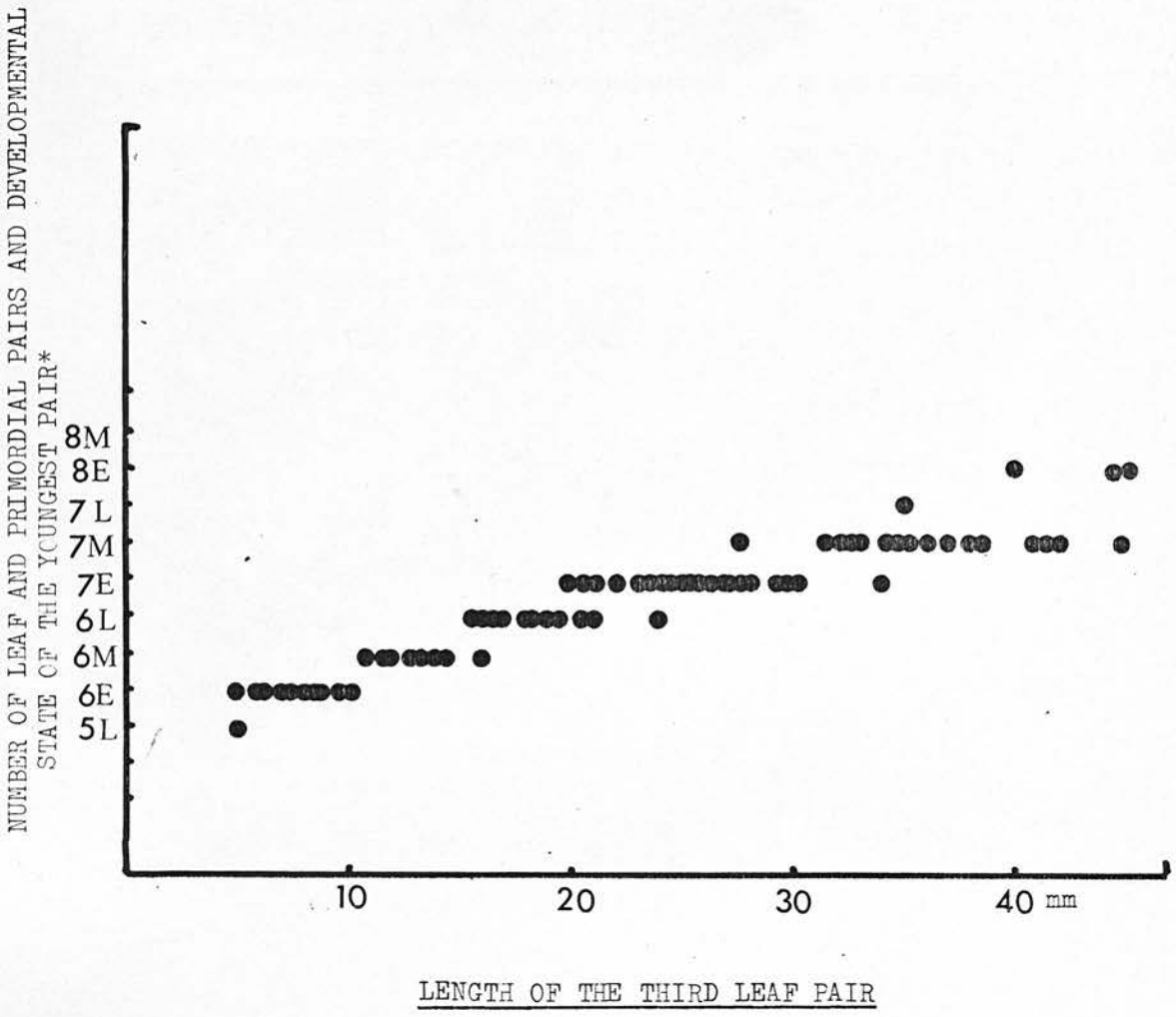


Fig. 4 Relationship between the length of the third leaf pair and the developmental state of the apex in 28 day old Silene SD plants. Each dot represents a single plant.

*E = early, M= medium, L=late.

population of plants at a similar developmental stage as well as the same chronological age.

A correlation was discovered between the length of the third oldest leaf pair which was still expanding and the developmental stage of the plant as a whole. It was shown that when the third oldest leaf pair of 28-day old plants was 20-30m m long the seventh primordial pair was just discernable as bumps on the flanks of the apex in the majority of plants (fig. 4). The plants could be quickly selected by measurement of the third oldest leaf pair with a small ruler. This was done and the remaining plants in the pots removed by cutting them just above soil level with scissors. Once this selective weeding was carried out approximately five plants were left per pot. These plants were then subjected to experimental treatments. Table 1 shows the effect of LD exposure on flowering of a population of plants selected by this method.

General Procedure Followed In Experiments Involving Apical Microtomy.

Plants were grown and selected when twenty-eight days old as described. Induction was commenced on that day and plants were induced by exposure to 7LD. The plants were then returned to SD. Control series were maintained in SD throughout sampling. A third series of plants was usually included in experiments. These plants were exposed to 3 LD and then returned to SD. With the exception of about 2% of plants 3 LD does not result in induction (table 1). These plants were included in order to record reversible phenomena associated with the initial stages of induction. Experimental plants were sampled daily usually 1 hour after the start of the high intensity light period. This time was chosen because of convenience and since samples were removed at a similar time each day any possible effects of diurnal rhythms would be minimised.

The actual sampling was carried out by severing the plant just above the level of the cotyledons and trimming it to a "Y" shape. This/

TABLE 1. The effect of LD treatment on induction in *Silene*. The plants were grown in SD until treatment which commenced when the plants were 28-days old (day 0) and returned to SD following treatment. The plants were scored for flower buds 3 weeks later. The sample size was at least 50.

Number of long days	1	2	3	4	5	6	7
Percent induction	0	0	2	66	90	94	100

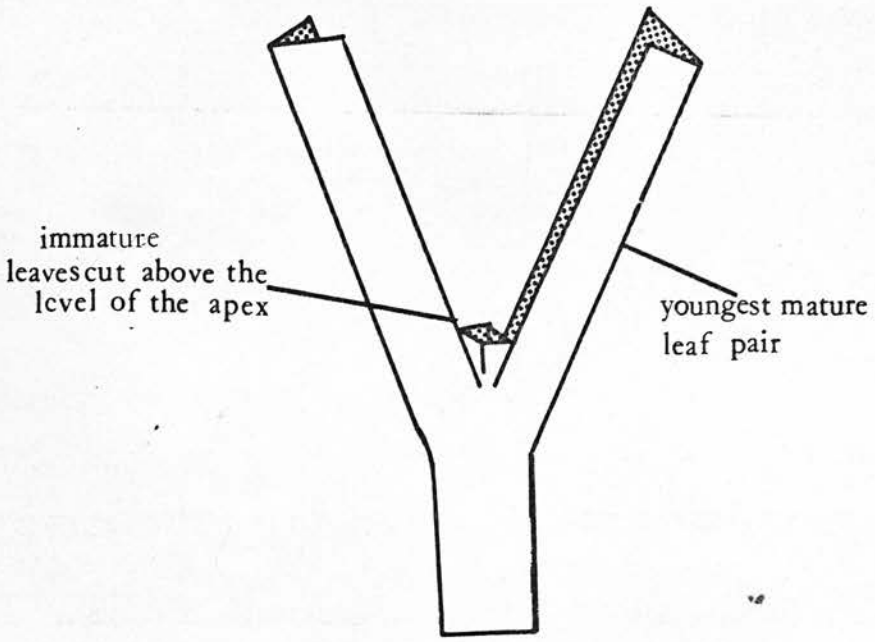


Fig. 5. Diagram showing the method of preparation of a plant sample prior to fixation for experiments involving tissue sectioning.

The youngest mature leaf pair has been trimmed to about 1 cm long, the internode immediately below the youngest mature leaf pair has been severed and the immature leaves have been cut just above the level of the apex.

During microtomy sections are cut in the plane of the youngest mature leaf pair which is preserved throughout the tissue preparation.

This was done by cutting off immature leaves just above the level of the apex, and cutting the youngest mature pair to about one centimetre long. Finally the main stem was cut just below the node of the youngest mature leaf. Fig 5 shows a diagram of such a prepared sample. Later long itudinal sections were cut through this leaf pair which was preserved in the sample. The leaves were numbered from the base of the plant, the oldest pair being numbered one. A note was, of course, taken of the leaf pair number preserved in the "Y" shape through which the tissue was later sectioned.

Fixation, Embedding and Sectioning.

Throughout most of this work the fixative used was ethanol/acetic acid (3/1, volume/volume). Samples were fixed for about two hours. Mitchell (1966/1968) demonstrated that this particular fixative preserved a greater quantity of nucleic acid and protein than others he tested; he also showed any fixation time between one and 24 hours was satisfactory. Following fixation, samples were transferred to 70% aqueous ethanol for at least one hour, dehydrated in absolute ethanol, and infiltrated with solutions of xylene in ethanol of increasing strength and finally to pure xylene. Once the samples were thoroughly infiltrated with pure xylene (at this stage the samples were completely transparent), flakes of paraffin wax with ceresin (BDH, melting point 54·5°C) were added to the sample tube, and the tube placed in an oven maintained at 60°C. After about twelve hours when the wax had melted, the molten xylene-wax solution was quickly poured off and molten pure wax at about 60°C added to the tube. This second change of wax was left for 24 hours and then changed for a third time. A further 24 hours was allowed to elapse before completing the embedding process. The samples were embedded in thin blocks allowing them to be seen inside the relatively opaque wax.

Long itudinal serial sections were cut using a Beck Rotary Microtome. Unless otherwise stated section thickness was 10μm and the sections were collected on cleaned glass slides coated with Gurr's glycerin albumen/

albumen adhesive. When sections were required for autoradiography, the sections were cut 5µm thick and collected on gelatin coated slides (see Page44).

Prior to staining sections were dewaxed by placing them in xylene for twenty minutes. The sections were then washed with absolute ethanol and hydrated with solutions of aqueous ethanol of decreasing strength.

Quantitative Histochemical Staining.

Total Nucleic Acid.

Total Nucleic Acids were stained in sectioned tissue using the gallocyanin chrom-alum method. This technique was selected because its properties as a specific nucleic acid stain are reasonably well documented, (Sandritter et al, 1954, 1963; Pakkenberg, 1962; Chen, 1966; Shires, 1967; Mitchell, 1968). In most of this work animal tissue was used but Chen (1966), and Mitchell (1968) demonstrated gallocyanin chrom-alum to be specific for plant nucleic acids by the use of nuclease enzymes. The stoichiometry and adherence to the Lambert-Beer Laws of spectophotometry were demonstrated for the stain nucleic acid complex in animal tissues by Sandritter et al (1963) when the stain is used at optimum conditions. Sandritter et al (1963) and Kiefer et al (1967) showed the stoichiometry of both nucleic acids with the stain is the same. Mitchell (1968) showed by the use of ribonuclease-treated sections that the binding of stain by both nucleic acids was similar and indirectly that the optical laws are obeyed. This was done both with a high R N A : D N A ratio in artichoke tuber tissue and with apical tissue from the pea apex where the R N A : D N A ratio is near unity. This was carried out by comparing cytophotometric values of stain density with the results of chemical extraction of nucleic acids.

In the present work the specificity of the stain for nucleic acid was checked by treating sections with nuclease enzymes and subsequently/

subsequently staining with gallocyanin chrom-alum. Fig. 6 shows photomicrographs of these sections. The results showed that if Silene apical sections were treated with neither deoxyribonuclease nor ribonuclease cytoplasm and nuclei were stained a deep blue (fig. 6a). If sections were incubated with deoxyribonuclease the cytoplasm and nucleoli formed a similar amount of stain complex to untreated sections but the nuclei stained only faintly (fig. 6b). Sections incubated with ribonuclease formed a stain complex with nuclei only (fig. 6c) and if sections were treated with both ribonuclease and deoxyribonuclease no colour reaction was detected (fig. 6d).

The stain was prepared by the method of de Boer and Sarnaker (1956) as summerised by Mitchell (1968). 600mg Gallocyanin (BDH Lab. Reagent Grade) was shaken for one minute in 200ml of water and the suspension filtered. The residue was extracted with boiling aqueous 5% chrom-alum for 30 minutes. After filtration and cooling the filtrate was adjusted to pH 1·6 with 1N hydrochloric acid.

Hydrated sections were placed in 0·02N hydrochloric acid for fifteen minutes, the sections were then stained overnight (18 hours) at 40°C. Excess stain was removed by washing the sections in 0·02N hydrochloric acid for fifteen minutes. This procedure was established as optimal for plant tissue by Mitchell, (1968). Finally the sections were dehydrated in ethanol, rinsed with xylene, and mounted in Canada balsam.

Measurements of relative stain density were later made at a wavelength of 575nm using a Barr and Stroud integrating microdensitometer.

Deoxyribonucleic Acid (DNA)

DNA was stained in sectioned tissue both by the Feulgen technique and also by the Gallocyanin chrom-alum method after prior treatment of the sections with ribonuclease.

Procedure When Staining DNA With Gallocyanin/

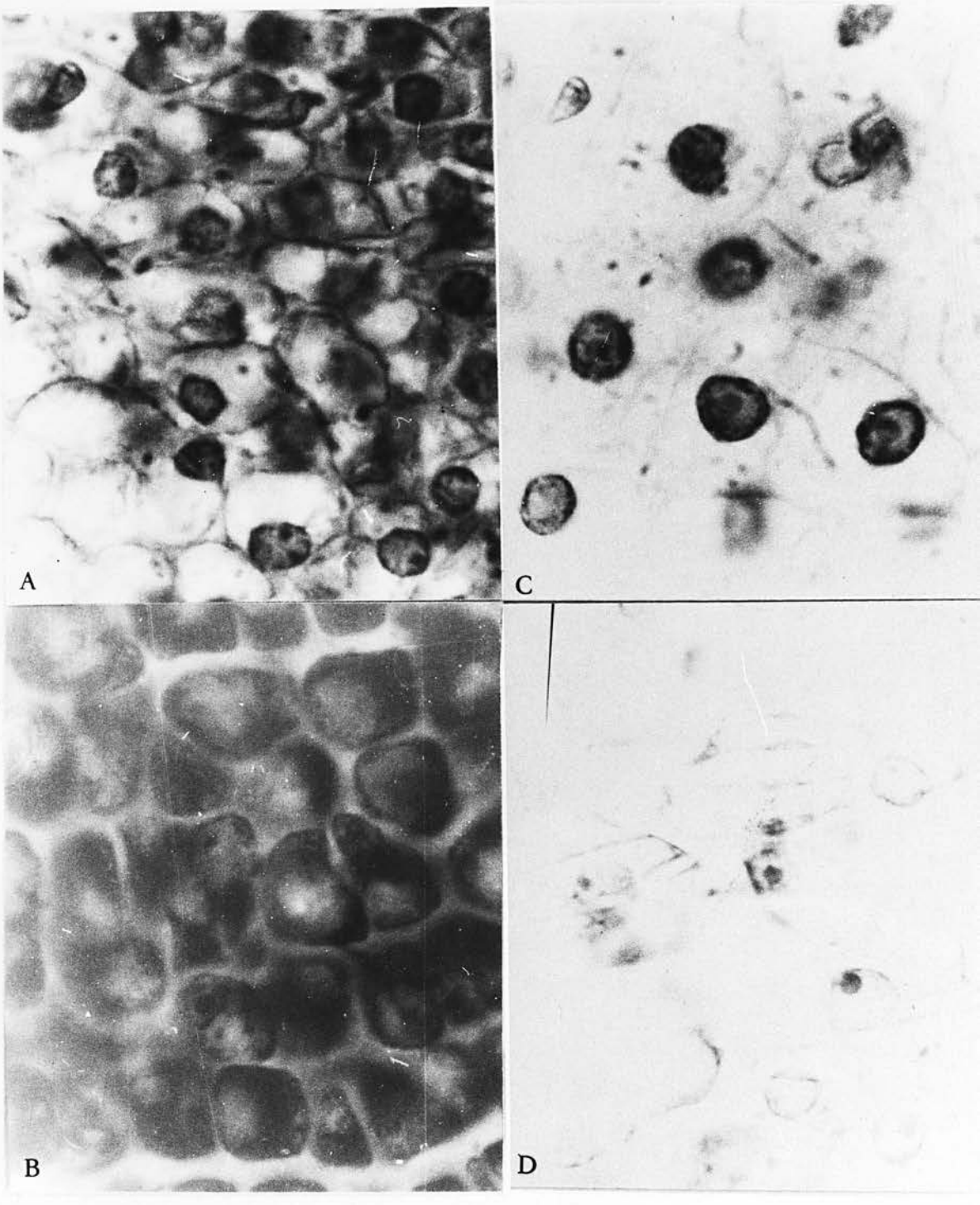


Fig. 6 *Silene* apical tissue stained with Gallocyanin

A. Tissue stained for RNA and DNA.

B. Tissue stained for RNA following deoxyribonuclease treatment.

C. Tissue stained for DNA following ribonuclease treatment.

D. Tissue stained following treatment with both ribonuclease and deoxyribonuclease.

Procedure When Staining DNA With Gallocynanin In order to remove RNA and so stain only DNA, hydrated sections were rinsed in water (adjusted to pH7 with sodium hydroxide) and then treated with ribonuclease solution (Sigma, 5 x recrystallized) at a concentration of 0·5 mg/ml for one hour at 60°C (Mitchell, 1968). Fig. 6c shows sectioned apical tissue of Silene stained with gallocyanin chrom-alum following RNA extraction by ribonuclease.

Feulgen Staining The Feulgen procedure was that described by Darlington and La Cour (1960) for sections and smears, the Leuco-basic fuchsin dye was prepared also according to Darlington and La Cour (1960). Hydrated sections were allowed to equilibrate in distilled water at 60°C, hydrolysed in 1N hydrochloric acid at 60°C for six minutes, and stained in leuco-basic fuchsin for two hours. Following staining the sections were rinsed in freshly prepared SO_2 water (5ml, 1N HCl; 5ml, 10% $K_2S_2O_5$; 100ml, H_2O) for three periods each of ten minutes. Finally the sections were rinsed in distilled water, dehydrated with ethanol, infiltrated with xylene and mounted in Canada balsam. Sections which were stained with leuco-basic fuchsin had previously been stained with 2 : 4 dinitrofluorobenzene (DNFB), a protein stain. This procedure was adopted to increase the potential information yield from one set of sections and so economise on time involved in tissue preparation and sectioning. Mitchell (1966) described conditions where DNFB acted as a quantitative protein stain and showed that the Feulgen procedure did not affect absorbance due to DNFB stain complex and the optical density of the Feulgen stain complex was not itself affected by DNFB staining. Optical density was measured at 565 nm (Feulgen) and 400 nm (DNFB).

Details of the DNFB procedure are described below in the section "Protein".

The absorbance at 565 nm due to DNFB stain/

TABLE 2. The effect of the Feulgen procedure on absorption at 400 nm by DNFB-treated cells, and the effect of DNFB-treatment on absorption at 565nm by Feulgen stained cells

Stain treatment	Absorption at 400nm	Absorption at 565nm
DNFB	26	0
Feulgen	1	7
DNFB + Feulgen	23	7

The results are each the mean of readings from areas 1, 2, 3, 4 and 5 in the 3 median sections of 3 vegetative apices each at the same developmental stage.

stain and that at 400 nm due to Feulgen stain was checked for Silene apical tissue. The results are shown in table 2 and show each stain has little effect at the wavelength of maximum absorbance of the other.

Ribonucleic Acid (RNA).

RNA was not stained directly, however values for RNA concentration were calculated by difference between optical density due to total nucleic acid-gallocyanin stain complex and that due to DNA alone.

A sample of ribonuclease-treated slides was included in each experiment and the mean DNA value for each area subtracted from the total nucleic acid value to give data on RNA.

Protein.

DNFB was used as a histochemical stain for total protein. It is well established (Sanger, 1949; Maddy, 1961) that protein treated with dinitrofluorobenzene forms dinitrophenol compounds with amino groups and sulphhydryl groups.

This method was used in conjunction with Feulgen staining as described earlier. It was assumed that initial fixation denatures all protein such that it was not extracted during subsequent treatment. Hydrochloric acid hydrolysis during the Feulgen procedure did not affect final stain concentration per apex (table 2) indicating the probable effectiveness of the fixation.

The staining solution contained 0·5g (0·32 ml) DNFB (BDH Lab. Reagent Grade) in 95 ml ethanol and 5ml 0·2 N sodium hyroxide (Jensen, 1962). Sections were dewaxed and partially hydrated in 70% aqueous ethanol then stained for about eighteen hours in DNFB solution at 65°C (Mitchell, 1966).

Measurements of optical density of DNFB stained sections were made at 400 nm.

Microdensitometry

Stain density in sectioned apical tissue was measured following/

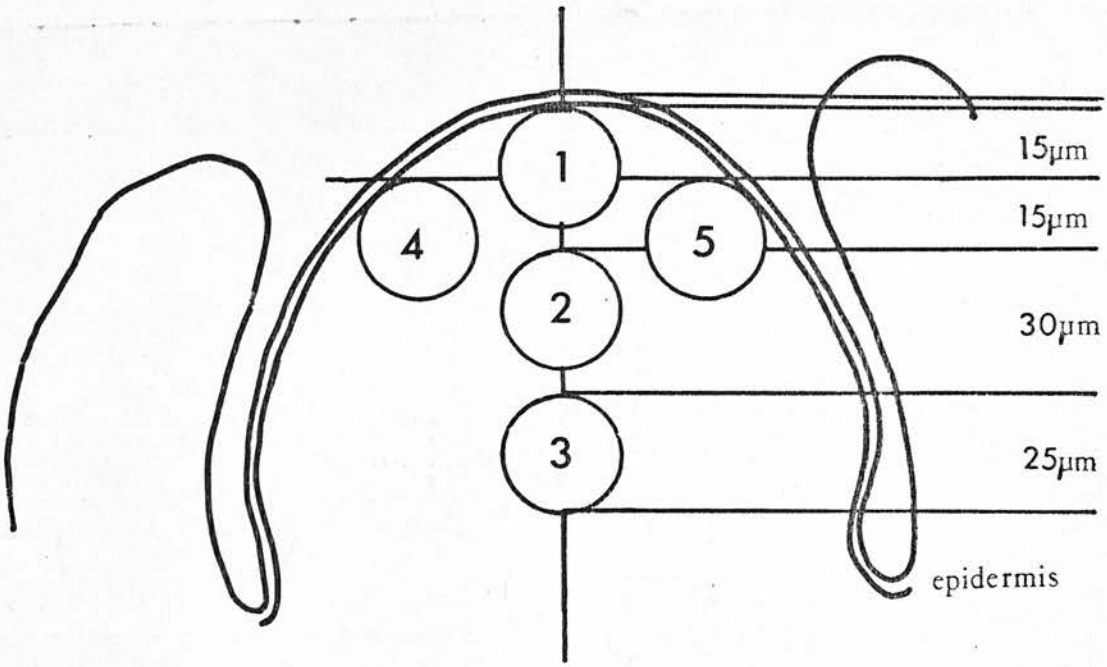


Fig. 7 Diagram of the median longtitudinal section of a typical vegetative Silene apex showing the position of the five 25 μm diameter areas in which stain density was measured in experiments involving quantitative staining.

Areas I, 4 and 5 are bounded by the inner epidermal wall.

In all experiments stain density was measured in areas 1, 2, 3, 4 and 5, in the 3 most median sections.

following gallocyanin, Feulgen, and DNFB treatments by means of a Barr and Stroud integrating microdensitometer. With this particular instrument it is only possible to measure absorbance in a circular area. There are a limited number of aperture sizes available and the most satisfactory was found to be one which gave an optical density reading of an area 25 μm in diameter. Readings were taken of five such areas within each of the three median longtitudinal sections of each apex.

The five areas were defined with respect to the extreme tip of the apex.

Fig. 7 illustrates these positions. Since areas four and five are essentially similar to one another the results from these two areas were taken together.

To check slide to slide variation in gallocyanin and Feulgen stained sections measurements were made of stain density of 3 or 4 single complete prophase nuclei in each apex. Since such nuclei contain a constant amount of DNA they act as an internal standard. If from any slide the optical density values for prophase nuclei were consistently different from the mean of all the slides, indicating the slide was unaccountably lightly or heavily stained, then all the absorbance values for that slide were "corrected" by the proportion which the prophase values differed from the mean prophase value. These variations were extremely infrequent and probably due to slight differences in treatment of samples (samples were harvested and prepared for sectioning daily and could not therefore be processed together).

Growth measurements.

Growth rates in the apex were measured by two methods; (a) direct observation of changes in cell number, cell size, and in volume of sectioned apices, (b) a thymidine labelling technique was used to obtain measurements of the cell cycle time.

Cell size. A measure of mean cell size was obtained by counting/

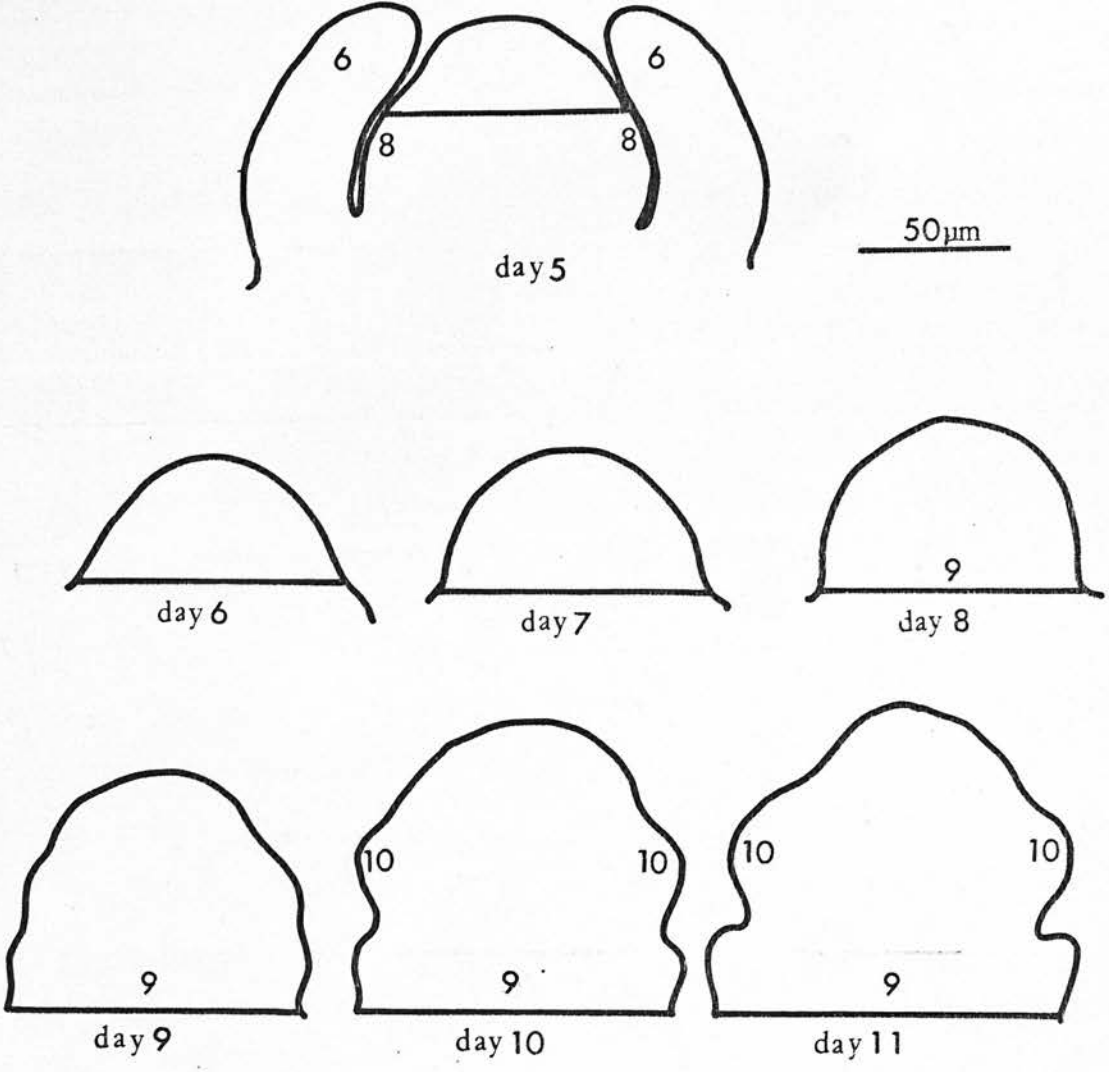


Fig. 8. Diagrams illustrating the method used for measuring growth at the apex. The diagrams are of median sections and show the enlargement of the apical tissue above leaf pair 8 from day 5-11. A base line has been drawn between the axils of leaf pair 8 and all the tissue above that line is measured from successive days sections. The numbers on the drawings represent the leaf number of primordial pairs. Pair 9 is at right angles to the plane of the sections.

counting the number of nuclei present in defined areas of sectioned tissue 10 μm thick. The nuclear number in the five areas (fig. 7) where optical density reading were taken was counted. These areas each represent a volume of tissue of 4,910 μm^3.

In practice it was not possible to count nuclei through the microscope of the integrating microdensitometer. The nuclear number in 25 μm diameter areas was instead counted using a light microscope fitted with a camera lucida.

A paper stencil with three circles corresponding to areas one, two and three and a construction line on which to place areas four and five was prepared. These were to the appropriate scale. The camera lucida image of a sectioned apex was then superimposed over the stencil and dots placed on the image position of nuclei in the circles on the paper stencil. This was done for the three median sections of each apex. Nuclei only partially present were included in the counts since this was convenient and the error introduced was assumed to be constant.

Mean cell size was calculated from the number of nuclei present in these areas each representing a volume of 4,910μm^3.

Apical Size and Cell Number. Data on rates of change of apical size and cell number were obtained by counting total cell number and volume of the apex above a fixed base line on successive days. The base line was a line drawn between the axils of a leaf pair or primordial pair as shown in fig. 8.

10μm long itudinal serial sections of the apices were cut. Care was taken to include the complete complement of sections through an apex. The sections were stained with Heidenhain's haematoxylin to stain the nucleoli black and the nuclei grey. The staining method used was that described by Johansen (1940), but the staining times were altered to give this effect to the nuclei.

TABLE 3. The error introduced by calculating the apical number from the apical volume and the cell number of the median section.

Sample	*Calculated cell number	**True Cell number	Difference as a percentage of the true cell number
Day 0 SD, above leaf pair 7	198	190	+4.2%
Day 3 SD, above leaf pair 7	1,247	1,409	-11.5%
Day 5 LD, above leaf pair 8	370	352	+5.1%
Day 6 SD, above leaf pair 8	770	723	+6.5%
Day 9 SD, above leaf pair 9	402	420	-4.5%

*Cell number calculated from the formula:

$$\text{Cell No} = \frac{\text{total apical volume}}{\text{volume of median section}} \text{. Nuclear number in the median section.}$$

** estimated by counting nuclei in each of the serial sections through the apex.

Hydrated sections were placed in mordant solution (Water 500ml; acetic acid 5ml; sulphuric acid 0·6ml; ferric ammonium sulphate 15g) for five hours. The slides were then washed with water and stained overnight in Heidenhain's haematoxylin No 2 solution (B.D.H.). The sections were then carefully destained in 2% aqueous ferric ammonium sulphate until the nuclei were grey, the nucleoli black, and the rest of the tissue almost colourless.

The sections were then rinsed in water, dehydrated in ethanol, rinsed in xylene and mounted in Canada balsam.

Outline drawings were prepared of camera lucida images of all the serial sections through each apex. A dot was positioned for each nucleus in the drawing of the median section. Nuclei were identified by the presence of the darkly stained nucleoli. There was generally a single nucleolus per nucleus but where there was more than one this was easily seen since the nuclei themselves were stained and in such cases the nucleus was recorded as a single dot.

Apical volume was calculated from the total area of the camera lucida drawings of serial sections, estimated by weighing the drawings. The cell number per apex was calculated from the cell number in the median section and the total apical volume by assuming cell concentration in the median section to be similar to that in the apex as a whole.

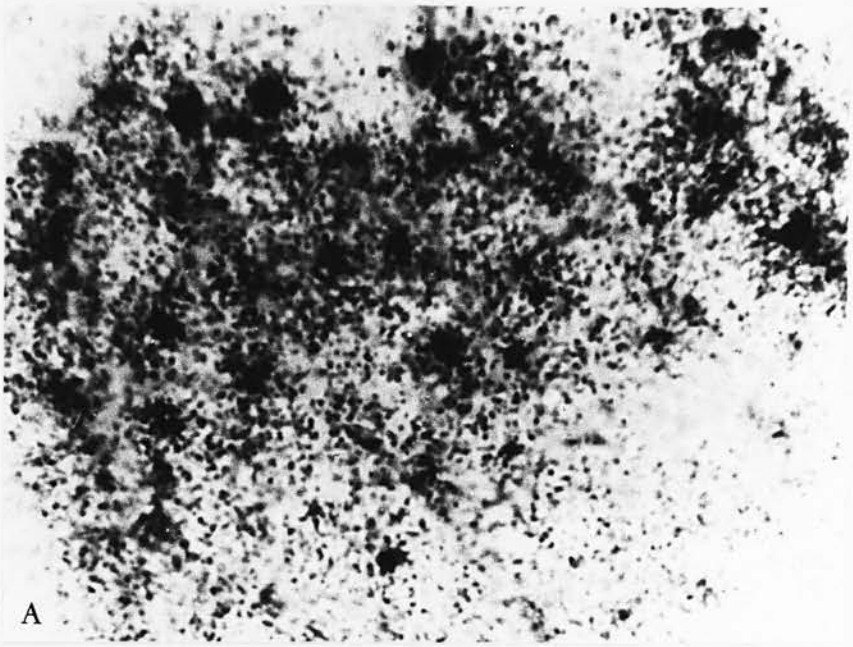
In a number of cases the total nuclear number per apex was counted and this assumption verified (table 3).

Experiments Involving The Use of Radiochemicals.

Tritiated uridine was applied to experimental plants to study the rates of synthesis of RNA during induction. Tritiated and ^{14}C thymidine were used to obtain a direct measure of apical cell generation times.

Radiochemicals used. The radio chemicals were supplied by the Radiochemical Centre, Amersham, England.

The nucleotide uridine -5 -H3 was used. It is converted/



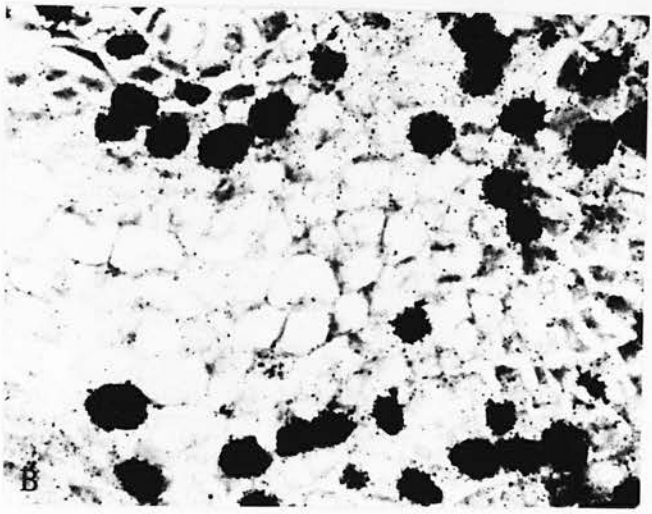


Fig. 9. Autoradiographs of Silene apical tissue:-

A. Showing incorporation of tritiated uridine into RNA.

B. Showing incorporation of tritiated thymidine into DNA (the nuclei).

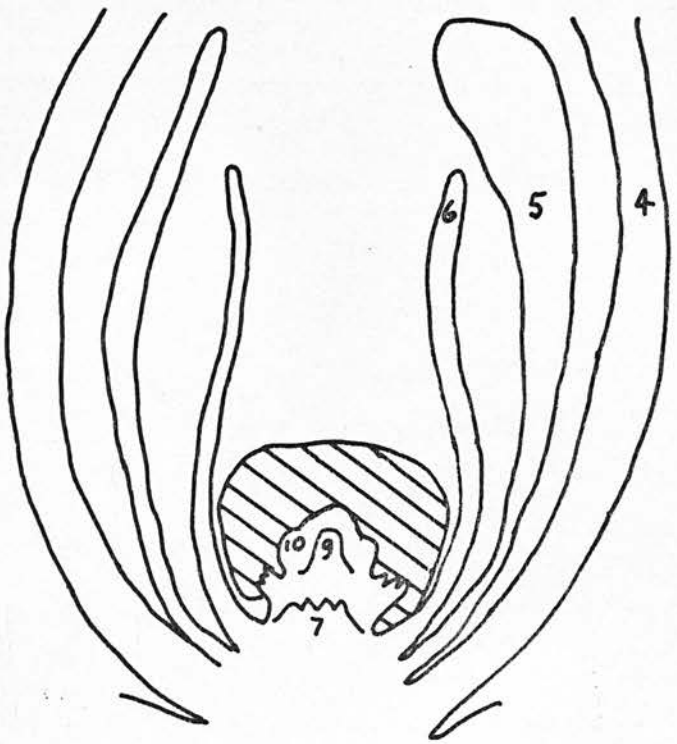


Fig. 10 Diagram of a long itudinal section of a Silene apex showing the method of application of radio-chemicals to the apex. The diagram shows the size of a 50 µl drop of liquid relative to the apex. The 3rd and 4th youngest leaf pairs, in this diagram leaf pairs 7 and 8 were removed by microsurgery prior to application of the liquid.

It is converted, in vivo, to nucleoside triphosphate and is then incorporated into RNA (Mahler and Cordes, 1967). Thymidine (Methyl - H3) and thymidine -2 - C14 were the radio-chemical forms of thymidine used. Thymidine is phosphorylated into thymidine triphosphate and then incorporated into DNA (Mahler and Cordes, 1967).

To check on the fate of labelled uridine and thymidine, sections of treated plants were incubated with deoxyribonuclease and ribonuclease. Autoradiographs were prepared. Photomicrographs are shown in fig. 9 and confirm that tritiated uridine is incorporated into RNA alone and tritiated and ^{14}C thymidine only into DNA.

Application of Radio Chemicals. Application of labelled compounds to plant shoot apices has proven a difficulty to previous investigators (Gifford, 1960; Gifford et al, 1963; Bernier, 1964; Nougarede, 1967). Various methods were tried, including application to the leaves, to the apex, and to the ends of cut leaves. Injection into the stem, application after pretreatment of the apex with various detergents, and application in solution with polyethylene glycol were also tried. The only satisfactory method was found to be a modification of a method described by Gifford (1960) and Bernier and Bronchart (1963). Two young leaf pairs were removed with fine forceps and the isotope applied as a aqueous drop (0·05ml. approximately) which completely immersed the apex and the newly cut surfaces. The leaf pairs removed were usually the third and fourth youngest pairs. Satisfactory labelling of the apex only occured if the drop was left on the apex for at least two hours. Examination of autoradiographs of sections of apices labelled for a shorter period indicated that the nucleotides were taken up through the severed leaves and then migrated to the apex. Fig. 10 illustrates the method of application. The surgery was performed under a stereomicroscope.

Any accidental damage which occured during surgery was apparent when the autoradiographs were examined and any visibly damaged apices/

apices were rejected. To check whether the removal of two leaf pairs from experimental plants caused any lasting effects upon growth some plants were allowed to grow on for a period after treatment. Subsequent growth was little affected by treatment and 7 LD plants eventually flowered showing that the removal of two leaf pairs did not seriously affect subsequent growth (provided the apex was not damaged).

When a dissected plant was left for some time before the application of label desiccation of the apex was prevented by placing a water-saturated cotton wool plug on the apex.

Fixation, tissue preparation and sectioning. In most cases radiochemicals were applied as a two-hour terminal label followed by immediate fixation. 80% aqueous ethanol was used as the fixative in the experiment on cell generation time. This fixative caused slight nuclear contraction. This reduction in nuclear size made subsequent autoradiographic identification of labelled nuclei easier.

Apart from this change in fixation the remainder of the embedding procedure was similar to that described earlier. Sections were cut 5 μm thick since thin sections increase the resolution of autoradiographic methods.

Autoradiography. All microscope slides used for autoradiography were prepared by "subbing" using the technique of Wall (1929). The "subbing" procedure was commenced by soaking new slides in cleaning solution (Potassium dichromate 100g; sulphuric acid 100ml; water 900ml) for twenty-four hours.

The slides were then rinsed first for about eighteen hours in running tap water and then in distilled water. Finally they were dipped in subbing fluid (0·5g chrom-alum; 5·0g gelatin; in 1 litre of water) at 25°C, and allowed to dry in a dust free place.

Sections were floated on warm water (40°C) and collected on these slides to which they stuck when dry. The sections were dewaxed/

dewaxed and hydrated and then dipped one by one into a 50% aqueous mixture of the appropriate nuclear emulsion maintained at 48°C in a vial. The dipping was carried out in the dark. The slides were allowed to drain, left to thoroughly dry, again in the dark, and then stored in light-tight boxes in the presence of desiccator at 4°C for about ten days.

The emulsion was then developed. The developer used was Ilford ID19 (Ilford Technical information sheet D20.3) diluted with an equal quantity of water.

The slides were agitated at 20°C in the developer solution for six minutes in the dark and then washed for one minute in a 2% acetic acid stopbath. The emulsion was then fixed in Ilford hypana fixer for six minutes at room temperature. Finally the autoradiographs were washed for two hours in running tap water and stained with methyl green-pyronin staining solution.

This post-staining method when used under appropriate conditions stained most of the sections light pink and nuclei blue-green allowing the autoradiographic grains to be easily seen.

The staining solution was made up according to Casselman (1959). The staining procedure was carried out by rinsing the emulsion-coated slides in distilled water, blotting them dry and placing them for one minute in the staining solution. The sections were again quickly rinsed in distilled water, dehydrated in acetone (since the stain is washed out in alcohol) rinsed in xylene, and mounted in Canada balsam.

Experiments involving tritiated uridine.

1μ Ci of tritiated uridine at a specific activity of 27 Ci. m mole^{-1} was applied in an aqueous drop of 0·05ml to each prepared apex. The uridine solution was applied two hours after the start of the high intensity light period and the plants sampled and fixed two hours later.

Sections were cut and the slides coated with Ilford K2 emulsion/

TABLE 4. The relationship between silver grain number and light scattering values of autoradiographs under dark-field illumination

*Number of silver grains	**light scattering value
175	8.4
94	4.5
75	3.5
30	1.4

*Estimated by counting the silver grains present in a microscope field.

**Value obtained for light scattering caused by silver grains under dark field illumination by means of a cadmium sulphide photocell.

emulsion, which records β - particles from tritium with high efficiency. An estimate of RNA synthetic rates in the apex was obtained by counting silver grains above apices of these autoradiographs once the emulsion had been developed and fixed and the sections stained and mounted.

Grain Counting. Since counting grains individually was extremely tedious an automatic method was devised. The method depended upon the scattering of light by grains under transmitted-light dark-field illumination on a Vickers Patholux microscope. Under this illumination silver grains appear as bright specks on a dark background. This is because the beam of light is so placed that it does not enter the objective lens, however when the light falls on a silver grain it is reflected and scattered from its original path, some into the optical axis of the microscope. Stained tissue merely absorbs some of the light of the appropriate wavelength but does not alter its path and so does not cause any light to enter the objective lens. The amount of light transmitted therefore is directly proportional to the number of grains in the optical axis.

The relative light scattering was measured in the median section by means of a cadmium sulphide photocell incorporated into the microscope. Electronic signal amplification was necessary because of the low amounts of current involved. Table 4 shows relative values for light scattering caused by different numbers of silver grains in the optical axis and confirms the linearity of the relationship between the number of silver grains present and the light scattering values obtained.

Unfortunately, because a limited number of objective lenses were available and it was not possible to reduce the field size on the Vickers Patholox microscope, measurements of relative grain densities within an apex were not possible.

Experiments involving tritiated and ^{14}C - thymidine.

The purpose of this experiment was to measure directly the cell/

cell generation time in apical tissue using a method similar to that of Wimber and Quastler (1963).

The experiment was carried out by applying ^{14}C - thymidine at "time zero" to prepared apices. After two hours the ^{14}C - thymidine was washed from the plants with water and a water saturated cotton wool plug placed on the apex until further treatment (except for early samples where tritium treatment followed directly). At hourly intervals from "time zero" to 30 hours tritiated thymidine was applied to plants. Two hours after the application of tritiated thymidine the plants were sampled and fixed.

Sections were cut and autoradiographs prepared which allowed identification of nuclei labelled with tritium, ^{14}C or with both isotopes.

Preparation of plants was commenced at the start of the high intensity light period. Because of the time involved in the preparation of a large number of plants the application of the first label was not possible until the end of the high intensity light period. 0·2 μ Ci ^{14}C - thymidine at a specific activity of 59 curies. mole $^{-1}$ was applied to each apex as an aqueous drop of 0·05ml. ^{14}C -thymidine was applied as the first isotope since few of the higher energy β^- - particles emitted by the ^{14}C isotope would terminate inside the nucleus than would be the case for the lower energy β-particles emitted by tritium. Since tritium was applied as a terminal label any deleterious effect upon the nucleus would be minimised. 2μ Ci of tritiated thymidine at a specific activity of 22 Curies. m mole $^{-1}$ in an aqueous drop of 0·05ml was applied to each apex.

The plants were fixed in 80% aqueous ethanol and longitudinal serial sections cut through the apex 5μm thick.

Autoradiography For Plants Labelled With Tritium and ^{14}C. The slides were coated with Ilford K2 emulsion at 48°C in a 50% aqueous mixture. This emulsion records tritium emissions with high efficiency, but due to its relative insensitivity records very few of the higher energy/

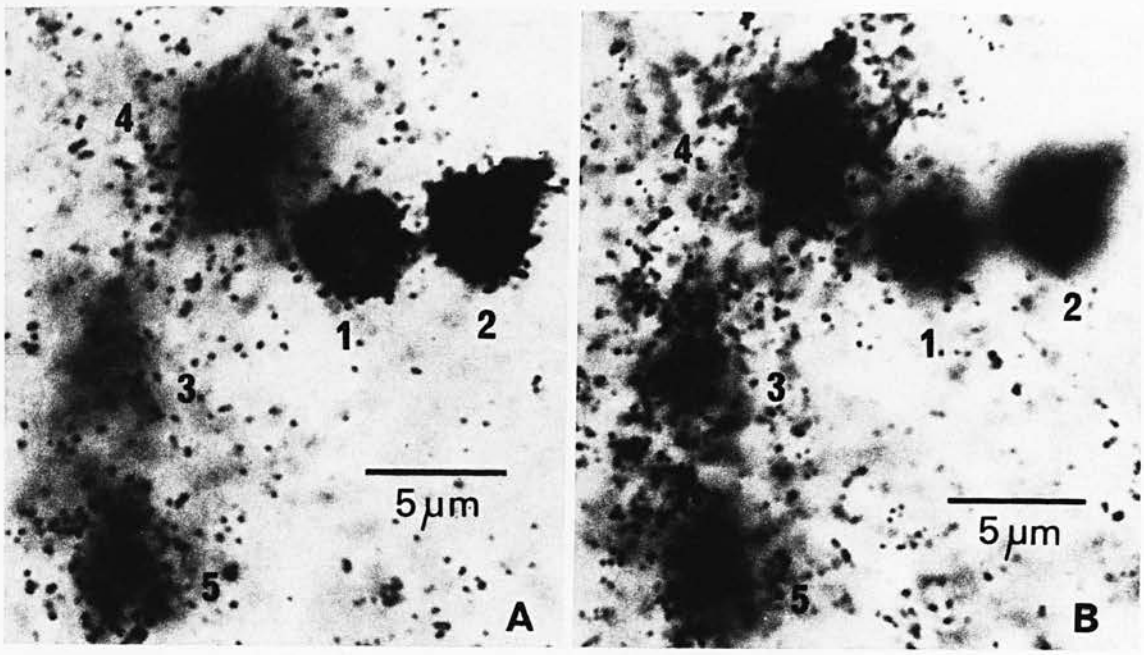


Fig. 11 Autoradiographs of labelled nuclei. A and B show the same field of view, A with the lower emulsion in focus and B with silver grains in the upper emulsion layer in focus. In A silver grains exposed by ^{3}H-labelled nuclei are in focus while those in the upper emulsion are indistinct. In B those in the lower emulsion are indistinct but those in the upper emulsion are in focus. Nuclei 1 and 2 are singly labelled with ^{3}H, nucleus 3 is singly labelled with ^{14}C and nuclei 4 and 5 which have exposed grains in both emulsion layers are thought to be doubly labelled.

energy ^{14}C β particles. After one hour this K2 emulsion had dried. A second layer of liquid emulsion was then applied to the slides. This second layer was of Ilford L4 emulsion applied at 42°C in a 75% aqueous mixture giving a relatively thick layer. This emulsion is more sensitive than K2 and will record β particles from ^{14}C as tracks of silver grains. β particles emitted by tritium travel a maximum of 3 µm and since relatively few penetrated the K2 emulsion layer to expose grains in the L4 layer the K2 emulsion was presumed to be about 3 µm thick (fig. 11). The emulsions were developed after one week's exposure and the sections were stained with methyl green-pyronin.

This isotope source was identified by the presence of grains in the different emulsion layers (which appear in different focal planes under a high power objective), and also by the more diffuse scatter of grains caused by a ^{14}C source. A further aid to source identification was that the L4 silver grains were slightly smaller than those in the K2 emulsion layer. Fig. 11 illustrates these points.

Micro Disc. Gel Electrophoresis of Proteins.

Apical protein extracts were subjected to polyacrylamide gel electrophoresis. A micro-gel method was used because this greatly increases the sensitivity of the method by concentrating the protein bands into a much smaller cross sectional area which will stain more darkly than would a less concentrated band in a conventional sized gel.

The micro-electrophoresis system described below was based on a sulphate-borate ion system developed by Neville (1971) for conventional sized gels. The micro method itself is a modification of systems described by Gainer (1971), Grossback (1965), and Hyden et al (1966).

The proteins were denatured into their constituent polypepide sub-units by sodium lauryl sulphate (SLS) extraction.

A stacking gel/

A stacking gel was used to preconcentrate the sample into bands before entry into the main gel. This increases the resolution of the method. The stacking gel had a final concentration of 4% acrylamide and the main gel a final concentration of 15% acrylamide.

Composition of gels and buffers.

The following stock solutions were prepared and stored for up to one month in the dark at 4°C. All chemicals used were BDH "analaR" grade, except for Sigma "Trizma" base (tris), Koch - light N, N, N^1, N^1 tetramethylethylenediamine (TEMED), and Eastman acrylamide and bisacrylamide which were both recrystalized from organic solvents before use. (see below).

Solution 1. Boric acid 1·24g; tris 2·42g; SLS 0·50g; to 500ml with water giving a pH of 8·64.

Solution 2. Tris 1·31g; sulphuric acid 0·29ml to 100ml with water giving a pH of 6·1.

Solution 3. Tris 10.35g; hydrochloric acid 2·21ml; to 250ml with water giving a pH of 8·47.

Solution 4. Acrylamide 3·0g; bisacrylamide 200mg; water 6·8ml.

Solution 5. Acrylamide 3·3g; bisacrylamide 30mg. water 6·7ml.

Solution 6. Ammonium persulphate 80mg; water 2·0ml.

Solution 7. TEMED 0·2ml; water 2·0ml.

The gel solutions and reservoir buffers were prepared from these stock solutions.

Lower gel Solution 3, 0·5ml; solution 5, 0·31ml; water, 0·135ml; solution 7, 7·5 µl.

This solution was degassed under a vacuum (to prevent air bubbles forming during polymerization) and then 0·05ml solution 6 added.

Upper gel. Solution 2, 0·5ml; solution 4, 0·1ml; water, 0·375ml; solution 7, 15 µl. This solution/

This solution was then degassed and 0·01ml solution 6 added.

Lower reservoir buffer. Solution 3 mixed 1: 1 volume/volume with water.

Upper reservoir buffer. Solution 1.

Recrystallization of Acrylamide and Bisacrylamide.

The monomers acrylamide and bisacrylamide were recrystallized from organic solvents before the preparation of the solutions just described. The method used was that of Loening (1967).

70g of acrylamide was dissolved in 1 litre of chloroform at 50°C. The solution was filtered hot without suction. The filtrate was cooled slowly (overnight) to 0°C and the crystals recovered by filtration in a chilled funnel. The crystals were washed briefly with cold chloroform and dried first in air and then in a vacuum.

Bisacrylamide was recrystallized from acetone, 12g being dissolved in one litre using a similar procedure.

Preparation of Capillary Gels.

10cm long pre cision-bore capillary tubes (Drummond 50 µl "microcaps") were stood overnight in 10% chromic/10% sulphuric acid cleaning fluid. The tubes were then thoroughly washed in distilled water and then immersed, under a vacuum, for two minutes in siliconizing fluid (2½% dimethyl dichlorosilene in carbon tetrachloride), dried by suction and stored in stoppered tubes until use.

Freshly prepared lower gel solution (see below) was introduced into the micro-gel tube from the bottom by mouth suction through a fine plastic tube attached to the gel capillary until the miniscus of the poly-acrylamide was 3cm. from the top of the capillary tube. The level was maintained by biting the plastic tube and the gel tube was positioned vertically by pushing the lower end into plasticine. The plastic tube was removed and the lower gel allowed to set at room temperature (20 - 22°C). Polymerization was complete in 15 - 30 minutes. Isobutanol/

Isobutanol was layered on top of the setting lower gel to flatten the meniscus. After polymerization the isobutanol was removed by means of a hand drawn glass tube attached to a 50 µl Hamilton syringe. The top of the lower gel was then washed with upper gel solution (see below) without ammonium per sulphate catalyst. This was removed and complete upper gel (stacking gel) solution was then injected through a clean glass tube until its meniscus was 1·5cm from the top of the tube. Polymerization of the upper gel took 10 - 20 minutes.

Prepared gels were stored for up to two hours without any apparent detrimental affects.

Preparation of Protein Sample.

The principal reasons for using a SLS based extraction medium was that no satisfactory method was found for homogenizing very small quantities of tissue in very small volumes of extracting medium. Using the SLS system it was possible to extract protein simply by heating intact excised apices in SLS extracting solution, (Gainer, 1971). It was shown empirically that extracted protein from ten apices gave a satisfactory band pattern following electrophoresis. These apices consisted of apical dome tissue and included no primordial tissue.

Apices were dissected from experimental plants using fine needles under a stereo-microscope and were then stored in 4 µl of extracting solution in small glass vessels until a sufficient number for an experiment was obtained.

The extracting solution contained 1% SLS, 6M urea, and 1% mercaptoethanol in water. Dissected apices were stored in this medium for up to 2 hours.

The protein extract was prepared for electrophoresis by heating at 90°C for 1 minute and a few grains of bromo-phenol blue dye powder was then added to the medium to act as a marker dye. The samples were then immediately applied on to the gel by means of a 5 µl Hamilton syringe.

Electrophoresis./

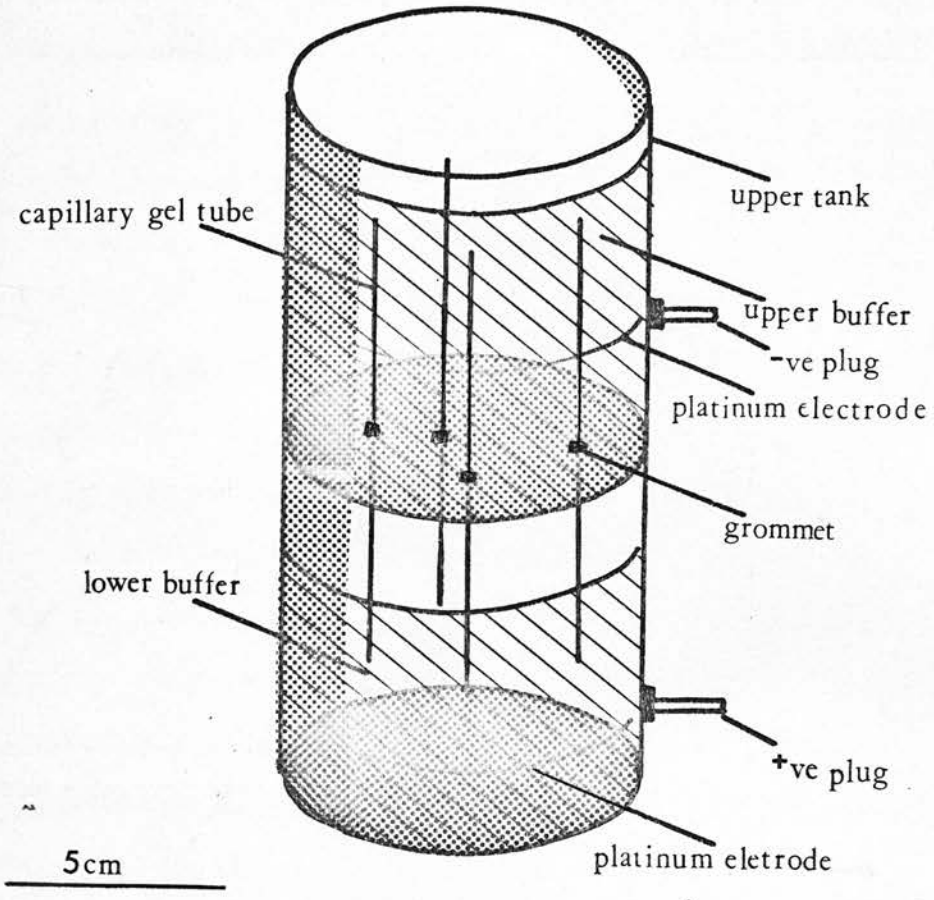


Fig. 12. Diagram showing the type of perspex gel tank used in the electrophoretic separation of protein extracts from Silene apical tissue.

Electrophoresis.

Electrophoresis was carried out in specially constructed perspex tanks each of which held four micro gel tubes (fig. 12). The upper reservoir held about 20ml buffer and the lower reservoir about 40ml buffer. The electrodes were platinum wire and the voltage source was a regulated high voltage Vokam Shandon power supply type 2541.

The gels were prepared for electrophoresis by cutting off the bottom centimetre of glass tube thus exposing 1cm of the lower gel to the lower reservoir buffer. The top of the upper gel was flushed with upper reservoir buffer with a 5 µl Hamilton syringe, then filled with this buffer and the tubes installed in the tanks. Loading with protein solution was also accomplished with a 5 µl Hamilton syringe. The urea and sucrose in the extracting solution made this solution more dense than the upper reservoir buffer and so facilitated loading.

The positive electrode was connected to the bottom reservoir and the negative electrode to the top and a constant potential difference of 120 volts maintained across the gels.

The marker dye was seen to concentrate quickly at the bottom of the 4% stacking gel and then to run as a fairly discrete band through the 15% main gel. When the marker dye was about 1·5cm from the bottom of the tube (about two hours after the start of electrophoresis) the power supply was switched off.

Staining and Destaining.

The gel tubes were removed from the tanks and the gels pushed out of the microcap capillary tubes by a close fitting stainless steel wire directly into staining solution. Coomassie brilliant blue solution (0·2% coomassie blue; 50% methanol; 7% Acetic acid; in water) was used to stain the protein in the gels. The gels were stained for 4 - 16 hours after which they were destained for 1 - 2 days in destaining/

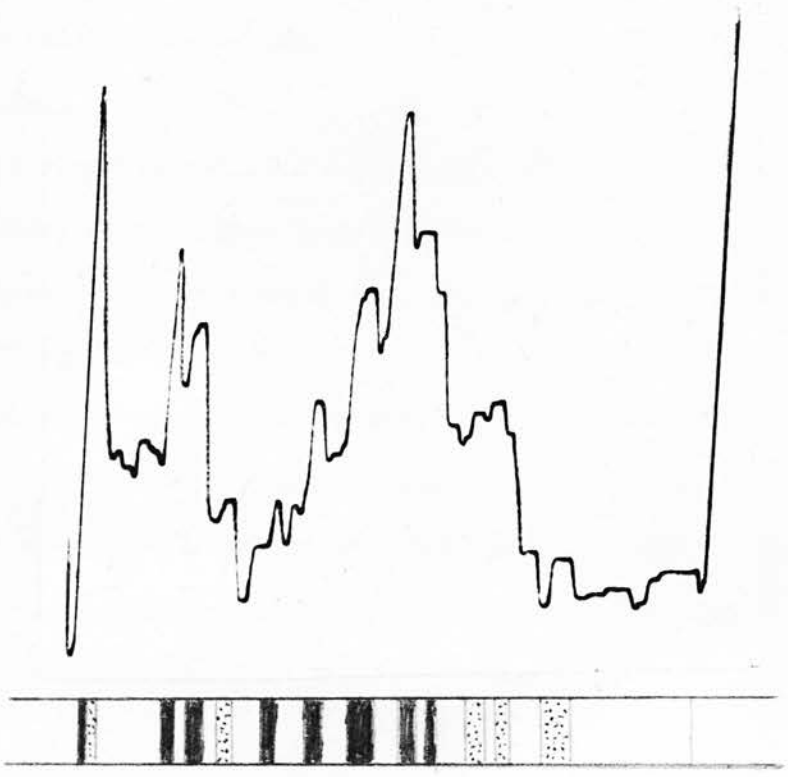


Fig. 12B Method of presenting the results of the electrophoretic separation on polacrylamide gels of the proteins of Silene apical domes.

A. A densitometric trace from a gel

B. Interpretative diagram prepared from a visual examination of the gel.

destaining solution (5% methanol; 7% Acetic acid, in water).

The gels were then sucked into precision bore tubes (Sherwood "BLu-tip" micro-hematocrit capillary tubes) of a slightly greater diameter than those in which the gels were run. The gels, in distilled water were then sealed in these tubes by heating the ends in a Bunsen flame, care being taken not to include any bubbles.

Scanning of Gels.

The gels were scanned in a Joyce-Loebl chromoscan. The gels, in capillary tubes, were aligned inside a conventional size gel carrier, surrounded with water and scanned with a slit light source using a post-gel filter N° 635 (635 nm).

To supplement the scans which were difficult to interpret because of the difficulty of loading similar quantities of extract on to different gels, drawings of the electrophoretic band patterns were prepared.

Fig. 12B illustrates the method involved in the preparation of these drawings of the electrophoretic band patterns.

PART III

RESULTS

Gross Morphological Events at the Apex During the 14 Days from the Start of Inductive Long Day Treatment

Plastochron

Silene plants were grown in short days and a uniform population, which were almost all in the early 7th plastochron, was selected when the plants were 28-days old (day 0). The plastochron-interval was measured by plotting the total number of leaf and primordial pairs present in samples of plants at various times from day 0 and calculating the interval from the slope of the line (fig. 13). This was done for plants maintained in SD and plants given 7 LD inductive treatment commencing on day 0.

This gave a value for the vegetative plastochron of $3\frac{1}{2}$ - 4 days during the 12-day period from day 0. The data from 7 LD plants indicate that the plastochron-interval was unaffected by the LD treatment during the limited period when 7 LD plants continued the production of leaf primordia (see below) and was also $3\frac{1}{2}$ - 4 days.

Morphological Development

Fig. 14 shows photomicrographs of the median longitudinal sections of a series of vegetative apices during the period of two plastochrons. The sections were cut in the plane of the even numbered leaf pairs. The development shown in fig. 14 is typical of growth at the apex of SD *Silene* plants during the 12-day period from day 0.

Fig. 15 shows photomicrographs of similar sections during days 0-12 of 7 LD Silene plants. As can be seen by comparison of fig. 14 with fig. 15 development at the apex appears very similar during the period of LD treatment and it was not until about day 8 when the apex of 7 LD plants begins to transform into a flower that differences appeared. The developmental sequence is summarized in fig. 16.

Out of a total of 87 LD plants measured 70 produced 2 pairs of leaves from day 0 before flower production and 17 produced 3 leaf pairs. Those which

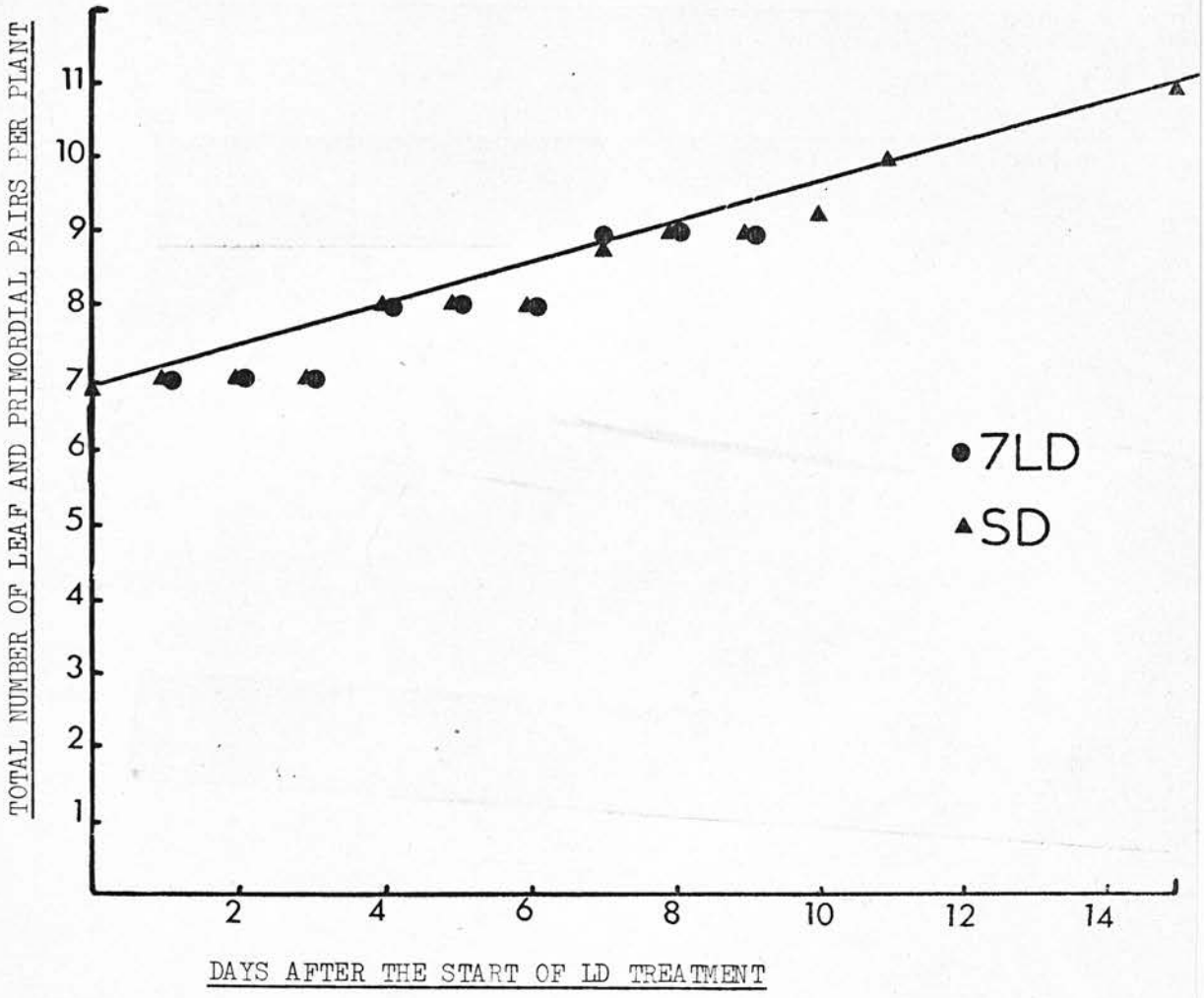


Fig. 13 The total number of leaf and primordial pairs present on SD and 7 LD plants from day 0. The plants were scored 1 hour after the start of the high intensity light period.

Each point is the mean of values from at least 5 plants.

7 LD plants commence flower morphogenesis once the 9th primordial pair has been produced.

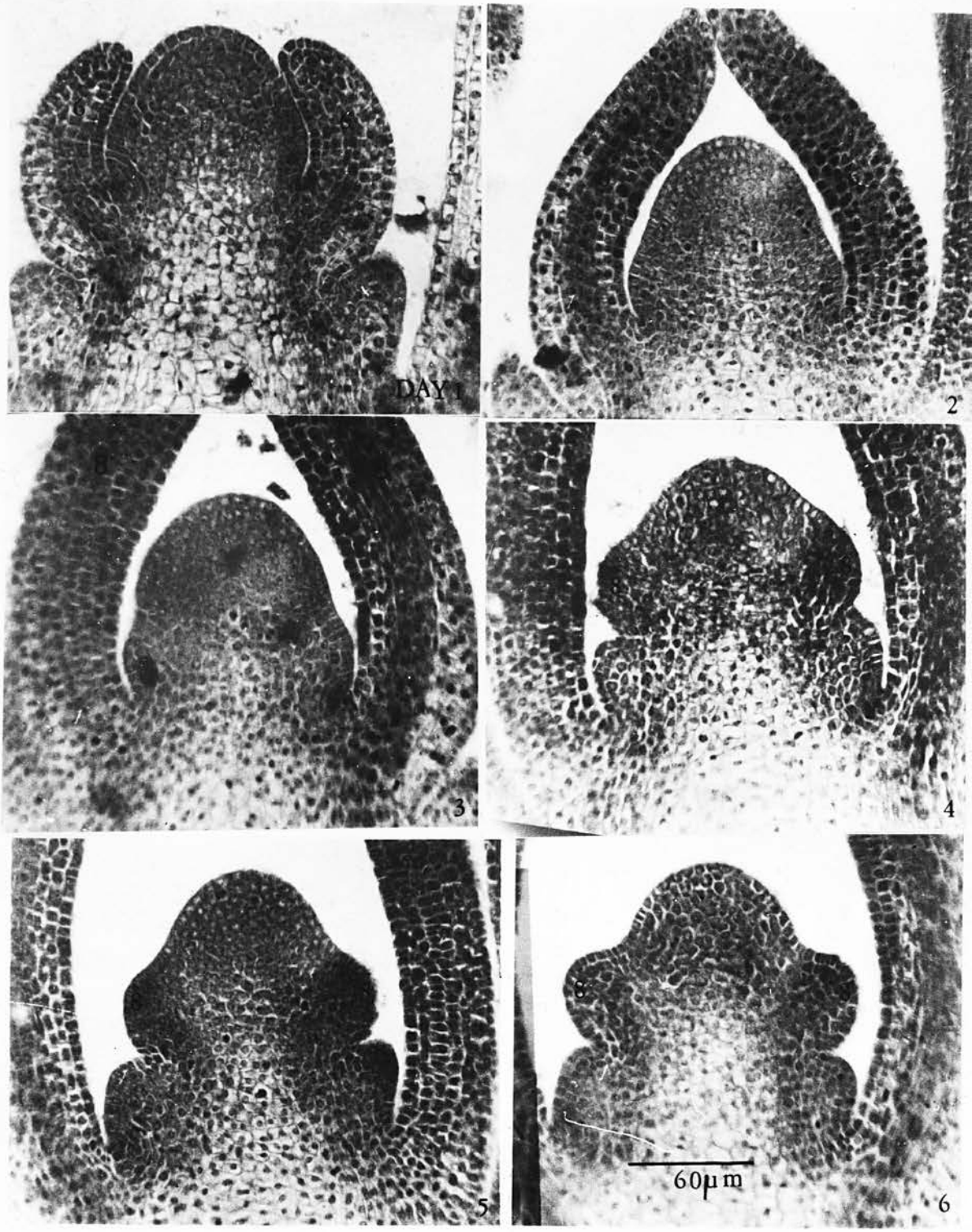


Fig. 14 Photomicrographs of the medium L.S. of vegetative Silene apices sectioned through the even numbered leaf and primordial pairs. The developmental sequence from day 1 (29 days old) to day 6 is shown. The leaf and plastochron pair is marked on the photographs.

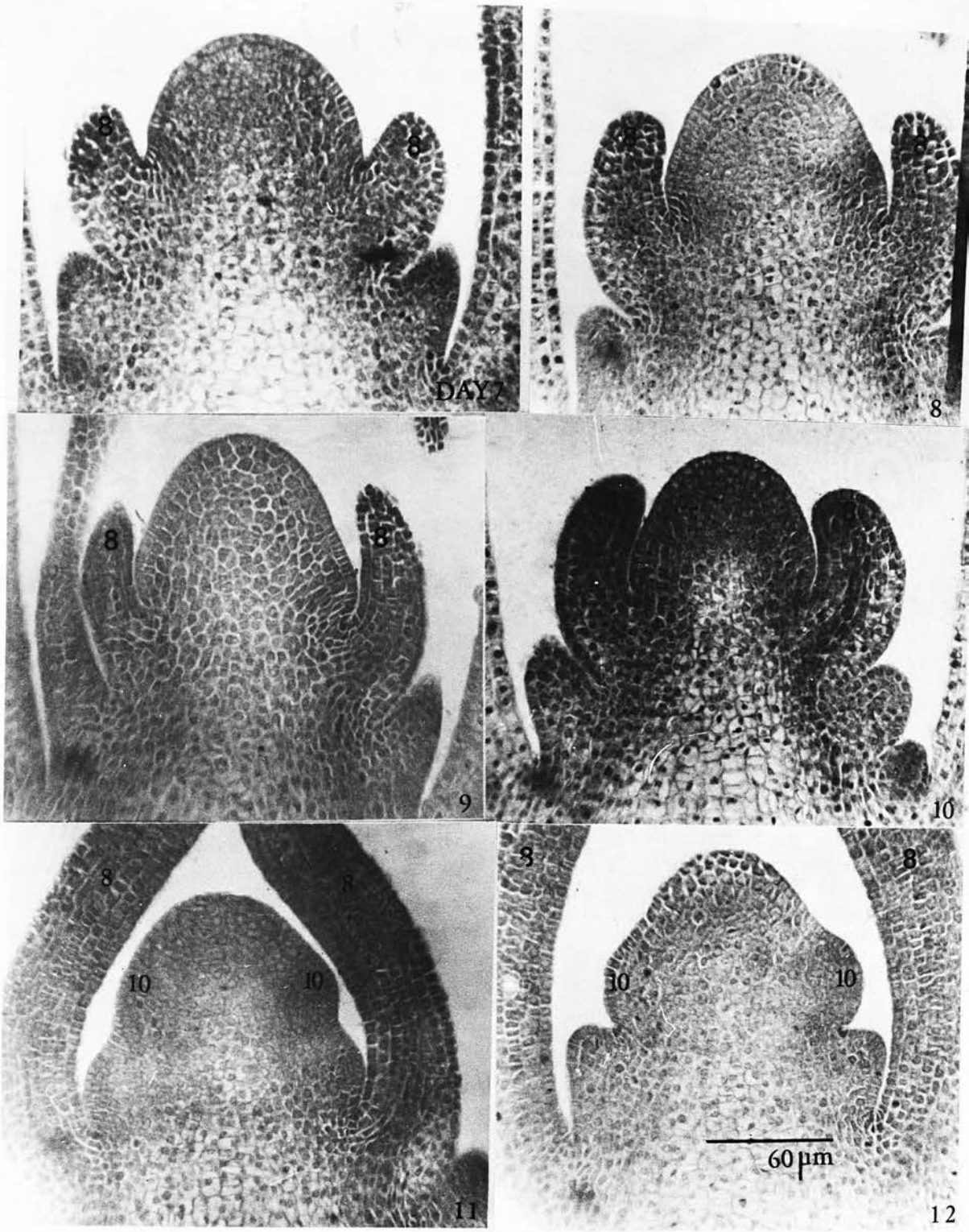


Fig. 14 (cont.) Photomicrographs of the median L.S. of vegetative Silene apices showing the developmental sequence from day 7-12. The leaf and plastochron pair number is marked on the photographs.

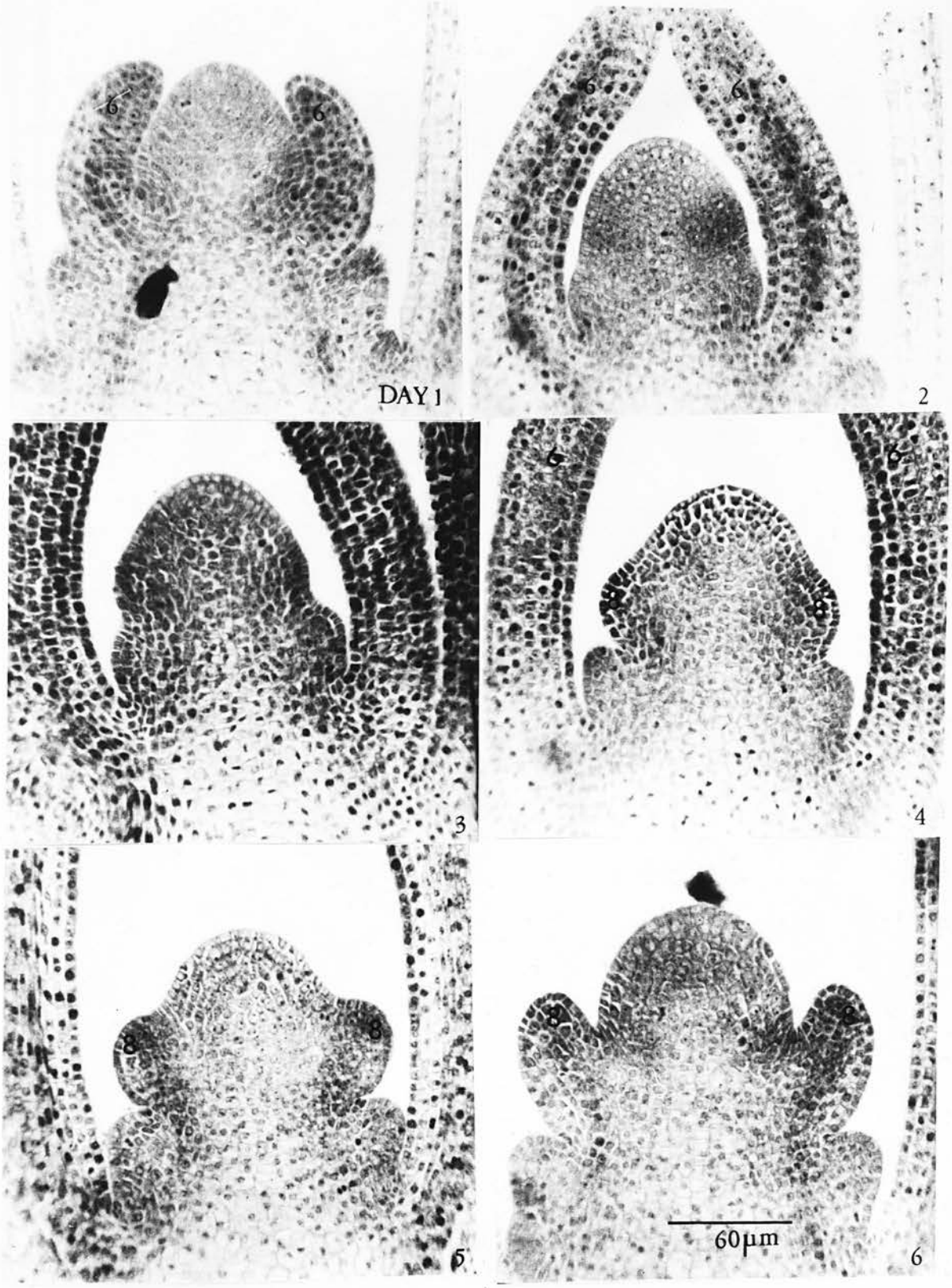


Fig. 15 Photomicrographs of median L.S. of 7 LD Silene apices from day 1-6. LD treatment commenced on day 0 and so these sections show the development in the apex during induction.

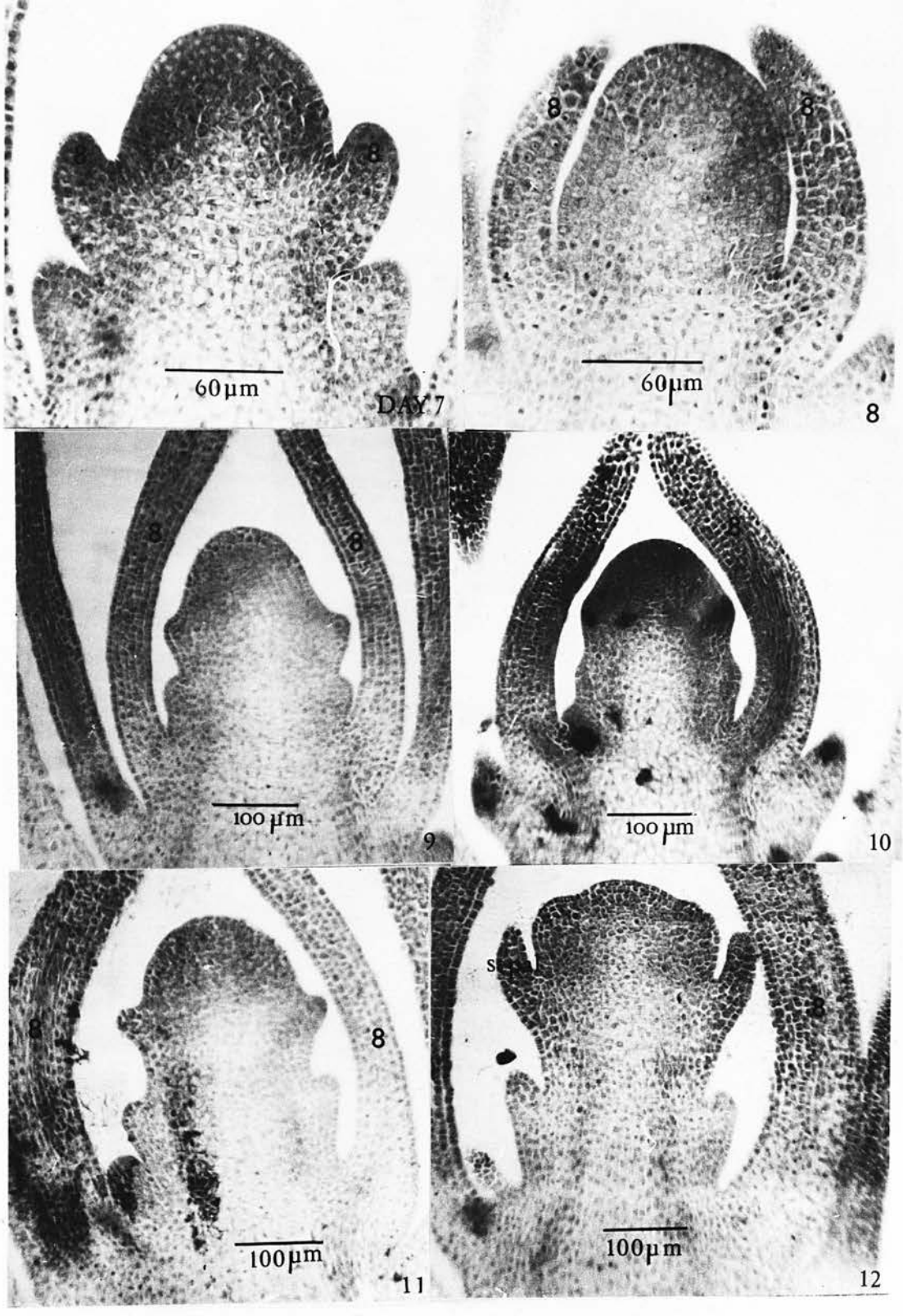


Fig. 15 (cont.) Photomicrographs of median L.S. of 7 LD Silene apices from day 7-12. These photographs show the development at the apex during early flower morphogenesis. Leaf and primordial pair numbers are marked on the photographs.

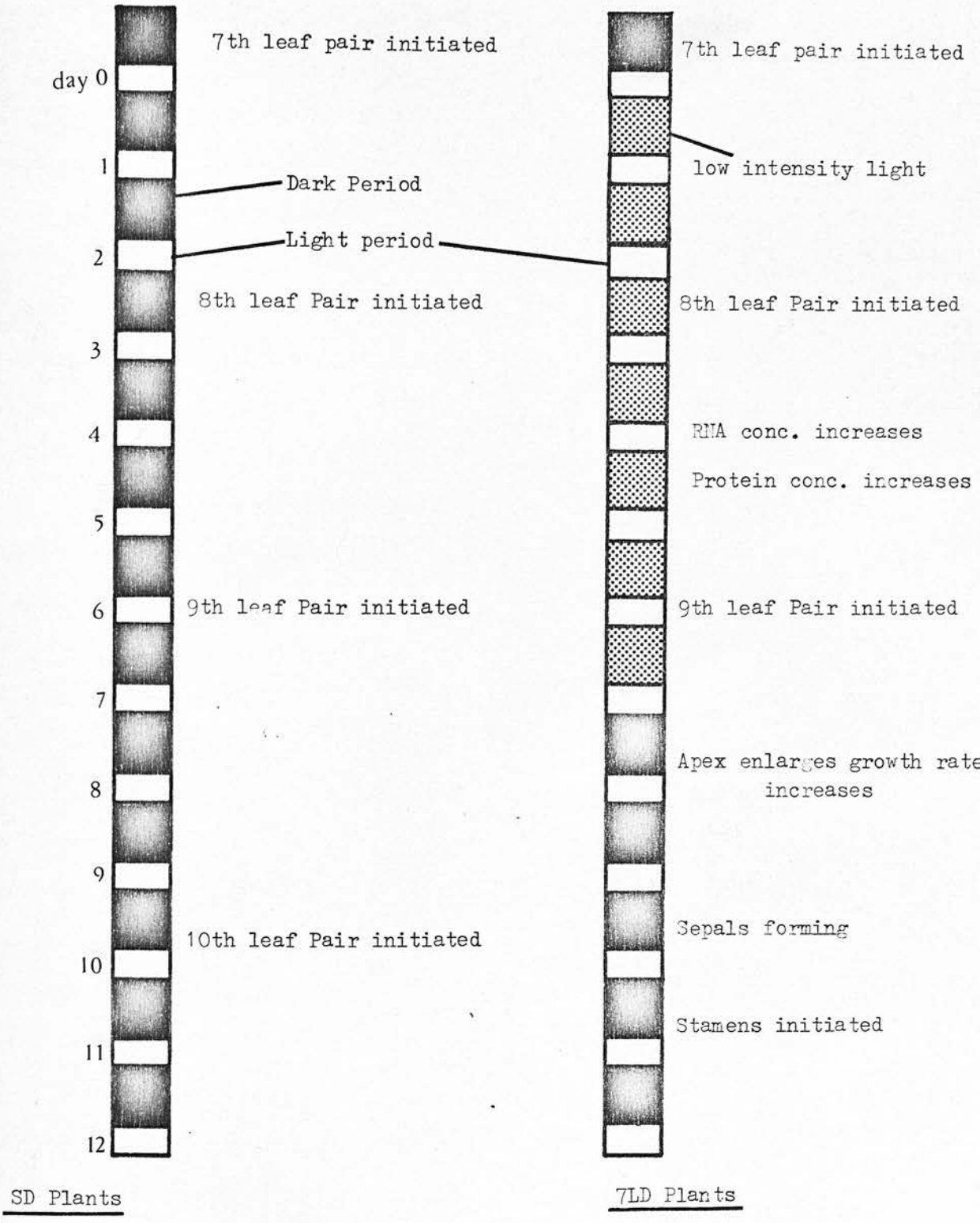


Fig. 16. Summary of main events in the SD and 7LD *Silene* apex from day 0-12.

produced 3 pairs appeared to be about one plastochron behind in development during early flower morphogenesis. Fig. 15 and 16 refer to 7 LD plants which produced 2 leaf pairs before flower morphogenesis.

Growth Rates in the Apex During Induction and Early Flower Morphogenesis

As has been described above the apices of SD and 7 LD plants appeared similar until about day 8 (fig. 14 and 15). From this time, however, there appeared to be an enlargement of the apex in 7 LD plants (fig. 15). To quantify this observation and to check whether the enlarged apex in induced plants was due to a greater production of tissue or to a re-distribution of growth or to a combination of both, the total amounts of tissue above leaf pairs 6, 7, 8 and 9 were measured during the 12 days following the start of LD treatment. Measurements were made on SD, 3 LD, 7LD plants and on plants exposed to LD throughout the period of sampling (continuous LD). Only the latter two treatments resulted in floral induction.

The results are shown in fig. 17. Each point represents the mean of readings from a sample of 3 plants. The values for the apical volume above leaf pair 6 and 7, and above leaf pair 8 on day 4 and 5, represent different parts of that total of tissue above the 6th leaf pair from one set of 3 plants per day. This is also the case for the values recorded for tissue above leaf pairs 8 and 9 from day 7.

It appears that the growth of the SD apex falls into 3 phases. There was an initial exponential phase. The increase in size of the apical dome above leaf pair 7 from day 0-3, above leaf pair 8 from day 4-7 and above pair 9 from day 7-10 all showed this initial exponential phase.(fig. 17). Following this exponential phase there was a lowering of the relative growth rate; this occurred over a 2-3 day period when a new primordial pair was differentiated within the tissue sample. This occurred from day 3-5 for the tissue above leaf pair 7, from day 7-8 for that above leaf pair 8, and from

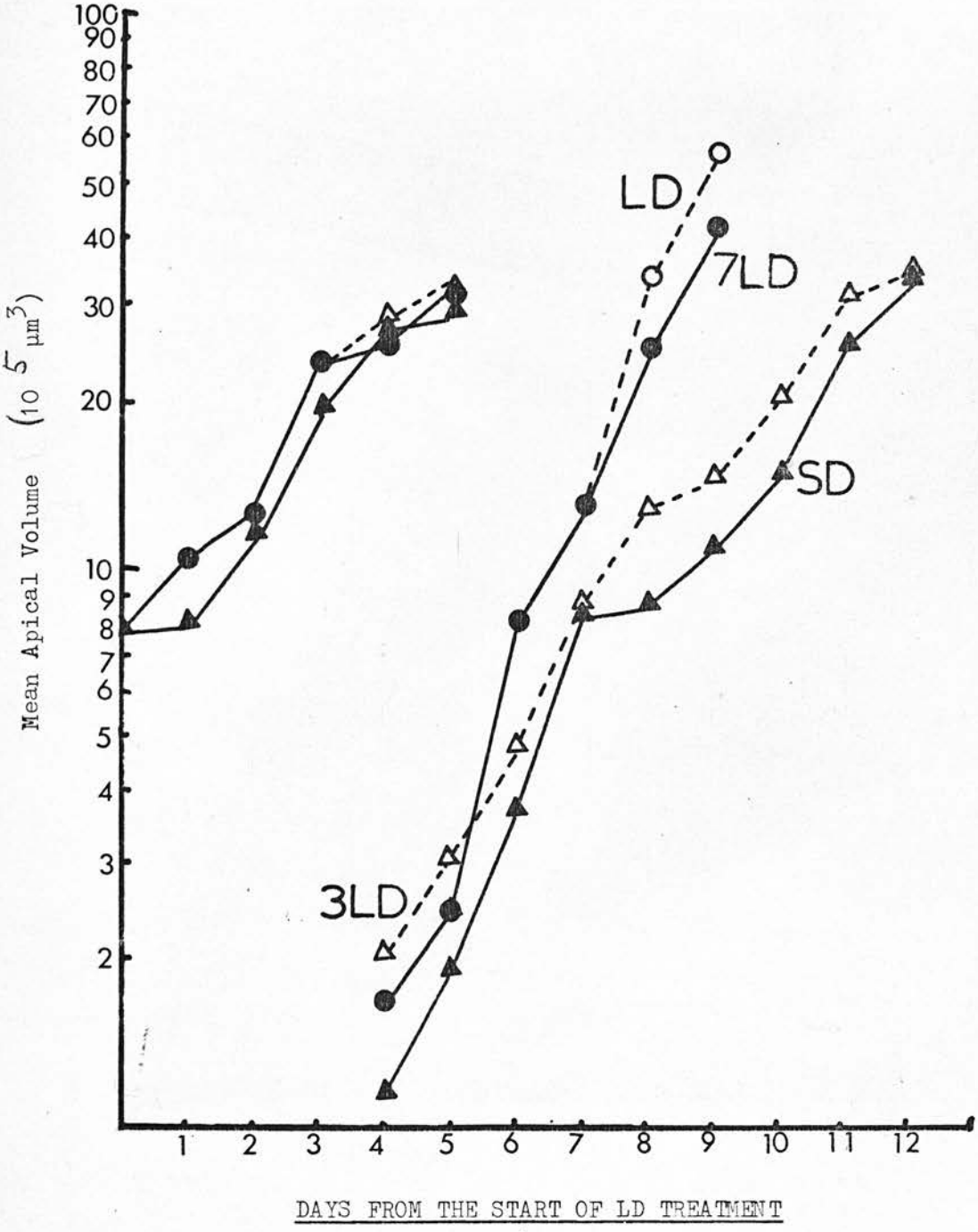


Fig. 17 Volume of the Silene shoot apex above primordial pairs 6 and 8 during the 12 days from the start of experimental treatment in plants maintained in SD, exposed to 3 LD to 7 LD and to LD throughout the period of sampling. Each point represents the mean of values from 3 plants.

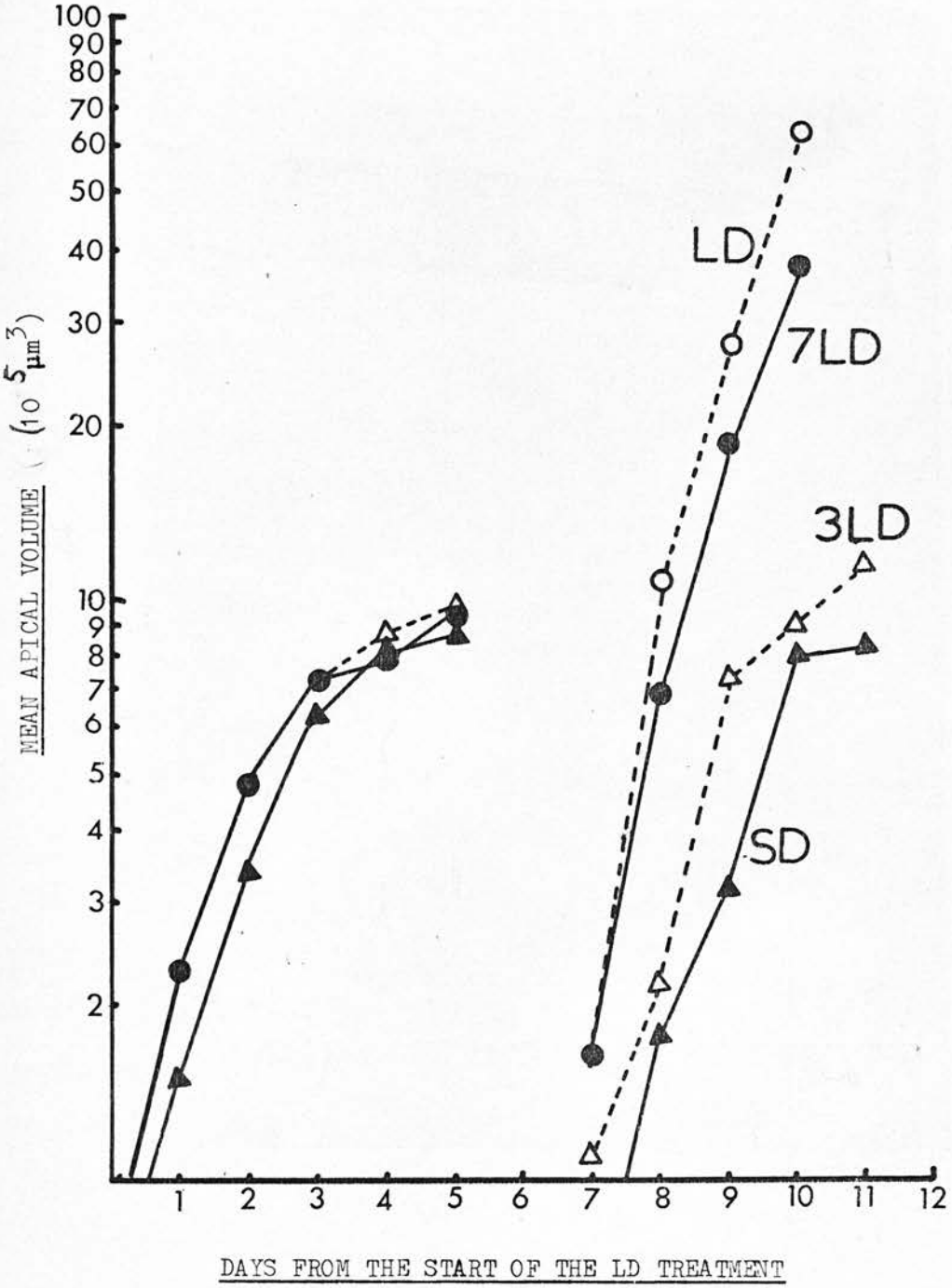


Fig. 17 (cont.) Volume of the Silene shoot apex above primordial pairs 7 and 9 during the 12 days from the start of experimental treatment in plants maintained in SD, exposed to 3 LD to 7 LD and to LD throughout the period of sampling. Each point represents the mean of values from 3 plants.

day 10-11 for that above leaf pair 9 (fig. 17). There then appeared to be a re-establishment of exponential growth (from day 1-5 in the tissue above leaf pair 6 and from day 8-12 in that above leaf pair 8) in the SD apex. The slope is however, less steep than that during the earlier exponential phase (fig. 17).

The rate of increase in cell number and volume of the apex in 7 LD plants and SD plants were very similar over the period from day 0-5. The data in fig. 17 suggests that the apical dome itself was growing exponentially throughout, with a volume doubling time of about 20 hours (calculated from the slope of the initial exponential growth phase). The lowering of the relative growth rate from this value was associated with the differentiation of a primordial pair within the sample of tissue being measured. This occurred on day 3, 7, and 10 and coincided with the slowing down of the relative growth rate, and caused a marked departure from the previous exponential phase. This reduction in the growth rate was probably due to the cessation or near cessation of growth of the tissue between the newly formed primordia (i.e. the nodal tissue). Fig. 18 shows a camera lucida drawing of a long itudinal section through the apical region of a vegetative plant on day 9. From this drawing of a typical plant it can be seen that the vertical distance between the leaf axils apparently does not increase down the stem. Since the tissue being considered (above leaf pair 8 on day 7-9 in SD plants) contained a relatively large part of nodal tissue which had apparently almost stopped growing this may account for the reduction in the relative overall growth rate during this period. As has been described exponential growth was then re-established (at a lower rate). This was probably due to the decrease in relative size of the more slowly dividing nodal tissue compared with the dividing tissue of the dome, and the now larger (2-3 day old) primordia. From fig. 17 there is an indication that when a second primordial pair was differentiated within the sample being measured there was again a reduction in the relative growth rate. This is shown in SD tissue above leaf pair 6 on day 4-5 when the 8th

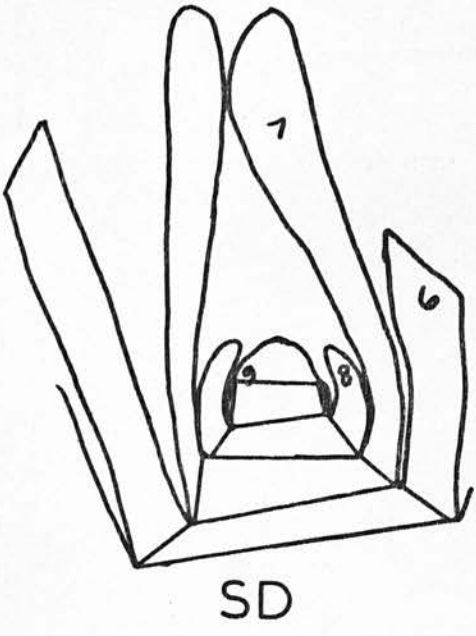


FIG 18

200µm

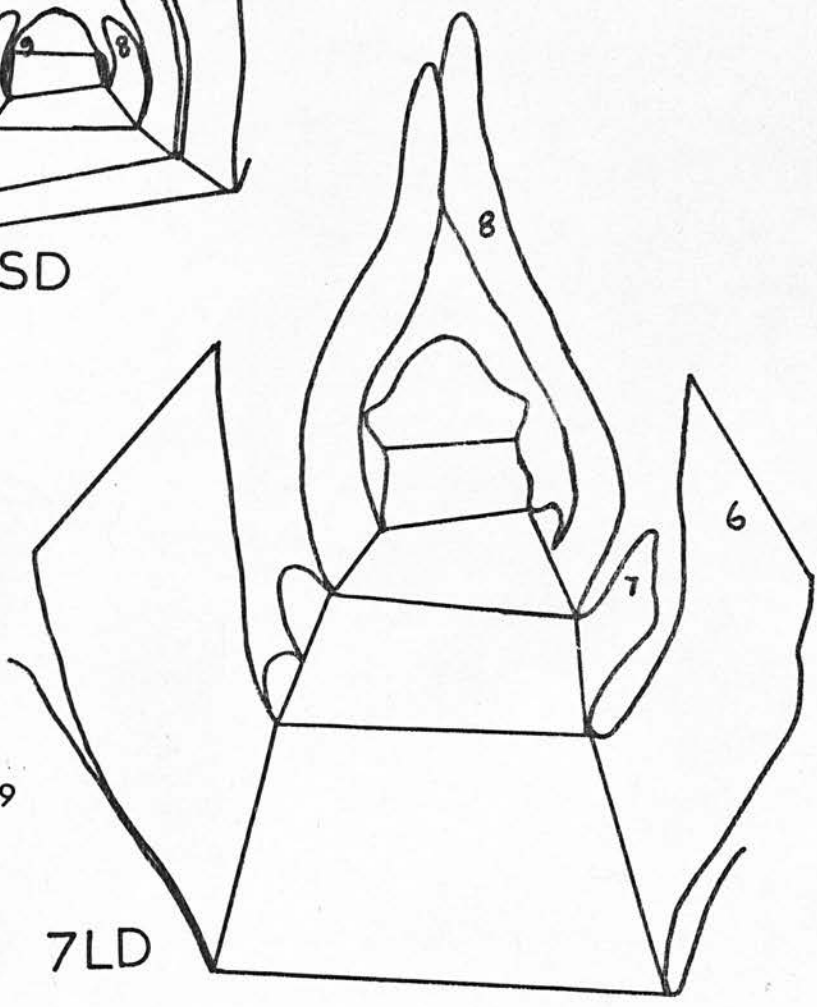


FIG 19

Fig. 18 and 19 Camera lucida drawings to the same scale of the median longitudinal section of Silene apices exposed to 7 LD and maintained in SD.

The plants were fixed 9 days after the start of experimental treatment and the sections cut in the plane of the even leaf and primordial pairs.

The numbers on the drawings represent the leaf pair number.

primordial pair was produced, and possibly in that tissue above leaf pair 8 on days 11-12 when the 10th primordial pair was cut off.

The growth rate of apical tissue in 7 LD plants began to diverge markedly from that in SD plants around day 6-7. The initial exponential growth phase of tissue above leaf pair 8 (day 4-6) in 7 LD plants was more or less similar to that in SD plants at most being perhaps very slightly greater. There was then (day 6-7) evidence of a slight reduction in growth rate in tissue above leaf pair 8 when the 9th primordial pair was cut off, however, this reduction in the growth rate was not so marked as in the vegetative apex. This continuation of almost undiminished exponential growth can be explained by reference to fig. 19. This fig. shows a camera lucida drawing of a sectioned 7 LD apex on day 9 (to the same scale as fig. 18). It can be seen from this drawing that the tissue between the young primordial pairs in the 7 LD plant did not slow down in its rate of enlargement when the young primordia were formed but went on increasing in size. The vertical distance between successive primordial pairs increases with increasing distance from the tip. This tissue includes nodal and inter-nodal tissue (one of the characteristic effects of LD treatment on Silene was an increase in internode length).

Following the slight break in exponential growth from day 7-8 in the tissue above leaf pair 8 in LD plants (fig. 17) an exponential growth rate was re-established. This re-established exponential growth rate in 7 LD plants was greater than in comparable tissue of SD plants, with an overall tissue doubling time similar to that in the dome itself and still about 20 hours. The growth rate of tissue above leaf pair 9 (day 7-10) in continuous LD and 7 LD plants showed a totally new characteristic; here the slope of the plot steepened to give a calculated volume doubling time of about 11 hours. Since this tissue (i.e. that above leaf pair 9, in which the absolute volume doubling time was reduced) was included in that sample above leaf pair 8 (from day 7-9) the increase in growth rate of the dome must contribute to the maintenance of

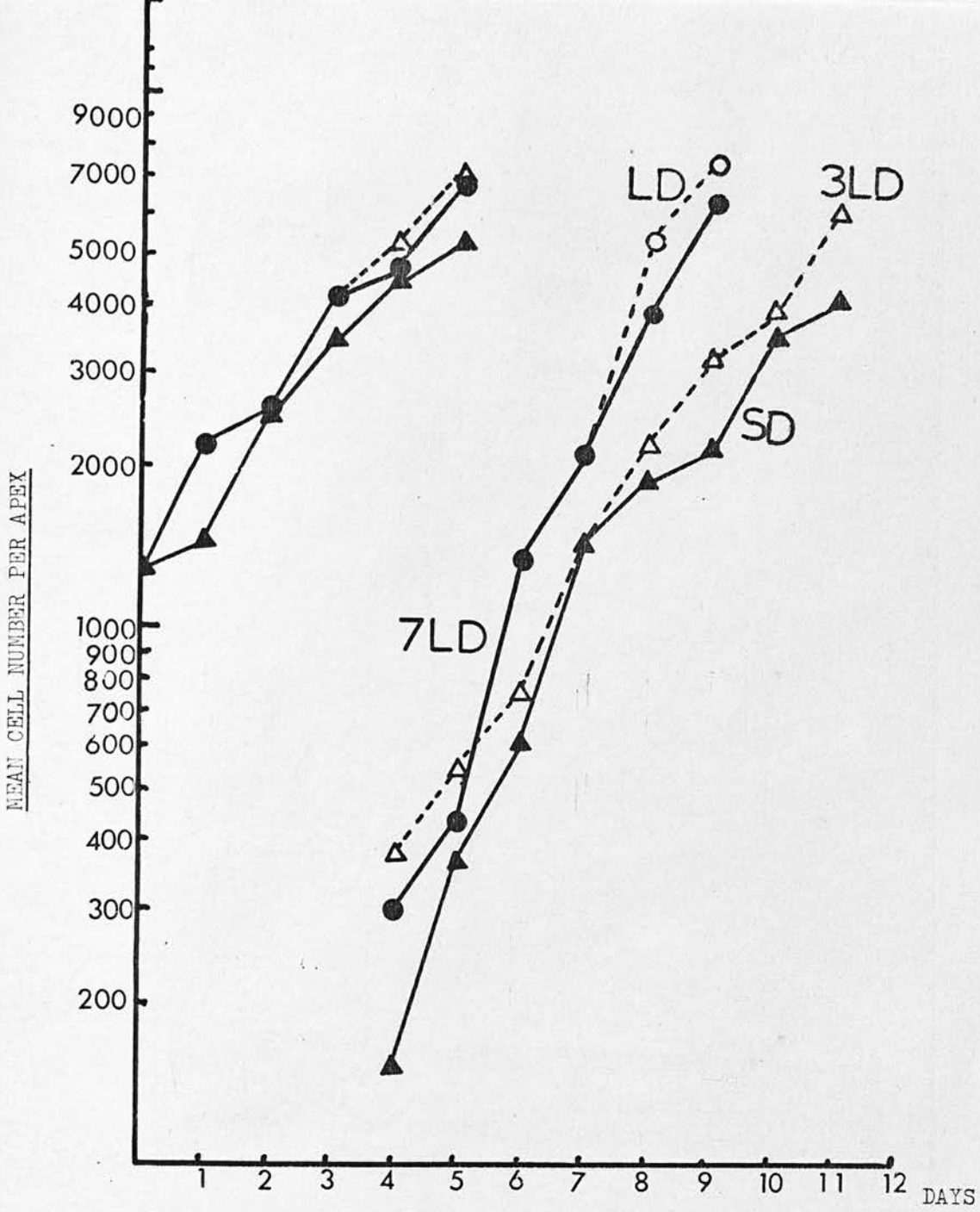


Fig. 20. Mean cell number of the Silene apex above primordial pairs 6 and 8 during the 11 days from the start of experimental treatment in plants maintained in SD, exposed to 3 LD, to 7 LD and to LD throughout the period of sampling.
Each point is the mean of values from 3 plants.

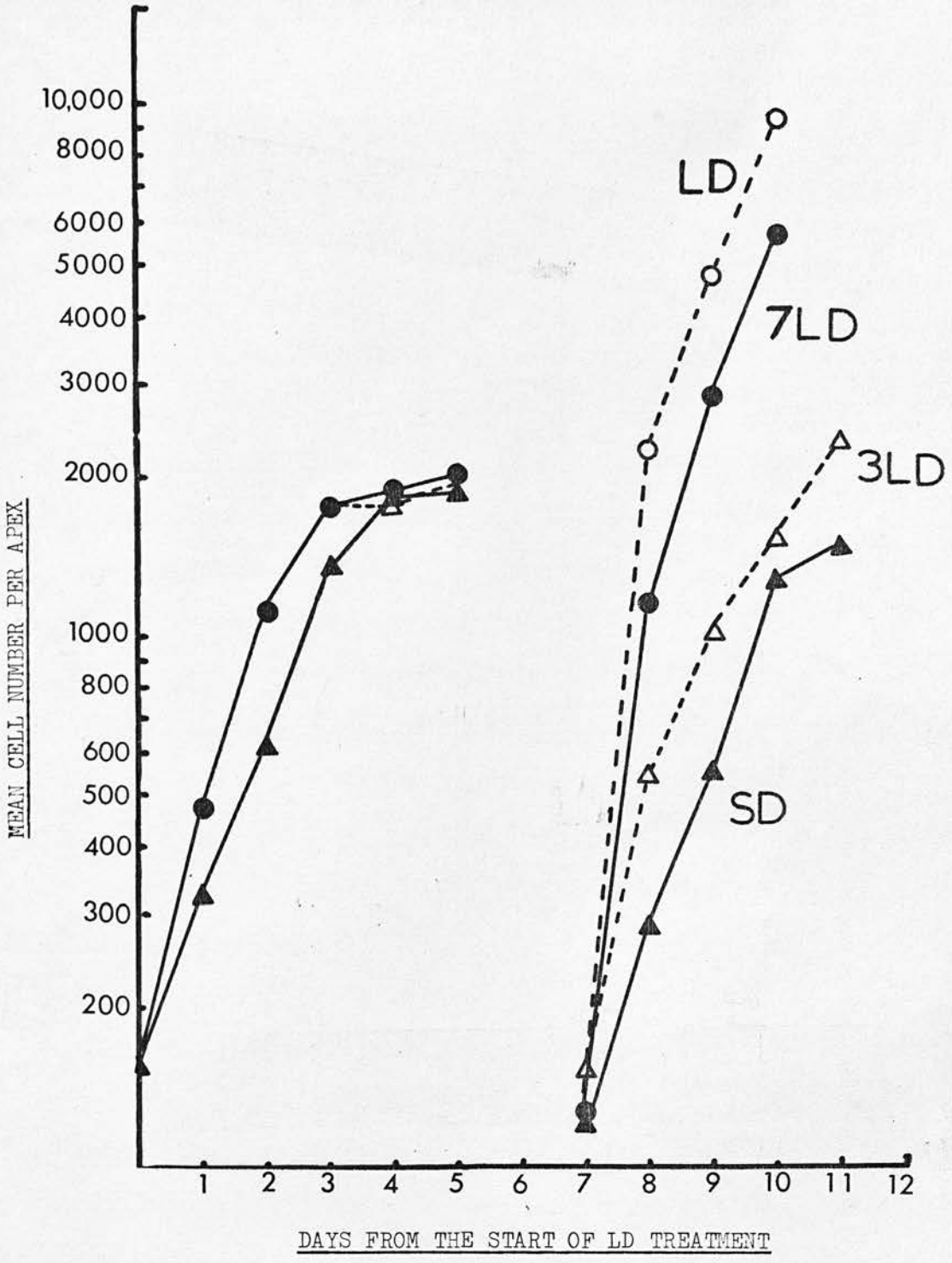


Fig. 20 (cont.) Mean cell number of the Silene apex above primordial pairs 7 and 9 during the 11 days from the start of experimental treatment in plants maintained in SD, exposed to 3 LD, to 7 LD and to LD throughout the period of sampling.
Each point is the mean of values from 3 plants.

the 20 hour value for the volume doubling of that total of tissue above leaf pair 8.

The data in fig. 17 suggests that the growth rate in apical tissue of continuous LD plants was slightly greater than that in 7 LD plants from day 7-10 (the two treatments diverge on day 7). The slope of the plot of the increase in apical volume in continuous LD plants was slightly steeper than that for 7 LD plants over this period, and consequently the absolute values for the apical volume of continuous LD plants on days 8 - 10 were greater than those for 7 LD plants.

The absolute values at any sampling time for the apical volumes of 3 LD plants are consistently greater than those for SD plants. The slopes of plots of apical size from SD and 3 LD plants against time, are, on the whole, similar. This suggests that there may have been an early effect of LD treatment perhaps on day 1 causing a short-lived stimulation of the growth rate in the apex. This would account for the apices of 3 LD plants and also those of LD plants during the first five days of this investigation being consistently bigger than SD apices, even though the growth rates (the slopes) were similar.

The total cell number per apex was measured using the same samples as those on which the apical volume measurements of fig. 17 were completed. This was done by counting the cell number in the median section and calculating the total apical cell number from the volume of the median section and the volume of the whole apex.

These results are shown in fig. 20. Comparison of this fig. with fig. 17 shows that changes in cell number during induction were very similar to the changes in apical volume and consequently the conclusions drawn above for volume doubling times apply equally to the mean cell generation time of the apical cells. The similarity of the two sets of data also suggests that the mean cell size of apical cells was not affected by any of the treatments

during the period of the investigation. To further check if cell size was unaffected by treatment the nuclear number within areas 1, 2, 3, and 4 and 5, (methods section, fig. 7) was counted. This was done in the 3 median sections of each of 3 plants per sample.

The results of this cell count data per unit volume are shown in table 5. There was apparently no effect of treatment on the nuclear number per unit volume, and hence cell size in any of the areas considered. This confirms that LD treatment does not affect cell size. In all plants there was a decrease in the mean number of nuclei present in the more central areas of the apex (areas 2 & 3) compared with the peripheral areas (1 and 4 & 5). This effect of an increase in cell size with distance from the exterior of the apex was also unaffected by LD treatment.

Conclusions from Apical Growth Data

As already stated the data of fig. 17 and fig. 20 were very similar since cell size was unaffected by treatment. Because of these similarities the general conclusions from the two sets of data (apical volume and apical cell number) will be considered together.

The principal points from these investigations into apical growth in SD and LD Silene plants are:-

1. Growth of the dome tissue in SD plants was exponential with a volume doubling time and mean cell generation time of about 20 hours.
2. The relative growth rate of tissue between the primordia (nodal tissue) in SD and 3 LD plants slowed down as soon as a primordial pair was differentiated.
3. Growth rates in the apex in LD and SD plants were similar until day 6.
4. Cell size was unaffected by LD treatment.
5. From day 7 there was a re-distribution of apical growth in induced plants; the development of that part of the apex between the newly formed 9th primordia pair apparently did not slow down to the same extent as comparable

TABLE 5. Nuclear number in regions 1,2,3, & 4+5 in vegetative and induced Silene apices.

	1	2	3	4 & 5
SD	10.6 (0.5)	10.0 (0.7)	8.3 (0.3)	10.8 (0.4)
3LD	10.5 (0.6)	9.8 (0.4)	8.1 (0.4)	10.6 (0.4)
7LD	10.3 (0.7)	9.4 (0.5)	8.4 (0.5)	10.3 (0.3)

The values shown are the mean of values over the period from day 1-13 obtained from the 3 median sections from each of 3 plants per treatment per day. Standard deviations are shown in brackets.

tissue, in SD plants. This contributed towards the maintenance of overall exponential growth in that tissue above leaf pair 8 in 7 LD plants.

6. The volume doubling time and the mean cell generation time shortened in induced plants in the dome tissue above leaf pair 9 to a value of about 11 hours.

7. Growth rates were slightly higher in continuous LD plants compared with 7 LD plants.

8. The apical size and cell number of 3 LD plants seemed to be consistently higher than that reached by SD plants from day 1, but growth rates themselves were similar. The difference may be due to an early ephe meral stimulation of growth in all LD treated plants.

Direct Cell Cycle Measurements.

The length of the cell cycle in the apex was measured directly using a double labelling technique. Measurements were obtained by this method for the length of the cell cycle in SD and LD apices on day 5-6 i.e. during induction, and for SD and 7 LD apices on day 8 - 9 i.e. at the beginning of the floral morphogenesis phase.

Apices were initially labelled with ^{14}C-thymidine and at various times later with ^{3}H-thymidine. When the time interval between the application of the two labels was short a high proportion of the nuclei in the S phase of the cell cycle incorporated both isotopes. As the time between applications of the two labels was increased an increasing number of those ^{14}C labelled nuclei had moved out of S and so there was a decreasing percentage of labelled nuclei incorporating both isotopes. Eventually, as the interval between the application of ^{14}C and ^{3}H-thymidine increased further, the percentage of labelled nuclei which were labelled with both isotopes again began to rise as cells labelled during S with ^{14}C re-entered S, one cell cycle later, to become labelled with ^{3}H-thymidine.

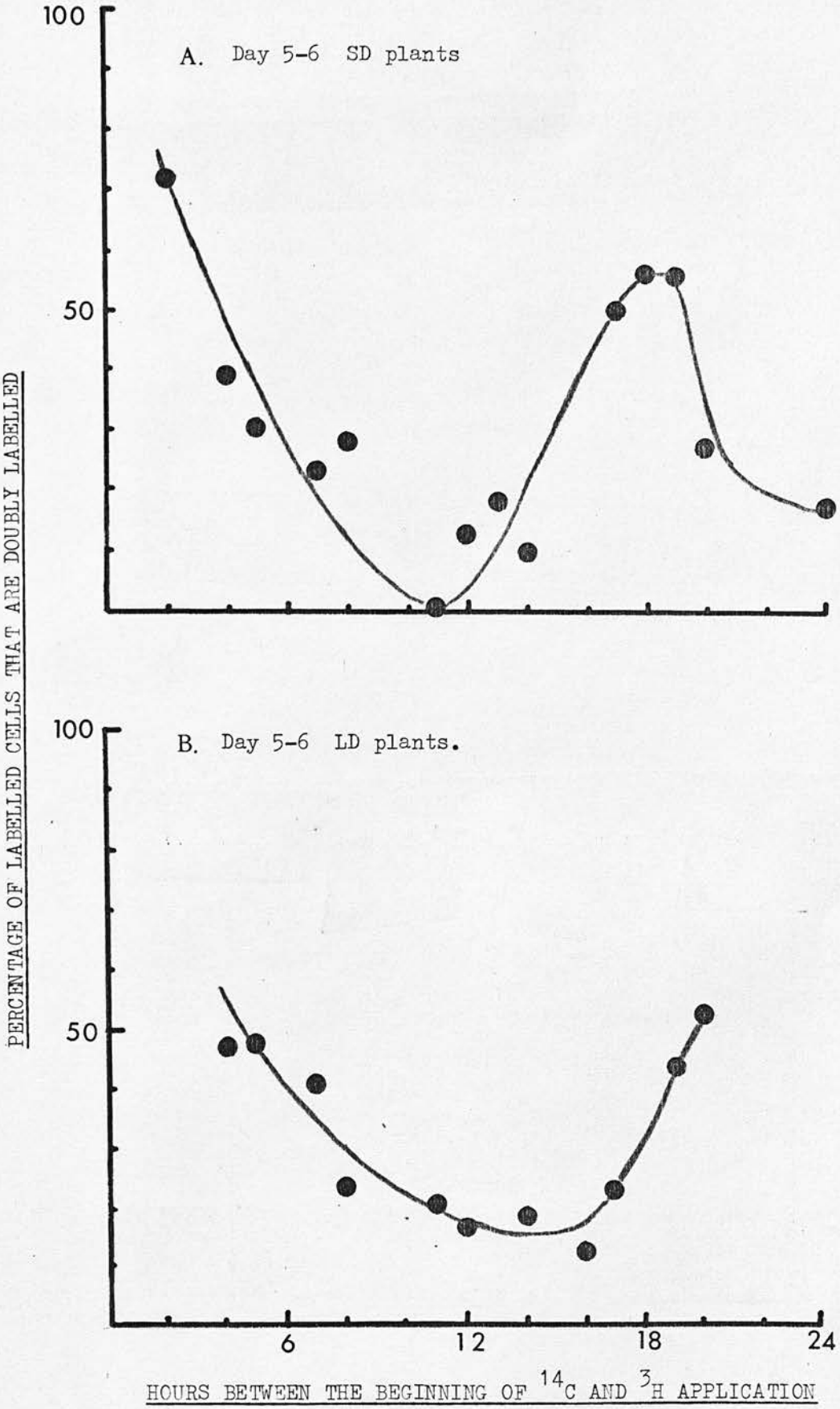


Fig. 21. The percentage of labelled cells that are doubly labelled following the application of ^{14}C-thymidine at 0 hours and ^{3}H-thymidine at intervals later.

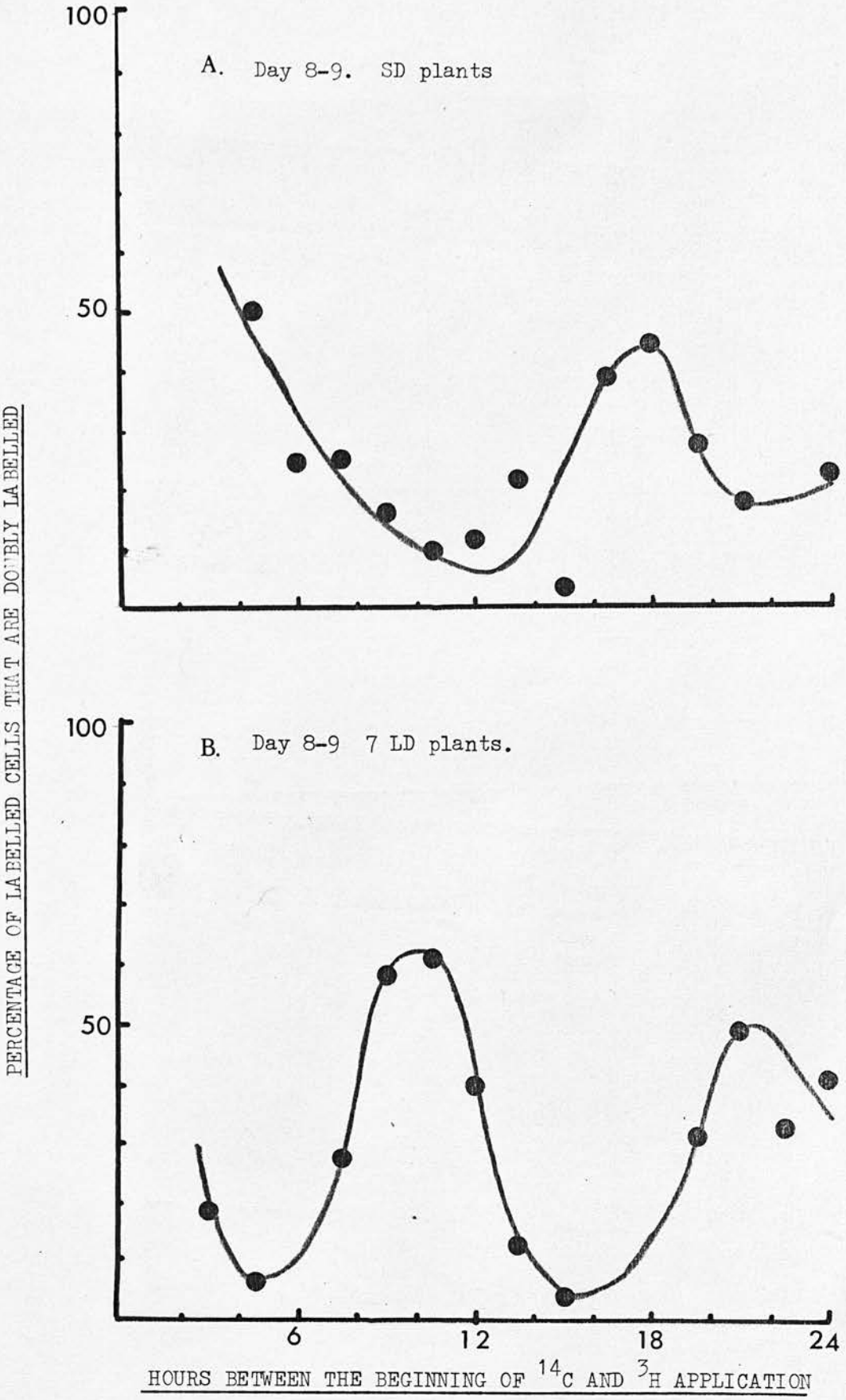


Fig. 22. The percentage of labelled cells that are doubly labelled following the application of ^{14}C-thymidine at 0 hours and ^{3}H-thymidine at intervals later.

TABLE 6. Cell cycle times in the Silene apical dome

	Experimental technique	
	Cell Accumulation	Double labelling experiment
SD apex day 1-12	20 hours	20 hours
LD apex during induction	20 hours	20 hours
LD apex during early morphogenesis	11 hours (day 7-10)	10 hours (day 8-9)

The values for the percentage of those nuclei labelled which were doubly labelled are shown in fig. 21 and 22. Those for samples obtained on day 5-6 in fig. 21 A and B and those for samples obtained on day 8-9 in fig. 22A and B.

Initially all the values fell from the theoretical value of 100% at time 0. to a minimum some time later. The values then rose to a new peak before falling of again. The time interval between the start of the experiment (time 0) and the second peak is equivalent to one cell cycle. This interval is around 18-19 hours in figs. 21A and 22A i.e. SD plants on day 5-6 and on day 8-9. Since values after 20 hours from LD plants on day 5-6 in fig. 21B are missing the second peak cannot be placed exactly but the data are sufficient to be certain it is not less than 20 hours. The length of the cell cycle in SD plants on day 5-6 and days 8-9 and in LD plants on day 5-6 (during induction) therefore appears to be similar. The situation is however different in LD plants on day 8-9. Here the values fell to a minimum 5 hours after the start of the experiment and rose to a peak around 10 hours after the start of the experiment. The values then fell to a second minimum around 15 hours and rose to a second peak around 20 hours after the start of the experiment. These values demonstrate that the length of the cell cycle in the 7 LD plants had fallen from around 20 hours on day 5-6 to around 10 hours on day 8-9. The overall results from this isotope labelling investigation are very similar to those obtained from the earlier experiments in which apical cell numbers were counted (fig. 17) and confirm that the cell cycle remained unaltered during floral induction but was shortened to about half its original value at the onset of floral morphogenesis.

Table 6 summarises the results obtained on the length of the cell cycle in the Silene apex during floral transition.

Changes in Nucleic Acid Concentration in the Apex During Floral Induction and Early Flower Morphogenesis.

Changes in the nucleic acid concentration in the Silene apex were followed during floral transition.

DNA

Fig. 23 shows the values for relative absorbance at 575nm due to DNA stain complex in sections stained with gallocyanin-chrom alum. Measurements were made in areas 1, 2, 3, and 4 and 5 (methods section fig. 7). The data in fig. 23 demonstrate that LD treatment had no effect on the DNA concentration within the apex. There was a decrease in DNA per unit volume with increasing distance from the extreme tip of the apex c.f. values from areas 2 and 3 (fig. 23 B and C) with those of areas 1 and 4 & 5 (fig. 23 A and D). This decrease in the concentration of DNA towards the interior of the apex was unaffected by floral induction and early flower morphogenesis. The experiment was repeated with similar results using Feulgen stain instead of gallocyanin. The results are shown in fig. 24.

The data in fig. 23 indicate there may have been an overall decrease in DNA concentration in the Silene apex during the sampling period. The absolute amount of this decrease was however very small and since this decrease was not recorded in the Feulgen experiment (fig. 24) it was probably not real.

It was shown (table 5) that cell size in the apex was unaffected by LD treatment and there was an increase in cell size towards the interior of the apex. Since this increase in cell size was similar in magnitude to the decrease in DNA concentration towards the interior, the mean DNA content per cell was constant throughout the apex, and also it was unaffected by LD treatment.

RNA

Changes in RNA concentration during transition in the Silene apex are shown in fig. 23 and 25. There was a certain amount of fluctuation in the values

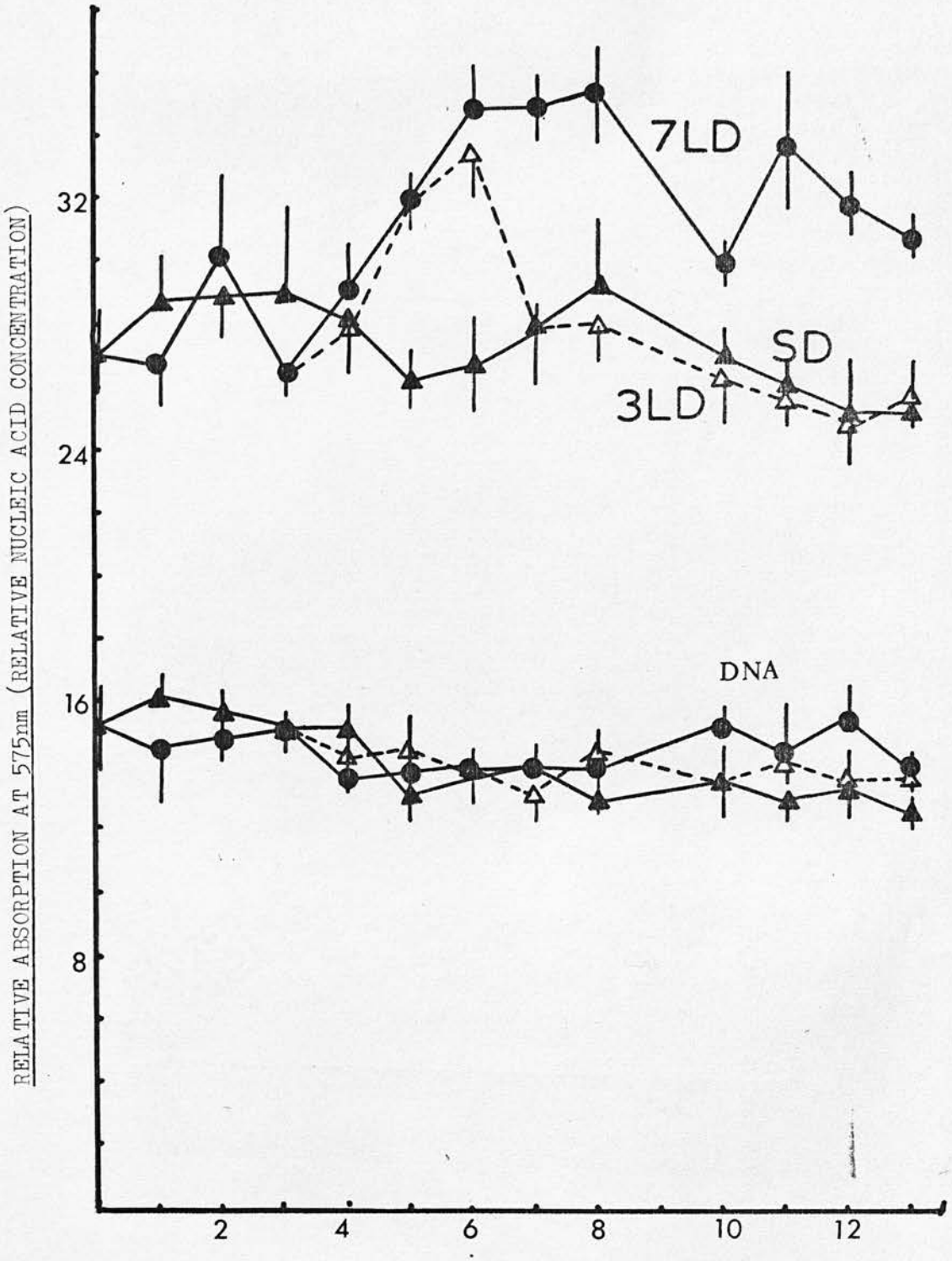


Fig. 23A Change in DNA and total nucleic acid concentration in area I of the Silene apex. Each point is the mean of readings in the 3 median sections in each of 3 plants per sample. Standard errors are shown as vertical lines.

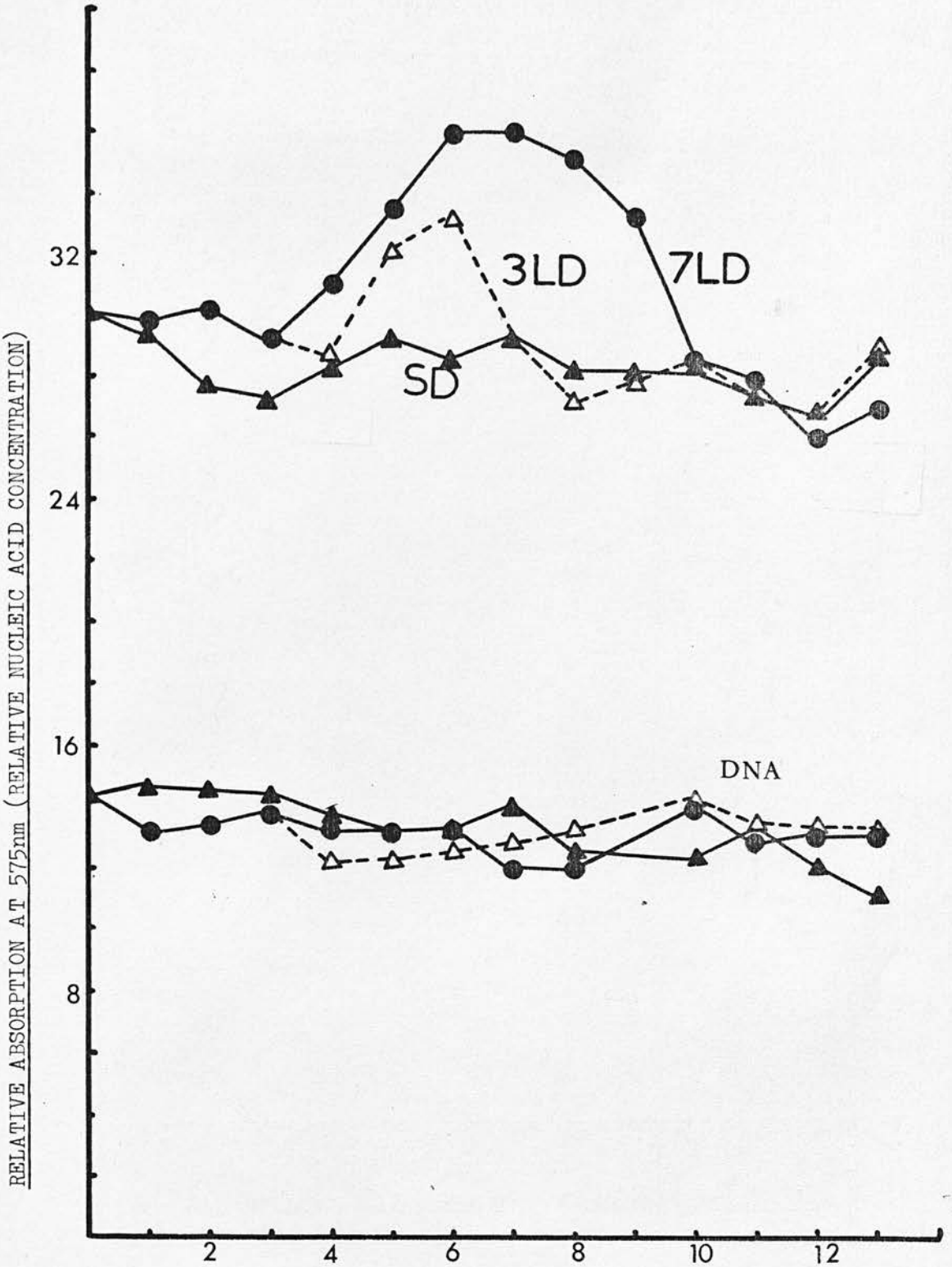


Fig. 23B. Change in DNA and total nucleic acid concentration in area 2 of the Silene apex. Each point is the mean of readings in the 3 median sections in each of 3 plants per sample.

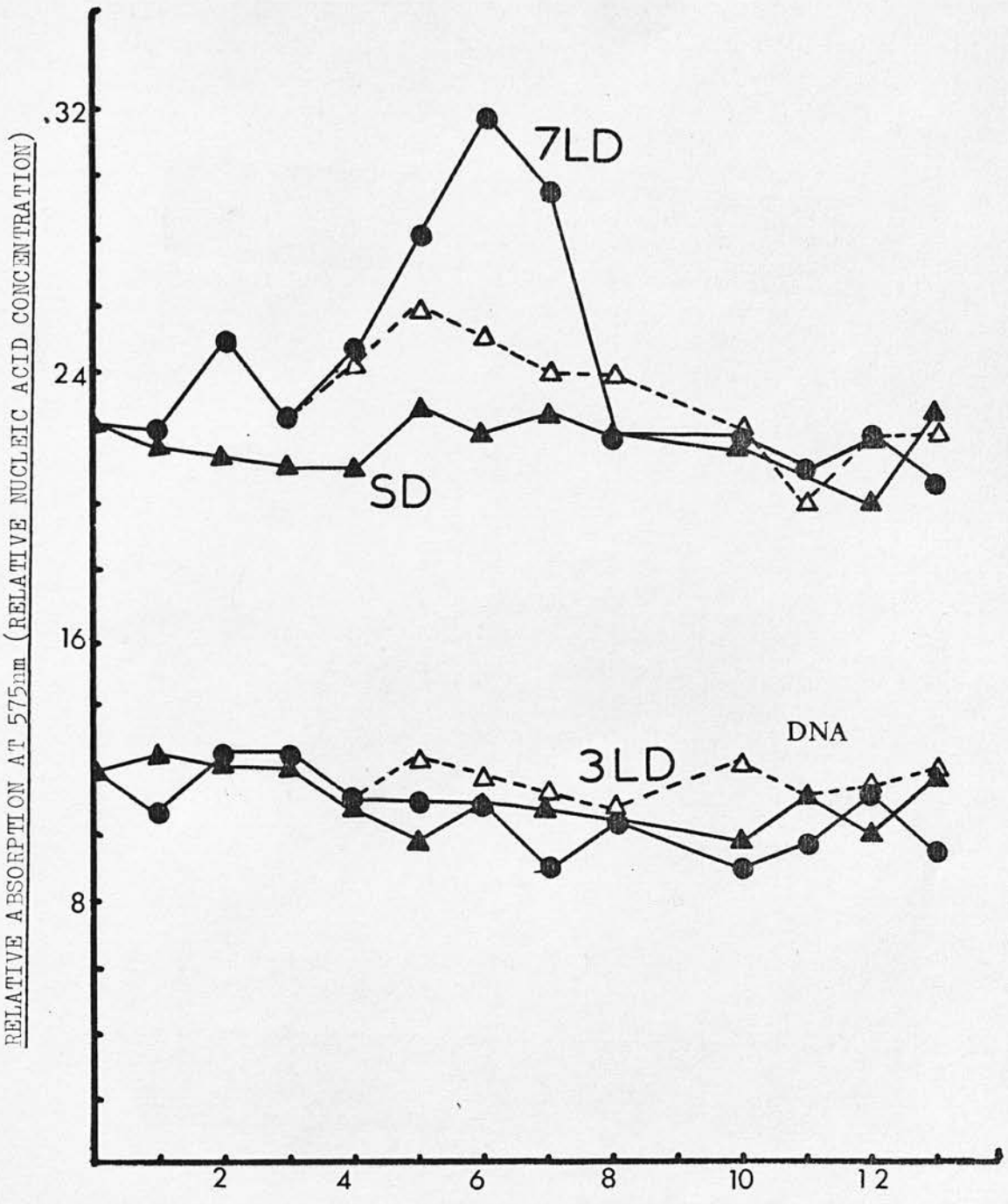


Fig. 23C. Change in DNA and total nucleic acid concentration in area 3 of the Silene apex. Each point is the mean of readings in the 3 median sections in each of 3 plants per sample.

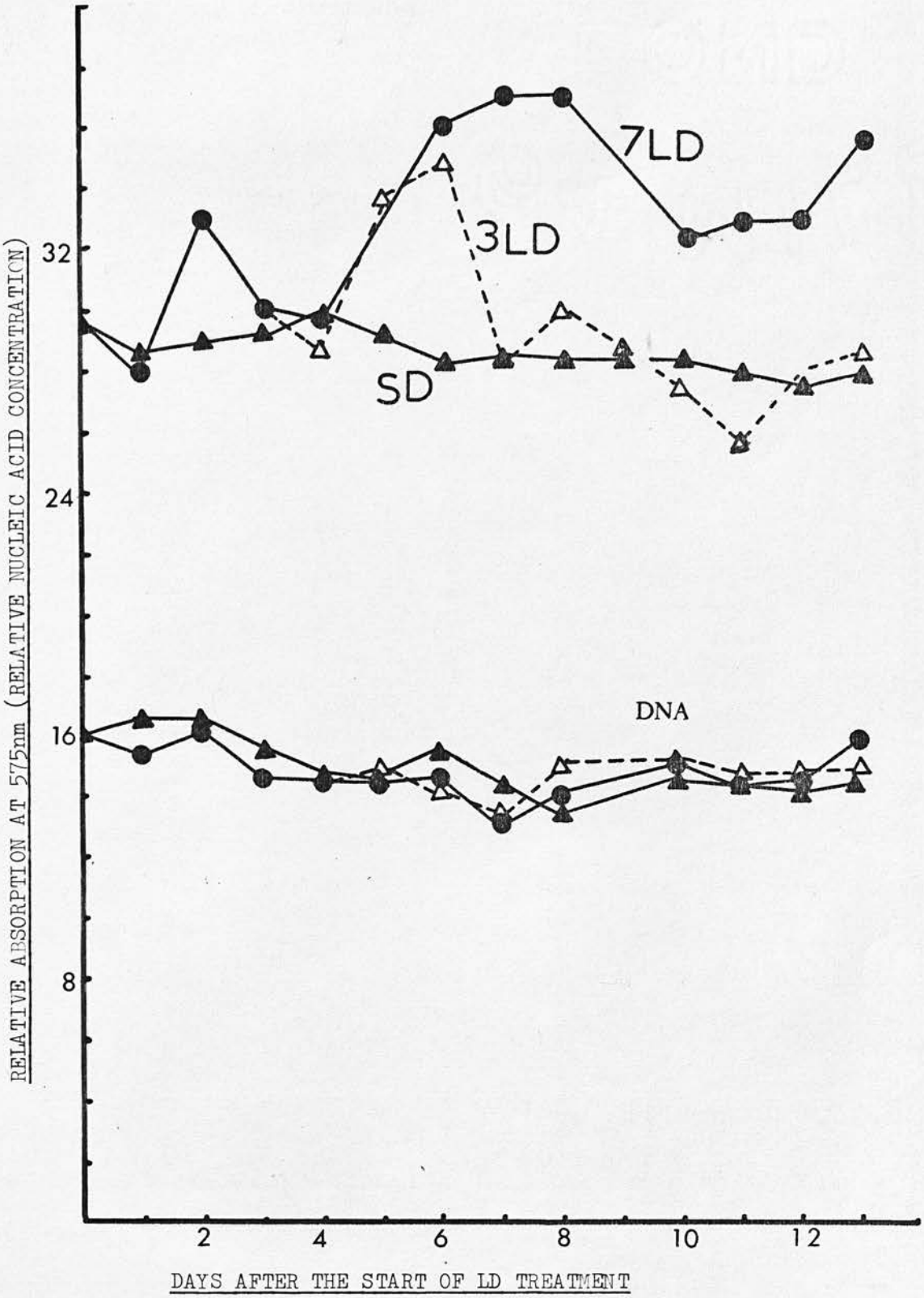


Fig. 23D. Changes in DNA and total nucleic acid concentration in area 4 and 5 of the Silene apex. Each point is the mean of readings in the 3 median sections in each of 3 plants per sample.

for RNA concentration in each of the areas measured in SD plants. These fluctuations seemed to follow no regular pattern and were not consistent from experiment to experiment c.f. fig. 23 and 25. The standard errors of these points overlapped indicating these fluctuations were not significant.

From day 0-4 no significant difference between RNA values from LD and SD treated plants was apparent (fig. 23). From day 5 there was an increase in the apical concentration of RNA in 7 LD plants (fig. 23). This increase in concentration in 7 LD apices from day 5 was consistently recorded in several independent experiments. The values for the RNA concentration in 7 LD apices when at a maximum was 50% higher than in SD apices (fig. 23). The difference in RNA concentration between 7 LD and SD apices was shown to be significantly different at the 0.01 level from day 5-13 by analysis of variance (using the individual data from areas 1 and 4 & 5 from which the mean values of fig. 23 are plotted). In the peripheral areas (area 1 fig. 23A, areas 4 & 5 fig. 23D) the increased concentration of RNA had decreased by day 9, however the difference in values between SD and 7 LD apices was still clearly maintained until day 13 when sampling was terminated. The increased concentration was not maintained over the same period in the more central areas of the apex. In area 2, the RNA concentration in the apices 7 LD plants had returned to that in SD plants by day 10 (fig. 23B) and in area 3 by day 8 (fig. 23C).

On days 5-6 there was an ephemeral increase in the concentration of RNA in the apical tissue of plants which had been exposed to 3 LD (fig. 23). This difference was shown to be significant on both day 5 and 6 at the 0.05 level by the t-test for the comparison of means. This rise mimicked that in plants receiving 7 LD during the initial period of the rise. By day 7, however, the concentration of RNA in the apices of these 3 LD plants had returned to that in SD apices and then remained at a similar concentration to that in SD plants throughout the rest of the sampling period.

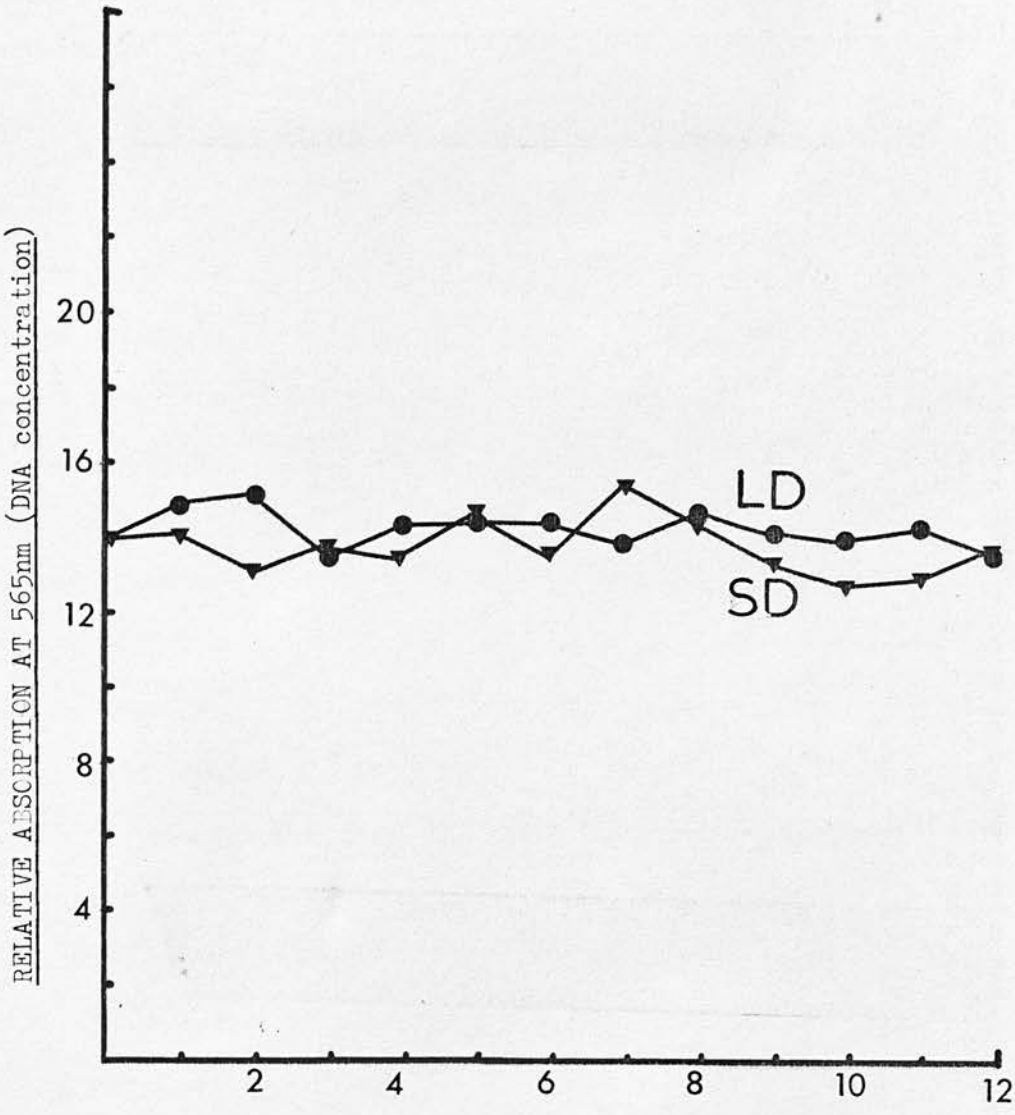


Fig. 24. Changes in DNA concentration in Silene apical tissue from plants maintained in SD, and exposed to 7 LD. Each point is the mean of readings in areas 1, 2, 3, 4, and 5 in the 3 median sections in each of 3 plants per sample. The sections were stained with Feulgen stain.

Fig. 25 shows the absorbance due to RNA stain complex in an experiment in which a sample of plants was included which were exposed to LD throughout the period of sampling (continuous LD). The major trends shown in fig. 25 resembled those shown in fig. 23. Again there was unexplained fluctuation in values from SD plants. As in fig. 23 the values for RNA in the 7 LD apices were higher than those in SD apices. This rise in RNA concentration in 7 LD apices commenced a day earlier, and the maximum difference between the SD and the 7 LD values was less, (being about 30%) than that shown in fig. 23. In the more central areas of the apex, the concentration of RNA in 7 LD plants again decreased towards the end of the sampling period to become similar to that in SD plants (on day 12 in area 2, fig. 25B, and on day 11 in area 3, fig. 25C). There is evidence from the data in fig. 25A and D that even in the peripheral areas of the apex the concentration of RNA in the 7 LD apex fell off towards the end of the experiment, approaching that concentration in the SD apex. In this experiment the ephemeral rise in the RNA concentration in the apices of 3 LD vegetative plants was again demonstrated. The combined 3 LD data from areas 1 and 4 & 5 (the peripheral areas) on both days 5 and 6 were significantly different (by t-test) at the 0.05 level from the SD values but not from the 7 LD values. As in fig. 23 this rise in 3 LD plants commenced simultaneously with the rise in 7 LD plants and after a further 3 days (by day 7) the RNA concentration had returned to that in SD apices. The increase in the concentration of RNA in 3 LD apices commenced, like that in 7 LD plants, on day 4 (fig. 25) which was a day earlier than in the experiment depicted in fig. 23. In the apical tissue of plants exposed to LD throughout the period of sampling there appeared to be a further increase in the concentration of RNA compared with plants induced by 7 LD. An analysis of variance was completed on the data from the summit area (areas 1) from day 8 - 13 (the treatments diverged on day 8) and the difference between the values over this period was shown to be significant at the 0.01 level.

Fig. 25 A-D. Changes in RNA concentration in the Silene apex in plants maintained in SD, exposed to 3 LD, to 7 LD and to LD throughout the period of sampling.
Each point is the mean of readings in the 3 median sections in each of 3 plants per sample.
Mean values for each area for DNA were subtracted from values for total nucleic acid to give the values shown.
Standard errors are shown in fig. 25 A by vertical lines.

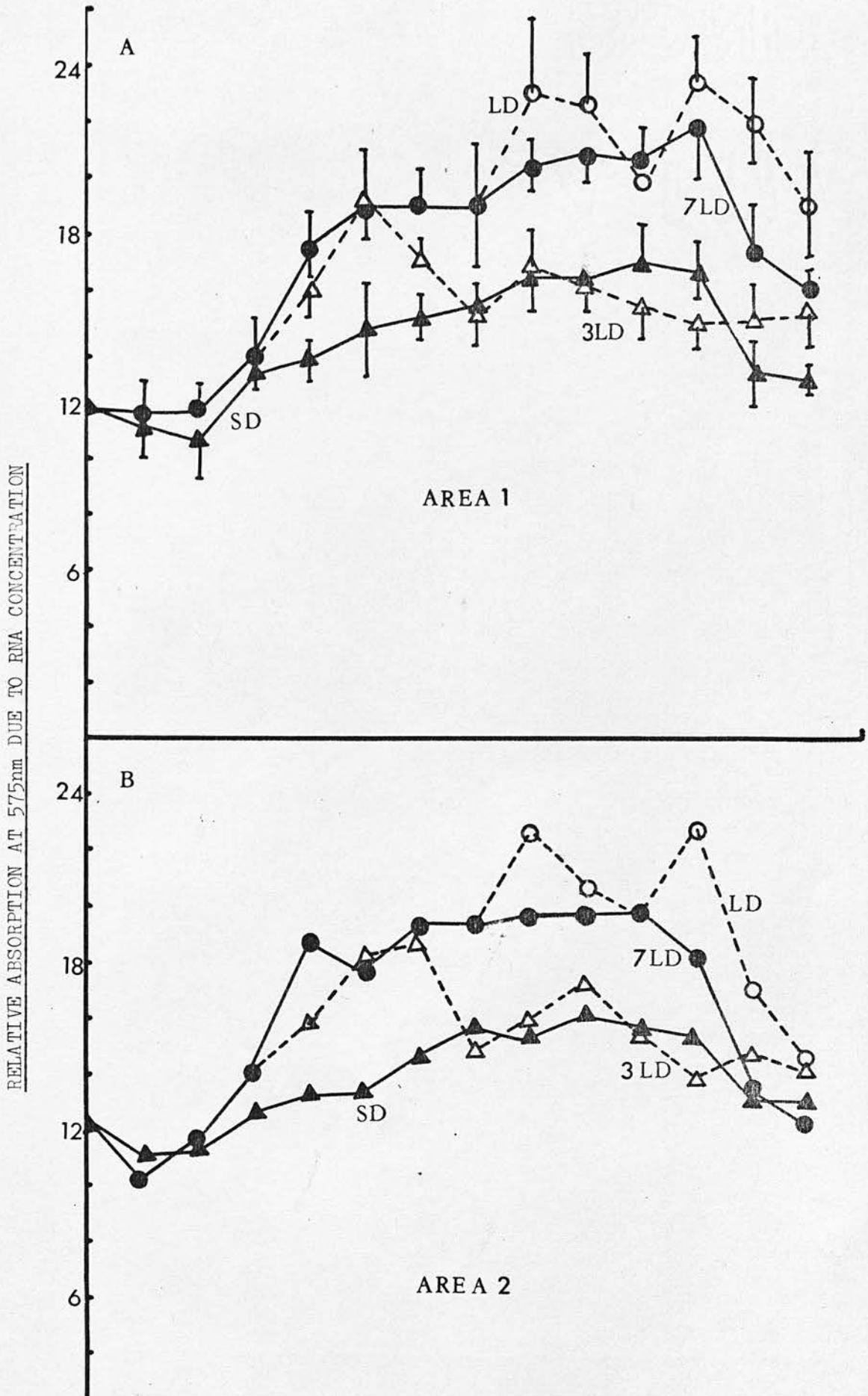


Fig. 25A and B. Change in RNA concentration in the Silene apex, in areas 1 and 2.

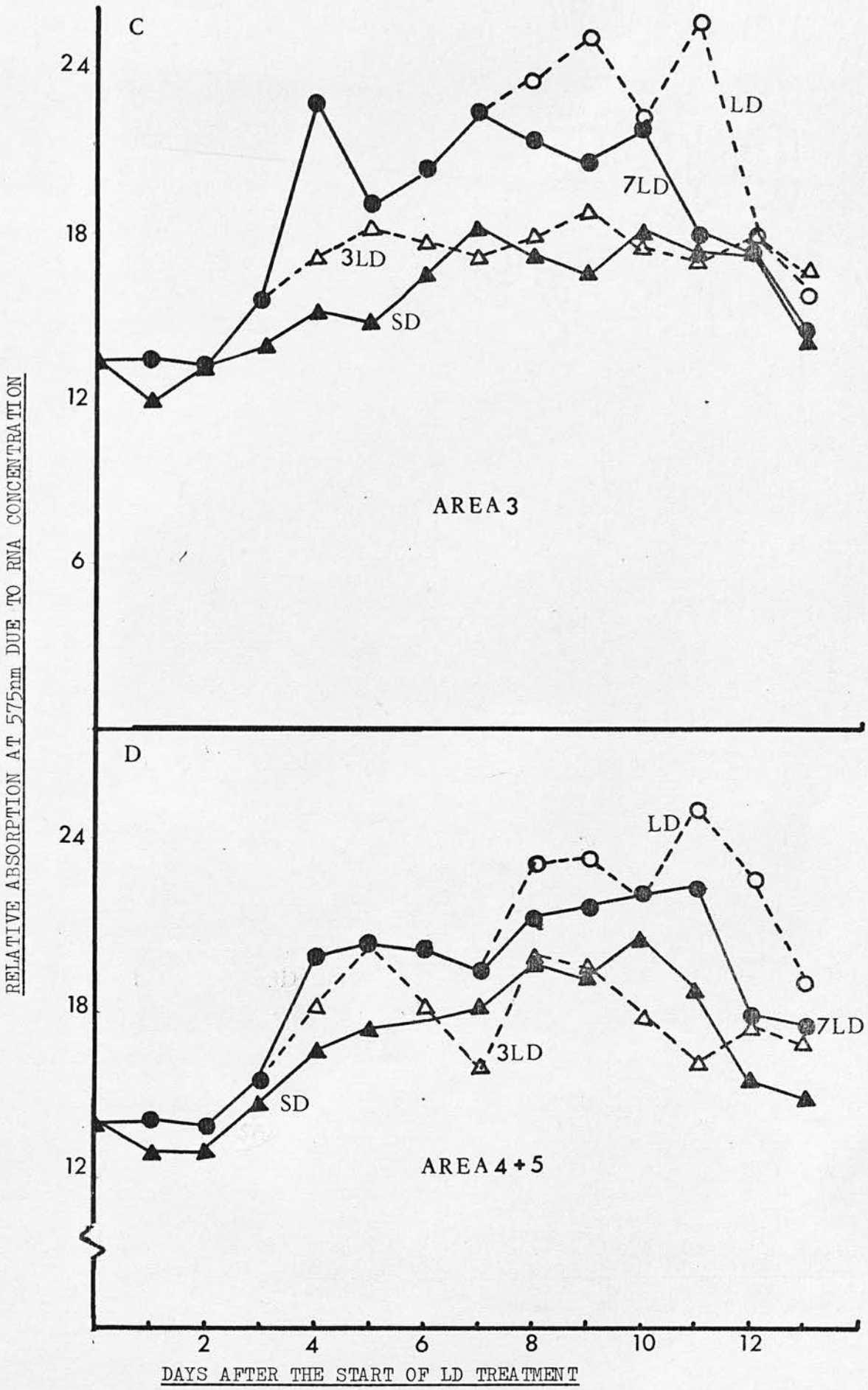


Fig. 25 C and D. Changes in RNA concentration in the Silene apex in areas 3, 4 and 5.

Summary of Data Presented on Nucleic Acid Concentration in the Silene Apex During Floral Transition.

1. The mean DNA concentration per cell was unaffected by LD treatment and was constant throughout the apex.
2. The concentration of DNA in the apical tissue decreased towards the interior; this effect again was unaffected by LD treatment.
3. From about day 4 there was an increase in the concentration of RNA in the apical tissue of all LD treated plants.
4. In 3 LD plants (which remained vegetative) the increase in RNA concentration in the apex was ephemeral. The concentration returned to that in SD plants after 2-3 days.
5. In induced plants the overall increase in RNA concentration in the apex was maintained during early flower morphogenesis.
6. This increase in RNA concentration in the apex of induced plants was greater towards the exterior of the apex.
7. The increase in RNA concentration in the apex during early flower morphogenesis (days 8-13) was more pronounced if the plants were maintained in LD during the period of early flower morphogenesis.

Interpretation of the Data on Growth Rates and Accumulation of Nucleic Acid in the Apex

Several possible interpretations could be placed on the results so far described. There was not a simple relationship between flowering and apical growth rate, or between flowering and accumulation of RNA in the apex. The evidence for this is; 1) the RNA concentration in the apex of 3 LD plants was increased in the absence of flowering and 2) in induced plants both the growth rate of the apex and the RNA concentration increased as the LD treatment the plants received increased (c.f. effect of 7 LD and continuous LD on RNA concentration and on growth rate).

There is a correlation between the concentration of RNA in the apex and the number of LD to which the plants were exposed. The lowest concentration of RNA was found in SD plants. In 3 LD plants the RNA concentration increased ephemerally following the 3 LD treatment. In 7 LD plants the RNA concentration rose further and this rise was maintained for a longer period, while in plants exposed to LD throughout the period of early flower morphogenesis (continuous LD) the RNA concentration, in the apex increased beyond that in 7 LD plants. This evidence therefore suggests that LD treatment had a direct effect on the concentration of RNA in the apex; an effect which may be independent of any effect of LD treatment on flowering.

A similar correlation exists between the growth rate of the apex and the number of LD to which the plants were exposed, with, however, the important exception of 3 LD plants. While the growth rate of the apex of 7 LD plants was much higher than SD plants and that of continuous LD plants yet higher, the growth rate in the apex of SD plants and 3 LD plants was the same. Because of this it can be stated there is not a direct relationship between LD exposure and growth rate - some other factor must therefore be involved.

With again the exception of 3 LD plants, there is a correlation between the RNA concentration in the apex and the subsequent growth rate of the apex. The growth rate being lowest in apices with a low RNA concentration (SD plants) and highest in apices with the highest RNA concentration (continuous LD plants). This suggests that the RNA concentration in the apex may determine the subsequent growth rate. If these two parameters are directly linked then some explanation for the 2 day rise in RNA concentration in 3 LD plants not being followed by an increase in growth rate would have to be found.

A further possible interpretation of the effects of LD treatment on RNA concentration and growth rate in the apex and on flowering is that the increase in RNA concentration is directly related to the morphogenetic switch

TABLE 7. Effect of short days, 7 long days and gibberellic acid on internode length

Treatment	Internode length (mm) on day 10		
	3rd internode	4th internode	5th internode
SD	3.7	-	-
SD + GA_3	11.0	16.6	1.7
7LD	11.3	13.7	-
7LD + GA_3	17.0	19.7	13.3

GA_3 was applied daily from day 0-6. The values are the mean of measurements from 3 plants per sample.

from vegetative to floral development at the apex. The increased growth may be a secondary effect concerned with flower production itself. It could be argued that exposure to 3 LD partially activates the morphogenetic switch (it will be described later that the effect of 3 LD treatment remains in the apex for up to 6 days). The major difficulty with this interpretation is that different inductive treatments (7 LD and continuous LD) have different effects on the RNA concentration and growth rate in the apex. If changes in RNA concentration depended solely on the morphogenetic switch itself then these different effects of different inductive treatments would not occur.

The following parts of the results section describe experiments which attempt to clarify these possible interpretations.

Gibberellic Acid (GA_3) Treatment

Aqueous GA_3 applied to the apex of the *Silene* plants caused internode elongation. Applications of various GA_3 concentrations were made to 28-day old SD plants and in no case did GA_3 treatment cause the plants to flower. In an attempt to give a GA_3 treatment which was as similar as possible to 7 LD treatment applications were made at the end of the high intensity light period on each of seven days commencing on day 0. 0.05 ml GA_3 solution at a concentration of 0.75 mg/ml was applied each day, with a fine syringe, to the apex of each treated plants. This caused rapid internode elongation in SD plants and a further increase (in addition to that caused by LD) in internode length in 7 LD plants. Table 7 shows values for internode length following GA_3 treatment of *Silene* plants. Because of this internode elongation SD plants had a similar overall appearance to 7 LD plants from the start of treatment to the end of the sampling period. These observations suggested there might have been a stimulation of growth at the apex of GA_3 treated plants in the absence of floral induction. Because of this possible dissociation of increased apical growth from induction, changes in nucleic acid concentration

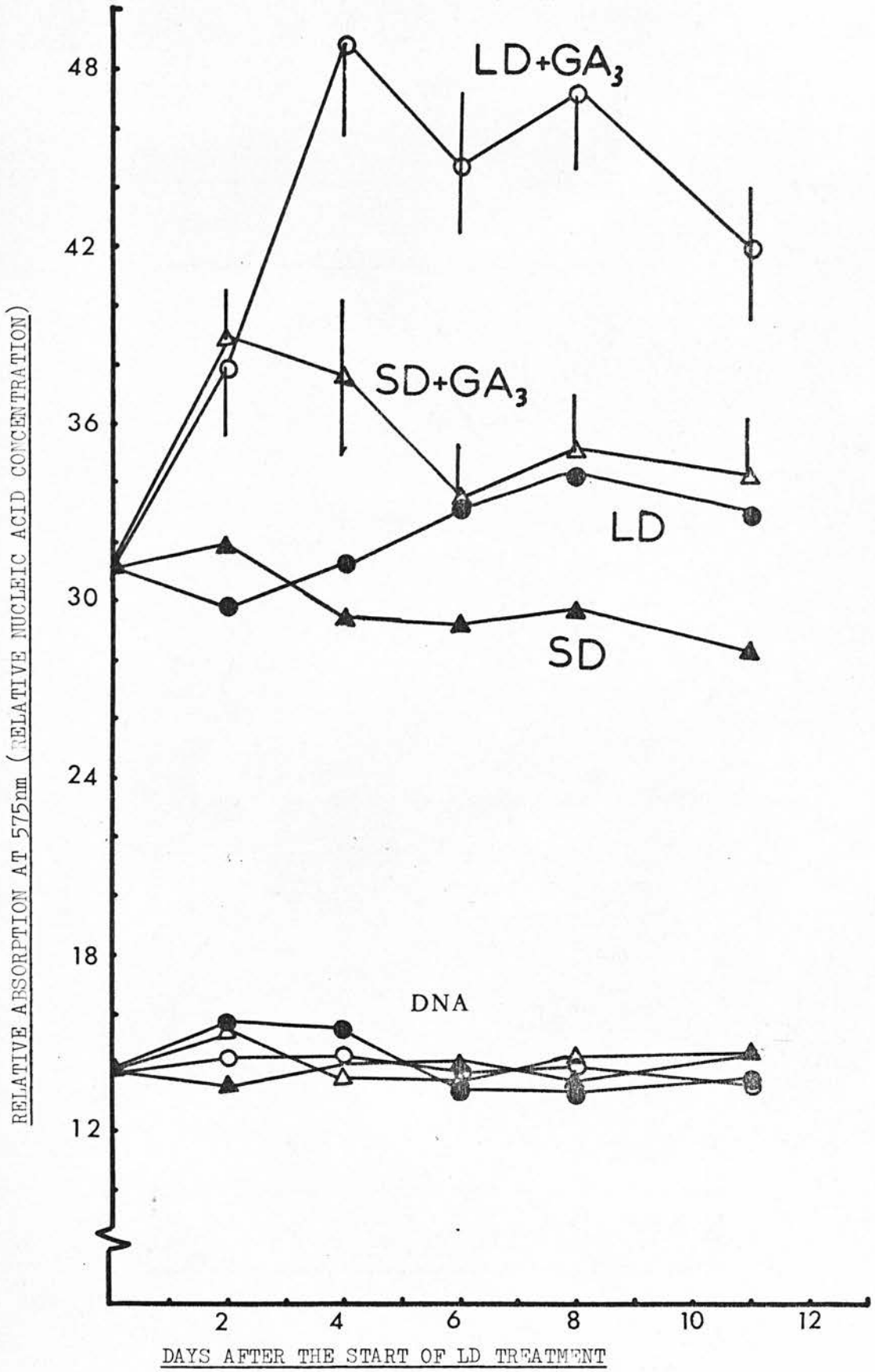


Fig. 26. Changes in DNA and total nucleic acid concentration in the SD and 7 LD Silene apex with or without the application of GA_3 on days 0-6. The values shown are the means from readings in areas 1, 2, 3, 4 and 5 in the 3 median sections in each of 3 plants per sample. Standard errors are shown for total nucleic acid values as vertical lines.

and the increase in the volume of tissue at the apex with time of SD plants and 7 LD plants treated with GA_3 from days 0 - 7 were examined.

The concentration of DNA was, once again, unaffected by treatment (fig. 26). Changes in RNA concentration following GA_3 treatment are also shown in fig. 26. By day 2 there was a rise in the concentration of RNA in the apex of both SD and 7 LD plants treated with GA_3 and by day 4 the concentration of RNA in the apex of GA_3-treated 7 LD plants was almost double that on day 0. In SD plants the increased RNA concentration in the apical tissue fell from a maximum on day 2, however an increase of about 20% over untreated SD plants was maintained throughout the period of sampling. The values from day 6, for RNA concentration from SD GA_3-treated plants are very similar to those from untreated 7 LD plants. The percentage increase in the RNA concentration in the apices of GA_3-treated 7 LD plants over untreated 7 LD plants was much greater than the comparable increase in SD plants. By day 11 the increase in 7 LD treated plants had also fallen - this may have been due to the cessation of GA_3 application 4 days earlier on day 7.

Changes in growth rates were estimated by calculating the volume of tissue above successive leaf pairs from camera lucida drawings of serial sections. These data are shown in table 8. These results show that GA_3 had no effect on the rate of accumulation of tissue at the apex in either SD or 7 LD plants and since the apical volumes on each sampling occasion of untreated and GA_3-treated plants were more or less similar this shows that GA_3 did not cause any appreciable stimulation of apical growth.

The concentration of RNA in the apex of SD plants was increased greatly by GA_3 treatment, however, the growth rate at the apex of untreated SD and GA_3-treated SD plants was the same. Likewise the RNA concentration in the apex of GA_3 treated 7 LD plants was greater than in untreated 7 LD plants, however

TABLE 8. Growth rate of the apex in the presence and absence of gibberellic acid

Days after start of long day treatment	Mean apical volume ($10^5 \mu m^3$) SD	SD+GA_3	7LD	7LD+GA_3	Above leaf pair
2	21.3	14.4	12.1	13.7	6
4	32.1	31.0	27.8	32.2	6
6	3.6	4.9	12.4	13.7	8
8	8.1	19.1	38.8	41.8	8
	1.1	4.3	14.6	18.4	9
11	49.6	33.2	$\gg$150	$\gg$150	8
	15.2	11.5	79.4	67.8	9

The values shown are the mean from 3 plants per sample.

TABLE 9. Rate of leaf initiation in plants in short days or given 7 long days at 13° and 20°

Treatment	SD 20°	SD 13°	7LD 20°	7LD 13°
Mean number of leaf pairs produced per day*	0.27	0.12	0.24	0.12
Length of vegetative plastochron** (days)	3.7	8.3	4.1	8.3
Mean number of leaf pairs produced by induced plants from day 0 to flower production.	-	-	2.2	1.7***

* Measured from days 0-15 (SD 20°) and 0-21 (SD 13°), and from day 0 to sepal formation which occurred on days 9 (7LD 20°) and 13 (7LD 13°). Sample size = 20.

**The interval between the initiation of successive leaf pairs.

***Sample size = 20.

the apices of these plants were also growing at the same rate. The RNA concentration in SD plants treated with GA_3 and in untreated 7 LD plants was similar, however the growth rates were very different. In these experiments there was therefore no correlation between RNA concentration and growth rate in the apex.

The data presented in table 8 and fig. 26 demonstrate that the rate of accumulation of RNA in the shoot apex could be stimulated independently of any effect upon floral induction and without a corresponding increase in the growth rate of apical tissue.

Cold-Grown Plants

If 28-day old (day 0) plants were transferred from standard growth conditions at 20°C to conditions with a lower temperature growth was slowed down. Table 9 shows the number of leaf and primordial pairs produced during a 2 - 3 week period from day 0 by plants transferred to a temperature regime of 14°C during the high intensity light period and 12°C during the low intensity light period or the dark period (12°C conditions); day-length and light intensity were the same as in standard conditions. As can be seen from table 9 the vegetative plastochron was increased from about $3\frac{1}{2}$ - 4 days at 20°C to 8·3 days at 12°C. This suggested apical growth rates were halved on transfer to the low temperature conditions. 100% induction was recorded in a sample of 25 plants given 7 LD treatment at low temperature and maintained in 12°C SD conditions from the end of LD treatment until flower bud production. It seemed therefore that low temperature had no effect upon the number of LD required for induction. One further gross measurement completed on these plants was to note the total number of leaf pairs produced by plants induced in 12°C conditions until flower production. These data showed that at 12°C on average one less leaf pair was produced during the period from the start of LD treatment to flower morphogenesis at the apex (table 9). This was probably because induction was unaffected by the low

TABLE 10. Growth rate of the apex, above the 8th leaf pair, in plants in short days or given 7 long days at 13° and 20°

Treatment		Mean apical volume ($10^5 \mu m^3$) Day 8	Day 10	Day 13
SD	13°	-	2.1	9.7
SD	20°	8.5	20.4	52.3
7LD	13°	9.4	23.0	41.5
7LD	20°	25.0	50.0	150

The values shown are the mean from 2 plants per sample.

temperature but since the length of the plastochron was increased fewer vegetative primordia could be produced before flower production. Changes in growth rates were estimated by calculating the volume of tissue above the 8th primordial pair on days 8, 10 and 13, from camera lucida drawings of serial sections. These data are shown in table 10. The values for tissue volume above the 8th primordial pair in 20°C SD and 20°C 7 LD plants (table 10) were very similar to those described at equivalent sampling times in an earlier experiment (fig. 17). The volume of tissue above the 8th primordial pair in 12°C SD plants was considerably less than that of the 20°C SD plants on all sampling occasions (table 10) and confirmed that growth rates were reduced at the lower temperature. The tissue volume above the 8th primordial pair in 12°C 7 LD plants was much greater than that for 12°C SD plants on all sampling occasions (table 10). This indicates that even at the lower temperature inductive LD treatment stimulated apical growth to a marked extent. The values for 20°C SD and 12°C 7 LD plants on each sampling occasion were barely distinguishable (table 10). The overall apical growth rate from day 0 - 13, at least above the 8th primordial pair, was similar therefore, under these two sets of environmental conditions, one of which (12°C, 7 LD) resulted in flower morphogenesis while under the other (20°C SD) the plants remained vegetative.

Nucleic Acid Concentration. As with all other treatments, the DNA concentration in the apex was unaffected by cold treatment (fig. 27). Changes in apical RNA concentration in 12°C treated plants is also shown in fig. 27. It can be seen from fig. 27 that placing SD plants in 12°C conditions resulted in a reduction of about 30% in the RNA concentration in the apical tissue. The values for RNA concentration in the apices of 12°C 7 LD plants showed only a very small increase (max. 10%) over those of 20°C SD plants through the period of sampling. This increase was very much less than occurred in the apices of 20°C 7 LD plants (fig. 23, fig. 25).

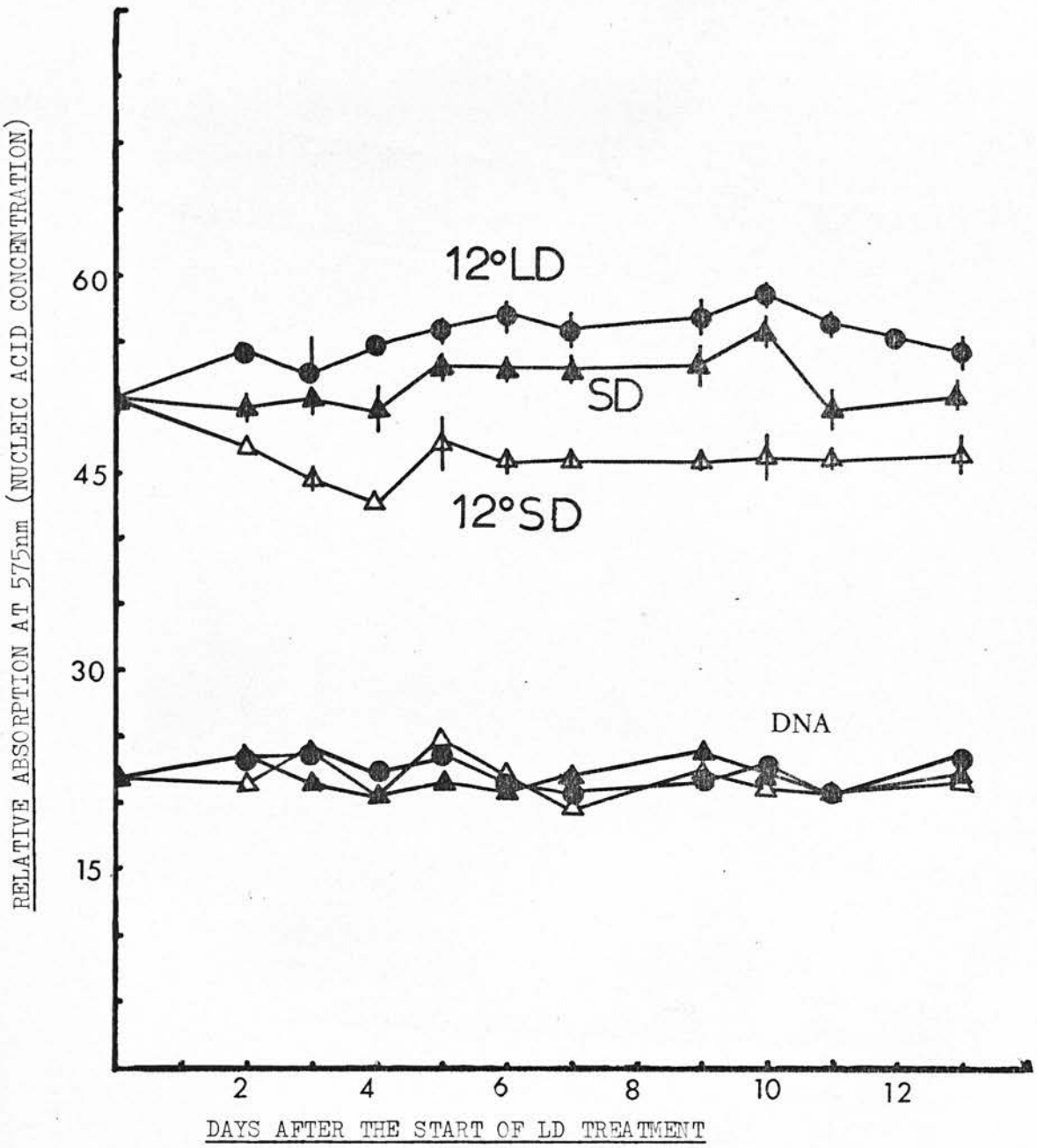


Fig. 27 Changes in DNA and total nucleic acid concentration in shoot apices of plants in SD at 20°C (SD), in SD at 12°C from day 0 (12° SD) or given 7 LD at 12°C from days 0-7 before transfer to SD at 12°C (12°C LD).

The values are the means from readings in areas 1, 2, 3, 4 and 5 in the 3 median sections in each of 3 plants per sample.

Sections were cut 16 µm thick and not 10 µm thick as in all other histochemical experiments.

Standard errors for total nucleic acid values are shown as vertical lines.

TABLE 11. Effect of long-day treatments, interrupted by short days, on flowering

Treatment	Number of plants	
	Floral	Vegetative
3LD/2SD/3LD	18	2
3LD/4SD/3LD	18	2
4SD/6LD	19	1
3LD/6SD/3LD	3	17
9SD/3LD	2	18

The plants were scored by the presence of flower buds in the induced plants.

The results from these experiments with different temperature regimes tend to support the hypothesis that increases in apical RNA concentration are directly linked to the increases in growth rate in the apex since:-

(1) When SD plants were transferred to cold conditions, which slowed down growth, the concentration of RNA in the apical tissue fell.

(2) There was no large rise in the rate of apical growth in 12°C 7 LD plants during early morphogenesis - nor was there a rise in RNA concentration in the apex.

(3) The growth rate of the apex of 12°C 7 LD plants and 20°C SD plants remained very similar - so did the concentration of RNA in the apex.

These conclusions are however still consistent with there being a direct correlation, within each temperature regime, between the RNA concentration and the subsequent growth rate in the apex. At 20°C a rise in the concentration of RNA precedes and is maintained during the increase in growth rate in the apex which accompanies floral morphogenesis in 7 LD plants. At 12°C the concentration of RNA in the tissue of the apex is higher in 7 LD plants than in SD plants. The growth rate in 7 LD plants is also higher than that in SD plants.

Fractional Induction

To further examine any reversible phenomena associated with the initial stage of induction experiments were carried out where 28-day old plants were exposed to two periods of 3 LD interrupted by up to 4 SD. This treatment resulted in about 90% of the plants becoming induced (table 11). A very similar percentage induction resulted from a single continuous period of 6 LD beginning when the plants were 28-days old (day 0), (table 11). If 6 SD were given to plants between two periods of 3 LD the first 3 LD period had little or no effect upon percent induction (c.f. results of 3 LD - 6 SD - 3 LD, and 9 SD - 3 LD plants; table 11) and 85% remained vegetative.

Nucleic acid concentration in the apical tissue of plants given two periods of 3 LD interrupted by various numbers of SD was examined. Again the concentration of DNA in the apex was unaltered by the various treatments (fig. 28). RNA concentration was examined in 3 LD treated vegetative plants, and in plants which were given two periods of 3 LD interrupted by 2, 4, or 6 SD. All the treatments commenced when the plants were 28-days old (day 0). Only those plants given 2 and 4 SD between the two periods of 3 LD became induced, while those which received 6 SD between the 3 LD periods and those given 3 LD alone did not (table 11). Fig. 28 also shows the values for relative absorption due to RNA. Samples were taken from each set of plants only when treatments diverged from one another, e.g. plants given two periods of 3 LD interrupted by 4 SD (3 LD - 4 SD - 3 LD) were treated similarly to 3 LD plants until the start of the second period of 3 LD; samples were therefore only taken from 3 LD - 4 SD - 3 LD plants from the start of the second period of 3 LD.

The concentration of RNA in the apical tissue of plants given a single period of 3 LD increased for a short time from day 5 - 7 (fig. 28). This increase was similar to that described in earlier experiments (fig.s 23 and 25). In plants which were given a second period of 3 LD after a 2 SD interruption the RNA concentration also rose. These plants became induced and the higher concentration of RNA was maintained in the apical tissue until sampling ceased on day 13 by which time flower morphogenesis was at an equivalent stage to that reached on day 10 in 7 LD plants. The concentration of RNA in the apical tissue of plants where 4 SD and 6 SD interrupted two 3 LD periods fell back to that concentration in SD control plants (fig. 28) following the increase in concentration on days 5 -7 which resulted from the first 3 LD exposure. The concentration of RNA in 3 LD - 4 SD - 3 LD plants increased a second time from day 11 just after the second 3 LD treatment had terminated. This second increase in the apical RNA concentration in these plants was maintained at least until day 17 when sampling was terminated. By this time the apices had

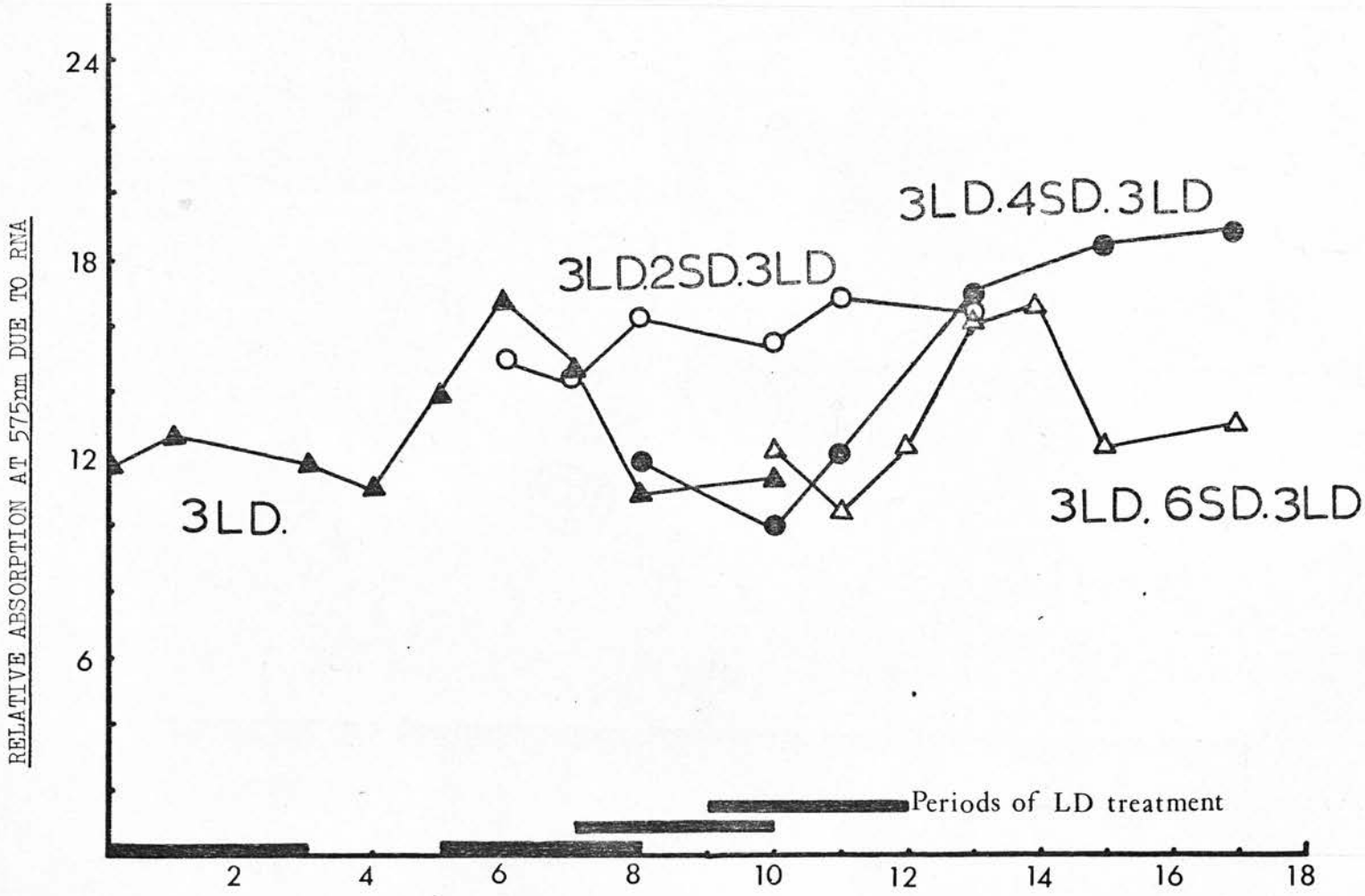


Fig. 28 Changes in RNA concentration in the Silene shoot apex in plants given 3 LD, 3 LD-2SD-3LD, or 3LD-6SD-3LD. All treatments began on day 0.

The values are the means of readings in areas 1, 2, 3, 4 and 5, in the 3 median sections in each of 3 plants per sample. A mean value of 14 for DNA has been subtracted from values for total nucleic acid to give the values shown.

become floral and were equivalent to the developmental stage reached on day 11 in plants induced by 7 LD. The RNA concentration in plants given 3 LD - 6 SD - 3 LD showed a different pattern. In these plants (which remained vegetative) following the second 3 LD period there was a second rise in the RNA concentration in the apex. This was very similar to the rise following the first 3 LD period (it was of a similar magnitude and was ephemeral).

The results from this experiment on fractional induction can be summarized as follows.

(1) Treatment of Silene plants with a single period of 3 LD caused a short-lived rise in the concentration of RNA in the apical tissue. This treatment did not result in flower induction.

(2) In plants given a second period of 3 LD after 2 SD the concentration of RNA in the apex was maintained at the increased level. This treatment resulted in flower induction.

(3) In plants given a second period of 3 LD after either 4 or 6 SD the RNA concentration fell back to that in SD apices after the 2 day rise which resulted from the first 3 LD exposure.

(4) Following the second 3 LD period in 3 LD - 4 SD - 3 LD plants there was a second rise in the RNA concentration in the apex, this second rise was maintained. This treatment resulted in flower production.

(5) Where a 6 SD period was given between two 3 LD periods there was a second ephemeral rise in the concentration of RNA in the apex following the second 3 LD treatment. These plants did not become induced.

These different effects on the concentration of RNA in the apex of 3 LD treated plants apparently depended on whether the treatment resulted in floral induction, and suggest that the maintained increase in apical RNA concentration in induced plants was concerned with the flowering process and was not a primary effect of LD exposure.

TABLE 12. The production of leaves over the period from the start of LD treatment (day 0) to flower production in plants induced by interrupted LD treatment.

Treatment	No of leaf pairs produced from day 0 to flower production
3LD/2SD/3LD	3.4
3LD/4SD/3LD	4.2
4SD/6LD	4.3

At least 18 plants were included for each treatment.

Growth rates in the apex were not themselves measured in these plants, however the total number of leaf pairs produced before flower production was counted (table 12).

Where 2 or 4 SD were given to plants between two 3 LD periods induction must have taken place some time during the second 3 LD period. Since induction was not 100% it is unlikely that in the majority of plants induction would take place before the 2nd or 3rd day of the second 3 LD period.

It was shown earlier that following 7 LD treatment of 28-day old (day 0) plants, the plants produced, on average, 2.2 leaf pairs from day 0 to flower production (fig. 13) and that the plastochron over this period is about $3\frac{1}{2}$ days (fig. 13). The majority (90 percent) of 7 LD plants in fact became induced by day 4 or 5 (table 1). Since 3 LD - 2 SD - 3 LD plants probably do not become induced until day 7 - 8 (3 days after day 4 or 5) then one would expect one extra leaf pair to be produced by these plants in the period prior to flower production since the plastochron is about $3\frac{1}{2}$ days. Similarly in 3 LD - 4 SD - 3 LD plants where induction takes place on day 9 - 10 yet a further leaf pair would be produced giving a total of (2.2. + 2) i.e. 4.2. The data of table 12 confirm these predictions and suggest that until flower induction actually takes place (the end of the second 3 LD period) the plants are growing as normal vegetative plants. The data on leaf production therefore suggest flower morphogenesis occurs in these plants as a single event just as it would take place in plants following a single continuous inductive period of 6 or 7 LD.

It is therefore likely that following fractional induction the sustained increase in the RNA concentration in the apex commenced just after induction and just prior to the increase in growth rate in the apical tissue which has been shown to accompany transformation of the vegetative apex into a flower at 20°C. As in earlier experiments the results show that

an ephemeral rise in the RNA concentration took place in the apex following LD treatment which was not sufficiently prolonged for induction to occur (i.e. 3LD treatment).

These results support the conclusion that this sustained rise was intimately concerned with the morphogenesis process, while the ephemeral rise which took place prior to and around the probable time of evocation is a temporary event which possibly results directly from LD treatment. This tends to support the hypothesis that induction is a stepwise phenomenon - certainly 3LD treatment does result in apical changes but not in induction, nor in any change in the normal vegetative plastochron. The initial 3LD period of full inductive treatment (7LD), is however, an essential phase of induction. After 6SD the effects of 3LD become dissipated showing no permanent change in the plant is produced by 3LD alone.

Incorporation of Tritiated Uridine into the Plant Apex.

This experiment was carried out to see whether the increase in RNA concentration in the apex which accompanies floral transition in Silene resulted from an alteration in the rate of RNA synthesis or breakdown relative to the rate of growth, or whether the rates of synthesis and breakdown relative to the growth rate remained unchanged.

An increase in the rate of incorporation of tritiated uridine into RNA could reflect either an increase in RNA synthesis or a decrease in the rate of RNA breakdown. No change in the rate of incorporation of tritiated uridine might either indicate the rate of RNA turnover relative to the growth rate in the apex is unchanged or the rate of RNA turnover relative to growth has increased or decreased provided the relative rates of synthesis and breakdown remain unchanged.

TABLE 13. Incorporation of ^{3}H-uridine into the Silene shoot apex

Treatment		Days after the start of LD treatment									
		1	2	3	4	5	6	7	8	9	10
SD	A	7.1	4.7	1.0	2.6	2.1	4.6	6.0	6.4	3.1	3.4
SD	B	3.2	3.2	0.8	1.1	1.3	3.3	2.8	2.5	1.7	2.3
Mean SD value		5.2	4.0	0.9	1.9	1.7	4.0	4.4	4.5	2.4	2.9
LD	A	8.8	3.1	2.5	8.0	3.3	5.0	6.0	8.3	2.8	3.5
LD	B	1.9	1.3	-	3.0	2.6	3.9	6.0	1.6	2.8	2.9
Mean LD value		5.4	2.2	2.5	5.0	3.0	4.5	6.0	5.0	2.8	3.2

The values were measured by light scattering values when autoradiographs were under dark field illumination.

Each individual value is the mean of readings in the 3 median sections.

Applications of tritiated uridine were made daily commencing four hours after the start of the high intensity light periods to the apices of 2 plants per sample. The plants were fixed 2 hours later and autoradiographs prepared of sectioned apices. Values for silver grain number per apex, measured by light scattering are shown in table 13.

In most cases there is much variation in the values from the 2 plants of each sample and also between successive days samples from the same treatment. The values obtained from different sections within one apex were very similar and so these variations are probably due to differential uptake of the isotope solution.

From day 4 the mean values for incorporation of tritiated uridine into 7 LD plants were higher than those for SD plants. The only period, however, where there was no overlap of data was from day 3-5. This result could indicate there was an increase in the rate of RNA synthesis or a decrease in the rate of RNA breakdown, relative to the growth rate, in the apical tissue of 7 LD plants over this period. This would be consistent with the pattern of RNA concentration previously presented where there was a sustained increase in RNA concentration in the apices of 7 LD plants from day 4-5 (figs. 23 and 25). The similarity of values for incorporation of tritiated uridine from day 6-10 in SD and 7 LD apices (table 13) indicate that during this period, when the concentration of RNA in the apices of 7 LD plants was higher than in SD plants and the growth rate of the apex greater, the rate of RNA turnover relative to the growth rate was similar to that in SD apices.

If the absolute rate of RNA turnover remained unchanged in 7 LD apices over the period when the growth rate doubled (days 8 - 10) then the values for RNA concentration and incorporation of tritiated uridine would fall since the RNA present would be diluted in the greater volume of tissue. Since the RNA concentration actually increases and the rate of incorporation of

^{3}H uridine relative to growth remains at least constant (and may in fact be higher in 7 LD apices compared with SD apices), then the absolute rate of RNA synthesis must have increased from day 8. A decrease in the absolute rate of RNA breakdown could give a similar result.

Gross Changes in the Protein Complement of the Apex During Floral Transition

Gross changes in accumulation of insoluble proteins in the Silene apex were followed histochemically by staining sections with D.N.F.B. Stain density was measured in the 3 median sections of each of the three plants per sample.

The values from this investigation are shown in fig. 29. As with the histochemical data on RNA accumulation in the apex the values for protein concentration in both the SD and LD apex show considerable day to day variation. From day 5 however the values for protein concentration in the apices of LD plants were consistently and significantly (0.01 level) higher than those from SD plants. This difference was maintained from day 5 (towards the end of the inductive period) until flower morphogenesis was well advanced on day 13 when sampling terminated. The difference was most pronounced, just prior to, and during the period when flower morphogenesis commenced (day 5-9), when the marked increase in growth rate occurs.

When the cell cycle time is halved as occurs around day 8-9 it is likely that in order to sustain this increased division and growth rate there must be an increase in synthesis of all cell chemicals. If the rate of synthesis remained constant then the more active growth would result in a dilution of the chemicals already present and a fall in concentration. Since the concentration of protein actually increases either the breakdown rate must be decreased or the rate of synthesis increased to a greater extent than the increase in growth rate.

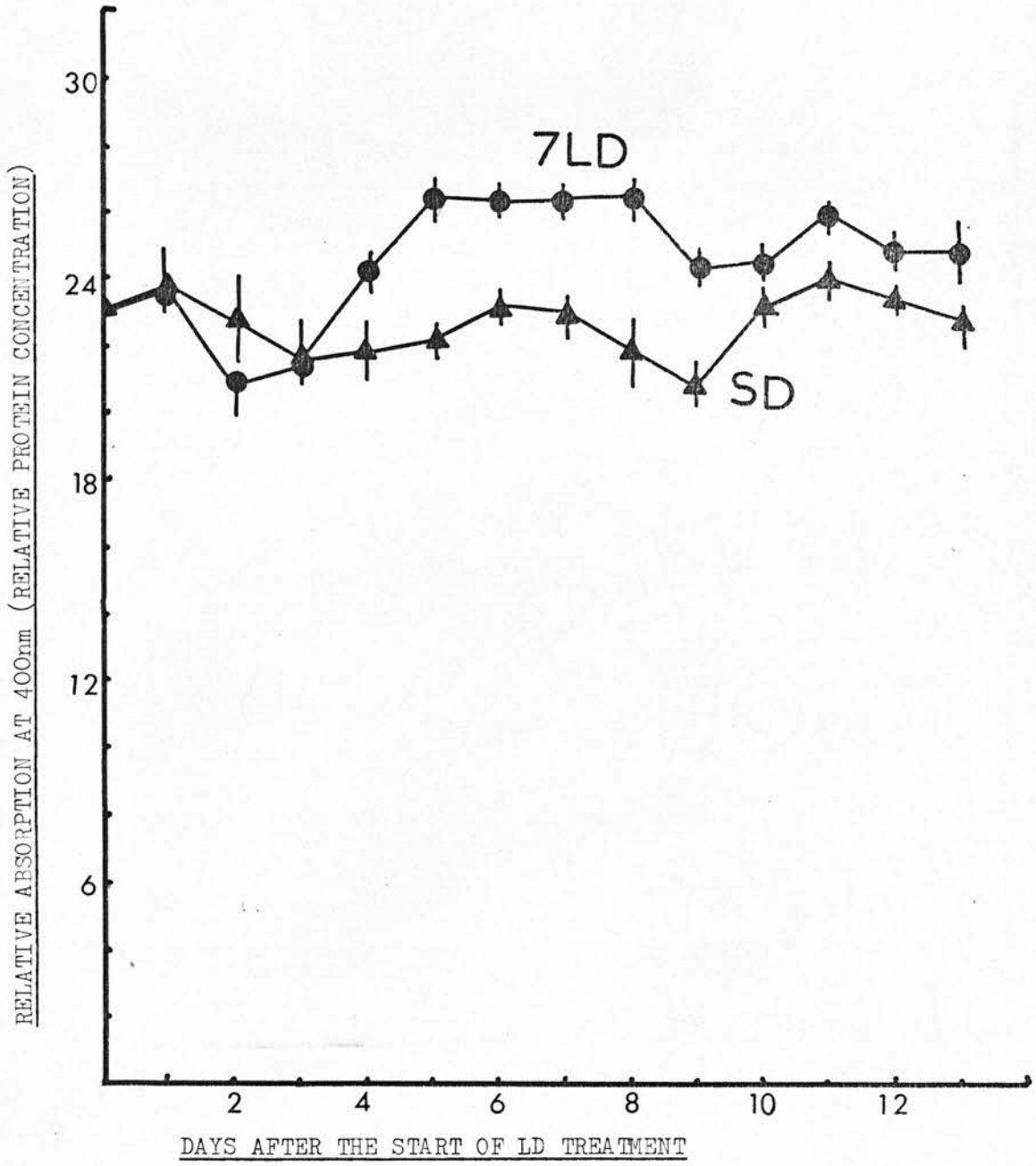


Fig. 29 Changes in the protein concentration in the Silene apex in SD plants and plants given 7 LD commencing on day 0.
The values shown are the mean of readings in areas 1, 2, 3, 4 and 5 in the 3 median sections in each of 3 plants per sample.
Standard errors are shown as vertical lines.

Following this study on gross protein accumulation an investigation was carried out to see whether changes in the protein complement accompany these changes in protein concentration in the apex during floral transition. SLS protein extracts from induced and vegetative apices were subjected to micro-polyacrylamide gel electrophoresis. The S.L.S extraction method breaks proteins into their constituent polypeptides.

Because of the small size of the apices (about 10 were included in each sample) and consequent small amount of protein extracted better resolution was obtained by running the protein sample on micro-gels (0·8 m.m. diameter) rather than on conventional sized gels (6 m.m. diameter). This however made photography of the gels extremely difficult and line drawing proved the most satisfactory method of recording the band pattern following electrophoresis.

The results of this investigation are shown in fig. 30. From these drawings it can be seen that no visible change was recorded in the protein band pattern during floral induction and in the early stages of morphogenesis. Considerable differences are apparent between protein extracts from different plant organs (c.f. those from stems, apices and roots) showing that the method can illustrate differences between samples.

The method however has severe limitations in that only 20-30 bands can be seen. This is obviously only a very small proportion of the total number of polypeptides in an organ such as an apex. The only conclusion therefore which can be drawn from the negative results obtained in this investigation is that there are no relative changes in absorbance of the most prolific proteins (those which can be seen by the electrophoretic method) present in the Silene shoot apex during either floral induction or the early stages of morphogenesis. It would appear therefore that during transition the rate of synthesis of the whole protein complement is stepped

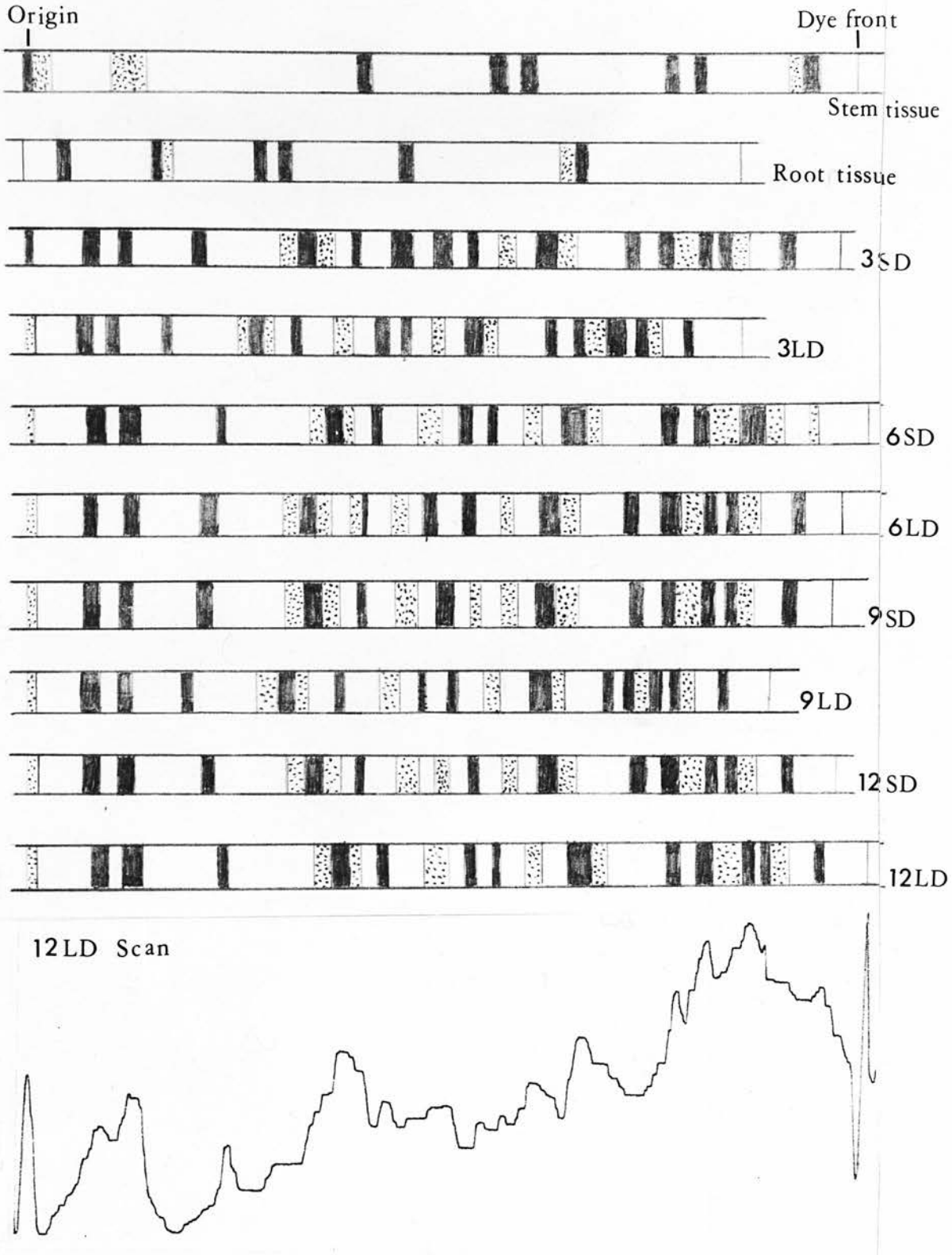


Fig. 30 Diagrams of the electrophoretic separation of proteins from Silene stem tissue, root tissue and apical tissue. LD apical tissue was from plants exposed to 7 LD. A typical scan (of the 12 LD gel drawn directly above) is shown to illustrate the method of presenting the results.

up with the relative quantities of at least the major proteins remaining unaltered.

PART IV

DISCUSSION

Discussion

The data presented demonstrate unequivocally there is an increase in growth rate in the apex during floral transition in Silene. This increase is manifest by both a decrease in the cell cycle time (fig. 21 & 22) and by an increase in tissue production (fig. 17) at the apex. The increase in cell division rate is not accompanied by a decrease in mean cell size (table 5) and so represents a true increase in growth rate. The majority (90%) of plants become induced by day 5 of LD treatment (table 1). Since there is no alteration in growth rate until day 8-9 (fig. 17) it is clear that during induction the growth remains unaltered from the vegetative situation. Indeed following the stage when the plant becomes fully committed to flower, and will do so if returned to short days, a further vegetative leaf pair is produced with no detectable alteration in the plastochron rate (fig. 13). It is only upon the commencement of the morphogenesis phase of flower formation on day 8 that the rate of apical growth alters (fig. 17). In Silene the first visible sign of flower morphogenesis at the apex is an enlargement of the apical dome prior to the formation of sepals. This enlargement begins to occur on day 8-9 and it is with this enlargement that the growth rate increase occurs. An enlargement of the apical tissue is a characteristic event during the early stages of transition, however as pointed out by Lyndon (1972) such an increase could be due either to an increase in relative growth rate or to a redirection of growth. The values from Silene on which the conclusion that apical growth rate is increased do not however merely reflect a re-direction of growth away from primordia and into the apical dome causing enlargement of the dome. The method adopted for monitoring growth relied on measuring the complete apex including the young primordia, above a fixed base line

(fig. 8) and so growth of the whole apex was followed. Further, an almost identical value for mean cell generation time to that calculated from the rate of cell accumulation was obtained from a totally independent study of the cell cycle using a thymidine labelling technique (figs. 21 and 22). The data therefore show that the increased growth is a reflection of cells dividing more quickly, as well as possibly also an increase in the proportion of cells dividing in the apex. Data on the accumulation of colchicine metaphases in the Silene shoot apex do indicate, a greater percentage of cells in the apex participate in division during flower morphogenesis than during vegetative growth (Lyndon - personal communication). And so this factor must also contribute to the overall increase in the growth of the induced apex.

Because of the timing of this increase in growth rate and the fact that it is clearly associated with the first visible signs of flower morphogenesis it is reasonable to conclude that the increase in growth rate is an intimate part of flower morphogenesis and occurs subsequently to the inductive process and that during the inductive and evocation processes in the Silene apex, a plant where the vegetative apex is directly transformed into a flower, the growth rate remains unaltered from the vegetative state. Data of Corson (1969) from Datura suggests there is an increase in apical growth following transition during flower morphogenesis, while data of Bodson (1975) from Sinapsis, suggest there is an increase in apical growth prior to transition. Bernier (1971) states that an increase in division rate is one of the first events to occur in the Sinapis apex following evocation i.e. the arrival at the apex of the floral stimulus. Though in the present study the first observed changes in the apex clearly associated with transition is an increase in RNA concentration which is evident 2-3 days prior to the increase in growth which accompanies transition there is evidence that exposure of Silene plants to a single LD results in a transient

but small increase in apical growth rate. This evidence is based on the fact that when changes in cell number and apical volume were recorded, during induction and prior to the large increase in growth associated with morphogenesis, values for apical cell number from LD plants were consistently greater than those from SD plants (fig. 17 and 19). This difference became manifest from day 1. More data would be needed to ascertain that this is an effect of a single LD however.

One further direct effect of LD exposure, whether or not sufficient LD are received for flowering, is the stimulation of internode elongation. The degree of internode elongation in Silene is more or less proportional to the number of LDs the plant receives over the range 1-14 LDs. This illustrates that LD treatment can have effects other than that on flowering - perhaps this transient increase in growth is such an effect. Those plants which were not returned to SD following 7 LD illustrate a similar trend. If plants are maintained in LD from 28-days old then eventual flowering takes place somewhat earlier than if plants were returned to SD after 7 LD. The values for apical cell number suggests that if plants were maintained in LD beyond 7 LD then apical growth continued at a higher rate than if plants were returned to SD. This would account for the hastening of flowering. Again this appears to be a direct effect of LD on the apex independent of that on induction.

In the Silene apex a sustained rise in the concentration of RNA in the apex is observed following exposure of plants to inductive LD conditions. This increase in concentration of RNA was first apparent on day 5 (when most plants were committed to flower) and was maintained until at least day 13 when sampling was terminated (figs. 23 and 25) and by which time flower morphogenesis was well advanced with sepals, stamens and petals being initiated (fig. 16). Since the growth rate doubles in 7 LD apices at the start of flower morphogenesis it is very likely that the rate of synthesis

of cell chemicals must also double to sustain this increase in growth. Indeed if the rate of synthesis of RNA in the apex did not increase to the same extent as the growth rate then the RNA concentration would fall. This, of course, does not happen and since there is an overall rise in RNA concentration this indicates that the synthetic rate of RNA is increased to a greater extent than is the growth rate.

Data obtained on RNA turnover rates obtained by labelling of LD and SD apices with tritiated uridine suggest that in LD plants there is an early stimulation in the rate of synthesis of RNA (from day 3-5) and that during flower morphogenesis the rate of synthesis per unit volume relative to growth rate is similar to or perhaps only slightly higher than that in vegetative apices (table 13). Because of technical difficulties these particular data are perhaps the most tenuous presented in the whole thesis, they do however suggest that there was no substantial increase in RNA synthesis relative to growth during the morphogenesis stage when the growth rate was increased. This being the case the data suggest that the rate of synthesis of RNA was stimulated to about the same extent or perhaps slightly more than the growth rate during the early stages of flower morphogenesis.

Following the start of LD treatment of 28-days old Silene plants the first observed increase in concentration of RNA in the apex takes place on day 5 (fig. 23). In the majority of plants (90%) induction is completed by this stage (table 1). It would therefore appear that the increase in RNA concentration may be intimately associated with successful induction and is, in some way, preparing the apex for the increase in growth which is about to take place. Observations on plants exposed to 3 LD and then returned to SD however, throw more light on the situation. These plants do not become induced; they do however consistently demonstrate an ephemeral increase in

apical RNA concentration which takes place on days 5-6; the concentration then returning to that in the SD apex. This suggests changes in RNA concentration in the apex may not be directly related to transition. Even though the first 3 LDs of induction do not result in flowering they do form an essential phase of the inductive process since if they are omitted from, for example, a 5 LD exposure the resultant 2 LD treatment does not induce flowering. Further it has been demonstrated that if two periods of 3 LD are interpolated by up to 4 SD then the effect of the initial 3 LD treatment persists and Silene plants proceed to flower in SD (table 11). Only if 6 SD are interpolated between two 3 LD treatments is the effect of the initial 3 LD exposure dissipated and the plants fail to flower. Because 3 LD treatment is an essential part of full inductive treatment any event following 3 LD treatment may therefore be associated with the first phase of induction.

These observations strongly suggest that flower induction is a stepwise phenomenon. It has been abundantly demonstrated that the floral stimulus is perceived by plant leaves and some substance(s) is (are) then translocated to the shoot apex where realization takes place (Lang 1965). There is evidence of the stepwise nature of evocation from data of Gressel et al (1970) who noted an increase in the rate of RNA synthesis in the Pharbitis apex before the presumed time of arrival of the floral stimulus at the apex. Also in Sinapis the first peak of mitotic index which typifies evocation takes place in vegetative plants which are exposed to conditions only just insufficient for induction to occur (Bernier et al, 1970). In all of these situations it is almost impossible to decide whether these phenomena are intrinsic to the processes of induction and evocation or whether they are parallel but independent events which result directly from the day-length treatment which is used to induce flowering. Certainly in Silene internode elongation appears to be a direct result of LD exposure which is independent of the flowering process.

Several experiments were carried out to determine whether the increase in growth rate which takes place in induced Silene plants during flower morphogenesis is itself an intrinsic part of the flowering process, and also whether the accumulation of RNA in the induced apex resulted directly from LD exposure, or from the arrival of the stimulus in the apex or whether it represented the preliminary stage of the increase in growth rate about to occur during early morphogenesis.

The external application of GA_3 mimicked the effect of LD exposure on internode elongation in Silene plants. In fact the external appearance of SD Silene plants following 7 daily applications of GA_3 was extremely similar to that of untreated 7 LD plants (table 7). GA_3 treatment also resulted in an immediate and sustained rise in RNA concentration in the apical tissue (fig. 26). This rise took place in both 7 LD and SD plants. This result suggests that the rise in RNA in untreated 7 LD plants may be (a) related to the internode elongation which possibly results from the internal release of a member of the gibberellin group of hormones or (b) a direct effect of any hormone released inside the plant as a result of LD exposure. There were however important differences between the effect of GA_3 and 7 LD on the RNA concentration in the apex. Firstly, GA_3 treatment caused an immediate rise in RNA concentration. This supports the view that the initial phase of the RNA increase in LD plants results from the build up of a hormone in the apex and took place only when a sufficient concentration of hormone had accumulated in the apex. The immediate rise in the GA_3 experiment possibly resulted from the application of a large amount of hormone on day 1. Secondly, GA_3 and 7 LD produce an additive effect on the RNA concentration in the apex. This suggests the mechanisms resulting from LD treatment and GA_3 application are different. Though GA_3 treatment of Silene plants caused both internode elongation and accumulation of RNA in the apex it did not,

in any way at all, alter apical growth. Clearly then an increase in RNA concentration in the apex of Silene is not necessarily followed by either an increased rate of apical growth or by flowering.

When Silene plants were transferred from 20°C SD conditions to 12°C SD conditions growth was greatly slowed down (table 9 and 10). DNA concentration per unit volume remained constant (fig. 27) indicating cell size was unchanged and the decrease in growth was due to an increase in the cell cycle time. As a consequence of this slowing down of growth the plastochron length increased (table 9). A fall in the concentration of RNA in the apex of these plants was also observed following transference to 12°C conditions (fig. 27). This fall suggests the rate of RNA synthesis had decreased relatively more than the growth rate, however, since growth did persist, although at a lower rate the RNA concentration was clearly sufficient to sustain it. When 28-day old plants were transferred from 20°C SD conditions to 12°C LD conditions the number of LD required for induction was not increased (indeed data of Lyndon show the number of LD required for induction at 12°C is actually less than that at 20°C - personal communication). In 12°C LD conditions - inductive conditions - the growth rate remained similar to that in 20°C SD conditions (table 10). It would appear that the fall in growth rate caused by the colder conditions was exactly compensated for by the stimulation in growth rate resulting from flower morphogenesis and so the apical growth rate of 12°C LD plants was more or less the same as that in 20°C SD plants. The concentration of RNA in the apex of these two sets of plants also remained very similar. (fig. 27). These observations suggest that the concentration of RNA in the apex is related to growth and in these plants where the growth rate was not increased there was no prerequisite for a higher RNA concentration to sustain it.

In the experiments where induction was brought about by exposing plants to two periods of 3 LD interrupted by various numbers of SD a sustained rise

in RNA concentration only occurred when the combination of 3 LDs and SDs resulted in flower morphogenesis. When flower induction did not take place the RNA concentration rose but only ephemerally, and after two days fell to that concentration in the SD apex (fig. 28). These results therefore further support the hypothesis that the sustained rise in RNA concentration in the apex in 7 LD plants is related to the growth rate increase which accompanies flower morphogenesis at 20°C.

In several situations it proved possible to increase the concentration of RNA in the Silene shoot apex experimentally without stimulating an increase in growth rate. Treatment of both SD and 7 LD plants with GA_3 resulted in an increase in the RNA concentration in the apex without causing any alteration in growth rate. When plants were exposed to 3 LD the RNA concentration increased transiently, however growth remained unchanged. In both these situations (GA_3 and 3 LD) one could postulate the increase in RNA concentration in the apex resulted directly from the arrival there of growth hormones. It also proved possible at 12°C to induce plants and to grow them on to the flower morphogenesis stage without either an increase in the RNA concentration in the apex or an increase in the growth rate at the apex. This experiment shows that successful floral initiation does not depend on either an increase in the RNA concentration or an increase in the growth rate of the apex.

Although situations where an increase in RNA concentration in the apex was not followed by an increase in growth rate were discovered, no technique was found which could stimulate an increase in growth rate in the apex in the absence of induction and which was not preceeded by an increase in RNA concentration. It therefore seems reasonable to conclude that at 20°C the increase in RNA concentration in the apex following inductive LD treatment is a necessary prerequisite for the increased growth rate of the apex during flower morphogenesis i.e. the increase in growth rate requires a higher

RNA concentration. It may well be that the initial stages of this increase in RNA concentration (like that in 3 LD plants) results directly from the internal release and build up of the flowering (and possibly other) hormones in the plant as a result of LD treatment and this concentration is then maintained in preparation for the increased rate of growth which is about to follow. In plants transferred to 12°C LD from 20°C SD the fall in RNA concentration in the apex observed in 12°C SD does not take place. This present hypothesis would account for this observation if in 7 LD plants at 12°C the arrival of hormone at the apex stimulated sufficient RNA synthesis to compensate for the fall observed in 12°C SD plants. Later this concentration of RNA - like that in 20° SD plants is sufficient to sustain apical growth at the rate of that in 20°C SD plants - but not at a higher rate. When the vegetative axillary buds of Cicer are stimulated to develop by the application of cytokinin the rate of synthesis of RNA is stimulated shortly after the application of the hormone. This stimulation in RNA synthesis is shortly followed by increased growth of the buds (Usciati, Codaccioni and Guern, 1972). Similar sequences of hormone application, followed by a stimulation of RNA synthesis followed by a stimulation of growth rate have been described in a variety of tissues (Jensen et al 1964, Hamilton et al, 1965; Zimmerer and Hamilton, 1965).

The increase in RNA concentration in the Silene apex following LD treatment is relatively large 25-50%. The magnitude of this increase suggests that ribosomal RNA is likely to be the major component in such a rise. Lance-Nougarède and Bronchart (1965), Bernier, (1971) and Healey (1964) observed a greater concentration of ribosomes in floral than vegetative apices. A high RNA concentration per cell suggests that the rate of protein synthesis is also high and indeed a high rate of protein synthesis might

be expected in rapidly growing tissue such as the shoot apex during early flower morphogenesis.

Changes in the gross protein concentration in the Silene apex during floral transition at 20°C follow a pattern very similar to that shown by RNA concentration. In 7 LD apices from day 5 the protein concentration in the apex is about 25% higher in LD plants than in SD plants (fig. 29). As with RNA this suggests that the rate of protein synthesis is increased to a greater extent than the growth rate over the period of early morphogenesis. Similar increases in the protein concentration in apices have been observed in other species during floral transition e.g. Sinapis (Bernier, 1969); Chenopodium album (Gifford and Tepper 1962), Pharbitis (Gifford, 1963). In spite of that fact that floral transition involves considerable structural and developmental changes in the apical cells, as well as the increase in growth, the protein band pattern following gel electrophoresis of apical extracts from vegetative and induced plants were indistinguishable. No change could be detected in the protein band pattern either during induction or during early flower morphogenesis even though the vegetative and floral apex were compared until morphogenesis was fairly well advanced with sepal, stamen and petal primordia formed. The interpretation of this result must take account of the fact that this method only allows comparison of the major proteins present. This group must represent only a very small proportion of the total number of proteins in the total complement, also no identification of those proteins present in the preparations was attempted. From the result however, it does appear that the major proteins are present in exactly the same proportions in the induced apex which is forming the flower as were present in the vegetative apex. There may of course be changes in the minor proteins in the apex which could not be detected by the method used.

The absence of any large changes in the protein complement tends to support the hypothesis that any changes which are observed in apical tissue during floral transition may be more concerned with the increase in growth rate and re-direction of growth which accompanies transition at 20°C rather than the morphogenetic switch from the production of green leaves to the production of a flower. If this is so then the selection of Silene, a plant where the apex is transformed directly into a flower, as an experimental plant would tend to minimise gross changes associated with changes in structure. Nevertheless even in Silene it has been shown that at 20°C the growth rate is radically increased upon flower morphogenesis. Such changes are likely to be greater in plants which produce an inflorescence rather than a terminal flower because of the large amount of supporting tissue present in the inflorescence structure.

In Silene plants grown at 20°C the plastochron in SD plants and in plants growing in LD (prior to morphogenesis of the flower) is similar being about 3.7 days. There is no gradual speeding up of the rate of leaf initiation prior to the increase in growth of the apex which heralds flower morphogenesis. At 12°C the rate of leaf initiation in both LD and SD plants was reduced to about 8.3 days. The average rate of growth of the LD apex apparently remained similar to that of 20°C SD plants. There was therefore a change in the relationship between leaf production and apical growth in these plants in 12°C LD conditions. In these plants (12°C LD) flowering took place more quickly relative to leaf production than it did in 20°C LD plants (on average 1.7 leaf pairs were produced by 12°C LD plants between the start of LD and flower production, while over the same period 2.2. leaf pairs were produced on average by 20°C 7 LD plants). These facts suggest that it may be an increase in growth rate of the apex relative to the rate of leaf primordial formation which is important in bringing about flowering. At 20°C the growth of the apex outstrips the rate of primordial production once 2 or 3 leaf pairs have been formed (from the start of LD treatment). On

transference of 28-day old SD plants to 12°C LD the leaf initiation rate was immediately reduced but growth of the apex remained more or less similar. The relationship between primordial production and apical growth had therefore been altered earlier in these plants. This may be the reason flower morphogenesis takes place relatively more quickly at 12°C than at 20°C with only 1 or 2 vegetative leaf pairs forming after the start of LD exposure at 12°C.

PART V

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The Cell Cycle in Vegetative and Floral Shoot Meristems Measured by a Double Labelling Technique

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Summary. A double labelling technique was used to measure the length of the cell cycle in the shoot apical meristem of *Silene coeli-rosa* L. plants kept in short days, in which they remained vegetative, or exposed to 7 long days, which induced flowering. The length of the cell cycle in the vegetative plants (those in short days throughout) was about 18 hrs. It was also 18 hrs, or somewhat longer, in plants which had been exposed to long-day conditions for 5 days, by which time 90% of the plants are committed to flower. When plants had been exposed to the full inductive period of 7 long days and had been transferred back again to non-inductive (short day) conditions, floral morphogenesis had just begun and the cell cycle had almost halved, to 10 hrs. The cell cycle was therefore unaltered during floral induction and shortened only at the onset of the growth of the flower itself.

Introduction

Measurements have shown when the apical meristem of a shoot begins to initiate flowers the rate of cell division is higher than when the shoot was vegetative. The rate of division has been measured in the shoot meristem of *Datura* (Corson, 1969) and *Sinapis* (M. Bodson, 1975) by the technique of metaphase accumulation in the presence of colchicine. The increased percentage of colchicine metaphases in the floral shoot apices indicated that the rate of division was higher than in the vegetative apices. The cell doubling time, measured from counting cell numbers in a series of apices in *Lupinus* and in vernalised rye (*Secale*), shortened on transition to flowering (Sunderland, 1961). Assuming all, or most, of the apical cells were dividing, this indicates a faster rate of cell division at the onset of floral initiation.

These experiments do not allow the distinction to be made between the events occurring in the apex during floral induction, when the apex is still producing leaves, during floral evocation, when the apex becomes committed to flower, and during realisation, when flower morphogenesis itself begins. The occurrence of peaks of mitotic index during induction (Bernier, 1971) is often interpreted as indicating an increased rate of cell division during induction.

The length of the cell cycle in the shoot apex can theoretically be measured by techniques involving labelling with radioactive substances. In practice there are several difficulties with the shoot apical meristem. Firstly, the cell cycle is relatively long especially in the slowly growing cells at the summit of the apical dome, so that even in experiments which last for several days the cell cycle of some of the apical cells cannot be measured (Michaux, 1969; Jacqmard, 1970).

Secondly, applied solutions are not readily absorbed by the intact shoot apex and usually each apex has to be exposed by dissection before label can be successfully administered (Bernier and Bronchart, 1963). This seriously limits the number of plants that can be used for an experiment and so allows only a small number of samples. Thirdly, the mitotic index is characteristically low in the shoot apex so that methods which depend on scoring the percentage of mitotic figures require many samples. Despite these difficulties Jacqmard (1970) succeeded in measuring the cell cycle and its component phases in most parts of the vegetative apex of *Rudbeckia*. However it is obvious that a more convenient method would be useful for measuring the cell cycle in the shoot apex, where the cell cycle is relatively long—often a day or more (Lyndon, 1973)—and the number of plants available for sampling may be limited. A method was therefore devised, similar to that of Wimber and Quastler (1963), which depends on labelling with [^{14}C]thymidine followed by [^{3}H]thymidine and which allows measurements to be made for several days with a minimum of samples.

The principle of the method is to label a cohort of cells, which are in the S phase of the cell cycle, with a pulse of [^{14}C]thymidine and then to allow these cells to proceed through the cell cycle. The progress of these cells is monitored by applying [^{3}H]thymidine at intervals as a terminal label. If the [^{3}H]thymidine is applied at the same time as the [^{14}C]thymidine then the cells will become doubly labelled with both ^{14}C and ^{3}H. If there is a sufficient interval between the application of [^{14}C]thymidine and [^{3}H]thymidine then all the ^{14}C-labelled cells will have moved out of S and into G2, M, or G1 and the ^{3}H will therefore label other cells, which are in S at that time and no doubly labelled cells will be found. When the ^{14}C-labelled cohort has completed a whole cell cycle and again reaches S, then the application of [^{3}H]thymidine will also label them and they will be observed as doubly labelled cells. The length of the cell cycle is therefore the interval between the application of [^{14}C]thymidine (at the beginning of the experiment) and this new occurrence of doubly labelled cells.

This method was used to measure the length of the cell cycle in the shoot apical meristem of *Silene* plants during vegetative growth, floral induction and the early stages of floral morphogenesis. *Silene* was chosen because, on induction, the apical meristem transforms directly into a terminal flower.

Materials and Methods

The plant used for this work was *Silene coeli-rosa* (L.) Godron (supplied as *Viscaria cardinalis* by Stewart's Ltd., Seedsmen, Edinburgh). This is a qualitative long-day plant in the conditions under which it has been grown in these experiments. Plants were routinely maintained in a short-day regime in a growth room at a constant temperature of 20° C. High light intensity (90–100 $W \cdot m^{-2}$ fluorescent light and 15 $W \cdot m^{-2}$ tungesten light) was given during 8 hrs daily from 9 a.m. to 5 p.m. For long-day conditions low intensity illumination (tungsten light, 2 $W \cdot m^{-2}$) was continued from 5 p.m. to 9 a.m. The plants were sown and all were reared in short-day conditions for 28 days. A uniform population of plants was obtained by retaining only those plants in which the 3rd leaf pair was between 20 and 30 mm long. These plants had just initiated, or were on the point of initiating, the 7th pair of leaf primordia. All experimental treatments were subsequent to this sampling of 28-day old plants. Plants were induced to flower by growing them for 7 days (days 28 to 35) in long-day conditions; the plants were then returned to short days.

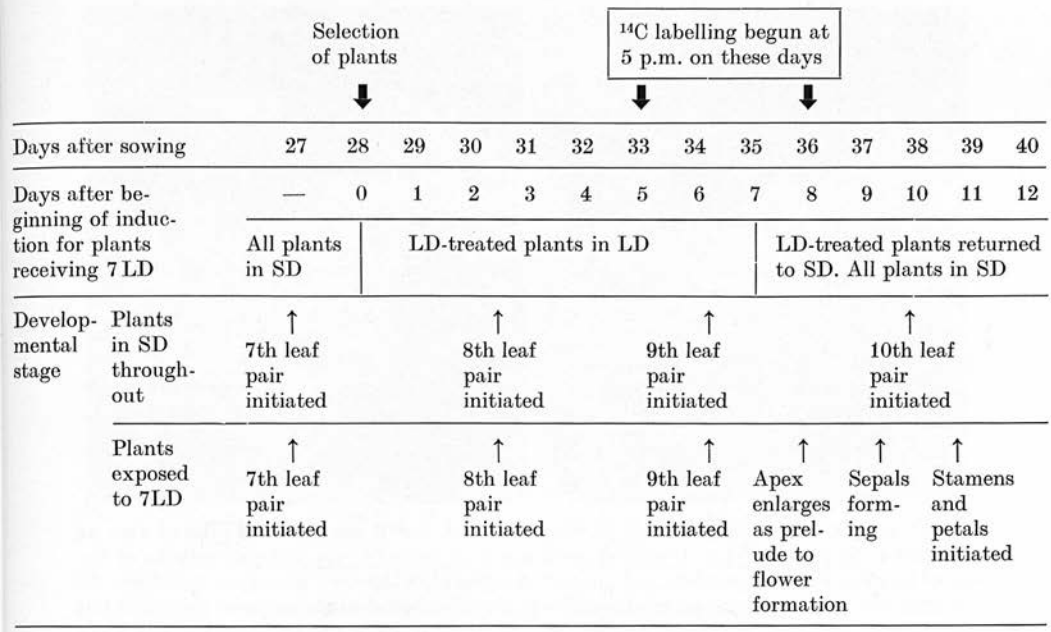

Fig. 1. The experimental design showing the development of the plants in relation to the times at which the cell cycle was measured

The labelling experiments were performed on plants on the 33rd and 36th day of growth (Fig. 1). On day 33 the plants in short days were vegetative; the plants being induced in long days were still making leaves although by this time 90% of them are committed to flower and will subsequently do so if returned to short days at this point. On day 36 the plants in short days were vegetative. The plants which had been in long days, and which had now been returned to short days, had become induced to flower and the very early stages of flower morphogenesis were about to commence. At this stage the apex is beginning to enlarge prior to flower formation. During the 33rd and 36th day of growth the plants to be used were carefully dissected to remove the young leaves near the shoot apex so that the apex was exposed. As each apex was prepared, a drop water was placed on the apex to prevent it drying out while further plants were being prepared. The [^{14}C]thymidine was applied to each apex after careful removal of the drop of water which had been previously placed on the apex. 0.2 μCi [^{14}C]thymidine (specific activity 59 Ci.mol^{-1}) was applied to each apex as an aqueous drop of 0.05 ml. After 2 hrs, excess [^{14}C]thymidine was carefully blotted off and the apex was washed with distilled water. A drop of water, held in place by a small piece of cotton-wool, was left on the apex to keep it moist until the application of [^{3}H]thymidine. [^{3}H]thymidine was applied at intervals as a terminal label. 2 μCi of [^{3}H]thymidine (specific activity 22 Ci. mmol^{-1}) in an aqueous drop of 0.05 ml was applied to each apex for 2 hrs. The [^{14}C]thymidine was in every case initially applied to the plants at 5 p.m. at the end of the high intensity light period and the [^{3}H]thymidine applied at intervals up to 24 hrs later. [^{14}C]thymidine was applied as the first isotope since fewer of the higher energy β particles emitted by the ^{14}C isotope would terminate inside the nucleus than would be the case for the lower energy β particles emitted by tritium. Since tritium was applied as a terminal label any deleterious effects would be minimised. A vegetative plant and an induced plant which had been treated with both radioisotopes were allowed to continue growth. The vegetative plant continued to produce leaves and the induced plant subsequently flowered showing that the apical meristem continued growth after treatment.

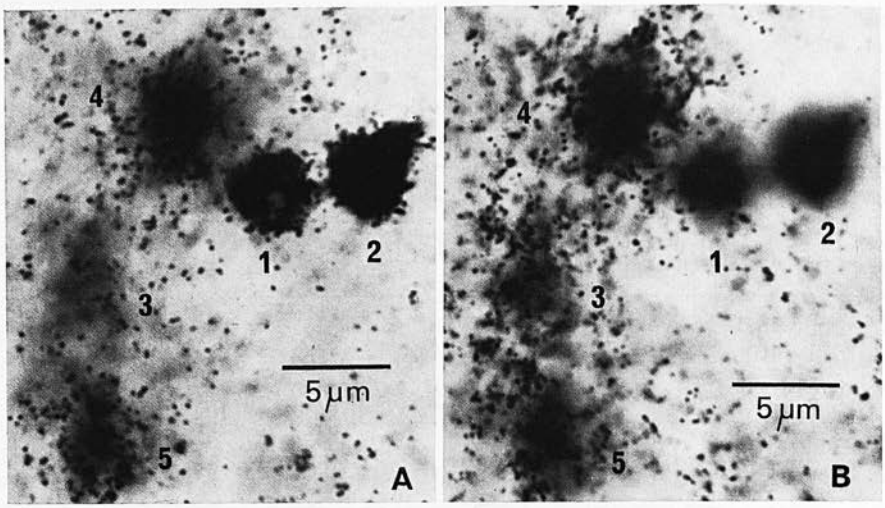

Fig. 2 A and B. Autoradiographs of labelled nuclei. A and B are the same field of view at different focal planes. When the lower emulsion is in focus (A) the autoradiographs of the nuclei labelled with ^{3}H are clear and autoradiographs of ^{14}C-labelled nuclei are indistinct. At a higher focal plane (B) the autoradiographs of the ^{3}H-labelled nuclei are now indistinct but the ^{14}C-labelled nuclei are clear. The ^{14}C also tends to produce tracks. Nuclei 1 and 2 are singly labelled with ^{3}H; nucleus 3 is singly labelled with ^{14}C; nuclei 4 and 5 are interpreted as being doubly labelled

The labelling was terminated by fixing the plants in 80% aqueous ethanol. The material was dehydrated, embedded in wax, and longitudinal serial sections 5 μm thick were cut through each apex. The sections were mounted on "subbed" slides (Rogers, 1967).

For autoradiography the slides were dewaxed with xylene and hydrated. They were then coated with Ilford K2 emulsion which records tritium emissions with high efficiency, but due to its relative insensitivity records very few of the higher energy ^{14}C β particles. The K2 emulsion was applied as a 50% aqueous mixture which resulted in an emulsion thickness of approximately 3 μm. About 1 hr after the K2 emulsion had dried a second layer of emulsion, this time Ilford L4, was then applied to the slides as a 75% aqueous mixture which gave a relatively thick layer of emulsion. Ilford L4 emulsion is more sensitive than K2 and will record β particles from ^{14}C as tracks of silver grains. Since β particles emitted by tritium travel a maximum of 3 μm, relatively few penetrated the K2 emulsion layer. After 1 week's exposure the emulsions were developed in Ilford ID19 developer, washed and then fixed in Ilford Hypana fixative. The sections were then stained through the emulsion with methyl green/pyronin (Casselman, 1959).

The isotope source was identified on the autoradiographs by the presence of silver grains in the different emulsion layers, which appear in different focal planes under a high power objective, and also by the more diffuse scatter of grains caused by a ^{14}C source (Fig. 2). A further aid to source identification was that the L4 silver grains were slightly smaller than those in the K2 emulsion layer and tended to appear as tracks. The numbers of nuclei, singly labelled with either ^{14}C or ^{3}H, or doubly-labelled with both ^{14}C and ^{3}H, were counted in the apical dome of each apex. Usually several non-adjacent, approximately median sections were scored for each plant.

Results

The values for the percentage of labelled cells which are doubly labelled for the experimental series are shown in Fig. 3. The values for the samples obtained

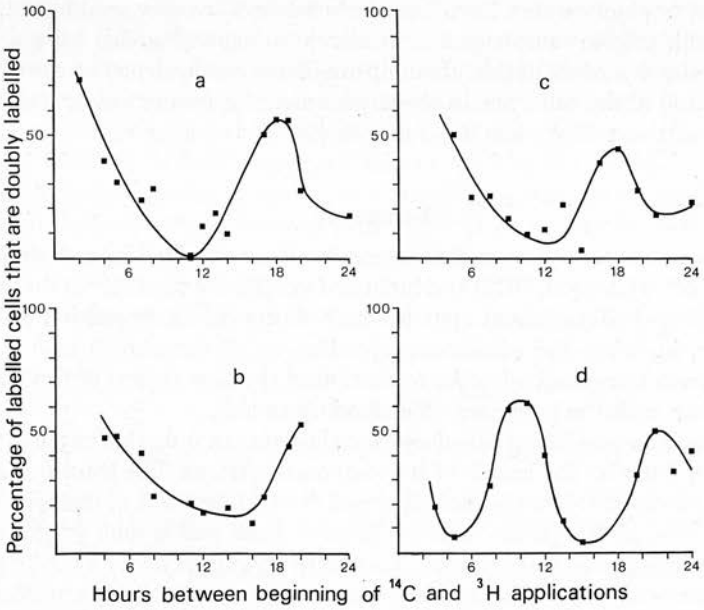

Fig. 3a—d. The percentage of doubly labelled cells at intervals after the application of [^{14}C]thymidine at 0 hrs. (a) Plants in short days, day 33 (vegetative). (b) Plants in long days, day 33 (induction completed for most plants). (c) Plants in short days, day 36 (vegetative). (d) Plants which have received 7 long days, day 36 (floral morphogenesis beginning)

on day 33 are shown in Fig. 3a and b, and on day 36 in Fig. 3c and d. In each case the values fall from a high value, presumably 100% at 0 hrs, to a minimum some hrs later. The values then rise to a new maximum, the period between the beginning of the experiment and the peak of the new maximum being the length of the cell cycle. In the vegetative plants on day 33 (Fig. 3a) the second maximum occurs at 18–19 hrs. The length of the cell cycle is therefore approximately 18–19 hrs. In the plants being induced in long days on day 33 (Fig. 3b) the values rise to a new maximum but the data are insufficient to say where the peak of this maximum occurs. It is however at least 20 hrs and the cell cycle in these plants undergoing induction was therefore at least as long as in the comparable vegetative plants in short days.

In the plants sampled at 36 days, those which had been in short days throughout and were vegetative (Fig. 3c) showed a new maximum of doubly labelled cells at about 18 hrs. The length of the cell cycle was therefore about 18 hrs. In the plants which had received 7 long days and had been returned to short days by the 36th day (Fig. 3d) the new maximum was attained very much sooner, at about 10 hrs. There was a second maximum at 21 hrs. The length of the cell cycle in these induced plants was therefore about 10 hrs, little more than half that of the comparable vegetative plants (Fig. 3d and c). The length of the cycle therefore appears to be similar, about 18–20 hrs, for vegetative plants on both the 33rd and 36th day of growth and for plants undergoing induction on their 33rd day of growth. In

contrast the plants which have been induced and are now committed to flower and in which floral morphogenesis is about to begin (Fig. 3d) have a cell cycle which is about one half of this, about 10 hrs. These results demonstrate that there is a shortening of the cell cycle in the shoot apex of *Silene* but only after induction is completed and only when floral morphogenesis is beginning.

Discussion

These values for *Silene* confirm the indications obtained from *Datura* (Corson, 1969), *Sinapis* (Bodson, 1975) and lupin and rye (Sunderland, 1961) that the length of the cell cycle in the shoot apex becomes shorter at the transition to flowering. However, in *Silene* the consequent speeding up of the growth rate is clearly a phenomenon associated with the formation of the new organs of the flower itself rather than with the processes which lead up to this.

It should be possible to calculate from the data not only the length of the whole cell cycle, but also the length of its component phases. The length of S is given by the distance between the ascending and descending limbs of the peak of doubly labelled cells, at the points midway between peak and trough values, when the 2 hrs labelling period is subtracted. This gives a value of about 4 hrs for S for both sets of short-day plants (Fig. 3a and c) and a value of about 2.5 hrs for the long-day plants on day 36 (Fig. 3d). Such a shortening of the length of S when the cell cycle shortens has been observed in vegetative apices of other plants (Lyndon, 1973).

The time at which mitosis occurs in the cohort of ^{14}C-labelled cells should be marked by the appearance of ^{14}C-labelled mitotic figures and also by a sudden doubling in the ratio of $^{14}C/^{3}H$ singly labelled cells. However, labelled mitotic figures were not unequivocally identified on the autoradiographs and the variations in the observed ratio of $^{14}C/^{3}H$ singly labelled cells were too great to allow meaningful conclusions to be drawn. The ratio in many samples was greater than 2, which is not theoretically possible, suggesting that there was an excess of ^{14}C-labelled cells, perhaps because the absorbed [^{14}C]thymidine was not adequately removed by the washing procedure, but persisted in the tissues so that there was a continued labelling of cells with ^{14}C after the initial period of application.

From the theory of the method it would be expected that when the ^{14}C-labelled cells once again reach S and become labelled with ^{3}H as well, the percentage of labelled cells which are doubly labelled in this new peak should reach 100%. It clearly does not do so in any of the samples measured (Fig. 3). One reason for this may be that 100% doubly-labelled cells would only be observed if there were no cell-to-cell variation in the length of the cell cycle, which presumably there is. A further reason that the doubly-labelled cells may not reach 100% could be the overestimation of singly labelled cells, for the reasons already discussed.

Despite the inadequacies of the present technique for measuring the phases of the cell cycle, it has proved satisfactory for measuring the length of the whole cell cycle. The double labelling method has considerable advantages for measuring the length of the cell cycle in tissues in which division is relatively slow and the

mitotic index is low, as in the shoot apex, and in tissues which are difficult to label. Relatively small numbers of plants may be used, since all the nuclei which are labelled contribute to the data and not only those which happen to be in mitosis. Also, sampling does not have to be so close as with methods which require the scoring of the percentage of labelled mitotic figures. Although there is some subjectivity involved in determining whether or not a nucleus is doubly labelled, the technique could possibly be improved by modification of the autoradiographic procedure so that cells, singly labelled with ^{14}C or ^{3}H, or doubly labelled, could be more easily distinguished from each other.

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