THE EFFECTS OF VERNALISATION ON GROWTH AT THE WHEAT SHOOT APEX

by

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ABSTRACT

Three genetic stocks of wheat, Triticum aestivum L. which differ by single genes for vernalisation requirement, were used in this study. Chinese Spring Hope 5A has no vernalisation requirement, Chinese Spring Hope 5D has a strong requirement and Chinese Spring Euploid is intermediate in requirement between the two. The growth of the shoot apex in the 3 unvernalised cultivars and in vernalised Chinese Spring Hope 5D was studied in an attempt to characterise the nature of the response of the plant to chilling. The plants were routinely grown at 18°C in an 18 hour daylength, vernalisation of the seeds was for 6 weeks at 4°C.

Vernalisation reduced the number of leaves produced on the main stem, the length of time between planting and ear emergence, and the number of tillers produced, to a level which was comparable to that in Chinese Spring Hope 5A. The reduction in time to ear emergence was mainly in the period from collar to double ridge formation.

There were few differences between the genetic stocks in the growth of the shoot apex up to collar initiation, which was initially regarded as the transition to flowering. However the mean volume of the apical dome and the mean cell volume over the first 10 days after planting, may have been greater in Chinese Spring Hope 5A and vernalised Chinese Spring Hope 5D than in Chinese Spring Hope 5D and Euploid. The relative rate of growth of the primordium may have increased in unvernalised plants of Chinese Spring Hope 5D upto the collar primordium whereas in vernalised plants the rate remained approximately constant. There appeared to be no particular size, rate of primordium initiation, volume relative growth rate or partitioning of the apical dome associated with collar initiation but there may have to be a maintenance of the rate of RNA accumulation (as in Chinese Spring Hope 5A and vernalised Chinese Spring Hope 5D) for early floral initiation to occur.

After collar formation in Chinese Spring Hope 5A and vernalised Chinese Spring Hope 5D, the mean volume of the apical dome increased rapidly up to double ridge formation, when the maximum dome volume was attained, but there was no such increase in Chinese Spring Hope 5D and Chinese Spring Euploid.

The rate of primordium initiation a few plastochrons after collar formation became inversely related to vernalisation requirement, an increase in rate being observed in Chinese Spring Hope 5A and vernalised Chinese Spring Hope 5D. In all the genetic stocks the volume relative growth rate of the apical dome tended to decline up to collar formation, and in Chinese Spring Hope 5D and Chinese Spring Euploid this decline was then maintained. However in Chinese Spring Hope 5A and vernalised Chinese Spring Hope 5D the decline in relative growth rate was halted and the rate tended to increase, suggesting that some substance could have built up during vernalisation which prevented the decline in relative growth rate, or vernalisation may have prevented the formation of some growth inhibitor . Associated with the increase in relative growth rate was a decrease in the length of the cell cycle, the reduction in length being mainly in the $G_1 + \frac{1}{2}S$ phase but $G_2 + \frac{1}{2}S$ and M were also reduced. Synchronisation of cell division was not detected at either planting, collar or double ridge formation in Chinese Spring Hope 5A.

Experiments in which plants were transferred from long to short days conditions at intervals suggested that perhaps double ridge formation should be regarded as the transition to flowering, since labile primordia were not committed to producing leaves or spikelets until double ridge formation.

The results suggest that the amount and partitioning of assimilates may be affected by vernalisation, and important in determining when floral initiation occurs, the involvement of growth substances is also implicated.

REGULATION 2.4.15

I declare that the work recorded in this thesis and the composition of the thesis is my own.

SIGNED

CONTENTS

PART 1

INTRODUCTION

The phenomenon of vernalisation was first observed in 1857 by Klippart, who described methods of treating moist winter wheat seed with cold so that the plants would head rapidly when they were planted in the spring. In the years up to 1918, George Klebs observed similar effects in plants of sugar beet and many other biennials. In 1918, Gassner extended these observations and his conclusions regarding the physiological difference between spring and winter rye were that spring rye is practically independant of any need to pass through a cold period before ear formation and elongation of the internodes (shooting) could occur, but flowering of winter rye was delayed unless it passed through a cold period either during germination or at some stage subsequent to germination. It was Lysenko in 1929 who gave a name to the phenomenon, he called the process jarovisation and in his English, French and German translations this became vernalisation (vernum - spring) (Chouard, 1960). Vernalisation has been extensively reviewed (Murneek and Whyte, 1948; Gregory, 1948; Chouard, 1960; Purvis, 1961; Schwabe, 1971) and further information can be obtained from these articles.

During the development of understanding of the process, vernalisation has been used with various meanings (Salisbury, 1963). The term has been used to imply any positive plant response to low temperature or any hastening of flowering in response to any environmental variable. The term vernalisation then became limited to the low temperature promotion of flowering and was not used for other responses like the breaking of dormancy in certain seeds or buds. Vernalisation is here restricted to the inductive promotion of flowering by low temperature. Vernalisation is therefore revealed by an after-effect, it is the preparatory process to flower initiation but is not the flower initiating process itself. By this definition, the cold treatment in Brussel sprouts is not regarded as vernalisation since cold sensitivity does not develop until a minimum size or age of the plant has been attained and formation of flower buds occurs during the cold treatment rather than later (Stokes and Verkerk, 1951).

There are three main classes of plants which have a vernalisation requirement (Schwabe, 1971). The first includes plants which begin their growth towards the end of autumn and over winter as seedlings; in these species the germinating seeds can be vernalised, for example winter cereals

The second group is the true biennials which enter winter as fairly large plants but flower in the next season, they are normally affected by chilling only in the later stages of their growth, for example Hyoscyamus niger. The third group is the perennials, in which branches on the old plant or offshoots from it are produced each season and have a chilling requirement, and these give rise to the flowering stems in the following year, for example Chrysanthemum.

The temperature and duration of chilling are important. Purvis (1948) suggested that the optimum temperature for vernalisation was between 1 and 6 or 7°C for winter rye, and Chujo (1966) showed that vernalisation in wheat could occur over the temperature -3 to +13°C but the most effective temperature was in the range 3 to 8°C. Purvis also observed that the time for complete vernalisation in Petkus winter rye was 40 - 45 days.

Much of the information on vernalisation in cereals has been obtained from the studies made by Gregory and Purvis using the winter and spring races of Secale cereale cv. Petkus (Purvis, 1961). Cold treatment of spring rye has no effect on the time to flowering but winter rye shows a great delay in ear formation and emergence unless exposed to low temperature during germination. The cold requirement of the cereals is not absolute since plants from unvernalised seeds will ultimately flower but only after a large number of leaves have been produced on the main stem.

Rye and wheat are quantitative long day plants in that they require long day conditions for flowering. However in winter rye, growth in short days for the first six weeks of growth can replace part of the vernalisation by hastening the onset of floral initiation (Purvis and Gregory, 1937). In continuous short days the plant produces the maximum leaf number on the main stem but, although differentiated, the ear does not emerge, and therefore the plant must be placed in long days in the later stages for extension of the internodes to occur. Winter wheat resembles rye in this respect, and Mc Kinney and Sando (1933) stated that winter varieties have growth optima under low temperature and short days during the initial growth phase, whereas spring varieties have growth optima at the higher temperatures and longer photoperiods.

Also Voss (1938) showed that short day conditions at constant high temperature induced "shooting" in winter wheat which showed that the thermo-phase was not obligatory in winter wheat.

Purvis (1934) introduced the concept that there is a minimum leaf number in rye which cannot be reduced. The validity of this was questioned by Holdsworth (1956) on the grounds that he was able to reduce the leaf number of Celosia argentea by withholding nitrogen. However Gott, Gregory and Purvis (1955) observed that with no added nutrients rye plants grown in sand came into ear with the same leaf number as plants which were supplied with nutrients. In rye no flower inducing treatment so far tested has reduced the leaf number below five. Between the minimum and maximum leaf number in rye, the primordia are known as labile (Purvis and Gregory, 1937) as they can produce either a leaf or a spikelet depending on the conditions. These primordia can be used in experiments where plants are transferred from long to short days at intervals, to determine when a plant is irreversibly committed to producing a certain leaf number, and therefore when a primordium is irreversibly committed to producing a leaf or a spikelet (see results page 122). The possibility of this being used to define the transition to flowering is considered in the results (page 131) and the discussion (page 153).

In most of the studies on flowering, plants have been used in which flowering is under the absolute control of day-length (for list of these plants see Salisbury, 1963; Evans, 1971) and hence the transition to the reproductive condition can be pinpointed. However in wheat, there is difficulty in deciding when the transition to flowering occurs. In this study, the transition to flowering is regarded as the formation of the collar, as all subsequent primordia will form spikelets. The first morphological sign that the plant has become reproductive is at double ridge formation, and therefore the possibility of this being regarded as the transition to flowering will be discussed.

It has been shown that it is the shoot apex which perceives the cold stimulus. In celery (Curtis and Chang, 1930) and beet (Choroboczek, 1934) it was shown that chilling the growing points alone (by passing cold water through rubber tubing wound around the tip of the plant while keeping the rest of the plant at ordinary glasshouse temperatures) was fully effective, leading to undelayed flowering. In Chrysanthemum, Schwabe (1954) demonstrated that vernalisation of the terminal growing points was as effective as vernalising the whole plant. Gregory and Purvis (1938a) showed that isolated rye embryos on sterile media could be vernalised almost as well as the whole grain (though with some lag response) and therefore the embryo and not the endosperm perceived the cold stimulus. Furthermore Purvis (1940) showed that apical fragments consisting of the apical dome and one leaf initial could be successfully vernalised. In wheat, Ishihara (1961) showed that the apical dome + two or three primordia, growing on an agar medium containing glucose and minerals, could be successfully vernalised. It is still not clear, however, whether it is the apical dome, the primordia, or both which is the most important in perceiving the stimulus. Wellensiek (1962) suggested that vernalisation requires dividing cells if it is to be effective. This was based on his observation in Lunaria, where rooted leaves may be chilled; the effect being perceived by meristematic cells formed at the base of the leaves. This may also be the explanation in Streptocarpus wenlandii, where if leaf cuttings were made from vernalised plants, the plantlets which arose from the centre of the leaf were the first to show signs of vernalisation, and the effect appeared to reach the main apex only later (Oehlkers, 1956).

In order to try and understand the mechanism of vernalisation, it was thought that studying the effects of chilling on growth at the wheat shoot apex would be an interesting approach, since the apex perceives the stimulus and it is also the part of the plant which reacts to the stimulus.

The first sign that an apex is becoming reproductive is an enlargement of the apex, described for wheat by Bonnett (1966) and Opatrná, Seidlová and Beneš (1964).

Kirby (1974) observed a doubling in the length of the spring wheat apex during the period of leaf formation, and an increase in the rate of primordium formation at about the time of collar formation, with spikelets being initiated approximately 3 times as fast as leaves. Vernalised winter cereals become reproductive at approximately the same time after planting as spring cereals, therefore an increase in the rate of primordium initiation, similar to that in spring cereals, might be expected in vernalised cereals at collar initiation. Indeed in rye, Sunderland (1961) observed an increase in the rate of primordium initiation several plastochrons (approximately 4) after the transition to flowering in vernalised plants, from 1.0 per day to 1.5 per day. In addition, primordia were produced at about double the rate in vernalised plants (1.0 per day) than in unvernalised (0.5 per day). Gott, Gregory and Purvis (1955) reported that the rate of primordium formation after floral initiation depended on the duration of vernalisation. Purvis (1934) observed that the rate of leaf initiation was not influenced by germination temperature. Purvis and Hatcher (1959) found that the rate of leaf emergence was increased in vernalised plants.

The only quantitative measurements of apical growth after vernalisation are those of Sunderland (1961) who studied apical fragments of winter rye. Cells were produced at a rate about 0.6 day higher in vernalised than in unvernalised seedlings. The average cell volume of the apex above the third primordium was greater in vernalised seedlings. In unvernalised plants the mean cell volume decreased after about 7 days from sowing and then remained about the same. In vernalised plants, the mean cell volume decreased about 4 days after planting, stayed about the same and then increased about 12 days after planting, just after the increase in the rate of primordium initiation.

The rates of cell division reported by Sunderland were only average rates which included the cells in the dome and in several primordia and embryonic internodes, but he suggested that these rates probably approximated to those in the apical dome, and the changes observed during development were indicative of corresponding changes in the dome.

Sunderland concluded that during vegetative development of the dome of unvernalised rye, the average rate of cell division and the average cell size both decreased, but the dome still increased in size because at the differentiation of each primordium a progressively smaller proportion of the cells in the apical dome was given over to the new primordium, and therefore a correspondingly larger proportion remained to constitute the new dome. With the onset of reproductive growth in vernalised rye, the size of the apical dome increased rapidly, there was a rapid rate of primordium initiation, the size of the primordium remained small relative to the dome and there was a large increase in the rate at which the cells multiplied and expanded. Sunderland therefore suggested that at the transition to flowering a large number of the cells in the apical dome which were dividing slowly started to divide rapidly. In rye the rate of cell division and expansion may remain high until most of the primordia have been produced and may then decline. In this thesis the growth of the apical dome and phytomers (primordia + axial tissue) will be studied separately.

In trying to understand the mechanism of vernalisation it is important to consider the environmental requirements for the process, the metabolic changes which occur during the cold treatment and also the theories which have been suggested to explain the process. For the vernalisation process oxygen is essential (Gregory and Purvis, 1938b). Seeds must have imbibed water and therefore be germinating for vernalisation to occur (Gregory and Purvis 1936) although it has been shown that grain maturing and ripening in the parent ear can be vernalised to a considerable degree (Gregory and Purvis, 1938a). In the cereals with no absolute cold requirement, vernalisation has proved effective at every stage from fertilisation until floral differentiation occurs in the absence of low temperature (Purvis, 1961). It is possible to vernalise growing plants of rye and in long days the older plants at the beginning of vernalisation, the shorter the period between the end of vernalisation and earing (Friend, 1953). However when floral initiation was reached cold no longer had any effect on the apex. Gott (1957) found that it was possible to vernalise winter wheat at any stage from the seed up to a six week old plant with 6-7 leaves on the main stem, despite earlier growth above the vernalisation temperature.

Purvis (1944) showed that no nitrogenous compounds additional to those present in the embryo were needed for vernalisation, and carbohydrate particularly in the form of 0.5% sucrose gave accelerated vernalisation but was not an essential substrate. This was confirmed in young plants of Chrysanthemum by Schwabe (1957).

Devernalisation can be achieved in a number of ways. The first is by high temperature treatment; the degree of de vernalisation varied inversely with the duration of the previous vernalisation treatment, and devernalisation was reversible, as a plant devernalised by high temperature could be vernalised again (Purvis and Gregory 1952). Devernalisation also occurs if vernalised grain is dried and stored (Gregory and Purvis, 1938b). Petkus winter rye vernalised for 6 weeks and air dried for 48 hours, retained the vernalised condition for over 6 weeks, but after 8 weeks became almost completely devernalised. Therefore it seemed that although the apex was still intact and viable, the vernalisation product was lost. Devernalisation can be achieved by interrupting the vernalisation. Gregory and Purvis (1938b, 1952) showed that discontinuous low temperature gave a lesser vernalisation response than continuous treatment, even with the same total exposure to cold. The response varied with the number of intercalated days at room temperature, and with equal periods of high and low temperature, there was no response.

Qualitative and quantitative changes in protein (Trione, 1966; Teraoka, 1967, 1968 and 1973; and Tomita, 1973) and RNA (Ishikawa and Usami, 1975; Ishikawa, Ishikawa and Usami, 1975; and Fukushi, Ishikawa and Sasaki, 1977) have been reported in wheat during vernalisation. There is an increase in the rate of nucleic acid synthesis in the seedlings of vernalised winter wheat compared to vernalised spring wheat (Filek, 1976), and an increase in the rate of DNA synthesis in the shoot apex of vernalised Arabidopsis thaliana (Besnard-Wibaut, 1977) and in barley seedlings during vernalisation (Shiomi and Hori, 1973). Dévay and Páldi (1976) observed a change in the forms of ribosomal RNA synthesised in winter wheat seedlings during vernalisation.

A ribon uclease enzyme (termed RNase 1) which was synthesised just under the conditions of vernalisation, and which appeared in the first few hours of vernalisation has been isolated (Dévay, 1965). Dévay hypothesised that low temperature not only inhibited the synthesis of the RNA which regulated vegetative growth but also as a consequence of RNase 1 activity disturbed the synthesis of RNA leading to a regulation of the ratio of various RNAs and the feasibility of their synthesis.

Many other effects on metabolic processes, either during or as a result of vernalisation, have been observed. Štefl, Trčka and Vrátný (1978) reported an accumulation of free proline in the shoot apex of wheat during vernalisation which may have a role in enzyme regulation. Changes in the activity of glutamate and malate dehydrogenase have been observed in winter wheat chloroplasts during vernalisation (Babenko and Nariĭchuk, 1976) and the activity of indoleacetic acid oxidase, which may be regulated by gibberellic acid, increased during cold treatment of winter wheat seedlings (Bolduc, Cherry and Blair, 1970).

Reports regarding the role of growth hormes in vernalisation are varied. Gibberellins had no direct effect on flower induction in winter rye and could not replace the low temperature treatment, any accelerating effect on flowering which was observed was at some post-inductive stage (Purvis, 1960). In Petkus winter rye, plants must reach a specific stage of development before they can be induced to flower by gibberellic acid - this can then affect the fate of labile primordia so that flowers, not leaves, are formed (Caso, Highkin and Koller, 1960). During the vernalisation of winter wheat the levels of auxins and gibberellins in the embryo increased and the levels of growth inhibitors decreased (Reda, Larsen and Rasmussen, 1978). There is an increase in indoleacetic acid, gibberellin and cytokinin levels in vernalised roots and shoots of wheat seedlings (El-Antably, 1976) and similarly, a greater accumulation of endogenous auxins and growth inhibitors has been observed in plants of winter wheat grown from vernalised seed rather than unvernalised (Khokhlova and Chuzhkova, 1976). Cytokinin activities which were detected in extracts of vernalised winter wheat grains, may act in a synergistic way with both auxins and gibberellins (Reda, 1976).

Reda also suggested that vernalisation is likely to involve meristem activation, induction of gibberellin synthesis, accumulation of RNA at the meristem and induction of DNA synthesis, ensuring that the plant is able to respond to optimal conditions of light and temperature to express floral evocation. In wheat and barley plants gibberellin can promote flowering in plants with a high vernalisation requirement where the chilling was insufficient to satisfy the high cold requirement (Suge and Yamada, 1965).

Suge (1977) observed a decrease in ethylene production in vernalised wheat seedlings but could not conclude whether this was truly related to the biochemical mechanism of vernalisation or whether it was a low temperature effect. Active phytochrome formation in winter wheat occurs in the second half of vernalisation (Dévay, 1967), and Friend (1965) suggested that cold may prevent the thermal reversion of active phytochrome 730 to inactive 660, which is a process accelerated by high temperature. The synthesis of unsaturated membrane phospholipids increased during vernalisation of winter wheat (De Silva, 1978). There was an enhanced accumulation of carbohydrate fractions in the leaves of winter wheat at $2^{o}C$ compared to $25^{o}C$, but these may be metabolic alterations associated with low temperature rather than a vernalisation response (Trione, 1966).

Theories to explain the vernalisation process were put forward as early as 1918 when Klebs discussed his results on the basis of nutrition, and suggested that the balance between assimilation and nitrogen uptake was in some way related to the attainment of the "ripeness to flower". In 1934, Lysenko developed "the theory of the phasic or stadial development of plants", which suggested that plants must progress in order through a series of developmental stages each subjected to environmental control (Murneek and Whyte, 1948). Purvis and Gregory (1937), Gregory (1948) and Gott, Gregory and Purvis (1955) developed a scheme to explain vernalisation in terms of a sequence of reactions involving hypothetical substances, the different stages of the scheme being affected by environmental conditions. Floral initiation would result from the build up, promoted by low temperature, of a certain level of one of these substances in winter cereals, but the required level of this substance was already present in spring cereals. A similar scheme was proposed for henbane by Lang and Melchers (1947).

Van de Sande Bakhuyzen (1947) interpreted the low temperature reaction as the production of a hypothetical enzyme "vernalase." A second phase follows, promoted by warm temperatures and short days and catalysed by "vernalase" in which a precursor is converted into a hypothetical substance "vernalin". When this substance reaches a critical concentration, ripeness to flower is achieved and the photoperiodic responses can then occur. If the critical level is not reached, conversion to other types of growth promoters were thought to take place by a number of back reactions.

Von Denffer (1950) suggested the role of low temperature is to prevent the formation of an inhibitor of flowering. However, if vernalisation does not result in the formation of some substance, it is difficult to explain devernalisation. Since vernalisation is less effective in the absence of oxygen, one would assume that the inhibitor would be produced in anaerobic conditions even at low temperature. Therefore spring rye would be expected to react to anaerobic conditions during growth in the same way as winter rye, but Gregory and Purvis (1938b) have shown that while flowering is slightly delayed by treating spring rye in this way, the effect is not comparable to that in winter rye.

Multiplication of the vernalisation effect can be shown by removal of the vernalised main shoot of rye and a succession of tillers; there was no diminution of the vernalisation effect in the crop of tillers allowed to grow (Purvis and Gregory 1937). In addition Schwabe (1954), in Chrysanthemum, found that after removing the main and many lateral shoots, after the seventh degree of branching there was no dilution or reduction of the vernalisation stimulus. He concluded that this suggested vernalisation caused a more permanent chemical change associated with the nucleus and cytoplasm of the apical meristem, which is self duplicating, rather than the production of a certain amount of substance which could be diluted by cell division and growth, and he suggested specific gene activation or suppression may occur. There is some evidence which suggests that a translocatable substance results from vernalisation treatment, which has been called "vernalin" (Melchers, 1939). Melchers found it was possible to graft a vernalised henbane plant to a non-vernalised plant, causing the non-vernalised plant to flower.

Cajlachjan (1956) performed similarly successful experiments with spring and winter varieties of rape and Abyssinian cabbage. In virtually all of these grafting experiments the donor plant was flowering at the time the graft was made, therefore the experiments did not necessarily prove that the primary vernalisation effect rather than the final flowering stimulus had been translocated. However in one group of experiments, Melchers made a vegetative biennial henbane plant flower in long days, by grafting on to it a vegetative short-day tobacco plant. In Chrysanthemum there was no evidence for any translocation of the cold stimulus in grafting experiments (Schwabe, 1954). In rye, Purvis and Gregory (1953) added an extract of vernalised embryos to unvernalised embryos in agar medium, but there was no conclusive effect of the passing on of the cold stimulus.

A series of chromosome substitution lines of wheat, Triticum aestivum L., of the variety Hope in Chinese Spring was developed by Sears (1953). This series consisted of 21 lines, each of twenty pairs of Chinese Spring chromosomes, plus a different pair of Hope chromosomes substituted in turn for the corresponding homologous pair in Chinese Spring. The variety Hope possesses no vernalisation response but the variety Chinese Spring possesses a moderately strong response. These substitution lines provide a means of studying the genetic basis for the vernalisation response character. It was found that homeologous group 5 of the chromosomes exerted the most significant effect on the expression of the vernalisation response, measured in terms of a reduction of leaf number on the main stem, and of particular significance w ere the chromosomes 5A and 5D (Halloran and Boydell ,1967). Using substitution lines for chromosomes 5A and 5D of Hope in Chinese Spring, single genes were located distally on the long arms of chromosomes 5A and 5D, which had a large effect on time to ear emergence (Law, Worland and Giorgi, 1976). They equated these genes with Vrn_1 and Vrn_3 which were identified by Pugsley (1972) as effecting vernalisation requirement . It appeared that genetic lines with no vernalisation requirement had a lower final leaf number and shorter time to ear emergence than lines with a vernalisation response, and hence vernalisation would reduce these parameters in a vernalisation requiring line.

In this study three genetic stocks of wheat (later referred to as cultivars) differing in their genes for vernalisation response were used. Since it is the shoot apex which perceives and reacts to the cold stimulus, it was thought that the growth of the shoot apex in these cultivars could be related to the genes for vernalisation response. In addition, by vernalising the cultivars it would be possible to determine whether cold had the same effects on growth at the shoot apex as the genes which conferred no vernalisation response.

Growth parameters of the shoot apex which were studied included rate of primordium initiation, volume of the apical dome, volume relative growth rate of the apical dome, cell volume, and the partitioning of the tissue of the apical dome. Cellular parameters studied included the amount of nucleic acid in the apical dome and the length of the phases of the cell cycle. These parameters were studied in the period up to and just after the transition to flowering to determine whether any differences which could be identified between the cultivars in the growth of their shoot apices could be related to predisposing some cultivars to flower earlier than others. It is also important in understanding the mechanism of vernalisation to determine whether any changes were sudden or gradual.

PART II

MATERIALS AND METHODS

A. The Plant Material

Three cultivars of wheat, Triticum aestivum L., with different vernalisation requirements (see page 51), were used in this study. These cultivars, provided by the Plant Breeding Institute, Cambridge, were:

(1) Chinese Spring Euploid

(2) Chinese Spring Hope 5A

(3) Chinese Spring Hope 5D

The ability of Triticum aestivum L. to tolerate the loss of a chromosome, allows the substitution of a single chromosome from one variety into another (Sears, 1953). Chinese Spring Hope 5A and Chinese Spring Hope 5D are single chromosome substitution lines, in which chromosomes 5A and 5D, respectively, from the variety Hope have replaced their homologues in the variety Chinese Spring. These substituted chromosomes have been shown to influence the vernalisation response of the variety Chinese Spring (Halloran and Boydell, 1967). Furthermore four genes, designated Vrn_1 to Vrn_4, affecting vernalisation requirement have been identified (Pugsley, 1972). Law, Worland and Giorgi (1976) suggested that Vrn_1 may be located on chromosome 5A and Vrn_3 on chromosome 5D, and that these genes may determine the vernalisation requirement of Chinese Spring Hope 5A and Chinese Spring Hope 5D, respectively.

Grains which had been harvested in 1977 were used for all the experiments except for the transfer experiments with vernalised Chinese Spring Hope 5D, in which 1978 grain was used.

B. The Methods

1. Conditions of plant growth

(a) Germination of grains

This procedure (M.D. Bennett-Personal communication) ensured that any residual dormancy in the grains was broken and that even germination occurred.

The grains were germinated on 2 layers of Whatman No. 1. filter paper soaked with 8 ml of tap water in 9 cm diameter plastic Petri dishes, with 10-15 grains per dish.

The dishes were kept at 22°C for 24 hours and then transferred to 4°C for 2-3 days. They were then returned to 22°C for a further 24 hours before the germinated grains were planted in pots. This germination procedure was carried out in the dark.

At this stage the germinated grains had roots 2-3 cm long and a coleoptile of 1-2 cm long.

(b) Vernalisation

The grains were germinated in the same way as described above except that they were kept at 4°C for 6 weeks instead of 2-3 days. During this time the Petri dishes were kept sealed with Sellotape to prevent evaporation of water, 5-8 grains were placed in each dish (instead of 10-15, see above) to allow for the growth which occurs during vernalisation. At the end of the 6 weeks at 4°C, the germinated grains had roots approximately 5 cm long and a coleoptile 2-4 cm long.

(c) Planting and growing procedure

Pots were filled with sand (Levenseat No. 13), over a layer of granite chips, which was moistened with tap water until water drained through the bottom of the pots. $3\frac{1}{2}$" square plastic pots were routinely used but in some experiments $2\frac{1}{4}$" square plastic pots were used so that a greater number of plants could be grown at once. These were used to grow plants for a maximum of six days and there was no evidence, at this early stage of development, that the pot size was affecting the growth of the plants.

The seedlings were planted, one per pot, with the roots just covered with the wet sand, and were placed in a controlled environment room.

50 ml of half strength Hoagland's solution (Hoagland & Arnon, 1938) were carefully poured on the surface of the sand in each pot, every other day, starting on the day after planting. Half strength solution was used because full-strength nutrient solution may cause chlorotic areas to develop in the leaves of wheat (Williams, 1960). The components of the Hoagland's solution are shown in Table 2.1.

Plants grown for longer than 30 days were given 50 ml of half-strength Hoagland's solution each day.

(d) Environmental conditions

All experiments were carried out at a temperature of 18 $\pm$ 2°C day and night.

The light intensity measured at sand level by a Lambda LI-185 Quantum/Radiometer/Photometer was 200 microeinsteins.m^{-2}. s^{-1} (150 W.m^{-2}, 15000 lux), and was provided by Phillips 65-80W warm white fluorescent tubes.

Tungsten bulbs were omitted from the growth room at the start of this study and it was therefore decided to continue without them. Incandescent light (which emits a greater proportion of energy in the far-red than fluorescent light does) hastens flowering in cereals (Friend, Helson and Fisher,1959), and in wheat this is brought about by acceleration of stem growth and the later stages of ear development rather than by earlier floral initiation (Friend, Helson and Fisher,1961). This suggests that in the stages being studied in this project, namely the vegetative phase and floral initiation, the incandescent light is less important than in the later stages. The ear emergence times observed were not the same as those reported by Law (1972) for plants grown with tungsten lights. However, the plants have responded to vernalisation in the desired way (by a reduction in leaf number and time to ear emergence) and <u>all</u> cultivars were grown without tungsten lights.

Table 2.1 Components of half-strength Hoagland's solution (from Hoagland & Arnon, 1938)

COMPONENT	STRENGTH IN SOLUTION
KNO_3	2.5×10^{-3}M
$Ca(NO_3)_2 \cdot 4H_2O$	2.5×10^{-3}M
$MgSO_4 \cdot 7H_2O$	1.0×10^{-3}M
KH_2PO_4	5.0×10^{-4}M
EDTA	1.7×10^{-6}M
MICRONUTRIENTS:	
H_3BO_3	5.2×10^{-5}M
$MnCl_2 \cdot 4H_2O$	1.0×10^{-5}M
$ZnSO_4 \cdot 7H_2O$	8.6×10^{-7}M
$CuSO_4 \cdot 5H_2O$	3.6×10^{-7}M
$H_2MoO_4 \cdot H_2O$	6.3×10^{-7}M

EDTA = Ethylene diamine tetracetic acid.

Therefore the omission of tungsten lights was not thought to be relevant to the results of this study.

The plants were routinely grown in 24 hour cycles consisting of 18 hours light (from 9.a.m. - 3.a.m.) and 6 hours dark, giving long day conditions. For those experiments in which short day conditions were required, the plants were given 8 hours light (from 9.a.m. - 5.p.m.) and 16 hours dark.

A humidifier was used to maintain a relative humidity of approximately 60-65% in all experiments.

2. Measurement of plant growth, and histological procedure

(a) Sampling

In the controlled environment room there was a gradient in light intensity between the centre and the edges (20% lower at the edges), so a random sampling procedure was used. The plants were all numbered working from left to right in the controlled environment room. The numbers were written on slips of paper, mixed up, and picked out at random. This determined the order in which the plants were sampled. The number and frequency of samples, and time of sampling are given in the results of individual experiments.

(b) Final leaf number on the main stem and time to ear emergence

The leaf number on the main stem of a sample of 6-10 plants was counted at ear emergence. At this stage it is difficult to distinguish between main stem and tiller leaves, so main stem leaves were labelled with jeweller's tags as they emerged. The number of days to ear emergence was measured from planting to the day on which the base of the ear had emerged past the ligule of the flag leaf.

(c) Number of emerged leaves

The number of emerged leaves on each plant was noted every day for a sample of plants (usually 10, never less than 7). A leaf was regarded as emerged on the day when it first protruded from the sheath of the last formed leaf. These data were used to compare the general growth of plants of one cultivar between experiments. The pattern of leaf emergence was also compared between cultivars.

(d) Dissection of plants

Plants were harvested by cutting the roots just below the sand surface and the leaves were removed, under a Watson Stereoscopic microscope where necessary, to expose the apex and allow the primordia to be counted (for method of counting primordia see page 37).

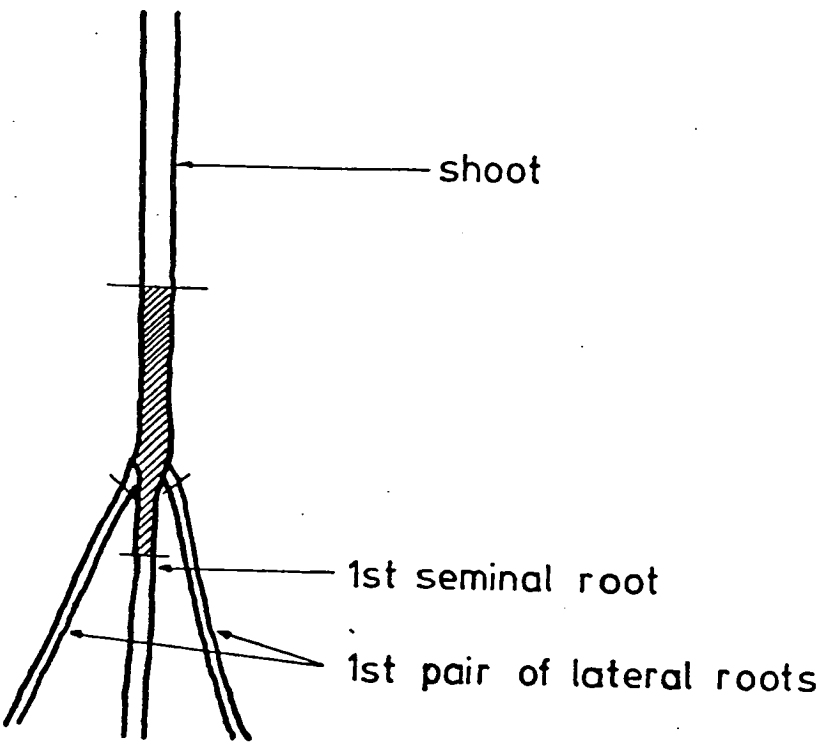
(e) Fixation, embedding and sectioning of apices

Plants were harvested by cutting the roots about 2 cm below the sand surface and the roots were then trimmed to aid orientation of the samples in the wax blocks (Fig. 2.1). The first pair of lateral roots grow perpendicular to the plane of the leaf insertions while the first seminal root grows in the same vertical plane as the leaf insertions. The seminal root was trimmed to approximately 1 cm in length while the lateral roots were trimmed to stumps. The sample was placed in the wax in such a way that the stump of one lateral root was above the stump of the other in the vertical plane. The apex lay in the same plane as the leaf insertions and was sectioned longitudually in this plane.

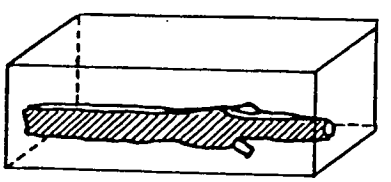
Throughout the study the fixative used was ethanol/acetic acid (3/1, V/V) and samples were fixed for about 24 hours. However, any fixation time between 1 and 24 hours is satisfactory and this fixative quantitatively preserves nucleic acid and protein (Mitchell, 1968). After fixation, samples were transferred to 70% ethanol for at least one hour, and if not required for processing immediately could be stored in this indefinitely.

The samples were then dehydrated in absolute ethanol, infiltrated with ethanolic solutions of xylene of increasing concentrations, and finally put in pure xylene. When thoroughly infiltrated with xylene the samples were almost completely transparent and pellets of Paraplast tissue-embedding medium (Lancer, Melting point 56°-57°C) were added to the sample tubes, which were then placed in an oven maintained at 60°C. After several hours, when the wax had melted, the xylene-wax solution was poured off and molten pure wax at 60°C was added to the tube. This wax was left for 24 hours, was changed again, and was then left for a further 24 hours. The samples were embedded in blocks and were orientated as described above.

FIG 2·1 (a) Method of trimming shoot and roots of plant for wax embedding.



(b) Orientation of sample in wax block.



Longitudinal serial sections, 10 microns thick, were cut in the plane of the leaf insertions, using a Beck rotary microtome. The sections were floated on water at about 40°C which flattened out the ribbon and they were then collected on slides which had been smeared with Gurr's glycerin albumen. The slides were placed on a dish-warmer to dry at least overnight before staining.

3. Quantitative Histochemical Staining

(a) Total nucleic acid

Sections were stained for total nucleic acid (DNA and RNA) using gallocyanin chrome-alum, which is a specific and quantitative stain for plant nucleic acids (Mitchell, 1968). There is a stoichiometric relationship between gallocyanin chrome-alum and nucleic acids, and the complex formed adheres to the Beer-Lambert Laws of spectrophotometry (Sandritter, Diefenbach and Krantz, 1954; Sandritter, Kiefer and Rick, 1963). The following procedure has been established as optimal for staining of plant nucleic acids (Mitchell, 1968).

Sections were dewaxed in xylene, hydrated with decreasing concentrations of aqueous ethanol solutions and placed in 0.02N hydrochloric acid for 15 minutes. The gallocyanin chrome-alum stain was prepared as described by Mitchell (1968) and the sections were stained overnight at 40°C. Excess stain was removed by washing the sections in 0.02N hydrochloric acid for 15 minutes and the sections were then dehydrated in ethanol, rinsed in xylene and mounted in Canada balsam.

The amount of stain in the sections was measured at a wavelength of 575 nm using the Vickers M 85 microdensitometer.

(b) RNA

Since the stoichiometry of both nucleic acids with gallocyanin chrome-alum is apparently the same, extractions with specific enzymes can be used to provide information on quantities of RNA and DNA separately (Kiefer, Kiefer and Sandritter, 1967).

The quantity of RNA can be measured directly by treating the sections with a deoxyribonuclease solution before staining (Kiefer, Kiefer and Sandritter, 1967). However, the DNA in sections of the wheat apex was not completely extracted by this method. Even after long treatments (approximately 2 hours) some nuclei still stained densely with gallocyanin chrome-alum. Therefore the following method was used to measure RNA. Before staining the sections, RNA was extracted with a ribonuclease solution: the amount of DNA was measured and was subtracted from the total amount of nucleic acid (see total nucleic acid, microdensitometry page 34).

For extraction of RNA, hydrated sections were treated with an aqueous solution of ribonuclease (sigma, 5X crystallised) at a concentration of 0.5 mg/ml for 1 hour at 60°C, rinsed with water and stained with gallocyanin chrome-alum (Mitchell,1968). Ribonuclease - treated and untreated sections in the same experiment were stained in separate dishes, to prevent contamination of control sections with ribonuclease. However they were stained at the same time with the same batch of stain.

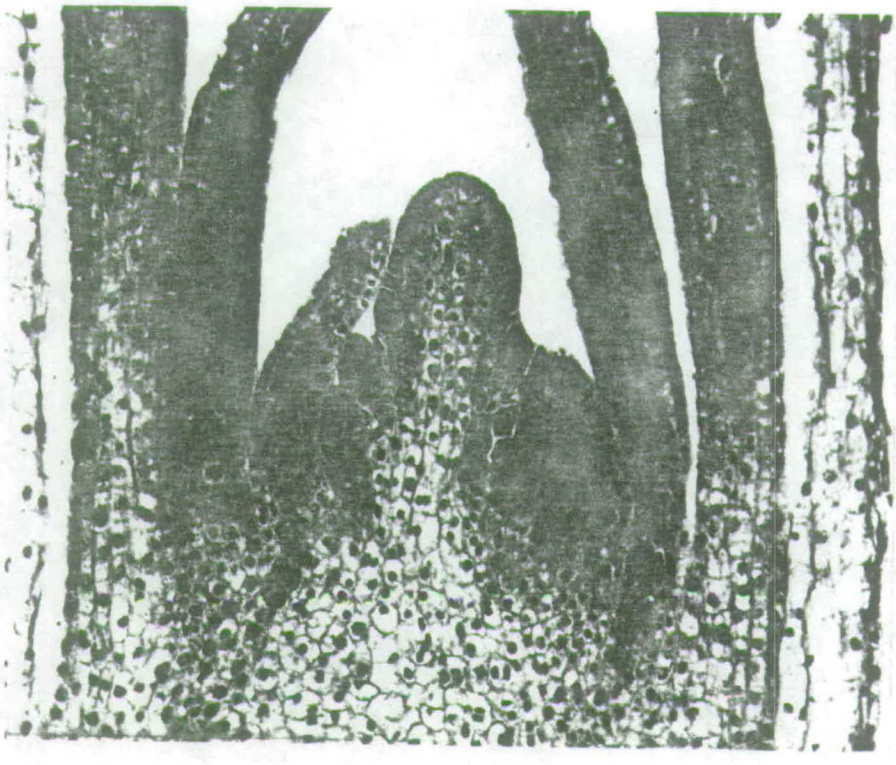
A sample of control and ribonuclease-treated sections was stained with methyl green/pyronin Y which differentially stains RNA and DNA (Kurnick, 1955). Hydrated sections were blotted dry, stained for 30 minutes in methyl green/pyronin Y (Casselman, 1959), rinsed in distilled water, blotted dry, dehydrated in acetone, rinsed in xylene and mounted in Canada balsam. The DNA stained blue-green and the RNA red in the control sections. In the ribonuclease-treated sections there was no red staining which showed that all the RNA was extracted by the above method (Fig 2.2).

(c) DNA

Sections were stained for DNA using the Feulgen method (McLeish and Sunderland, 1961) which depends on the reaction of decolourised basic fuchsin with aldehyde groups, formed by splitting off purines from the deoxy-sugars by acid hydrolysis (Pearse, 1960).

FIG 2.2 Photomicrographs of median longitudinal sections of wheat shoot apices
(a) stained with methyl green/pyroninY
(b) stained with methyl green/ pyroninY following ribonuclease treatment

(a)



(b)

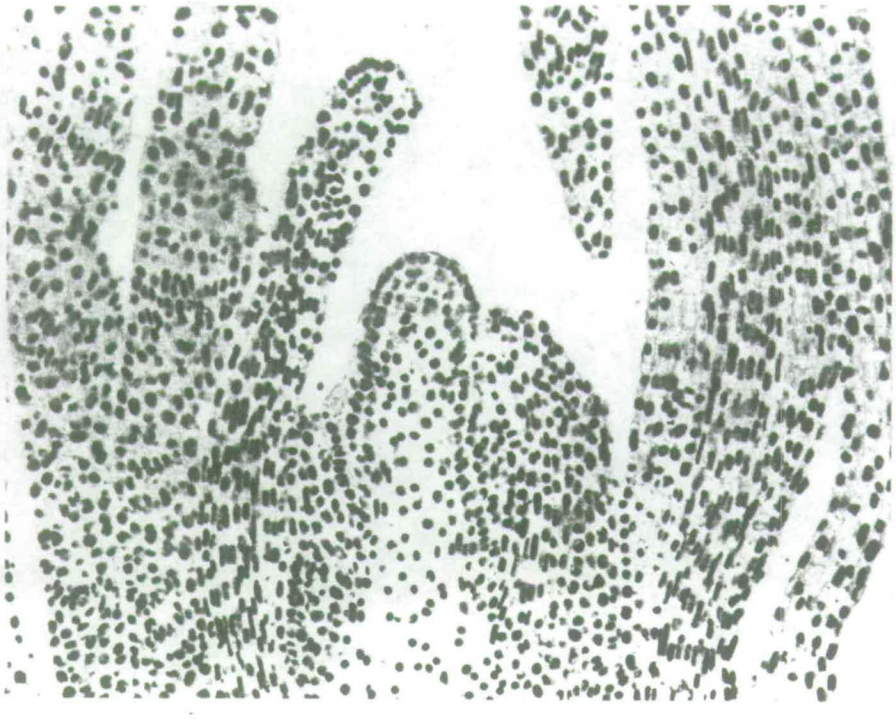
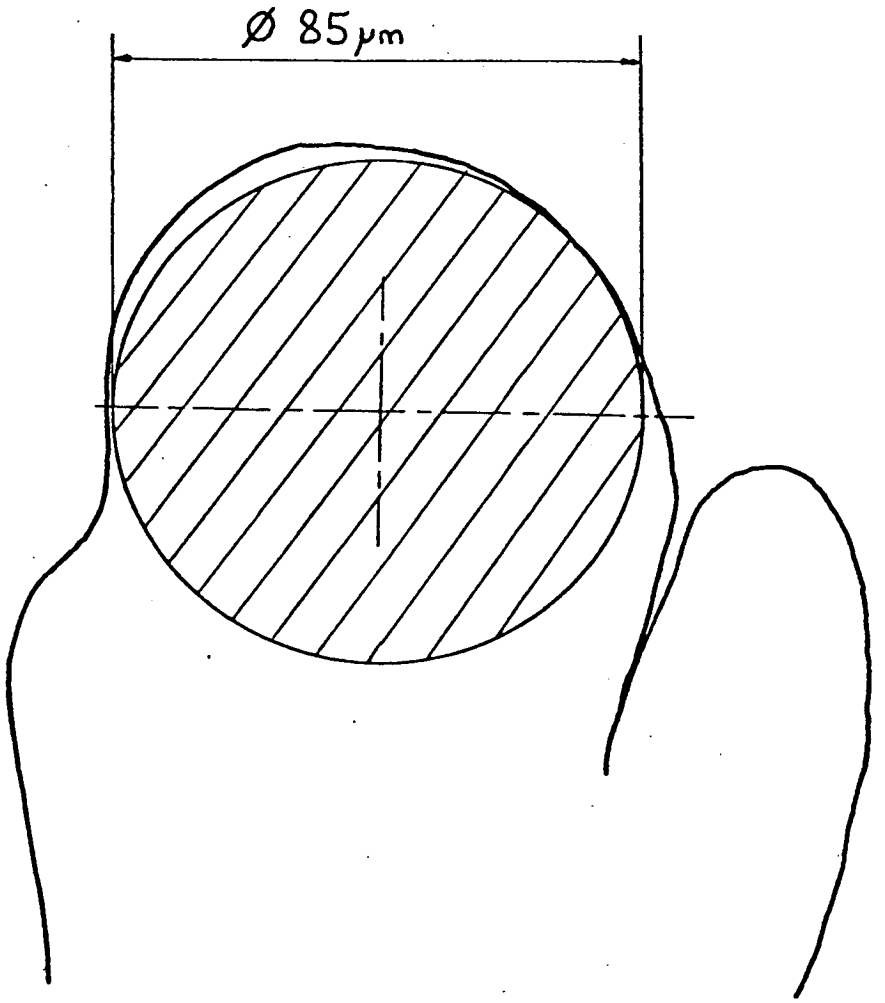


Table 2.2 The mean prophase values (in arbitrary units) measured in Feulgen - stained, and ribonuclease treated, gallocyanin-stained apices. The mean (of 3-5 nuclei) prophase value for each sectioned apex in a batch and the mean (of 5-9 apices) prophase value of all the sectioned apices in that batch are shown.

Staining procedure	Batch number of apices (n)	Mean prophase value of all apices in batch (n) (with standard errors)	Mean prophase value for individual apic in batch (n)
Feulgen	1	6.14 ± 0.08	6.13 6.39 5.96 6.00 6.24
	2	7.56 ± 0.10	7.39 7.37 7.91 7.45 7.66
Gallocyanin chrome-alum	1	10.69 ± 0.11	10.47 10.70 10.16 10.95 10.95 10.76 10.22 10.92 11.04
	2	12.10 ± 0.12	11.73 12.30 11.71 11.89 12.30 12.42 12.34
	3	9.90 ± 0.14	9.70 9.82 10.02 10.01 9.10 10.47 10.16 9.88

FIG 2·3 Camera lucida drawing of the median longitudinal section of a vegetative wheat shoot apex showing the position of the aperture on the apical dome. The area of stain density measured is shown by the diagonal lines.



Sections were dewaxed in xylene, hydrated with decreasing concentrations of ethanol solutions, hydrolysed in 5N hydrochloric acid at 25°C for 25 minutes (Fox, 1969) and rinsed with distilled water. The sections were stained in the Feulgen reagent (Darlington and La Cour, 1960) for 2 hours at room temperature. After staining, the sections were rinsed in 3 changes of SO_2 water (5 ml N hydrochloric acid, 5 ml 10% $K_2S_2O_5$, 100 ml distilled water) for 10 minutes each change, rinsed in distilled water, dehydrated with ethanol, rinsed with xylene and mounted in Canada balsam.

The amount of stain in the sections was measured at a wavelength of 565 nm using the Vickers M85 microdensitometer.

It was hoped that individual nuclei could be measured in Feulgen stained sections of apices to obtain information on the proportion of cells in the different phases of the cell cycle (for explanation see later section on synchrony). However, this was impractical since the nuclei were too close together in the apical dome to be measured individually. The total amount of DNA in the apical dome could be measured from these sections and was converted to the same C basis (see below) as the sections which had been treated with ribonuclease and stained with gallocyanin chrome-alum. In each set of sections of the plants sampled on a particular day, some were stained with gallocyanin chrome-alum and some with Feulgen, allowing a comparison to be made between the amount of DNA in the apical dome measured by the different techniques.

Staining by the Feulgen or gallocyanin chrome-alum methods may differ between batches of sections processed at different times. All values were therefore converted to a common basis for comparison (Lyndon, 1970a). This was done using prophase nuclei which gave the absorption value corresponding to the 4C amount of DNA (1C being the amount of DNA in a gamete). 3 - 5 prophase nuclei were measured in each sectioned apex, selecting whole nuclei (these look spherical when the microscope is focussed up and down) where the nuclei were well-spaced in the leaf tissue surrounding the apical dome. The mean prophase value for any one sectioned apex was within 6% of the mean prophase value for all the sectioned apices (5 - 9) processed at the/

same time (Table 2.2). Prophase values for the gallocyanin-stained sections were obtained from the sections which had been treated with ribonuclease before staining.

The total DNA values obtained for the apical dome were therefore converted into multiples of C. The total nucleic acid values were also expressed as multiples of C, so different batches of sections could be compared even if they had stained to different extents. This is valid since the stoichiometry of both DNA and RNA with gallocyanin chrome-alum is apparently the same (Kiefer, Kiefer and Sandritter, 1967).

Some sections were stained with dinitrofluorobenzene before staining with Feulgen, which provided a means of measuring the amount of protein in the tissue as well as the amount of DNA (Mitchell, 1967). However, the dinitrofluorobenzene staining procedure was not successful for the sections of the wheat apex. There was a large variation in the density of staining of sections (visible even with the naked eye) processed at different times, and since there was no internal standard, comparisons could not be made. The glycerin albumen sticking the sections on the slides also tended to take up the stain, which affected the results. Therefore the procedure would have to be modified for consistent results to be obtained for the wheat apex.

(d) Microdensitometry

The stain density in the apical dome of the sectioned tissue was measured using the Vickers M85 scanning microdensitometer. The measurements were made in an area delimited by a mask of the appropriate size. Masks were selected in which the aperture covered as much of the apical dome as possible without including any background areas not in the section. The aperture sizesused corresponded, on the sections, to diameters of 65 microns for young plants and 85 microns for older plants. Measurements made with the 65 micron diameter aperture were multiplied by 1.71 so that all measurements were expressed on the same unit area basis. The aperture was placed centrally over the apical dome with its upper edge as near to the tip of the apical dome as possible (Fig 2.3). Measurements were made of the three most median longitudinal sections of each apex.

4. Growth of the shoot apex

(a) Number of primordia

The number of primordia was counted from the median section of each apex. A primordium was regarded as present when there was an inflexion in the curve of the apical dome, opposite but higher than the last formed primordium (Fig.2.4).

The number of primordia was plotted against time and the rate of primordium initiation and the plastochron, which is the length of time between the initiation of successive primordia, were calculated from linear regression analysis (see results section, page 58).

(b) Volume of the apical dome and the phytomer

The shoot apex is here defined as that region of the axis which lies above the plane drawn normal to the axis and passing through the axil of the youngest leaf to overtop the apical dome (Rogan and Smith, 1974). The apical dome is defined as the part of the apex which lies above the youngest primordium (Fig.2.5). The axil P of primordium (n) was defined as the point of inflexion of the curve along the flank of the apical dome (Fig. 2.5). Originally the apical dome was delimited by drawing a line between the axil of the primordium (n) and the axil of primordium (n-1). However this was invalid since a leaf primordium eventually formed a collar round the apex, and therefore some of the tissue on the opposite flank of the apical dome to that on which the primordium was initiated, was contributing to the tissue of the primordium The length x (Fig. 2.5) was measured, for a primordium which was visible on both sides of the apex, on successive days, and length x was then plotted against time. The day on which the primordium was initiated was known (from the graph of number of primordia against days after planting) and therefore by extrapolation the length of x at the initiation of the primordium could be read off from the graph.

Length x on initiation was greater than zero, which confirms the idea that tissue on that flank of the apical dome was contributing to the primordium even at its initiation. It would have been impractical to do this analysis for every primordium in each experiment and therefore a general method for delimiting the apical dome was devised. Since x was never longer than y (Fig.2.5) and at initiation of the primordium was approximately the same as y, a line PQ was drawn across the apex,

FIG 2·4 Camera lucida drawings of median longitudinal sections of the vegetative wheat apex. A primordium (n+1) was present when there was an inflexion in the curve of the apical dome opposite but higher up than primordiu

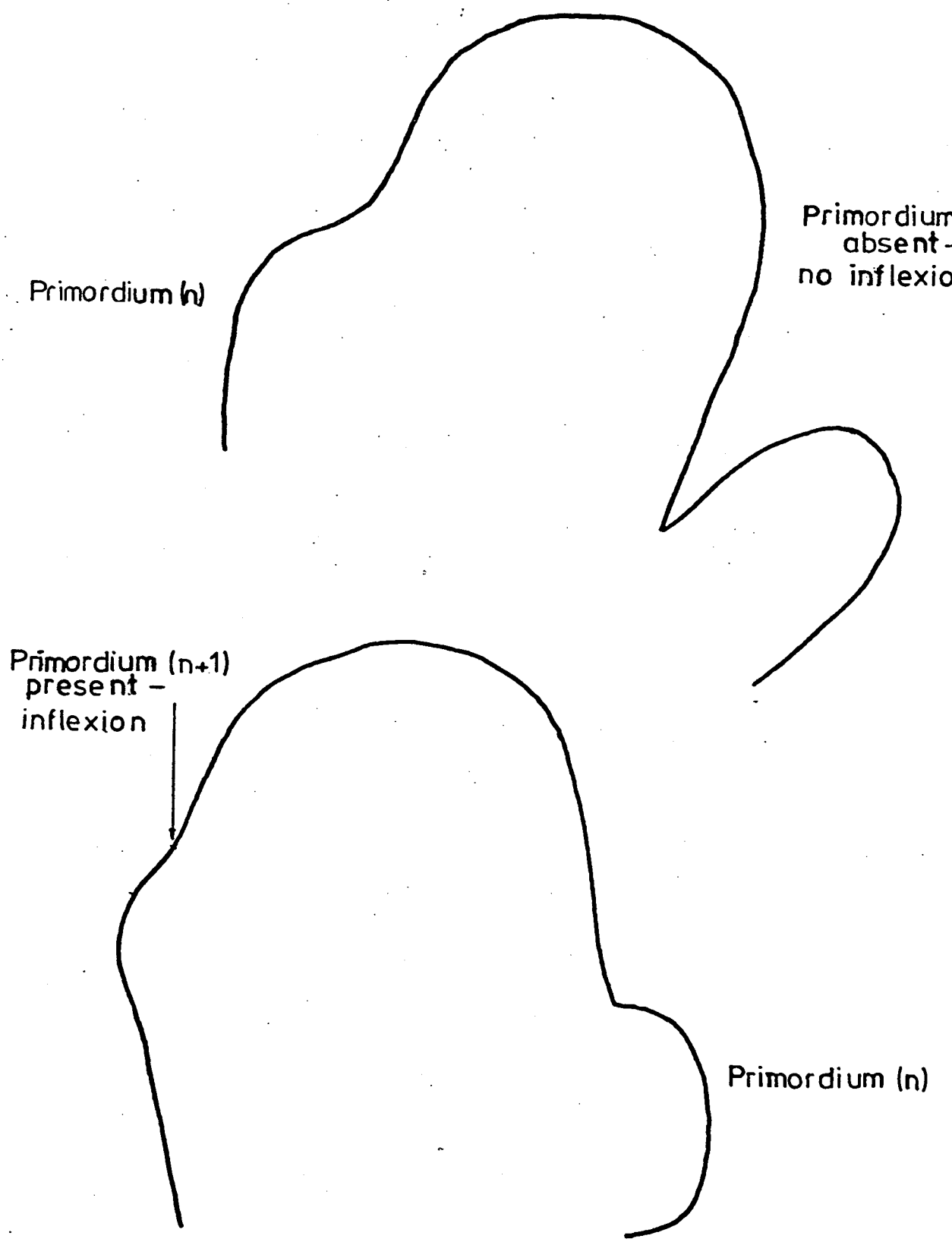
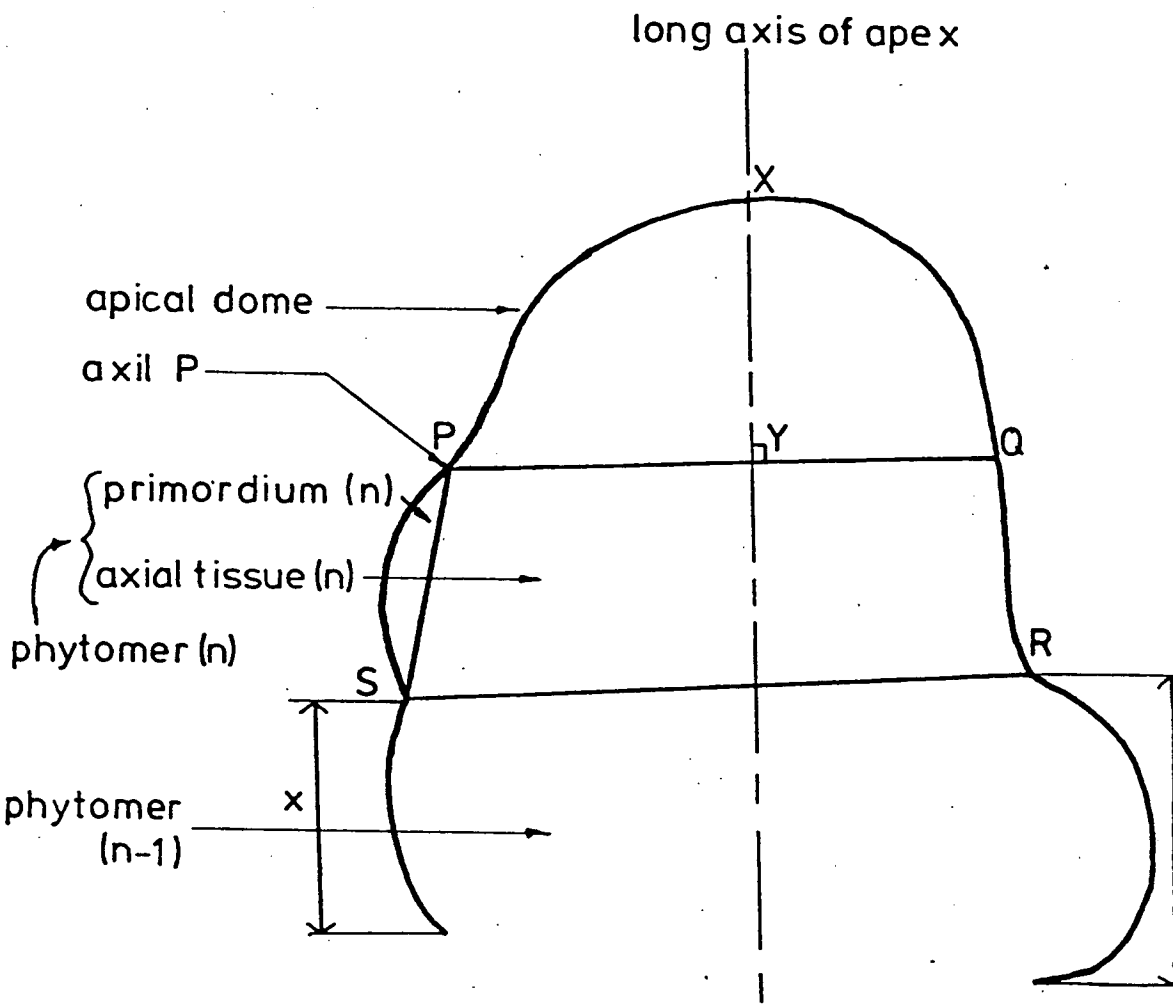


FIG 2·5 Camera lucida drawing of the median longitudinal section of a vegetative wheat apex showing the delimitation of the apical dome and phytomer.



PQ = diameter
XY = height

from the axil P of primordium (n), perpendicular to the line drawn down the long axis of the apex. The apical dome was therefore defined as that part of the shoot apex which was distal to line PQ.

The phytomer (n) is primordium (n) + the associated axial tissue (Fig.2.5). It was delimited by the lines PQ and RS, the latter being drawn across the axil of primordium (n-1) which was visible on both sides of the apex.

Outline drawings were made at a standard magnification, on paper of uniform thickness, of camera lucida images of the median longitudinal section of the apical dome and phytomer. The drawings were weighed and since the weight of a standard area of paper and the thickness of the sections was known, the weights could be converted into volumes. The median section was regarded as being representative of the shoot apex and reflects the amount of tissue present in the apex (Abbe, Phinney & Baer, 1951). The error involved in calculating the volumes from the median sections rather than from all the serial sections of the apex was determined.

The volumes of the apical domes of 35 apices and the volumes of the phytomers of 25 apices were obtained by drawing and then weighing all the serial sections through these apices. Apices of different cultivars at varying stages of development were measured. The volume of the whole apical dome or phytomer was plotted against the volume of the median section of the dome (Fig. 2.6a) or phytomer (Fig. 2.6b) and, by regression analysis, were shown to be linearly related. The volume of the apical dome or phytomer could then be calaculated, from this graph, for any median volume. The error between the observed and calculated apical dome and phytomer volumes is shown in Table 2.3a. The range of errors was not related to the age of the apex or to the cultivar. The resultant error in calculating the volume relative growth rate per plastochron (for method see pages 76 to 89) from the volumes of median sections is shown in Table 2.3b. These errors should be considered when interpreting the results. The reason for using median sections is that for the number of apices involved in these experiments, drawing and weighing all the serial sections would be an extremely lengthy process.

FIG2.6 Volume of the whole as a function of the volume of the median section of (a) the apical dome (b) the phytom
Regression lines shown are based on values for all cultiv

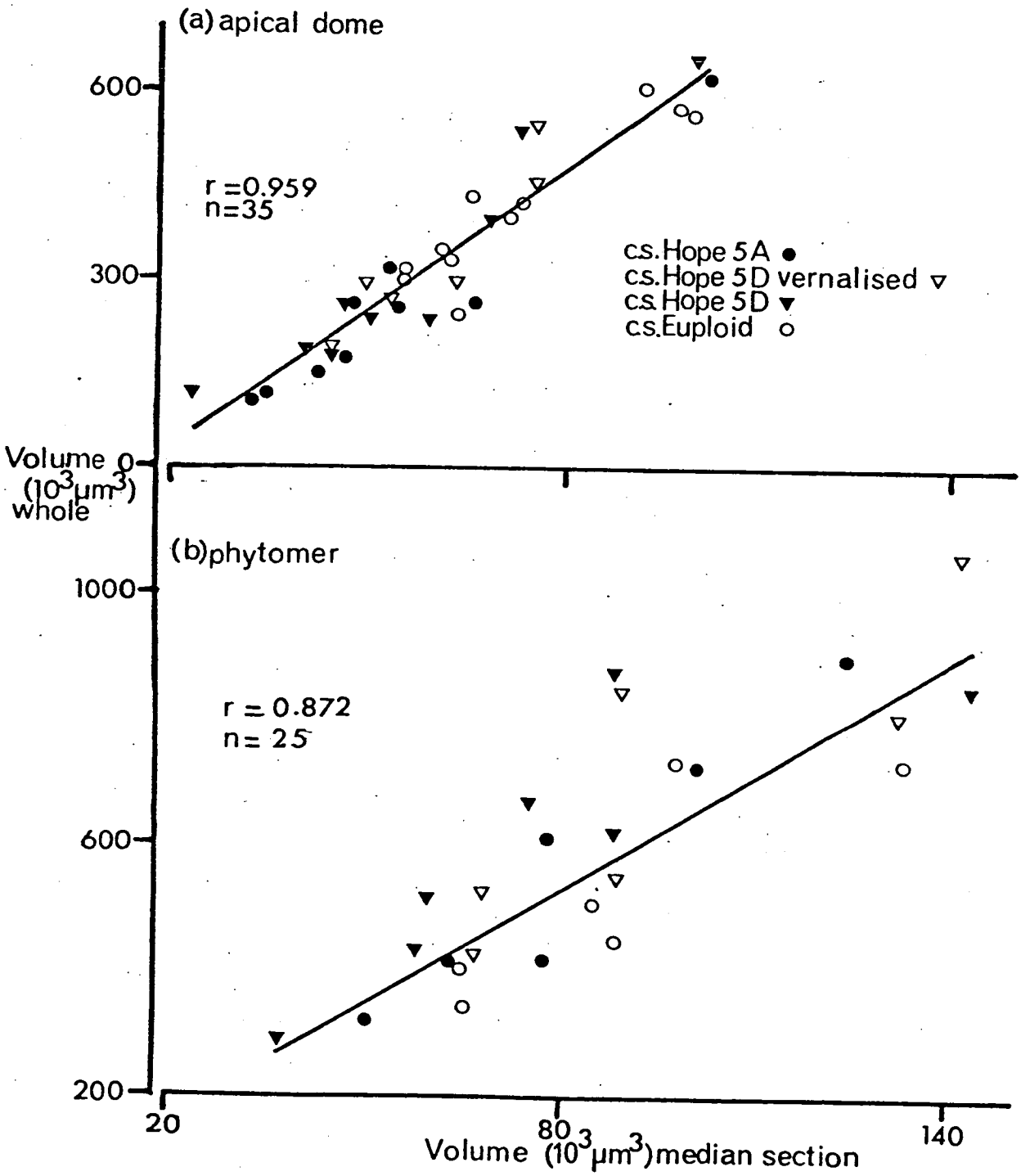


Table 2.3a Range of error and mean error involved in calculating the volume of the apical dome and phytomer from the volume of the median section.

	Range of error (%)	Mean error (%)
Apical dome (35 apices)	+ 2.11 to + 45.20 - 1.98 to - 48.40	± 11.94
Phytomer (25 apices)	+ 1.75 to + 35.44 - 3.00 to - 27.57	± 14.11

b The subsequent error in the calculation of the volume relative growth rate of the apical dome using the volume of the median section. Each growth rate is based on the volumes of 6-10 apices.

Volume relative growth rate per plastochron

Calculated from the volume of the median section (x)	Calculated from the volume of all sections (y)	Difference as a percentage of (y)	Mean error (%)
0.96	0.85	+ 12.94	
0.91	1.04	- 12.50	
0.68	0.76	- 10.53	± 10.85
0.92	0.81	+ 11.96	
0.74	0.79	- 6.33	

(c) Cell number and cell volume

Cells were counted in the median longitudual section, with the aid of a camera lucida, by marking on the drawing of the apical dome all nuclei which were apparently whole or more than half present. The mean cell volume was calculated by dividing the volume of the median section of the apical dome by the cell number in the median section.

(d) Dimensions of the apical dome (Fig 2.5)

The diameter and height of the apical dome were measured from the median sections. The diameter of the apical dome is the length of the line PQ which delimits the apical dome and the height is the length of the line XY drawn perpendicular to PQ up to the highest point on the apical dome.

5. Measurement of the length of the cell cycle

(a) Possible methods

A number of methods, which are independent of apical volume data, can be used to measure the length of the cell cycle in the shoot apex. These include a ^{14}C - and ^{3}H - thymidine double labelling technique (Miller and Lyndon, 1975; Nougarède and Rembur, 1977) and the method of accumulation of metaphasesas a result of colchicine treatment in a number of dicotyledons (Denne, 1966; Corson, 1969; Lyndon, 1970b; Bodson, 1975; Rembur and Nougarède, 1977). In wheat, the shoot apex is less accessible than in these dicotyledonous plants. The apex is situated at the base of the plant (at sand level) and is completely enclosed by the leaves. It is necessary in both the double-labelling and colchicine methods, that the apex survives for at least the approximate length of the cell cycle after the application of the chemical. The double labelling technique was tried after exposing the apex by partially removing the leaves but the apex did not survive for the length of time necessary for results to be obtained. The most practical method seemed to be to immerse the plants in a colchicine solution up to the level of the shoot apex, in the hope that the colchicine would penetrate to the apex (Rolinson, 1976). The principle behind this method is that the colchicine does not disturb the entry of cells into mitosis, but prevents the cells from passing out of mitosis by arresting the cells at the metaphase stage. It is assumed that anaphase and telophase nuclei will disappear (which did in fact happen in wheat, page 98) (Evans, Neary and Tonkinson, 1957).

The number of cells accumlated at metaphase over a given period of colchicine treatment would then be equivalent to the division rate of the cells. The length of the cell cycle can be calculated using the following formula (Lyndon, 1970b):

$$T = \frac{100 \log_e 2}{D}$$

T = Length of the cell cycle in hours
D = Rate of accumulation of colchicine metaphases per hour (%).

This formula is a modification (based on the assumption that all cells are dividing) of that used by Evans, Neary and Tonkinson (1957). For further explanation of calculation, see results section (page 95).

(b) Application of colchicine

Plants with their roots intact were removed from the pots by immersing the pots in water and easing the roots out of the sand. Sand sticking to the roots was carefully washed off and the roots were placed between two sheets of blotting paper to remove excess water. Plants were placed in beakers of suitable sizes to accomodate the root systems, with 3 or 4 plants per beaker. The plants were immersed, up to the level of the shoot apex, in a 0.05% colchicine solution (M.D. Bennett-personal communication) containing 1% dimethyl sulphoxide (which aids penetration of a solution through membranes).

Plants were sampled at intervals over the approximate length of the cell cycle (calculated from apical volume data). The roots and all the leaves up to the one overtopping the apex were removed. The apices were fixed in ethanol/acetic acid (3/1, V/V) for at least one hour, taken through 70%, 50% and 30% ethanol to water and were then stained using lacto-propionic orcein (Dyer,1963).

The apices were treated in 1N hydrochloric acid at 60°C for 5 minutes and rinsed in water. The apical dome was removed from each apex and placed in a drop of lacto-propionic orcein on a glass slide for 2 minutes to stain. A coverslip was applied and the cells were tapped out using a mounted needle. The slide and coverslip were then squashed between 2 layers of filter paper. These preparations were temporary but were adequate for scoring the cells. To make the preparations permanent the coverslip was smeared with glycerin albumen before being placed on the material. After squashing the apical dome, the coverslip with attached cells was floated off the slide in 45% acetic acid, taken rapidly through 60%, 80%, 95% and absolute ethanol and xylene before mounting in Canada balsam.

This method has the advantages that it is a quick staining procedure and it gives a good contrast between the cytoplasm and chromosomes.

The preparations were examined and the number of cells in interphase and in each stage of mitosis was counted.

6. Synchronisation of cell division

Synchrony can be detected from the distribution of cells in the different phases of the cell cycle, as indicated by the DNA contents, measured by microdensitometry of Feulgen-stained nuclei. If the distribution remains constant over a period of time, the cells are dividing asynchronously rather than synchronously. The problem in investigating synchrony is to know what stage, or stages, of development of the plant to study. Chinese Spring Hope 5A was used for these experiments as it has the most rapid rate of development and does not require vernalisation.

A preliminary experiment was carried out to get some indication of the stages in development of the plant at which synchronisation may be occurring. Samples, each of two plants, were taken every day up to 21 days after planting. This time span included collar and double ridge formation. The samples were processed, and permanent squash preparations were made by the procedure (slightly modified as explained below) described under application of colchicine (page 44). After fixation, when the apices had been taken to water, they were stained using the Feulgen method (page 33), then the apical domes were excised and placed in a drop of 45% acetic acid on a slide. After squashing, when the coverslip had floated off the slide in 45% acetic acid, it was taken through 2 rinses (each 10 minutes) of SO_2 water (page 33) before it was placed in 60% ethanol.

The amount of DNA per nucleus was measured at a wavelength of 565nm using a Vickers M85 Scanning microdensitometer. For each slide a number (4 - 7) of half telophase and prophase nuclei was measured to obtain values for the 2C and 4C amounts of DNA respectively (Table 2.4). For any slide each half telophase or prophase value was within 10% of the mean of all the half-telophase and prophase values. The 2C value for each slide was apparently always less than half the 4C value.

Table 2.4 Examples of the half-telophase and prophase values (in arbitrary units) used to produce a C scale for each slide.

Slide number	Measurements of half-telophase		Mean of half-telophases (with standard errors)	Measurement of prophases		Mean of prophases (with standard errors)
1	42.34	41.47	42.73	90.99	88.36	88.81
	43.86	41.79	± 0.55	87.40	88.71	± 0.76
	44.17					
2	42.04	42.04		89.78	88.63	89.12
	43.18	43.51	43.56	87.24	89.83	± 0.53
	46.12	44.01	± 0.53	90.10		
	44.04					
3	32.39	31.86	31.38	67.41	63.01	65.83
	33.37	29.60	± 0.49	66.23	66.66	± 0.97
	30.63	30.33				
	31.45					
4	38.47	42.33		86.32	83.42	84.60
	43.51	43.51	40.63	84.28	84.50	± 0.61
	38.01	37.95	± 1.13			

This is probably because the chromosomes in the half-telophase nuclei were tightly clumped, and the microdensitometer tends to give lower readings for these nuclei than for those in which the chromosomes are more spread out.

Since there was variation in staining between slides, nuclei were expressed as a C value and hence results could be combined for one day and compared between days. The difference between the 2C and 4C values for any one slide was divided by 5 and this amount was taken as one category, equivalent to 0.4C. A scale was then devised from 1.2C to 4.8C (consisting of 9 categories) and for each category the range of absorbance values was known. Each nucleus on a slide could then be ascribed to a C category.

On each day, the number of nuclei was plotted against C value. The proportion of cells in the G_1 (gap -:pre-DNA Synthesis) and G_2 (gap-:post-DNA Synthesis) phases of the cell cycle was worked out. Since the number of nuclei in the S phase (DNA synthesis-:3C nuclei) could not accurately be determined by microdensitometry alone, these nuclei were partitioned equally to the 2C and 4C populations (Francis and Lyndon, 1978). The G_1 population was therefore regarded as all nuclei with less than the 3C amount of DNA, and the G_2 population as all nuclei with greater than the 3C amount of DNA. The ratio G_2/G_1 was worked out for each day and any change in this ratio was regarded as an indication that synchronisation of the cells might be occurring.

Intensive sampling was carried out over the days on which synchronisation of the cells was suspected. Two plants were sampled every 8 hours, over the course of the cell cycle (calculated from growth data and colchicine measurements). These were fixed, stained and permanent squash preparations were made as previously described in this section. The amount of DNA per nucleus was measured on the Vickers M 85 scanning microdensitometer, and histograms of the frequency of nuclei with different C values were produced. The ratio G_2/G_1 was calculated for each sampling time, and a change in this ratio during the course of the cell cycle would indicate that synchronisation of the cells was occurring.

7. Transfer of plants from long days to short days

The aim of these experiments was to determine at what stage in its development a primordium becomes committed to producing either a leaf or spikelet. Wheat plants grown in short days will produce a greater total leaf number on the main stem, than plants grown in long days (Pugsley, 1966). Therefore if plants are transferred from long to short days at different stages of development, the final leaf number may be altered and the lability (Purvis and Gregory,1937) and time of commitment of primordia may be determined. Since the response of wheat to short days is strongly conditioned by variety (Pugsley, 1966), transfer experiments were done with unvernalised plants of all 3 cultivars of wheat and vernalised plants of Chinese Spring Hope 5D.

In each experiment 10 plants were grown in continuous long days and 10 in continuous short days as controls (for growth conditions see page 25). In addition 3 plants were transferred at intervals (from 2 days to a week depending on the cultivar and the stage of development of the plant) from long day to short day conditions, in the period up to and including double ridge formation. Transfers from long to short days (rather than from short to long days) were carried out, because the fate of the primordia in continuous long days was already known (from the final leaf number), and from measurements made on sections (page 37), the number of primordia initiated by each cultivar on each day, at least up to day 20, had been calculated. The final leaf number on the mainstem was counted (for method see page 27) for the transferred plants at the stage when the ear was differentiating and the final leaf number was irreversibly determined.

8. Dry weights

Dry weights of 10 plants of each experimental cultivar were measured on the twenty-first day after planting. This was the day which marked the limit of growth measurements.

The plants were harvested by immersing the pots in water and easing out the roots. The sand sticking to the roots was carefully washed off and excess water removed by placing the roots between two/

sheets of blotting paper. The plants were then divided up for the dry weight determinations. The roots were cut off at the root/stem junctions, leaves 1 - 5 were cut off at the base of their leaf sheaths, and the coleoptile tiller, tiller 1 and tiller 2, which arise in the axils of the coleoptile, leaf 1, and leaf 2, respectively, were cut off at their bases. Measurements of dry weight were therefore made of the roots, leaves 1 - 5 (separately), coleoptile tiller, tiller 1 and tiller 2 and the shoot apex + stem. The plant material was placed on a sheet of blotting paper on a metal tray, dried overnight in an oven at 90°C and then weighed on an Oertling electric balance, accurate to within 0.1 mg.

9. Photography

All photographs of slides were taken on Ilford Pan F film, using a Zeiss Photomicroscope.

PART III RESULTS.

The results are divided into 4 sections. Section A describes the final leaf number and days to ear emergence in the vernalised and unvernalised cultivars. It is essential to appreciate these external characteristics which distinguish between the cultivars before any description of the more specialised growth measurements can be thoroughly understood.

The first part of section B continues these generalised observations in describing the morphology of the shoot apex and the relative times of attainment of the various stages of development in the different cultivars. This must be explained in order to understand why and when the subsequent apical growth measurements were made. The remainder of Section B is a detailed study of the growth of the shoot apex. Section C deals with the cellular parameters of the shoot apex which may be changing as a result of vernalisation and/or at the transition to flowering.

Section D considers another aspect of defining the transition to flowering by approaching the problem with primordium commitment experiments.

The results section concludes with section E which reports general aspects of the plant growth including tillering and leaf emergence.

A. The effects of the genes for vernalisation requirement on final leaf number and days to ear emergence

The total number of leaves on the main stem at ear emergence was counted. The number of days to ear emergence was measured from planting to the day on which the base of the ear had emerged past the ligule of the flag leaf. The data (based on 15-20 values from 2-3 experiments for each cultivar) for each unvernalised and vernalised cultivar are shown in Table 3.1a.

In the unvernalised plants, the final leaf number and days to ear emergence increased in the order C.S. Hope 5A, C.S. Euploid and C.S. Hope 5D. After vernalisation, the final leaf number and days to ear emergence were reduced in C.S. Euploid and C.S. Hope 5D, while in C.S. Hope 5A the final leaf number was unchanged and the days to ear emergence increased by approximately 5%. If the reduction in days to ear emergence is regarded as a measure of vernalisation requirement, C.S. Hope 5A has no requirement, C.S. Hope 5D has a strong requirement and C.S. Euploid has an approximately intermediate requirement. There was variation in the final leaf number and days to ear emergence in C.S. Hope 5D, particularly in the vernalised plants, which seemed to depend on the year in which the grain was harvested (Riddell and Gries, 1958, observed that older grains may produce fewer leaves on the main stem than younger ones).

Table 3.1b. shows the values for days to ear emergence in the three unvernalised cultivars as measured by Law (1972). The values differed from those shown in Table 3.1a, but the increase in days to ear emergence was in the same order of the cultivars.

The resemblence between vernalised Chinese Spring Euploid and vernalised C.S. Hope 5D, and unvernalised C.S. Hope 5A, in the final leaf number and days to ear emergence, suggested that in the growth of their shoot apices the vernalised cultivars might more closely resemble the unvernalised C.S. Hope 5A than their own unvernalised cultivars.

The data which will be presented are for unvernalised C.S. Hope 5A, C.S. Hope 5D and C.S. Euploid, and vernalised C.S. Hope 5D. C.S. Hope 5D was chosen as the vernalised cultivar, as it has the strongest vernalisation response.

Table 3.1 (a) Mean Leaf number on the main stem and mean days to ear emergence (with associated standard errors) for the three unvernalised and vernalised cultivars.

Cultivar	Total leaf number on the main stem	Days to ear emergence	Reduction in days to ear emergence	Ye in wh grain harve
Unvernalised:				
C.S. Hope 5A	7.0±0.0	49.6±0.3	-	1977
C.S. Euploid	10.0±0.0	83.8±1.5	-	1977
C.S. Hope 5D	12.7±0.2	105.0±0.6	-	1977
	13.1±0.2	117.2±1.9	-	1978
Vernalised:				
C.S. Hope 5A	7.0±0.0	52.4±0.5	-2.8	1977
C.S. Euploid	7.5±0.2	53.0±0.3	+30.8	1977
C.S. Hope 5D	7.0±0.0	52.0±0.5	+53.0	1977
	9.0±0.1	74.5±2.7	+42.7	1978

(b) Mean days to ear emergence in unvernalised plants as measured by Law, 1972. Plants were grown at 18°C in continuous light.

Cultivar	Mean days to ear emergence
C.S. Hope 5A	41.92
C.S. Euploid	56.58
C.S. Hope 5D	91.92

The total number of leaves on the main stem was also used, in retrospect, to identify the collar primordium (see page 53), which, at its initiation was indistinguishable from leaf primordia. All primordia initiated after the collar primordium developed into spikelets, and therefore the initiation of the collar was regarded as the transition to flowering.

B. Growth of the shoot apex

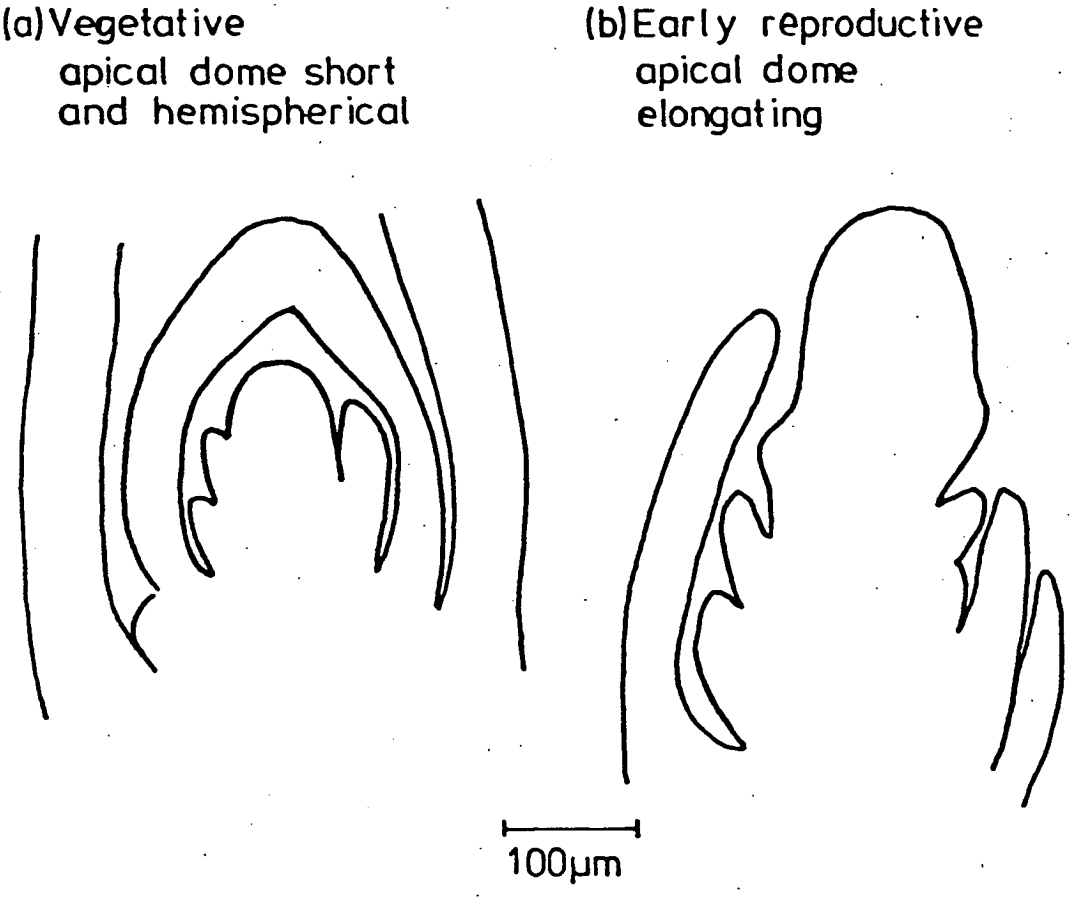
1. Morphological development

The development of the wheat shoot apex has been fully described (Barnard, 1955; Bonnett, 1966), however it was necessary to know the temporal details of development of the cultivars being studied. The following information was obtained from longitudinal sections of shoot apices and by dissecting plants to expose the apices.

There were four primordia in the embryo of the wheat cultivars in all the grains examined. At planting, all the cultivars always had four primordia already initiated, except for vernalised C.S. Hope 5D, which always had five primordia, the fifth primordium being initiated during the vernalisation. At this vegetative stage, the apical dome was short and hemispherical (Fig 3.1a). The first sign of the onset of the reproductive condition was the rapid elongation of the apex with primordia being formed in quick succession in alternating order along the two opposite flanks of the apical dome (Fig 3.1b). Buds in the axils of these primordia then grew rapidly giving rise to a double structure composed of a leaf and bud primordium-the double ridge (Fig 3.1c). The upper ridge then developed into a spikelet primordium and the lower ridge (leaf primordium) was completely suppressed. However at the base of the spike (the whole inflorescence), one lower ridge developed into the collar (Bonnett, 1966), which marked the point of transition between the vegetative and reproductive conditions.

Figs 3.2 - 3.4 are camera lucida drawings of median longitudinal sections of the shoot apices of the different cultivars of wheat on the 8th, 16th and 20th days after planting. On day 8 (Fig 3.2), the shoot apex was vegetative and at a similar stage of development in all the cultivars. However, by day 16, C.S. Hope 5A (Fig 3.3a) and vernalised C.S. Hope 5D(Fig 3.3b) were at a more advanced stage of development than C.S. Hope 5D (Fig 3.3C) and C.S. Euploid (Fig 3.3d).

FIG 3.1 Camera lucida drawings of median longitudinal sections of representative wheat shoot apices at different stages of development



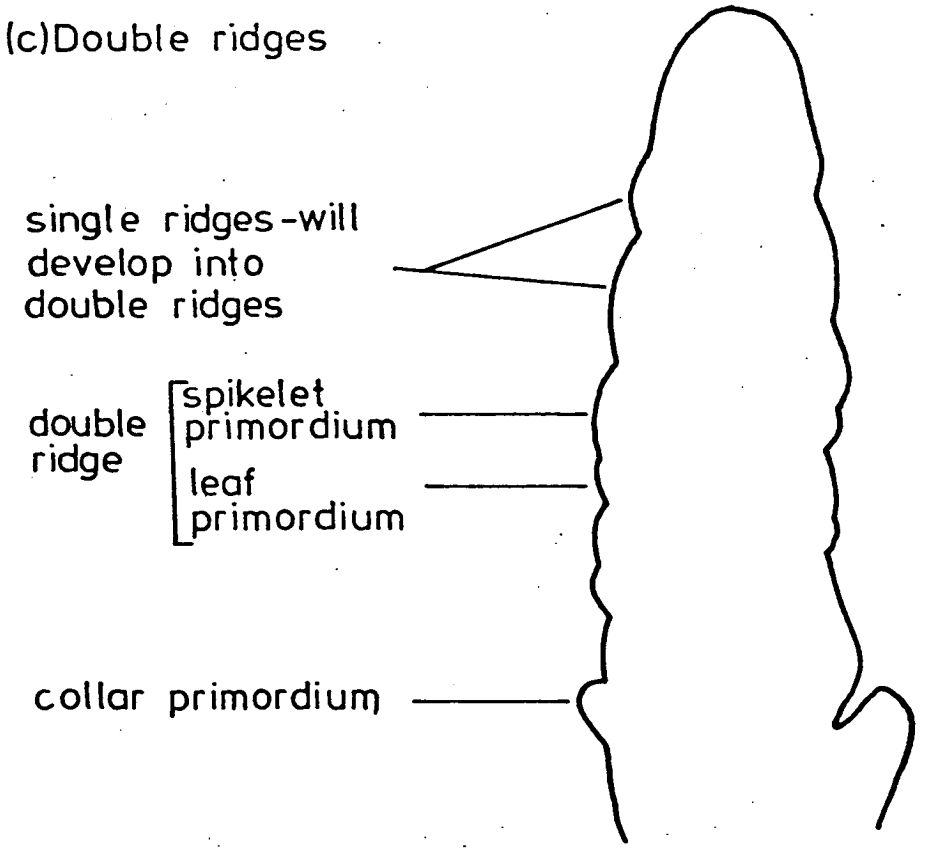
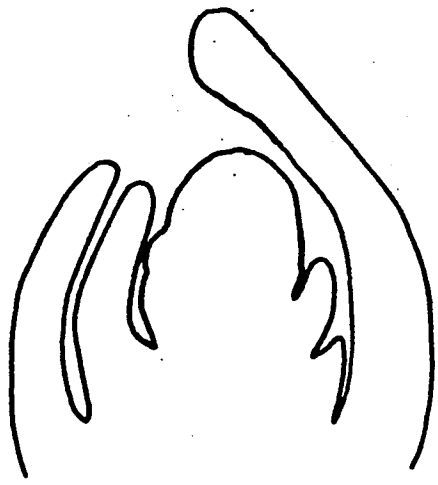
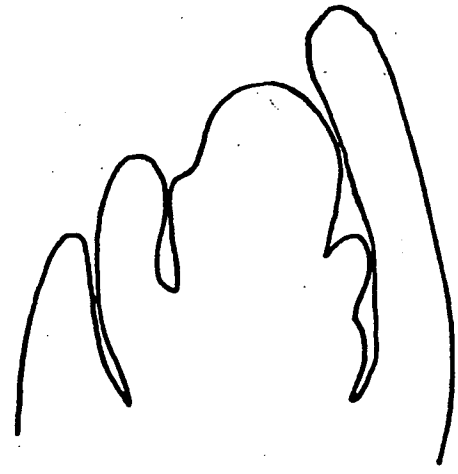


FIG 3.2 Camera lucida drawings of representative median longitudinal sections of wheat shoot apices on the 8th day after planting

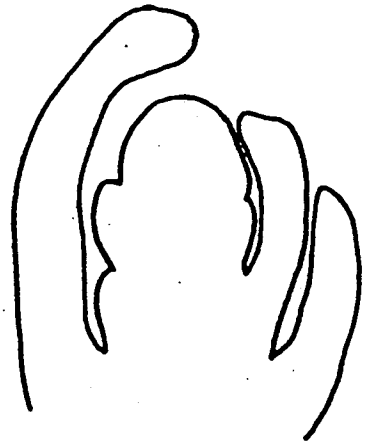
(a) C.S.Hope 5A



(b) C.S.Hope 5D vernalised



(c) C.S.Hope 5D



(d) C.S. Euploid

100 microns

FIG 3.3

Camera lucida drawings of representative median longitudinal sections of wheat shoot apices on the 16th day after planting.

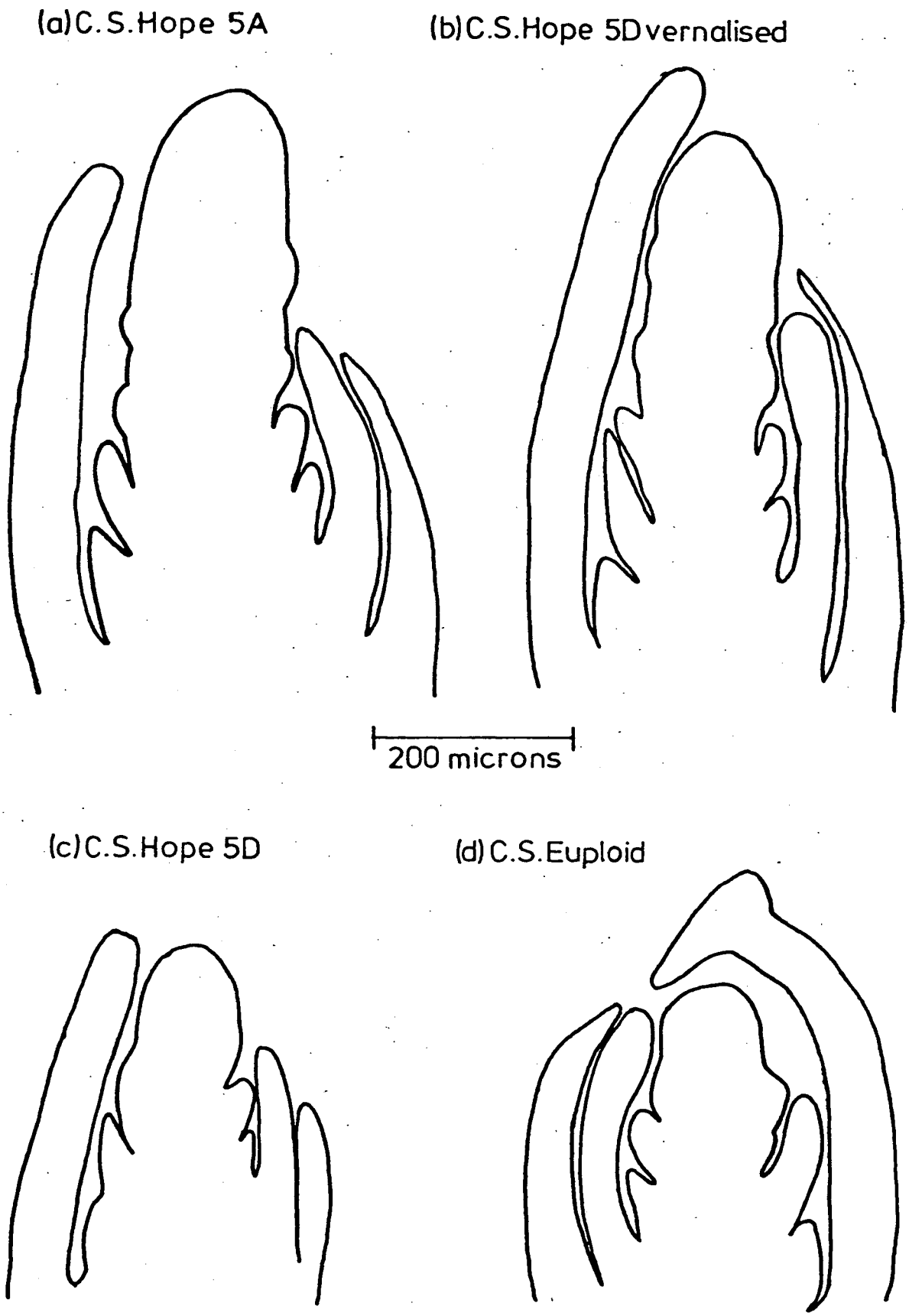
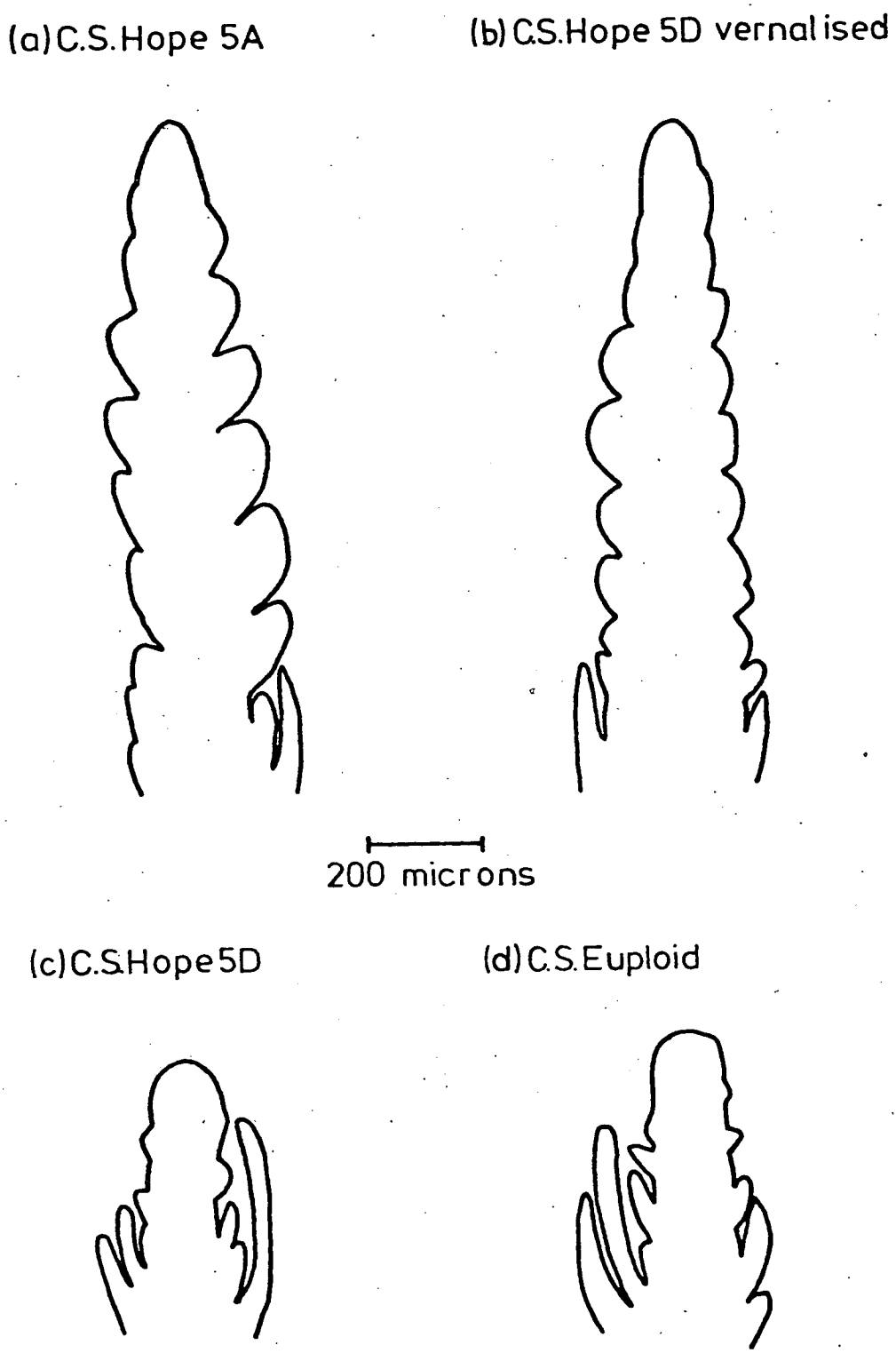


FIG 3.4 Camera lucida drawings of representative median longitudinal sections of wheat shoot apices on the 20th day after planting.



In C.S. Hope 5A and vernalised C.S. Hope 5D the apical dome had elongated and there was a build up of primordia on the shoot apex, which indicated that the apex had switched to the reproductive condition. In C.S. Hope 5D and C.S. Euploid the apical dome had increased in size compared to day 8 but the apex was still vegetative. On day 20, in C.S. Hope 5A (Fig 3.4a) and vernalised C.S. Hope 5D (fig 3.4b), double ridges were visible at the base of the spike but more distally, the lower ridges had been completely suppressed leaving just the spikelet primordia. In C.S. Hope 5D (Fig 3.4c), and more markedly in C.S. Euploid (Fig 3.4d), the shoot apex had increased in size, and primordia were beginning to build up on the apex indicating the switch to the reproductive condition.

Figs 3.2 - 3.4 therefore illustrate that C.S. Hope 5A, and C.S. Hope 5D when vernalised, undergo the transition to flowering earlier than unvernalised C.S. Hope 5D and C.S. Euploid.

2. Rate of primordium initiation

The number of primordia initiated by each cultivar was counted each day from the median longitudinal sections of shoot apices. The mean (of 3-6 apices) number of primordia was plotted against days after planting for each experiment (Fig 3.5-3.8). Regression analysis was carried out on these values, with separate regression lines for the parts of the graphs which appeared to have different slopes. The slopes of the regression lines were the rates of primordium initiation (Table 3.2). The mid-point of the plastochron in which the collar was formed, and the first appearance of double ridges are marked on the graphs.

In C.S. Hope 5A (Fig 3.5), the rate of primordium initiation remained constant at least up to collar formation. In experiment 01 (Fig 3.5a), there may have been an increase at collar formation but since this was not reflected in experiment 02 (Fig 3.5b), where the rate of primordium initiation was constant up to day 16, it may not be significant that these two events coincided. From day 16 (Fig 3.5b) (by which time 8 primordia had been initiated since planting), which was just before double ridge formation, the rate of primordium initiation was approximately four-fold higher.

FIGS 3.5 and 3.6 Mean number of primordia as a function of days after planting. Collar formation (↓C) was after the 7th leaf in both cultivars (Table 3.1a). Standard errors are shown for expts 02, 11, 12, if not shown, they were zero.

FIG 3.5 C.S. Hope 5A

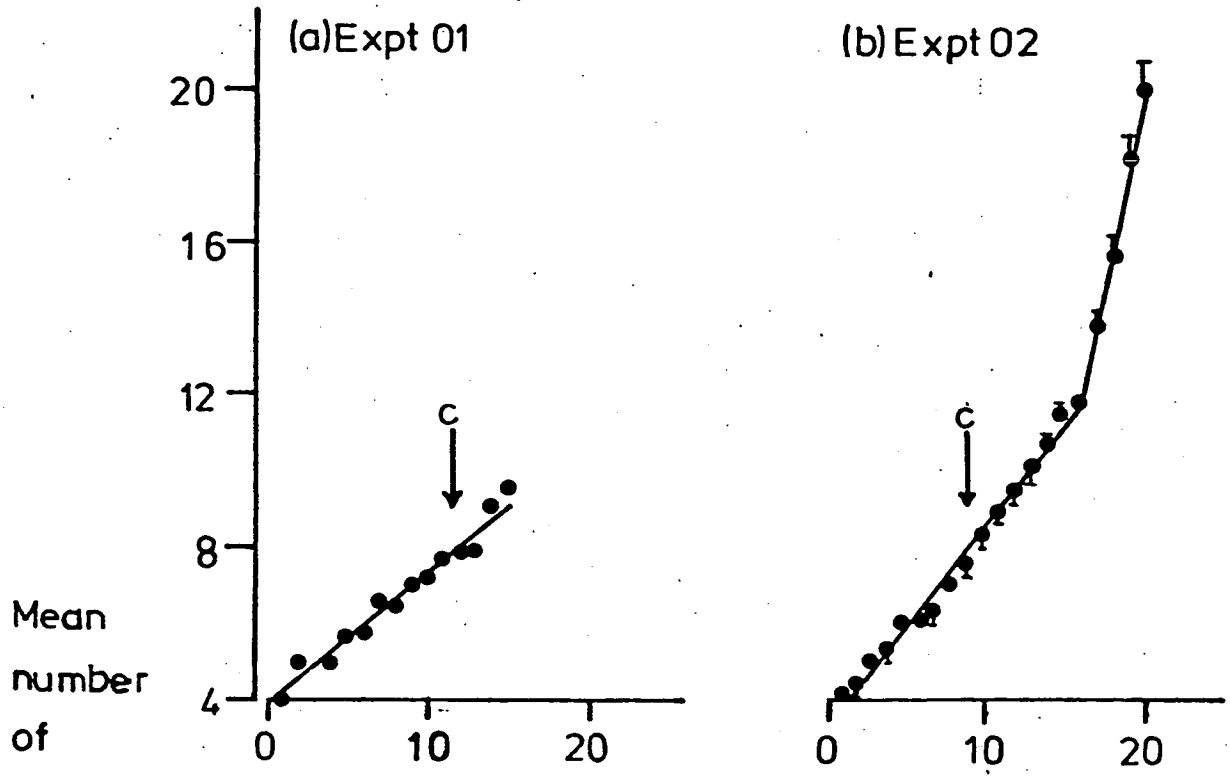


FIG 3.6 C.S. Hope 5D vernalised

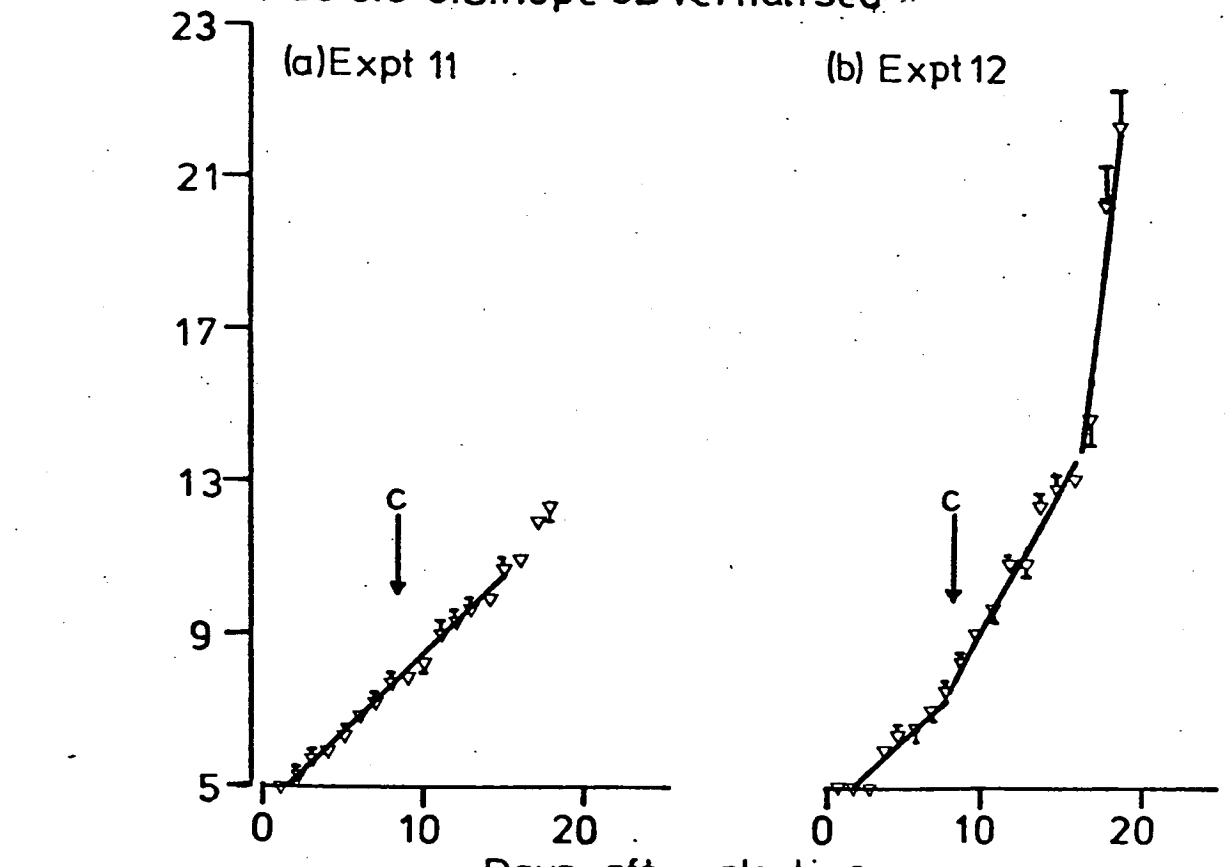


FIG 3.7 Mean number of primordia as a function of days after planting in C.S.Hope 5D. Collar formation ($\downarrow^{c}$, $\downarrow^{c}$ indicates expected time) was after the 12th or 13th leaf. Standard errors are shown for experiments 21, 22 and 24, where they are not shown they were zero.

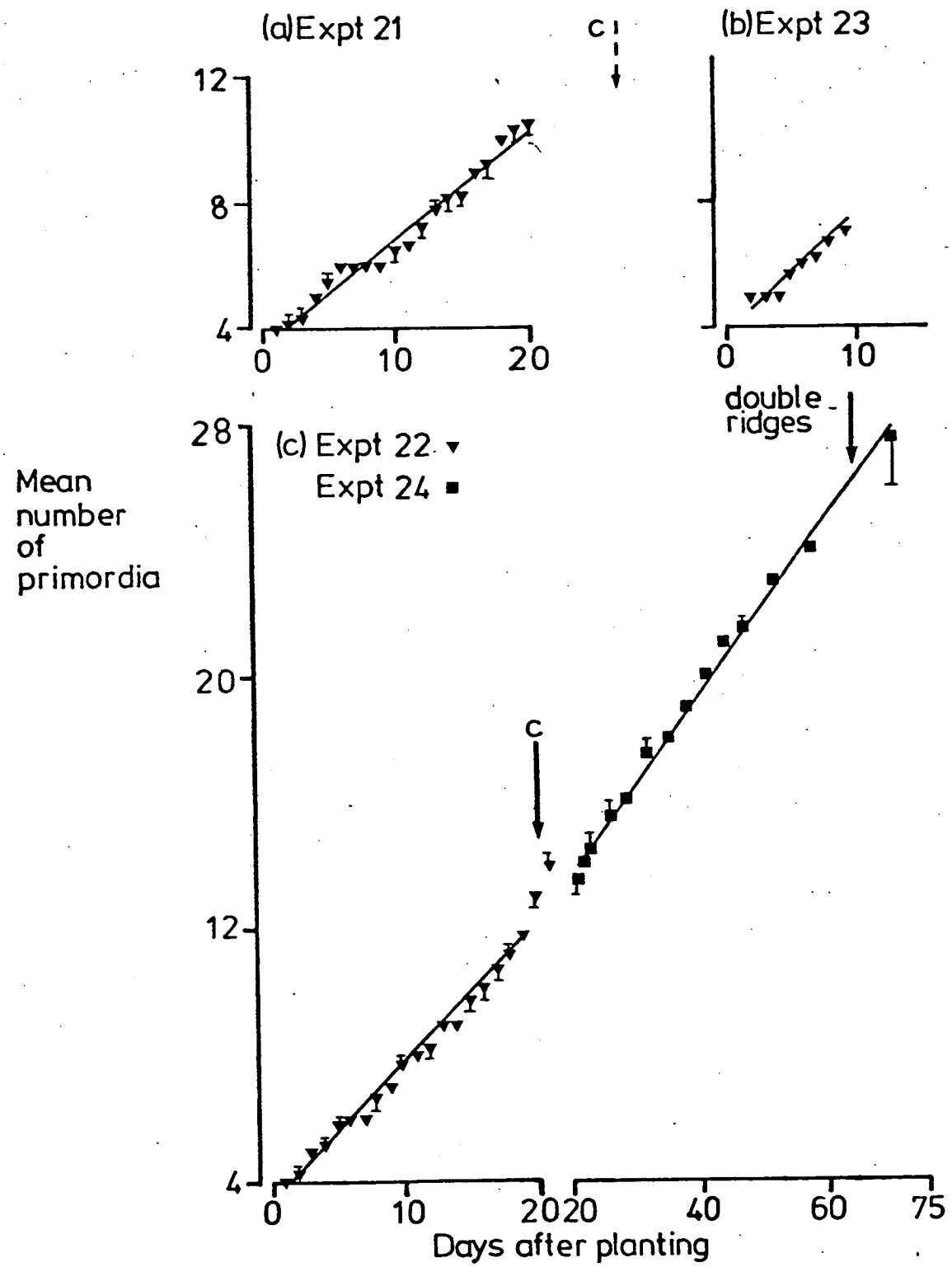


FIG 3.8 Mean number of primordia as a function of days after planting in C.S.Euploid. Collar formation($\downarrow^{C}$, $\downarrow^{C}$ indicates expected time) was after the 10th leaf. Standard errors are shown for expt 32, if not shown they were zero.

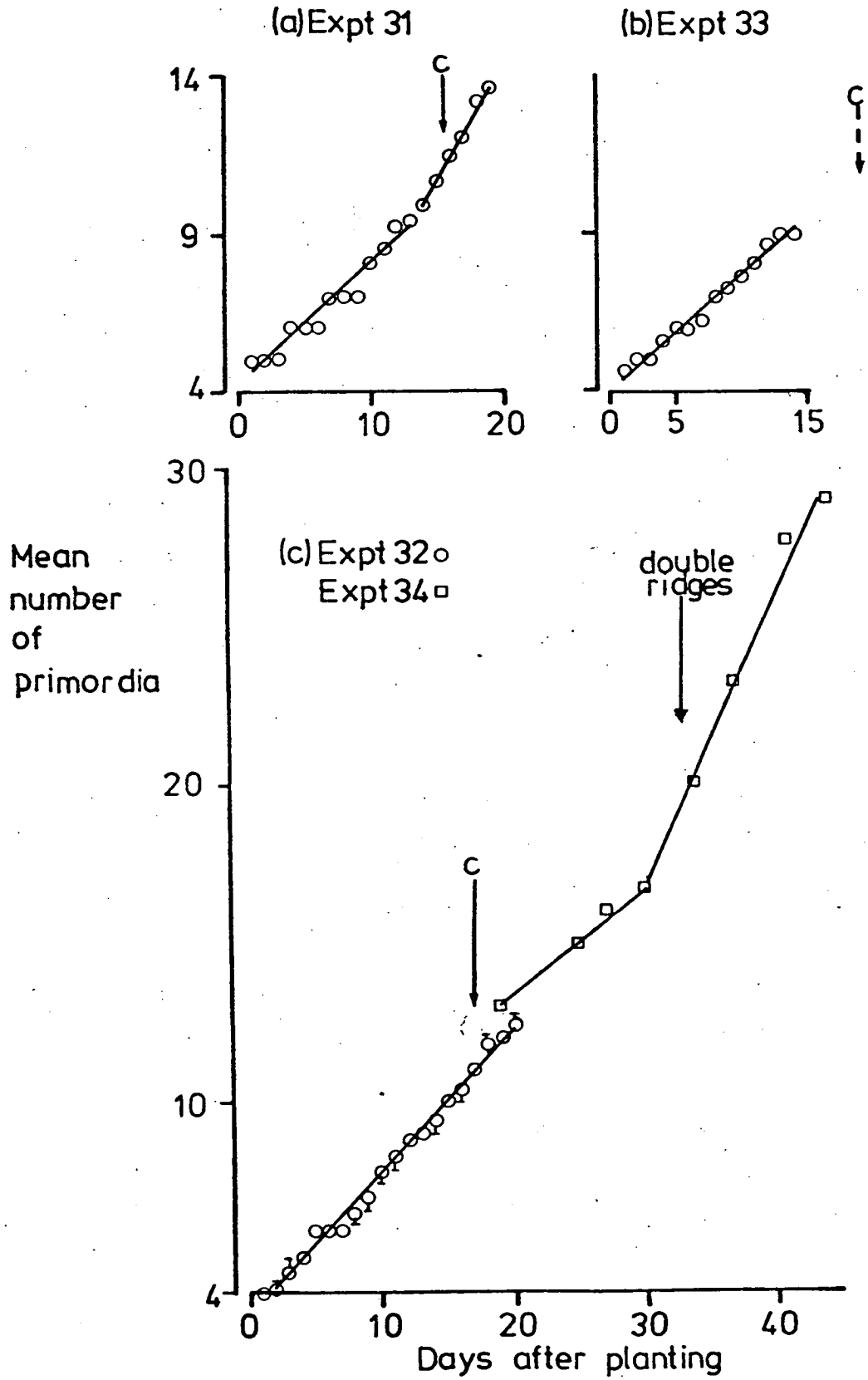


Table 3.2. Rates of primordium initiation. The values for rate of initiation are the regression coefficients of the lines shown in FIGS. 3.5-3.8.

Cultivar	Experiment	Period (Days)	Primordium numbers	Rate of initiation of primordia. day^{-1}	Plasto-chron (Days)
C.S. Hope 5A	01	1-15	5-10	0.36	2.78
	02	1-16	5-12	0.53	1.89
	02	17-20	13-20	2.12	0.47
C.S. Hope 5D	11	1-15	6-11	0.41	2.44
Vernalised	12	1-8	6-7	0.39	2.56
	12	9-15	8-13	0.77	1.30
	12	16-19	14-22	3.35	0.30
C.S. Hope 5D	23	1-9	5-7	0.35	2.86
	21	1-20	5-10	0.34	2.94
	22	1-19	5-12	0.42	2.38
	24	21-70	13-27	0.29	3.45
C.S. Euploid	33	1-14	5-9	0.36	2.78
	31	1-13	5-9	0.39	2.56
	31	14-19	10-14	0.75	1.33
	32	1-20	5-12	0.45	2.22
	34	19-30	13-17	0.33	3.03
	34	31-44	18-29	0.93	1.08

$$\text{Plastochron} = \frac{1}{\text{Rate of initiation of primordia}}$$

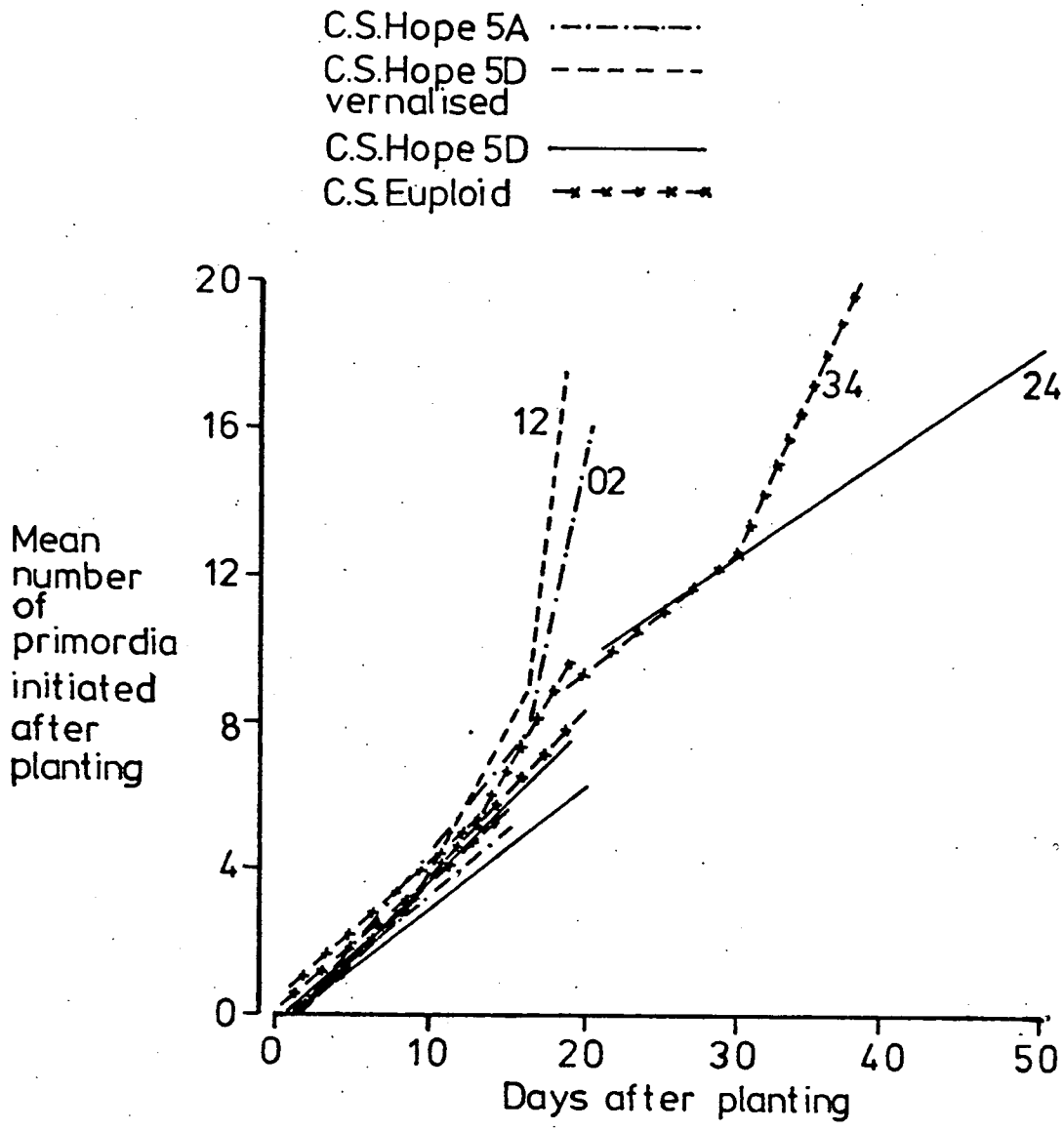
Similarly, in vernalised C.S. Hope 5D (Fig 3.6), the rate of primordium initiation was constant up to collar formation. In experiment 12 (Fig 3.6b), the rate then increased about two-fold, but as this was not the case in experiment 11 (Fig 3.6a), it may not be related to collar initiation. From day 16 (when 8 primordia had been formed since planting), which was just before double ridge formation, the rate of primordium initiation increased about four-fold, as was the case in C.S. Hope 5A.

In unvernalised C.S. Hope 5D (Fig 3.7 a,b,c,), the rate of primordium formation was apparently constant up to day 20, by which time 6 (experiment 21) or 8 primordia(experiment 22) had been formed since planting. In Fig 3.7c, the results of experiment 24 are plotted with those of experiment 22, and since the first two values (days 21 and 24) in experiment 24 follow the pattern of experiment 22, the experiments were considered together. On day 20, just before collar formation, the rate of primordium initiation seemed to increase for approximately five days and then the rate was lower for the remainder of the experiment. There was no apparent increase in the rate around double ridge formation comparable with that in C.S. Hope 5A and vernalised C.S. Hope 5D (cf. Figs 3.5b and 3.6b).

In C.S. Euploid (Fig 3.8 a,b,c,), the rate of primordium initiation was constant until about collar formation. At this point in experiment 31 (Fig 3.8a), the rate of primordium initiation increased. In Fig 3.8c, the values for experiment 34 are plotted with those of experiment 32. In this case, it is not clear whether the results of experiment 34 follow directly on from experiment 32, and therefore the apparent decrease in the rate of primordium initiation at collar formation may not be significant. From day 30, which was just before double ridge formation, the rate of primordium initiation increased by about two-to three-fold (Fig 3.8c). The rate of initiation increased less than in C.S. Hope 5A (Fig 3.5b) and vernalised C.S. Hope 5D (Fig 3.6b), but more than in unvernalised C.S. Hope 5D (Fig 3.7c).

The regression lines from all the experiments are shown in Fig 3.9. Over the first 15 days, the rate of primordium initiation was similar in all the experiments except for experiment 12 (vernalised C.S. Hope 5D) in which there was an increase in the rate of primordium initiation on day 8. After 7 to 12 primordia had been formed since planting, there was a difference in the rate of primordium initiation, it increased with decreasing vernalisation requirement.

FIG 3.9 Mean number of primordia initiated after planting as a function of days after planting. Combined data from FIGS 3.5–3.8.



C.S. Hope 5D, which has the highest vernalisation requirement, had the slowest rate of primordium initiation, whilst C.S. Hope 5A, which does not require vernalisation, had the fastest rate of initiation. C.S. Euploid, which has an intermediate vernalisation requirement, had an intermediate rate of primordium initiation. After vernalisation, C.S. Hope 5D resembled C.S. Hope 5A in its rate of primordium initiation.

3. Volume of the apical dome, cell number and cell volume

(a) Change in the mean volume of the apical dome

The change in the mean volume of the apical dome with time is a parameter which was used to compare the growth of the shoot apex in the different cultivars.

The volume of the median longitudinal section of the apical dome (for justification see methods page 40) was measured, each day, from camera lucida drawings of 3-6 apices of each cultivar. The mean volume of the median section of the apical dome was plotted against days after planting for each experiment (Fig 3.10 and 3.11).

In C.S. Hope 5A (Fig 3.10a), the mean volume of the apical dome remained constant or increased gradually up to about collar formation in both experiments. There was then a large increase in the volume of the apical dome in both experiments, and in experiment 02, the volume of the apical dome continued to increase up to day 16, which was just before double ridge formation. From collar formation up to day 16, the apical dome increased in volume about four-fold. After day 16, the mean volume of the apical dome started to decrease, and therefore the volume on day 16 was probably the maximum dome volume.

In vernalised C.S. Hope 5D (Fig 3.10b), the pattern of increase in the apical dome volume was very similar to that in C.S. Hope 5A. In experiments 11 and 12, the mean volume of the apical dome remained constant or increased gradually up to just past collar formation. There was then a three-fold increase in the volume of the apical dome, in experiment 12, up to day 18.

FIG 3.10 Mean volume of median section of apical dome as a function of days after planting. Standard errors are shown for all experiments. c=collar formation.

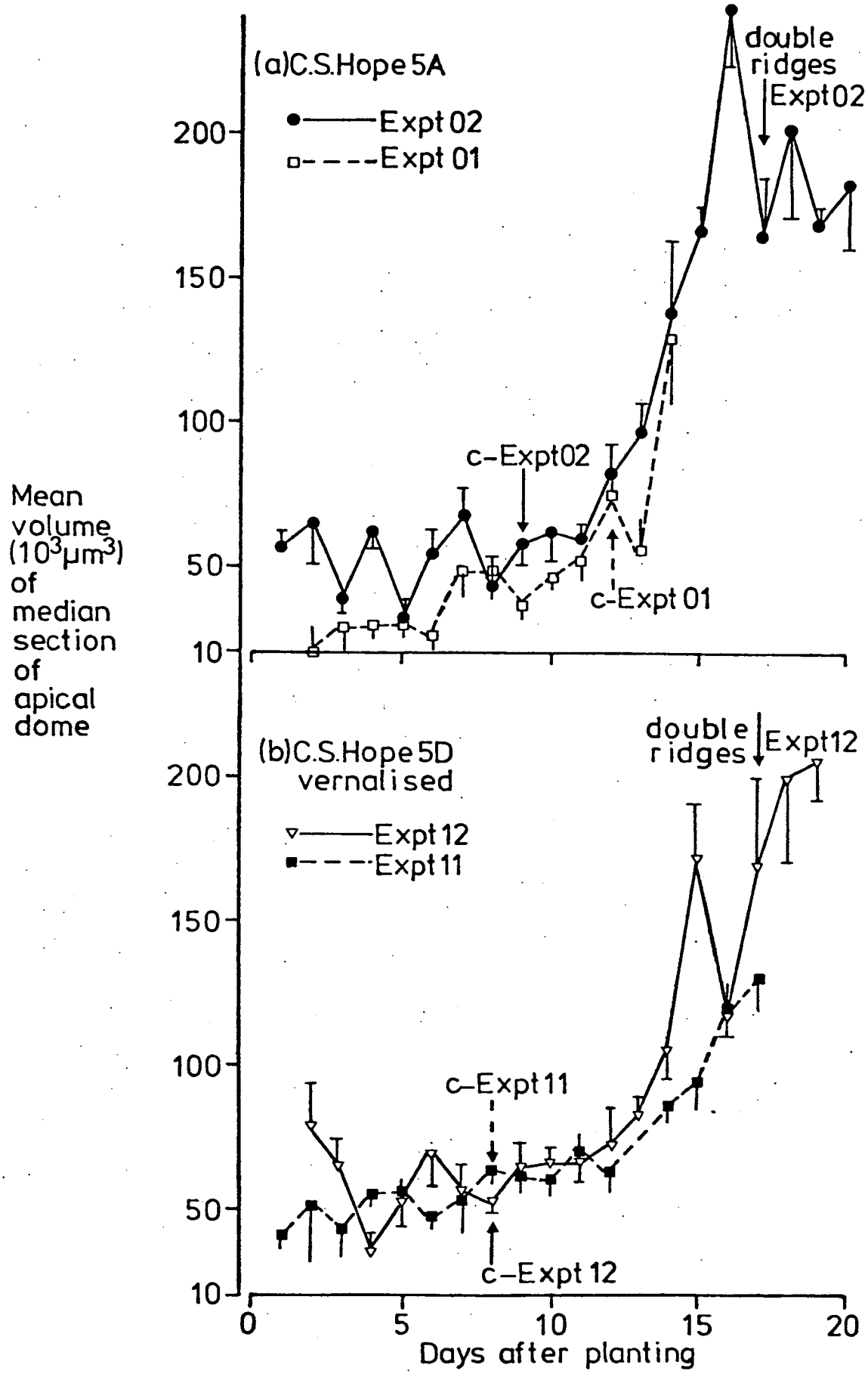
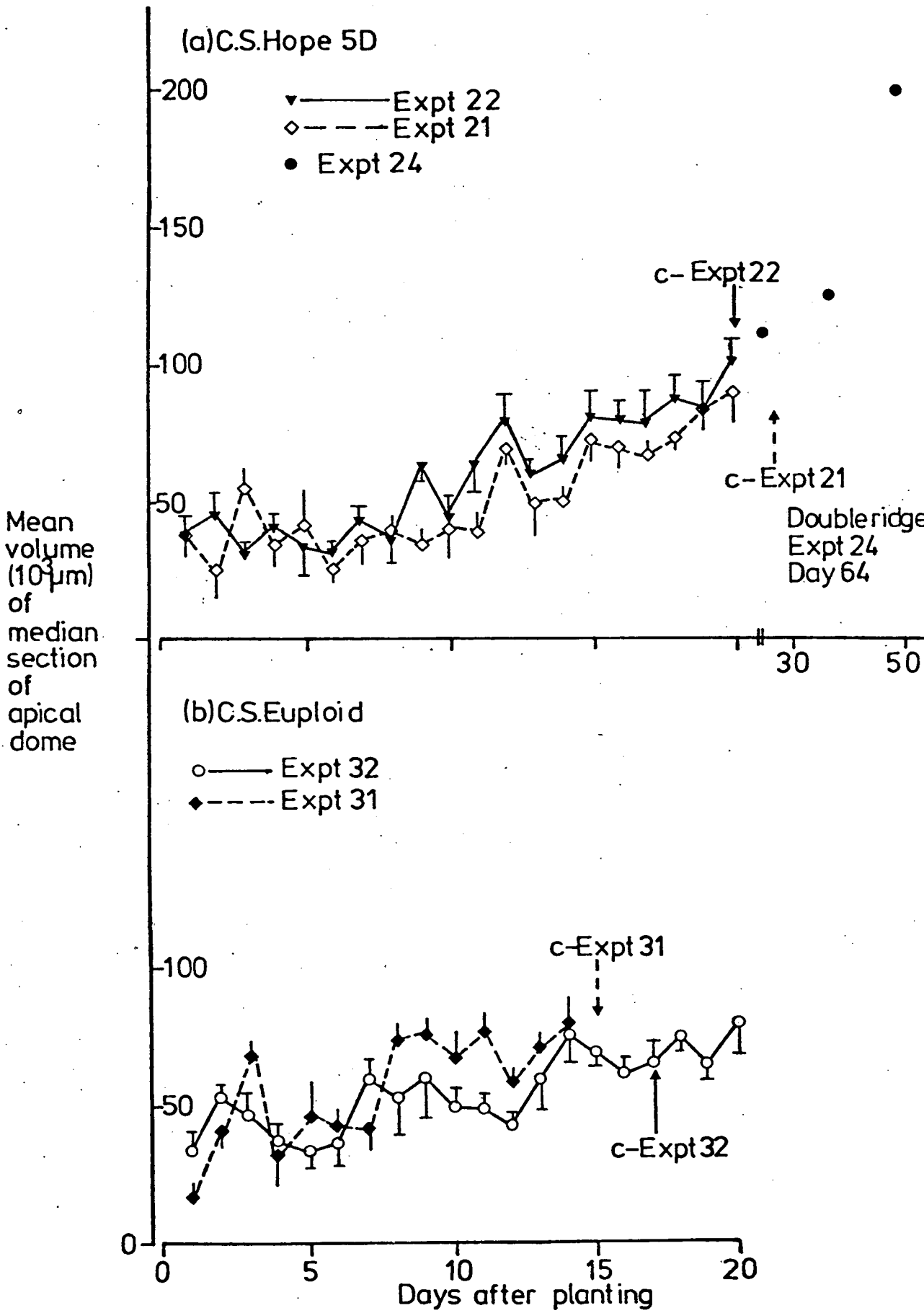


FIG 3.11 Mean volume of median section of apical dome as a function of days after planting. Standard errors are shown for experiments 21,22,31,32. c=collar formation.



It was not possible to tell from the graph whether the volume on day 19 was the maximum dome volume, but if the vernalised C.S. Hope 5D was behaving in a similar way to C.S. Hope 5A, the maximum volume would be attained around the time of double ridge formation.

In both unvernalised C.S. Hope 5D and C.S. Euploid (Figs 3.11a and b), the increase in apical dome volume followed the same trend in the different experiments for each cultivar. The mean volume of the apical dome increased gradually, by about two-fold up to collar formation.

C.S. Hope 5A and vernalised C.S. Hope 5D behave similarly with respect to the change in the mean volume of the median section of the apical dome with time. The volume of the apical dome remained constant or increased gradually up to collar formation, and then sharply increased up to about double ridge formation. In unvernalised C.S. Hope 5D and C.S. Euploid the mean volume of the median section of the apical dome increased gradually up to collar formation, which indicated that this period of growth may be similar to the growth of the C.S. Hope 5A and vernalised C.S. Hope 5D up to collar formation, but continued over a greater length of time. Detailed information is not available regarding the change in volume of the apical dome after collar formation in C.S. Hope 5D and C.S. Euploid. However data obtained in experiment 24 for C.S. Hope 5D indicated that the volume of the apical dome continued to increase at a similar rate both pre-and post-collar formation and that there was no sudden increase in apical dome volume as seen in C.S. Hope 5A and vernalised C.S. Hope 5D.

In C.S. Hope 5A and vernalised C.S. Hope 5D the large increase in the volume of the median section of the apical dome (Fig 3.10a and b) occurred at approximately the same stage after planting as the large increase in the rate of primordium initiation (Fig 3.5b and 3.6b). In C.S. Hope 5D the volume of the median section of the apical dome increased at the same rate after collar formation (Fig 3.11a) and therefore there was no change which could be correlated with the decrease in the rate of primordium initiation at this stage (Fig 3.7c). In C.S. Euploid no data were obtained on the pattern of increase of the volume of the median section of the apical dome after collar formation.

(b) Dimensions of the apical dome

The dimensions of the apical dome were measured to give further information on the way in which the volume of the apical dome was changing with time in the different cultivars.

The height and diameter of the apical dome were measured from median longitudinal sections of the shoot apices, and were plotted against days after planting (Fig 3.12 and 3.13). The results shown are for experiments 02, 12, 22 and 32, each point is the mean of 3-6 values.

In C.S. Hope 5A (Fig 3.12a) and vernalised C.S. Hope 5D (Fig 3.12b) the diameter of the apical dome increased gradually throught the experiments by about 1.5-fold. However the height increased gradually until just after collar formation and then suddenly increased, up to about double ridge formation, by 2.5-fold in C.S. Hope 5A and 2.0-fold in vernalised C.S. Hope 5D.

In C.S. Hope 5D (Fig 3.13a) and C.S. Euploid (Fig 3.13b), both dimensions of the apical dome increased gradually throughout the experiments by about 1.5-fold.

Therefore, in the period up to collar formation in all the cultivars, both the height and diameter of the apical dome increased gradually, and therefore were contributing to a similar extent to the increase in the mean volume of the apical dome. However, after collar formation in C.S. Hope 5A and vernalised C.S. Hope 5D, the diameter of the apical dome continued to increase gradually but the height suddenly increased, indicating that it was the change in height that was contributing most to the sudden increase in the mean volume of the apical dome.

The values shown for C.S. Hope 5D (Fig 3.13a) for experiment 24 after collar formation, indicate that the height and diameter continued to increas at about the same rate as before collar formation, at least up to approximately day 55. Then the height increased fairly suddenly so that it exceeded the diameter.

FIG 3.12 Mean dimensions of the apical dome as a function of days after planting. Standard errors are shown.

● Diameter ○ Height

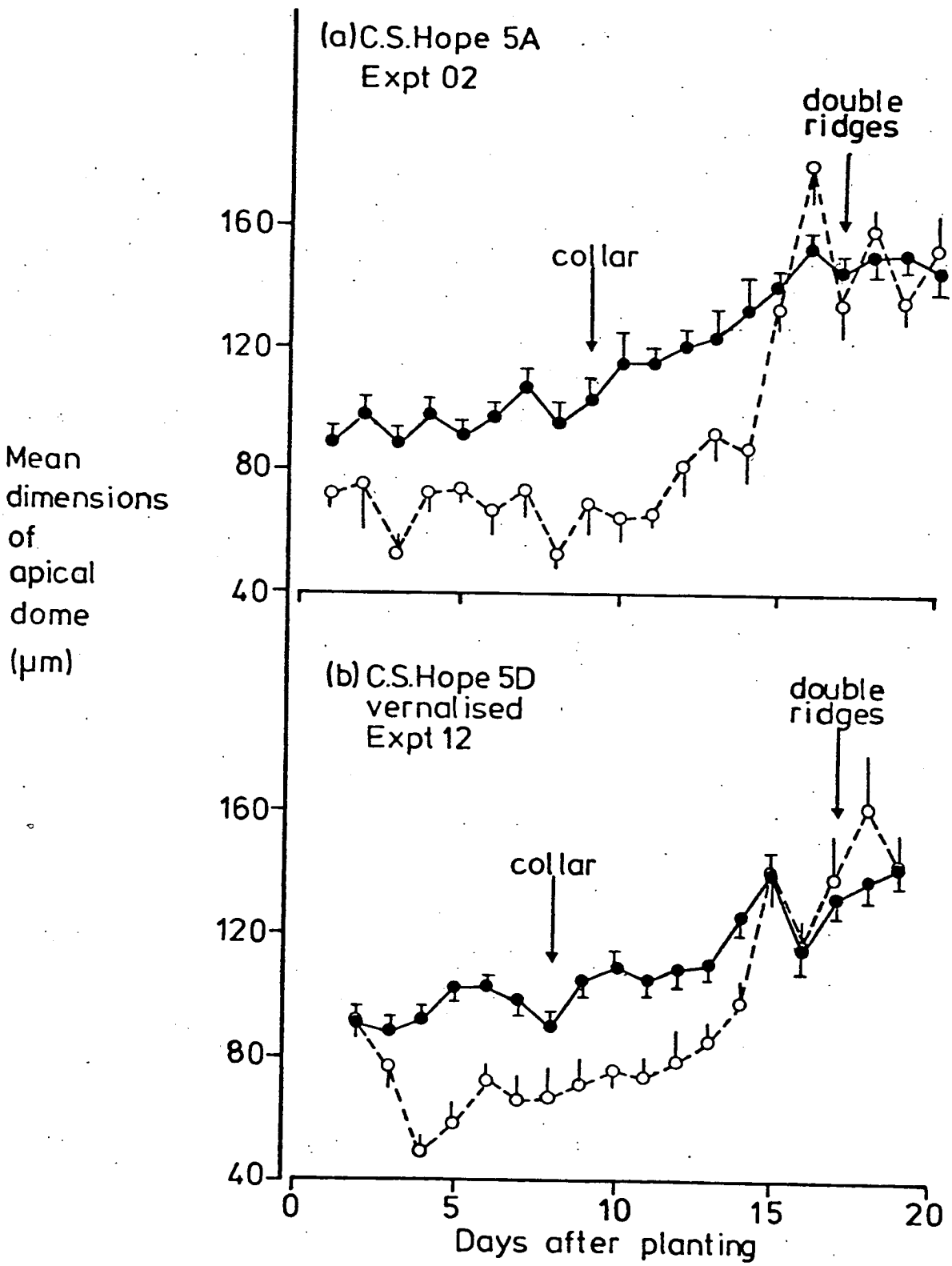
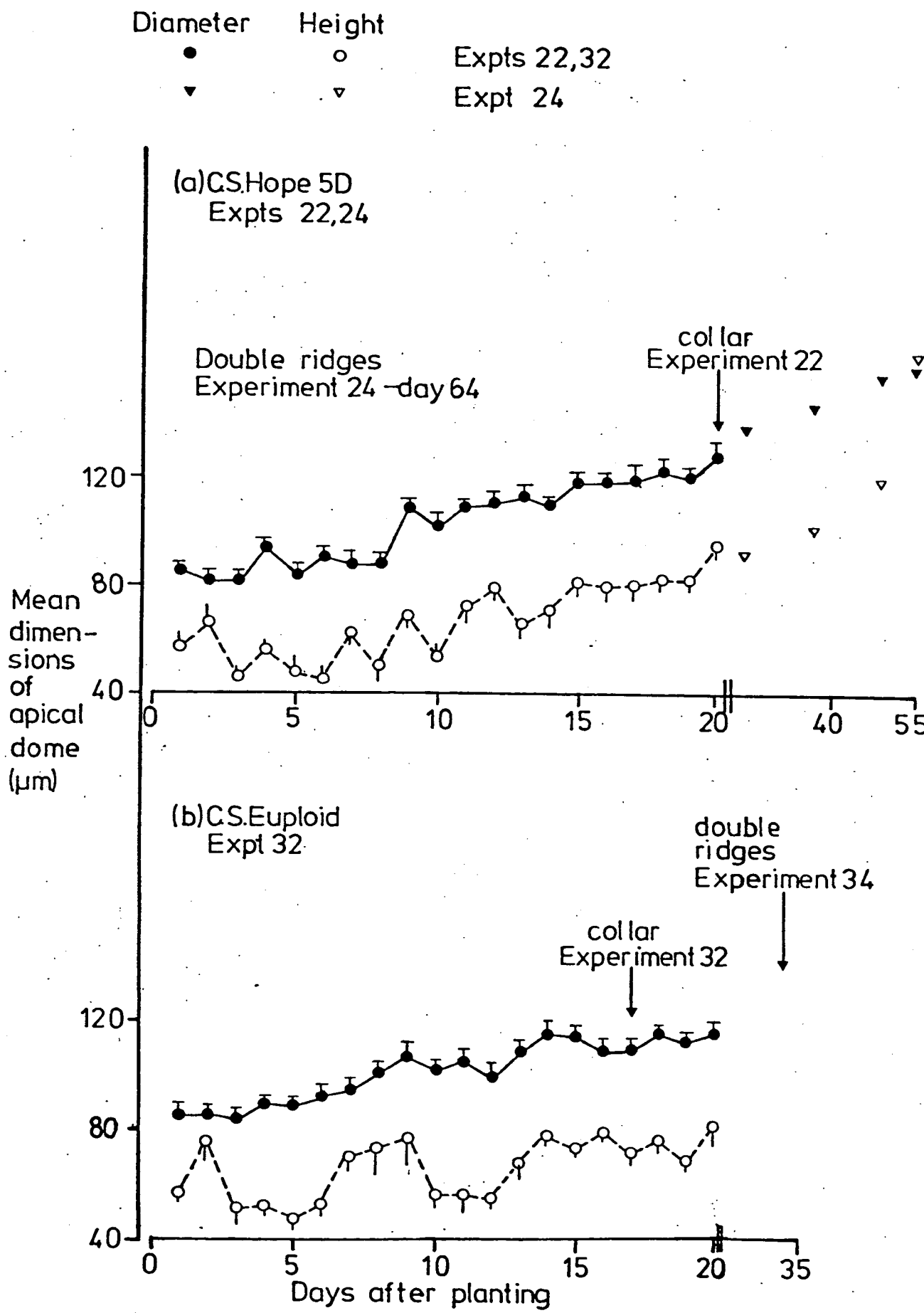


FIG 3.13 Mean dimensions of the apical dome as a function of days after planting. Standard errors are shown for experiments 22 and 32.



This was similar to the pattern of increase in the height of the apical dome, just before double ridge formation in C.S. Hope 5A (Fig 3.12a) and vernalised C.S. Hope 5D (Fig 3.12b). It therefore appeared that in the unvernalised C.S. Hope 5D, the height of the apical dome exceeded the diameter of the apical dome just before double ridge formation but the increase in height was less sudden than in the vernalised C.S. Hope 5D.

(c) Mean cell volume in the apical dome

The number of cells in the apical dome was counted in the median longitudinal sections of the shoot apices for experiments 02,12,22 and 32. The mean cell volume (for 3-6 apices) was calculated by dividing the mean volume of the median section of the apical dome by the mean cell number in the median section, for each day. The mean cell volume was plotted against days after planting for each cultivar (Fig 3.14).

In all the cultivars, the mean cell volume fluctuated from day to day. In C.S. Hope 5A (Fig 3.14a) and vernalised C.S. Hope 5D (Fig 3.14b), the mean cell volume was more or less constant up to day 15, and then seemed to be tending to increase. The mean cell volume was measured, separately, in the two-layered tunica and the corpus of the apical dome (Barnard, 1955) in C.S. Hope 5A, and was plotted against time (Fig 3.14a). It appeared that the mean volume of the cells of the corpus increased throughout the experiment, but the mean volume of the cells of the tunica remained more or less constant up to day 16 and than increased slightly. Therefore, the increase in volume of the cells of the corpus was contributing most to the increase in the mean cell volume in the apical dome. However as the shape of the apical dome was changing, it may have been that in the later stages of the experiment the corpus was occupying a relatively greater proportion of the apical dome.

In C.S. Hope 5D (Fig 3.14c) and C.S. Euploid (Fig 3.14d) the mean cell volume was approximately constant throughout the experiments.

The mean cell volumes were compared, between the cultivars, over the period of the graphs (Fig 3.14a-d) where the mean cell volumes remained more or less constant from day to day. The mean of the mean cell volumes was calculated for the first 10 days after planting, and these means were compared (Brownlee, 1953) (Table 3.3).

FIG 3.14 Mean cell volume in the median section of the apical do
as a function of days after planting.

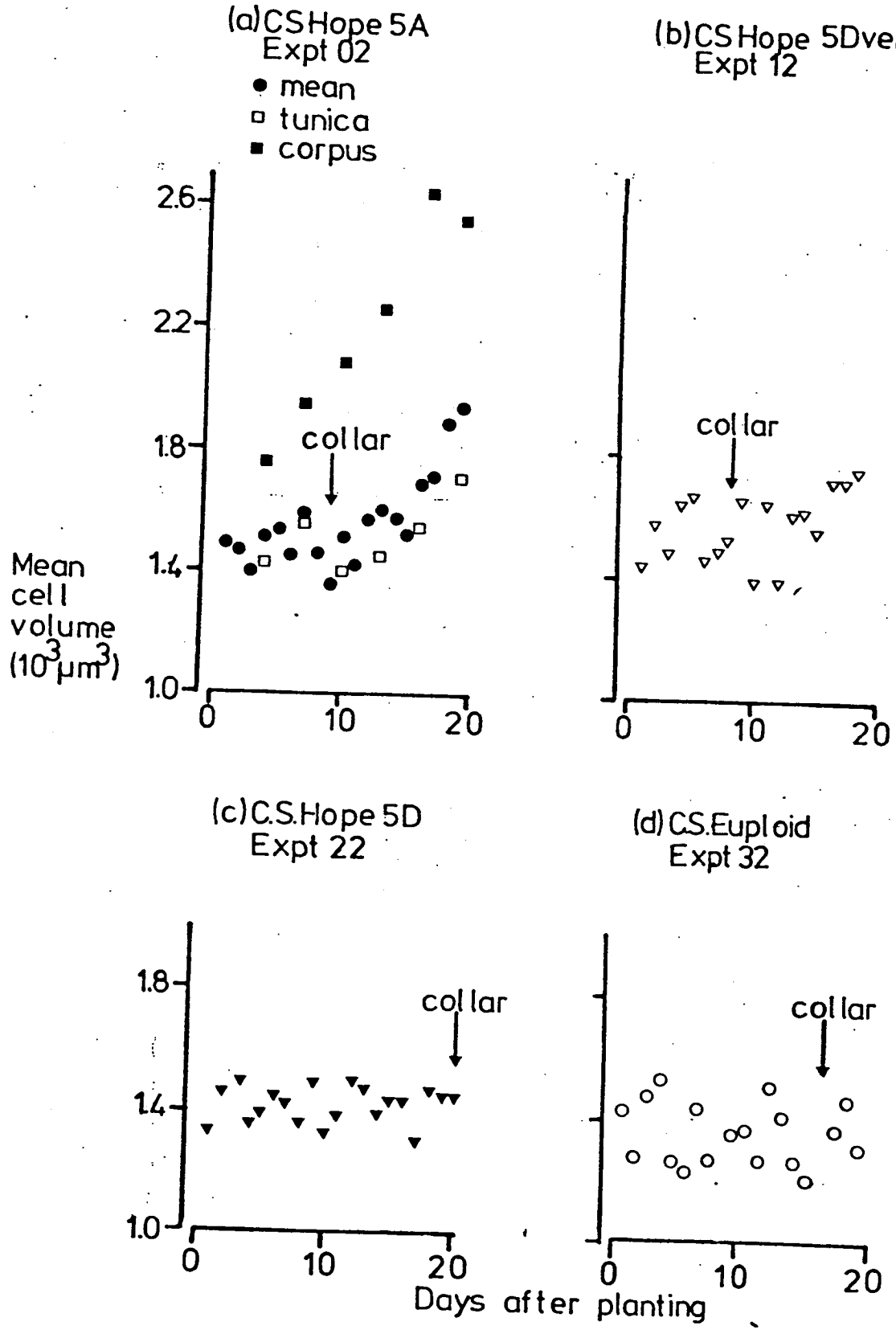


Table 3.3. The mean cell volume (with standard errors) in the median section of the apical dome and the mean volume (with standard errors) of the median section of the apical dome over the first 10 days after planting for experiments 02,12,22 and 32.

Cultivar	Mean Cell Volume ($10^3 \mu m^3$)	Mean volume of the apical dome ($10^3 \mu m^3$)
C.S. Hope 5A (5A)	1.48 ± 0.02	53.89 ± 3.89
C.S. Hope 5D (5DV) vernalised	1.53 ± 0.03	60.22 ± 4.18
C.S. Hope 5D (5D)	1.42 ± 0.02	40.71 ± 3.16
C.S. Euploid (EUP)	1.35 ± 0.03	46.41 ± 3.22

Result of the comparison of the mean cell volumes

	5A	5D	EUP
5DV	nsd	**	**
5D	*	-	nsd
EUP	**		

Result of the comparison of the mean volume of the apical dome

	5A	5D	EUP
5DV	nsd	**	*
5D	*	-	nsd
EUP	nsd		

nsd - means not significantly different

** - means significantly different at 1% level

* - means significantly different at 5% level.

The mean cell volume was significantly greater in the vernalised C.S. Hope 5D than in the unvernalised C.S. Hope 5D. The mean cell volume in C.S. Hope 5A was not significantly different from that in the vernalised C.S. Hope 5D, but these were both significantly greater than the mean cell volume in C.S. Hope 5D and C.S. Euploid, while the C.S. Hope 5D and C.S. Euploid were not significantly different from each other.

Therefore vernalisation appears to increase the mean cell volume in the apical dome by 7.7%, to a volume which is comparable to that in the cultivar which has no vernalisation requirement. The means of the mean volumes of the median sections of the apical domes (from Figs 3.10-3.11) were also calculated for the different cultivars over the first 10 days after planting (Table 3.3). The means were then compared by the same method as the mean cell volumes (Table 3.3). The mean volume of the median section of the apical dome was significantly greater in the vernalised C.S. Hope 5D than in the unvernalised C.S. Hope 5D over this period. Vernalisation increased the mean apical dome volume by 47.9%. Therefore although the 7.7% increase in the mean cell volume after vernalisation may have contributed to the increased apical dome volume, the major factor involved was an increase in the cell number. There was little difference in the C amount of DNA in the apical dome in the vernalised and unvernalised C.S. Hope 5D (Table 3.14), which showed that although there was a difference in cell volume between the two cultivars, the cells were distributed in the various phases of the cell cycle in a similar way.

The mean apical dome volume in C.S. Hope 5A was not significantly different to that in the vernalised C.S. Hope 5D, but both were significantly greater than in C.S. Hope 5D. Although the mean volume of the apical dome was significantly greater in vernalised C.S. Hope 5D than in C.S. Euploid, the mean apical dome volume in C.S. Hope 5A was not significantly greater than in C.S. Euploid (Table 3.3). However, the value for the mean apical dome volume for C.S. Euploid was intermediate between the volume for C.S. Hope 5A and C.S. Hope 5D which might be expected as it is intermediate in its vernalisation requirement between C.S. Hope 5A and C.S. Hope 5D.

Vernalisation increased the mean cell volume in the apical dome and the mean volume of the apical dome over the first 10 days after planting.

This increase in the apical dome was not solely brought about by the increase in cell volume but was almost entirely due to an increase in cell number.

(d) Volume relative growth rate

A number of methods were considered for determining the volume relative growth rate of the apical dome in each plastochron. The first problem encountered was that if the volume of the apical dome was plotted as a function of days after planting (Fig 3.15 - 3.16), on any one day there were apices with different numbers of primordia. Richards' method (1956) depends on obtaining an accurate estimate of the size of the phytomer at initiation and this could not be precisely determined with out first calculating the relative growth rate (see section 4, page 100), therefore this method was not practical. Hussey (1971) developed a method of placing apices on a developmental scale according to the axillary distance of the youngest primordia, and a modification of this method was used to try and calculate the volume change in a plastochron, however the relationship between axillary distance and volume was not consistent for wheat, particulary when the apex had undergone the transition to flowering. However, to calculate the volume relative growth of the apical dome in a plastochron it was necessary in some way to determine the minimum and maximum volume of the apical dome in each plastochron. If the volumes were simply plotted against primordium number (to eliminate the difficulty of apices with different numbers of primordia on any one day), the minimum and maximum values would have to be derived from the single lowest and single highest volumes. Therefore a method was devised, based on that used by Abbe, Phinney and Baer (1951).

The volume of the apical dome and phytomer of all the apices in plastochron (n) was plotted alongside the volume of the apical dome of all the apices in plastochron (n-1), for each plastochron, in all cultivars. Fig 3.17 shows the plot for experiment 02 (C.S. Hope 5A) for the first 5 plastochrons.

FIG 3.15. Volume of median section of apical dome as a function of days after planting. Number of primordia initiated by each apex is indicated.

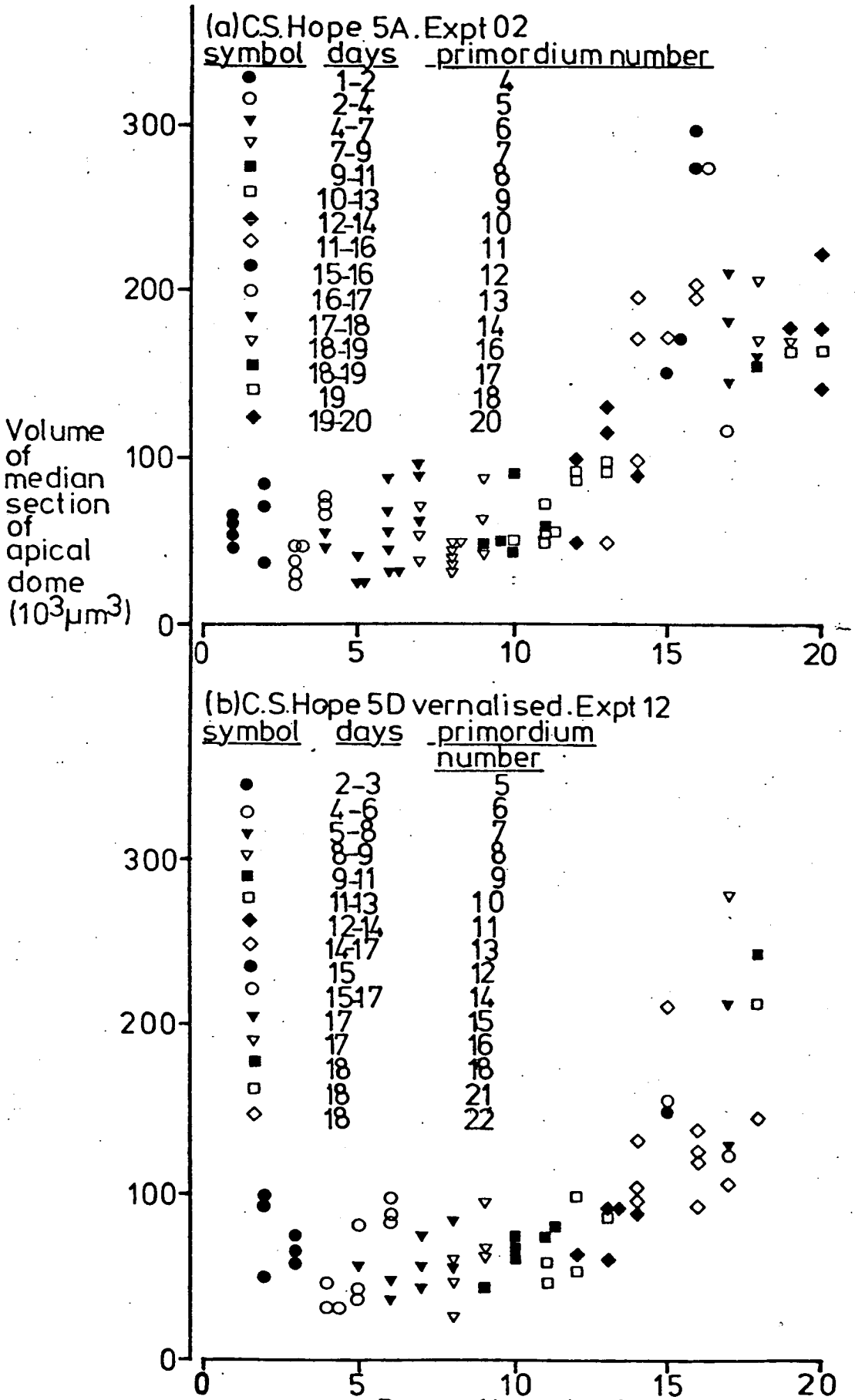


FIG 3.16. Volume of median section of apical dome as a function of days after planting. Number of primordia initiated by each apex is indicated. .

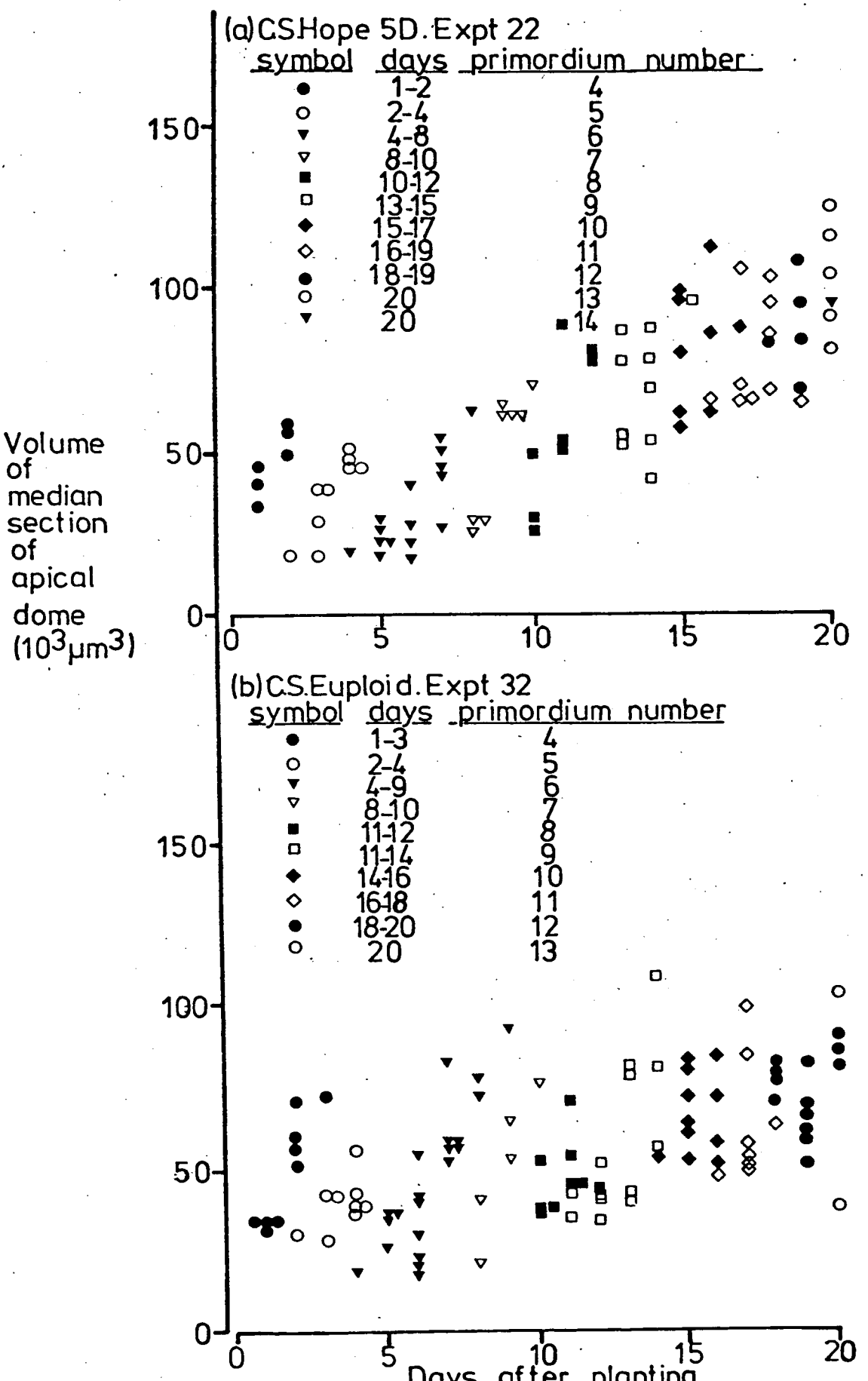
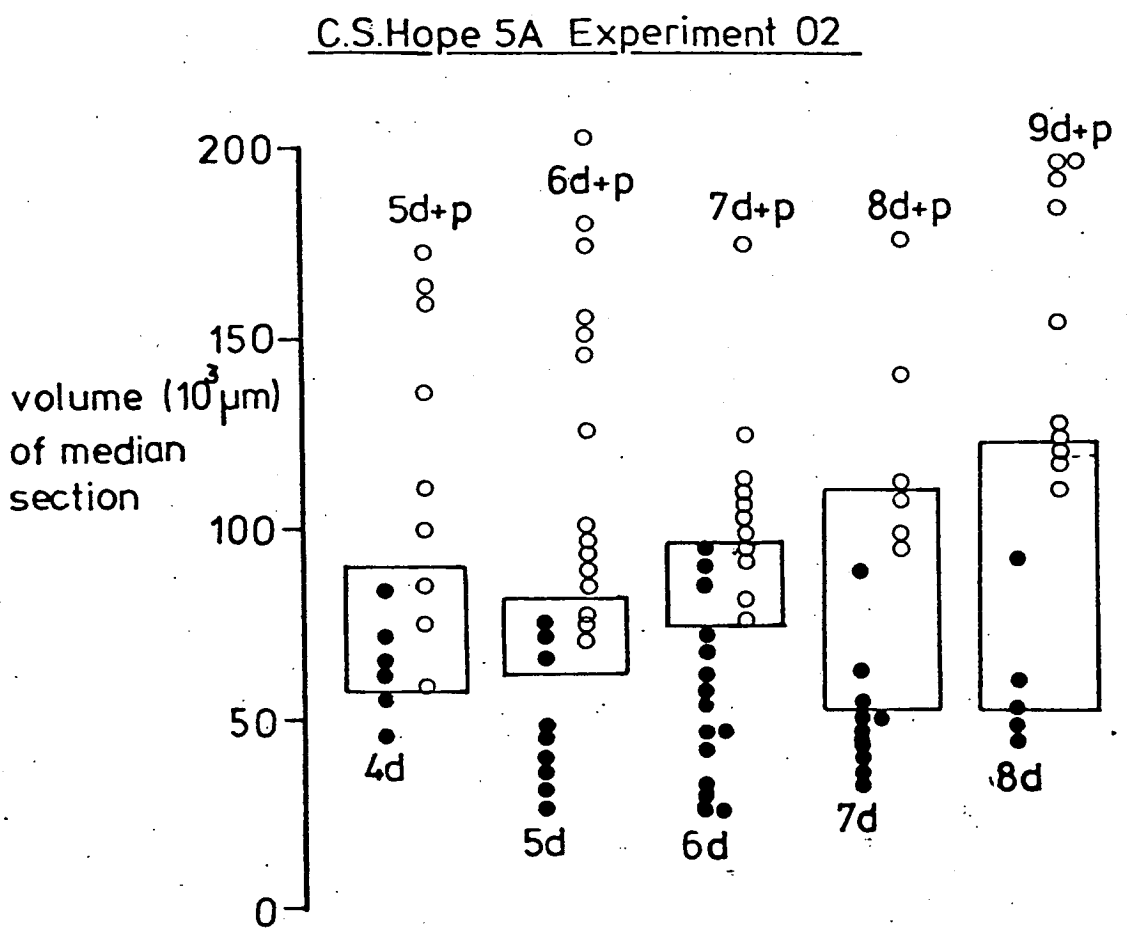


FIG 3.17 Method of determining the mean minimum and mean maximum apical dome volume for each plastochron.



The numbers eg. 4d, 5d+p, refer to the number of primordia initiated by the apices

d=apical dome p=phytomer

delimits values used in calculations

There was some overlap between the volume of the apical dome at the end of plastochron (n-1) and the volume of the apical dome + phytomer at the start of plastochron(n). The maximum dome volume in any plastochron was therefore calculated by taking the mean of the values which overlapped in the paired plots (delimited by Fig 3.17). If there was little overlap, the three highest values of the volume of the apical dome, and the three lowest values of the volume of the apical dome + phytomer were used (if there were less than 6 values in total for the volume of the apical dome,and less than 6 values in total for the volume of the apical dome + phytomer, the highest two and lowest two values respectively were used. This was for the later plastochrons in C.S. Hope 5A and vernalised C.S. Hope 5D, where there were few apices with the same number of primordia, Fig 3.15). The minimum dome volume for plastochron(n) was calculated by taking the mean of the apical dome volumes of the apices which were used as phytomer + dome in the calculation of the maximum volume for plastochron(n-1). The minimum (of 3-4 values, occasionally 2) and maximum (of 5 - 7 Values, occasionally 4) apical dome volumes calculated for experiments 02, 12, 22 and 32 in this way, are shown in Table 3.4 (Maximum and minimum dome volumes could not be calculated for experiments 01, 11, 21 and 31, as there were insufficient values, as described above, for the volume of the apical dome and phytomer for each plastochron).

The natural logarithms of these minimum and maximum apical dome volumes were plotted against the primordium number just initiated. Therefore against primordium number 6, the mean maximum volume for plastochron 5, and the mean minimum volume for plastochron 6, were plotted (Fig 3.18 - 3.21). Logarithmic plots were used so that the relative growth rates could be read directly off the graphs. Lines were fitted, by regression analysis, to the natural logarithms of the mean minimum and mean maximum values for each cultivar. (The bends in the lines were positioned according to which line best fitted the points which appeared by eye to be involved in a change in slope).

The volume relative growth rate per plastochron is the difference between the natural logarithms of the mean minimum, and mean maximum volumes of the apical dome, in that plastochron.

Table 3.4 Mean minimum and mean maximum volume of the apical dome calculated by the method shown in FIG 3.17.
The plastochron is the interval between the initiation of the indicated primordium and the next primordium

Plastochron Minimum volume ($10^3 \mu m^3$) of the median section of the apical dome at the beginning of the plastochron

	C.S.Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
5	38.47	*	21.54	24.53
6	32.46	33.11	19.69	23.57
7	38.09	46.06	27.66	31.19
8	--47.47--	--45.15--	35.16	38.09
9	53.46	58.56	46.06	36.60
10	69.41	49.90	64.72	54.05
11	79.84	74.44	67.36	--51.42--
12	160.77	-	76.71	60.34
13	-	96.54	85.63	-
			-- --	

Plastochron Maximum volume ($10^3 \mu m^3$) of the median section of the apical dome at the end of the plastochron

	C.S.Hope 5A	C.S. Hope 5D vernalised	C.S.Hope 5D	C.S. Euploid
5	72.97	97.51	46.53	54.05
6	88.23	101.49	65.36	85.63
7	71.52	82.27	72.97	74.44
8	--90.92--	--86.49--	91.84	70.11
9	120.90	96.54	108.85	97.51
10	123.97	103.54	112.71	85.63
11	212.72	-	116.75	--102.51--
12	287.15	156.02	123.97	-
			--- --	

* 5th primordium formed during vernalisation

\- insufficient data

--- plastochron in which collar is initiated

FIG 3.18 Natural logarithm of the mean minimum and mean maximum volume of the median section of the apical dome as a function of the primordium number just initiated in C.S.Hope 5A. The values used for the regression analyses are:
minimum 5-11 and 11-12
maximum 6-11 and 11-13

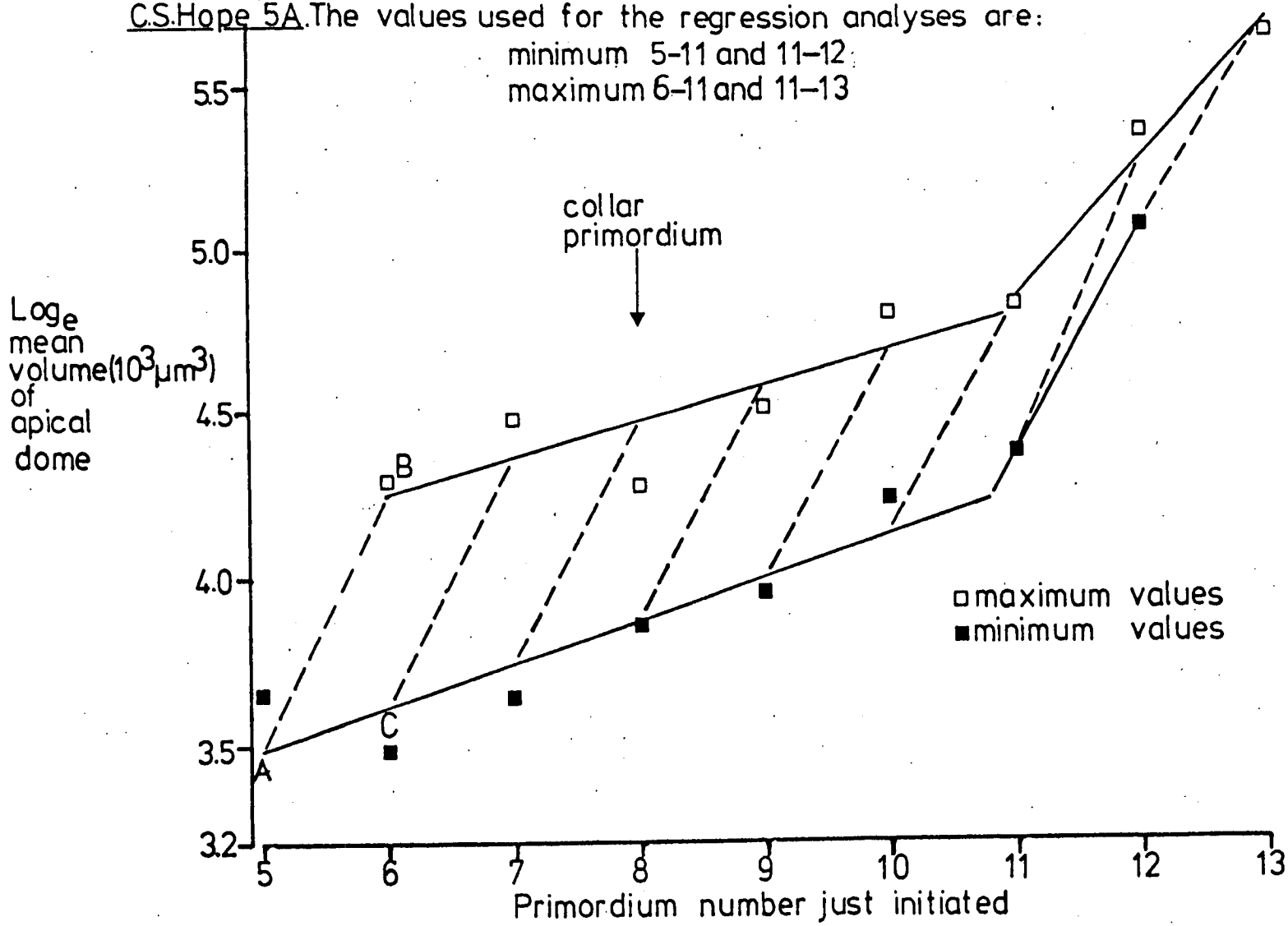


FIG 3.19 Natural logarithm of the mean minimum and mean maximum volume of the median section of the apical dome as a function of the primordium number just initiated in vernalised C.S. Hope 5D. The values used for the regression analyses are:
minimum 6–13
maximum 6–10 and 11–13

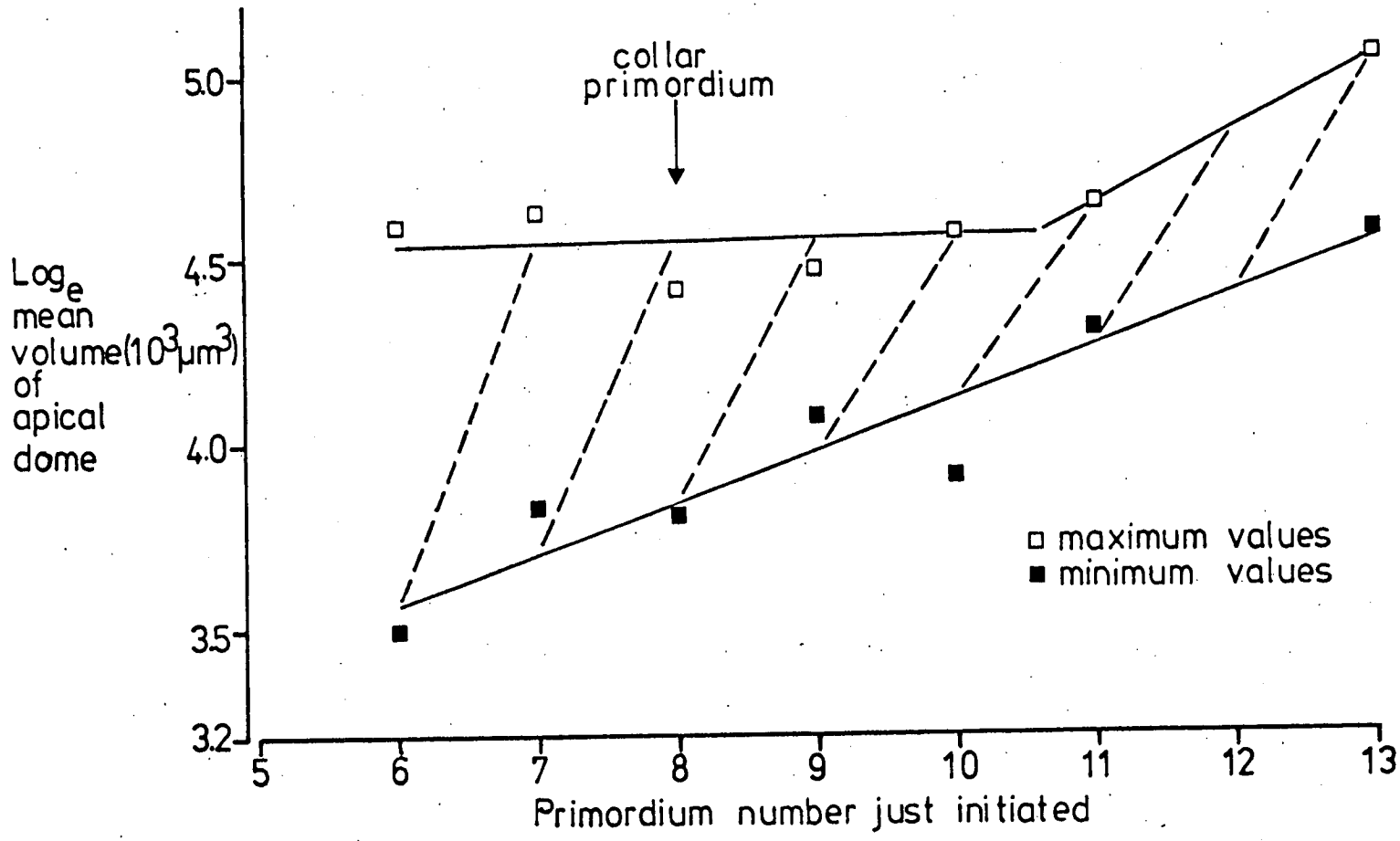


FIG 3.20 Natural logarithm of the mean minimum and mean maximum volume of the median section of the apical dome as a function of the primordium number just initiated in C.S.Hope 5D. The values used for the regression analyses are:
minimum 5-10 and 10-13
maximum 6-10 and 10-13

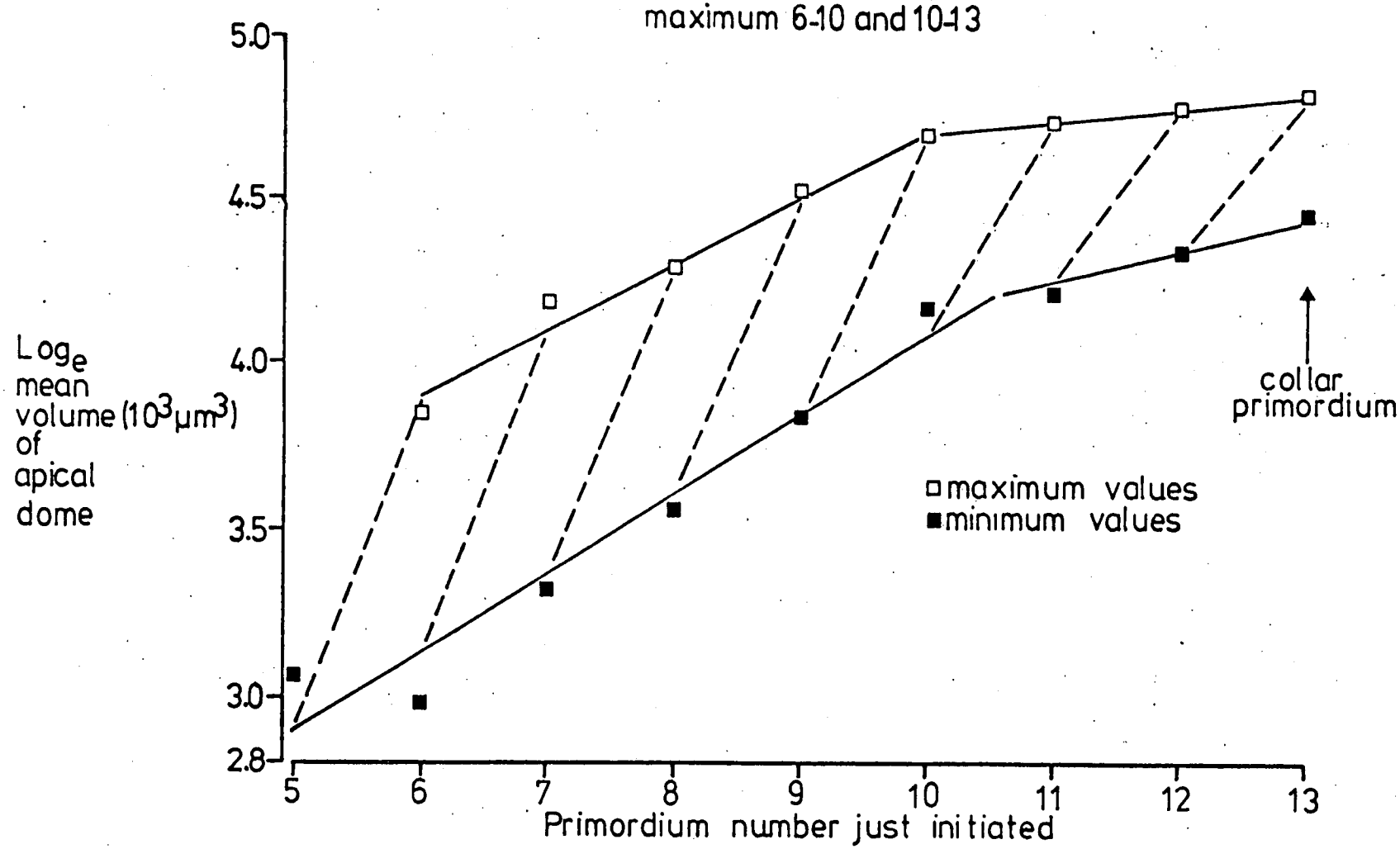
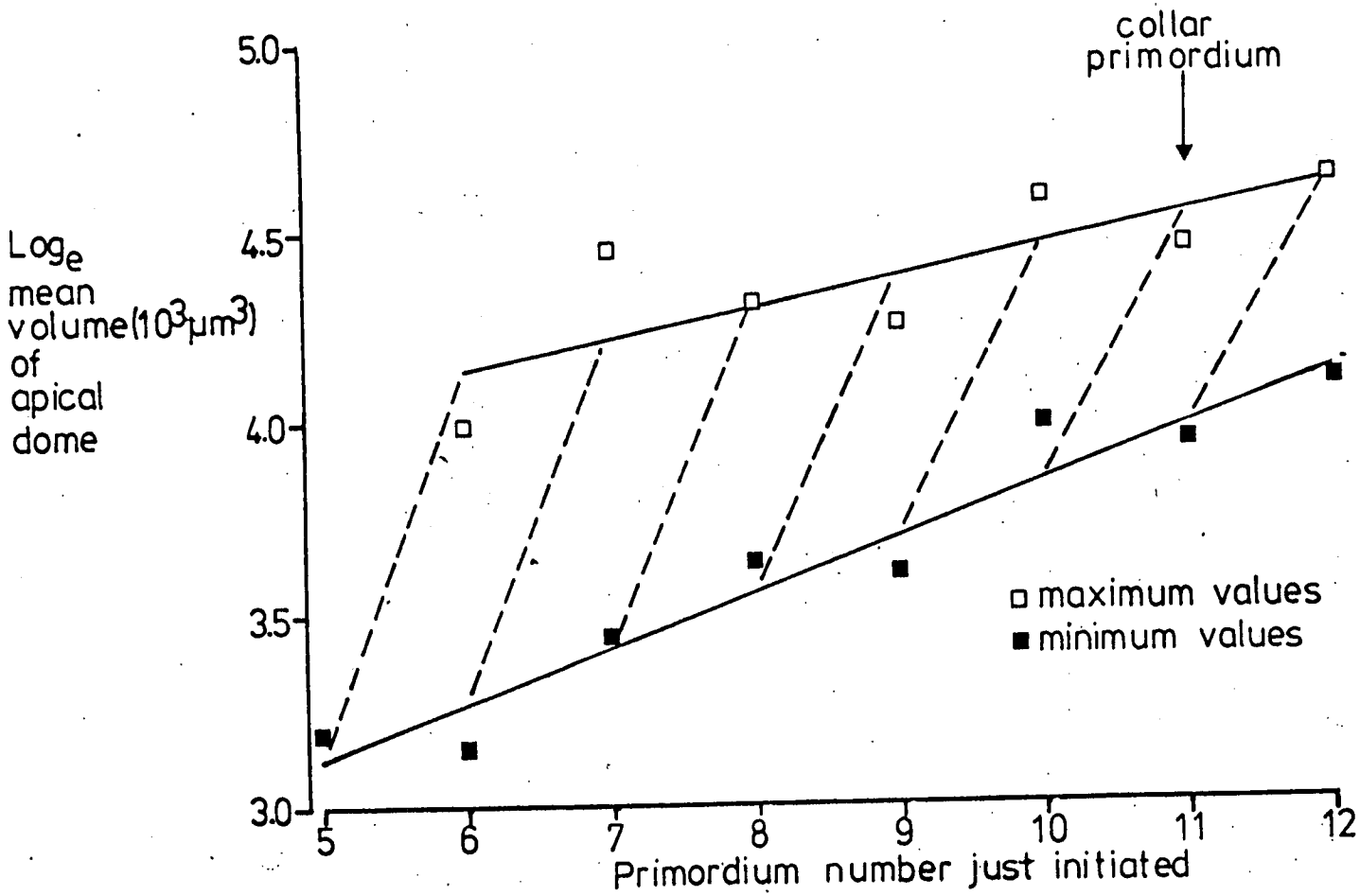


FIG 3.21 Natural logarithm of the mean minimum and mean maximum volume of the median section of the apical dome as a function of the primordium number just initiated in C.S.Euploid. The values used for the regression analyses are:

maximum 6-12
minimum 5-12



In Figs 3.18 - 3.21, this was obtained from the slope of the lines joining the mean minimum and mean maximum volumes, given by the regression lines, in a plastochron (for example, line AB-Fig 3.18). If the slope of these lines remained constant, the relative growth rate per plastochron was remaining constant. However if, the slope of the lines steepened, the relative growth rate per plastochron was increasing, and if the slope decreased, the relative growth rate per plastochron was decreasing. The volume relative growth rate per plast ochron for each cultivar is shown in Table 3.5.

In C.S. Hope 5A (Fig 3.18, Table 3.5) and vernalised C.S. Hope 5D (Fig 3.19, Table 3.5), the volume relative growth rate per plastochron tended to decrease very gradually down to the fifth plastochron after planting (plastochron 9 in C.S. Hope 5A and plastochron 10 in vernalised C.S. Hope 5D). The volume relative growth rate may then have increased slightly.

In unvernalised C.S. Hope 5D (Fig 3.20, Table 3.5) and C.S. Euploid (Fig 3.21, Table 3.5), the volume relative growth rate per plastochron decreased gradually down to plastochron 12 in C.S. Hope 5D and plastochron 11 in C.S. Euploid. The experiments were not continued far enough to determine the pattern after collar initiation.

The volume relative growth rate per day for each plastochron is shown in Table 3.6b (This is obtained by dividing the values for volume relative growth rate per plastochron -: Table 3.5, by the plastochron-: Table 3.6a).

In C.S. Hope 5A, since the plastochron was the same up to plastochron 12 (Table 3.6a), the relative growth rate per day followed the same pattern as the relative growth rate per plastochron up to that point, with the relative growth rate tending to decline up to plastochron 9 and then it may have increased. However, the length of the plastochron was reduced in plastochron 12, so the relative growth rate per day increased.

Table 3.5. Volume relative growth rate. plastochron^{-1} for each cultivar derived from FIGS 3.18-3.21.

Plastochron Number	Volume relative growth rate.plastochron^{-1}			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S.Euploid
5	0.78	*	1.00	1.01
6	0.75	0.98	0.96	0.95
7	0.73	0.85	0.93	0.88
8	--0.71--	--0.71--	0.90	0.82
9	0.69	0.58	0.86	0.76
10	0.73	0.52	0.65	0.71
11	0.90	0.59	0.53	--0.63--
12	0.63	0.66	0.47	-

The plastochron is the length of time between the initiation of the indicated primordium and the next primordium.

* Could not be calculated, the 5th primordium was initiated during vernalisation.

- Could not be calculated as there was insufficient data.

----indicates the plastochron in which the collar was initiated.

Table 3.6 (a) Plastochron, obtained from Table 3.2.
At the points where the rates of primordium initiation changed in C.S. Hope 5A and vernalised C.S. Hope 5D, the plastochron was readfrom FIGS 3.5b and 3.6b (it is the length of time between the points where 50% of the plants have (n) primordia and 50% have (n+1) primordia).

Cultivar	Plastochron number	Plastochron (days)
C.S. Hope 5A	5-11	1.89
	12	1.75
C.S. Hope 5D Vernalised	6	2.56
	7	1.50
	8-12	1.30
C.S. Hope 5D	5-12	2.38
C.S. Euploid	5-12	2.22

(b) Volume relative growth rate.day^{-1} for each cultivar.

Plastochron number	Volume relative growth rate day^{-1}			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S.Euploid
5	0.41	*	0.42	0.45
6	0.40	0.38	0.40	0.43
7	0.39	0.57	0.39	0.40
8	--0.38--	--0.55--	0.38	0.37
9	0.37	0.45	0.36	0.34
10	0.39	0.40	0.27	0.32
11	0.48	0.45	0.22	--0.28--
12	0.84	0.51	0.20	-
			- --	

* 5th primordium initiated during vernalisation
\- insufficient data available
--- indicates the plastochron in which the collar was initiated. The plastochron is the length of time between the initiation of the indicated primordium and the next primordium

In vernalised C.S. Hope 5D, the volume relative growth rate per day increased in plastochron 7 owing to the decrease in the length of the plastochron (Table 3.6a), then as with the relative growth rate per plastochron, the relative growth rate per day tended to decline down to plastochron 10 and then appeared to increase slightly. In C.S. Hope 5D and C.S. Euploid, the plastochron remained the same (Table 3.6a) and therefore, as with the relative growth rate per plastochron, the relative growth rate per day decreased gradually throughout the experiments.

In all cultivars (except vernalised C.S. Hope 5D from plastochron 6 to 7, see above) the relative growth rate per plastochron and per day tended to decrease up to collar formation. In C.S. Hope 5A and vernalised C.S. Hope 5D this decline in relative growth rate continued up to the fifth plastochron after planting and then the relative growth rate tended to increase, to a greater extent in C.S. Hope 5A than in the vernalised C.S. Hope 5D. It did not appear that therewas a particular value for the volume relative growth rate associated with collar formation. In the unvernalised C.S. Hope 5D and C.S. Euploid, the volume relative growth rate was lower at collar formation than in C.S. Hope 5A and vernalised C.S. Hope 5D.

The experiments were not continued long enough with C.S. Hope 5D and C.S. Euploid to determine whether the relative growth rate continued to decline after collar formation. However volume relative growth rate can be calculated by another method which requires fewer samples, and this was therefore used to calculate the volume relative growth rate for several apices sampled after collar formation in C.S. Hope 5D. The method assumes that in the course of a plastochron the apical dome grows by an amount which is equivalent to the volume of the youngest phytomer (Sunderland and Brown, 1956).

It gives only an approximate answer as it also assumes that the apex is growing in a steady state from one plastochron to the next. By this method,

Volume relative growth rate per plastochron $= \log_e$ volume $(a + b) - \log_e$ volume a

where a = volume of the apical dome in plastochron n

b = volume of phytomer (n)

both measured at any stage in plastochron n.

The volume relative growth rates calculated in this way are shown in Table 3.7. Relative growth rates were calculated for all cultivars for comparison with the values shown in Tables 3.5 and 3.6b.

In C.S. Hope 5D and C.S. Euploid, particularly, the values for relative growth rate per plastochron and per day were similar when calculated by the different methods. The relative growth rate per plastochron and per day decreased up to collar formation and then in C.S. Hope 5D appeared to stay about the same for the next 4 or 5 plastochrons(Table 3.7),before continuing to decline. In contrast, in C.S. Hope 5A, the relative growth rate per day decreased down to just past collar formation and then appeared to increase. In vernalised C.S. Hope 5D there was no indication in Table 3.7 of the increase in relative growth rate in the third or 4th plastochrons after collar formation seen in Tables 3.5 and 3.6b.

There are therefore differences in the trends in the volume relative growth rate of the apical dome which may be associated with the vernalisation requirement of the cultivars. In C.S. Hope 5A (Tables 3.5, 3.6b and 3.7), and to a lesser extent in vernalised C.S. Hope 5D) (Table 3.5, 3.6b), the decline in relative growth rate was arrested just after collar formation. However in C.S. Hope 5D (Table 3.7) the volume relative growth rate may have been stabilised for a few plastochrons after collar formation but then continued to decline.

Table 3.7 Volume relative growth rate calculated by the method of $\log_e$ volume (a+b) - $\log_e$ volume (a). Each value is a mean of values for 2-8 apices.

(a)

Plastochron number	Relative growth rate . plastochron^{-1}			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
5	1.13	*	0.99	1.03
6	0.86	0.99	0.92	0.86
7	0.76	0.95	0.87	0.78
8	--0.76--	--0.82--	0.80	0.74
9	0.70	0.69	0.67	0.65
10	0.56	0.70	0.68	0.66
11	0.38	0.62	0.67	--0.60--
12	0.32	-	0.50	0.62
13	0.33	0.45	--0.51--	-
14	0.27	-	0.40	-
15	-	-	0.50	-
16	-	-	0.47	-
18	-	-	0.53	-
22	-	-	0.39	-
23	-	-	0.31	-

(b)

Plastochron number	Relative growth rate . day^{-1}			
	C.S. Hope 5A	CS.Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
5	0.60	*	0.42	0.46
6	0.46	0.39	0.39	0.39
7	0.40	0.42	0.37	0.35
8	--0.40--	--0.63--	0.34	0.33
9	0.37	0.53	0.28	0.29
10	0.30	0.54	0.29	0.30
11	0.20	0.48	0.28	--0.27--
12	0.68	-	0.21	0.28
13	0.70	0.35	--0.15--	-
14	0.57	-	0.12	-
15	-	-	0.15	-
16	-	-	0.14	-
18	-	-	0.15	-
22	-	-	0.11	-
23	-	-	0.09	-

* The 5th primordium was formed during the vernalisation
\- Insufficient data available
---- Collar formation
Plastochron number is the number of primordia initiated by the apices used in the calculation

(e) Cell doubling times

(1) Cell doubling time calculated from the volume relative growth rate

The volume doubling time in each plastochron was calculated from the volume relative growth rate per day in each plastochron (Table 3.6b):

$$\text{volume doubling time (days)} = \frac{\log_e 2}{\text{Volume relative growth rate. day}^{-1}}$$

The cell doubling time would be the same as the volume doubling time if the mean cell volume remained constant throughout the experiments. However if the mean cell volume increased the cell doubling time would be longer than the volume doubling time, and if the mean cell volume decreased the cell doubling time would be shorter than the volume doubling time.

In C.S. Hope 5A and vernalised C.S. Hope 5D (Fig 3.14 a & b) the mean cell volume was more or less constant up to day 15 (whichcorresponded to plastochron 11 in C.S. Hope 5A (Fig 3.5b) and plastochron 12 in vernalised C.S. Hope 5D (Fig 3.6b))and then increased. Therefore the volume doubling times were adjusted in the later plastochrons to produce the cell doubling times.

The volume doubling time in plastochron 12 in C.S. Hope 5A was multiplied by 1.65/1.50, which was the mean cell volume in plastochron 12/mean cell volume in plastochron 11. In vernalised C.S. Hope 5D, volume relative growth rate.day $^{-1}$ was not available after plastochron 12 so no adjustment had to be made.

In C.S. Hope 5D (Fig 3.14c) and C.S. Euploid (Fig 3.14d) the mean cell volume was constant throughout the experiments, so the cell doubling time was the same as the volume doubling time.

The cell doubling times are shown in Table 3.8. The trend in the cell doubling time in each cultivar was, of course, necessarily opposite to the trend in the relative growth rate per day. If the relative growth rate decreased, the cell doubling time increased and vice-versa. In C.S. Hope 5A, the cell doubling time gradually increased up to plastochron 9 and then appeared to decrease. In vernalised C.S. Hope 5D, after an initial high doubling time, this decreased, then gradually increased up to plastochron 10 where it appeared to decrease again. In C.S. Hope 5D and C.S. Euploid, the cell doubling time gradually increased throughout the experiments.

In C.S. Hope 5A, (and possibly in vernalised C.S. Hope 5D) this increase in the rate of cell division in the later plastochrons can be related to the increase in volume relative growth rate of the apical dome (Tables 3.5, 3.6b, 3.7) and therefore to the increase in the rate of primordium initiation (Fig 3.5b & 3.6b). However in C.S. Hope 5D and C.S. Euploid, the rate of cell division decreased with time causing a decline in the relative growth rate but without decreasing the rate of primordium i nitiation at least up to collar formation (Figs 3.7c, 3.8c).

(11) Cell doubling time calculated from the colchicine method

This method, which was independent of the measurements of the volume of the apical dome, was used as a check of the values obtained for the cell doubling time shown in Table 3.8. The cell doubling time was measured on day 5 (which corresponded to plastochron 6 in all cultivars) and on day 17 (which corresponded to plastochron 14 in C.S. Hope 5A, plastochron 16 in vernalised C.S. Hope 5D, and plastochron 11 in C.S. Hope 5D and C.S. Euploid), in all the cultivars. These days were chosen primarily to determine whether there was a decrease in cell doubling time in C.S. Hope 5A and vernalised C.S. Hope 5D after plastochrons 11 or 12, and whether there was an increase in doubling times in C.S. Hope 5D and C.S. Euploid, as suggested by Table 3.8.

Table 3.8 Cell doubling time in each plastochron for all cultivars. These values were derived from data in Table 3.6b.

Plastochron number	Cell doubling time (days) C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
5	1.69	*	1.65	1.54
6	1.73	1.82	1.73	1.61
7	1.78	1.22	1.78	1.73
8	--1.82--	--1.26--	1.82	1.87
9	1.87	1.54	1.93	2.04
10	1.73	1.73	2.56	2.17
11	1.39	1.54	3.15	--2.48--
12	0.91	1.36	3.47	-
			- --	

* 5th primordium initiated during vernalisation.
\- insufficient data available.
--- indicates plastochron in which collar was initiated.

The plastochron is the length of time between the initiation of the indicated primordium and the next primordium.

The percentage of metaphase nuclei was plotted against the duration of the colchicine treatment (Figs 3.22-3.25). The graphs were all roughly S-shaped , implying that at first the colchicine had not penetrated fully to the apex, and in the later stages colchicine was inhibiting the entry of cells into mitosis. After 5 or 6 hours of colchicine treatment, anaphase and telophase nuclei were still observed but after 11 or 13 hours of colchicine treatment the anaphase and telophase nuclei had disappeared in all the cultivars (Figs 3.26). The percentage of prophase nuclei was scored at each sampling time and was found to be more or less constant throughout the experiments in all the cultivars (Fig 3.26). Therefore the colchicine did not appear to be inhibiting the entry of cells into prophase, but since the rate of metaphase accumulation declined towards the end of the experiments, there may have been a general inhibition of the cell cycle.

Therefore it appeared that the colchicine was able to penetrate to the apex at least after about 6 hours, but towards the end of the experiment it was having an in hibitory effect. The percentage increase in metaphases over a given period is equivalent to the rate of cell division. This was measured over the period of the graphs where the rate of increase in the percentage of metaphases was the most rapid. In all the graphs this was between 5 and 11, or 6 and 13 hours of colchicine treatment.

The cell doubling times were calculated from these rates as described in the methods (page 44), and are shown in Table 3.9. These cell doubling times could be compared to the cell doubling times shown in Table 3.8.

In C.S. Hope 5A and vernalised C.S. Hope 5D, there was a reduction in the cell doubling time after plastochron 11 compared to plast ochron 6, measured by either method. In C.S. Hope 5D and C.S. Euploid, the cell doubling time had increased in plastrochron 11 compared to plast ochron 6, when derived from the volume relative growth rate. When measured by the colchicine method the cell doubling time increased in C.S. Euploid but appeared to decrease in C.S. Hope 5D.

FIGS 3.22–3.25 The percentage of metaphase nuclei (with associated standard errors) as a function of duration of colchicine treatment. The plastochron is the length of time between the initiation of the indicated primordium and the next.

FIG 3.22 C.S.Hope 5A

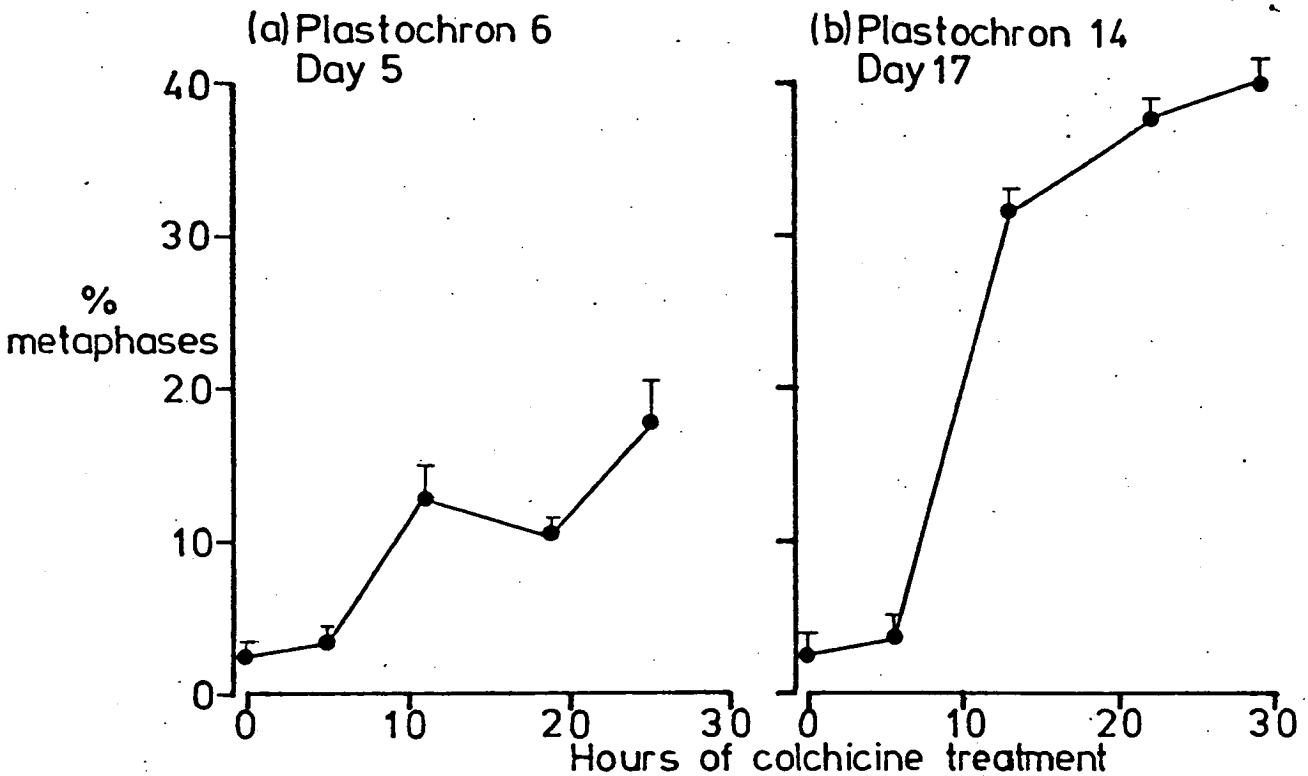


FIG 3.23 Vernalised C,S.Hope 5D

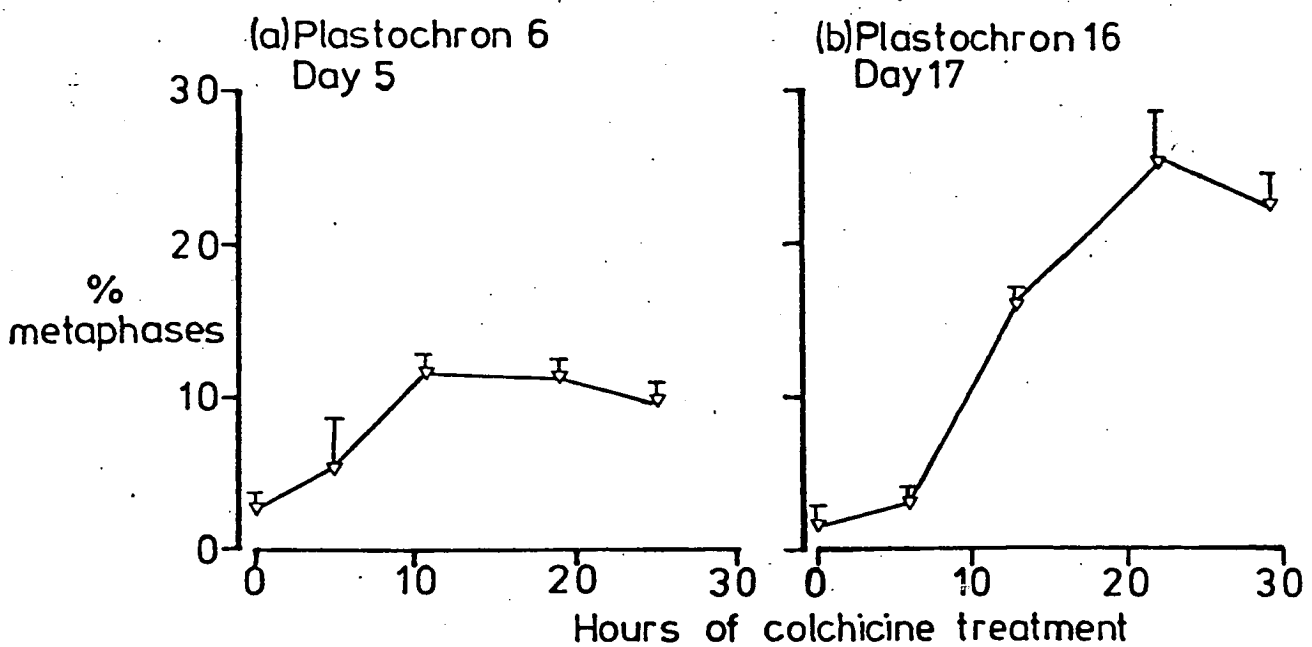


FIG 3.24 C.S.Hope 5D

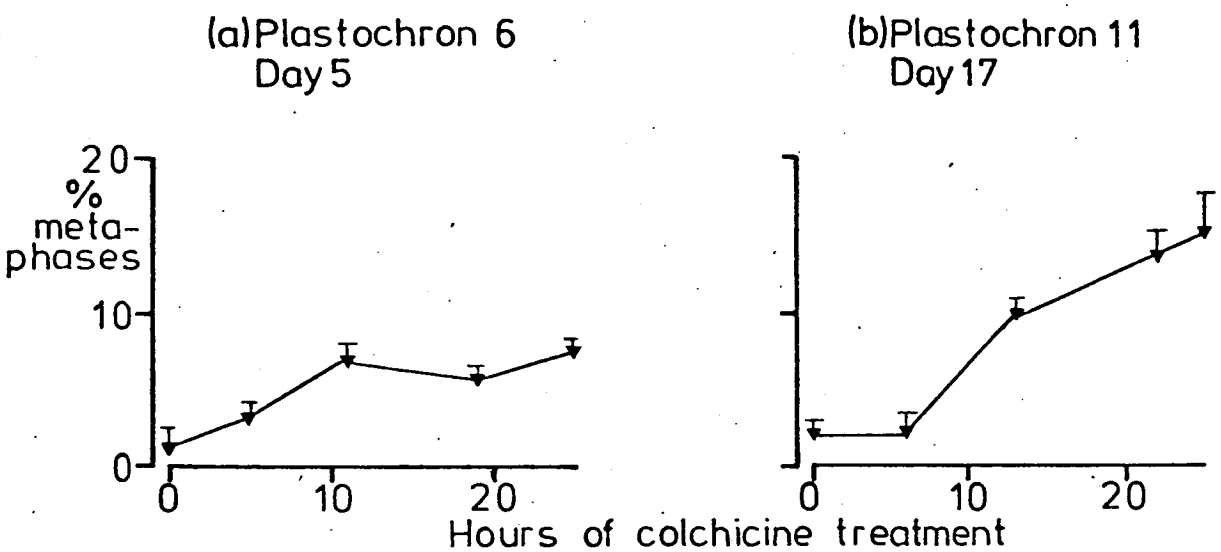


FIG 3.25 C.S.Euploid

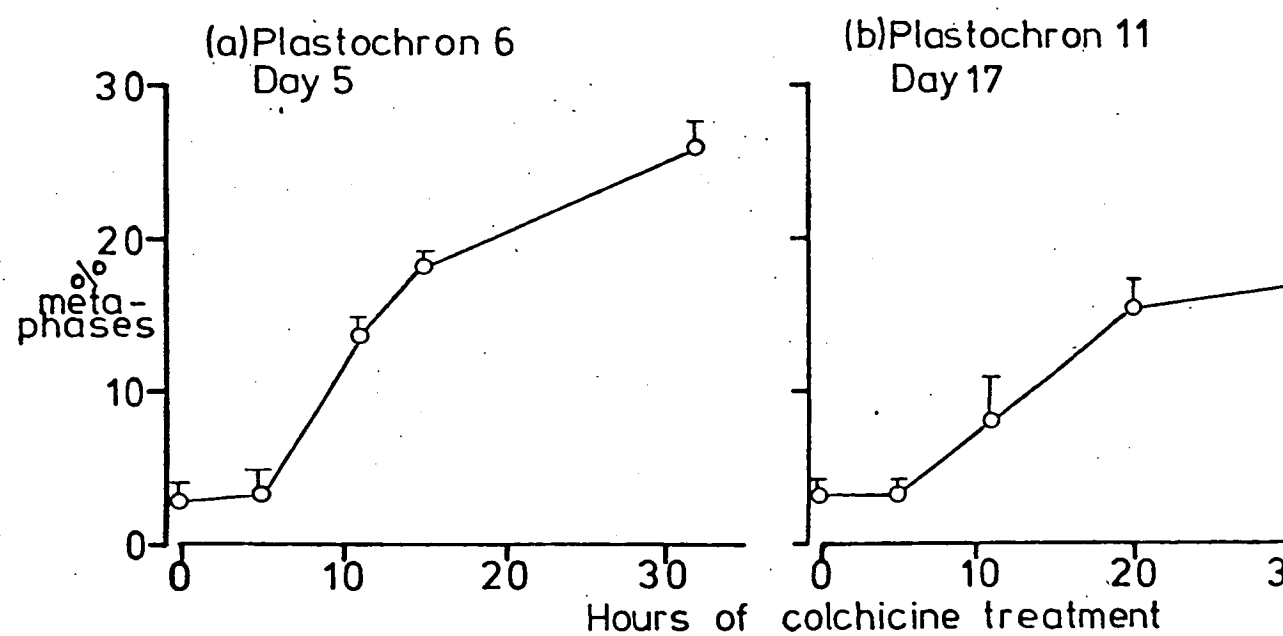


FIG 3.26. Percentage of prophase, and anaphase + telophase nuclei as a function of duration of colchicine treatment.

- ■ prophase nuclei
- □ anaphase + telophase nuclei

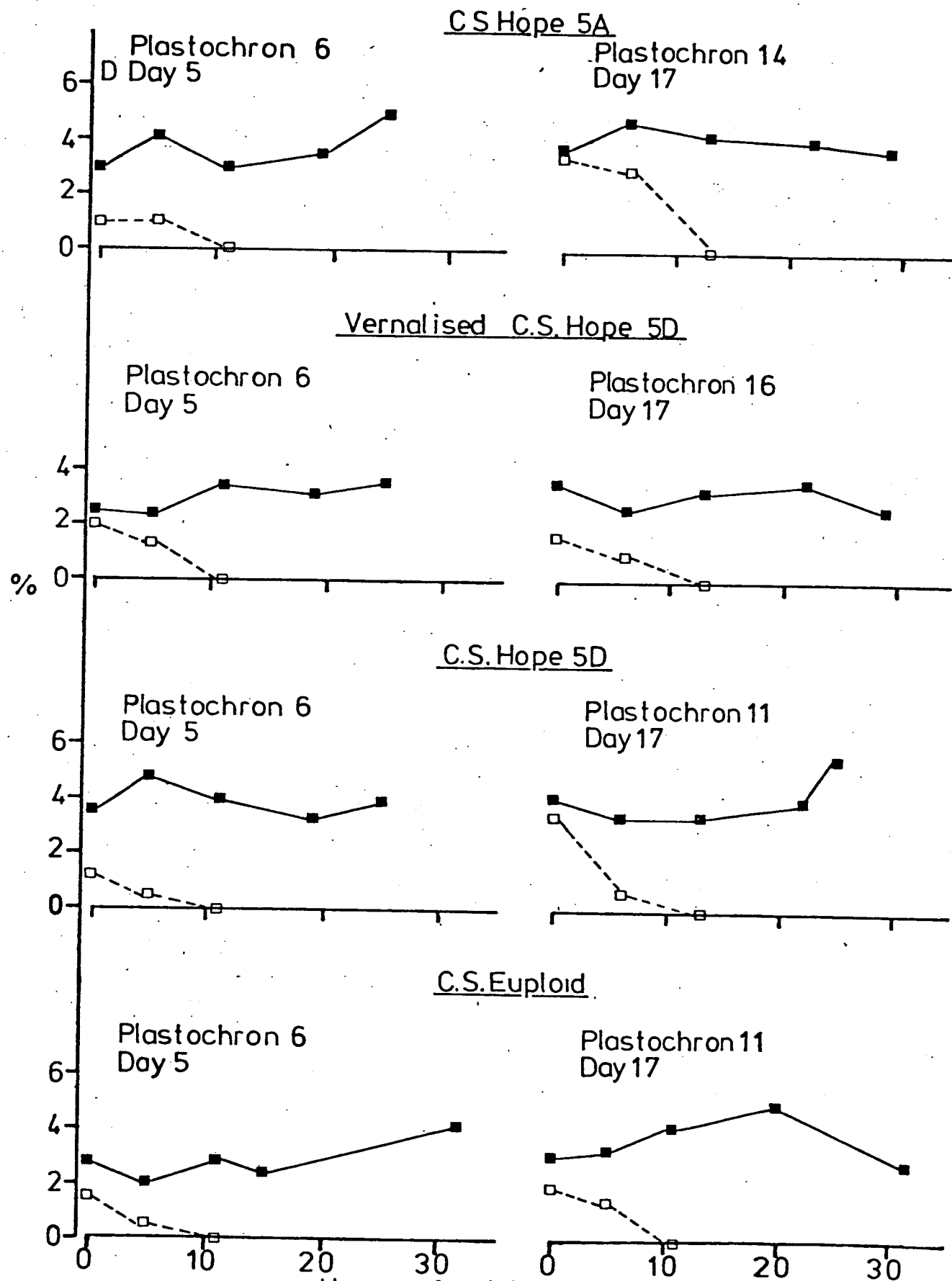


Table 3.9 Cell Doubling time in days as measured by the colchicine method

Cultivar	Plastochron in which plants were treated with colchicine	Rate of Metaphase accumulation % . hour^{-1}	Cell doubling time (days)
C.S. Hope 5A	6	1.70	1.70
	14	3.99	0.72
C.S. Hope 5D Vernalised	6	1.03	2.80
	16	1.86	1.55
C.S. Hope 5D	6	0.67	4.31
	11	1.11	2.60
C.S. Euploid	6	1.69	1.71
	11	0.77	3.75

The plastochron is the interval between the initiation of the indicated primordium and the next primordium.

Since the percentage of metaphases observed in plastochron 6 in C.S. Hope 5D (Fig 3.24a) was very low (never > 7.5%), the colchicine may have been having an inhibitory effect throughout the experiment giving a long cell doubling time.

Therefore although the values may differ when measured by the two methods, a similar trend in doubling time was observed. In all the cultivars (except for C.S. Hope 5D by the colchicine method) the doubling time was of the order of 15-28 days in the early plastochrons, about half this in the later plastochrons in C.S. Hope 5A and vernalised C.S. Hope 5D, and probably longer than 24 days in the later plastochrons in C.S. Hope 5D and C.S. Euploid.

4. Partitioning of the apical dome

The partitioning of the tissue of the apical dome at the end of a plastochron, between the apical dome and the phytomer at the start of the next plastochron, is important in understanding the way in which the volume of the apical dome is changing.

In Fig 3.18, the distance BC, which is the difference between the $\log_e$ volume of the apical dome at the end of plastochron 5 and the $\log_e$ volume of the apical dome at the beginning of plastochron 6, gives a measure of the proportion of the apical dome being cut off as a phytomer at the end of the plastochron. Therefore, if the lines for the $\log_e$ mean minimum and $\log_e$ mean maximum volume of the apical dome remain the same vertical distance apart throughout the experiment, the proportion of the apical dome being cut off as phytomer is the same at the end of each plastochron. If the lines converge the proportion being cut off as phytomer is decreasing and if the lines diverge the proportion being cut off as phytomer is increasing.

The proportion of the apical dome being cut off as a phytomer at the end of a plastochron can be calculated using the following formula:

$$P = \frac{Vmax(n) - Vmin(n+1)}{Vmax(n)}$$

$$= 1 - \exp \log_e Vmin(n+1) - \log_e VMax(n)$$

P = Proportion of apical dome being cut off as phytomer

Vmax(n) = Mean maximum volume of the apical dome at the end of plastochron (n)

Vmin(n+1) = Mean minimum volume of the apical dome at the start of plastochron (n+1)

The proportion cut off at the end of each plastochron is shown in Table 3.10a and the volume of each phytomer at its initiation Vmax(n) - Vmin(n+1) is shown in Table 3.10b.

In C.S. Hope 5A (Table 3.10), the proportion of the apical dome being cut off as a phytomer (P) at the end of the plastochron decreased very gradually down to plastochron 10 and then decreased to a greater extent in pastochron 11. The volume of the phytomer at initiation showed the opposite trend down to phytomer 11 in that it was tending to increase, and then decreased at phytomer 12. The volume of the phytomer at initiation was able to increase in volume despite the decrease in P because the mean volume of the apical dome increased (Fig 3.10a). In plastochron 12, P approximately halved and therefore the volume of the phytomer at initiation decreased even though the mean volume of the apical dome continued to increase.

In vernalised C.S. Hope 5D (Table 3.10), P decreased gradually down to plastochron 10 and then may have increased in plastochrons 11 and 12. The volume of the phytomer at initiation also decreased down to phytomer 11 and then increased. Therefore in this case the volume of the phytomer at initiation changed in the same way as P. The increase in both these parameters at the end of plastochron 11 was due to the large increase in the mean volume of the apical dome at this point (Fig 3.10b).

In C.S. Hope 5D (Table 3.10), P decreased throughout the experiment, while the volume of the phytomer at initiation increased up to phytomer 10 and then tended to decrease. The volume of the phytomer was able to increase even though P decreased due to the increase in the mean volume of the apical dome (Fig 3.11a). However in the later plastochrons P must have become sufficiently small that the volume of the phytomer decreased.

Table 3.10.(a) Proportion of the apical dome cut off as a phytomer at the end of each plastochron (P). Derived from data from FIGS 3.18-3.21.

Plastochron Number	Proportion of the median section of the apical dome cut off as a phytomer at the end of the plastochron(P)			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
5	0.47	0.63	0.53	0.58
6	0.47	0.58	0.52	0.55
7	0.46	0.51	0.50	0.52
8	--0.44--	--0.43--	0.48	0.49
9	0.43	0.36	0.46	0.46
10	0.38	0.32	0.38	0.43
11	0.18	0.36	0.35	--0.39--
12	-	0.41	0.32	-

(b) Volume ($10^3 \mu m^3$) of the phytomer at its initiation also derived from FIGS 3.18-3.21.

Phytomer number	Volume of the median section of the phytomer at initiation			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Euploid
6	33.14	57.60	26.30	36.22
7	36.63	53.86	31.26	37.46
8	--39.88--	--48.67--	36.73	38.53
9	42.91	41.11	43.49	39.39
10	47.29	34.02	50.80	39.50
11	48.70	32.73	43.19	--40.17--
12	34.60	46.29	41.21	39.31
13	-	63.90	--38.80--	-

- insufficient data available

--- plastochron in which the collar was initiated or phytomer which would become the collar.

In C.S. Euploid (Table 3.10), P decreased gradually throughout the experiment but the volume of the phytomer remained more or less constant. The volume of the phytomer was able to remain the same even through P decreased because the mean volume of the apical dome increased (Fig 3.11b).

To determine whether the conclusions being drawn regarding the volume of the phytomer, from these derived data, were correct, the volume of the phytomer was plotted against phytomer number (Fig 3.27), and the lower limit of these values (taken as the volume at initiation) was considered. In C.S. Hope 5A (Fig 3.27a), the volume of the phytomer increased gradually down to phytomer 9 and then tended to decrease. In vernalised C.S. Hope 5D (Fig 3.27b), the volume decreased down to phytomer 9 and then may have increased again. In C.S. Hope 5D (Fig 3.27c), the volume of the phytomer increased and then tended to decrease at phytomer 11. In C.S. Euploid (Fig 3.27d), the volume of the phytomer remained more or less constant. The trends observed in these data are therefore similar to those in Table 3.10b.

It appears that the change in volume of the apical dome may be brought about rather differently in the various cultivars. In C.S. Hope 5D and C.S. Euploid the volume relative growth rate declined up to collar formation and therefore the volume of the apical dome increased because P decreased. However in C.S. Hope 5A, in the first plastochrons, P and the relative growth rate changed very little and therefore some other factor must have been important in the increase in volume of the apical dome. However in the later plastochrons, the relative growth rate increased and P decreased leading to the large increase in the volume of the apical dome. In vernalised C.S. Hope 5D, P tended to decrease and al the relative growth rate declined a little over the first plastochrons, the volume of the apical dome increased. In the later plastochrons the relative growth rate may have increased with little change in P. The factors responsible for the increase in volume of the apical dome will be discussed more fully in the next section.

FIG 3.27 Volume of the median section of the phytome
as a function of phytomer number.

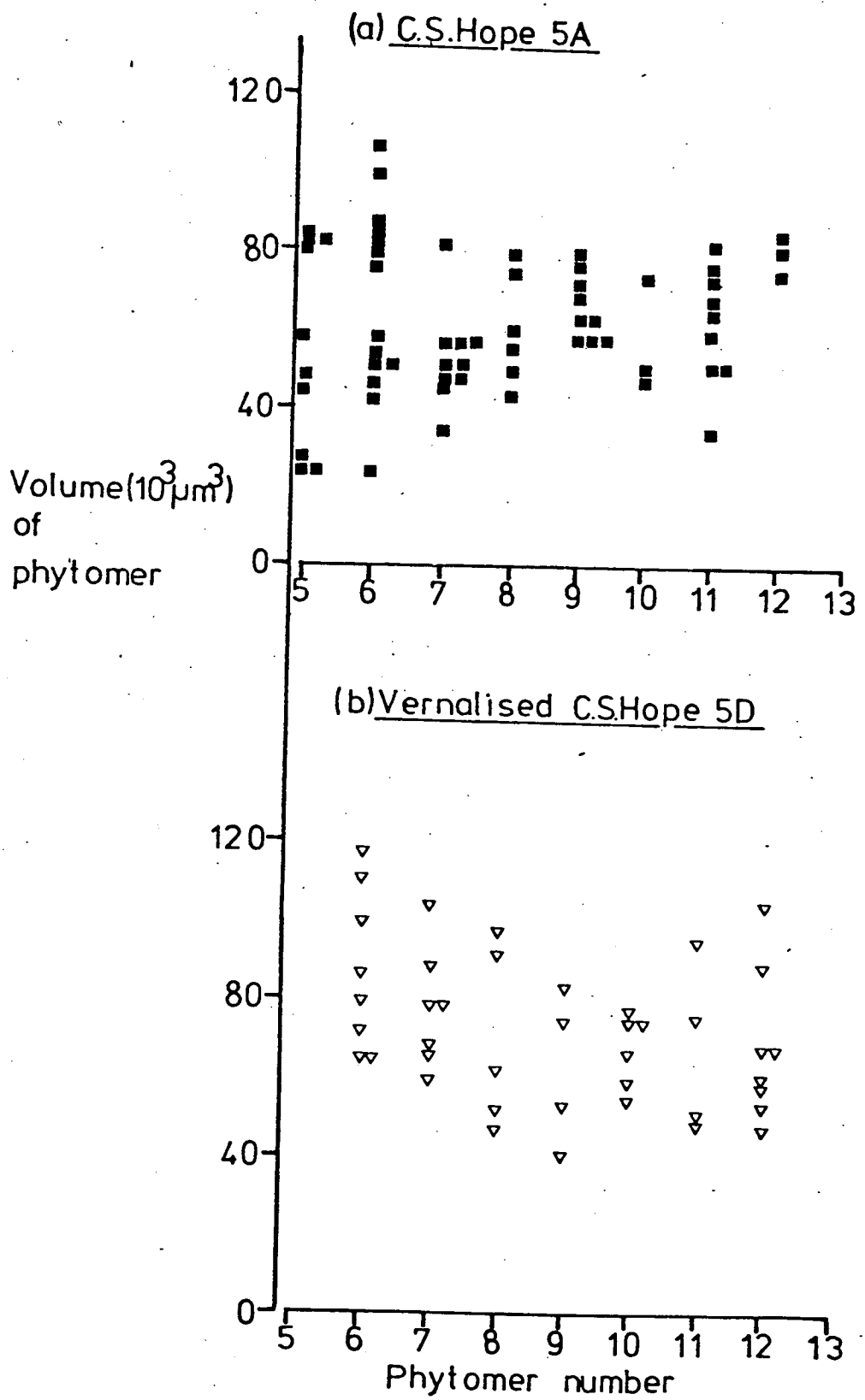
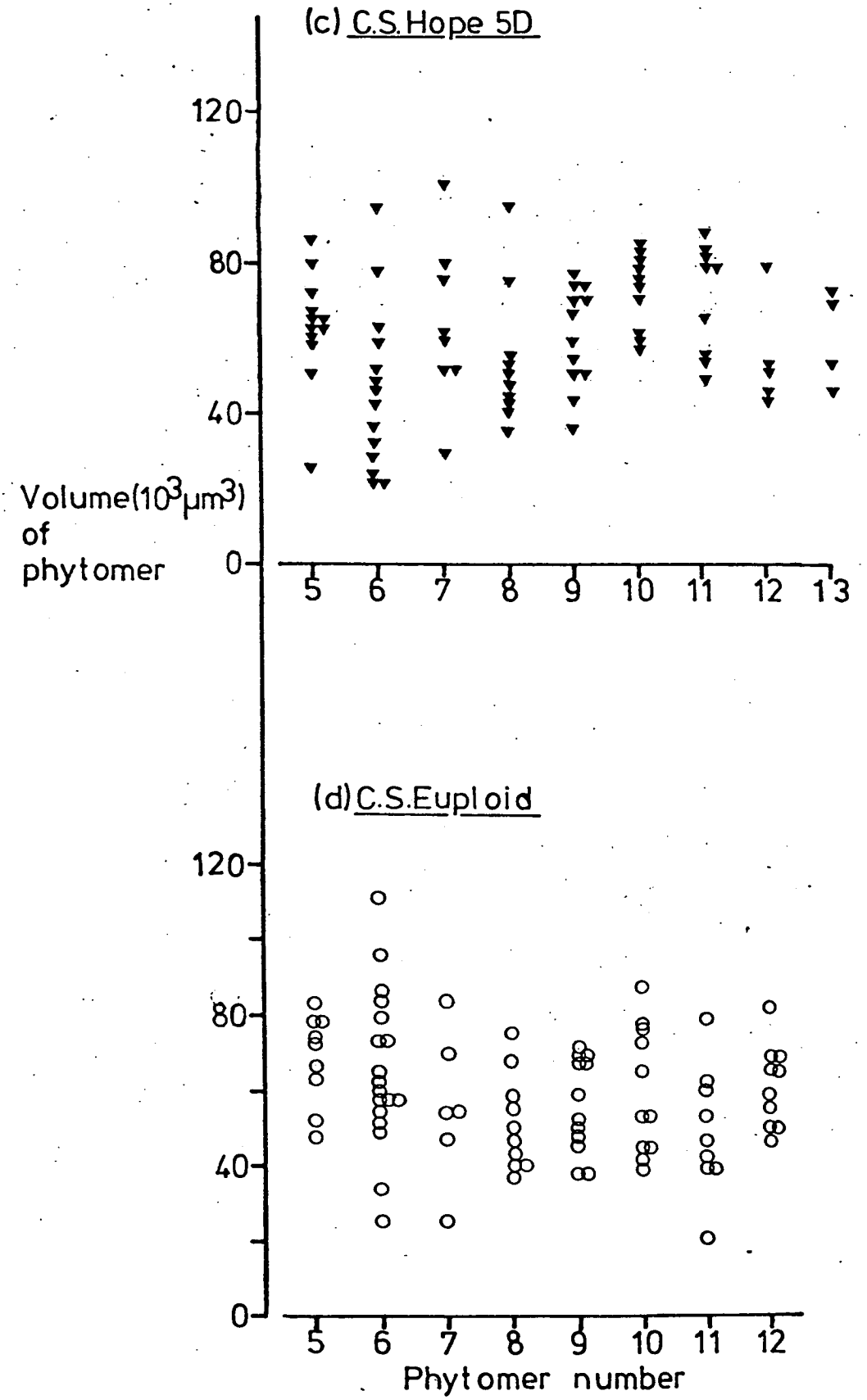


FIG 3.27


(c) C.S. Hope 5D
120
80
40
0
Volume($10^3 \mu m^3$) of phytomer
5 6 7 8 9 10 11 12 13
(d) C.S. Euploid
120
80
40
0
5 6 7 8 9 10 11 12
Phytomer number

Summary of the way in which the mean volume of the apical dome is changing

The factors which should be considered in understanding the growth of the shoot apex are.

(1) The rate of primordium initiation and hence the plastochron (Figs 3.5 - 3.8, Table 3.2)

(11) The change in volume of the apical dome (Figs 3.10-3.11, 3.18-3.21)

(111) The volume relative growth rate per plastochron-R_p (Figs 3.18-3.21, Table 3.5)

(1v) The proportion of the apical dome cut off as phytomer (P) at the end of a plastochron, and the volume of the phytomer at initiation (Figs 3.18 - 3.21, Table 3.10)

In C.S. Hope 5A, up to plastochron 9, the mean volume of the apical dome was increasing (Fig 3.18) but R_p changed very little (Table 3.5). P did not decrease to any marked extent over this period (Table 3.10) and so was not responsible for the increase in the mean volume of the apical dome. Therefore as none of the above factors could explain the increase in volume of the apical dome, some other explanation was needed (Lyndon, 1977). In Fig 3.18, the distance BC represents the $\log_e$ multiple (F) by which the apical dome must increase in plastochron 6 to restore it to the same size as it was at the end of plastochron 5. F is related to P as follows:

$$P = 1 - \frac{1}{\exp F}$$

If $R_p = F$, the size of the apical dome would remain constant from plastochron to plastochron. However in Fig 3.18, R_p was greater than F, that is, the apical dome had grown by a greater amount than was necessary to restore the volume of the apical dome to the same maximum as it had in the preceding plastochron. Consequently, the mean volume of the apical dome increased (Fig 3.18) and therefore it was possible for the volume of the phytomer at initiation to increase.

After plastochron 9 in C.S. Hope 5A, R_p tended to increase (Table 3.5) due to an increase in the volume relative growth rate per day (R_D) (not because of an increase in the plastochron - this remained the same and then shortened), and P decreased to a considerable extent in plastochron 12 as did the volume of the phytomer at initiation (Table 3.10). Therefore, in C.S. Hope 5A, in the later plastochrons, although R_p was still greater then F, the large increase in the mean volume of the apical dome was observed (Fig 3.18) because the volume relative growth rate increased and P decreased.

In vernalised C.S. Hope 5D, up to plastochron 10, R_p tended to decrease (Table 3.5). However P and the volume of the phytomer at initiation decreased (Table 3.10) over these plastochrons, and R_p was greater than F, so the mean volume of the apical dome increased (Fig 3.19). In plastochrons 11 and 12, R_p may have increased (Table 3.5) due to a slight increase in R_D and R_p was still greater than F. Therefore although P and the volume of the phytomer at initiation may have increased (Table 3.10) the mean volume of the apical dome could still increase, and to a greater extent than in the earlier plastochrons because of the increase in R_p.

In unvernalised C.S. Hope 5D, R_p tended to decline right up to collar formation (Table 3.5) due to a decline in R_D. However P decreased and R_p was greater than F, so the mean volume of the apical dome increased (Fig 3.20). This allowed the volume of the phytomer at initiation to increase, even though P decreased, at least up to plastochron 10 (Table 3.10). In table 3.7 it appeared that the relative growth rate remained about the same after collar formation, and the mean volume of the apical dome continued to increase at about the same rate (Fig 3.11a). It was therefore likely that R_p would continue to be greater than F to allow the apical dome to increase in volume.

In C.S. Euploid, R_p decreased up to collar formation (Table 3.5) However P decreased, the volume of the phytomer at initiation remained about the same (Table 3.10) and R_p was greater than F, so the mean volume of the apical dome increased (Fig 3.21).

Table 3.11 Summary of the factors responsible for bringing about the increase in the mean volume of the apical dome in the different cultivars.

Cultivar	C.S.Hope 5A		C.S. Hope 5D Vernalised		C.S. Hope 5D	C.S. Euploid
Plastochrons	5-9	10-12	6-10	11-12	5-12	5-11
Increase in Rp	-	✓	-	✓	-	-
Decrease in P	-	✓	✓	-	✓	✓
Rp F	✓	✓	✓	✓	✓	✓

- Factor not involved in increase in volume of apical dome

✓ Factor involved in increase in volume of apical dome

The ways in which the change in mean volume of the apical dome is brought about are summarised in Table 3.11. $R_p > F$ in all the cultivars at all the stages. They was only an increase in R_p in C.S. Hope 5A and to a lesser extent in vernalised C.S. Hope 5D in the later plastochrons. In C.S. Hope 5D and C.S. Euploid up to plastochrons 12 and 11 respectively, and in vernalised C.S. Hope 5D up to plastochron 11, the important factors for the increase in volume of the apical dome were a decrease in P but R_p remaining >F. However in C.S. Hope 5A up to plastochron 10, it was only $R_p > F$ which was important.

C. Cellular parameters associated with the transition to flowering

1. Cell Cycle

(a) Synchronisation of cell division

Experiments were carried out to determine whether there was any synchronisation of cell division in the apical dome, at any stage of development, and particularly associated with the transition to flowering.

A preliminary experiment was carried out, sampling daily (except at the beginning) over the first 21 days after planting in C.S. Hope 5A. The amount of DNA per nucleus was measured, by microdensitometry, in Feulgen-stained squash preparations of the apical domes, and the number of nuclei was plotted as a function of nuclear DNA content (Fig 3.28). The proportions of nuclei in the G_1 and G_2 phases of the cell cycle were obtained from these diagrams (as described in the methods page), and the ratio of G_2 to G_1 nuclei was calculated (Table 3.12). The stages in development at which synchronisation of cell division might be anticipated were just after planting (day 1), collar formation (day 9) and double ridge formation (day 17) (Fig 3.5b) Table 3.12 shows that there was a large change ($\pm$100%) in the ratio G_2/G_1, between days 1 and 5, days 8 and 9, and days 15 and 16/17, which supported the idea that synchronisation of cell division might be occurring at these stages. Therefore intensive sampling was carried out on days 1, 8, and 16 to determine whether synchronisation of cell division was occurring.

FIG 328 Nuclear DNA contents of interphase nuclei in squash preparations of the apical dome in C.S. Hope 5A. N = number of nuclei measured.

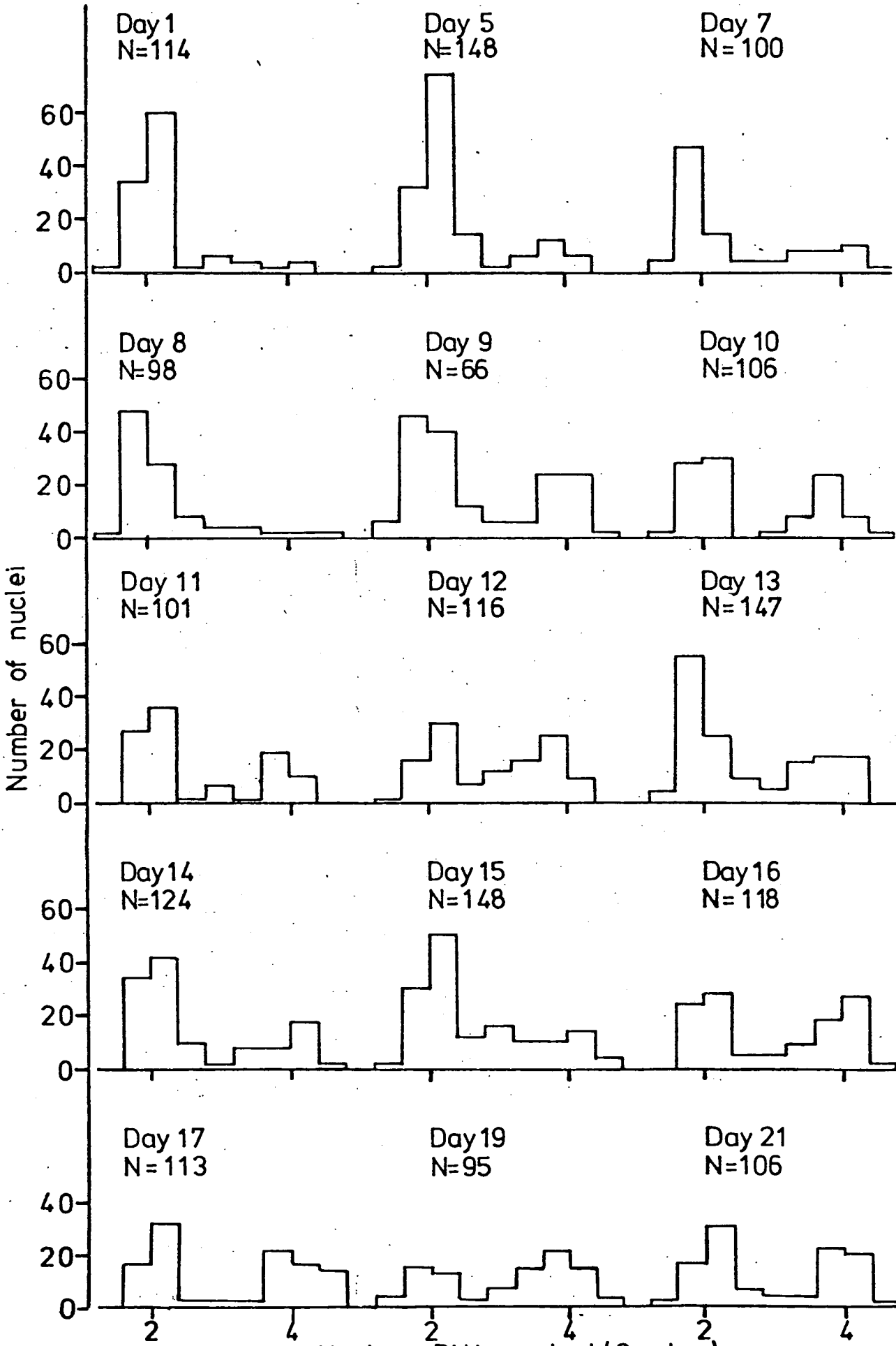


Table 3.12. The proportions of nuclei in the G_1 and G_2 phases of the cell cycle and the ratio of G_2 to G_1 nuclei in squash preparations of the apical dome of C.S. Hope 5A.

Day after planting	G_1	G_2	$\frac{G_2}{G_1}$
1	0.89	0.11	0.12
5	0.83	0.17	0.21
7	0.70	0.30	0.43
8	0.90	0.11	0.11
9	0.65	0.35	0.54
10	0.58	0.42	0.72
11	0.66	0.34	0.52
12	0.52	0.48	0.92
13	0.35	0.35	0.54
14	0.70	0.30	0.43
15	0.69	0.31	0.45
16	0.50	0.50	1.00
17	0.48	0.52	1.08
19	0.41	0.59	1.44
21	0.53	0.47	0.89

Samples were taken at 8 hour intervals, for the approximate length of the cell cycle (see Table 3.8), starting at 0800 hours on days 1, 8, and 16. The amount of DNA per nucleus was measured in squash preparations of the apical domes, and the number of nuclei was plotted against nuclear DNA content (Fig 3.29). The proportions of nuclei in G_1 and G_2 and the ratio G_2/G_1, are shown in Table 3.13. The values did vary from one sampling time to another, but if synchronisation of cell division was occurring, any peak in the ratio G_2/G_1, would be followed by a corresponding trough in the ratio. Since there were no clear changes in the ratio in a wave-like pattern, it appeared that there was no synchronisation of cell division in the apical dome just after planting, or at collar or double ridge formation.

(b) Length of the different phases of the cell cycle

Assuming that the cells of the apical dome were dividing asynchronously, the length of the different phases of the cell cycle could be calculated, if the length of the cell cycle and the proportion of cells in G_1, G_2, S and mitosis were known. Owing to the age gradient of the population (for each cell passing through G_2, there will be 2 cells entering G_1), the proportion of cells in any particular phase is not necessarily equal to the proportion of the cell cycle spent in that phase. Therefore the formulae of Nachtwey and Cameron (1968), which take into account this age gradient (assuming it is exponential), were used to calculate the length of the phases of the cell cycle.

It was explained in the methods (page 47) that the number of nuclei in S could not be accurately determined by microdensitometry alone so were partitioned equally to the 2C and 4C populations. Therefore the proportions of G_1 and G_2 nuclei in Tables 3.12 and 3.13 represent $G_1 + \frac{1}{2}S$ and $G_2 + \frac{1}{2}S$. A method which can be used to determine the number of nuclei in S is an ^{3}H-thymidine labelling technique (Mak, 1965), where the radio-chemical is applied to the shoot apex for about two hours, the apical dome is stained, squashed and autoradiographs are made. The number of labelled nuclei can then be counted, and this labelling index gives the proportion of nuclei in the S phase of the cell cycle.

FIG 3.29 Nuclear DNA contents of interphase nuclei in squash
arations of the apical dome in C.S.Hope 5A . N = numbe
of nuclei measured.

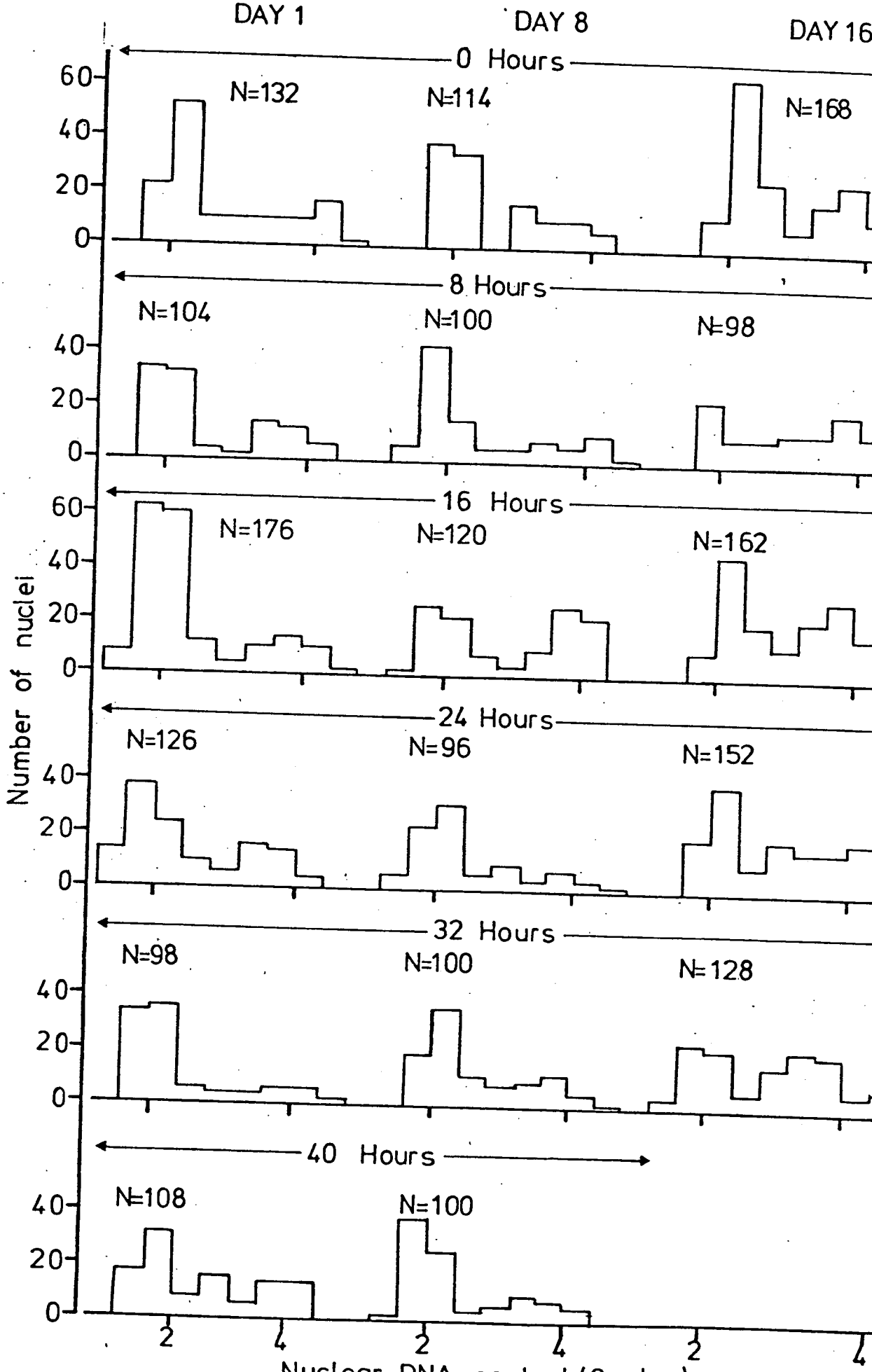


Table 3.13. The proportions of nuclei in the G_1 and G_2 phases of the cell cycle and the ratio of G_2 to G_1 nuclei in squash preparations of the apical dome of C.S. Hope 5A, **at various sampling times on days 1, 8 and 16 after planting.**

	Hours after start of sampling	G_1	G_2	G_2/G_1
Day 1	0	0.67	0.33	0.49
	8	0.68	0.32	0.47
	16	0.78	0.22	0.28
	24	0.71	0.29	0.41
	32	0.80	0.20	0.25
	40	0.61	0.39	0.64
Day 8	0	0.70	0.30	0.43
	8	0.71	0.29	0.41
	16	0.50	0.50	1.00
	24	0.76	0.24	0.32
	32	0.69	0.31	0.45
	40	0.73	0.27	0.37
Day 16	0	0.63	0.37	0.59
	8	0.51	0.49	0.96
	16	0.51	0.49	0.96
	24	0.53	0.47	0.89
	32	0.50	0.50	1.00

A preliminary labelling experiment was carried out with C.S. Euploid at the vegetative stage of development and a labelling index of 10.6% was obtained. Since the C amount of DNA in the apical dome was similar in all cultivars, the labelling index would probably be comparable in C.S. Hope 5A.

The mitotic index in the apical dome in C.S. Hope 5A was measured in median longitudinal sections of the apical domes. The number of prophase, metaphase, anaphase and telophase nuclei was counted, at intervals up to 16 days after planting, and expressed collectively as a percentage of the total number of cells (Fig 3.30). The mitotic index appeared to remain more or less constant over the period of the experiment and therefore a mean value of 5.4% was calculated for C.S. Hope 5A. Having calculated the mitotic index, the percentage of cells in $G_1 + \frac{1}{2}S$ could be calculated, using a mean of all the proportions of $G_1 + \frac{1}{2}S$, and $G_2 + \frac{1}{2}S$ for each day. The length of the cell cycle had been calculated from apical volume data (Table 3.8) and these values were used for the calculations. Day 1 corresponded to plastochron 4 (Fig 3.5b), and as there was no cell cycle time available for this plastochron, the value for plastochron 5 was used. Day 8 corresponded to plastochron 7 and day 16 to the beginning of plastochron 12 (Fig 3.5b). The length of the different phases of the cell cycle is shown in Fig 3.31.

On all days mitosis occupied a small proportion of the cell cycle. On days 1 and 8 the proportion of the cell cycle occupied by $G_1 + \frac{1}{2}S$ and $G_2 + \frac{1}{2}S$ was similar, with $G_1 + \frac{1}{2}S$ being longer than $G_2 + \frac{1}{2}S$ on both days. On day 16 the length of the cell cycle was approximately half the length on days 1 and 8. This could be brought about by a shortening of all the phases of the cell, or of only one phase, with the others remaining the same length. The histograms (Fig 3.31) show that while M approximately halved on day 16 and $G_2 + \frac{1}{2}S$ was reduced slightly, $G_1 + \frac{1}{2}S$ was reduced approximately 60% compared to days 1 and 8, and therefore was mainly responsible for the reduction in the length of the cell cycle.

/

FIG 3.30 Mitotic index in the median section of the apical dome as a function of days after planting in C.S Hope 5 Standard errors are shown.

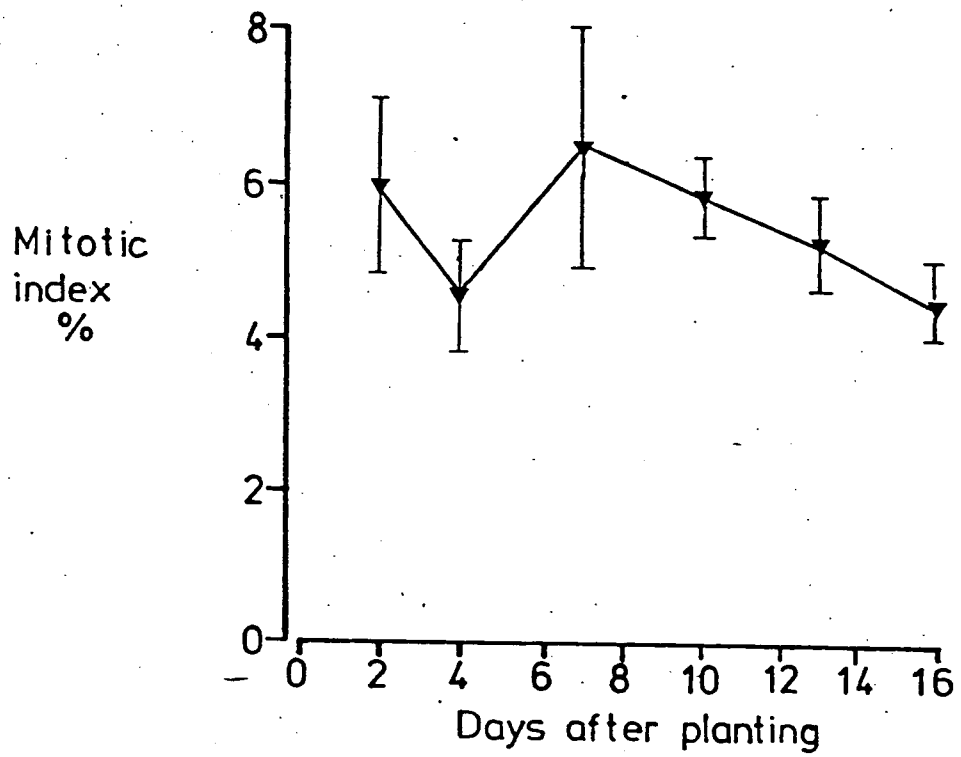
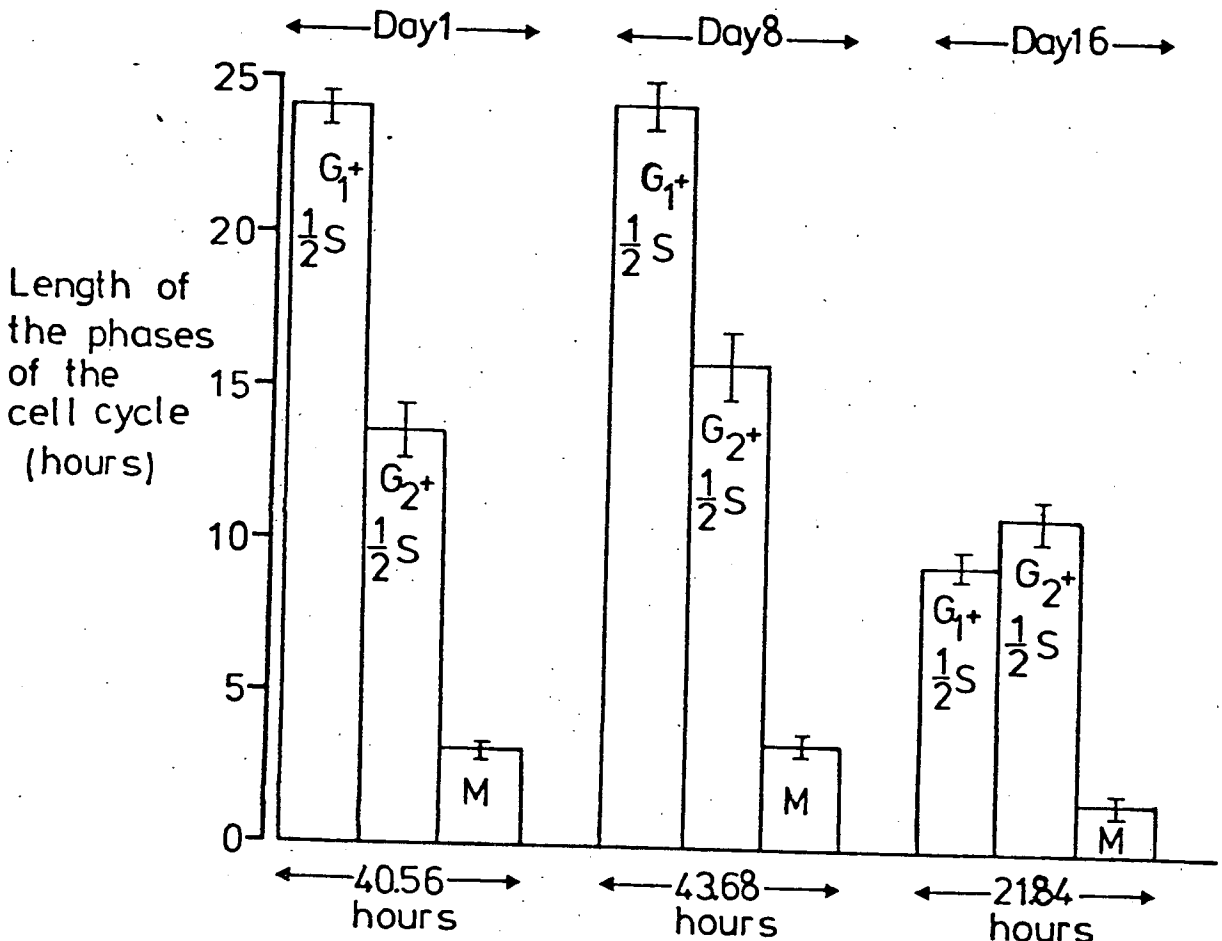


FIG 3.31 Length of the different phases of the cell cycle (with standard errors) on days 1, 8, and 16 after planting in C S Hope 5A.



	Day1	Day8	Day16
$G_1 + \frac{1}{2}S$	24.00 h	24.48h	9.36h
$G_2 + \frac{1}{2}S$	13.44h	15.84h	10.80h
M	3.12h	3.36h	1.68h

2. Nucleic Acids

Microdensitometric measurements were carried out to determine whether there were any changes in the amount of RNA or DNA in the apical dome associated with the transition to flowering in any of the cultivars.

The total nucleic acid and DNA absorbance were measured, in gallocyanin-stained sections, in an area of 5675 microns^2 of the apical dome. The readings were converted to a C basis and were plotted against days after planting (Figs 3.32 and 3.33).

The C amount of DNA in the apical dome (Figs 3.32 and 3.33) remained more or less constant throughout the experiments in all the cultivars. Some Feulgen-stained sections were measured for DNA absorbance in an area of 5675 microns^2 in the apical dome, on days 4, 8, 12, 16 and 20 after planting, and the mean DNA C amount for each cultivar was calculated. The C amount of DNA measured by the gallocyanin and Feulgen methods could then be compared (Table 3.14). In all the cultivars the C amount of DNA measured by the Feulgen method was within $\pm$ 8% of the DNA C amount measured by the gallocyanin method. This indicated that both methods gave an accurate measure of DNA content, giving a mean DNA C amount of 81.11 $\pm$ 0.88 for all the cultivars (both methods).

The C amount of RNA was the difference between the C amount of total nucleic acid and the C amount of DNA for each day (from Figs 3.32 and 3.33). The C amount of RNA in 5675 microns^2 of the apical dome could be converted into the C amount per cell since the mean cell volume was known for each day (Fig 3.14).

$$\text{C amount of RNA per cell} = \frac{\text{C amount of RNA in 5675 um}^2}{\text{number of cells in 5675 um}^2}$$

The number of cells was calculated by dividing the volume of the apical dome measured (56750 um^3 - since thickness of sections was 10 um) by the mean cell volume. A mean of the cell volumes had been calculated for the first 10 days after planting in all the cultivars (Table 3.3a) and so was used for these calculations, but after day 10 the mean cell volume for each day was used.

FIGS 3.32 and 3.33 The C amount of total nucleic acid and DNA in an area of 5675 μm^2 of the apical dome (with standard errors) as a function of days after planting. ▼NA=Total nucleic acid ▽DNA=Deoxribonucleic acid only

FIG 3.32

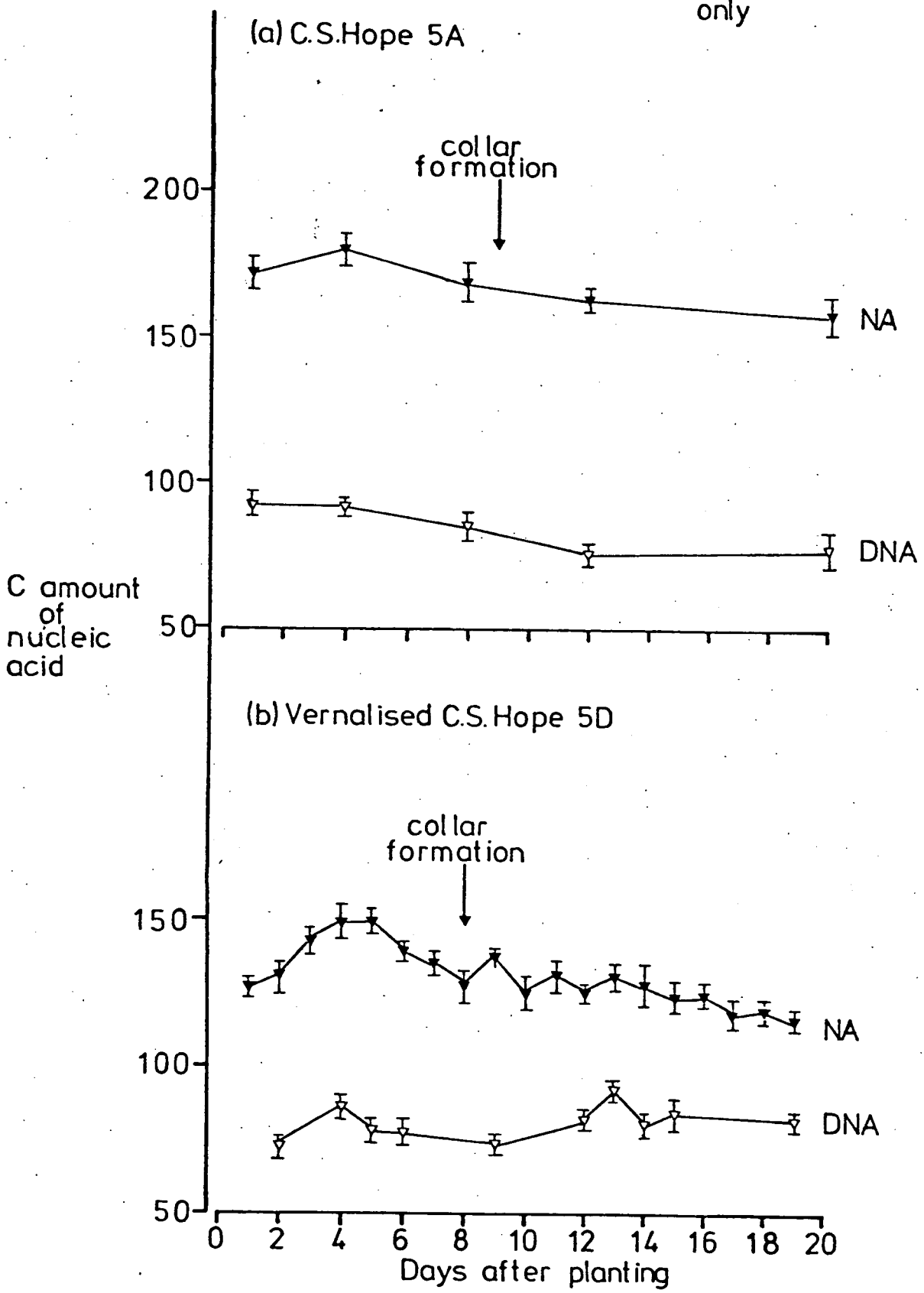


FIG 3.33

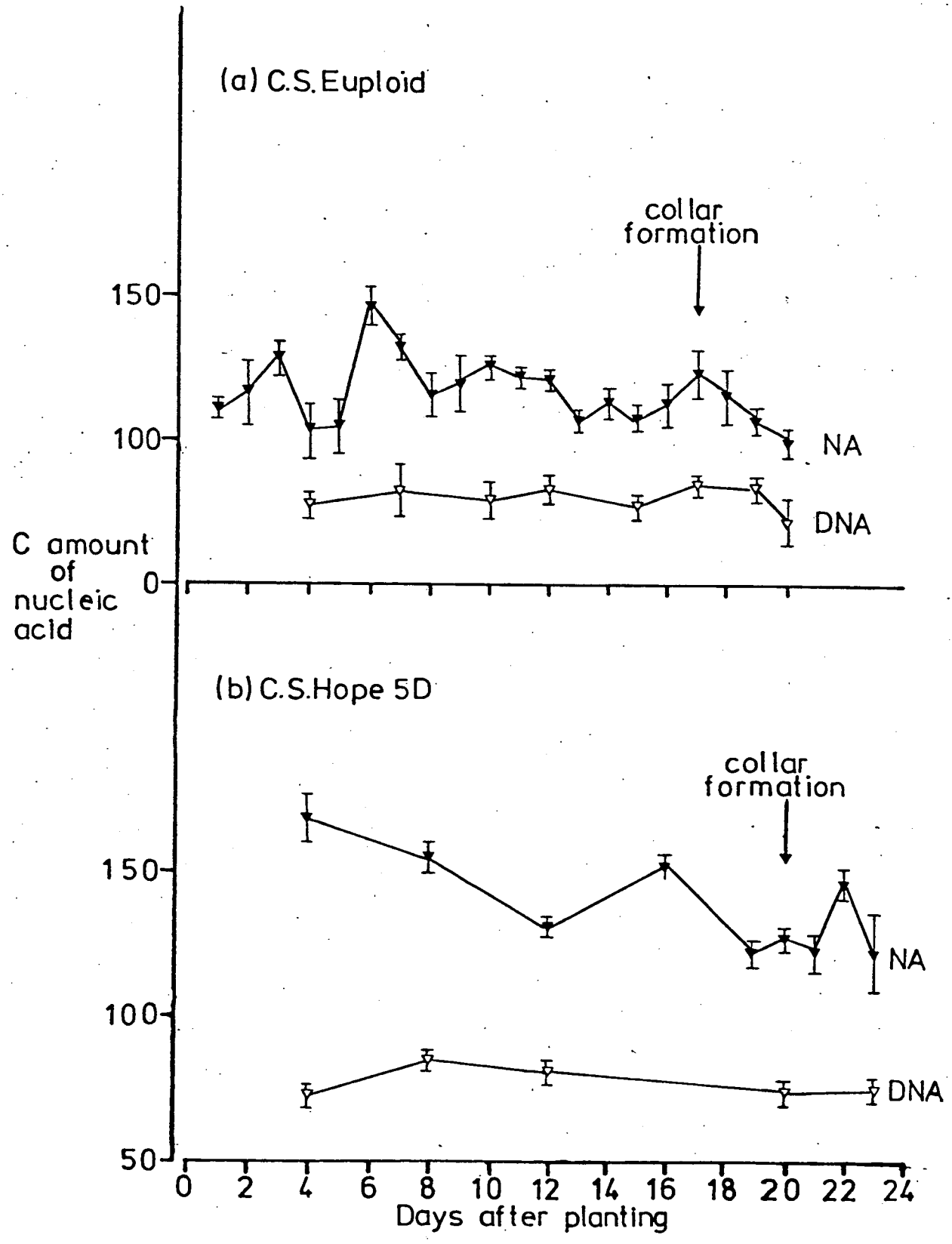

(a) C.S. Euploid
collar formation
150
100
NA
DNA
C amount of nucleic acid
0
(b) C.S. Hope 5D
collar formation
150
100
50
NA
DNA
0 2 4 6 8 10 12 14 16 18 20 22 24
Days after planting

Table 3.14. The DNA content in an area of ($5675\mu m^2$) of the apical dome measured by the gallocyanin and Feulgen methods. The gallocyanin values were a mean, for each cultivar, of the DNA values shown in FIGS. 3.29 and 3.30. The Feulgen values were a mean, for each cultivar, of values obtained for days 4, 8, 16 and 20 after planting (each of which was the mean of values for the three most median sections of 3 sectioned apices).

	DNA CONTENT (c)	
	Gallocyanin after ribonuclease	Feulgen
C.S. Hope 5A	86.36 ± 3.59	79.75 ± 3.47
C.S. Hope 5D Vernalised	81.02 ± 1.79	79.70 ± 3.45
C.S. Hope 5D	77.75 ± 2.36	81.34 ± 2.53
C.S. Euploid	81.16 ± 0.98	81.79 ± 0.60

The C amount of RNA per cell was plotted against days after planting (Fig 3.34).

In C.S. Hope 5A (Fig 3.34a), the C amount of RNA per cell was constant up to collar formation and then tended to increase. In vernalised C.S. Hope 5D (Fig 3.34b) the C amount of RNA per cell was also more or less constant up to collar formation and then decreased slightly. In unvernalised C.S. Hope 5D (Fig 3.34c), the C amount of RNA tended to decline up to collar formation, but in C.S. Euploid (Fig 3.34d) was more or less constant up to collar formation. Therefore it appears that vernalisation may prevent the decrease in the C amount of RNA per cell before collar formation in C.S. Hope 5D.

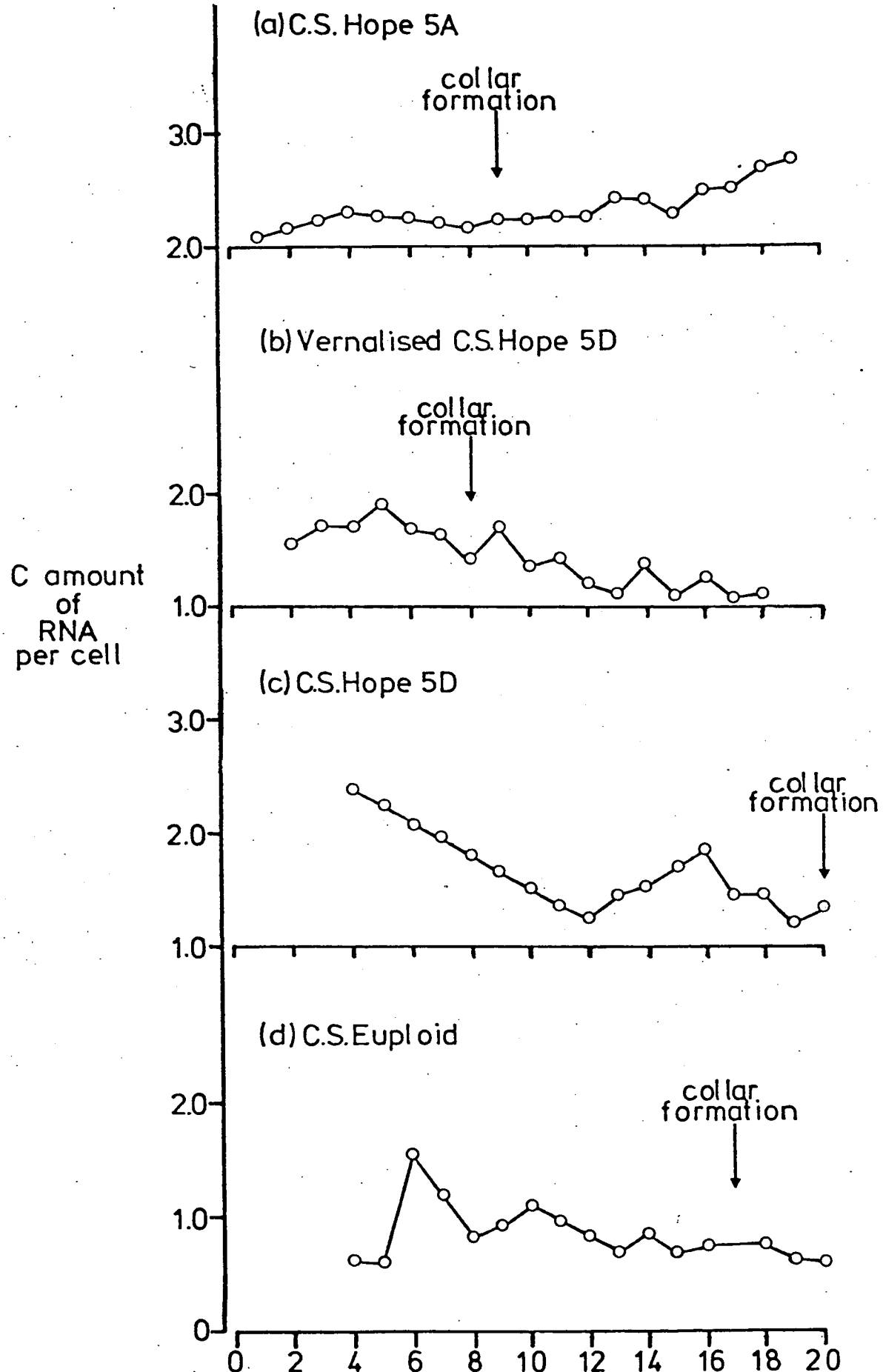
Fig 3.34 also indicated that the C amount of RNA per cell may generally be higher in C.S. Hope 5A than in the other cultivars. The vernalised and unvernalised C.S. Hope 5D had similar C amounts of RNA per cell which were higher then in C.S. Euploid. Vernalisation did not appear, therefore, to have affected the C amount of RNA per cell but may have prevented the decline in the C amount of RNA per cell in the period up to collar formation.

D. Commitment to the reproductive condition

Plants were transferred from long days to short days at varying stages of development to try and determine when the primordia became irreversibly committed to producing a leaf or a spikelet.

In all cultivars a greater final leaf number was produced by plants grown in continuous short days rather than continuous long days. This suggested that transfer of plants from long days to short days at certain stages, would increase the leaf number by altering the fate of labile primordia. This information could then be used to decide whether collar formation really should be regarded as the transition to flowering or whether this was at some other stage of development.

FIG 3.34 The mean C amount of RNA per cell in the apical dome as a function of days after planting The C amount of RNA was calculated from the difference between the C amount of total nucleic acid and the DNA in FIGS 3.32 and 3.33.



Samples of 3 plants were transferred at intervals from long day to short day conditions. In addition 10 plants of each cultivar were grown to ear emergence in continuous long days and 10 in continuous short days, to determine the final leaf number produced on the main stem in these conditions. The final leaf number on the main stem was counted in the transferred plants at the stage of ear differentiation. The final leaf number was plotted against the number of primordia which had been initiated in long days at the time of transfer to short days (Figs 3.36 - 3.37). The number of primordia initiated in long days at the transfer times was obtained for C.S. Hope 5A from Fig 3.5b for C.S. Hope 5D from Fig 3.7c and for C.S. Euploid from Fig 3.8c. The seed used in the transfer experiments with vernalised C.S. Hope 5D was harvested in 1978, whereas the data shown in Fig 3.6b is for 1977 seed. Therefore the number of primordia initiated in long days by the 1978 vernalised C.S. Hope 5D was measured at intervals, by dissecting samples of 3 plants and counting the number of primordia. The mean number of primordia was plotted against days after planting (Fig 3.35) and the best curve (fitted by eye) was drawn through these points. An approximation of the mean number of primordia initiated at each transfer in vernalised C.S. Hope 5D could than be read from this graph.

For each cultivar there was a minimum number of leaves which could be produced depending on the conditions, and the primordium numbers from the minimum +1, to the maximum +1 leaf numbers, inclusive, were therefore labile primordia (Table 3.15a). It is apparent from Table 3.15a, that the minimum and maximum leaf numbers increased with increasing vernalisation requirement (in the order C.S. Hope 5A, C.S. Euploid, C.S. Hope 5D). It therefore followed that the number of the first primordium which would always form a spikelet, irrespective of the conditions, also increased with the increasing vernalisation requirement (Table 3.15b). Vernalisation reduced the minimum leaf number in C.S. Hope 5D so that it was similar to C.S. Hope 5A, but the maximum leaf number was relatively unaffected. Therefore vernalisation increased the number of labile primordia in C.S. Hope 5D from 3 to 6 (Table 3.15a).

FIG 3.35 Mean number of primordia as a function of days after planting in vernalised C.S. Hope 5D (1978 grain) grown in continuous long days.

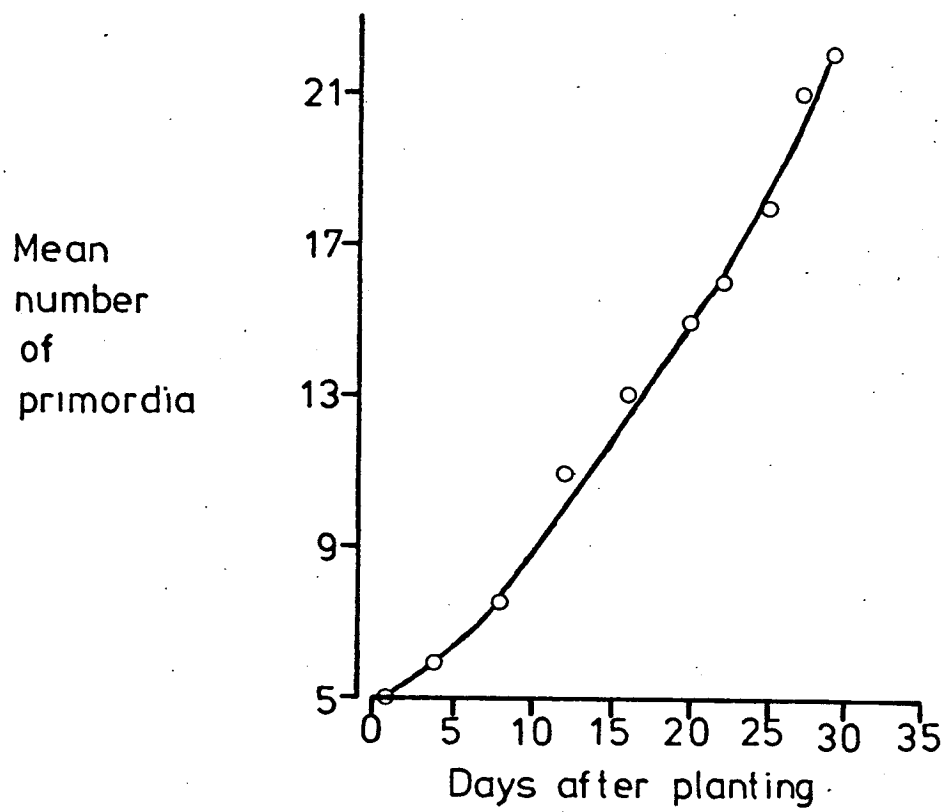


Table 3.15(a) The primordia which were labile in each cultivar.

Cultivar	Minimum Leaf No.	Maximum Leaf No.	Labile Primordia
C.S. Hope 5A	7	9	8 can produce leaf or collar 9 can produce leaf, collar or spikelet. 10 can produce collar or spikelet
C.S. Hope 5D Vernalised	8	13	9 can produce leaf or collar 10) 11)can produce leaf, collar or 12)spikelet 13) 14 can produce collar or spikelet
C.S. Hope 5D	12	14	13 can produce leaf or collar 14 can produce leaf, collar or spikelet 15 can produce collar or spikelet
C.S. Euploid	9	12	10 can produce leaf or collar 11)can produce leaf, collar or 12)spikelet 13 can produce collar or spikelet

(b) The first primordium irreversibly committed to producing a spikelet irrespective of the conditions:-

Cultivar	Number of the first primordium irreversibly committed to producing a spikelet.
C.S. Hope 5A	11
C.S. Hope 5D Vernalised	15
C.S. Hope 5D	16
C.S. Euploid	14

In figs 3.36 and 3.37, the period after its initiation in long days during which the fate of a potential collar or spikelet primordium can be altered to form a leaf, is given by that part of the graph, after its initiation, in which values may reach or lie above the final leaf number on the main stem which corresponds to the primordium number in question. In addition, the period after its initiation in which a potential spikelet primordium may form a collar is given by that part of the graph, after its initiation, which is above the final leaf number on the main stem corresponding to one less than the primordium number in question. The data derived from Fig 3.36 and 3.37 in this way are shown in Table 3.16.

In C.S. Hope 5A (Fig 3.36 and Table 3.16) the fate of the labile primordia could be altered from 1 to 3 plastochrons after their initiation in long days. Where the final leaf number exceeded the final leaf number in continuous short days, it appeared that the long days were making the primordia more flexible, and this occurred after only 2 days of growth in long day conditions.

In vernalised C.S. Hope 5D (Fig 3.36 and Table 3.16) there were more labile primordia than in C.S. Hope 5A, and in general the fate of these could be altered 1-7 plastochrons after their initiation . Primordium 9 (which formed a leaf in continuous long days) formed a collar when the plants were transferred to short days 9-11 plastochrons after the initiation of primordium number 9. Therefore at this stage (after 24-27 long days) the short days had a hastening effect on flowering. In addition, primordium number 13 could become a leaf if transferred to short days when 15 primordia had been initiated, indicating an increased flexibility of the primordia at this stage.

In C.S. Hope 5D (Fig 3.37 and Table 3.16) the fate of the labile primordia could be altered for at least 11-12 plastochrons after their initiation in long days. Therefore although there were fewer labile primordia than in the vernalised C.S. Hope 5D they were able to change their fate for a much longer period after their initiation. Since the final leaf number for most of the experiment exceeded the final leaf number in short days, it appeared that short days had a more marked delaying effect on flowering, when the plants had already been growing in long days for about 12 days.

FIGS 3.36 and 3.37 Mean final leaf number on the main stem (with standard errors) as a function of number of primordia initiated at time of transfer to short days (where standard errors are not shown, they were zero).

c=collar formation
dr=double ridge formation
in continuous long days

LD and SD indicate the number of leaves formed in continuous long days and continuous short days respectively

FIG 3.36

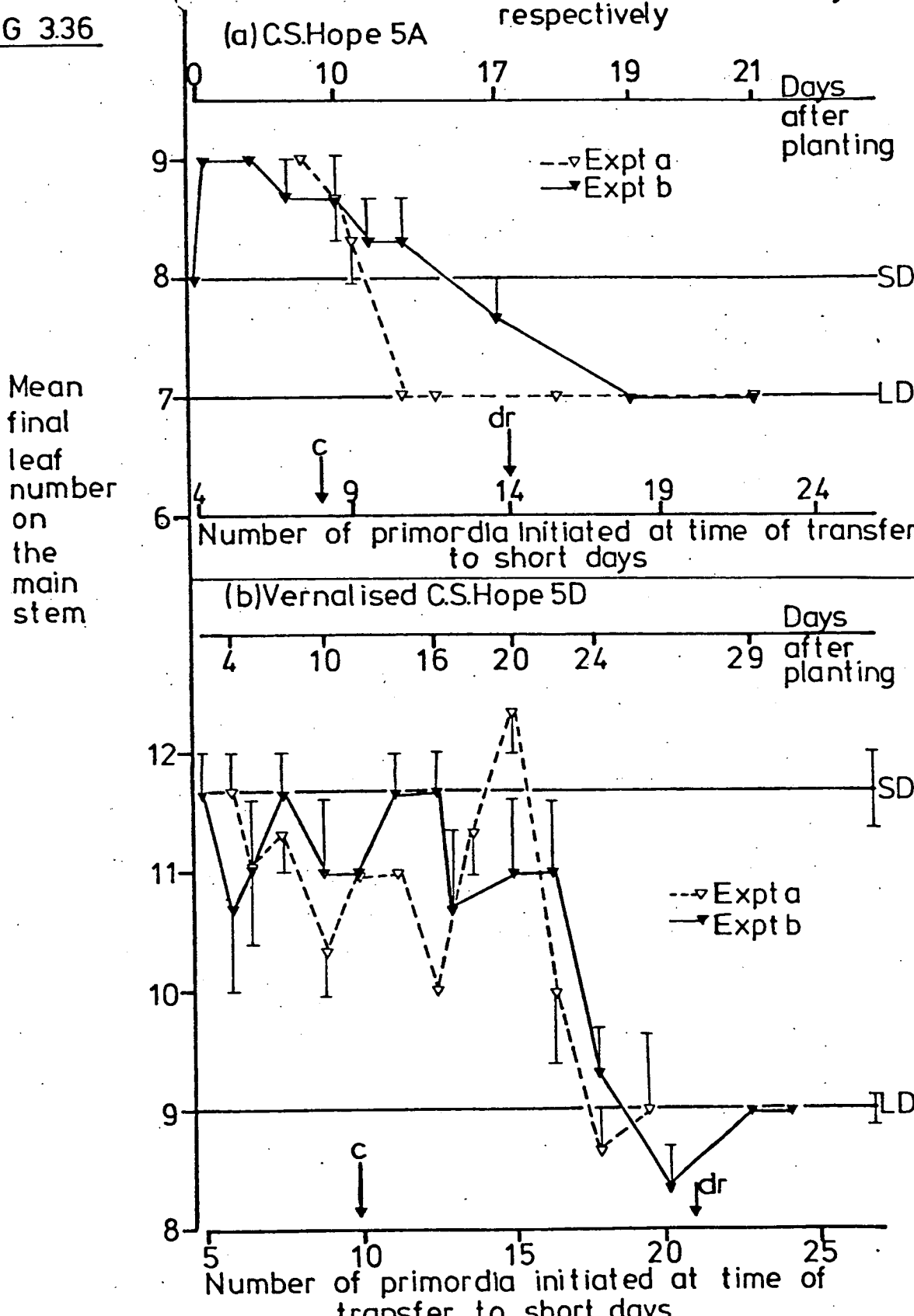


FIG 337

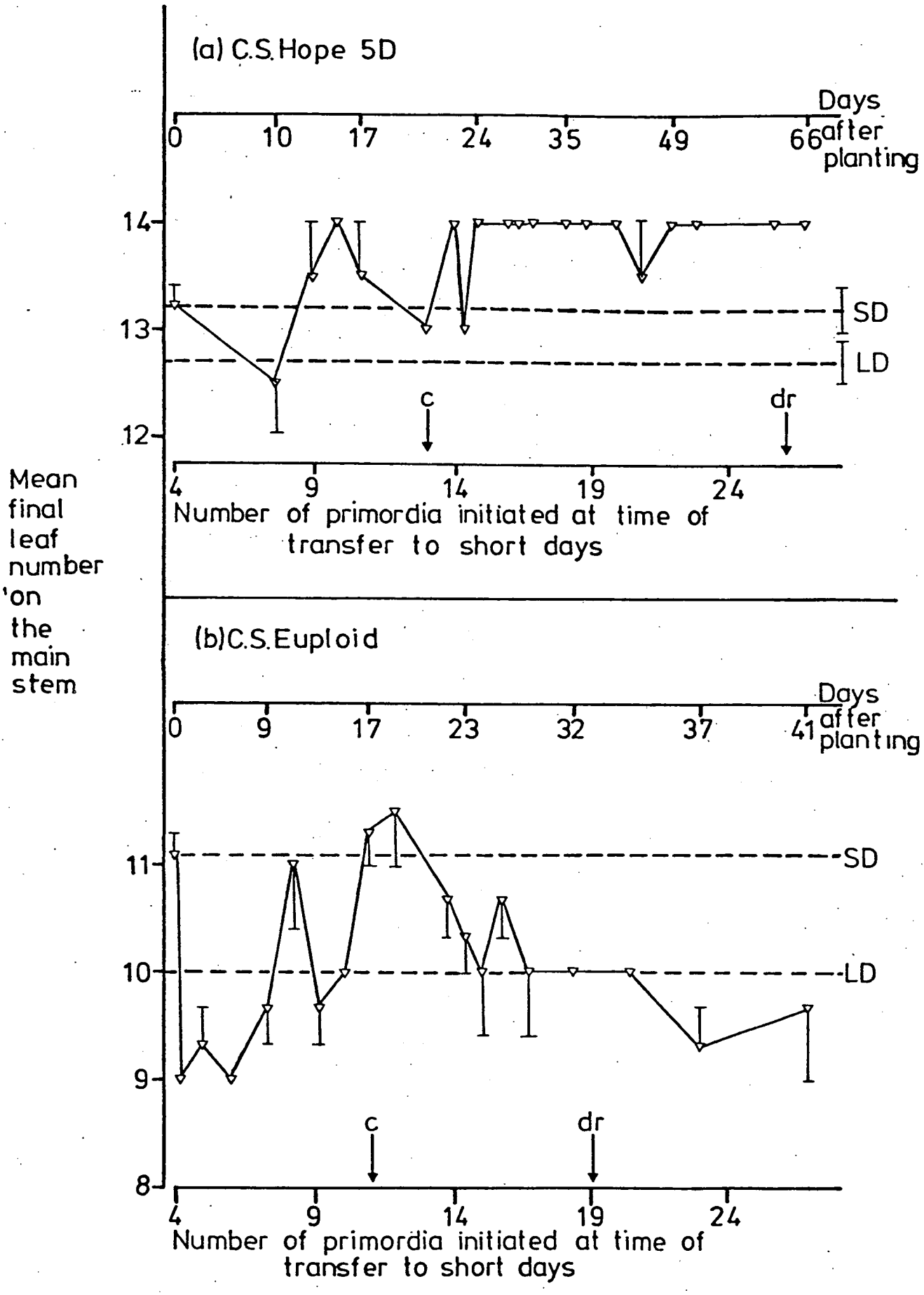

(a) C.S. Hope 5D
Days after planting
0
10
17
24
35
49
66
14
13
12
SD
LD
c
dr
4
9
14
19
24
Number of primordia initiated at time of transfer to short days
Mean final leaf number on the main stem
(b) C.S. Euploid
Days after planting
0
9
17
23
32
37
41
11
10
9
8
SD
LD
c
dr
4
9
14
19
24
Number of primordia initiated at time of transfer to short days

Table 3.16. Data derived from FIGS 3.36 and 3.37 regarding the period after their initiation during which the fate of labile primordia may be altered.

Cultivar	Primordium number	Normal fate in continuous long days	Can become a leaf if transferred at primordium number (n)	Can become a collar if transferred at primordium number(
C.S. Hope 5A	8	collar	11(mean of the 2 experiments)	-
	9	spikelet	11	11
	10	spikelet	-	11
C.S. Hope 5D Vernalised	9	leaf	-	18-20
	10	collar	17	-
	11	spikelet	16	17
	12	spikelet	15	16
	13	spikelet	15	15
	14	spikelet	-	15
C.S. Hope 5D	13	collar(in 30% of plants)	at least up to 26	-
	14	collar(in 70% of plants)	at least up to 26	at least up to
	15	spikelet	-	at least up to
C.S. Euploid	10	leaf	-	20-26
	11	collar	13	-
	12	spikelet	12 or 13	13
	13	spikelet	-	12 or 13

In C.S. Euploid (Fig 3.37b and Table 3.16) the fate of the labile primordia could, in general, only be altered for 1 or 2 plastochrons after their initiation in long days. However primordium 10 could become a collar if the plants were transferred to short days either after 2-9 long days, or 10-16 plastochrons (after 34-40 long days) after the initiation of primordium 10 in long days.

The number of labile primordia, and the stages at which they were irreversibly committed varied between the cultivars. However in none of the cultivars was the fate of the labile primordia determined by collar formation. In C.S. Hope 5A, vernalised C.S. Hope 5D and C.S. Euploid the final leaf number did not exceed the number normally produced in continuous long days, when transferred to short days after double ridge formation, however the experiment was not continued far enough in C.S. Hope 5D to see if this was so. These findings pose the question whether collar formation really should be regarded as the transition to flowering or whether double ridge formation is a more accurate definition of the transition to flowering, at which stage the plant cannot produce a greater number of leaves.

E. The effects of vernalisation on growth of leaves, tillers and the whole plant

1. Leaf emergence

The mean number of emerged leaves was counted for a sample (7-10) of plants, each day, up to the 20th day after planting. The mean number of emerged leaves was then plotted against days after planting for each experiment for each cultivar (Figs 3.38 and 3.39).

All the experiments for each cultivar followed a similar pattern, so a common line was drawn through the points. The day on which 50% of the plants had a certain number of emerged leaves was read from the graphs (Table 3.17a). For each leaf number (except 1, in which it was the same as C.S. Hope 5A) the leaves emerged earlier in vernalised C.S. Hope 5D than in the other cultivars. This is possibly because during vernalisation these leaves would have grown by a small amount and therefore at planting were at a more advanced stage of development than in the unvernalised cultivars. For leaf numbers 3 and 4, the leaves tended to emerge earlier in C.S. Hope 5A than in C.S. Hope 5D and C.S. Euploid. However there appeared to be no obvious difference between the patterns of leaf emergence in C.S. Hope 5D and C.S. Euploid.

FIGS 3.38 and 3.39 Mean number of emerged leaves as a function of days after planting

FIG 3.38

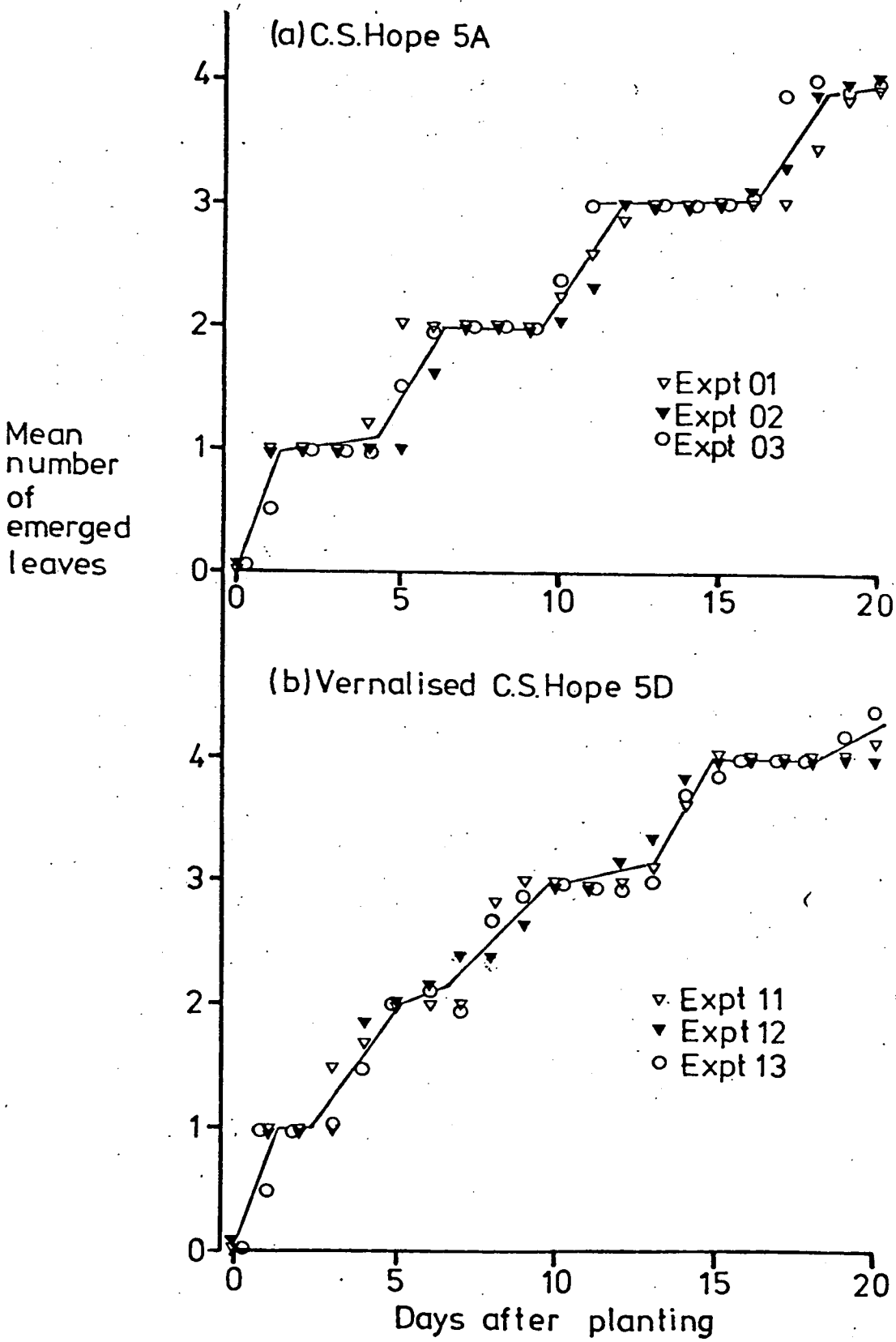


FIG 3.39

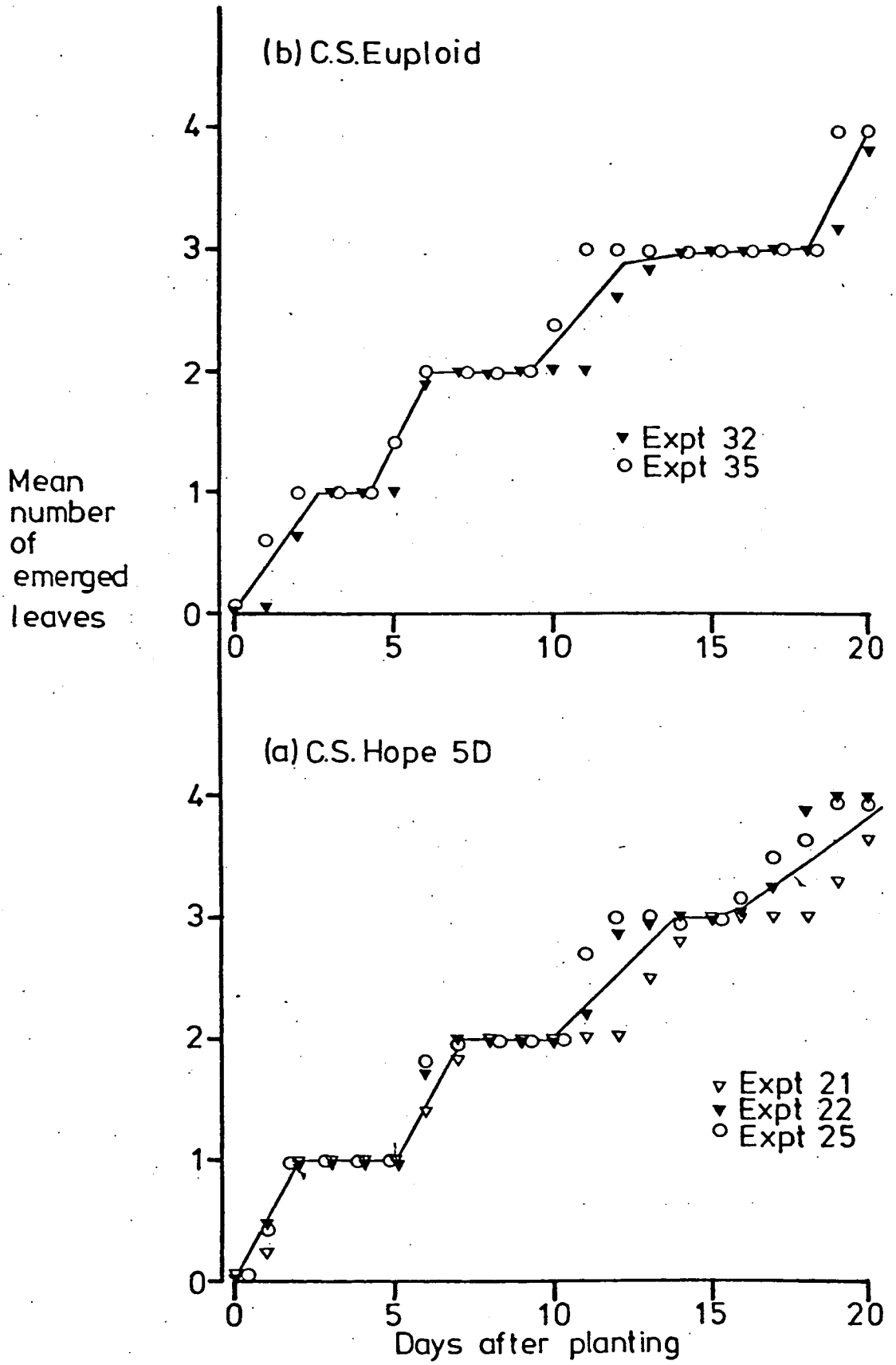

(b) C.S. Euploid
Expt 32
Expt 35
(a) C.S. Hope 5D
Expt 21
Expt 22
Expt 25
Mean number of emerged leaves
Days after planting
0
1
2
3
4
0
5
10
15
20

Table 3.17. The day after planting on which 50% of the plants had (n) emerged leaves, and the interval between the emergence of successive leaves. Data read from FIGS. 3.33-3.34.

(a)

	Day after planting on which 50% of the plants had (n) emerged leaves			
Leaf number (n)	1	2	3	4
C.S. Hope 5A	0.7	5.2	10.7	17.3
C.S. Hope 5D Vernalised	0.7	3.7	7.9	13.8
C.S. Hope 5D	1.0	6.1	11.9	18.4
C.S. Euploid	1.3	5.2	11.0	19.0

(b)

	Days between the emergence of successive leaves		
Leaf numbers	1-2	2-3	3-4
C.S. Hope 5A	4.5	5.5	6.6
C.S. Hope 5D Vernalised	3.0	4.2	5.9
C.S. Hope 5D	5.1	5.8	6.5
C.S. Euploid	3.9	5.8	8.0

The days between the emergence of successive leaves were calculated from Table 3.17a (Table 3.17b) to determine whether the leaves were actually emerging faster in vernalised C.S. Hope 5D or whether the graph was just displaced to the left of those for the other cultivars. However each leaf interval was shorter in vernalised C.S. Hope 5D than in the other cultivars indicating that the leaves were emerging at a faster rate.

2. Dry weight of the plants

The dry weights of the roots, leaves 1-5 (separately), the coleoptile tiller, tiller 1, tiller 2 and the shoot apex + stem were measured in 10 plants of each cultivar, on the twenty-first day after planting, (Table 3.18).

The dry weight of each part of the plants was very similar in all the cultivars. A difference which was observed was that the coleoptile tiller was present in only 50-60% of the plants of C.S. Hope 5A and vernalised C.S. Hope 5D but was always present in C.S. Hope 5D and C.S. Euploid at this stage.

However, the mean dry weight of the whole plants was very similar, particularly the vernalised and unvernalised C.S. Hope 5D.

3. Tillering

The number of tillers produced by each cultivar was counted at ear emergence (Table 3.19).

The number of tiller was similar in C.S. Hope 5A and vernalised C.S. Hope 5D, and also similar in C.S. Hope 5D and C.S. Euploid. Vernalisation therefore appeared to reduce the number of tillers produced by a plant.

Table 3.18 Mean dry weights (with associated standard errors) of a sample of 10 plants on the twenty-first day after planting.

	Mean Dry Weight (mg)			
	C.S. Hope 5A	C.S. Hope 5D Vernalised	C.S. Hope 5D	C.S. Eupl
Roots	185±9	183±3	180±11	190±7
Apex+leaf 6 + stem	11±1	9±2	9±0	11±1
Leaf 5	8±1	7±1	8±1	5±1
Leaf 4	55±3	50±3	50±3	54±3
Leaf 3	57±2	60±1	50±2	62±2
Leaf 2	32±1	30±1	28±1	31±1
Leaf 1	20±1	16±1	18±1	21±1
Coleoptile tiller	7±3	6±2	16±4	15±2
Tiller 1	47±3	51±3	52±4	52±3
Tiller 2	10±1	12±2	19±2	17±2
Whole Plant	442±23	424±21	429±17	458±6

Table 3.19. The number of tillers(with standard errors) present at ear emergence. A tiller was regarded as present when it protruded beyond the ligule of the leaf which subtended it.

Cultivar	Number of tillers present at ear emergence	Sample size
C.S. Hope 5A	2.6 ± 0.5	10
C.S. Hope 5D Vernalised	2.4 ± 0.1	27
C.S. Hope 5D	5.3 ± 0.3	9
C.S. Euploid	4.5 ± 0.5	6

4. Growth of the phytomer

The mean volume of the median section of each phytomer was calculated for several days following its initiation for experiments 01, 11, 21, and 31. The natural logarithms of these mean volumes were plotted against days after planting, and linear regression analysis was carried out on these points. The slope of the regression lines gave the relative growth rate of the phytomer (Table 3.20a). A similar analysis was carried out for each primordium (phytomer minus axial tissue) in the vernalised and unvernalised C.S. Hope 5D, and the relative growth rates of the primordia are shown in Table 3.20b. In C.S. Hope 5A the volume relative growth rate of the phytomer remained about the same up to collar formation and then appeared to decrease. In vernalised C.S. Hope 5D the relative growth rate of the phytomer remained more or less constant throughtout the experiment but with a higher value for phytomer 7. In C.S. Hope 5D the relative growth rate of the phytomer decreased down to phytomer 9 and then may have increased. In C.S. Euploid similarly, the relative growth rate appeared to decline down to phytomer 9 and then increased. In all the cultivars except vernalised C.S. Hope 5D the relative growth rate of the later phytomers was lower than that of the first phytomers. There did not appear to be any particular relative growth rate associated with the collar phytomer.

The volume relative growth rate of the primordium appeared to remain about the same with increasing primordium number in both the vernalised and unvernalised C.S. Hope 5D (Table 3.20b). Although the volume relative growth rate of each primordium tended to be higher in unvernalised C.S. Hope 5D, if a mean was calculated of the relative growth rates for primordia 6-10 for each cultivar, they were not significantly different when tested by the method of Brownlee (1953). Since the volume relative growth rate of the phytomer (Table 3.20a) tended to decrease in the later plastochrons in C.S. Hope 5D but the relative growth rate of the primordium remained about the same (Table 3.20a), the growth in the phytomer must have been biased towards the growth of the primordium. However in the vernalised C.S. Hope 5D both the relative growth rate of the phytomer (Table 3.20a) and the primordium (Table 3.20b) remained about the same.

The volume of the axial tissue of the phytomer was plotted against days after planting but no significant correlation could be obtained by regression analysis.

Table 3.20. Volume relative growth rate per day of median section of (a) phytomer (b) primordium.

Cultivar	Phytomer number	Relative growth rate.day^{-1} of phytomer	Correlation coefficient	n
C.S. Hope 5A	5	0.27	0.647	15
	6	0.24	0.842	23
	7	0.27	0.700	14
	8	--0.22--	0.775	12
	9	0.09	0.647	12
	11	0.11	0.578	12
C.S. Hope 5D Vernalised	6	0.13	0.898	17
	7	0.27	0.935	20
	8	--0.13--	0.968	21
	9	0.12	0.991	17
	10	0.14	0.812	15
C.S. Hope 5D	6	0.16	0.962	24
	7	0.14	0.767	16
	8	0.13	0.997	22
	9	0.05	0.978	14
	10	0.06	0.942	21
	11	0.08	0.883	9
C.S. Euploid	6	0.23	0.958	12
	7	0.21	0.815	12
	8	0.09	0.802	14
	9	0.08	0.837	18
	10	0.18	0.874	15
	11	--0.12--	0.558	11

(b)

	Primordium number	Relative growth rate day^{-1} of primordium	Correlation coefficient	n
C.S. Hope 5D Vernalised	6	0.55	0.971	17
	7	0.63	0.989	20
	8	--0.51--	0.934	21
	9	0.37	0.951	17
	10	0.57	0.924	15
C.S. Hope 5D	6	0.77	0.980	24
	7	0.62	0.977	16
	8	0.63	0.973	22
	9	0.45	0.983	14
	10	0.78	0.960	21

n = number of apices
--- indicates the phytomer which would become the collar.

PART IV DISCUSSION

The main results of the foregoing experimentation are discussed under five main headings, namely the effects of vernalisation on: the course of the major stages of apical development, the growth of the shoot apex before and after collar initiation, the transition to flowering, and other effects of vernalisation.

A final section outlines the main conclusions of the study and indicates lines for future work.

A. The effects of vernalisation on the length of the major developmental stages of the wheat cultivars.

The ultimate effect of vernalisation was to reduce the number of leaves formed on the main stem and the number of days to ear emergence, after planting, in responsive cultivars (C.S. Hope 5D, C.S. Euploid). These parameters were comparable to those in the non-vernalisation requiring cultivar (C.S. Hope 5A).

The reduction in the length of time to ear emergence in vernalised C.S. Hope 5D was broughtabout mainly by a reduction in the number of days from collar initiation to double ridge formation. The number of days from planting to collar initiation was approximately halved in vernalised plants but the number of days from collar initiation to double ridge formation, after vernalisation, was approximately one fifth the number of days between these stages in unvernalised plants. However the period from double ridge formation to ear emergence was relatively unchanged. This is in agreement with the observations that in Australian wheats vernalisation reduced the interval from germination to double ridge appearance but the time from this to anthesis was not affected (Halse and Weir, 1970). Similarly in some Indian and exotic cultivars of wheat, the accelerating effect of vernalisation was most pronounced during the period from planting to double ridges (Garg and Chinoy, 1964). Vernalisation may not, therefore, be affecting spikelet differentiation and stem elongation indicating that spikelet initiation may be under a separate control from spikelet differentiation.

If the number of days to ear emergence is counted from the beginning of the vernalisation treatment, it was found that only in C.S. Hope 5D was there an actual reduction in the total number of days to ear emergence compared to unvernalised plants. This illustrates the difficulty of deciding whether vernalisation had had an accelerating effect on growth in the different cultivars. Since vernalisation has such a large effect on the number of days from collar initiation to double ridge formation, perhaps the length of this period could be used to determine the vernalisation response of a particular cultivar. For example in C.S. Euploid, the total time to ear emergence was increased if the 6 weeks

vernalisation was included, but if the length of time between collar initiation and double ridge formation had been measured, perhaps this would have given a better idea of the vernalisation response of the cultivar.

B. The effects of vernalisation on the growth of the shoot apex before collar initiation

The points to be considered here are whether there is anything happening in the apex, before collar initiation, as a result of vernalisation, and if so whether this predisposes the plant to flower, and in what way?

(a) Apical dome and cell volume

Over the first 10 days after planting, the mean volume of the apical dome was greater in vernalised than in unvernalised plants of C.S. Hope 5D. In addition, the mean volume of the apical dome was, in general, inversely related to the vernalisation requirement, C.S. Hope 5A having the largest apical dome volume and C.S. Hope 5D the smallest.

The mean cell volume was also slightly greater in vernalised plants of C.S. Hope 5D and in C.S. Hope 5A than in the other unvernalised cultivars. However the larger cell volume in vernalised plants was not totally responsible for the larger apical volume, therefore there must have been a greater number of cells in the vernalised apical dome. The larger cells in vernalised C.S. Hope 5D could have been brought about by an inhibition of cell division although such inhibition could not be complete because a new primordium was initiated during the vernalisation process. Alternatively there could be a direct promotion of cell expansion during vernalisation.

It is interesting to note that the mean cell volume of 1.4×10^3 microns^3 for the tunica and 2.1×10^3 microns^3 for the corpus on day 10 in C.S. Hope 5A, which has a similar mean cell volume in the apical dome to vernalised C.S. Hope 5D, is much lower than the values reported by Williams (1960) for Spring wheat of 2.9×10^3 microns^3 for the tunica and 2.8×10^3 microns^3 for the corpus on the 11th day after planting. This indicates that the mean cell volume probably varies considerably at least between cultivars and therefore the different cell sizes

observed may be irrelevant to the interpretation of vernalisation effects.

The mean cell size tended to increase in C.S. Hope 5A and vernalised C.S. Hope 5D just after collar initiation, therefore it could be that the cell size is important at some later stage and that the cells have to reach a critical size at double ridge formation (measurements were not made of cell size in C.S. Hope 5D and C.S. Euploid after collar initiation). Unfortunately there does not appear to be any information regarding this in the literature.

(b) Is a critical size of the apical dome attained?

In the cultivars studied, the apical dome did not have to be at a particular volume, height or diameter for floral initiation to occur. The values of these parameters at collar initiation increased with increasing vernalisation requirement, with vernalised C.S. Hope 5D having similar values to C.S. Hope 5A. Therefore it seemed that the size of the apical dome at floral initiation was irrelevant and was determined only by how far into the plants' development floral initiation occurred (and therefore how long the apical dome had to grow).

The idea of a critical size of the apical dome has, however, been supported by Williams and Williams (1968), who observed that floral initiation occurred in wheat when the apical dome had attained a critical value, and even though the dimensions of the apex may have varied, as in different photoperiodic treatments, the volume was always the same. In Chrysanthemum, Horridge and Cockshull (1979) observed a critical volume for the apical dome when the first bract was initiated, and in barley, Nicholls and May (1963) reported that the apex must reach a critical length for the appearance of spikelets. However, also in barley, according to Dale and Wilson (1979) the transition of the apex to produce spikelet primordia occurred with widely different volumes of the apical dome.

The evidence therefore indicates that the size of the apical dome at floral initiation may or may not be critical, according to the species, but in the plants studied in this thesis it is not.

(c) Rate of primordium initiation

The rate of primordium initiation was similar in all the cultivars before collar formation. However in rye, Sunderland (1961) observed that primordia were produced at about double the rate in vernalised compared to unvernalised plants during vegetative development, but this is based on only 2 values for vernalised rye as the transition to flowering occurred just 4 days after planting. In spring wheat, the rate of primordium initiation increased at collar formation, with spikelet primordia being produced about 3 times as fast as leaf primordia (Kirby, 1974).

However the rate of primordium initiation did not increase until several plastochrons after collar formation in C.S. Hope 5A and vernalised C.S. Hope 5D. Similarly, in vernalised rye, the rate of primordium initiation increased about 4 plastochrons after the transition to flowering (Sunderland, 1961). In unvernalised C.S. Hope 5D, the rate of primordium initiation appeared to decrease just after collar formation, with C.S. Euploid (intermediate vernalisation requirement) having a rate of primordium initiation intermediate between that of C.S. Hope 5A (and vernalised C.S. Hope 5D) and C.S. Hope 5D.

Therefore there is no change in the rate of primordium initiation associated with collar formation and since the rate of initiation a few plastochrons after collar initiation is very different between cultivars, perhaps a particular rate of primordium initiation is not a pre-requisite for flowering.

(d) Volume relative growth rate of the apical dome

There was no particular volume relative growth rate of the apical dome associated with collar formation. The values were inversely related to the vernalisation requirements of each cultivar, the highest relative growth rate being in C.S. Hope 5A (and vernalised C.S. Hope 5D) and the lowest in C.S. Hope 5D, with the value for C.S. Euploid again being intermediate. In the period up to collar formation, the volume relative growth rate tended to decline in all the cultivars, the extent by which this declined again being cultivar dependent. It appeared that the volume relative growth rate of the apical dome at collar formation was greater in vernalised C.S. Hope 5D and C.S. Hope 5A than in C.S. Hope 5D and C.S. Euploid because floral initiation occurred earlier in the plants' development, and the relative growth rate had therefore only declined over a limited number of plastochrons.

A decrease in the relative growth rate during vegetative development was also suggested by Sunderland's results with rye (1961) in which measurements were made on small portions of the apex, which included the apical dome and one or more primordia together with the axial tissue. In contrast, Abbe, Phinney and Baer (1951) found that the relative growth rate of the apex increased exponentially during vegetative development in maize.

Therefore there is no particular relative growth rate associated with the floral transition in the wheat cultivars.

(e) Partitioning of the apical dome and relative growth rate of the phytomer

Although the actual size of the apical dome at floral initiation may not be important, it could be that the partitioning of the apical dome at this stage into primordia, stem tissue and new dome material is critical.

It did not appear that there was a particular value for the proportion of the apical dome which was cut off as the collar phytomer, this proportion being inversely related to the vernalisation requirement of the cultivars. There was a decrease in the proportion of the apical dome cut off as phytomer in the period up to collar formation and, as with the volume relative growth rate of the apical dome, the decrease was most marked in C.S. Hope 5D and C.S. Euploid as collar initiation occurred later in these plants' development than in C.S. Hope 5A and vernalised C.S. Hope 5D.

The volume of the collar phytomer at initiation in all the cultivars was in the range $38-49 \times 10^3$ microns. If the volume of the phytomer had to be at this particular size to develop into the collar this would have occurred at an earlier phytomer number in C.S. Hope 5D since this volume had already been attained by phytomer 9,4 plastochrons before the collar phytomer was initiated. However for all the cultivars the volume of the collar phytomer at initiation was approximately in the middle of the range of volumes of all the phytomers measured at initiation. There may have to be 2 factors acting in conjunction to enable a phytomer to develop into the collar, and hence for future phytomers to become spikelets. These could be a particular volume of the phytomer ($38-49 \times 10^3$ microns^3) but for this size to be attained when the apical dome is at a particular volume, but this does not seem to be the case as the apical dome has varying volumes at collar initiation in the different cultivars.

Regarding the volume relative growth rate of the phytomer, there was a range of relative growth rates from approximately 0.08-0.22 for the collar phytomer, and therefore there did not appear to be a critical relative growth rate associated with the collar phytomer. In vernalised C.S. Hope 5D and C.S. Hope 5A there was no apparent trend in the growth rate of the phytomer up to collar initiation, whereas in unvernalised C.S. Hope 5D and to a lesser extent in C.S. Euploid the relative growth rate tended to decline towards the collar phytomer.

In vernalised C.S. Hope 5D the relative growth rate of the primordium showed no trend, either, up to the collar primordium, indicating that the relative rates of growth of the primordium and axial tissue were similar for each primordium and portion of axial tissue up to the collar phytomer. However, in C.S. Hope 5D, the relative growth rate of the primordium remained about the same until collar formation while the relative growth rate of the phytomer declined, indicating that the relative growth rate of the axial tissue probably declined.

Therefore, there may have been a difference between the unvernalised and vernalised C.S. Hope 5D in the relative rates of growth of the primordium and axial tissue until collar formation, in that the growth rates of the axial tissue in the unvernalised plants tended to decline. The relative growth rate of the apical dome also declined over this period and therefore the growth rate of the primordium relative to the axial tissue and the apical dome must have increased.

(f) RNA content

At the cellular level, the amount of RNA per cell in the apical dome tended to decline up to collar formation in unvernalised C.S. Hope 5D. Since the cell doubling time increased over this period, the rate of accumulation must have been slowing down. In C.S. Euploid the amount of RNA per cell remained approximately constant until collar formation but as the cell doubling time tended to increase over this period, the rate of RNA accumulation was probably slowing down, as in C.S. Hope 5D. However in vernalised C.S. Hope 5D and C.S. Hope 5A the amount of RNA per cell remained more or less constant up to collar initiation, as did the cell doubling time and hence the rate of RNA accumulation.

There did not appear to be a particular amount of RNA per cell in the apical dome associated with collar initiation. The data suggest, however, that there may have to be no decline in the rate of RNA accumulation for early floral initiation to occur as in vernalised C.S. Hope 5D and C.S. Hope 5A.

C. The effects of vernalisation on the growth of the shoot apex after collar initiation.

(a) Volume of the apical dome

The first indication that the apex had become reproductive in the vernalised plants was a four-fold increase in the mean volume of the apical dome from day 11 up to day 19, the increase beginning before the spikelet primordia could be morphologically distinguished. This increase in apical dome volume occurred over the period from a few days after collar initiation to about double ridge formation. A similar increase was observed in C.S. Hope 5A in which it appeared that the maximum dome volume was attained at double ridge formation, aswas found for barley by Kirby (1977).

In unvernalised C.S. Hope 5D, the mean volume of the apical dome increased at a similar rate both pre- and post collar initiation. Vernalisation appeared to have increased the rate at which the mean volume of the apical dome increased after collar formation and since there was no such increase in unvernalised C.S. Hope 5D the increase does not appear to be essential for double ridge formation to occur and indeed does not appear to be particularly relevant to the switch to the floral state.

(b) (i) Volume relative growth rate of the apical dome

In unvernalised C.S. Hope 5D, the volume relative growth rate of the apical dome declined down to collar initiation, remained static for a few plastochrons and then continued to decline. However in vernalised C.S. Hope 5D, the decline in the volume relative growth rate was halted about 3 plastochrons after collar formation and the growth rate then appeared to increase.

Vernalisation may therefore, have resulted in the build up of some substance which maintained and even increased the volume relative growth rate of the apical dome at collar initiation, or it may have prevented the production of some growth inhibitor which caused the decline in relative growth rate in unvernalised plants of C.S. Hope 5D. However an increased relative growth rate was not essential for flowering in the wheat cultivars as ear development and emergence did eventually occur in unvernalised C.S. Hope 5D despite the continuing decline in the growth rate.

(ii) Cell cycle in the apical dome

The length of the cell cycle decreased several plastochrons after collar formation in vernalised C.S. Hope 5D (and in C.S. Hope 5A), which may be deduced from the increased volume relative growth rate at this stage. From data for C.S. Hope 5A it appeared that the decrease in the length of the cell cycle was attributable mainly to a reduction in the length of the $G_1 + \frac{1}{2}S$ phase of the cell cycle but $G_2 + \frac{1}{2}S$ and M were also reduced. Similarly, Francis and Lyndon (1978), found that in shortening of the cell cycle in Silene after floral induction there was more shortening of G_1 and S than G_2 and M.

The results indicate that there is no one control point in the cell cycle because all the phases were reduced to some extent. Van't Hof (1966) found that the cells in meristems of cultured pea roots which had been temporarily starved of carbohydrates, were arrested in G_1 and G_2, and Van't Hof later proposed (1973) that the cell cycle is regulated by two principal control points - one in G_1 (regulating the G_1 to S transition) and another in G_2 (controlling the G_2 to M change). In addition Bernier, Bronchart, Jacqmard & Sylvestre (1967) suggested from their observations on Sinapis that gibberellic acid may act at the points of entry of cells into S and M. It is interesting to consider how the cell cycle could be so controlled. Gibberellin has been shown to reduce the length of the cell cycle in seedlings of watermelon primarily by shortening the S phase but the G_1 and G_2 periods were also shortened (Brent Loy, 1977). Van't Hof (1968) in experiments on cells in the proliferative phase in the root meristems of Pisum found that kinetin slightly increased G_2 and certainly G_1 durations, and IAA appeared to act primarily on the duration of S. IAA + kinetin produced all three results.

Further work is required to determine the effects of growth regulators on wheat and whether they are involved in the vernalisation process.

(c) Partitioning of the apical dome and its relationship to volume relative growth rate

In vernalised C.S. Hope 5D, for the first 6 plastochrons after planting, the volume relative growth rate of the apical dome declined but the apical dome was able to increase in volume because the proportion of the apical dome cut off as phytomer at the end of the plastochrons progressively decreased as well. However by plastochron 11, the volume relative growth rate increased which meant the apical dome increased to a much greater extent.

The mean volume of the apical dome in C.S. Hope 5A increased in a similar way to vernalised C.S. Hope 5D, but P continued to decrease after plastochron 10, so together with the increased growth rate, brought about the large increase in apical dome volume.

In unvernalised C.S. Hope 5D, the volume relative growth rate declined throughout but the mean volume of the apical dome was able to increase because P gradually decreased throughout the vegetative phase. This pattern of growth may also have been maintained after collar initiation.

It appears that the normal pattern in vernalisation requiring cultivars is for a declining growth rate throughout ., but with changes in partitioning of the apical dome which allow the apical dome to increase in volume. However, in vernalised C.S. Hope 5D and in C.S. Hope 5A although P declined more or less throughout as in C.S. Hope 5D and C.S. Euploid, the decline in growth rate was halted and an increase in the volume relative growth rate led to a large increase in volume.

The observations on relative growth rate and partitioning of the apical dome suggest that the volume relative growth rate may be affected by vernalisation but a particular volume relative growth rate is not essential for floral initiation to occur.

Partitioning of the apical dome appeared to be relatively unaffected by vernalisation but the relative rates of growth of the primordium and axial tissue in the phytomer may have been affected by vernalisation, in that the relative growth rate of the primordium was maintained and the relative growth rate of the axial tissue and apical dome declined in unvernalised plants up to the collar phytomer, while in vernalised plants the relative growth rates did not appear to change.

Observations on volume relative growth rate in relation to partitioning of the apical dome have been made in a number of species. During the vegetative development of the shoot apex in Agropyron repens, the change in partitioning of the apex was responsible for the increase in apical dome volume (Smith and Rogan, 1979). Similarly in the vegetative development of rye, the relative growth rate declined but the apex could increase in volume probably by a decrease in the proportion of the apical dome being cut off as a phytomer (Sunderland, 1961). However, when vernalised rye plants became reproductive, there was a large increase in the rate at which the cells divided and expanded in the apex (pointing to an increased growth rate), together with the rapid formation of primordia. This is similar to the pattern seen in C.S. Hope 5A and vernalised C.S. Hope 5D, with an increase in the rate of primordium initiation several plastochrons after collar initiation, together with a rapid increase in the volume of the apical dome, and an increase in the volume relative growth rate and rate of cell division.

The results in this study suggest that perhaps growth rate and partitioning are under separate control because the trend in declining volume relative growth rate in C.S. Hope 5D was halted by vernalisation (and the growth rate even increased) but partitioning of the apical dome did not appear to be affected. The observations by Schwabe (1971) that an auxin antagonist affects the phyllotaxis of the apex but not the relative growth rate of the apical dome, is consistent with the idea that partitioning and growth rate are under separate control. So how could the growth rate be controlled? If the first 2 leaves of tomato were removed during the early part of the vegetative phase, the relative growth rate of the apical dome increased, and since nitrogen and other elements were supplied in adequate amounts to intact plants it appeared that the apical dome was competing with the young leaf primordia for carbon assimilates (Hussey, 1963).

In excised apices the growth rate may be controlled by sugar concentration (Ball and Soma, 1965) or growth substances (Hussey,1971).

This evidence is consistent with the idea that distribution of assimilates could affect rates of growth in the phytomer and the apical dome in the wheat cultivars and also suggests that growth substances are involved.

D. The transition to flowering

The transition to flowering has been identified with collar initiation in these cultivars. However, at initiation, the collar primordium cannot be distinguished from the leaf or spikelet primordia, and can only be recognised in hindsight by counting the total leaf number produced on the main stem. Therefore should the transition to flowering be taken as some later stage eg. double ridges, where the spikelet primordia can be morphologically distinguished from leaf primordia, or is there no clear cut stage in its development at which the plant has undergone the transition to flowering?

Many of the plants which have been used in studies on flowering can be photoperiodically induced to flower and therefore the transition to flowering can be precisely pinpointed.

An increase in RNA concentration has been observed in the shoot apex of a number of such plants following the transition to flowering eg. Sinapis (Bernier,Bronchart and Kinet, 1970), Lolium temulentum (Evans, Knox and Rijven, 1970) and Silene (Miller, 1976; Miller and Lyndon, 1977). In addition an increase in the rate of cell division often occurs after floral induction (Corson, 1969; Bodson, 1975; Miller and Lyndon, 1975), and in Sinapis, synchronisation of cell division occurred, associated with floral induction (Jacqmard and Miksche, 1971). It was thought that if similar changes in RNA concentration and rate and synchronisation of cell division could be observed in the wheat cultivars, they might aid the identification of the transition to flowering.

In vernalised C.S. Hope 5D, although the length of the cell cycle decreased, it was not a sudden change and there was no increase in the amount of RNA per cell in the apical dome. In C.S. Hope 5A, there may have been an increase in the RNA content just after collar initiation but neither that nor the length of the cell cycle (which did decrease) changed suddenly, and there did not appear to be any synchronisation of cell division at either collar or double ridge formation.

Neither RNA content nor rate and synchronisation of cell division could be used to pinpoint the transition to flowering in the wheat cultivars. Therefore have the events associated with evocation not occurred in these cultivars? It has been pointed out by Heslop-Harrison J. and Heslop-Harrison Y. (1970) that always using plants which are photoperiodically sensitive may lead to a failure to distinguish between environmental control of induction and the endogenous control. A sudden and total alteration in development from vegetative to floral growth is not clearly exhibited in day-length neutral plants, pointing to a gradual advancement towards the reproductive state. This would appear to be similar to the pattern in C.S. Hope 5A and vernalised C.S. Hope 5D where the rate of cell division gradually increased as the plants became reproductive and in C.S. Hope 5A the RNA content gradually increased. However the events of evocation seemingly always observed in photoperiodically sensitive plants did not appear to be essential to flowering in the wheat cultivars therefore could some of these events be photoperiodic effects? In unvernalised C.S. Hope 5D there was apparently no increase in the rate of cell division but the plants were still able to flower.

It has been suggested that the transition to flowering is the irreversible commitment to the reproductive condition (Schwabe, 1959). It was therefore thought that if a stage in the plants' development could be found at which the primordia were irreversibly committed to producing spikelets, the transition to flowering could possibly be pinpointed. This was investigated by means of experiments in which plants were transferred from long to short day conditions. In none of the cultivars were the plants irreversibly reproductive if transferred to short days at the time of collar initiation in long days, and it appeared that it was closer to double ridge formation that the primordia became irreversibly committed to producing a leaf or a spikelet.

In agreement with this, Thorne, Ford and Watson (1968) claimed that spikelet number and final leaf number in wheat could be altered before double ridges but not after. However Seidlova-Blumova (1961), transferred plants from long to short days and observed that the apex was most susceptible to the new photoperiod and hence to the abnormalities of the ear caused by the transfer, in the period from double ridges to the differentiation of the spikelet primordia. This suggests that if the plants are transferred to short days after double ridge formation, abnormalities of the ear can occur but there is no reversion of spikelet primordia to leaves.

It is interesting to note that vernalisation reduced the length of time from planting to double ridge formation (particularly the time from collar initiation to double ridge formation when in long days), and this was also the approximate period over which the primordia were labile. Since this is so, is shifting the collar really irrelevant and is double ridge formation the critical point in the plants' development? Perhaps in the wheat cultivars the transition to flowering should be regarded as double ridge formation and not collar initiation.

The different wheat cultivars reacted in varying ways to the transfer from long to short days. Vernalisation increased the number of labile primordia in C.S. Hope 5D because the minimum leaf number was reduced but the maximum leaf number was approximately the same as in unvernalised plants. Some primordia which became spikelets after vernalistion if the plants were grown in long days, became leaves if the plants were switched to short days at any stage up to double ridge formation. Therefore vernalisation reduced the leaf number produced on the main stem but only if the plants were grown in a favourable photoperiod. In C.S. Hope 5A, the number of labile primordia was much smaller than in vernalised C.S. Hope 5D, which is an example of the genes and cold treatment having a different effect on the apex. (However it should be remembered that C.S. Hope 5A has not received a cold treatment and it is not known whether such treatment might have increased the number of labile primordia, as in vernalised C.S. Hope 5D).

Purvis (1944) showed that carbohydrate, particularly in the form of 0.5% sucrose accelerated vernalisation in rye. Therefore it could be that vernalisation increased the availability of carbohydrate to the shoot apex (which might be a facility naturally present in the spring cultivar - C.S. Hope 5A). Carbohydrate could be a modifier of flowering affecting the floral stimulus in some way (Fontaine (1973) suggested that the rate of movement of the floral stimulus could be affected by the sugar level and the number of leaves present). In long days there is a greater irradiance for the synthesis of carbohydrates than in short days. Even if a plant was vernalised (increasing the availability of carbohydrate to the apex), if placed in short days, there would be less photosynthates thus nullifying any increase in carbohydrate which there might be because of vernalisation, and therefore altering the fate of those primordia which can develop into either spikelets or leaves. Dale and Wilson (1979) suggested that the fate of a primordium once initiated was dependent on the distribution of available metabolites including growth substances, within the apex, between primordia of different ages and the apical dome.

E. Other effects of vernalisation

(a) Dry weight and number of tillers

The dry weight of the cultivars was very similar on the 21st day after planting. The coleoptile tiller was not present in all vernalised C.S. Hope 5D and C.S. Hope 5A plants but was present in all the plants of C.S. Hope 5D and C.S. Euploid. This was, however, the only indication at this stage that vernalised C.S. Hope 5D and C.S. Hope 5A would produce fewer tillers than C.S. Hope 5D and C.S. Eupliod. It is possible that the same number of tiller buds were initiated in vernalised plants as in unvernalised, but failed to develop at a later stage because of e.g. lack of carbohydrates or suppression by auxin. In accordance with the idea of carbohydrates being important, perhaps in unvernalised plants more carbohydrate is transported to the tillers than in vernalised plants leading to a greater number of tillers being produced. Alternatively, if more tillers are present in unvernalised plants they could act as a sink for carbohydrates. Kirby (1973) observed that the leaf and internode growth and final size were greater in a non-tillering single gene mutant of barley than in a freely tillering spring barley genotype.

He proposed that the differences were due to changes in internal competition for assimilates brought about by the absence of tillers. Kirby also considered the possibility that the gene for non-tillering increased the potential growth rate of the main shoot apex and therefore its competitive ability which might lead to a suppression of tillering. However if the shoot apex of the uniculm-mut ant was removed, tillers were not produced indicating that the uniculm-mutant phenotype was not an indirect effect because of suppression of tillers.

(b) Leaf emergence

Vernalisation decreased the number of days to the appearance of leaf numbers 1-4 in C.S. Hope 5D and decreased the days between the emergence of successive leaves.

Purvis and Hatcher (1959) observed an accelerated rate of leaf emergence in vernalised cereals and a reduction in the length of the first leaf. The rate of leaf emergence has been shown to be affected by a number of factors. Dale and Wilson (1978) reported that leaf emergence rate varied between cultivars and was inversely related to the number of leaves produced on the main stem, the greater the final leaf number, the slower the rate of leaf emergence. This was so for vernalised and unvernalised C.S. Hope 5D, but the relationship was less clear cut for C.S. Hope 5A and C.S. Euploid. Kirby and Eisenberg (1966) found that the rate of leaf emergence varied between cultivars and treatments in barley, with a lengthening photoperiod increasing the rate of leaf emergence.

Since both vernalisation and photoperiod can increase the rate of leaf emergence it could possibly be that carbohydrate balance is involved. However this would not really explain why C.S. Hope 5A had a slower rate of emergence than vernliased C.S. Hope 5D. Perhaps the rate depends on a combination of carbohydrate availability and cultivar.

F. Conclusions and indications of further work

The growth of the shoot apex from planting to collar initiation was not affected in any major way by vernalisation. It was not until just before double ridge formation that the effects of vernalisation were manifest at the apex, when the rate of primordium initiation increased, the volume relative growth rate increased and the labile primordia became committed to producing leaves or spikelets. This suggested that the transition to flowering should be regarded as double ridge formation rather than collar initiation. Another indication that the period from collar to double ridge formation was critical in the plants' development was that the reduction in the length of time to ear emergence in vernalised plants was largely brought about by a reduction in this period of growth. Whatever was happening at the apex during vernalisation was not evident at the apex until this period between collar and double ridge formation, which suggested that whatever changes were brought about by vernalisation could not be expressed until the plant was grown in a warmer temperature for a minimum time (i.e. up to the earliest moment that the collar could be initiated).

Most of the events at the shoot apex normally associated with evocation which have been largely observed in photoperiodically induced plants, were not evident in the wheat cultivars. The change which was observed, namely an increase in relative volume/growth rate was not a sudden change, thus suggesting that the transition to the reproductive condition is a gradual process.

The results suggest that growth substances and the amount and partitioning of assimilates are important in vernalisation and the flowering process. The data are consistent with the possibility of a build up of some substance (growth substance?) during vernalisation which prevented the decline in volume relative growth rate of the apical dome, and even allowed the rate to increase.

The foregoing results and discussion have indicated 2 main lines of work which could be pursued. These are to examine (a) the effects of growth substances on the wheat plants and (b) the partitioning of assimilates in the plants.

The effects of exogenous applications of growth substances to the wheat plants could be investigated and combined with an analysis of the naturally-occuring growth substances present in the plants. The levels of growth substances could also be measured at intervals during vernalisation. The plants could be grown at temperatures below 18°C which often increases root growth relative to that of the stems and may consequentlyincrease the level of cytokinins, these being produced in the roots.

The amount of assimilates available to the apex could be varied, for example by growing the plants in various light intensities. The effects on apical growth could be observed in an attempt to relate development to assimilate availability.

It would also be interesting to remove the main shoot apex of the plants to determine whether tiller buds were being supressed or just not produced in C.S. Hope 5A and vernalised C.S. Hope 5D. The result of such studies could have implications of significance to any interpretatic involving growth substances and assimilates, and would enable competitive effects between apices to be assessed.

PART V

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