STUDIES ON TUBERISATION IN THE POTATO, *SOLANUM TUBEROSUM* L.

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SUMMARY

Tuber initiation in a late maincrop variety of cultivated potato (Solanum tuberosum L.) was studied. The two principal areas of investigation were firstly, whether the response of tuberisation to daylength is direct and truly photoperiodic or whether it is mediated by the growth of the tops (haulms) and secondly, the hormonal nature of the hypothetical tuberising stimulus proposed by several workers; studies were also carried out on the histology of the stolon tip immediately prior to the appearance of visible swelling.

Experiments in which plants grown in inductive short days were subjected to light break treatment showed that such treatment causes partial inhibition of tuberisation and that the effect of daylength is a genuinely photoperiodic one. The most likely explanation of the effect of light break treatment was thought to be control of tuberisation by a hormonal stimulus, the formation of which is regulated by photoperiod, among other factors.

Studies on the nature of the proposed hormonal stimulus provided good evidence for the involvement of endogenous gibberellins, as an influence acting to inhibit or delay tuberisation: tuberisation was partially inhibited by repeated applications of gibberellic acid and promoted when the synthesis of endogenous gibberellins was inhibited by CCC, when these compounds were applied either to the plant as a whole or directly to the site of tuberisation, the stolon tip. The level of endogenous gibberellin-like substances in the stolon tip was found to be inversely correlated with inductive daylength conditions and with the degree of advancement of the developmental state of the stolon tip along the path towards tuberisation, the level of

gibberellin-like substances falling as the tip began to tuberise. This correlation extended to the period immediately prior to the onset of visible swelling, in which starch deposition takes place in the tissues of the tip; this deposition occurred in a consistent sequence in the various tissues. It seemed unlikely from the results of the present studies that abscisic acid forms part of the tuberising stimulus (although endogenous growth inhibitor(s) appeared in the stolon tip at tuberisation), although it may act indirectly through effects on overall growth. There was also no evidence to support the suggestion that cytokinins promote tuberisation.

The present work provides support for the theory that the tuberising stimulus consists of a balance between endogenous gibberellins and growth inhibitors (the identity of the latter being unknown). Tuberisation appears to be promoted by environmental conditions or treatments which cause a lowering of the ratio of endogenous gibberellins to growth inhibitors at the stolon tips, although other factors may also be involved.

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N.B. Where the work of other authors is dealt with at any length in parts of the text not dealing specifically with the literature, these paragraphs are indented at the left hand margin.

SECTION I. GENERAL INTRODUCTION

The purpose of the present investigations was to make some contribution to our knowledge of the mechanism of tuber initiation in the potato, Solanum tuberosum, L. The work was mainly concerned with the effect of daylength on tuberisation, and the line of investigation followed led into studies on the contribution of various growth substances in the process; these studies made up the major part of the work.

(i) Review of the relevant literature

A substantial body of literature exists on the induction of tuberisation in the potato, but before briefly reviewing this it is necessary to examine the exact nature of the tuber itself, and its position in the life-cycle of the potato plant.

The potato plant is produced by the sprouting of a bud on a tuber emerging from dormancy. When the plant reaches a certain stage of development, stem structures called stolons are produced from around the bases of the aerial stems. The stolons, which develop in acropetal succession from the base of the plant, are axillary shoots with a hook-like tip, showing negligible leaf expansion and a diageotropic growth habit. It is on these structures that tubers are produced, either at their tips or at the tips of side buds or branches on them.

Histological examination (Artschwager, 1924; Plaisted, 1957; Booth, 1963) has revealed readily-observable differences between the tip of a stolon showing extension growth and the tip of a stolon which is beginning to form a tuber.

The first macroscopic visible sign of tuber initiation is an increase in the diameter of the stolon in the region just behind the tip (i.e. in the region of the sub-apical meristem). The work of Booth (1963) suggests that this is brought about at the cellular level by a change in polarity, first in the direction of cell enlargement and subsequently in the plane of cell division. At first, the cells in the pith and cortex become shorter and wider in the region of the sub-apical meristem, and their volume increases. This initial change is followed by cell division throughout the pith, and also in the cortex and vascular tissues, the divisions taking place in "random" directions for the most part, although most of the divisions in the peripheral cortex appear to take place radially (Artschwager, 1924). Plaisted (1957) states that the growth of the tuber, at least to a size of about 200g. fresh weight, is due more to an increase in cell number than to changes in cell size, and that the rate of growth is highest in tissues inside the vascular ring, that is in the pith and in the storage parenchyma derived by cell division in the internal phloem. The perimedullary zone produces the largest proportion of the tissue of the mature tuber.

In the early stages, growth of the tuber occurs at all points along both the longitudinal and the short axis, with resulting maintenance of the original length/diameter ratio. Later, while growth in width continues more or less equally at all points on the major axis, longitudinal growth of the basal part of the tuber decreases relative to the apical part (Sadler, 1961).

These microscopic visible changes in the stolon tip raise two questions:- firstly, what changes in the metabolism of the pith

and cortical cells of the stolon tip are responsible for initiating the visible changes and, secondly, by what environmental conditions are these changes brought about.

Most of the work on tuberisation in the potato has been concerned with the second of these questions; the responses of the plant to differing environmental conditions have been studied in great detail.

As pointed out by Gregory (1965), the development of the potato tuber may be divided into three phases. At first, changes take place in the stolon tip which result in tuber initiation. Following this, the tuber passes into the stage of tuber enlargement, which is simply a form of growth, and is characterised by extensive meristematic activity and accumulation of reserve food materials, especially starch. Finally, the haulms (tops) die down and the tuber matures and passes into the dormant period, the cycle being completed by the breaking of dormancy, which shows itself by the sprouting of the "eyes" (each consisting of one second order and two third order buds, except in the case of the terminal bud) to produce shoots.

Each of these stages in the growth of the tuber have different optimum conditions, and these conditions also vary with the species and variety of potato. For instance, natural short day conditions bring about earlier tuber initiation than do long day conditions, but the final total yield is often found to be better under natural long day conditions. This is partly because long days tend to bring about better stolon development, growth and branching, leading to a greater number of tubers. Also, under field conditions, where day-

length effects cannot be separated from those due to total light received by the plant, long days provide a better supply of photosynthate (which leads to greater tuber growth); in addition, long days lengthen the duration of vegetative growth and active tuber formation and growth (eg Werner, 1940; Wassink and Stolwijk, 1953; Bodlaender, 1958; Krug, 1960). In some of the older literature, many authors do not clearly differentiate between the different phases of tuber development, but to do so is essential if observations are to be useful.

In the present work, the only stage of tuberisation to be considered is the first one, that of tuber initiation, in which extension growth in the stolon ceases and is replaced by the swelling which is the first visible sign of tuber initiation.

In the normal course of development, for tuber initiation to occur the plant must be in an induced state for tuber formation (Gregory, 1965), induction being attained by exposing the plant for a period of time to a suitable environment (although there are exceptions to this in cases of abnormal tuber formation:- aerial tuberisation, de Vries, 1878; Vöchting, 1887; Bates, 1943; Denisen, 1953; Gregory, 1956; secondary tuber formation, Krijthe, 1955; Lippert, Rappaport and Timm, 1958; premature tuber formation, Schacht, 1856; Wellensiek, 1923, 1924, 1929; Harvey, Reichenberg, Lehner and Hamm, 1944; Krijthe, 1948; van Schreven, 1949).

Gregory (1965) has pointed out that as conditions for induction approach the optimum, less time is required for its occurrence, and that although the production of the induced condition is a reversible process (Gregory, 1954), once the tuber is initiated a point is

reached where the process becomes irreversible. Chapman (1958), however, has shown that under certain conditions, tubers can begin to "grow out" ie to sprout from the eyes soon after they are formed.

It is generally agreed (Slater, 1963) that the time from emergence of the potato plant to the formation of tubers is least under the following conditions (at least for cultivated varieties of S. tuberosum):- short days, low temperatures, high total daily radiation and low mineral nutrient supply.

These different environmental variables interact strongly, but some separation of their effects is possible.

There is a large literature on the effects of daylength on tuberisation in potato (eg Garner and Allard, 1923; McClelland, 1928; Doroschenko, Karpechenko and Nesterov, 1930; Schick, 1931; Werner, 1940; Driver and Hawkes, 1943; Pohjakallio, 1953; Wassink and Stolwijk, 1953; Kopetz and Steineck, 1954; Steineck, 1955, 1956 a and b, 1958; Chapman, 1958; Bodlaender, 1958; Krug, 1960). Almost all workers are agreed that the onset of tuberisation in the cultivated potato is promoted by short days and delayed by long days (including natural summer days). Except in some wild South American species, which have an obligate requirement for short days in order to tuberise, (eg S. demissum, S. acaule, S. bukasovii) short days are not essential for tuberisation, although they cause earlier tuber formation, as they have been found to do in most species of plants which produce tubers (see Gregory, 1965): the effect is quantitative, not qualitative.

The extent of the daylength dependence effect varies considerably

between varieties (eg Schick, 1931) and even between seedlings of single progenies (Kopetz and Steineck, 1954; Steineck, 1956b, 1958). Kopetz and Steineck have suggested that the variation is due to genetically-determined differences in the critical daylength (ie the daylength separating the inductive and the non-inductive daylength ranges as defined by Kopetz, 1937). Generally, the difference in time of tuber initiation in long days and short days is greater in maincrop, and especially late maincrop varieties than it is in early varieties, since the latter are adapted to forming tubers during the longest days of summer. Above the critical daylength, which is high for early varieties and lower for late varieties, tuber growth is inhibited and a large development of foliage is found; below the critical daylength, tuber growth is stimulated and top growth is inhibited (Kopetz and Steineck, 1954; Bodlaender, 1958). Bodlaender (1963) has pointed out that in potato the "critical daylength" is an inexact term, since it covers a range of daylengths above which plants develop long-day characteristics and below which short day characteristics, but within which gradual differences occur.

In short days, stem elongation terminates earlier, and the plants die earlier than under long day conditions. Leaf and stem weight as well as the final number of leaves per stem are lower but leaflets are larger. The leaf/stem weight ratio is higher under short than under long days (Bodlaender, 1963). Top weight is higher under long days irrespective of day or night temperature (Gregory, 1954). In the early stages of development, tuber weight is higher in short days than in long days, although, as mentioned above, plants grown in long days

eventually surpass the short day plants and reach a higher final production despite their later tuber initiation (Bodlaender, 1963).

Temperature has also been found to exert an important effect on tuberisation (eg Bushnell, 1925; Arthur, Guthrie and Newell, 1930; Beaumont and Weaver, 1931; Werner, 1934; Stelzner and Torka, 1940; Davis, 1941; Driver and Hawkes, 1943; Gregory, 1954; Went, 1959; Borah and Milthorpe, 1959, 1962; van Hiele, 1955; Bodlaender, 1960). Tuber formation starts earlier at low than at high temperatures (Bodlaender, 1963); a high night temperature (26°C) depresses tuber formation under both a high and low day temperature. The optimum temperature for tuberisation in terms of yield in most varieties appears to be about 17°C; above and below this temperature, tuberisation is partially inhibited, and tubers are very rarely, if ever, formed above 30°C (Gregory, 1965). The number of tubers per plant is larger at low than at higher temperatures (Borah and Milthorpe, 1959, 1962; Bodlaender, 1960; Gregory, 1954) and bigger tubers are generally formed at higher than at lower night temperatures (Bodlaender, 1960). High temperatures are favourable for stem growth (eg Gregory, 1954, found that stem elongation increased with rising night temperature, with a maximum at 23°C under both low and high day temperatures and short and long days, and that with high day temperature, elongation occurred at a faster rate); they are unfavourable for leaf expansion. Maximum tuber yields are therefore found at intermediate temperatures.

Night temperature appears to exert a greater effect on growth and

tuberisation than does day temperature (eg Gregory, 1954). The influence of temperature, like that of daylength, is found to exhibit inter-specific and inter-varietal differences.

By growing potato plants (S. tuberosum, variety Kennebec) under inductive and non-inductive environments, Gregory (1954) sought to determine the relationships between photoperiod, day temperature and night temperature on growth of tops and tuber initiation. As mentioned above, he found that stem elongation appears to increase with rising night temperature, with a maximum at 23°C, under both low and high day temperatures and both short and long days; with high day temperature, elongation occurred at a faster rate. On the other hand, short days and low night temperatures were found to arrest the growth of the plant, and caused it to mature earlier. At intermediate night temperatures, there appeared to be little effect of daylength on rate of elongation at both high and low day temperatures, but at both high and low night temperatures, rate of elongation was significantly faster under long days.

Gregory found that in short days, there is a wide range of day and night temperatures where a relatively uniform number of tubers are produced, but that high night temperatures (about 26°C) depress tuber formation under both high and low day temperatures. In long days, the temperature range for tuber formation was found to be greatly restricted by a requirement for low temperatures at night, that is as night temperature increases, tuber formation becomes more dependent on short days. In long days, tuber formation is inhibited in a wide range of both day and night temperatures.

Similar results have also been obtained by Bodlaender (1960) with the variety of S. tuberosum Gineke.

Total daily radiation, as distinct from photoperiod, also affects growth and tuberisation of the potato plant. Pohjakallio (1951) found that a decrease in light intensity from 67 to 33 per cent of full daylight resulted in a decrease in the dry weight of the whole plant (38%), in an increase in that of the shoots (57%) and a sharp decrease in tuber weight (80%). Similar results have been found by Bodlaender (1963), who has also shown that stem elongation was much more pronounced at low than at higher light intensities in the range 2,000 to 16,000 lx and that leaf weight was highest at high light intensities. Tuber yields were also larger at higher than at lower light intensities. Tuber formation started earlier, maximum stem length was reached earlier and the plants died earlier at higher light intensities. There was a greater total production of dry matter at high than at low light intensities, and a larger percentage of the dry matter was used in tuber production.

These results have also been confirmed by the work of Slater (1963), which is described more fully in Section IV. Slater found that there was no difference in the dry weight accumulation between long and short days with any one amount of daily radiation; he also found that differences in other components of top growth were small and variable with any one amount of daily radiation. As the amount of daily radiation was increased, the difference in time to tuber initiation in long or short days decreased. These results suggest that the effect of daylength and total daily radiation are separate, and that the effect of daylength on tuberisation is a true photoperiodic one, and not a result of the production of different amounts

of photosynthate under different daylengths. This conclusion is confirmed by an experiment in which tuberisation was delayed by a light break in the middle of the night given to plants grown in short day conditions (see Section IV); similar results have also been found with light break treatment in a wild species (Mokronosov and Lundina, 1959) although these are the only two experiments which have used a light break treatment and few details are available. Borah and Milthorpe (1959) have also found that tuber initiation occurred sooner with increase in the mean daily radiation over the range 70-370 cal./cm.^{2}/day.

Bodlaender (1963) sums up the interactions of temperature, light intensity and daylength as follows:-

High temperature generally stimulates stem growth and inhibits leaf and tuber development, especially at low light intensities. As light intensity increases, stem elongation is inhibited and the optimum temperature rises. The influence of high temperatures may be counteracted to some extent by short days, leading to shorter stems, larger leaves and earlier tuber formation. With very low light intensities and long days, stems become very elongated and very few tubers are produced. Temperature, light intensity, and sometimes also daylength, show optimum curves for the various growth components.

It therefore appears from the literature that low temperature, high light intensity and short days accelerate the development of potato plants - stem elongation terminates early, tuber initiation begins early and the plants die early. The plants have small stems and large leaves and tuber growth is stimulated. On the other hand,

high temperature, low light intensities and long days promote stem elongation but inhibit leaf expansion and delay tuber formation.

There is therefore a great deal of information concerning the effect of environmental conditions on tuberisation. Much less information, however, is available concerning the first of the questions posed at the beginning of the Introduction, namely, what changes in the metabolism of the pith and cortical cells of the sub-apical region of the stolon tip are responsible for initiating the visible changes which take place at tuber initiation?

Many different theories have been advanced to explain how tuber initiation is brought about; these fall into two groups. The first group comprises those theories which attribute tuberisation to a high carbohydrate level. De Vries (1878~~a and b~~) suggested that the formation of tubers was due to an increase in the concentration of nutrient substances as a result of loss of moisture. Wellensiek (1924~~a and b~~, 1929) has also proposed this explanation for tuber formation on sprouts of old tubers; he considered that an increase in the concentration of metabolites arose from the loss of water by the tubers during storage, and especially following de-sprouting. Oortwijn-Botjes (1927), however, was able to obtain tuberisation of the last-formed sprouts of successively de-sprouted tubers stored in darkness and constantly supplied with water, thus throwing doubt on Wellensiek's explanation. Vöchting (1887, 1900) and Molliard (1915, 1920) also proposed that the formation of tubers was caused primarily by the concentration of metabolites such as sugar.

A great deal of work appears to confirm the idea that tuberisation is associated with high carbohydrate levels in the plant. Factors which raise the carbon/nitrogen ratio also favour tuberisation. These results have been found both in experiments with whole plants (eg Wellensiek, 1929; Werner, 1934; Driver and Hawkes, 1943; Borah and Milthorpe, 1959, 1962; Milthorpe, 1962; Headford, 1962; Bodlaender, 1963) and with cultured pieces of plants (Bernard, 1902; Magrou, 1938, 1939; Gregory, 1956; Mes and Menge, 1954; Okazawa, 1955; Borah, 1959) although Chapman (1958), using cultured node-pieces, found that varying the nitrogen level of the medium exhibited no effect on the rate of tuberisation.

There have been various speculations on the effect of respiration rate on the growth of the plant and the connection of this with tuberisation.

Driver and Hawkes (1943) suggested that excessive respiration, brought about either by long days or by high temperatures, diminished the quantity of carbohydrates available for translocation to the underground parts and therefore reduced the growth of tubers.

Bushnell (1925) proposed that the failure of several American potato varieties to form tubers at temperatures of 26-29°C was due to the much greater increase in respiration rate than in photosynthetic rate with increase in temperature and therefore to a small amount of carbohydrate being available for tuber initiation; he substantiated his idea by his finding that plants grown at high temperatures had a low carbohydrate content compared with plants grown at lower temperatures. Other workers, however, were not satisfied with Bushnell's conclusions. Slater (1963) has pointed

out that in the potato, maximum vegetative growth occurs at 25°C and that losses from respiration at this temperature are usually only about one fifth of the income from photosynthesis. Also, according to the calculations of Burton (1966) from the results of Winkler (1961), it would seem that the leaves of a turgid potato plant might produce a net surplus of carbohydrate up to a temperature of nearly 34°C, and certainly at 29°C, the temperature at which Bushnell found no tuber formation.

The key to the question appeared to be that top growth is potentially faster the higher the temperature, over the range considered, and tends to consist disproportionately of stem growth. Therefore, although there may be a net exportable surplus of carbohydrate in the functional leaves, it is all used in the young plant for top growth, especially stem and branch growth.

Bodlaender (1963) has come to a similar conclusion. He showed that the ratio of leaf weight to stem weight fell with rising temperature, and, coupling this with the fact that tuber initiation was earlier at low temperature than at high temperature, he suggested that temperature might exert its effect by differentially influencing different organs and the partitioning of nutrients.

Gregory (1954) used the ratio $\frac{\text{weight of tubers}}{\text{weight of tops}}$ to compare the conditions for optimal weight of tops with those for optimal tuber weight; these ratios are also a measure of the efficiency of the plant in converting photosynthate into tuber growth (see Table 1). He found that the ratio of $\frac{\text{weight of tubers}}{\text{weight of tops}}$ fell as the night temperature was raised with both short and long days and with both high and low day temperatures. The data suggests (see Table 1)

TABLE 1. Ratio for weight of tubers to weight of tops as affected by day length, day and night temperature in *S. tuberosum*, variety Kennebec (after Gregory, 1954).

Night temp (°C)	SD, day temp. (°C)		LD, day temp. (°C)	
	17	30	17	30
10	6.87	3.15	0.54	0
17	2.22	1.16	0.03	0
23	0.29	0.05	0	0
26	0	0	0	0

SD = 8 hour photoperiod
LD = 16 hour photoperiod

that a far greater proportion of the photosynthate formed in the aerial parts of the plant is used for top growth rather than for tuber growth with high temperatures and long days, conditions which are less favourable for tuberisation. The data also indicate that there is no relation between plant size and tuber yield. It therefore appears that the growth of the tops and the initiation of tubers are intimately inter-related and that it is not simply the overall carbohydrate level in the plant which is important, but its distribution between tops and tubers.

It appeared that a fuller understanding of the growth of the potato plant and the ways in which it is influenced by temperature might be obtained by following the correlations in growth of the different organs during ontogeny. Borah and Milthorpe (1962) did this and showed that the growth of the whole plant before tuber initiation was maximum at 25°C and that the difference between photosynthesis and respiration per unit weight of tissue was greatest at this temperature. In the face of this result, Bushnell's suggestion that there is little carbohydrate remaining for growth at high temperatures does not hold, at least up to 25°C. Borah and Milthorpe showed that there is more assimilate available at 25°C than at lower temperatures, but that a greater proportion of this was used in stem, root and stolon growth at this temperature. They concluded that this, together with the faster growth rate of all tissues, implied a lower concentration of translocated carbohydrate at the stolon tips of the plants grown at high temperatures, and suggested that tuber initiation was associated with those conditions which lead to a higher concentration of soluble sugars in the stolon tip; this condition could also be expected under conditions of high

rather than low radiation. The results they obtained suggest that tuber initiation was indeed associated with those conditions which lead to a high concentration of soluble carbohydrates at the sites of tuber production. Borah and Milthorpe, in contrast to many other workers, dismissed the influence of photoperiodic effect as negligible, emphasising the "substrate" stimulus as the only factor of importance. (This may have been due to the fact that they used Arran Pilot, a first early variety, for many of their experiments; such varieties do not normally, as mentioned before, exhibit such a marked response to daylength as do maincrop and especially late maincrop varieties.)

Burt (1961, 1964a) measured the changes in the concentration of soluble sugars at the stolon tips in an experiment in which groups of plants grown at 25°C were transferred for one week to temperatures of either 3°C or 9°C. At the end of the low temperature treatment, the soluble sugar content of the stolon tips rose markedly, then returned to normal, and tubers were formed. Burt, like Borah and Milthorpe (1962), considers that tuber formation is associated with a high concentration of soluble sugar in the stolon tip. He also found starch in stolon apices.

Lovell and Booth (1967), however, found that delay in tuber initiation brought about by gibberellic acid application was not associated with a low concentration of carbohydrates in the stolon; gibberellic acid treatment gave longer stolons which, in the first 3 weeks after treatment, had a higher total sugar content than those of the untreated plants. They did find, nevertheless, that starch synthesis in the stolons was closely correlated with the onset of tuber formation, and that "gibberellic acid treatment

delayed tuber formation and hence delayed the onset of starch deposition". In translocation experiments in which $^{14}CO_2$ was fed to single leaves of plants just starting to tuberise, treatment with gibberellic acid a few days earlier almost stopped translocation to the stolons and tubers, the predominant site of accumulation in untreated plants, and switched translocation to the upper shoot and shoot apex. Although these results may appear to be consistent with the carbohydrate theory of tuber initiation, Lovell and Booth do not interpret them in this way (see below).

Many other workers (eg Vöchting, 1900; Werner, 1934; Driver and Hawkes, 1943) have also attributed tuberisation to an accumulation of nutrients above the level needed for respiration and growth. The opinion general among them is that tuber formation has a lower "priority" on available photosynthate than does shoot growth, and that favourable conditions for tuberisation exert their effect by supplying photosynthate in excess of the demands of the shoot.

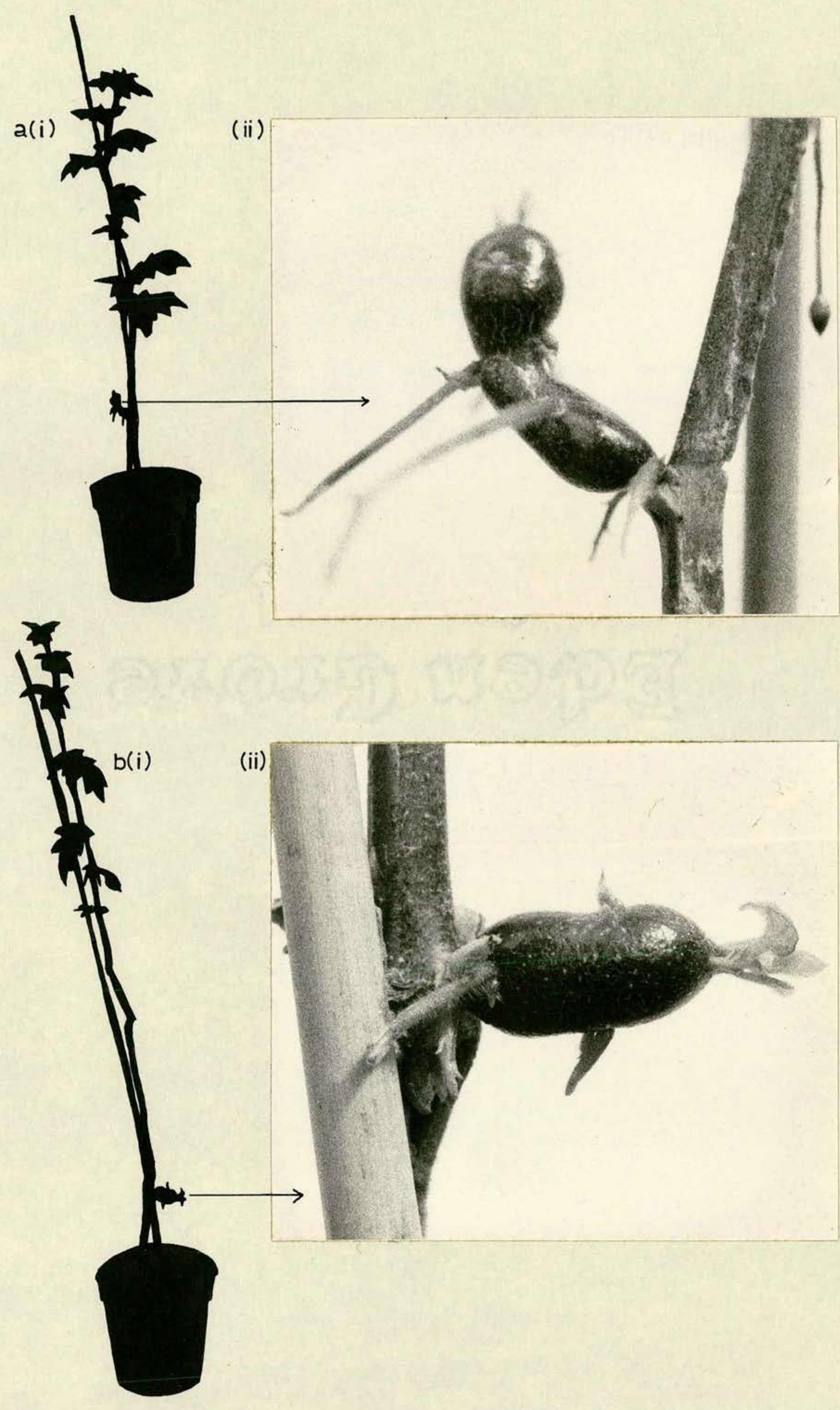
The idea that the distribution of carbohydrate can be achieved by competition between sinks of varying activities does not, however, account for all the facts. Tuberisation is not necessarily confined to the underground parts of the plant. Under certain conditions (if the plant is damaged or is grafted on to a stock of another *Solanum* species so that normal tuberisation cannot take place), tubers can be formed on the aerial parts (see Fig. 1). It appears that, under certain conditions, any bud, even one near the shoot apex, can elongate to form, instead of a leafy branch, a stolon and subsequently a tuber.

The other group of theories of tuberisation propose the involvement

FIGURE 1. Abnormal tuber formation at nodes on aerial parts of plants consisting of potato scion grafted on to tomato stock.

a. (i) diagram of whole plant No. 1
   (ii) detail showing tuber in leaf axil

b. (i) diagram of whole plant No. 2
   (ii) detail showing tuber at node.


a(i)
(ii)
b(i)
(ii)

of a specific hormone-like, transmissible factor for tuberisation and consider that, while carbohydrate is obviously necessary for tuber growth, the actual stimulus is not a certain level of sugars, but a more specific tuber-forming substance.

Several early workers have proposed from studies on various tuberous plants, the existence of a special tuberising stimulus (eg Doposcheg-Uhlár, 1911; Zimmerman and Hitchcock, 1936; Dostál, 1945). The principal recent exponents of the hormonal theory of tuberisation in the potato are Gregory (1956), Chapman (1958) and Madec (1963).

Gregory and Chapman, using cuttings from Kennebec and Triumph potato plants respectively, grown under different environmental conditions, found that induced cuttings from all parts of the plant formed tubers from axillary buds at the lower nodes, whereas non-induced cuttings formed vegetative shoots from axillary buds when the plants were subsequently kept in non-inducing conditions. In Chapman's experiments, inducing and non-inducing conditions consisted of short and long days respectively, the long days having a supplementary daylight period of very low intensity light, which Chapman considers to have been more or less photosynthetically inactive.

In Gregory's experiments, both daylength and temperature were different in inducing and non-inducing conditions, which consisted of short days with low temperatures and of long days with higher (night) temperatures respectively.

Gregory and Chapman have interpreted their results from these experiments to mean that the proposed tuber-forming stimulus is

present throughout the plant, but only under inducing conditions. They have also concluded that the stimulus moves mainly in a basipetal direction: this is indicated by the fact that it was almost always the basal bud of a cutting which developed first into a tuber no matter from which part of the plant the cutting was taken. Also, sub-apical cuttings were found to require a longer induction time than apical cuttings.

Gregory has also shown that the induced state can persist in an induced cutting, even after some time in non-inducing conditions, although the induced state was found to disappear fairly rapidly from intact plants which were placed in a non-inducing environment. He has also demonstrated a quantitative relationship between the duration of induction and the amount of tuber-inducing factor produced in the plants.

Gregory (1956) conducted other experiments which demonstrated that tubers could also be induced on one-node stem-pieces without leaves, grown in sterile culture. He first demonstrated that sugar was necessary for the production of tubers using one-node pieces taken from induced plants and grown on agar with different sucrose concentrations. No tubers were formed on pieces grown on agar alone, but tubers were formed on the pieces in all the sucrose treatments after about 4 days, the size of tubers formed being in proportion to the amount of sucrose supplied. He then showed that sugar alone was not sufficient to bring about tuber initiation; for this, he used node-pieces from non-induced plants. Vegetative shoots were formed after about 4 days in the cultures given suitable sucrose concentrations, but tubers were only formed after a very long time (about 26 days), contrasting with the response in

stem-pieces from induced plants, which began to show tubers after only 4 days. These experiments showed that a single axillary bud, without the leaf, from an induced plant, is capable of forming a tuber provided it is supplied with sucrose. Gregory points out that with the non-induced stem cuttings planted in agar alone, not only tuber formation was absent, but shoot development as well. When sucrose was added to the medium, however, shoots developed rapidly, indicating that sucrose is required for shoot development also. He considers that the fact that buds from non-induced stem pieces developed shoots, and those from induced stem-pieces formed tubers when sucrose was added is indicative that sugars are required for the growth of the shoot and of the tuber, but not responsible for the actual tuber induction. Similar results have also been obtained by Okazawa (1955) with sprouts of different physiological ages: in the presence of sugars, segments of young sprouts do not tuberise, but those of aged sprouts do form tubers.

Gregory considers that in the intact plant the axillary buds may be in an induced state, but that tuber initials do not normally develop into visible tubers because all the available photosynthate is translocated to the actively-growing tubers beneath the ground: that this may be the case is suggested by the observation of aerial tubers in field-grown potato plants affected by diseases interfering with the movement of food to underground parts.

Gregory (1956) has pointed out that one of the most important pieces of evidence for a flowering hormone is derived from grafting experiments in which an induced plant can cause the formation of flowers when grafted on to a non-induced plant, even when both plants are then kept under non-inducing conditions. (see eg

Zeevaart and Lang, 1963; Salisbury, 1963). Gregory found that non-induced potato stocks on to which induced scions were grafted produced tubers when kept under non-inducing conditions, but that non-induced scions did not produce this result; the control stocks formed no tubers, but instead developed shoots from their leaf axils. This result appears to indicate that a tuberising hormone, similar to the flowering hormone, is produced in potato tops when grown under suitable environmental conditions. Chapman (1958) has found similar results with grafted cuttings. In addition, he found that when scions grown in long days (non-induced) were grafted on to tuberised stocks which had been grown in short days (induced), the tubers began to grow vegetatively again, forming stolons from the eyes of the tubers. It seems from the results of all these experiments that tuber-formation could be due to the translocation of some tuber-forming material from the induced scions to the stocks.

Chapman has also conducted experiments (Chapman, 1958) in which different parts of the plant were subjected to different daylength treatments to attempt to locate the site of production of the stimulus. He found evidence suggesting that the stimulus is formed in the apical part of the shoot (results of differential daylength treatment given to the terminal part alone were the same as when given to the whole plant) and that there is very little lateral movement of the stimulus on its way down the stem (differential daylength treatment of plants with two stems resulted in unilateral tuber formation on the half of the stolon system below the stem given short day treatment). He also found that plants which had had the basal leaves removed tuberised first.

Okazawa and Chapman (1962) have shown that the stimulus still travels from the growing point to the stem base even if the growing points are trained downwards so that they are below the level of the base.

Madec and Perennec (1959) found that tomato scions were incapable, when grafted on to potato stocks, of causing the initiation of tubers on the stocks. They also found that the subsequent grafting of induced foliage of potato on to the tomato foliage of such grafted cuttings (resulting in a potato/tomato/potato double-grafted cutting) allows them to form tubers. This result has been confirmed by Okazawa and Chapman (1963). Okazawa and Chapman also found, however, that the tuberising stimulus from a double-grafted potato scion could only pass through the tomato interstock if this did not bear young leaves; when the interstock bore young leaves, tuberisation of the potato stock was inhibited whether or not there was a supply of tuber-forming stimulus from the potato top scion. They also found that tomato and eggplant scions with young leaves were capable of <u>inhibiting</u> tuberisation in potato stocks. It therefore seems that it is impossible to induce tuberisation in potato by using scion tissues which are not of potato, suggesting that the stimulus which brings about tuberisation is specific to potato, but that something is produced in the young leaves of other Solanaceous plants which can inhibit tuberisation in potato.

Another type of experiment which has been carried out following up the analogy of the flowering hormone is one by Madec (1961) in which 0.2-0.35 of raw, filtered sap, extracted with a press from induced plants of the variety Bintje, was injected into the

medullary space of the basal internode of non-induced cuttings of the variety Ackersegen. This is comparable to an experiment carried out by Harada (1960) to investigate the flowering hormone in *Chrysanthemum* and *Rudbeckia*. Madec found that tubers were produced much sooner in plants injected with sap from induced plants than in the controls, in which the injection consisted of sap from non-induced plants. Other experiments have also suggested that the inducing substance is thermo-stable, since sap of induced plants remained active after boiling for 5 minutes. These results are perhaps not wholly convincing because sap was injected into a space which is normally empty, and this alone might conceivably cause some effect on the plant's metabolism. Simmonds (personal communication) was unable to stimulate tuberisation by this method. Edelman (1963) has pointed out that the results of Madec's experiments could be explained on the basis that sap from the induced plants inhibited growth of the cuttings, thereby deflecting photosynthate into tuber formation. Madec (1963) doubts this, however, since tomato foliage and non-induced potato leaves would not substitute for induced potato leaves. It is also conceivable that tuberisation could be brought about by the presence of a high level of photosynthate in the injected sap of induced plants, but the volumes used were so small that this seems unlikely.

Two other types of experiment also lend support to the theory of a hormonal tuberising stimulus. The first of these are the experiments of Mokronosov and Lundina (1959) and Slater (1963) (mentioned earlier in the context of the effects of daylength and light intensity and discussed in more detail in Section IV), in which a light break treatment given to plants grown in short days

was found to prevent or delay tuber initiation without much detectable effect on the growth of the tops. It is difficult to explain these results on the basis of carbohydrate levels in the plant.

In the second type of experiment, Burt (1964b) and Nösberger and Humphries (1965) have demonstrated that the removal of developing tubers depressed the net assimilation rate (E_A) of the parent plant. These results suggest that the movement of carbohydrates to the tuber is caused by a "pull" to an active sink (the developing tuber) such as would be expected if a hormonally-induced metabolic change had occurred there. This is difficult to explain on the theory that tuberisation is the result of the build-up of high levels of carbohydrates at the stolon tips, caused by conditions which depress the growth of the shoot, because this suggests a "push" of carbohydrate from the leaves to a passive sink (the tuber) which would then presumably continue even if the tubers were removed.

Lovell and Booth (1967) have also interpreted the results of their studies on the basis of hormonal control of the initiation of tuberisation. Gibberellic acid treatment was found to switch translocation of labelled carbohydrate from the stolons and tubers to the upper shoot and shoot apex. Although gibberellic acid treatment had little obvious morphological effect on the shoot apical region during the period of the experiment, it was clear from observation of plants similarly treated and allowed to continue growth that they later showed enhanced elongation of the upper shoot region. The increased metabolic activity of shoot apical regions brought about by gibberellic acid treatment might

have been expected to act as a sink for labelled carbohydrate. They consider, however, that it is unlikely, since the new more-active shoot sink would have been competing with tuber sinks already in operation, that the appearance of the shoot sink would have cut down translocation to the tuber sink as markedly as was found to be the case. They have suggested, instead, that the development of the shoot sink following gibberellic acid treatment is accompanied by, rather than results in, a switching-off of the tuber sinks by changing the nature of growth at the stolon tip, such that starch deposition and tuber bulking is suppressed, leading to the eventual outgrowth of the tuber as a stolon. In summary, the translocation pattern is held to reflect the activity of the various sinks, this activity being hormonally controlled.

This idea that the level or distribution of carbohydrate and its connection with the state of dormancy is controlled by some other factor is also supported by the results of van Schreven (1956) who has observed tuberisation of sprouts in darkness after feeding a series of different substances to the mother tubers. He found that early formation of daughter tubers on sprouts was correlated with an increased ratio of soluble carbohydrates to soluble nitrogenous substances in the mother tubers and in the sprouts. This ratio was found to be higher in tubers which had been stored at a high temperature than in those stored at a low temperature. Despite this, feeding of sugars (glucose, sucrose) or of nitrogenous substances (tyrosine, asparagine, glutamic acid) were both found to retard tuberisation of the sprouts. None of the substances fed greatly affected the dates of tuberisation of the sprouts of tubers which had been stored at either low or high temperatures, but the

times of tuberisation of sprouts on mother tubers stored at the different temperatures were markedly different. This suggests that none of the substances used is the real stimulus which brings about tuber initiation, but that this is some other factor or factors which is produced, or destroyed, at different rates in mother tubers stored at different temperatures.

There seems, therefore, to be a strong body of evidence to suggest that tuber initiation is brought about by a tuber-forming stimulus, manufactured in the tops and translocated to the stolons in the growing plant (or made, or stored and destroyed, and translocated to the sprouts in stored mother tubers).

Some workers, however (eg Borah and Milthorpe, 1959; Milthorpe, 1962; Headford, 1962; Burton, 1966) have remained unconvinced of the existence of a specific tuber forming stimulus.

Burton (1966), for instance, considers that the available evidence, with the possible exception of that of Madec (1961) is not inconsistent with tuber initiation as a result of the translocation of excess carbohydrate to the stolons and has suggested (Burton, 1963) that tuber formation follows the cessation of axial growth in the stolon as a result of accumulation of a substance or substances produced during the operation of the respiratory cycles, its concentration being related to the rate of respiration and therefore to the supply of carbohydrate substrate to the stolon tip. He considers that this suggestion may be reconciled to the results of Gregory, Chapman and Madec if it is postulated that the substances are produced both in the foliage and in the stolon in the course of respiration. Their accumulation in the stolon may be a result of

both their translocation as such from the foliage or their excessive production at the stolon tip, following the accumulation in it of carbohydrate translocated from the foliage. It is difficult to reconcile this suggestion with the inhibiting effect of high temperatures on tuberisation, since at high temperatures, respiration would be expected to proceed more rapidly, and therefore to lead to the production of a greater amount of Burton's proposed substance. Burton (1966), however, appears to have modified this theory to include gibberellins and inhibitors of axial growth in his "substances which are related in amount to the activity of the respiratory cycles", although he does not make it clear how he considers that the balance of these substances is affected under different environmental conditions.

It could be argued that the results of Gregory and Chapman with grafted potato plants in which tubers were found on non-induced stocks after the grafting on to them of induced scions, may be explained by the possible presence in induced scions of large amounts of carbohydrate available for use in tuber growth. It is difficult to understand, however, if this were the case, why tuberisation could not then be brought about by tomato scions (Madec, 1963) grown in suitable environmental conditions, unless the balance of different types of carbohydrate produced in tomato foliage was in some way unsuitable. Even more difficult to explain on the basis of carbohydrate levels, is the observation of Okazawa and Chapman (1963) that a tomato interstock with young leaves did not allow the induction of tubers by an induced potato scion double-grafted on to it, unless the available carbohydrate was used for growth by the young leaves on the interstock.

There remains one other factor to be considered. Madec and Perennec (1962) have pointed out that the photoperiodic, or other environmentally controlled, induction of tuberisation may be modified and supplemented by the inducing effect of the mother tuber. It is quite possible for young tubers to be produced in the complete absence of foliage (eg Gregory, 1956) or before emergence of the foliage above the ground (see Gregory, 1965). Also, Madec and Perennec (1959), in their grafting experiments of tomato on potato, in which grafting was followed by propagation of cuttings, have shown that the mother tuber participates in the tuberisation of the plant and can by itself induce it. They demonstrated that grafted cuttings separated very early from the mother tuber and propagated, had not tuberised by the time grafted cuttings separated much later had initiated tubers; the enlargement of tubers was assured by the products of photosynthesis supplied by the tomato foliage, which was inactive in inducing the formation of tubers. Madec and Perennec (1959, 1962) have also shown that the effects of the foliage and of the mother tuber on tuberisation are additive. This suggests, they propose, that the tuberisation factor synthesised by the foliage is the same as that produced during "incubation" of the mother tuber.

Madec (1963) thinks that it is possible to combine the two main groups of theories which have been formulated to explain tuberisation of the potato, and that these are in fact complementary and not incompatible. He has pointed out what should perhaps by now be obvious: that, "after the preliminary induction of the faculty to tuberise, the growth of the tubers also depends on

the supply of necessary metabolites; it is not surprising therefore that the factors acting on this supply could have effects on the manifestation of tuberisation". He considers that it is at this point that factors such as water supply, mineral nutrition and photosynthesis exert their effect; he further proposes that temperature and even photoperiod may have their effects to some extent on this part of the tuberisation process.

It therefore appears possible and likely, although all workers are not in agreement on this point, that tuberisation is brought about by some sort of stimulus, which can be supplied to the stolons from both the tops, when grown in suitable inductive conditions (of which photoperiod and temperature seem to be the most important), and/or from the mother (seed) tuber. The influence from the latter appears to be always promotive of tuberisation. The nature of the stimulus, and the methods of its production in the plant and its action in initiating tubers are, however, very incompletely understood.

(ii) Plan of the work

From the foregoing review of the literature it may be seen that there are, among others, two principal areas where our knowledge needs to be expanded. The first of these is the question of whether the response of tuberisation to daylength is direct and truly photoperiodic, or whether it is mediated by the growth of the tops. The second is that of the hormonal nature of the hypothetical tuberising stimulus.

After some preliminary experiments which were carried out to establish growing and treatment conditions for the material

(Section III), the work was mainly concerned with these two areas of investigation. Some histological examination of the stolon tip immediately prior to and at the time of the onset of visible swelling was also carried out (Section VI).

The photoperiodic nature of the daylength response of tuberisation was investigated in the experiments described in Section IV; Section V comprises experiments concerning the hormonal nature of the stimulus.

Various growth-active substances have been suggested as being concerned in the initiation of tuberisation, either as the tuberising stimulus itself, or as a component of it, or as an antagonist of its action. Possible contributions of these substances are discussed in Section V (i) which comprises, as well as an introduction to the experiments involving growth-active substances carried out in the present work, a review of the substantial literature on this subject. It was felt that such an introduction and literature review was necessary in the case of Section V in order to point out the reasons for the various lines of investigation followed in this part of the work. It was also felt that this introduction should be placed immediately prior to the experiments to which it refers, both to preserve the logical sequence of the thesis, and also because it is particularly relevant only to that section (V). For this reason it does not form part of this General Introduction.

This policy is also pursued in the other sections concerned with experimental results (IV and VI), although the introductions involved are much shorter than that for Section V. In Section IV,

the introductions concerned with the different types of experiment are given separately in their relevant positions with respect to the descriptions of the experiments.

Throughout the thesis, the details of the results of each experiment or group of closely related experiments are discussed in the account of the experiment(s) in the appropriate section. The more general implications of the results and the ways in which these may be related to the results of the other experiments carried out, and also to those of other workers, are further discussed in Section VII (General Discussion).

SECTION II. MATERIAL AND GENERAL METHODS

(i) Material

a. Requirements

So as to be certain of obtaining material at all times, it was considered advisable to use a fairly common variety of cultivated potato (*Solanum tuberosum* L.) which could be purchased commercially, rather than a wild potato species, even though some of these have an obligate requirement for short days for tuberisation (see Section I), because this would have to be grown entirely in the Botany Department.

Because of their relatively clear-cut tuber initiation response to daylength, a late maincrop variety of *S. tuberosum* was considered to be most useful for the present work (see Section I).

Other considerations were availability of virus-tested stocks, and a fair resistance to the commoner potato diseases. A fairly long tuber with well-spaced eyes was also required, so that the maximum number of tuber pieces of uniform size containing one eye could be obtained from each tuber, in view of the growing technique established in Section III (see below).

b. Variety selected

The commercial variety which best seemed to fulfil these requirements was "Up-to-Date" (UTD).

Initial experiments were carried out using Golden Wonder (GW), which is also a late maincrop variety, but difficulty was experienced in obtaining it virus-free so that it was not used in later experiments some other varieties were also used

initially (Kenyan varieties Roslin Samura and 3751/5 produced at the Scottish Plant Breeding Institute at Pentlandfield, Midlothian), but these were found to grow less uniformly under controlled environment conditions than did Up-to-Date.

c. Suppliers and storage

Some material was kindly supplied by the Agricultural Scientific Services Official Seed Testing Station at East Craigs, Corstorphine, Edinburgh, from their virus-free stocks of Up-to-Date. Material was also obtained from Buchan Potato Growers Ltd, 20 Commerce Street, Fraserburgh, Aberdeenshire. The highest quality commercially-available grade (FS) of virus-tested tubers was purchased.

The remainder of the material used was grown either in the glass-house or in the garden at the Botany Department of the University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh, from the stocks from the above two suppliers, or from stored tubers produced in the Botany Department in the previous year.

Each experiment used material from a single source, and there was therefore no variation within experiments which was due to the source. To determine whether there was any possible variation between experiments due to the source, graphical analyses were carried out, plotting various parameters against age of the plant for plants grown from tubers from various sources. The results of these analyses are given in Section VIII.

Initially, potato tubers were stored in polythene bags, with or without dry sand, in cardboard boxes in a cold room maintained

continuously at a temperature of $2^{o}C$ ($\pm 1^{o}C$). Some rotting was found under these conditions and the sprouting eyes tended to turn black, presumably due to frosting. A small wooden potato store was therefore constructed out of doors, with a heater and thermostat arranged so that the temperature never fell below $5^{o}C$. The tubers were laid on metal trays with a space of about 150 cm between the potatoes on one tray and the tray above for circulation of air. These conditions of storage seemed to be more successful, although the potatoes tended to sprout during the spring and early summer months. After a large harvest of stocks in the garden or glasshouse, when this store was full, some of the material was stored temporarily in cold frames out of doors. Each experiment used material which had all been stored under one set of conditions.

(ii) General growing conditions

The general growing methods employed were developed from the results of the preliminary experiments described in Section III.

The plants for all the experiments were grown under controlled environment conditions, achieved by the use of large walk-in type growth rooms with facilities for controlling automatically the daylength, temperature and humidity.

a. Lighting conditions

Light regimes

Three light regimes were employed. During the time after planting when the plants were being grown up in preparation for an experiment (referred to as the growing period), the plants were given the maximum possible amount of light for photosynthesis

and the light period (day) consisted of 18 h of fluorescent (Fl) light, supplemented by tungsten (T) light to supply the longer wavelengths. The remaining 6 h of the 24 h cycle consisted of darkness (night). This regime will be referred to as high intensity long days (HI LD).

After the growing period, which lasted for about 35 days, by which time the plants had reached a height of about 10-15 cm, they were transferred to the experimental conditions. These were of two types:

1. Non-inductive long days

and 2. Inductive short days

In the non-inductive long days, the day consisted of 8 h of fluorescent plus tungsten light, followed by 10 h of low intensity tungsten light, making a total of 18 h of light; this was followed by a 6 h dark period. This regime will be referred to as low intensity long days (LI LD). It was used for long day conditions during the experimental period so that plants in short days and long days received approximately the same total amount of high intensity light.

The intention of this was to eliminate as far as possible any overall nutritional effects (due to photosynthesis) of the day-length differences and thus ensure that any differences between plants grown in short days or long days were due to a direct response to photoperiod. The studies of Hofstra, Ryle and Williams (1969) on the effects of daylength extension with low intensity light on growth have shown that the energy supplied by such supplementary light can make a significant contribution.

to carbon assimilation. Nevertheless, the regime used was certainly preferable in this respect to the high intensity long day regime.

Although both long day regimes may be regarded as non-inductive to tuberisation, tubers do eventually form after many weeks on plants kept in such conditions, the response to daylength being (see above) quantitative and not qualitative in cultivated varieties of S. tuberosum. The time taken to initiate tubers is, however, usually a great deal longer than that required under the conditions referred to as inductive.

The inductive regime used during the experimental period consisted of a day of 8 h fluorescent plus tungsten light, followed by a night of 16 h darkness. This regime will be referred to as short days (SD).

The combination of low intensity long days and short days used during the experimental period is referred to for convenience as "differential daylength conditions".

The different daylength regimes are shown in Fig 2.

Types of Light used

The fluorescent lights used were Mazda 5 ft 65/80 W T 12 "Warm White" tubes, and the tungsten lights were 40 W bulbs. The emission spectra for fluorescent light alone and for fluorescent and tungsten light together are shown in Fig 3. The total light energy supplied by fluorescent plus tungsten sources together was 80.3-129.1 W/m^2 in the wavelength range 400-700 nm at soil level in the pots on the growing table; the intensity of the tungsten

FIGURE 2. Daylength regimes used in growing period and experimental (differential daylength) period.

HI LD = high intensity long days
LI LD = low intensity long days
SD = short days
Fl = fluorescent light
T = tungsten light

GROWING PERIOD

18h FL+T

6h dark

(HI LD)

EXPERIMENTAL PERIOD

8h FI+T

10h T

6h dark

(LI LD)

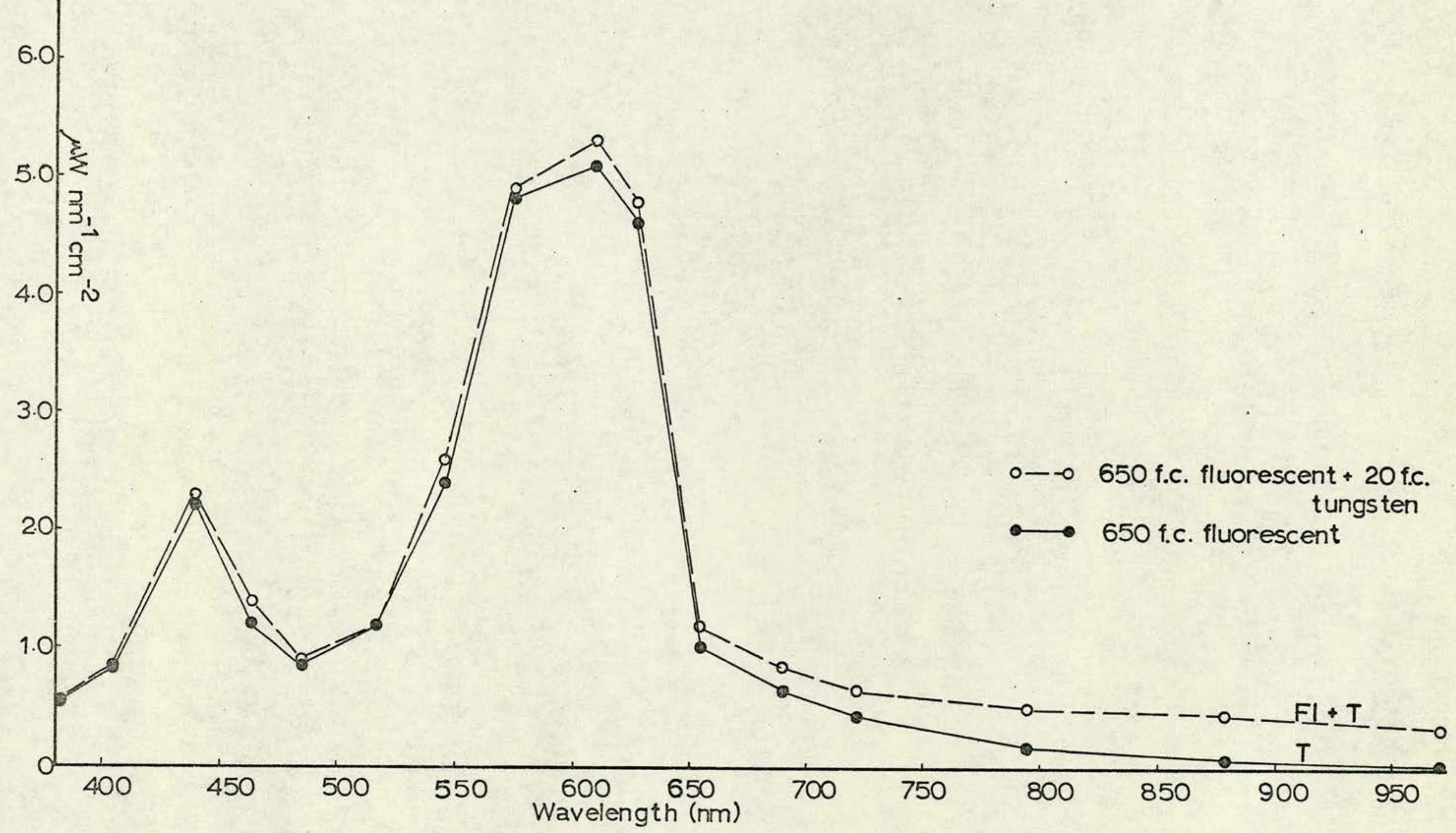
8h FI+T

16h dark

(SD)

FIGURE 3. Emission spectra of fluorescent and fluorescent plus tungsten light sources.

T = tungsten light alone
Fl + T = fluorescent and tungsten light together



μW nm⁻¹ cm⁻²
6.0
5.0
4.0
3.0
2.0
1.0
0
400
450
500
550
600
650
700
750
800
850
900
950
Wavelength (nm)
650 f.c. fluorescent + 20 f.c. tungsten
650 f.c. fluorescent
Fl + T
T

lights alone at this level was about 6.1 W/m^2 in the range 400-700 nm.

These intensities fell slightly as the lights aged. Replacement of fluorescent lights was carried out regularly (after approximately 3000 hours burning time), and the tungsten bulbs were replaced individually as necessary. The illumination level varied to some extent over the surface of the table, but plants were routinely randomised to overcome the effect of any environmental gradients.

b. Temperature

A temperature of 20°C (±1°C) during the day and 17°C (±1°C) during the night was employed in the high intensity long day and short day light regimes. In the low intensity long day regime, the temperature during the first 8 hours of the day, when both fluorescent and tungsten lights were on, was 20°C (±1°C) and the temperature during the rest of the 24 hour cycle (tungsten extension of day and the night) was 17°C (±1°C) so as to be comparable with the short day regime. In a few experiments, higher temperatures (30°C day-27°C night, both ±1°C) were used.

c. Humidity

The relative humidity of the growth rooms was automatically controlled at a minimum value of approximately 65%. A check was carried out using Weatherley's leaf disc method (Weatherley, 1950) to ensure that conditions employed in both temperature regimes were not such as to cause water strain in the plants. At 20°C-17°C, the results of this check were found to be entirely satisfactory, with relative water content values greater than 90%. Some slight water strain was found in plants

grown at 30°C-27°C, relative water content values being between 80 and 85% no matter how well watered the plants were; these plants were not, however, visibly wilted.

d. Growing media and nutrients

The standard growing procedure used washed river sand (Quartzog B, supplied by British Industrial Sand, Glasgow) as the medium. The tuber pieces were planted in sand in plastic trays 30.5 cm x 58.5 cm (35-40 pieces per tray) and watered with 1 l of Hoagland's No 1 solution (Hoagland and Arnon 1938; see Section VIII) weekly, water being given in addition when necessary. (Unless otherwise stated, "water" means distilled water). After two weeks the successfully sprouted pieces, which had also rooted, were transferred to sand in small plastic pots (12 cm top diameter and 11 cm high). Drainage in both trays and pots was provided by a bottom layer of large and a layer of small granite chips. Plants in pots were given 100 ml each of Hoagland's No 1 solution weekly throughout the growing and experimental periods. 100 ml of water was also given every 1-2 days as necessary.

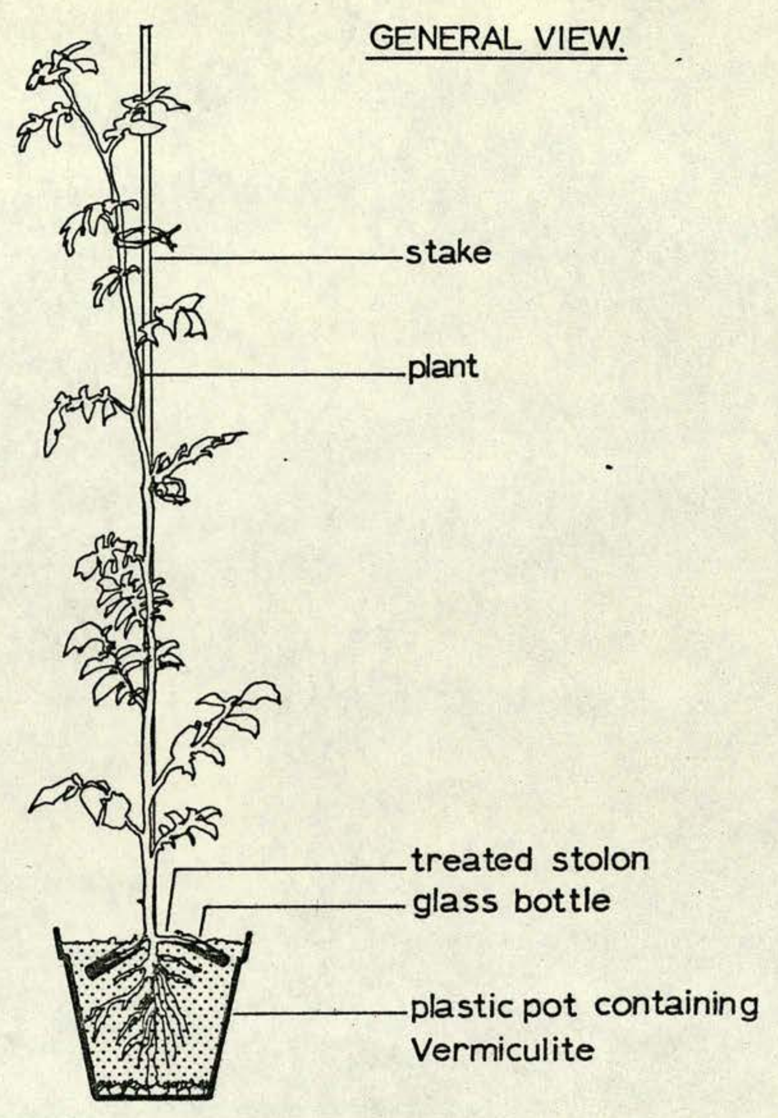
For stolon feeding experiments (Section V (iii)), the plants were grown, after transfer from trays to small pots, in "Vermiculite" or "Vermipeat" (supplied by Vermipeat Ltd, 1 Bath Road, Britton, Bristol, or by Alexander Products Ltd, Burnham-on-Sea, Somerset) instead of sand. With this method, it was easier to obtain stolons free from the substratum and undamaged for treatment.

At the beginning of the experimental period the stolons were freed from the substratum, and two unbranched stolons about 10 cm long were selected on each plant. The terminal 2-3 cm of these stolons (which were still attached to the parent plant) were

placed in small glass bottles containing the treatment solution. The bottles were cylindrical (5 cm long x 1 cm diameter) with a narrow neck (0.6 cm diameter) which prevented the solution inside from running out of the bottle, even if this was lying horizontally. This was of advantage when fitting the stolons into the bottles. The bottles were painted on the outside with black Valspar gloss paint to reduce the light level to that enabling tuberisation to take place, and also to inhibit algal growth. 1.5 or 2.0 ml of the appropriate solution was placed in each bottle and aerated with laboratory air through a Pasteur pipette connected to a small Austin Dymax II pump. The stolon to be treated was then inserted in the bottle, which was partly embedded in the Vermiculite (see Fig 4). Every 1-2 days the stolons were carefully removed from the bottles and air was again bubbled through the solutions to prevent rotting of the stolons due to lack of oxygen or build-up of carbon dioxide. When the stolons were removed from the bottles on these occasions, the progress of tuberisation in each stolon was noted. The solutions were replaced several times during the course of each experiment. Details of the experiments are given in Table 25 (Section V (iii)).

For checks on stolon growth (see Section III), the plants were grown for the first 3 weeks in Vermiculite instead of sand (to allow transplanting with a minimum of damage) and then transferred to polythene basins containing Hoagland's No 1 solution diluted 1:10. The nutrient culture set-up is shown in Fig 5. The plants were supported by Hoffman clips in which the stems were inserted after being wrapped in cotton wool; they were also each contained within a wire spring of diameter about 120 cm, which could be

FIGURE 4. Arrangement used in stolon feeding experiments (Section V (iii)).


GENERAL VIEW.
stake
plant
treated stolon
glass bottle
plastic pot containing
Vermiculite

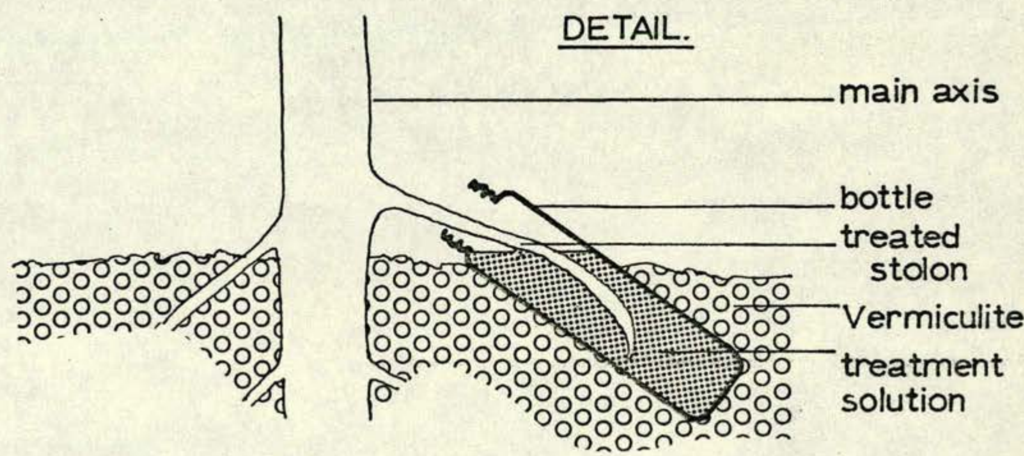
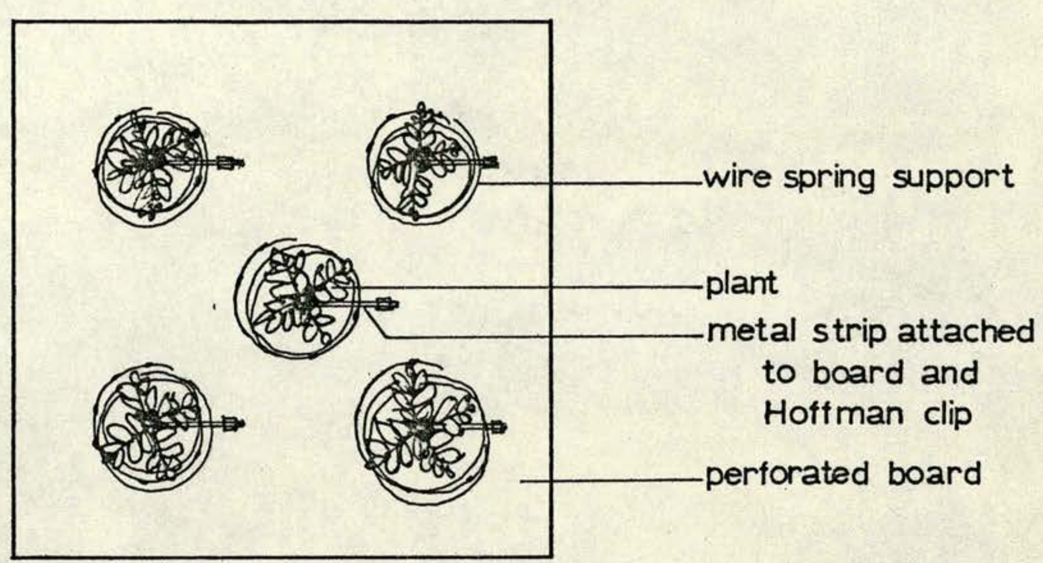
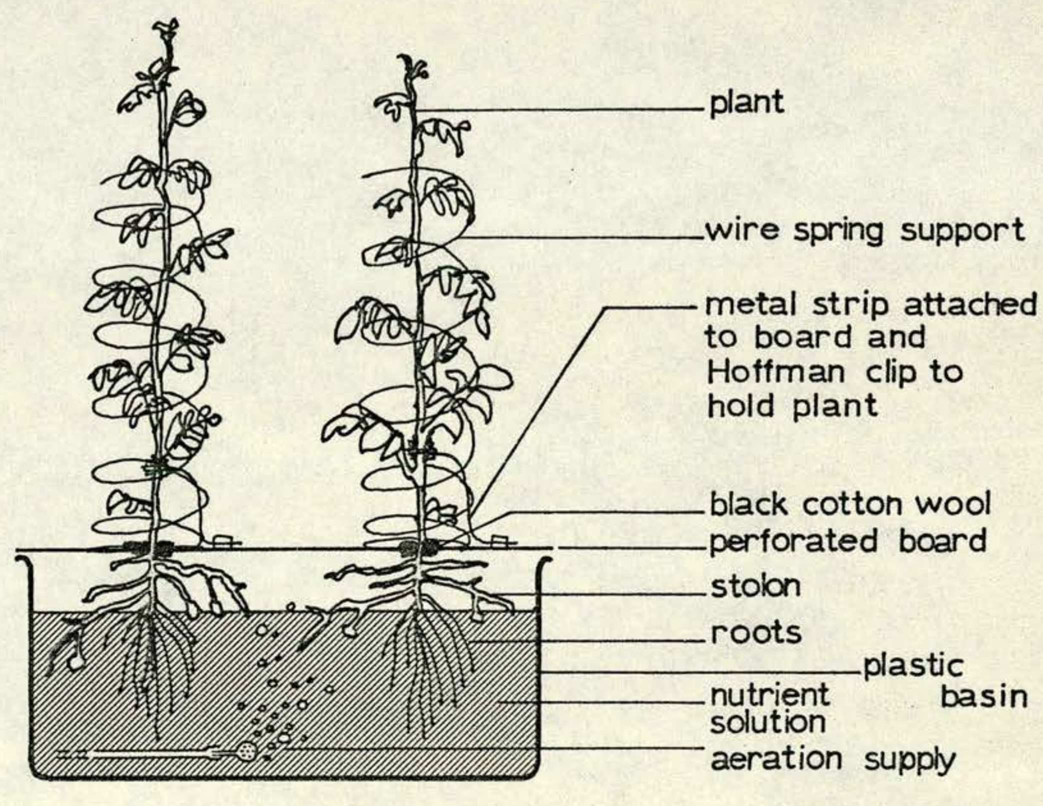

DETAIL.
main axis
bottle
treated
stolon
Vermiculite
treatment
solution

FIGURE 5. Arrangement used for growing plants in nutrient culture.

Nutrient solution consisted of Hoagland's solution (see Section VIII) diluted 1:10 with water.

PLAN.





SECTION.

extended as the plant elongated. To prevent algal growth, and so as not to inhibit tuberisation of stolons, light was excluded by means of a board across the top of the basin, perforated to allow the plant roots to reach the solution. The solution was topped up every second day and replaced several times during the experiment; it was continuously aerated with laboratory air using a small Austin Dymax II pump. The holes in the board were loosely plugged with black cotton wool to help in elimination of light.

e. Harvesting procedure

A harvesting procedure was developed in which the following measurements were made:- top height, node number (counting the apical cluster of very small leaves as one), dry weight of tops, and occasionally of roots, stolon number, tuber number, number of tuberising stolons and sometimes tuber fresh weight.

(N B Harvest dates are referred to throughout the work by the number of days after the start of the experimental period (ie the period during which differential daylength treatment was given). Plants at a 14 day harvest are therefore 49 days old, having been grown up for 35 days and then given 14 days of differential daylength treatment during the experimental period.)

When it was realised that starch deposition in stolons which were not visibly tuberised might be useful (see Section VI), this information was also recorded.

Flowering (presence or absence of flowers or visible flower primordia) was also recorded throughout the work, but this is not included in the tables of results as the data showed no apparently meaningful trends, flower initiation occurring

irrespective of environmental conditions.

(iii) Methods used in the light break experiments (Section IV)

a) General procedures

In all the experiments, the plants were grown for 35 days in high intensity long days. After this, control plants were grown in low intensity long days or short days and treated plants in short days with a short period of night break light given at or near the middle of the night. The plants were harvested 14 days after the beginning of the light break treatment, and in those cases where light breaks were not continued until the harvest date, the plants were transferred at the end of the cycles incorporating light breaks to low intensity long days for the remainder of the experimental period. Details are given in Table 5 (Section IV).

b) Light break treatments (see also Table 5)

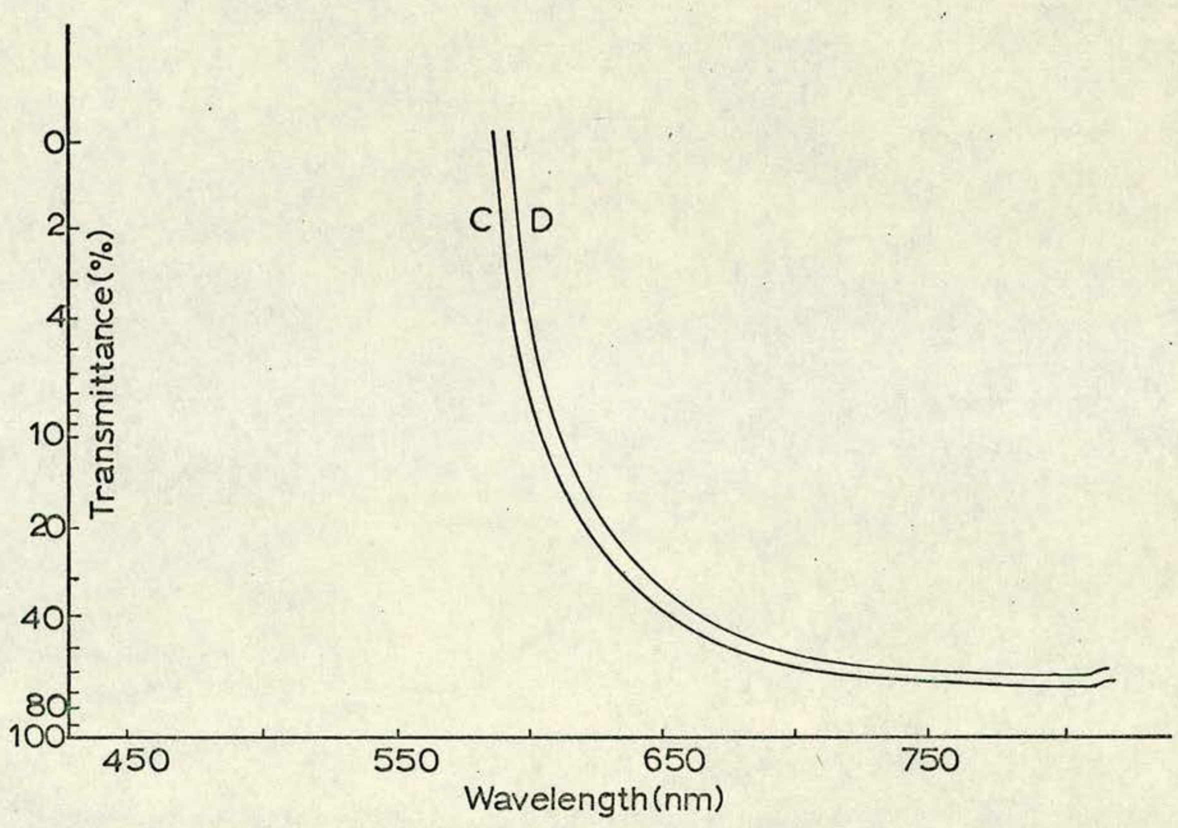
Lights and filters

The filters used for the coloured light breaks were red (flashed ruby) and blue (flashed blue) glass plates obtained from Cunningham, Dickson and Walker Ltd, Northfield Broadway, Edinburgh; transmission curves are given in Figs 6 and 7.

Administration of light break treatment

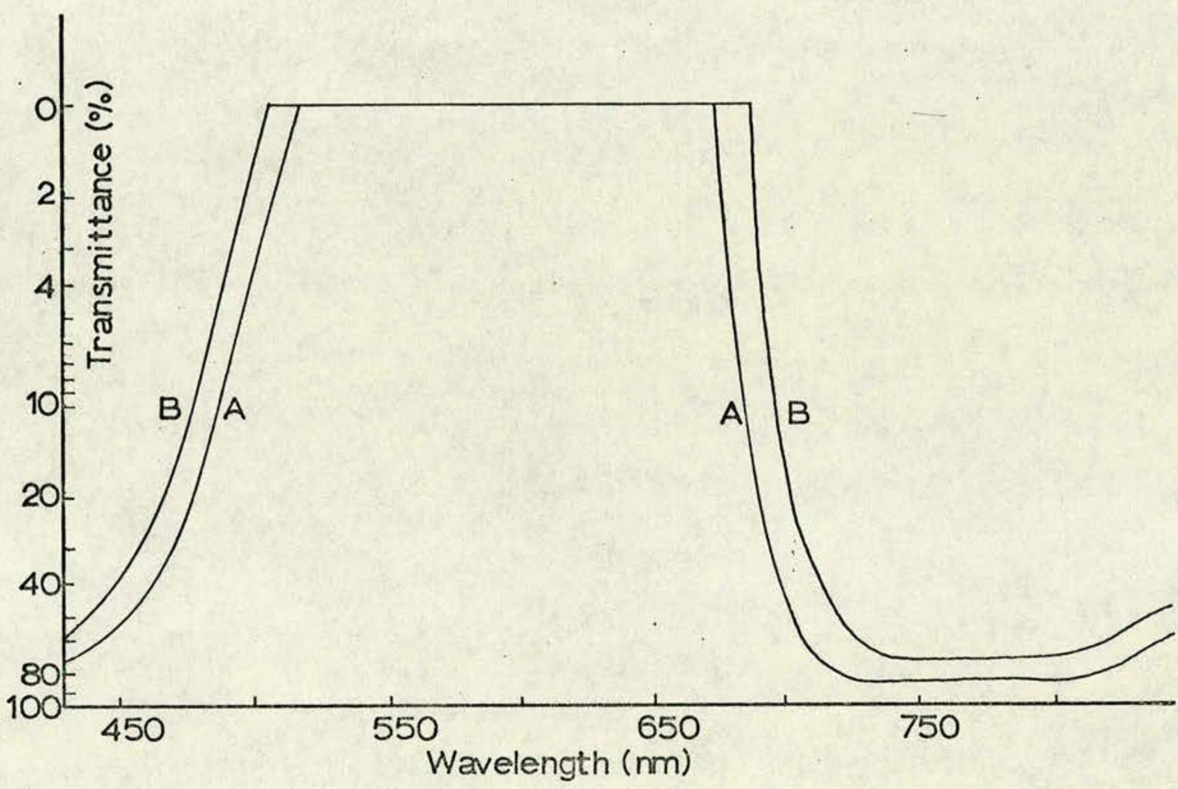
During the period when light break treatment was given the plants (in small pots, as described in Section II (ii)) were placed in the large metal cans used in some of the preliminary experiments (see Section III). The cans were painted black on the outside and silver on the inside, and each can accommodated 4 plants in pots (see Fig 8). For the coloured light breaks, the glass plate filters, which were slightly larger than the mouths of the cans, were placed on top of the cans. Each plate was fitted with a

FIGURE 6. Transmittance of red glass plate used to provide the red light source and, together with blue plate (see Fig 7), the far-red source for light break treatments (Section IV).



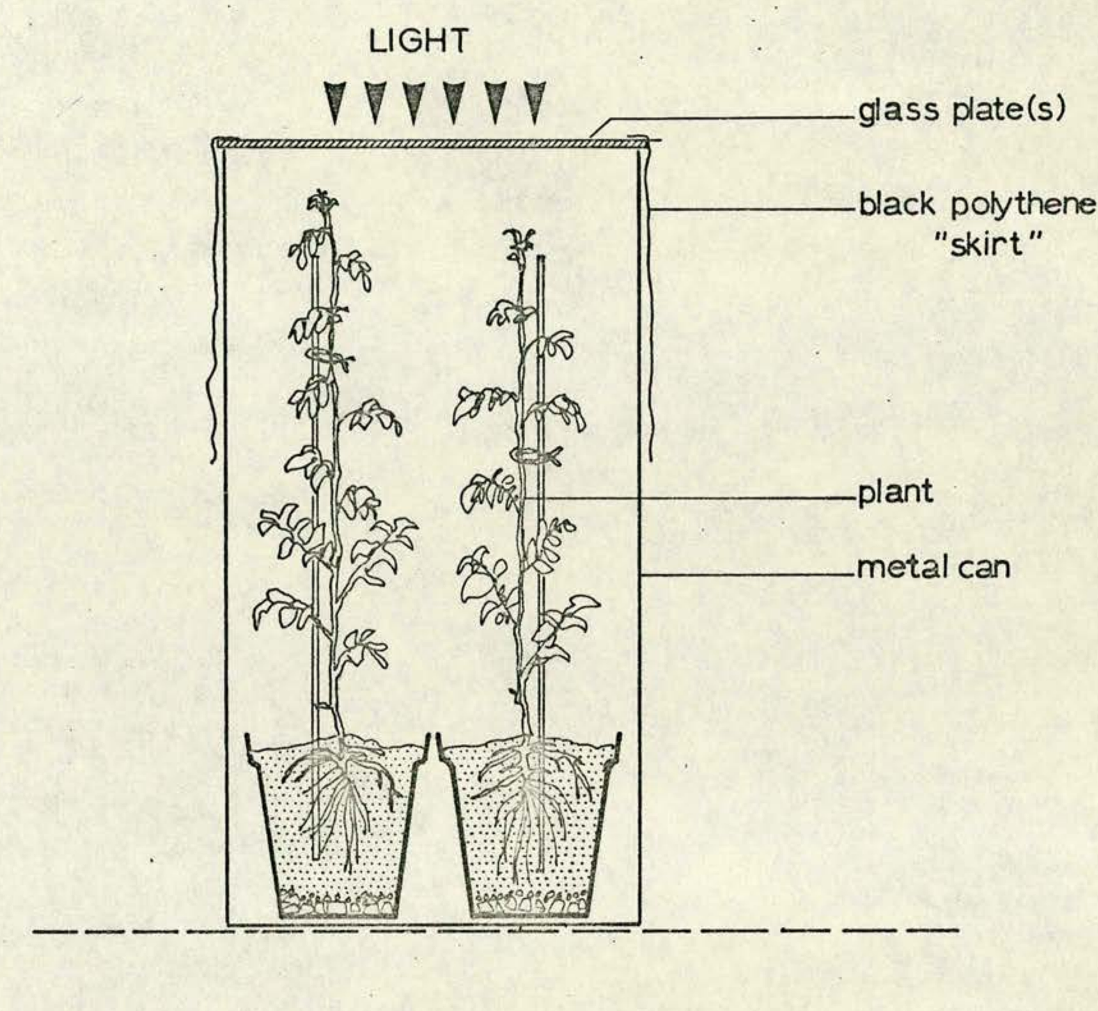
C, D : transmittance curves for two separate red glass plates

FIGURE 7. Transmittance of blue glass plate used to provide the blue light source and, together with red plate (see Fig 6), the far-red source for light break treatments (Section IV).



A , B : transmittance curves for two separate blue glass plates

FIGURE 8. Arrangement used for giving coloured light break treatments (Section IV).


LIGHT
glass plate(s)
black polythene
"skirt"
plant
metal can

black polythene "skirt" which hung down round the sides of the can when the filter was in position, to ensure against any penetration of white light. No filter was used over the cans containing plants being given white light breaks, and closely-fitting black polythene covers were placed over cans containing short day control plants.

Light breaks were controlled either by a pre-set time clock, in the case of the simpler experiments, or manually, if it was necessary to change filters. With automatically-controlled treatment, the plants were prepared prior to the start of the dark period and the filters removed again before the beginning of the light period. In experiments 4 and 5, the plants given light break treatment were kept in normal short day conditions in the day and transferred at night to a small culture room for light break treatment so as not to disturb other material for which normal short days were required.

Checks were made on the operation of the automatically-timed light breaks by exposing photographic bromide paper together with a thermograph which registered a rise in temperature when the tungsten lights came on in experiments 1-3, or by means of a Solarimeter fitted to a recording integrator (Lintronic/Agromet Mk III) in experiments 4 and 5.

When manually-operated light breaks were given, great care was taken to allow no stray light to enter the growth room containing the plants during their night period. The room containing the growth chambers was locked and darkened, and operations were carried out with the aid of a small torch fitted with a green

filter, considered physiologically "safe" (Murray, 1968). All filters and covers were removed after the last light break treatment in each night period in readiness for the automatically-timed commencement of the next light period (day). In experiments with manually-operated light breaks in which the plants' "night" corresponded to the natural day, the plants were either grown up entirely in this light regime, or were subjected to several days of continuous light at the end of the growing period.

SECTION III PRELIMINARY EXPERIMENTS

(i) Introduction

This part of the work was conducted principally to determine the most suitable growing conditions for the material and to plan, by observation of tuberisation under various conditions and at various times after planting, a time schedule for future experiments. It also contributed to the final choice of the variety to be used.

Before undertaking these experiments, growth of potato plants kept under controlled environment (growth room) conditions was compared with that of plants in the glass house. The material was shown to grow and tuberise satisfactorily in the growth rooms; as many tubers were formed as in the plants grown in the glass-house, although total and mean tuber fresh weight were lower at harvest after 5½ months.

(ii) Comparison of top growth and tuberisation of plants grown at high and low temperatures under growth room conditions

The first of the preliminary experiments was carried out to determine whether the results of other workers (see Section I) on growth and tuberisation with different temperatures would be confirmed using the growth room conditions available. The variety Golden Wonder was used, although it was not virus-tested, because it was a readily-available late maincrop variety and stocks of Up-to-Date had not yet been obtained.

The conditions used were short days with two temperature regimes: low (20°C day - 17°C night) and high (30°C day - 27°C night). The plants were grown from whole tubers planted in large metal cans about 30 cm square by about 60 cm high provided with broken crocks covering drainage holes in the bottom and three-quarters

filled with John Innes Compost No 2. Watering was with tap water as required. Two plants from each treatment were harvested at approximately weekly intervals, beginning about a month after the appearance of the first sprouts, 9 days after planting.

The results are given in Table 2 and the appearance of typical plants in Figs 9 and 10. Values of all parameters were generally greater at low temperature (except shoot number, which was dependent on the number of eyes sprouted). Leaves were larger, flatter and lighter green at low temperature (see Figs 9 b and 10 b); also, stems were thicker and tubers formed earlier. These results are in agreement with those of other workers (see Section I), showing that a late maincrop variety behaved in the expected way with the controlled conditions available.

It was decided that this method of growing plants was unsuitable for future work, which would require larger numbers of plants. Very few cans could be accommodated on the growing tables. The plants themselves were large and required strong staking to prevent them becoming tangled together, and they were difficult to move from room to room. They were also very variable due to the differing weights of and the different numbers of eyes sprouted on the mother tubers. An alternative growing method was therefore necessary.

(iii) Trial of methods to obtain smaller and more uniform plants

To try to obtain smaller and more uniform plants, a trial was conducted using small and medium-sized pieces of tuber (Golden Wonder). The small pieces consisted of cylinders 1 cm high and 2.5 cm in diameter, extracted from the mother tuber with a cork

TABLE 2. Results of first preliminary experiment (Section III (ii)). Differences in top, stolon and tuber growth in SD at high (HT) and low (LT) temperatures in plants grown in cans from whole tubers.

Harvest date	38 DAYS		45 DAYS		53 DAYS		59 DAYS		64 DAYS	
Temperature	LT	HT	LT	HT	LT	HT	LT	HT	LT	HT
No. of shoots	21	13	12	7	10	9	12	10	6	5
Top fwt. (g)	254.2	114.1	153.9	110.3	172.7	145.3	174.5	57.5	170.4	85.3
Top dwt. (g)	-	-	-	-	9.4	8.6	9.2	3.7	9.7	5.0
Leaf area (cm^2)	-	-	-	-	2796	2567	3230	1114	3203	1383
No. non-tub. stolons	55	23	23	15	37	24	29	18	16	25
Tub. Stolon No.	11	9	8	5	9	6	10	4	17	11
Tuber fwt.(g)	20.3	0.3	39.2	14.3	49.1	30.7	76.4	17.2	109.5	43.6
Replication	2	2	2	2	2	2	2	2	2	2

Harvest date is given in this experiment as days from planting. Sprouting occurred approximately 9 days after planting.

LT = 20°C day/17°C night; HT = 30°C day/27°C night

For other abbreviations, see Section VIII.

FIGURE 9. Results of first preliminary experiment (Section III (ii)).
a. Appearance at harvest, 64 days after planting (55 days after sprouting) of plant grown at low temperature.
b. Appearance at harvest, 59 days after planting (48 days after sprouting) of third and fourth leaves of plant grown at low temperature. Note large size compared to leaves from plant grown at high temperature (Fig 10).

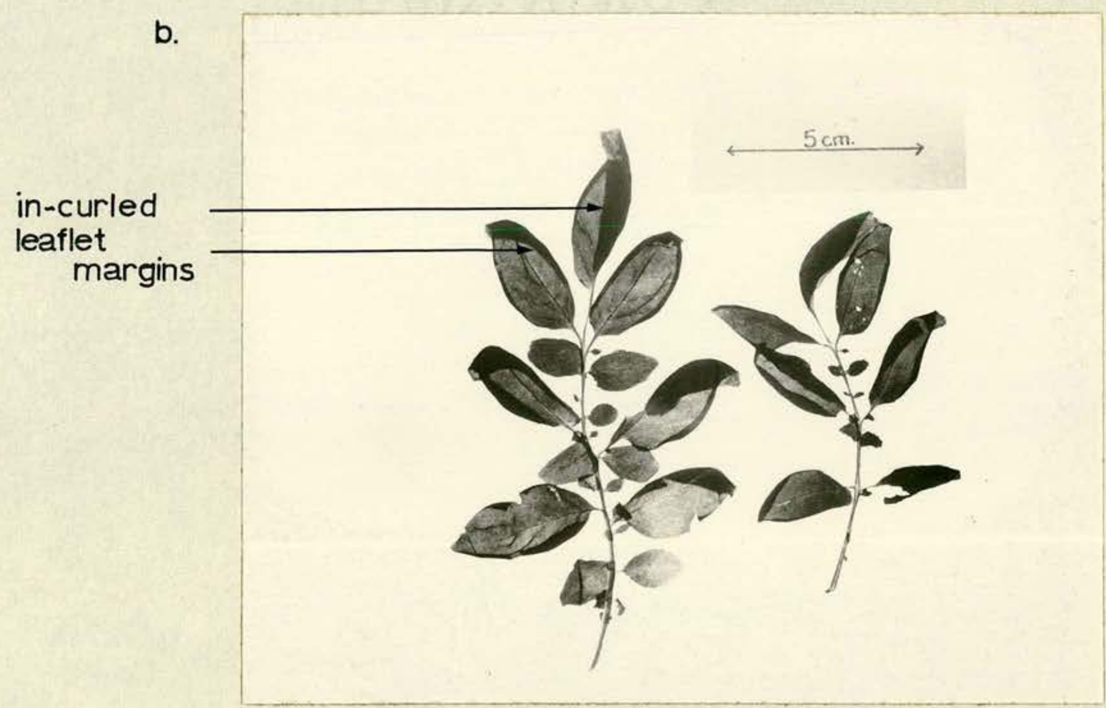
a.

b.

FIGURE 10. Results of first preliminary experiment (Section III (ii)).
a. Appearance at harvest, 64 days after planting (55 days after sprouting) of plant grown at high temperature.
b. Appearance at harvest, 59 days after planting (49 days after sprouting) of third and fourth leaves of plant grown at high temperature. Note small size compared to leaves from plant grown at low temperature (Fig 9) and in-curled leaflet margins.

a.

b.



borer, and each containing one eye. The medium-sized pieces consisted of pieces from tubers cut into quarters; the pieces were trimmed to approximately equal weights, and all eyes but one were excised (see Fig 11).

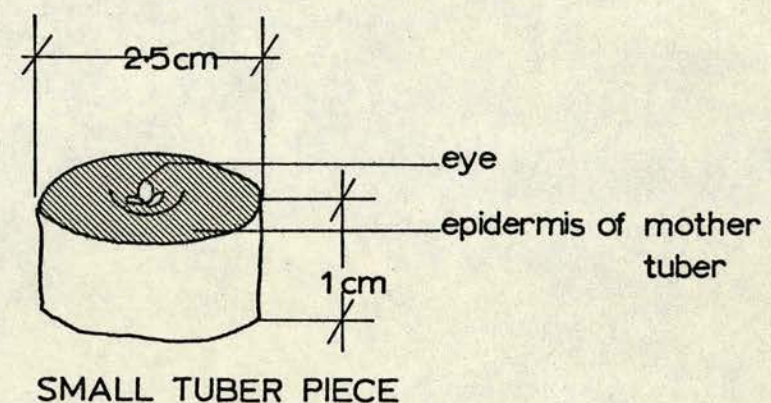
18 pieces of each size were sprouted and grown throughout the experiment (35 days) in either short day or low intensity long day conditions, so as to combine observation of tuberisation under the different daylength conditions with the trial of size of tuber piece. Six pieces of each size were grown under high intensity long day conditions.

The tuber pieces were grown in trays and small pots as described in Section II (ii) d. Only one shoot was allowed to develop; this was achieved by removing any shoots which developed from lateral buds in the eyes.

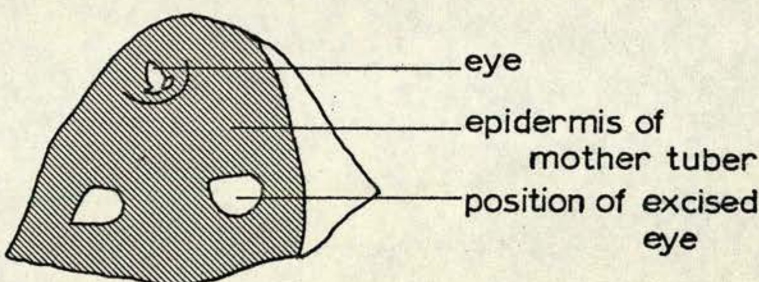
These procedures resulted in smaller and much more uniform plants than had been obtained from whole tubers grown in cans (see Fig 12). The size of tuber piece used had very little effect on the growth of the young plants; this being the case, it was decided to use the smaller pieces for future experiments, since they were less wasteful of material and quicker to prepare.

At harvest 35 days from planting, tubers had formed on almost all the plants grown in short days, but on none of those grown in either long day regime, confirming the inductive nature of the short day conditions and the non-inductive nature of both types of long day conditions. Low intensity long days produced taller plants with thinner, more flexible stems than did short days or high intensity long days.

FIGURE 11. The two sizes of tuber piece used in second preliminary experiment (Section III (iii)).



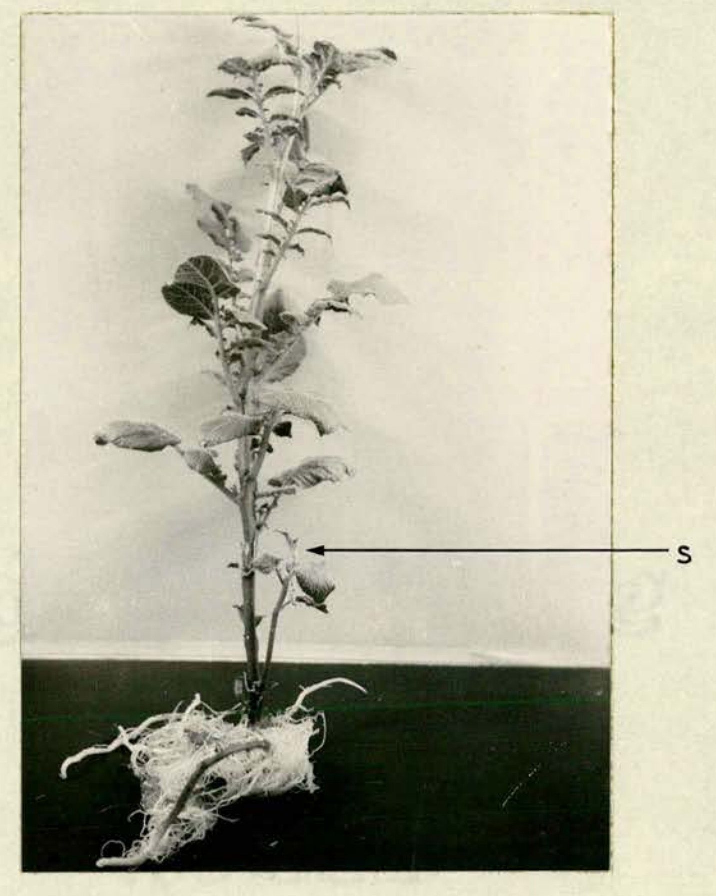
SMALL TUBER PIECE



MEDIUM - SIZED TUBER PIECE

FIGURE 12. Appearance of potato plant grown in small pot for 35 days in high intensity long day conditions at low temperature and then transferred to short day conditions at low temperature for 7 days.

Small shoots such as that labelled "s" were routinely removed as soon as they appeared above the soil.


S

Gregory (1956) and Chapman (1958) found that about two weeks was necessary for tuber initiation; it therefore appeared that it was not necessary for differential daylength treatment to extend over the whole time of the experiment. It was decided to use an initial growing period of non-inductive long days before commencing differential daylength treatment. High intensity long days were thought most suitable for this growing period for two reasons. Firstly, plants grown in high intensity long days were shorter and less flexible and it was thought that this would make it easier to ensure that each plant received an equivalent amount of incident light and to administer treatments, for example in the projected light break experiments (Section IV) and the projected growth substance application experiments (Section V (ii)); in the former, plants would be placed inside cans with filters on top, and in the latter, drops of liquid would be applied to the apex. Secondly, more high intensity light would mean less likelihood of lack of carbohydrate for tubers due to low photosynthetic activity.

Such an initial growing period of high intensity long days, followed by differential daylength treatment consisting of a varying number of short day cycles, was used in the next experiment.

(iv) Investigations into the minimum number of short day cycles required for and the effect of changing the number of short day cycles given upon tuberisation

Having developed a suitable growing method, it was next necessary to establish a basic time schedule for tuberisation experiments, based on a knowledge of the minimum number of short day cycles required to induce tuberisation and of the effect on tuberisation of increasing the number of such cycles above the necessary

minimum. The object of these investigations was to determine the optimum number of short day cycles for the induction of tuberisation; this would in turn determine the timing and number of harvests used in investigations of the effects of factors other than daylength on tuberisation.

Two similar experiments were conducted, one using Golden Wonder and the other Up-to-Date and the Kenyan varieties (see Section II (i)). The plants were grown by the standard method described in Section II (ii) d and then given either low intensity long days or a number of short day cycles (3, 6, 9, 12, 15, 18 or 21) followed by low intensity long days if necessary until harvest. The plan of the experiments is given in Table 3.

The results of these experiments, for the varieties Golden Wonder and Up-to-Date, are shown in Figs 13 and 14. The responses of both varieties were very similar for all the parameters measured.

Generally, with increasing number of short day cycles, decreases were found in top height, node number and top dry weight (the last being very small and due mainly to a decrease in stem dry weight). The trends tended to become more clear-cut at later harvests. With increasing number of short day cycles, tuber number and tuber fresh weight increased.

In Up-to-Date, tubers were detected with 3 short day cycles at the 21 day harvest, but a minimum of 6 short day cycles were required at the 14 day harvest. Because of the increase in tuber number and fresh weight with increasing number of short day cycles, it was considered desirable to use a basic inductive treatment of more than the minimum number of cycles. In Up-to-Date, the

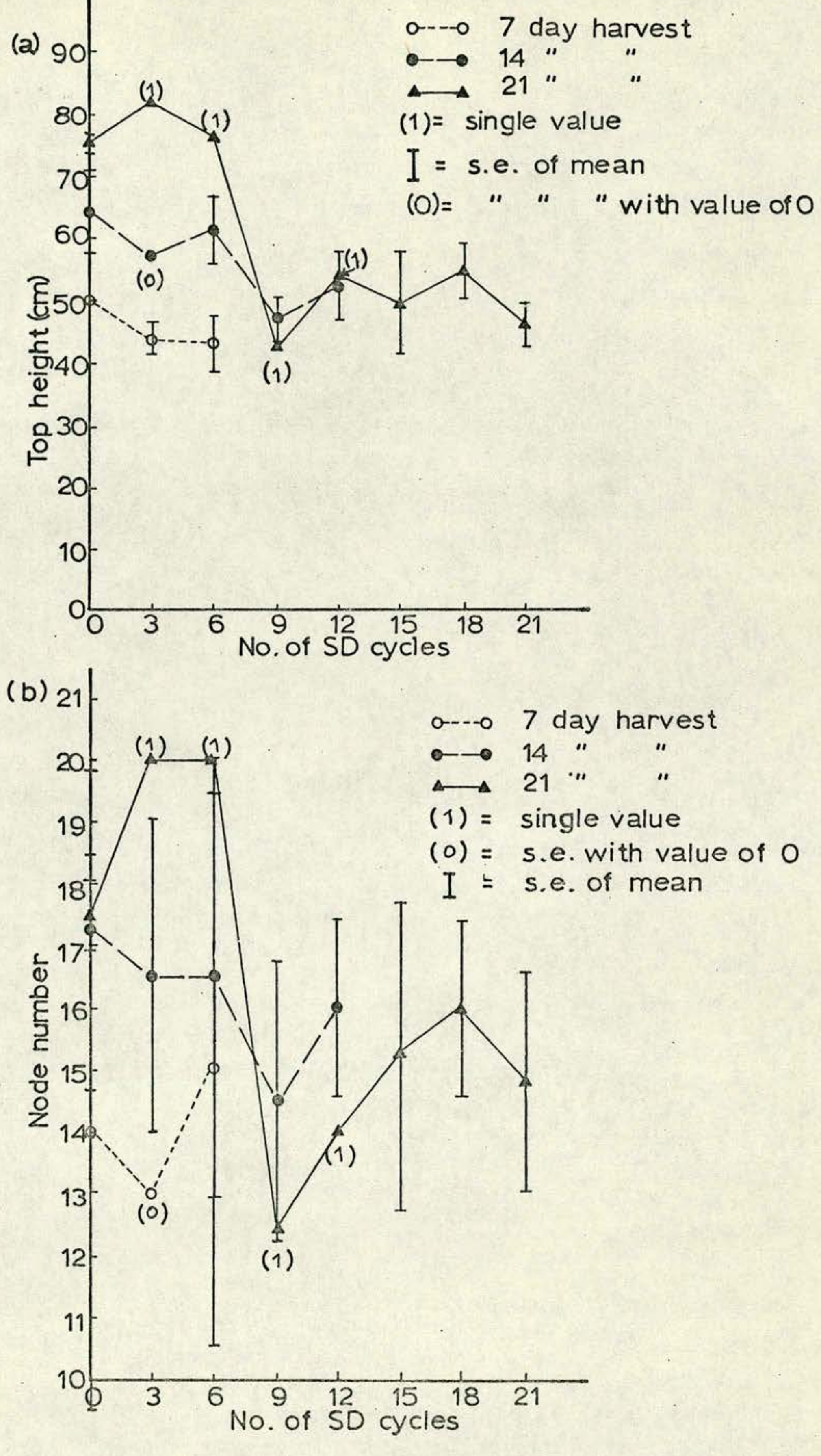
TABLE 3. Plan of third & fourth preliminary experiments (Section III (iv)). Number of plants given a certain number of SD cycles and harvested on certain dates.

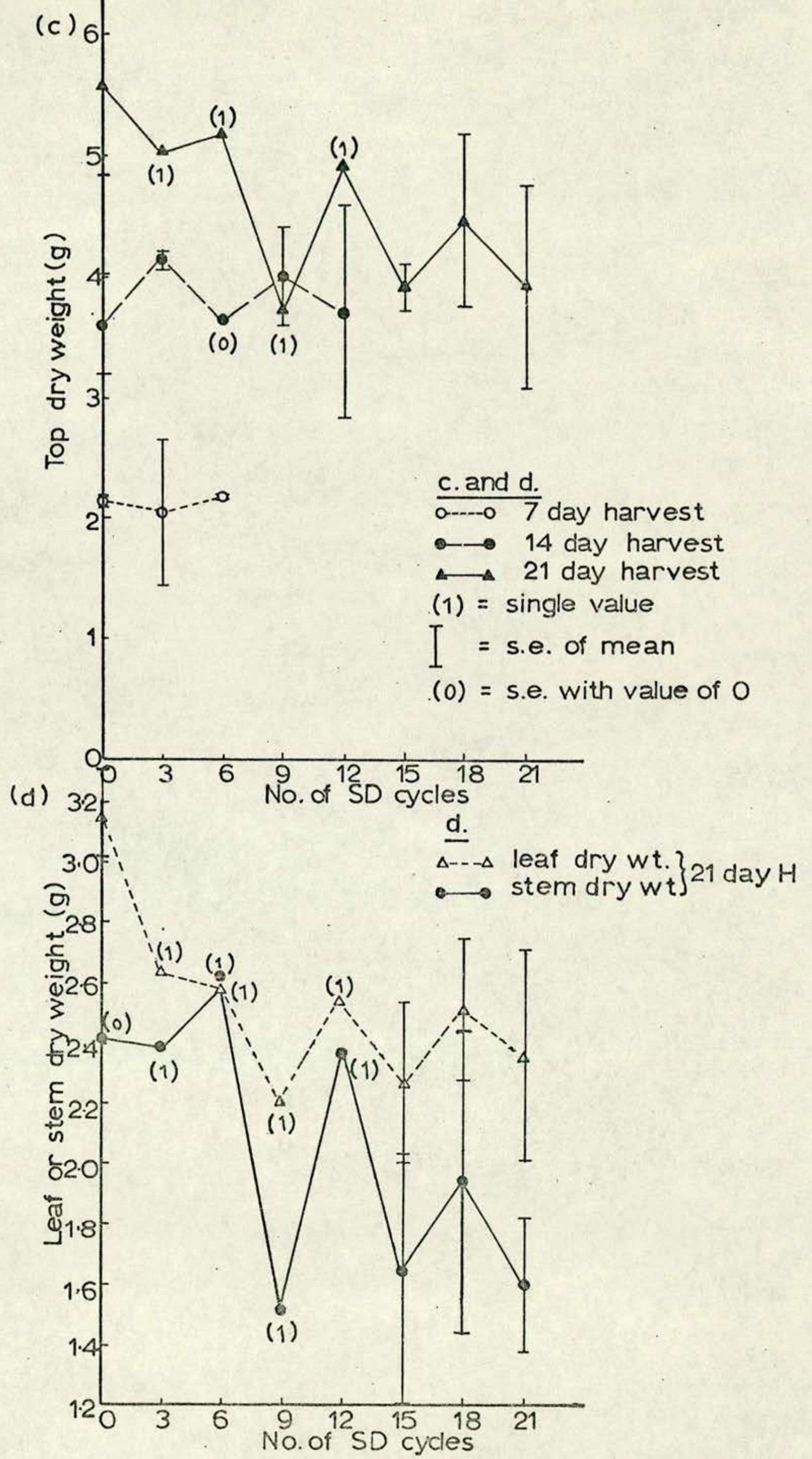
No of SD cycles	Harvest date (days)					
	7		14		21	
	GW	UTD	GW	UTD	GW	UTD
0	2	2	2	2	2	2
3	2	2	2	2	1	1
6	2	2	2	2	1	1
9			2	2	1	1
12			2	2	1	1
15					2	1
18					2	1
21					2	1

GW = Golden Wonder
UTD = Up-to-Date
Date of harvest is given from start of differential daylength (experimental) period.

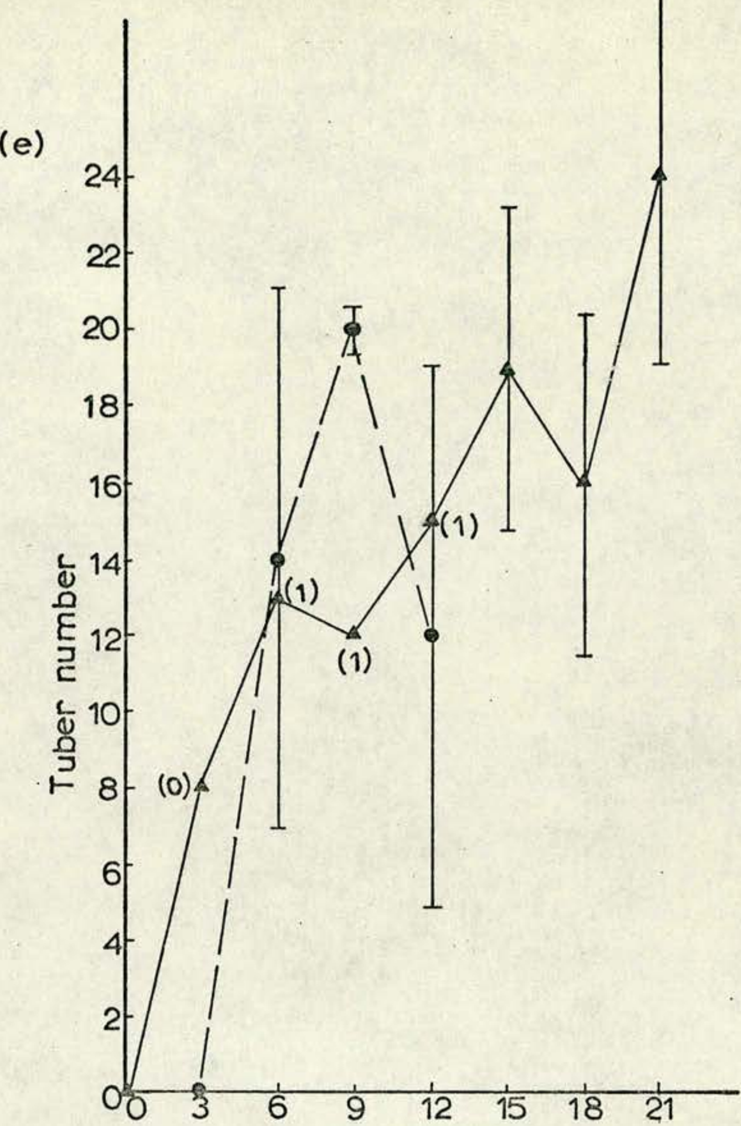
FIGURE 13. Results of third preliminary experiment (Section III (iv), using Golden Wonder). Changes with increasing number of short day cycles in
a. top height
b. node number
c. top dry weight
d. leaf and stem dry weight (taken separately)
e. tuber number
f. tuber fresh weight.

Points are means of two values unless otherwise indicated. Standard errors (s.e.) of means are shown.


(a)
o---o 7 day harvest
●—● 14 " "
▲—▲ 21 " "
(1)= single value
I = s.e. of mean
(O)= " " " with value of O
Top height (cm)
No. of SD cycles
(b)
o---o 7 day harvest
●—● 14 " "
▲—▲ 21 " "
(1) = single value
(o) = s.e. with value of O
I = s.e. of mean
Node number
No. of SD cycles



(c)
Top dry weight (g)
6
5
4
3
2
1
0
(1)
(1)
(1)
(0)
(1)
c. and d.
7 day harvest
14 day harvest
21 day harvest
(1) = single value
= s.e. of mean
(0) = s.e. with value of 0
0 3 6 9 12 15 18 21
No. of SD cycles
(d)
Leaf or stem dry weight (g)
3·2
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
d.
leaf dry wt.
stem dry wt
21 day H
(0)
(1)
(1)
(1)
(1)
(1)
(1)
(1)
(1)
0 3 6 9 12 15 18 21
No. of SD cycles



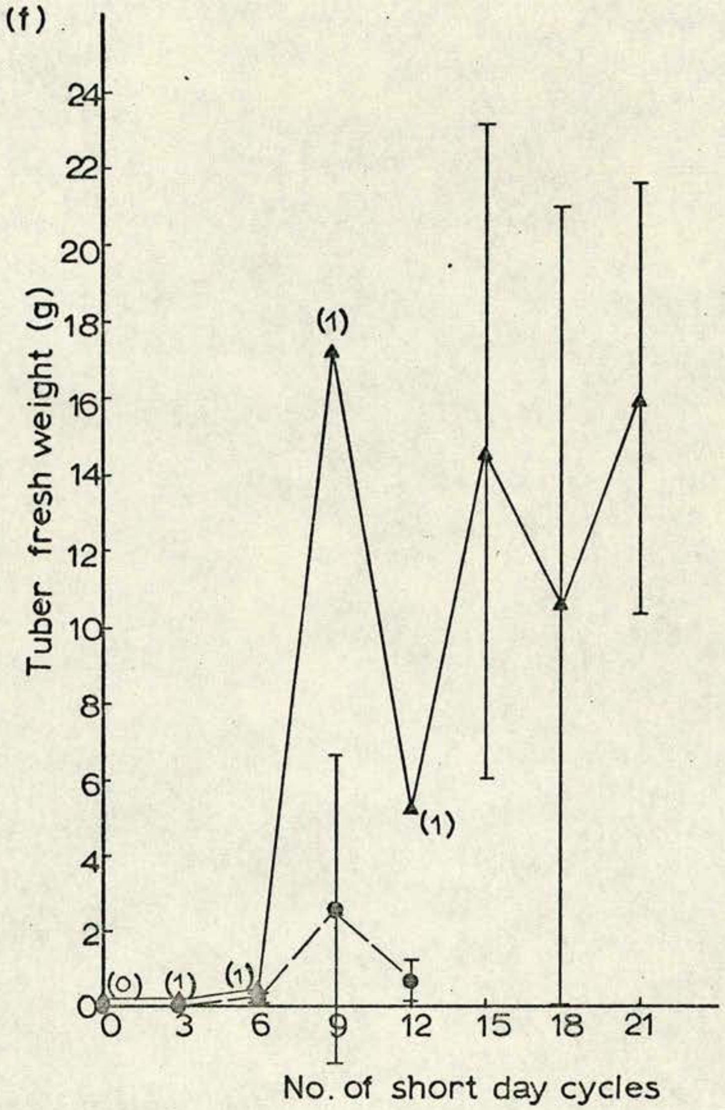
●—● 14 day harvest

▲—▲ 21 day harvest

(1) = single value

I = s.e. of mean

(o) = s.e. with value of 0



●- -● 14 day harvest

▲—▲ 21 day harvest

(1) = single value

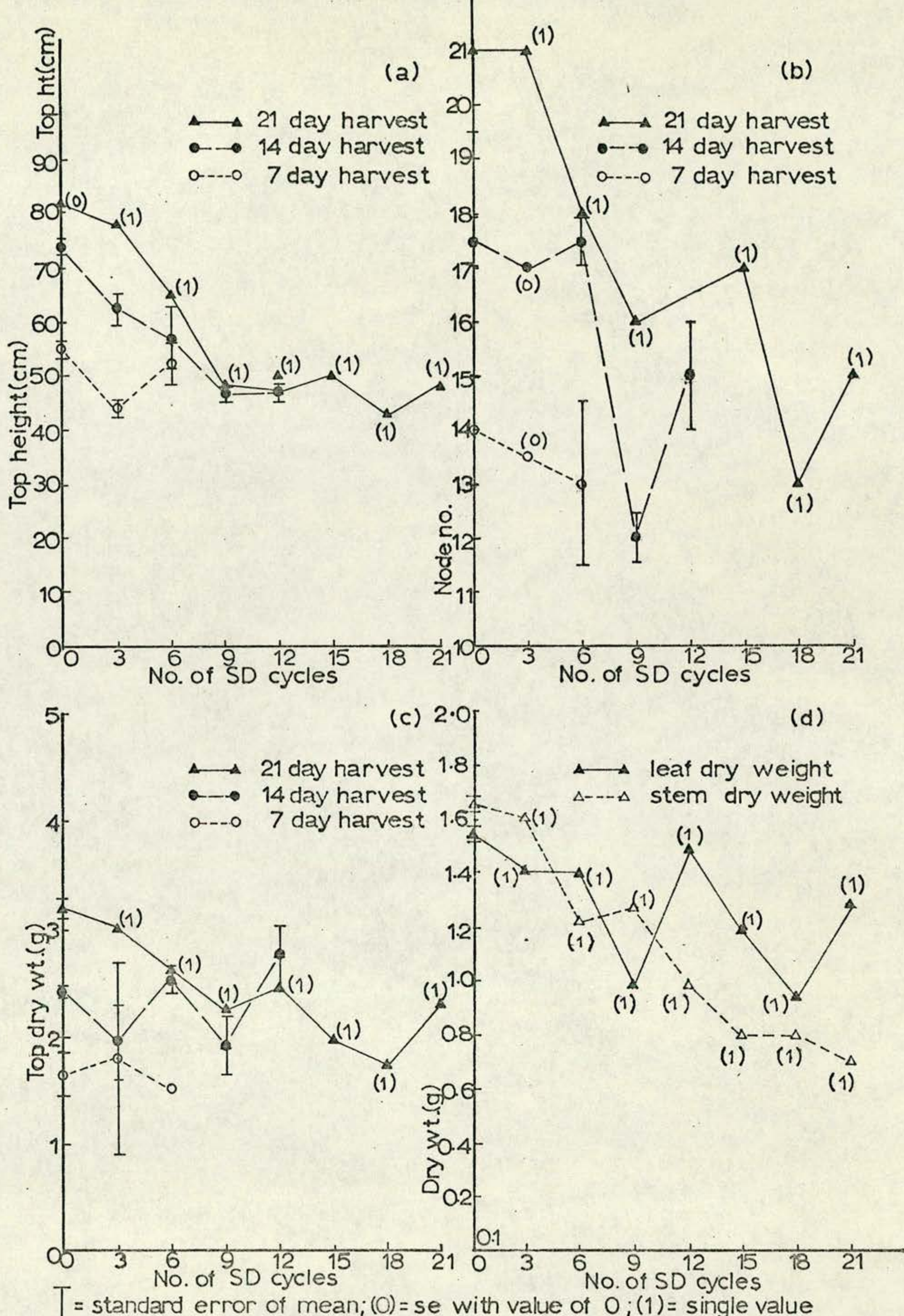
I = s.e. of mean

(0) = s.e. with value of 0

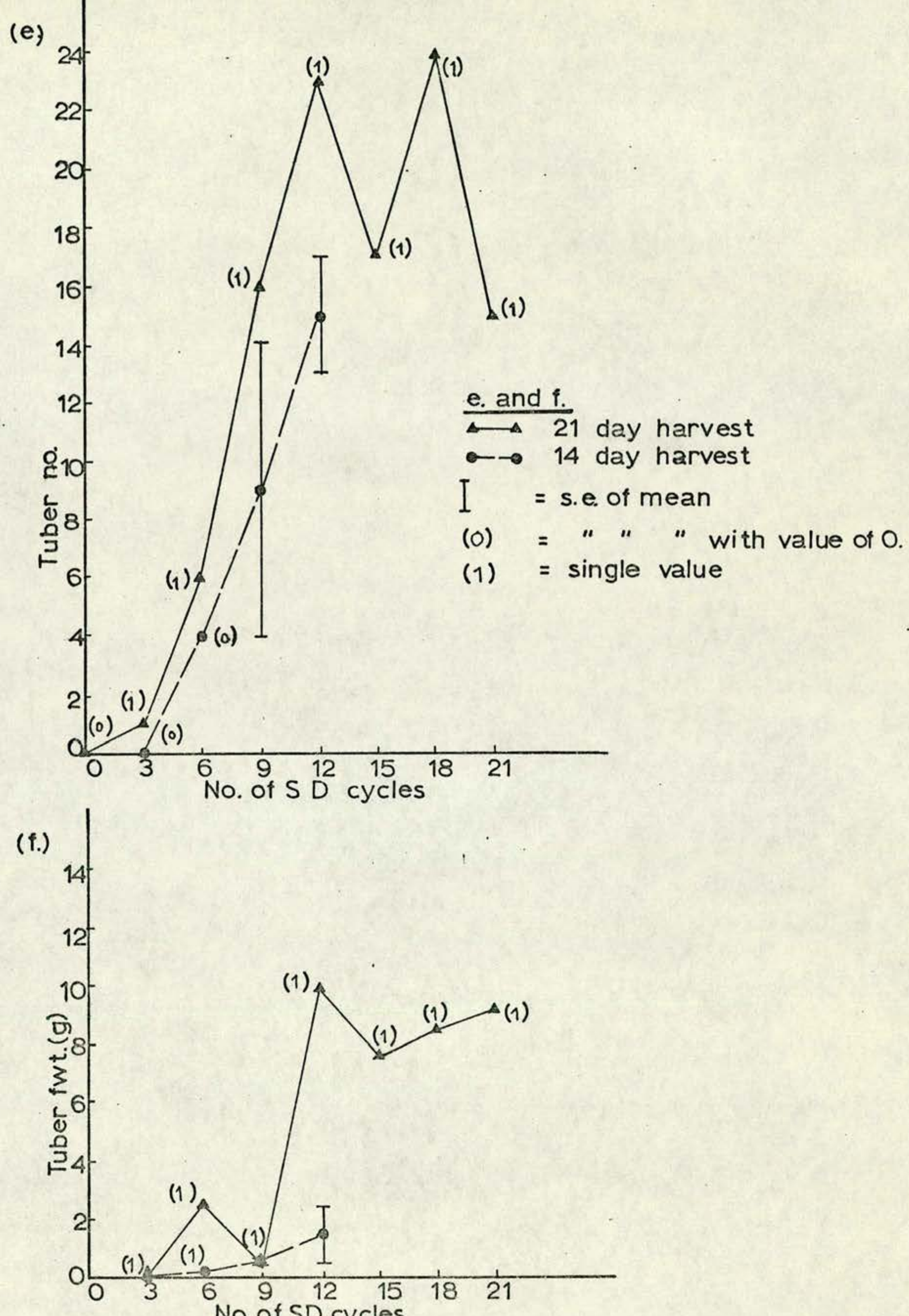
FIGURE 14. Results of fourth preliminary experiment (Section III (iv), using Up-to-Date). Changes with increasing number of short day cycles in

a. top height
b. node number
c. top dry weight
d. leaf and stem dry weight (taken separately)
e. tuber number
f. tuber fresh weight

Points are means of two values unless otherwise indicated. Standard error (s.e.) of means are shown.



= standard error of mean; (0) = se with value of 0; (1) = single value



(e)
Tuber no.
No. of S D cycles
e. and f.
21 day harvest
14 day harvest
= s.e. of mean
(0) = " " " with value of 0.
(1) = single value
(f.)
Tuber fwt.(g)
No. of SD cycles

increase in tuber number and fresh weight started to decrease after about 12 short day cycles (see Figs 14 e and f) and it was therefore thought unnecessary to give many more cycles than this. 14 short day cycles was accordingly selected as a convenient number for the standard inductive treatment.

The Kenyan varieties showed much less clear-cut trends, probably due to their uneven early growth, and this, together with the difficulty of obtaining a continuous supply of tubers, led to their rejection as experimental material.

The results with Golden Wonder and Up-to-Date are in agreement with those of Gregory (1956) and Chapman (1958). Although 14 short days were not strictly necessary for tuberisation, as few as 3 cycles being sufficient for some response if harvest was delayed until 21 days, 14 short days was decided on as a more suitable, and reliable, inductive treatment.

(v) To examine the effect of age of the plants at the beginning of the differential daylength treatment upon the number of short day cycles required for tuberisation

The object of this investigation was to determine whether the time taken to initiate tubers is affected by the age of the plant at the beginning of the differential daylength treatment (experimental period). If this were the case, it would be necessary to standardise the length of the growing period.

Two similar experiments were carried out using Up-to-Date plants which were grown for 35, 40 or 45 days in high intensity long day conditions and given 3, 6 or 9 short day cycles and then kept in low intensity long days until harvest at day 14 (ie 14 days from start of differential daylength treatment). The replication

levels were 4 (2 for long day controls) and 3 in the first and second experiments respectively.

Similar results were obtained in both experiments (see Fig 15: results of second experiment). All parameters, including tuber number, were greater in the older plants. Top height decreased with increasing number of short day cycles, but node number, where it was recorded (in the first experiment), and top dry weight appeared unaffected. Fewer short day cycles were required to bring about tuberisation in older plants (see Fig 15 c).

On the basis of these results, it was found necessary to use a constant length of growing period; material was therefore grown for 35 days prior to the start of the experimental period.

The basic time schedule developed as a result of these experiments and those in (iv) above consisted of a growing period of 35 high intensity long days, followed by an experimental period of usually 14 days in which differential daylength treatment, consisting of either short days (inductive) or low intensity long days (non-inductive) was given. Harvest was usually carried out 14 days from the beginning of the experimental period, although in some experiments where sufficient material was available, additional harvest dates were also used.

It was also found in the above experiments that the material showed a fairly high level of variability, and this was encountered throughout the work, although all possible attempts were made to minimise and counteract it, ie standardised planting, growing and feeding conditions, randomisation of experimental

FIGURE 15. Results of one run of fifth preliminary experiment (Section III (v)). Changes with increasing number of short day cycles and different ages of plant in

a. top height
b. top dry weight
c. tuber number

Points are means of three values ~~unless otherwise indicated.~~ Standard errors (s.e.) of means are shown.

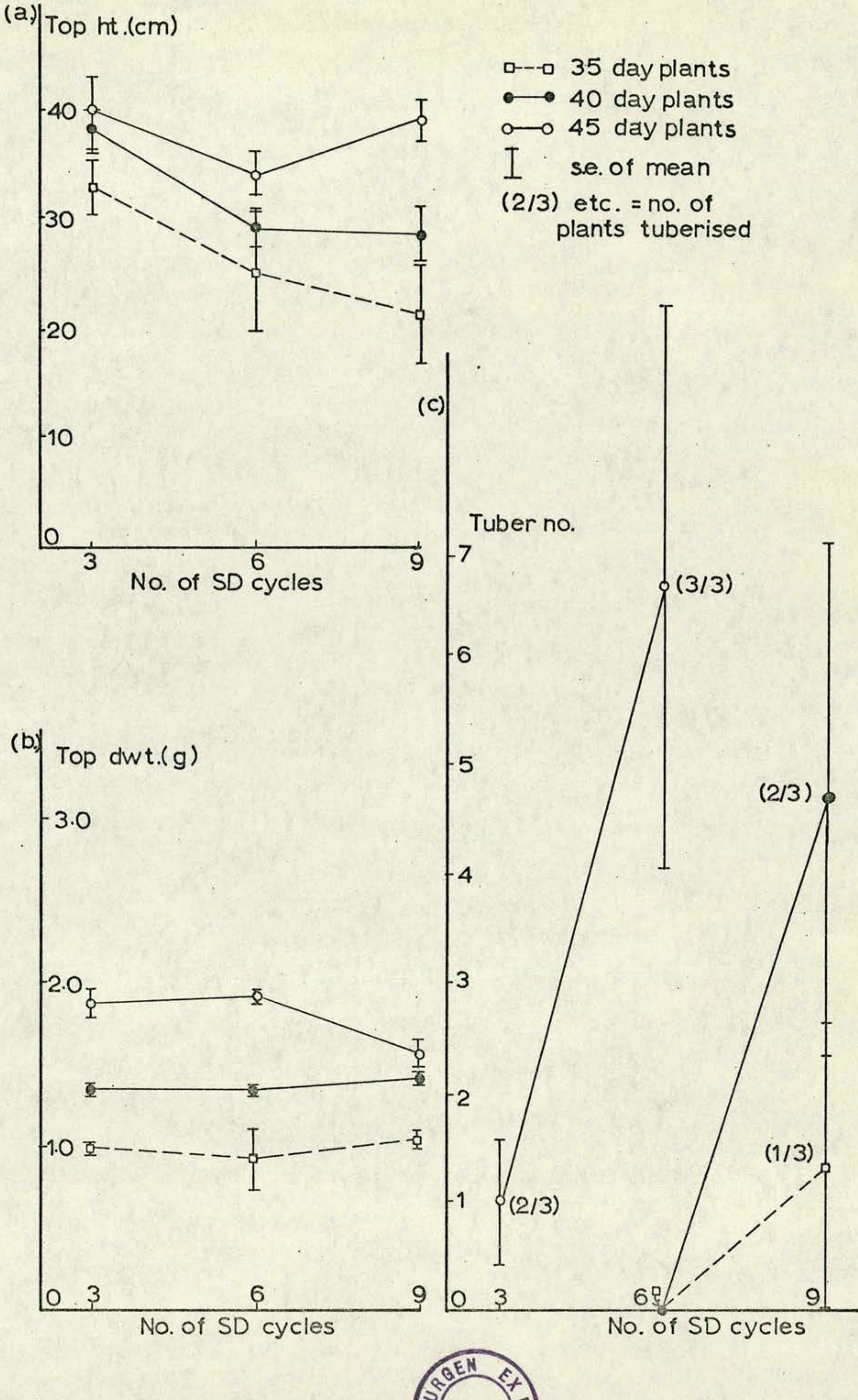


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TABLE 4. Results of topping experiment (Section III (vi)). Effect of decapitation on top, stolon and tuber growth at 14 day harvest for plants grown in SD.

Treatment	Topped	Intact	S.A.	
			p	LSD
Top dwt.(g)	1.6	2.2	0.05	0.4
Stolon no.	7.0	8.3	ns	
Tuber no.	4.7	2.5	0.05	2.0
Tub. stolon no.	2.3	1.8	ns	
Tuber fwt.(g)	5.0	1.2	0.005	0.4

S.A.= Results of statistical analysis. Level of significance of variance ratio (p) and least significant difference (LSD) are quoted.
For other abbreviations, see Section VIII.

The level of replication was 4.

treatments, and as high levels of replication as growing space would allow.

<u>(vi) Investigation of the effect on tuberisation of removal of the growing point and apical leaf cluster (topping experiment)</u>

As mentioned in Section I, the experiments of Chapman (1958) in which different parts of the plant were given different daylength treatments have suggested that the stem apex mediates the photoperiodic control of tuberisation. To discover if this were the case for Up-to-Date, 16 plants were grown in the usual way (see Section II (ii) d) for 35 days, after which time half of them had the small apical cluster of leaves, including the growing point, removed. 4 topped and 4 intact plants were then transferred to either short or low intensity long days. After 14 days all the plants grown in short days and half of those grown in long days were harvested; the remainder were harvested after 18 days.

Top height and node number were, as expected, greaterin the intact plants as was top dry weight; stolon number was unaffected. No tubers were formed after 18 days in the plants kept in long days. After 14 short days, tuber number and tuber fresh weight were both greater in topped plants. The results for plants grown in short days are shown in Table 4.

These results suggest that something is formed in the shoot apex which inhibits or delays tuberisation, although it is likely that this is not all that is involved (see Section VII). This interpretation is consistent with the results of Okazawa and Chapman (1962) with plants with forked stems given differential daylength and pruning treatments (see Section V (i)). It also explains a result which they were unable to account for on their

interpretation (when both long day and short day shoots of forked plants had the growing points removed, tuberisation was greatly hastened). It is also consistent with the results of Chapman (1958) with differential daylength treatments and with pruning of plants kept in long days (in which removal of the apex led to earlier tuberisation) although not with those of a similar experiment with plants in short days. This discrepancy may have been due to differences in age of the plants used or in the number and position of the young developing and fully expanded leaves removed in addition to the stem apex; on neither of these points is Chapman explicit. The possible mechanism of control exerted by the shoot apex and young leaves is investigated in Section V and discussed in Section VII.

(vii) Preliminary experiments examining stolon growth

a. To investigate the effect on stolon number of differential daylength treatment during both the growing period and the experimental period

It is generally accepted (Gregory, 1965) that long days enhance the development, growth and branching of stolons. Such differences were not found, however, with differential daylength during the experimental period alone (the procedure normally used). This experiment was carried out to determine whether differential daylength treatment during both the growing period and the experimental period would cause differences in stolon number in Up-to-Date.

24 plants were grown for 49 days, 12 in low intensity long days and 12 in short days (20°C day - 17°C night throughout). Significantly more stolons were formed on the plants grown in long days (mean 10.4, s e 0.34) than on plants grown in short

days (mean 7.9, s e 0.31). Stolons on plants given long days were also found to be generally more highly branched (due to the development of more axillary buds into side shoots) and longer.

These results confirm that stolon development in Up-to-Date behaves in the same way as it does in other varieties. It was to be expected that such differences would not appear with the normal experimental schedule, since stolons begin to develop during the 35 day growing period, when all plants receive the same high intensity long day light regime.

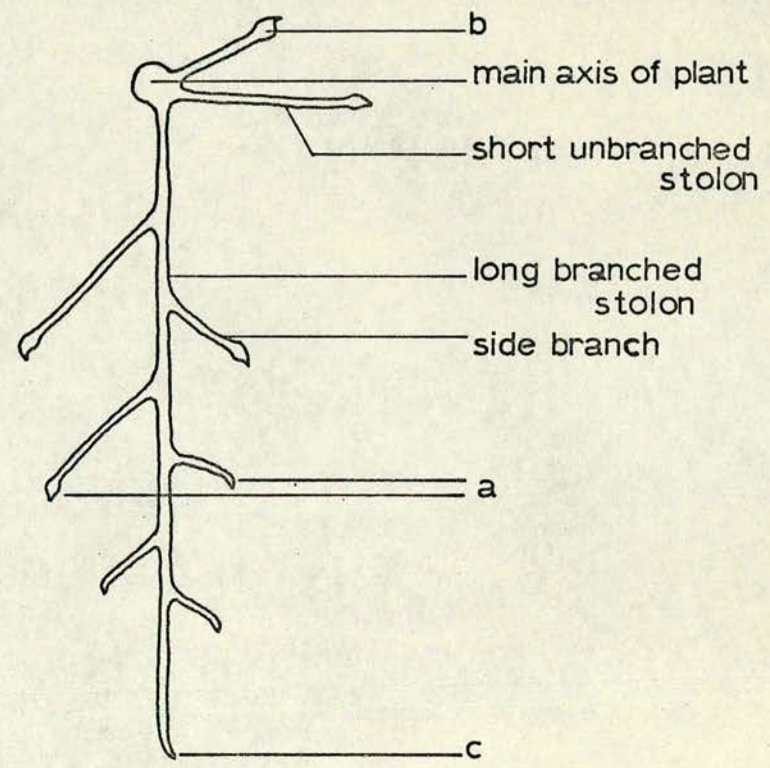
b. Investigation of the order in which different types of stolon tip tuberise under inductive (short day) conditions

Stolon tips may be divided into three categories as shown in Fig 16. The object of this experiment was to ascertain whether all stolon tips on an induced plant begin to tuberise at about the same time, or, if this were not the case, the order in which tuberisation occurs in the different types of stolon tips. 20 plants were grown in Vermiculite and then in nutrient culture (see Section II (ii) d) so that the stolons could be continuously observed without damage. After 35 cycles high intensity long days at low temperature, the last 14 of which were spent in nutrient culture, the light regime was changed to short days at low temperature (see Section II (ii)).

The stolons were observed regularly for signs of tuberisation, which began 9 days after the start of the experimental period (ie after 9 short day cycles). After this, tuber number and tuber fresh weight increased fairly linearly with time for a period of rather more than a week. The first stolon tips to tuberise were those of side branches of long branched stolons

FIGURE 16. Different types of stolon tip (as found in Section III (vii) b)

a = tip of side bud or branch of long branched stolon
b = tip of short unbranched stolon
c = terminal bud of long branched stolon.



b
main axis of plant
short unbranched stolon
long branched stolon
side branch
a
c

("a" in Fig 16). 3 or 4 days later, visible signs of tuberisation were noted at the tips of short, unbranched stolons (b) and after another 4 or 5 days, the terminal buds of long branched stolons (c) began to tuberise.

In an induced plant, therefore, the stolon tips do not all tuberise at exactly the same time, although the times at which they do so are not very far apart in the life of the plant, nor is the order of tuberisation of the tips a random one. There are two possible explanations for this.

It is possible that the arrival of the tuber-forming stimulus at the stolon tip brings about immediate tuberisation, but that the growth characteristics of the different types of stolon tip cause different rates of transport of the stimulus from the tops to the stolon tips. Alternatively, it is possible that there is no great difference in the time of arrival of the stimulus at the different types of stolon tip, but that it is the differing conditions which the stimulus encounters when it reaches the tips which cause the order of tuberisation of the different types of tip.

Further investigation of the conditions at the stolon tip and the effect of various factors on these was obviously necessary to try to discover why all stolon tips on an induced plant do not tuberise at the same time, and some experiments on these topics are described in Section V.

SECTION IV INVESTIGATION OF THE PHOTOPERIODIC BASIS OF TUBERISATION - LIGHT BREAK EXPERIMENTS

(i) Introduction

As has been discussed in the General Introduction (Section I), there is general agreement that the time from emergence of the potato plant to the formation of tubers is least under short days, among other conditions, although the response varies with variety and species. As well as exhibiting delayed tuber initiation in long days, the plants also have much greater haulm growth under natural illumination conditions. This response to differences in daylength has been interpreted in two different ways by different authors. Some, including Gregory (1956), Chapman (1958) and Okazawa and Chapman (1962) have explained their results on the basis of the presence of a "tuber-forming hormone" and others eg Burt (1961), Borah and Milthorpe (1962) have explained the differences brought about by daylength as resulting from differences in growth of the haulms which in turn leads to different substrate supply to the stolon tip. In the experiments (described in Section I) of both Gregory and Chapman, very little information is given concerning the differences in top growth in the inducing and non-inducing conditions. Although the results of these experiments point, as concluded by the authors, to a specific tuber-forming hormone, it has been pointed out (Slater,1963) that they do not rule out the possibility that the differences in time to tuber-initiation are caused by the differences in top growth and therefore in supply of metabolites to the stolon tips. He has also mentioned the fact that many workers have failed to consider the differences

in total daily radiation between different daylength treatments. He has himself carried out an experiment (variety unspecified) to examine the relationship between daylength and tuber initiation with different amounts of total daily radiation. The following treatments were used:-
long days (18 hours) with 36, 72 or 108 cal/sq. cm./day and
short days (9 hours) with 36, 54 and 72 cal/sq. cm./day.
He found that there was no significant difference in the dry weight accumulation between daylengths with any one amount of daily radiation and any differences in top growth prior to tuber initiation were small and variable. He also found that as the amount of daily radiation was increased, the difference in time to tuber initiation decreased. He has concluded that the photoperiodic effect does occur independently of differences in the total daily radiation and that his results are compatible with a hormone hypothesis.

Slater (1963) has also carried out a further experiment with S. tuberosum var. Arran Pilot and the wild species S. demissum in which a light break was given in the middle of the dark period in short days. Details of the experiment are not given but it is assumed that the light break consisted of white light only, since no coloured light treatments were mentioned. Tuber initiation was found to be delayed by the light break treatment for two weeks in the Arran Pilot material and no tuberisation at all took place with such treatment in the S. demissum material (which has an obligate requirement for short days in order to tuberise). Slater also states that the results obtained could not be accounted for by differences in haulm growth between the treatments.

Similar results were obtained by Mokronosov and Lundina (1959), who showed that a short light interruption of the dark period nullified the promotive effect of short days on tuberisation in S. demissum.

These are the only experiments which have been carried out to try to establish that the tuberisation process itself is photoperiodically controlled, and not regulated by the effect of differing daylengths on top growth, and hence distribution of assimilate to the stolons.

In view of the unsatisfactory state of our knowledge on this point, the experiments in the present section were performed to establish whether or not the tuberisation response, for the variety, Up-to-Date, was genuinely photoperiodic. Since it was thought likely that the phytochrome system might mediate the photoperiodic response, red (R) and far-red (FR) in addition to white (W) light break (LB) treatments were given. The effect of blue (B) light breaks was also investigated. In the first experiment, a white light break treatment was given, in the second both red and red plus far-red light breaks, and in the third white, red and red plus far-red light break treatments were used. In the final experiments (4 and 5) blue light breaks were given.

The general methods used in these experiments (growth and harvest of plants and light break treatments) are given in Section II (Material and General Methods). Table 5 gives details of the light breaks used in each experiment (timing, duration, number, timing method, types of lights and filters used) and also the level of replication employed and the dates of the harvests.

TABLE 5. Details of light break treatments used in light break experiments 1 - 5 (Section IV).

LB Experiment	1	2			3		4	5
Colour of LB	W(T)	W(Fl)	R	R + FR	R	R + FR	B	B
Time of LB (h)	8	8	8	8	8.5	8.5	8	8
Duration of LB (min)	10	5	5	5 + 15	5	5 + 15	15	15
No. of LB	14	7	7	7	6	6	14	8
Timing method	A	M			M		A	A
Light source	6.1 W/m² T	80.3 - 129.1 W/m² Fl		80.3 - 129.1 W/m² Fl + 95.6 - 144.4 W/m² Fl + T	1000 W T at top of cans		17.5 W/m² Fl	
Filters	-	-	1R	1B + 1R	1R	1B + 1R	1B	1B
Replication	12	16(8)			4(3)		8	8
Harvest date (days)	14	14			14		14	14

Colour of LB: W = white light (T = tungsten, Fl = fluorescent); R = red; FR = far-red; B = blue.
Time of LB = Time of light break, measured from start of dark period.
No. of LB = Number of consecutive light breaks.
Timing method: A = automatic; M = manual.
Intensity of light source is given before passing through filters (W/m² = Watts per square metre; T = tungsten; Fl = fluorescent (Warm White).)
Filters: R = red glass; B = blue glass (see Section II).
Bracketed values for replication represent number of plants used per treatment in LD (long day) controls.
Harvest dates are given from start of experimental (light break) period.

(ii) The experiments; results and discussion

First Light break experiment

In this experiment, a white light break was given at the middle of the night period, which time appears to be the most sensitive for most photoperiodic effects (eg Salisbury and Bonner, 1956; Borthwick and Downs, 1964; Fredericq, 1964).

[An experiment was in fact carried out to confirm that this was the case for S. tuberosum var "Up-to-Date", in which light breaks were given at 3, 6, 8, 10 and 13 hours after the beginning of the 16 hour dark period to plants grown in short days, but very few tubers at all were formed on any of the plants; perhaps due to slow development of the plants or to delay in banking up the sand around the bases of the plants during the early stages of the growing period, which may have caused slow stolon initiation.

The only plants to tuberise in the experiment were short day controls (2 plants out of 16) and plants given a light break at 3 or 6 hours from the beginning of the dark period (3 out of 16 and 2 out of 16 respectively); plants given light break treatment later in the dark period did not tuberise. The numbers of tubers were so small that these results could not be regarded as meaningful; they did point, however, in the direction that light breaks given at or after the middle of the dark period may have been more effective in inhibiting the short day tuberisation response than light breaks given before the middle of the dark period.

It was unfortunately impossible to repeat this experiment due to lack of time, since it occupied 8 weeks, two of which required a

growth chamber exclusively devoted to this experiment.7

In the present experiment (No 1), in which the light break was given at the middle of the dark period, all the parameters measured at harvest, except for those pertaining to tuberisation, were unaffected by the light break treatment (ie top height, node number, top dry weight, root dry weight and stolon number - see Table 6). There was a significant difference between the short day control plants and those grown in short days with light break treatment for tuber number, number of tuberising stolons and tuber fresh weight. All three parameters were greater in the plants given short days without light break treatment. The percentage of plants tuberised was also greater in the short day controls. Despite the large reduction, none of the three parameters was affected relative to the short day controls as greatly by light break treatment as by low intensity long days, in which no tubers at all were formed in this experiment. The effect on tuberisation caused by the light break was quantitative, not qualitative. This is also true, generally speaking, of the daylength response of tuberisation; in some experiments, low intensity long day controls were found to have produced a few tubers, although always less than plants grown in short days during the experimental period.

These results confirm those of Slater (1963), the light break being found to cause some inhibition of tuberisation, and being unaccompanied by any measurable differences in haulm elongation or dry matter production as far as the parameters recorded were concerned. This suggests that the effect on tuberisation is a direct photoperiodic one, unmediated by differential growth of the haulm under different daylength conditions. It is, however,

TABLE 6. Results of first light break experiment. Differences in top, stolon and tuber growth in LI LD and SD with and without light break treatment at harvest after 14 days.

Treatment	LDC	SDC	SD + LB	S.A.	
				p	LSD
Top ht.(cm)	48.2	40.8	35.8	ns	
Node No.	19.8	19.1	18.8	ns	
Top dwt.(g)	1.78	1.65	1.78	ns	
Root dwt.(g)	0.62	0.61	0.58	ns	
Stolon No.	6.7	8.3	9.3	ns	
Tuber No.	0	7.6	1.8	0.001	2.33
Tub. Stolon No.	0	4.8	1.4	0.001	1.02
Tuber fwt.(g)	0	1.7	0.1	0.001	0.66
No. plants tub.	0/12 (0%)	12/12(100%)	8/12(66%)	-	
Replication	12	12	12	-	

S.A. = Results of statistical analysis for SD and SD + LB treatments. Level of significance of variance ratio (p) and least significant difference (LSD) are quoted.

Treatments: LDC = long day controls; SDC = short day controls; SD + LB = short days plus light break.

For other abbreviations see Section VIII.

quite possible, although Slater does not mention this, that the light break causes differences in the growth of the haulm which have not been measured in either his or the present experiment; for example treatments could lead to differences in the levels of various metabolites being supplied to the underground parts, but in amounts too small to affect top growth.

Having shown an effect of light break treatment on tuberisation in Up-to-Date, it was next considered desirable to attempt to demonstrate the involvement of phytochrome.

Second light break experiment

Hillman (1967) has pointed out the increasing support for the idea that the basic timing mechanism in photoperiodism is closely related to the endogenous circadian rhythms observable in most organisms (Cumming, Hendricks and Borthwick, 1965; Takimoto and Hamner, 1965) and the loss in support for the "reversion-as-timer" hypothesis, which proposed that dark reversion from the Pfr to the Pr form of phytochrome represents the basic mechanism in photoperiodic timing, and which once seemed attractive (Hendricks, 1959; Borthwick, 1964). He goes on to say, however, that although the literature suggests that phytochrome itself is not part of the photoperiodic timing mechanism in higher plants, "it remains the most important, if not the only, transducer of light effects on that mechanism".

It therefore seems likely that phytochrome is involved in the control of the photoperiodic response of tuberisation in potato.

The present experiment was accordingly carried out to obtain

evidence for the possible involvement of phytochrome in the mediation of the inhibiting effect (see above) of light break treatment on tuberisation. This was attempted by trying to show an inhibition of tuberisation by a red light break and a reversal of this effect by a subsequent short period of exposure to far-red light.

The experiment included white, red and red plus far-red light break treatments (for details see Table 5). Light breaks were given at 8 hours from the beginning of the 16 h night period, and the illumination was provided by fluorescent lights for the white and red light breaks and fluorescent plus tungsten lights for the far-red treatments.

The results of the experiment are given in Tables 7a and b. The white light break treatment caused a significant decrease in top height in comparison to the short day controls, but there was no effect of the red light break. The red plus far-red treatment caused an increase in top height and also in node number above that of the short day controls and the plants in the red light break treatment. This enhancement of top growth by the red plus far-red treatment was the only reversal of a light break effect to be found in either of the experiments where this was investigated; there appeared to be no accompanying reversal of the effect on tuberisation (see below). Node number was unaffected by any treatment except the red plus far-red and top and root dry weight and stolon number were unaffected by any treatment.

Although there were too many zeroes in the tuberisation data to allow a valid analysis to be performed on them, all the light

TABLE 7a. Results of second light break experiment. Differences in top, stolon and tuber growth in LI LD and SD with and without white, red or red plus far-red light break treatment at harvest after 14 days.

Treatment	LDC	SDC	SD+WLB	SD+RLB	SD+R+FRLB	S.A.	
						p	LSD
Top ht.(cm)	50.9	19.4	17.2	19.1	25.5	0.001	1.11
Node No.	19.0	17.1	17.2	17.3	18.4	0.05	0.83
Top dwt.(g)	1.51	1.82	1.65	1.65	1.75	ns	
Root dwt.(g)	0.31	0.39	0.42	0.41	0.42	ns	
Stolon No.	3.5	4.3	5.1	5.4	4.9	ns	
Tuber No.	0	0.63	0.06	0.19	0.13	-	
Tub. stolon No.	0	0.63	0.06	0.19	0.13	-	
Tuber fwt.(g)	0	0.21	0.04	0.02	0.05	-	
No. plants tub.	0/8 (0%)	9/16 (56.3%)	1/16 (6.3%)	3/16 (18.8%)	2/16 (12.5%)	-	
Replication	8	16	16	16	16	-	

P.T.O. for key

S.A. = Results of statistical analysis (not including LDC data). Level of significance of variance ratio (p) and least significant difference (LSD) are quoted).

Treatments: LDC = long day controls; SDC = short day controls; SD + WLB = short days plus white light break; SD + RLB = short days plus red light break; SD + R + FRLB = short days plus red light break followed by far-red light break.

For other abbreviations, see Appendix(VIII)

TABLE 7b. Results of second light break experiment (continued). Raw data for differences in tuberisation in LI LD and SD with and without white, red or red plus far-red light break treatment at harvest after 14 days.

Treatment	Parameter	Raw data (Replicates)															
SDC	Tuber no.	1	1	0	1	0	2	0	1	1	0	1	0	1	0	0	1
	Tub.stolon no.	1	1	0	1	0	2	0	1	1	0	1	0	1	0	0	1
	Tuber fwt.(g)	0.10	0.37	-	0.13	-	0.59	-	0.10	2.12	-	0.10	-	0.10	-	-	0.41
SD + WLB	Tuber no.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Tub.stolon no.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Tuber fwt.(g)	-	-	-	-	0.79	-	-	-	-	-	-	-	-	-	-	-
SD + RLB	Tuber no.	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
	Tub.stolon no.	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
	Tuber fwt.(g)	-	-	-	-	-	-	-	0.10	-	-	-	0.10	-	-	-	0.10
SD + R + FR LB	Tuber no.	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
	Tub. stolon no.	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
	Tuber fwt.(g)	-	-	0.10	-	-	-	-	0.71	-	-	-	-	-	-	-	-
LDC	Tuber no.	0	0	0	0	0	0	0	0								
	Tub.stolon no.	0	0	0	0	0	0	0	0								
	Tuber fwt.(g)	-	-	-	-	-	-	-	-								

break treatments appeared to cause a reduction in tuber number (from 0.63 to 0.06-0.19), number of tuberising stolons (from 0.63 to 0.06-0.19), tuber fresh weight (from 0.21 to 0.02-0.05g) and percentage of plants tuberised (from 56.3% to 6.3-18.8%). Because of the small numbers of tubers formed and the resulting lack of statistics, it was felt that these results could be more clearly seen in the form of the raw data, as presented in Table 7b. It was thought that the low level of tuberisation, which was found throughout all the treatments in the experiments, was due to slow development of the plants, since their early growth was supervised with great care because of the difficulty experienced in the experiment to establish the best time for the light break (see above).

A number of reasons may be put forward to explain the lack of reversal of the red effect by far-red.

Firstly, it is possible that the far-red sources used were inadequate in some respect. They may not have been selective enough with respect to wavelength, admitting too much red light, and therefore making reversal of the red effect impossible, or they may not have been intense enough, the filter cutting out too much light.

There are two reasons why this explanation seems unlikely. The same far-red filters (see Section II for details) were used by Murray (1968) with success in Phaseolus, although it is conceivable that the response to Pr/Pfr ratios in potato may differ from that in bean. Also, the promotion of top growth by the red plus far-red light break treatment in the present experiment

suggests that the plants were capable of responding to the far-red source used as a light break in the opposite way from their response to white light (which brings about a high Pfr/Pr ratio in the plant) as a light break. The response to the far-red light break is also that which would be expected from the literature, far-red light being usually found to promote stem elongation (Salisbury, 1963).

The second possibility to explain the lack of response of tuberisation to far-red light break treatment is that the time (3-4 minutes) spent in changing the filters between the red and far-red treatments was long enough for the Pfr-mediated inhibitory reaction to become irreversible. The time taken for this operation was unavoidable using manually-applied light breaks in an experiment of this size, since the black polythene covers used for plants not receiving light break treatment had to be fitted over 8 cans randomly distributed over the growing bench and blue glass filters added to a further 4 cans also randomly distributed, on top of the red plates already covering them during the first part of the light break treatment. This procedure had to be performed in darkness with the aid of only a point of very low intensity physiologically-inactive green light from a heavily-masked torch, and was therefore carried out mainly by feeling for the positions of the cans, covers and filter plates.

The time required for the inhibitory act of Pfr in plants of short day response type varies greatly from species to species. The results of Kasperbauer, Borthwick and Hendricks (1963) with <u>Chenopodium rubrum</u> have shown that flowering of small seedlings of one strain of this plant

is inhibited by a red light break in the middle of the night, this inhibitory action being reversed by a far-red irradiance immediately afterwards. If darkness intervened between the red and far-red treatments, the level of flowering declined as the period of darkness increased and required 70 minutes of darkness to reach zero. It has since been found, however, by Downs (personal communication quoted in Borthwick, 1964) that in one selection of *Chenopodium album*, flowering usually failed even when the far-red treatment followed the red within one minute. Also, Fredericq (1964) found with *Pharbitis nil* that far-red reversal of the effect of a red light break on flowering did not take place when two minutes of darkness intervened between the two treatments; this indicated that the flower-inhibiting action of Pfr was completed in slightly more than a minute.

The period required for Pfr action to prevent flowering thus ranges from less than one minute to more than an hour in different plants. It is thus possible that in the present experiment, the time between the red and far-red treatments was enough for the effect of red light treatment to become irreversible, if potato is a plant in which only a very short period of time is required.

The third possibility to explain the lack of reversal is, of course, that phytochrome is not involved in the response.

A further experiment (No 3) was carried out to try to determine which of these alternative explanations was the correct one.

Third light break experiment

This experiment used smaller numbers of plants than did the

previous one, and this made it possible to cut down the time spent in changing the filters between the red and far-red treatments to about 30 seconds or less. A higher intensity light source for the light breaks was also used, consisting of a 1000W tungsten photoflood bulb positioned close above the coloured glass filters covering the cans containing the plants. The red content of this light was also smaller compared to the far-red content than that in the light used for the light breaks in the previous experiment, in which the source consisted of fluorescent plus tungsten light. (For details of light sources, see Section II; for other details of the experiment, see Table 5).

The results of the experiment are presented in Table 8. Top height was found to be significantly reduced by both red and red plus far-red treatment, no reversal being found with far-red in this experiment. Node number, top dry weight, root dry weight and stolon number were all unaffected by either light break treatment.

Tuber number and number of tuberising stolons were reduced by both the red and the red plus far-red treatments, although all the plants in all short day treatments carried some tubers, and tuber fresh weight was not found to be significantly affected, probably because of the high variance in the data. Although some tubers were formed on the long day controls, these plants showed the lowest levels of tuberisation of all; tuber number and number of tuberising stolons were significantly less in these plants than in not only the short day controls, but also the plants given short days and light break treatment. The results show once again that the reduction in tuberisation caused by the light

TABLE 8. Results of third light break experiment. Differences in top, stolon and tuber growth in LI LD and SD with and without red and red plus far-red light break treatment at harvest after 14 days.

Treatment	LDC	SDC	SD+RLB	SD+R+FR LB	S.A.	
					p	LSD
Top ht.(cm)	48.8	45.3	39.8	38.6	0.005	2.48
Node No.	18.0	17.3	17.8	17.7	ns	
Top dwt.(g)	1.33	1.26	1.27	1.32	ns	
Root dwt.(g)	0.42	0.40	0.47	0.43	ns	
Stolon No.	9.7	9.8	7.0	12.3	ns	
Tuber No.	0.3(1.0068)	6.3(1.2156)	3.5(1.1434)	3.0(1.1205)	0.005	(0.0611)
Tub. stolon No.	0.3(1.0155)	4.5(1.1696)	2.8(1.1196)	3.0(1.1178)	0.005	(0.0489)
Tuber fwt.(g)	0.11	0.90	1.21	0.35	ns	
No. plants tub.	1/3 (33.3%)	4/4(100%)	4/4(100%)	4/4(100%)	-	
Replication	(1 rotted) 4	4	4	4	-	

Treatments:- LDC = long day controls; SDC = short day controls; SD + RLB = short days plus red light break; SD + R + FR LB = short days plus red followed by far-red light break.
Bracketed values are log transformed values, and LSD values obtained from these. For other abbreviations, see Section VIII.

break treatment (compared to short day conditions) was quantitative, not qualitative, and less pronounced than that found in low intensity long days.

It was again impossible to demonstrate a reversal of the inhibiting effect of red light-break treatment on tuberisation by the subsequent exposure to far-red light even with the conditions used in this experiment. If anything, the subsequent far-red treatment enhanced the inhibitory effect of the red light break on tuberisation.

These results are in agreement with those of the previous experiment, red light breaks causing inhibition of tuberisation, and no reversal of the effect of a red light break being found with subsequent far-red treatment.

Although it is less likely than in the previous experiment (due to the modifications made in the present experiment), it is still possible that the far-red light source was inadequate for the potato, or that the time between red and far-red treatments was sufficient for the Pfr-mediated reaction to be completed; of these two possibilities the latter is probably the more likely.

To summarise the results obtained from the first three light break experiments:-

It has been established that light breaks given in the middle of the long night of plants grown in short days reduce tuberisation. This effect was found with either white or red light. Although the light break treatments reduced tuberisation, they did not prevent the response, and the inhibition caused was less than

that caused by low intensity long days as compared to short days (which is also not always a complete inhibition in *S. tuberosum*, but was usually almost complete with the experimental schedules used).

It has also been shown that there is very little effect of the light break on growth of the tops. Neither white nor red light breaks had any effect on node number or top (or root) dry weight. In experiments 2 and 3 top height was reduced by light break treatment, but this was not the case in experiment 1, and tuberisation was nevertheless inhibited in the latter experiment, where no effect whatever of the light break was detected in the tops. Therefore, the light break treatment caused reduction in tuberisation even where there was no detectable effect on the growth of the tops as measured by top height, node number and top dry weight. The effect on tuberisation would therefore appear to be a genuine direct photoperiodic effect, and not due to differences in total growth of the tops. This result does not, of course, entirely rule out differences in supply of assimilates from the tops to the stolons in the plants treated and not treated with light breaks.

As mentioned above, Hillman (1967) has stated that phytochrome remains the most important, if not the only, transducer of light effects on the mechanism of photoperiodic timing in higher plants. Since both white and red light breaks brought about a reduction in the tuberisation response, this would suggest the participation of phytochrome. Hanke, Hartmann and Mohr (1969) have drawn this conclusion from similar results in studies on the flowering of *Sinapis alba*, although they, as was the case in the present experiments, were unable to detect any far-red reversibility of

their effect. It is felt that it is most likely that the inability to demonstrate far-red reversibility of the effect of white or red light break treatments on tuberisation was due to some deficiency in technique, most probably the unavoidable time spent between the red and the far-red treatment in changing the covers and filters.

However, it was necessary to consider the possibility that phytochrome might not be involved, and the next two experiments were carried out with the intention of investigating whether some other photoreceptor system was involved.

Fourth and fifth light break experiments

At the time when these experiments were conceived, Mohr (1959, 1964) had put forward evidence for a photoreaction possibly unrelated to phytochrome which was thought to control a number of plant responses, most of which are also controlled by phytochrome. The responses appeared to be irreversible, and the action spectra showed fairly sharp peaks in the blue (440 nm) and the far-red (725 nm). The basic observations which led to this concept (of what he named a "high energy reaction", separate from phytochrome) were obtained from examination of the light-dependent enlargement of the cotyledons of the mustard seedling (Sinapis alba), which is an example of a graded photoresponse which can be easily and accurately measured. This "high energy reaction" differed from the phytochrome response in that it showed a characteristic intensity dependence, whereas the photoequilibrium of the phytochrome system at a particular wavelength does not depend on intensity.

To investigate the possibility that such a system with a peak in the blue region of the spectrum might be involved in the tuberisation response in potato, two experiments were carried out using a blue light break of 15 minutes duration, applied 8 hours from the beginning of the night period. Details of the experiments are given in Table 5 and the results are presented in Table 9.

The blue light break treatment was not found to have any effect on the growth of the tops, except for a small increase in node number found in the second experiment; this was probably not meaningful. Top height, top dry weight and stolon number were all unaffected.

In both experiments, however, the light break treatment caused an inhibition of tuberisation. In the first experiment (No 4), both tuber number and the number of tuberising stolons was reduced. Tuber fresh weight was not recorded, as all the tubers were very small. In the second experiment (No 5), tuber number was again reduced. The number of tuberising stolons and tuber fresh weight, while appearing to be reduced (see Table 9) were not found to be significantly affected, probably because of a high level of variance in the data. In both experiments the percentage of plants tuberised was much greater in the short day controls than in the plants given the light break treatment.

When these experiments were performed, it was thought informative to examine the starch deposition, if any, in non-visibly tuberised stolons, as this had been found to increase prior to visible tuberisation (see Section VI). This was accordingly included in the harvesting procedure. It was not possible to perform an

TABLE 9. Results of fourth and fifth light break experiments. Differences in top, stolon and tuber growth in SD with and without blue light break treatment, at harvest after 14 days.

Treatment	Experiment 4				Experiment 5			
	SDC	SD + BLB	S.A.		SDC	SD + BLB	S.A.	
			p	LSD			p	LSD
Top ht.(cm)	31.2	34.2	ns		52.5	53.3	ns	
Node No.	14.8	14.8	ns		20.4	21.3	0.05	0.81
Top dwt.(g)	1.00	0.87	ns		2.26	2.55	ns	
Stolon No.	7.5	6.3	ns		12.6	10.8	ns	
Tuber No.	2.6	0.5	0.001	1.07	5.9	1.5	0.05	3.82
Tub. stolon No.	2.6	0.5	0.001	1.07	5.5	1.4	ns	
Tuber fwt.(g)	-	-	-		0.21	0.06	ns	
Non-tub. stolons								
with starch	1.3	0.9	-		1.5	0.2	-	
no starch	3.9	5.2	ns		5.6	9.2	ns	
% with starch	33.3	17.3	-		26.8	2.2	-	
No. plants tub.	8/8(100%)	2/8(25%)	-		7/8(87.5%)	3/8(37.5%)	-	
Replication	8	8	-		8	8	-	

SDC = short day controls; SD + BLB = short days plus blue light break.
For other abbreviations, see Section VIII.

analysis on the data for the number of visibly untuberised stolons containing starch, as there were too many zeroes in the data, and no differences were found between the numbers of untuberised stolons without starch. It appeared, however, that a higher proportion of untuberised stolons contained starch deposits (which appear immediately prior to visible tuberisation - see Section VI) in the short day controls than in the plants given the blue light break treatment. This amplifies the above data showing inhibition of tuberisation by the light break treatment.

From the results of these experiments, it therefore seems that a blue light break is as effective in causing partial inhibition of tuberisation as a white or red light break. This result can be explained in more than one way.

> Recently, Mohr (1969) has stated that while for some years it seemed necessary to postulate, even in the wavelength range above 550 nm, the existence of a separate photoreceptor besides phytochrome in order to account for the phenomena of morphogenesis (Mohr, 1959, 1964), there are now, nevertheless, good arguments suggesting that the active photoreceptor is in all cases exclusively phytochrome, at least above 550 nm (eg Hartmann, 1966, 1967 a, b and c). Mohr (1969) considers that there seems no alternative at the moment to the conclusion that phytochrome is the photoreceptor of the "high energy response" in the far-red range, with either Pfr or one of the short-lived intermediates between Pr and Pfr as the specific effector molecule (Linschitz, Kasche, Butler and Siegelman, 1966; Linschitz and Kasche, 1967; Spruit, 1966), most likely Pfr (Hartmann,

1967 b and c). He also thinks, however, that there are "a considerable number of blue light dependent photoresponses where an explanation on the basis of phytochrome seems to be excluded." He discusses four clear-cut examples, in three of which he considers that the photoreceptor may be a flavoprotein, and in the fourth of which phytochrome is, in addition, involved. He concludes that if a response shows a strong effect of short wavelength light and only a slight effect of long wavelength light (eg anthocyanin synthesis in milo seedlings (Downs and Siegelman, 1963)) it seems reasonable to postulate a simultaneous action of phytochrome and a flavoprotein. Also, there are still workers who consider that an alternative photoreceptor system to phytochrome may be activated by both blue and far-red light (eg Esashi, 1969, thinks that such a system, as well as a phytochrome system, may be required to explain his observations on photoperiodically-controlled tuberisation and sprouting of tuberous buds in *Begonia*)

It is conceivable that the inhibitory effect of the blue light break on tuberisation in the potato may be due to such a photoreceptor, instead of or as well as phytochrome. It does seem unlikely, however, that such a photoreceptor *only* is involved, if Mohr's conclusions are correct, since tuberisation is inhibited not only by a blue, but also by a red light break, which Mohr now considers to be exclusively due to phytochrome mediation.

The results of the blue light break experiments on tuberisation in potato described above are also explicable on the basis of phytochrome mediation alone:-

Both species of phytochrome, Pr as well as Pfr, absorb in the blue and the ultraviolet regions of the spectrum (eg Siegelman and Butler, 1965; Hillman, 1967). Although the absorption coefficients and the relative quantum efficiencies of the photoconversions are much lower than in the red or far-red regions of the spectrum, photoequilibria are nevertheless established if the irradiation is continued for some time (Butler, Hendricks and Siegelman, 1964; Pratt and Briggs, 1966). The ratio of Pfr/Pr as measured in vitro (Pratt and Briggs, 1966) appears to be between 1% and 35% depending on the particular wavelength used; in any case, Pfr will be formed under the influence of blue and near ultraviolet light, and short wavelength light can therefore bring about the same responses as does red light.

The results of all the light break experiments can therefore be interpreted in terms of a phytochrome response, since similar effects can be achieved by light breaks in the red and in the blue regions of the spectrum, phytochrome being known to absorb light in both these regions. The only factor which would lead one to suppose that phytochrome was not involved is the lack of reversal of the white and red light break effects by far-red light, and it is considered that this was probably the result of the intervening time between the two types of treatment.

(iii) Conclusions

It may therefore be concluded that tuberisation in S. tuberosum variety Up-to-Date shows a genuine photoperiodic response, being inhibited by a light break in the middle of the inductive long

night, and this effect not being due to overall growth of the tops. It is a quantitative not a qualitative response, and light breaks were not found to be such effective inhibitors of tuberisation as long days. It is considered likely, in view of the fact that white, red and blue light breaks all caused partial inhibition of tuberisation, that phytochrome may be involved, but it was unfortunately impossible to determine this definitely because of inability to demonstrate a reversal of the light break effect by subsequent exposure to far-red light. This inability was, however, considered to be due to the shortcomings of the techniques used.

SECTION V. INVESTIGATIONS CONCERNING THE INVOLVEMENT OF GROWTH-ACTIVE SUBSTANCES IN TUBERISATION

(i) Introduction

As discussed above, the results of the light break experiments described in Section IV suggest the participation of a tuberising "stimulus" in the control of tuberisation, although they do not eliminate the possibility that the distribution of various metabolites to different parts of the plant may be regulated by the light break treatment. The latter type of situation also, however it occurs, usually implies the involvement of one or more plant growth substances.

As mentioned in Section I, the involvement of a specific tuber-forming stimulus has been proposed by a number of workers (eg Gregory, 1956; Chapman, 1958; Madec, 1963). This may not be a single substance; it is more likely to be made up of several growth substances. Several groups of such substances have been suggested as being involved in the tuberisation response.

Some workers have considered it likely that ethylene may be involved because of its effect on the direction of cell expansion (Burg and Burg, 1965) and evidence has been put forward supporting this idea (Burton, 1952; Catchpole and Hillman, 1969); it was felt, however, that the evidence is unconvincing, and the possible involvement of ethylene was not included in the topics to be investigated in the present work.

Growth substances likely to be involved on the basis of their relevant properties

In a choice of which substances are most likely to be involved, it is necessary to consider their properties, especially with

respect to site of formation, mobility in the plant, and effects on the processes taking place in the stolon tip at tuber initiation.

1. Properties relevant to changes at the stolon tip

As already discussed in detail (Section I), the principle visible cellular changes which take place at tuber initiation are, firstly, a loss in the polarity of cell enlargement so that medullary and cortical cells in the region of the sub-apical meristem of the stolon tip become shorter and wider with little accompanying cell division, and, secondly (slightly later), a promotion of cell division in the same region of the stolon tip, most of the divisions taking place in "random" directions (see Fig 50c).

Another change which takes place in the stolon tip immediately prior to those just described, is that starch deposition commences in the cells of the medulla and cortex (Lovell and Booth, 1967; Palmer and Smith, 1969a; for details of the process see Section VI).

The growth substances likely to be involved in the control of tuber initiation might therefore be expected to be substances which are known to exert an effect on cell enlargement and/or cell division, especially in the sub-apical meristem and/or substances known to affect starch metabolism.

Vegetative extension growth of the stolon, of course, also requires cell enlargement and cell division, but mainly in a different direction. The cells in the sub-apical meristem of the non-tuberised, growing stolon normally elongate in a direction parallel to the long axis of the stolon, and most cell divisions take place at right angles to this axis (Sadler, 1961; Booth, 1963). What seems to be necessary for tuber initiation is for

this type of cell elongation and cell division to cease and to be subsequently replaced first by cell enlargement to produce shorter, wider cells in the medulla and cortex, and then by cell divisions in the "random" directions characteristic of the tuber (Booth, 1963).

In view of the above events in the tuberising stolon tip, the growth substances most likely to be involved are auxins, gibberellins, naturally-occurring growth inhibitors and cytokinins, for the reasons given below.

a. Auxins: Thimann (1969) points out that the most characteristic action of auxin is to promote cell enlargement. Auxin also causes cell division in some tissues (eg Snow, 1935; Söding, 1936). In view of these two effects, auxins might be expected to perform some role in tuber initiation, although it is perhaps relevant to point out that the site of auxin action on cell elongation is generally in the elongation zone of the stem, not in the sub-apical meristem.

b. Gibberellins: On the basis of its effects at the terminal portion of stems, and especially the sub-apical meristem, gibberellin appears an even more likely candidate for involvement in the control of tuberisation. One of the most dramatic of its effects is its ability to induce "bolting" of the dwarf stems of rosette plants in the absence of the appropriate environmental conditions which normally bring it about (Lang, 1956). This has been shown to be due to an activation of cell division in the normally inactive sub-apical meristem (Sachs, Bretz and Lang, 1959). Lang (1960) has shown that normal

bolting is correlated with a marked increase in the level of endogenous gibberellins, suggesting that these are the normal controlling agents. The same sort of control also seems to be involved in caulescent plants (Sachs, Lang, Bretz and Roach, 1960).

Sachs et al (1959) have also found that in plants such as Hyoscyamus, the final cell length is unaffected by gibberellin. In other cases, gibberellin has been found to stimulate both cell division and cell elongation (Lockhart, 1956, 1960; Cleland, 1964; Arney and Mancinelli, 1966), although the major effect is still the induction of cell division and other evidence (eg Brian and Hemming, 1958) also suggests only a minor role in the control of cell elongation.

Gibberellin has also been shown to affect starch metabolism. It can induce the de novo synthesis of α-amylase in the aleurone layer of barley endosperm (eg Paleg, 1960; Varner and Ram Chandra, 1964; Varner, Ram Chandra and Chrispeels, 1965).

Gibberellin, therefore, appears to be capable of controlling all three processes which characterise tuber initiation, especially starch synthesis and cell division in the sub-apical meristem.

c. Growth inhibitors: Naturally-occurring growth inhibitors similar to and perhaps including abscisic acid might also be expected to be involved on the basis of the activities of growth inhibiting substances on these three processes.

The work of Sachs et al (1960), mentioned above, has shown that the synthetic growth retardant Amo-1618 brings about its inhibition by suppressing cell division in the sub-apical

meristem, and that its action can be overcome by applied gibberellin. It is possible that other growth inhibitors such as abscisic acid also operate in this way, especially in view of the many opposing interactions of gibberellins and abscisic acid (eg Thomas, Wareing and Robinson, 1965; Sondheimer and Galson, 1966; Aspinall, Paleg and Addicott, 1967; Chrispeels and Varner, 1966, 1967; Madison and Rappaport, 1968).

Of particular relevance for tuber initiation is the inhibition by abscisic acid of the enhanced synthesis of α-amylase in barley caused by gibberellic acid (Chrispeels and Varner, 1966, 1967); this can be overcome by more gibberellic acid. It is thus possible that gibberellic acid and abscisic acid could together control starch metabolism at the stolon tip.

Abscisic acid has also been found to be a highly active growth inhibitor in the Avena coleoptile test, which is mainly dependent on cell elongation (see Wareing, 1969).

d. Cytokinins: These substances would also appear to be capable of exerting the type of control required in tuber initiation. The most characteristic property of the cytokinins is the stimulation of cell division (see Miller, 1961; Letham, 1967). High levels of cytokinins are found to be associated with times and regions of intense cell division in plant tissues (Letham, 1963; Bottomley, Kefford, Zwar and Goldacre, 1963).

Cytokinins can also promote cell expansion (Miller, 1956; Kuraishi and Okumura, 1956; Scott and Liverman, 1956; Arora, Skoog and Allen, 1959; Kuraishi, 1959; Haber and Luippold, 1960; Katsumi, 1962), inhibit cell elongation (Vanderhoef and

Key, 1968; Brian and Hemming, 1957), and cause lateral expansion of cells (Katsumi, 1962; Hashimoto, 1961). They may also be involved in the control of starch metabolism; they have been shown to inhibit α- amylase activity (Mann, Yung, Storey, Pu and Conley, 1967; Sprent, 1968) and to promote starch synthesis (Wang, 1961; Möthes, 1964) although they can also induce starch degradation (Boothby and Wright, 1962).

All four groups of growth substances therefore seem capable of exerting control on the processes taking place in the stolon tip at tuber initiation; information is rather less extensive on the sites of formation and the transport of these substances.

2. Site of formation and mobility

The results of the topping experiment described in Section III suggest that the factor involved in delaying tuberisation is formed in the stem apex or very young leaves of the tops, since topping led to a promotion of tuberisation.

Chapman (1958) has interpreted his results to mean that the tuberising stimulus is formed by the active growing point, since results of differential daylength treatment of different parts of the plant showed that the results of treatment given to the terminal leaf cluster (including the growing point) alone were the same as those of treatment to the whole plant; this was not the case with treatment of the basal leaves alone. Okazawa and Chapman (1962) have carried out experiments in which plants with forked stems were treated differentially with respect to daylength or subjected to girdling or pruning treatments. They have concluded that the growing points are involved in the production of both the stimulus which promotes tuberisation and the growth substances which inhibit

it, although the results could also be interpreted as indicating that the inhibitor of tuberisation is formed in the shoot apex, and the stimulus promoting tuberisation in the mature leaves.

The substances involved in the control of tuberisation must be capable of fairly rapid movement, suggesting that they move in the vascular system, in either xylem or phloem or both. Their movement would also be expected to be non-polar, since they appear to move from the shoot apex or leaves to the base of the plant (basipetally), and thence from the base of the stolon to its tip (acropetally).

Auxins are formed in the stem apex and are mobile in the plant so that they would appear to be able to fulfil the above conditions; endogenous auxin, however, is probably transported in a polar fashion in intact plants and is generally found to move basipetally (eg Went, 1928; Skoog, 1938; Jacobs, 1952; Scott and Briggs, 1960).

It seems that the sites of synthesis of gibberellins are those regions which normally have the highest levels of gibberellins, eg the apices of stem and root and the young leaves (Jones and Phillips, 1966). They appear to be capable of quite rapid movement (Zweig, Yamaguchi and Mason, 1961; McComb, 1964) in both the phloem (Kluge, Reinhard and Ziegler, 1964) and the xylem (eg Phillips and Jones, 1964); their movement has generally been found to be non-polar (Kato, 1958; Clor, 1967; Jones and Phillips, 1966).

Goldsmith (1969) considers that the situation of the cytokinins may be similar to that of the gibberellins; although some workers

have found very little movement (eg Möthes, 1960; Thimann, 1963; Sachs and Thimann, 1964), others have found evidence for movement (eg Osborne and Black, 1964; Osborne and McCready, 1965).

In the case of the naturally-occurring growth inhibitors, some studies have suggested that they are formed in the mature leaves (Wareing, 1954; Waxman, 1957) and are transmitted from there to the shoot, demonstrating their mobility in the plant.

From the above account it therefore appears that gibberellins, growth inhibitors, cytokinins and auxins are all capable of being involved in the control of tuberisation.

Effects of these groups of growth substances on the tuberisation in potato

An auxin, probably indole acetic acid, has been found in potato tissues (Booth and Wareing, 1958). While it appears that auxin, together with gibberellin, controls typical stolon growth (Booth, 1963), the evidence for a direct role of auxin in the formation of the tuber itself is conflicting.

Borah (1959), in experiments with stem cuttings in sterile culture, found that indole acetic acid accelerated tuber formation in Media with a suitable sucrose concentration. Van Schreven (1956) has studied the effect of auxins on premature tuber formation (ie the formation of secondary tubers by mother tubers which have completed their dormancy and are germinating). He applied indole acetic acid, naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid to the sprouting tubers and found that earlier tuber initiation was induced by all these substances, when applied at low concentrations.

Tizio (1964a) found that both naphthalene acetic acid and indolyl butyric acid promoted tuberisation in cuttings, and has suggested that the effect is an indirect one brought about through an effect of auxin on root development. Gausman, Corbett and Struchtemeyer (1958) injected a number of growth-active substances (2,4-dichloroanisole, 2,3,5-triiodobenzoic acid, indole acetic acid, naphthalene acetic acid and 2,2-dichloropropionic acid) into stolons. They found that all these substances, especially indole and naphthalene acetic acids, caused delayed tuber initiation.

Other workers have produced evidence suggesting that auxins are not involved in the control of tuberisation (eg Dostál, 1945 with Ficaria verna and Ito and Kato, 1951, with potato).

The involvement of auxin in tuberisation is therefore somewhat uncertain. It was decided to carry out an experiment using the growth promoters indole acetic acid and gibberellic acid applied to the whole plant alone and in combination and to examine the effect of such treatments on tuberisation to try to resolve this question.

More convincing evidence has been obtained for the involvement of endogenous gibberellins in tuberisation, as an influence tending to inhibit or retard the process. Okazawa (1959), using ethanolic extracts from various parts of potato plants of varieties Irish Cobbler and Norin No 1, has demonstrated the presence of several kinds of gibberellin-like substances in potato tissues. Okazawa also found that when gibberellin was applied to potato plants, even under short day conditions, no tuber formation occurred. He concluded that endogenous gibberellin may play some physiologically important role in controlling tuber formation.

Retardation or prevention of tuberisation by gibberellins has also been found by Dyson and Humphries (1966) with the variety Majestic, and with the variety Bintje using stem cuttings (Tizio, 1964b, 1966; Perennec, 1966) cultured *in vitro*. Tizio has shown that the delay in tuberisation caused by treatment with gibberellic acid was directly correlated with the concentrations used. McCorquodale and Moorby (1968) have also found that gibberellic acid treatment at concentration of 0.1 and 1.0 ppm delayed tuberisation in excised stolon tips grown *in vitro*, the delay being greater with the higher concentration.

Lovell and Booth (1967) have suggested a possible mechanism of gibberellin action on tuberisation. In experiments using plants treated or not treated with gibberellic acid, they have demonstrated a correlation (in both groups of plants) between tuber initiation and the appearance of starch in the stolons, although no such correlation was found with sugar level. When untreated plants were fed with $^{14}CO_2$, almost all the translocated ^{14}C accumulated in the tubers, whereas in plants pre-treated with gibberellic acid a few days before the feeding of $^{14}CO_2$, practically no ^{14}C was detected in the stolons or tubers but accumulated in the shoot, particularly at the apex. Lovell and Booth proposed that applied gibberellic acid may act by altering the activity of the various metabolic sinks (principally the shoot apex and the tubers) and therefore the pattern of translocation. This does not explain, however, what happens at the stolon tip of a gibberellin-treated plant to cause an inhibition of starch deposition, or whether this is a cause or a result of the altered sink activities and pattern of translocation. The studies of Sachs et al (1959, 1960) on the effect of gibberellins on the

sub-apical meristem, described above, suggest that changes in the pattern of translocation of metabolites are probably not all that is involved (see Section VII, General Discussion).

Okazawa (1960) in studies on the endogenous gibberellin content of the leaf blades of potato plants, found that with low temperatures (13°C) and short days (8 hr), both conditions favourable for tuberisation, lower levels of natural gibberellins occurred than in plants grown at higher temperatures or in long days. Racca and Tizio (1968), using Bintje, have also followed changes in the content of gibberellin-like and anti-gibberellin-like substances in shoots and roots during different periods of the vegetative cycle. They found that before tuberisation, shoots showed activity of both types of substance. They showed that when tuberisation began there was a decrease in gibberellin-like activity in the shoots, accompanied by an increase in activity of anti-gibberellin-like substances, especially in the acidic fractions. With the progress of tuberisation, the levels of both classes of compound fell markedly. Similar extracts made of the roots gave very different results. Before tuberisation, large quantities of anti-gibberellin-like substances were produced by the roots, but this activity disappeared almost completely when the tubers began to grow. After this time, root extracts showed the presence of gibberellin-like substances.

These results all suggest that endogenous gibberellins may be involved in some way as an anti-tuberising influence in the potato plant.

In contrast to these results, Razumov (1960), working with S.demissum and S.acaule, was unable to observe inhibition of

tuberisation with gibberellic acid treatment in short days, and concluded that the inhibition of tuberisation in long days was a secondary effect, due to the promotion of shoot growth by the applied gibberellin, and that gibberellin plays no direct part in tuberisation. Claver (1960) has concluded that gibberellin does not inhibit the formation of tubers "when the factors of tuberisation are present"; in later papers (Claver, 1966 and 1970), however, he seems to support the view that gibberellins are one of the factors which control tuberisation.

While the position is by no means clear, therefore, it nevertheless appears that gibberellins are probably involved in the control of tuberisation and that further study of their role is required.

It would also seem valuable to investigate the effect of 2-chloroethyltrimethylammonium chloride (CCC) on the process of tuberisation, since this compound has been shown to be an inhibitor of gibberellin synthesis eg in Fusarium moniliforme (Kende, Ninnemann and Lang, 1963; Ninnemann, Zeevaart, Kende and Lang, 1964; Barnes, Light and Lang, 1969) and in embryos of Avena fatua (Simpson, 1966).

The effects of CCC on the growth of whole potato plants has been investigated by Dyson and Humphries (1963, 1966), Dyson (1965) and Humphries and Dyson (1967), while McCorquodale and Moorby (1968), Palmer and Smith (unpublished, quoted in Palmer and Smith, 1969b) and Tizio (1969) have examined its effects on cultured pieces of plants grown in vitro.

CCC was consistently found to hasten tuberisation when applied

to whole plants. Tizio, using sections of sprouts of tubers of the variety Bintje also found that CCC promoted tuberisation, and that gibberellic acid reversed its effects. McCorquodale and Moorby (1968), using stolon tips, found no such promotive effect of CCC (see Section V (iii)). Palmer and Smith, also using stolon tips, found that stolon growth was inhibited by CCC treatment, but that tubers were not initiated in the absence of kinetin (see Section V (iii)).

It was felt that further investigations of the effect of CCC on whole plants and on stolons might prove useful to amplify further work on the role of gibberellins.

Okazawa (1959), in addition to demonstrating the presence of endogenous gibberellins, has shown the presence of a natural growth inhibitor in the tissues of potato plants and has found an apparently antagonistic action of this substance to that of gibberellin in bioassay. Booth (1963) demonstrated, in extracts of stolon tips which had just begun to tuberise, a chrometographically separable substance which inhibited the _Avena_ straight growth test. This growth inhibitor was found in tubers throughout their growth and storage, and its level only began to fall some time after the commencement of sprouting; the level was variable during the growing season, but rose markedly at the onset of leaf senescence. Although Booth did not compare the levels of inhibitor in potato shoots, grown under long and short days, he has pointed out that the results of Okazawa (1960) which show high levels of gibberellin-like substances under long day conditions, can equally well be used to demonstrate a significantly higher level of growth inhibitor in short days. A smaller

increase in growth inhibitor level was found when plants grown at 13°C were compared with plants at 18°C. In both cases the higher level was correlated with the condition favourable to tuberisation. Booth has also confirmed Okazawa's finding (1959) that gibberellic acid could overcome the inhibition in bioassay due to the growth inhibitor.

Racca and Tizio (1968), as mentioned above, have also found a rise in the level of anti-gibberellin-like substances in the shoots at the onset of tuberisation. Smith and Rappaport (1969) have also found evidence for the appearance of a growth inhibitor in extracts of young tubers, based on the fact that gibberellin activity in tuber extracts as determined by the dwarf pea bioassay showed a considerable increase when the extracts were diluted; a much smaller increase in activity on dilution was found in non-tuberised stolons.

The above evidence all suggests that a growth inhibitor appears in potato plants prior to tuberisation, and it seems likely that this growth inhibitor has some role in the control of tuberisation; its identity, however, remains unknown.

A suggestion that one of the inhibitors involved may be abscisic acid has come from work on the emergence from dormancy of potato tubers. These studies are relevant to the control of tuberisation if tuber initiation and tuber sprouting (emergence from dormancy) are considered as opposite processes; it is possible, however, that this is not strictly correct and that tuber initiation and the onset of dormancy in the young tuber are quite separate processes.

Hemberg (1949, 1952) found that potato peelings contain both neutral and acid growth inhibiting substances and that the acid, but not the neutral, growth inhibitors disappear when the potato leaves the rest period; he concluded that these substances are of importance as regulators of the rest period in potato. This theory has been supported by other workers (Blommaert, 1954; Varga and Ferenczy, 1957). Blommaert demonstrated by chromatography on extracts from resting potatoes an acid growth inhibitor which was evidently identical with the "β-inhibitor" complex of Bennet-Clark and Kefford (1953); this growth-inhibitor was not found in extracts from non-resting potatoes. Brian, Hemming and Radley (1955) have shown that treatment of resting potatoes with gibberellic acid breaks their rest and this treatment also results in a decrease in the amount of "β-inhibitor" in the tubers (Boo, 1961). Many workers hold the view that abscisic acid, ABA (Addicott et al, 1968) is probably the important inhibiting component of the "β-inhibitor" complex (Wareing, Eagles and Robinson, 1964; Blumenthal-Goldschmidt and Rappaport, 1965; Smith and Lyon, 1966; Milborrow, 1967).

A role of abscisic acid in promoting tuberisation is also tempting to consider in view of the interactions mentioned above, in many different physiological processes with gibberellin, which substance appears to be involved as an influence acting to inhibit or delay tuberisation. These interactions and their possible significance for the tuberisation process are discussed in detail in Section VII.

The work of El-Antably, Wareing and Hillman (1967) provides more direct

evidence for the involvement of abscisic acid. They carried out experiments in which plants of two varieties of S.tuberosum and of a clone of S.andigenum (which normally required short days for tuberisation) were grown under long days. The plants were given a 20 mg/l daily foliar spray of an aqueous solution of abscisic acid. Tuberisation under long day conditions in all three groups of plants was promoted by abscisic acid treatment, although Smith and Rappaport (1969), working with whole plants, and Claver (1970), using tuber-sprouts, have not been able to demonstrate such a promotive effect (see Section VII).

Other experiments have also been performed (McCorquodale and Moorby, 1968; Smith and Rappaport, 1969; Palmer and Smith, 1969b) in which abscisic acid was applied to stolon tips, without finding any promotive effect of abscisic acid on tuberisation; in some cases, indeed, the compound appeared to have an inhibiting effect on tuberisation. These results are also discussed in Section VII.

It therefore appears that, while it seems likely that a growth inhibitor or inhibitors is involved in the control of tuberisation, the identity of this inhibitor is uncertain; the evidence concerning the possibility that the growth inhibitor involved is abscisic acid is conflicting and, as in the case of the probably involvement of the gibberellins, it was considered that more work was needed in this area.

The remaining group of substances which have been suggested as being involved in the initiation of tuberisation is that of the cytokinins (Courduroux, 1966; Palmer and Smith, 1969a and b, 1970; Smith and Palmer, 1970).

In addition to the effects of cytokinins on cell division and cell enlargement mentioned above, the effect of cytokinins on mobilisation of various substances may also be important for tuberisation, since tuberisation presumably required the establishment of an active metabolic sink in the sub-apical region of the stolon which mobilises to this locus the substrates required for the synthesis and accumulation of starch and other materials. There are several reports to substantiate the mobilising effect of the cytokinins on various substances, bringing about their movement to treated areas in leaves (eg Möthes and Engelbrecht, 1959; Möthes, 1960; Gunning and Barkley, 1963) in seedling stems (Seth and Wareing, 1964), and between leaves, and leaves and buds (Penot, 1963, 1964).

Some direct evidence has also been put forward for a possible role of cytokinins in tuberisation.

Okazawa (1969, 1970) has demonstrated the presence of endogenous cytokinin in potato tuber tissue and has shown that the levels of two such cytokinins rose at the beginning of tuber formation in developing tubers; this rise was followed by an increase in tuber fresh weight. The activities then fell, and remained at an almost constant low level for some time before finally falling to undetectable levels. It is not clear however, whether the cytokinin levels rose before or immediately after the onset of tuberisation.

Palmer and Smith (Palmer and Smith, 1969a and b, 1970; Smith and Palmer, 1970) consider that they have demonstrated a requirement for cytokinins in the tuberisation of excised stolons grown _in vitro_ and obtained from etiolated sprouts of tubers of _S. tuberosum_,

variety Norgold Russet. Three cytokinins have been used in the system:- 6-furfurylaminopurine (kinetin), N_6-benzyladenine and 6-benzylamino-9-(tetrahydropyran-2-yl) purine at concentrations of 0.25, 2.5 and 25 mg/l (Palmer and Smith, 1969a). Tuber formation occurred earlier with cytokinins.

Using kinetin-8-^{14}C, it was found that more labelled material appeared at the locus of tuber formation than in other parts of the stolon; this occurred before visible signs of tuberisation (Smith and Palmer, 1970). The basal portion of the stolon also accumulated substantial amounts of labelled material.

Studies on the effect of inhibitors of protein and nucleic acid synthesis on tuberisation (Palmer and Smith, 1970) and incorporation studies with labelled uridine and leucine (Smith and Palmer, 1970) have led to the conclusion that kinetin-induced tuber formation may be due to the stimulation of metabolic processes not associated with the synthesis of specific proteins. It was also shown that stolons pre-incubated in kinetin prior to incubation on a basal medium without kinetin did not form tubers, but did so on a basal medium with kinetin. Kinetin is only required in the basal medium for 3 to 4 days in order to induce tuber formation, which can then progress on a basal medium only.

It has also been demonstrated (Palmer and Smith, 1969b) that abscisic acid can, at suitable concentrations, markedly inhibit stolon elongation and kinetin-induced tuber initiation (see discussion in account of abscisic acid stolon feeding experiment in Section V (iii)). This inhibition was not found when the

stolons were previously grown for 7 days on a medium containing kinetin but no abscisic acid, and Palmer and Smith have deduced from this that abscisic acid is ineffective once the initiation process has been completed. They have suggested several alternatives for the mode of action of abscisic acid:- on the formation of specific proteins, in preventing the uptake and transport of kinetin to the locus of action, or in inhibiting the cell division which would normally occur in the sub-apical regions in the presence of kinetin. They have proposed that in potato, the importance of abscisic acid and other endogenous growth inhibitors may be to inhibit the activity of gibberellins and to arrest stolon elongation, allowing the tuber-inducing hormones, cytokinins, to exert their effect.

It was felt that, in view of the above findings, further work on the effect of cytokinins on tuberisation might be valuable. This was restricted to stolon feeding experiments; an experiment with whole plants was not carried out using exogenous cytokinin because of the doubts as to its mobility in the plant from its site of application.

The present work

From the preceding account, it is clear that more work is required to elucidate the possible role in tuberisation auxins, gibberellins, naturally-occurring growth inhibitors and cytokinins, and it was for this purpose that the experiments described in this section were carried out.

The experiments fall into three groups:-

Group A: Experiments in which growth-active substances were applied to the growing point or roots of intact plants and their effects on growth and tuberisation observed. An account of these experiments is given in Section V (ii) - Growth Substance Application Experiments.

Group B: Experiments in which growth-active substances were fed to individual stolons on intact plants and their effects on the tuberisation of these stolons, and on the rest of the plant, observed. These experiments are described in Section V (iii) - Stolon Feeding Experiments.

Group C: Extractions of endogenous gibberellin-like substances from stolon tips in various developmental states. These studies are described in Section V (iv).

(ii) Group A: Growth-active substance application experiments

a. Introduction and general methods

The purpose of these experiments was to determine the tuberisation response of potato plants of variety Up-to-Date to applications to the intact plant (via the shoot apex or roots) of various growth-active substances, before going on to examine the involvement of such substances at the site of tuberisation, the sub-apical region of the stolon.

The compounds used, chosen for the reasons given in part (i) above, were the following:- gibberellic acid, GA_3 (Hopkin and Williams): 7 experiments, and the inhibitor of its synthesis 2-chloroethyltrimethylammonium chloride, CCC (BDH): 3 experiments;indole acetic acid, IAA, alone and in combination with gibberellic acid: 1 experiment; synthetic racemic abscisic acid, ABA (kindly supplied by Dr B V Milborrow of Shell Research Ltd, Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent): 1 experiment. It has been pointed out by El-Antably et al (1967) that it may be provisionally assumed that the results of tests with the racemic mixture of abscisic acid are equally applicable for the naturally occurring d-form.

The compounds were dissolved in water (CCC), methanol (gibberellic acid experiment 1), or a 50:50 solution of ethanol and water (all other experiments). Control plants were given corresponding applications of the appropriate solvent. The details of the experiments are given in Tables 10 and 20. Basic growing conditions were as described in Section II (ii), with slight modifications of the schedule to overcome difficulties of growing space in the growth rooms. Low temperatures (see

TABLE 10. Time schedules and treatments used in growth substance application experiments with gibberellic acid, GA_3, and gibberellic acid/indole acetic acid, GA_3/IAA (Section V (ii)).

Experiment	Age at E (days)	Age at A (days)	Harvest date (days from E)	Replication	GA_3 or IAA application: Amount applied (µg/plant)	Solvent	Size of drop (µl)	Daylength régimes after E
GA_3 1	49	1 date:- 49	14	4	10	CH_3OH	50	SD and LILD
GA_3 2	35	1 date:- 35	14	7	1.0, 10, 100, 1000	50:50/ C_2H_5OH: H_2O	50	SD (+LDC)
GA_3 3	35	1 date:- 35	14, 20	3	0.01, 1.0, 10	50:50/ C_2H_5OH: H_2O	20	SD (+LDC)
GA_3 4	24, 35	1 date:- 22	14, 18 (24 day plants); 7, 14 (35 day plants)	3	1.0, 10	50:50/ C_2H_5OH: H_2O	20	SD (+LDC)
GA_3 5	35	1 date:- 0	14	7	≡ 1000µg/ Tuber piece	0.5 ml C_2H_5OH $+H_2O$;	Bathing solution	SD and LILD
GA_3 6	35	5 dates:- 35+1, 35+3, 35+7, 35+9, 35+14	6, 12, 15	5	1.0, 10	50:50/ C_2H_5OH: H_2O	20	SD (+LDC)
GA_3 7	35	4 dates:- 35+1, 35+4, 35+8, 35+14	8, 15, 20	5	1.0, 10	50:50/ C_2H_5OH: H_2O	20	SD
GA_3/IAA	35	4 dates:- 35, 35+2 35+5, 35+7	9, 12	5	100 GA_3; 100 GA_3 + 100 IAA; 100 IAA.	50:50/ C_2H_5OH: H_2O	20	SD

Key on next page

TABLE 10.

Age at E = Age of plants at beginning of experimental period (days)

Age at A = Age of plants at application of growth substance (days)

Harvest date is quoted, as in all experiments in the main body of the work, as days from start of experimental period.

With more than one treatment date, all treated plants received treatment on each of these dates.

SD = short days

LILD = low intensity long days

LDC = long day controls

Section II (ii)) were generally used, except in the third CCC experiment (which used high temperatures during the experimental period) and the abscisic acid experiment, which used a constant temperature of 20°C in the short day growth room. Treatment solutions were applied directly to the growing point with a micropipette (20 or 50 μl), except in the fourth gibberellic acid experiment, in which the compound was applied as a bathing solution to the unsprouted tuber pieces immediately after excision and before planting, and the CCC experiments, in which a soil drench was used (because CCC has been found to be less effective and to cause scorching of the foliage when applied to the tops (Krug, 1963)).

b. The experiments, results and discussion

First experiment with gibberellic acid

As may be seen from Table 11, top height was considerably increased by gibberellic acid in all treated plants. Top dry weight was only affected (increased) in plants kept in short days. Number of nodes, stolons and tubers, and tuber fresh weight and percentage of plants tuberised were all unaffected by treatment.

The stem elongation without increase in node number found in treated plants was what was expected from the results of other workers (eg Rappaport, Lippert and Timm, 1957, Humphries and French, 1960; Dyson and Humphries, 1963, 1966); this indicated that the substance was penetrating the plant tissues and, once within the plant, capable of exerting a characteristic physiological effect. The lack of effect on tuberisation was, however, surprising in view of the findings of other workers

TABLE 11. Results of first GA_3 application experiment. Differences in top, stolon and tuber growth in LIID and in SD with and without gibberellic acid at harvest after 14 days.

Treatment	LIID				SD			
	No GA_3	10µg GA_3/plant	S.A.		No GA_3	10µg GA_3/plant	S.A.	
			p	LSD			p	LSD
Top ht.(cm)	42.4	63.0	0.05	14.19	40.8	60.5	0.05	12.2
Node no.	12.5	15.2	ns		11.5	14.5	ns	
Top dwt.(g)	0.75	0.94	ns		0.65	0.99	0.005	0.15
Stolon no.	8.5	6.0	ns		4.5	5.0	ns	
Tuber no.	0.5	1.5	ns		2.5	3.0	ns	
Tuber fwt.(g)	0.1	0.1	ns		0.23	0.35	ns	
No. plants tub.	2/4 (50%)	2/4 (50%)	-		4/4 (100%)	4/4 (100%)	-	
Replication	4	4	-		4	4	-	

For abbreviations, see Section VIII

(eg Okazawa, 1959; Dyson and Humphries, 1966). Further experiments were therefore carried out to try to discover the reason for the discrepancy. It was possible that the concentration of gibberellic acid applied may have been unsuitable, or that the compound was applied too late in the plants' development, or that repeated applications might be necessary.

Second experiment with gibberellic acid

This experiment and the next were carried out to discover whether unsuitable concentration was the reason for the lack of effect in the first experiment of gibberellic acid on tuberisation. The present experiment used one application of gibberellic acid at 4 concentrations (the range extending above that used in experiment 1, see Table 10) applied in ethanol and water instead of methanol, because of the slight deformation of apical leaves found with the latter in the first experiment.

As can be seen from Table 12, the two higher gibberellic acid concentrations (100 and 1000 μg/plant) markedly increased top height, but the other concentrations (1 and 10 μg/plant) had no effect. The lack of effect at 10 μg/plant was surprising in view of the increase obtained with this concentration in experiment 1. It was thought that this might have been due to the loss due to run-off of some of the 50 μl drop from the apex before absorption, which was not immediate, as was the case with methanol; for this reason later experiments used smaller drops (20 μl). Node number was again unaffected (as expected from the probable mode of action of gibberellic acid - see Section V (i)). Top dry weight was increased by treatment at concentrations of 10, 100 and 1000 μg/plant. Stolon and tuber numbers were unaffected,

TABLE 12. Results of second GA_3 application experiment. Differences in top, stolon and tuber growth in LIID and in SD without and with GA_3 at 4 concentrations (2 of them higher than that used in the previous experiment) at harvest after 14 days.

Treatment	LDC	SDC	SD + GA_3 (μg/plant)				S.A.	
			1	10	100	1000	p	LSD
Top ht.(cm)	45.3	41.7	40.3	44.7	60.6	71.9	0.001	10.47
Node no.	17.7	16.5	18.7	17.8	17.1	18.1	ns	
Top dwt.(g)	2.21	2.12	2.35	2.74	2.67	3.27	0.005	0.49
Stolon no.	5.0	8.6	8.3	6.6	7.6	6.3	ns	
Tuber no.	0	4.1	6.0	8.5	8.2	7.3	ns	
Tub. stolon no.	0	3.0	4.3	5.0	4.3	4.3	ns	
Tuber fwt.(g)	0	0.1	2.5	3.1	4.8	1.3	0.005	2.25
No. plants tub.	0/7 (0%)	7/7 (100%)	7/7 (100%)	7/7 (100%)	6/6 (1 rotted) (100%)	4/4 (3 rotted) (100%)	-	
Replication	7	7	7	7	7	7	-	

For abbreviations, see Section VIII

but gibberellic acid caused an increase in tuber fresh weight at 1, 10 and 100 μg/plant, although not at 1000 μg/plant; the low value with 1000 μg/plant treatment may have been caused by the very large requirements of the tops (mean height 71.9 cm compared to 41.7 cm for the short day controls) for photosynthate, overcoming the demands of the tubers.

Once again, even with higher concentrations, there was no retarding effect of gibberellic acid treatment on tuberisation; the increase in tuber fresh weight was thought to be an indirect effect due to increased photosynthesis because of enhanced top growth.

Third experiment with gibberellic acid

This experiment used a lower range of concentration (see Table 10); for the reasons explained above, a 20 μl drop of solution was used. One application was again given, and there were two harvests, because tuberisation was not very far advanced in the short day controls after 14 days; the blocks consisting of the tallest plants were harvested first.

The results are given in Table 13, and Fig 17 shows the appearance of underground parts of plants harvested after 20 days. Top height was increased at both harvests by 10 μg/plant gibberellic acid (in agreement with the result of experiment 1) but was unaffected by lower concentrations; the reduction found with 0.01 μg/plant after 14 days was not thought to be a real effect, but may have been due to the fact that one of the plants in the treatment was stunted, perhaps due to some damage to the apex, although its dry weight appeared normal. Node number was unaffected, as in experiments 1 and 2. Top dry weight was also unaffected. This is

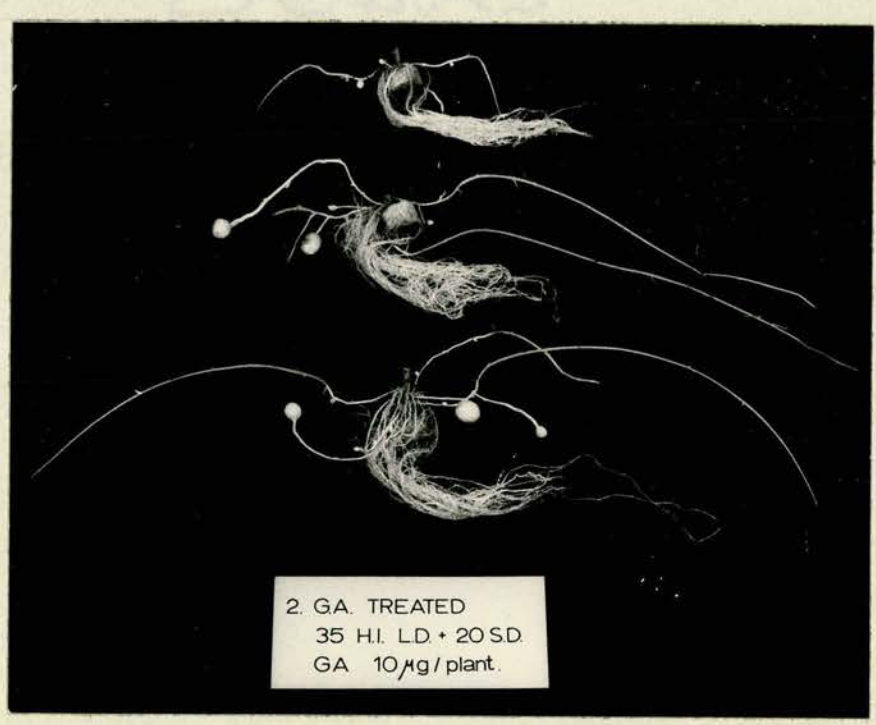
TABLE 13. Results of third GA_3 application experiment. Differences in top, stolon and tuber growth in LILD and in SD without and with GA_3 at 3 concentrations at harvest after 14 or 20 days (see also Fig. 17).

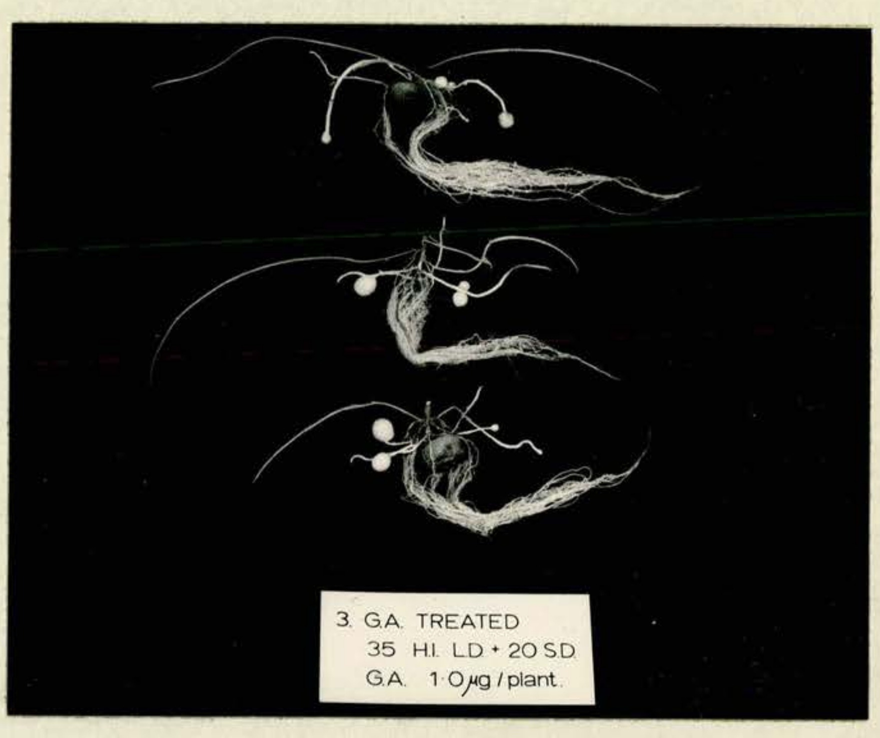
Treatment	14 day harvest							20 day harvest						
	LDC	SDC	SD + 0.01μg GA_3	SD + 1.0μg GA_3	SD + 10μg GA_3	S.A.		LDC	SDC	SD + 0.01μg GA_3	SD + 1.0μg GA_3	SD + 10μg GA_3	S.A.	
						p	LSD						p	LSD
Top ht.(cm)	39.5	40.2	34.2	35.1	52.0	0.001	5.11	29.7	29.3	24.5	27.3	46.5	0.005	5.39
Node no.	15.3	18.3	17.7	17.3	19.0	ns		15.0	15.0	12.3	12.3	15.7	ns	
Top dwt.(g)	1.35	1.77	1.37	1.24	1.97	ns		1.36	1.58	1.51	1.48	1.91	ns	
Stolon no.	6.0	8.3	7.3	13.7	6.7	0.001	2.96	5.3	9.7	9.0	8.0	6.3	ns	
Tuber no.	0	0.7	2.0	7.0	1.0	ns		0	4.0	7.7	6.7	6.7	ns	
Tub. stolon no.	0	0.7	1.0	4.3	1.0	ns		0	3.0	5.0	3.7	3.3	ns	
No. plants tub.	0/3 (0%)	2/3 (66.7%)	2/3 (66.7%)	3/3 (100%)	2/3 (66.7%)	-		0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	-	
Replication	3	3	3	3	3	-		3	3	3	3	3	-	

Underlined values of p and LSD are for analyses of variance which included the data for the long day controls; other values did not include these. For abbreviations, see Section VIII.

FIGURE 17. Results of gibberellic acid application experiment 3. Appearance of underground parts of plants grown in either short days (SD) or low intensity long days (LI LD), treated or untreated with gibberellic acid, and harvested after 20 days.

1. S.D. CONTROL
35 H.I. L.D. + 20 S.D.
NO G.A.


2. G.A. TREATED
35 H.I. L.D. + 20 S.D.
GA 10 μg / plant.


3. G.A. TREATED
35 H.I. L.D. + 20 S.D.
G.A. 1·0μg / plant.

4. G.A. TREATED
35 H.I. L.D. + 20 S.D.
GA 0·01μg / plant.

5. L.D. CONTROL
35 H.I. L.D. + 20 L.I. L.D.
NO G.A.

consistent with the results of the second experiment at 1μg/plant, but inconsistent with results of experiments 1 (for plants in short days) and 2 for 10μg/plant, where an increase was observed. It is possible that some plants may have lower assimilation rates than others and that in the former, the increase in top height with gibberellic acid treatment may be achieved at the expense of stem thickness, so that no increase in dry weight is found; alternatively, the lower level of response may have been due to incomplete penetration of all the applied compound, as discussed above.

Stolon number after 20 days was unaffected by treatment (as found in the previous experiments), but after 14 days an increase in stolon number was found with 1.0μg/plant gibberellic acid. This was surprising since stolon formation usually takes place before the end of the growing period, although it is possible that third order lateral buds which would not normally elongate to produce stolons were stimulated by the treatment to do so; the effect may have been due to chance and the small number of replicates. Tuber number and number of tuberising stolons were unaffected by gibberellic acid treatment. There were apparent increases in both these parameters with treatment at 0.01 or 1.0μg/plant after 14 days, and in tuber number with all three concentrations after 20 days, but these were not found to be significant, perhaps because of the high variability of the data. There was certainly no evidence of a retarding effect of the treatment on tuberisation.

The results of the first three experiments therefore show no such effect of gibberellic acid on the tuberisation at

concentrations from 0.01 to 1000 μg/plant. It was conceivable that gibberellic acid (GA_3), while capable of causing stem elongation in potato plants of variety Up-to-Date, was not the appropriate gibberellin to affect tuber formation. Such differences in the specificity of gibberellins to affect various physiological processes in the same plant have been found by Michniewicz and Lang (1962). In potato, however, the workers who have demonstrated retardation of tuberisation with gibberellin treatment have done so (although with other varieties) using GA_3, so that this was thought unlikely. Another possible explanation might have been that the treatment was given too late in the plants' development, by which time short days exerted too strong an influence to be overcome by gibberellin.

Fourth experiment with gibberellic acid

For the reasons given above, gibberellic acid treatment was given in the present experiment to younger plants, 22 days old (for schedule, see Table 10). Alternative times of transfer to differential daylength treatment were used to see if leaving the plants in long day conditions for some time after treatment had any effect. Two harvest dates were used for each group of plants to ensure detection of the first stages of tuberisation.

The results are presented in Table 14. In plants 24 days old on transfer to short days (Table 14 a) top height after 14 days was increased by treatment at 10 μg/plant, but not at 1 μg/plant (as in experiments 1 and 3). There was, however, no effect of treatment on top height after 18 days (second harvest); this may have been because of the small number of replicates, or perhaps, when gibberellic acid is applied to fairly young plants, the

TABLE 14a. Results of fourth GA_3 application experiment. Differences in top, stolon and tuber growth in LIILD and in SD without and with GA_3 at 2 concentrations in plants harvested at 2 different dates.
a) plants 24 days old at start of experimental period.

Treatment	14 day harvest						18 day harvest					
	LDC	SDC	SD + 1.0 $\mu g GA_3$	SD + 10 $\mu g GA_3$	S.A. p	S.A. LSD	LDC	SDC	SD + 1.0 $\mu g GA_3$	SD + 10 $\mu g GA_3$	S.A. p	S.A. LSD
Top ht.(cm)	36.0	32.8	29.0	43.2	0.005	7.42	38.5	39.8	31.3	38.3	ns	
Node no.	14.3	14.7	13.7	14.3	ns		16.0	16.7	14.3	15.3	ns	
Top dwt.(g)	0.99	1.10	1.47	1.80	0.05	0.47	1.37	1.11	1.61	1.56	ns	
Stolon no.	7.0	8.7	9.0	6.3	ns		6.0	7.0	8.0	7.7	ns	
Tuber no.	0	4.7	5.3	4.0	0.05	0.93	0	8.3	11.0	7.7	ns	
Tub. stolon no.	0	4.0	4.3	3.3	ns		0	4.7	6.0	4.7	ns	
No. plants tub.	0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	-		0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	-	
Replication	3	3	3	3	-		3	3	3	3	-	

Underlined values of p and LSD are for analyses of variance which included the data for the long day controls; other values did not include these.
For abbreviations, see Section VIII.

TABLE 14b. Results of fourth GA_3 application experiment (continued)
b) plants 35 days old at start of experimental period.

Treatment	7 day harvest						14 day harvest					
	LDC	SDC	SD + 1.0 μgGA_3	SD + 10 μgGA_3	S.A.		LDC	SDC	SD + 1.0 μgGA_3	SD + 10 μgGA_3	S.A.	
					p	LSD					p	LSD
Top ht.(cm)	33.8	28.5	27.3	35.3	ns		39.2	38.3	20.8	34.7	0.05	9.84
Node no.	16.0	16.0	16.0	15.7	ns		16.3	19.7	18.0	18.0	ns	
Top dwt.(g)	1.39	1.06	1.26	1.68	0.05	0.39	1.54	1.61	2.00	2.20	ns	
Stolon no.	5.0	6.0	5.0	4.7	ns		6.0	6.0	6.0	6.7	ns	
Tuber no.	0	0	0	0.67	-		0	0.67	8.3	5.0	0.05	3.97
Tub. stolon no.	0	0	0	0.67	-		0	0.67	2.7	2.3	ns	
Tuber fwt.(g)	-	-	-	-	-		0	0.1	2.3	1.3	0.05	1.29
No. plants tub.	0/3 (0%)	0/3 (0%)	0/3 (0%)	1/3 (33.3%)	-		0/3 (0%)	2/3 (66.7%)	3/3 (100%)	3/3 (100%)	-	
Replication	3	3	3	3	-		3	3	3	3	-	

Underlined values for p and LSD are for analyses of variance which included the data for the long day controls; other values did not include these.
For abbreviations, see Section VIII.

controls make enough growth after 18 days to even out any difference caused by treatment. There was no effect of treatment on node number. Top dry weight, while unaffected after 18 days (perhaps for the reasons given above for top height), was increased after 14 days by 10μg/plant although not by 1μg/plant gibberellic acid treatment. Stolon number was unaffected at either harvest date. After 14 days, tuber number was greater with 1 than with 10μg/plant gibberellic acid, but the results of neither treatment differed from those of the controls. Number of tuberising stolons was unaffected. A similar lack of effect of treatment on tuberisation was found after 18 days. At both harvests, all the plants grown in short days had tuberised.

In the case of the plants 35 days old on transfer to short days (Table 14 b), there was no effect of treatment after 7 days (first harvest) on top height or node number. Top dry weight was increased by 10, but not by 1μg/plant gibberellic acid treatment, as was found in the 24 day old plants after 14 days (their first harvest). At the second (14 day) harvest of the 35 day old plants, top height was less with 1μg than with 0 or 10μg gibberellic acid treatment, but this result was probably due to chance and the small number of replicates. There was no evidence for an increase in top height with treatment; gibberellic acid had no effect on node number or top dry weight, as was also found at the second (18 day) harvest in the 24 day old plants. This lack of effect found at the later harvests in both groups of plants, may have been due to the small number of replicates, or, more likely, to the loss with time and continued growth of the evidence of growth promotion which had taken place a considerable time

before harvest. Once again, as in the 24 day old plants, the 35 day old plants showed no retardation of gibberellic acid treatment on tuberisation; if anything, treatment promoted tuberisation in terms of number of tubers, of plants tuberised, and (where recorded) of tuber fresh weight. The promotion of tuberisation in these plants, as opposed to the lack of effect in the plants transferred after 24 days to differential daylength conditions, is not explicable by differences in top growth (which were very small), and may have been due to differences in timing of gibberellin-induced changes in carbohydrate metabolism; if gibberellin promotes starch breakdown (see Section V (i))it is possible that the older plants,having had a longer time from treatment, may have had available a larger supply of soluble carbohydrates on transfer to short days.

In summary, although there was very little effect on top growth, top dry weight at the first harvest in both ages of plant was increased by 10 μg/plant gibberellic acid, as in experiments 1 and 2. Once again, as in the previous experiments, there was no retarding effect of treatment on tuberisation.

<u>Fifth experiment with gibberellic acid</u>

To investigate the possibility that treatment had still not been given early enough in the life of the plant in experiment 4, gibberellic acid was applied to the plants in this experiment at the earliest possible moment, as a bathing solution into which the unsprouted tuber pieces were placed immediately after excision and prior to planting. The pieces remained in the solution for either 2 or 4 hours, controls being treated similarly but without gibberellic acid in the solutions, which were aerated from a

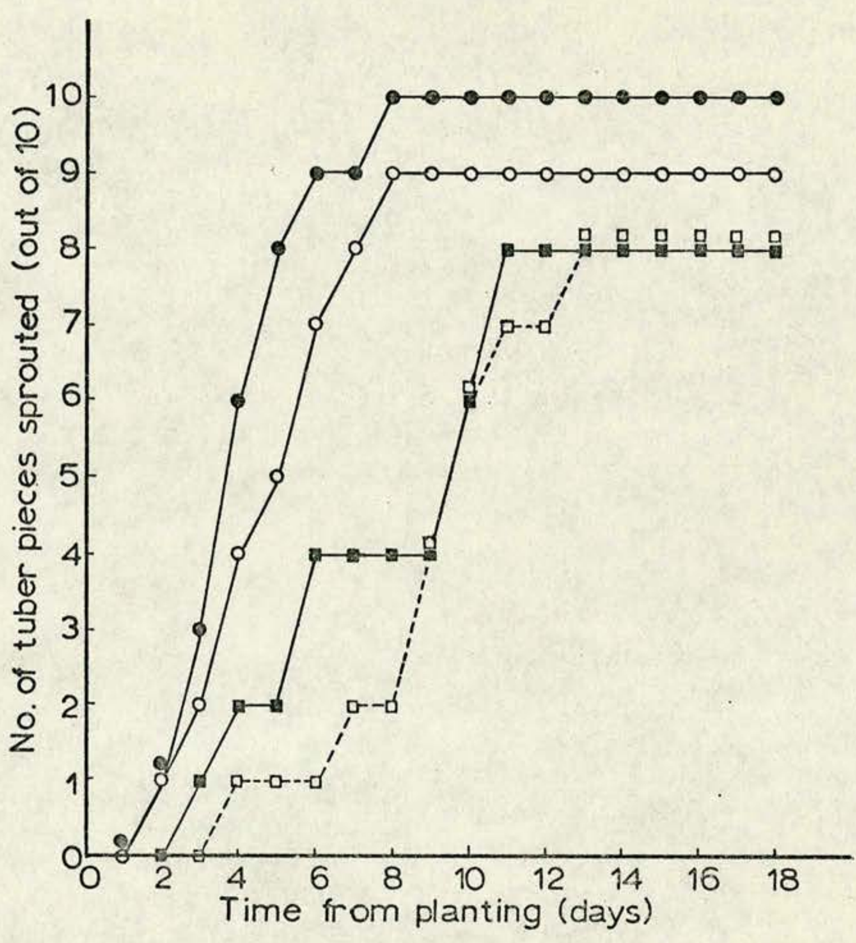
compressed air supply throughout treatment. A high concentration of gibberellic acid was used (see Table 10) to ensure adequate uptake of gibberellic acid.

Gibberellic acid treatment increased the rate of sprouting (Fig 18), in agreement with the results of other workers (eg Dyson and Humphries, 1963). This showed that the compound was absorbed into the tissues of the buds and that it then exerted a physiological effect.

The results are given in Table 15. No effect of gibberellic acid treatment was found on the growth of tops or stolons. Tuber number in short days in both soaking treatments appeared to be increased by gibberellic acid, but the differences were not significant, probably because of the high variability in the data. The only significant effect of treatment was on tuber fresh weight, which was increased with both soaking times; this was probably a result of the accelerated sprouting and resulting faster development of plants from treated pieces. It is possible that all the applied gibberellic acid was used up in accelerating sprouting, none being left to affect top height or tuberisation; it is strange, however, that Dyson and Humphries (1963, 1966) brought about a delay in tuber initiation with similar treatments applied prior to planting.

The remaining possibility to explain the lack of retarding effect was that one application of gibberellic acid was insufficient to bring this about.

FIGURE 18. Gibberellic acid application experiment 5. Graphs showing sprouting of tuber pieces with time; pieces soaked for 2 or 4 hours in solutions with (+) and without (no) gibberellic acid.



No. of tuber pieces sprouted (out of 10)
0
1
2
3
4
5
6
7
8
9
10
O
2
4
6
8
10
12
14
16
18
Time from planting (days)
2 hours soaking +GA_3
4 hours soaking +GA_3
2 hours soaking no GA_3
4 hours soaking no GA_3

TABLE 15. Results of fifth GA_3 application experiment. Differences in top, stolon and tuber growth in plants grown in LILD or SD from tuber pieces soaked before planting for one of 2 times in solutions with or without GA_3.

Treatment	2h. soaking				4h. soaking				S.A. (SD plants)	
	No GA_3		+ GA_3		No GA_3		+ GA_3			
	LD	SD	LD	SD	LD	SD	LD	SD	p	LSD
Top ht.(cm)	39.0	37.3	41.0	37.6	25.7	38.0	43.0	33.1	ns	
Node no.	18.0	17.6	21.0	19.2	12.0	18.0	20.0	17.4	ns	
Top dwt.(g)	1.89	2.14	2.40	2.15	1.55	1.97	1.61	2.14	ns	
Stolon no.	7.0	9.0	12.0	7.1	11.0	7.7	4.0	6.7	ns	
Tuber no.	0	4.0	0	5.9	0	2.9	0	4.1	ns	
Tub. stolon no.	0	3.0	0	3.0	0	2.2	0	2.7	ns	
Tuber fwt.(g)	0	0.2	0	1.3	0	0.3	0	1.1	0.005	0.65
No. plants tub.	0/1 (0%)	7/7 (100%)	0/1 (0%)	7/7 (100%)	0/1 (0%)	7/7 (100%)	0/1 (0%)	7/7 (100%)	-	
Replication	1	7	1	7	1	7	1	7	-	

For abbreviations, see Section VIII. Statistical analysis is for short day plants only.

Sixth experiment with gibberellic acid

This and the seventh experiment were carried out to investigate the above possibility, gibberellic acid at concentrations of 1 and 10 μg/plant being applied to treated plants on several dates (see Table 10). Three harvest dates were used to ensure detection of the first visible signs of tuberisation. Root dry weight was recorded in addition to the usual measurements because of the suggestion (Tizio, 1964 a) that tuberisation may be related to the degree of development of the root system.

At all three harvests (see Table 16), 10 μg/plant gibberellic acid treatment caused a marked increase in top height. Although 2 applications of 1 μg/plant gibberellic acid (6 day harvest) were insufficient to increase top height, this was achieved after 4 or 5 applications at this concentration (12 and 15 day harvests). Node number was unaffected until the 15 day harvest, when it was increased by both concentrations of gibberellic acid. Top dry weight was unaffected at the 6 day harvest, but was increased by both concentrations of gibberellic acid at the 12 day, and by the higher (10 μg/plant) concentration at the 15 day harvest. Root dry weight was entirely unaffected.

Stolon number was slightly greater at the 15 day harvest in the plants given 10 μg gibberellic acid than in those given 1 μg, although neither value differed from that in the short day controls. Otherwise, it was unaffected. No tuberisation was observed until the 15 day harvest, when gibberellic acid treatment caused a marked reduction in terms of number of tubers and tuberising stolons, and tuber fresh weight (although the data contained too many zeroes for valid statistical analysis). At the 15 day harvest 5/5 short

TABLE 16. Results of sixth GA_3 application experiment. Differences in top, stolon and tuber growth in LILD and in SD without or with repeated applications of GA_3 at 2 concentrations, at harvest after 6, 12 or 15 days.

Treatment	6 day harvest						12 day harvest						15 day harvest					
	LDC	SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.		LDC	SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.		LDC	SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.	
					p	LSD					p	LSD					p	LSD
Top ht.(cm)	18.0	15.9	18.4	22.3	0.05	3.63	24.3	29.5	39.9	52.6	0.001	6.70	28.0	26.3	49.2	63.9	0.001	6.84
Node no.	14.3	13.0	14.2	14.2	ns		17.0	17.5	17.8	18.5	ns		17.5	16.6	18.6	19.8	0.001	1.03
Top dwt.(g)	0.69	0.70	0.97	0.77	ns		0.99	1.16	1.42	1.42	0.005	0.14	1.06	1.15	1.33	1.55	0.05	0.25
Root dwt.(g)	0.32	0.29	0.35	0.35	ns		0.38	0.31	0.37	0.23	ns		0.35	0.30	0.36	0.28	ns	
Stolon no.	13.3	11.8	12.0	11.0	ns		13.0	14.5	12.3	8.5	ns		14.3	11.0	8.4	13.3	0.05	3.79
Tuber no.	0	0	0	0	-		0	0	0	0	-		0	3.4	1.2	0	-	
Tub. stolon no.	0	0	0	0	-		0	0	0	0	-		0	2.6	1.2	0	-	
Tuber fwt.(g)	0	0	0	0	-		0	0	0	0	-		0	0.5	0.1	0	-	
No. plants tub.	0/3 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	-		0/3 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	-		0/3 (0%)	5/5 (100%)	4/5 (80%)	0/5 (0%)	-	
Replication	3	5	5	5	-		3	5	5	5	-		3	5	5	5	-	

For abbreviations, see Section VIII.

day control plants had tuberised, 4/5 of those given 1 μg and 0/5 of those given 10 μg gibberellic acid.

Repeated applications of gibberellic acid were therefore found to retard tuberisation. The lack of effect on root growth suggests that it is not through this that gibberellic acid exerts its effect on tuberisation. It also seems unlikely, although it remains a possibility, that the influence of gibberellic acid is exerted only in an indirect way by an effect on overall top growth. Top growth was markedly increased by treatment in the present experiment, but this effect was also found in experiments 1-3, where there was no accompanying retardation of tuberisation.

Seventh experiment with gibberellic acid

This experiment was very similar to the previous one, and was carried out to try to confirm the results obtained in the latter. The harvest dates used were slightly later, because no tuberisation was found at the two earlier harvest dates in experiment 6. Details are given in Table 10, and the results in Table 17.

Both gibberellic acid concentrations (1 and 10 μg/plant) caused a marked increase in top height at all three harvests. Node number, as in experiment 6, was unaffected until the third (18 day) harvest, when it was increased by both concentrations of gibberellic acid; this suggests that after a long period of treatment, or the build-up of a high level of gibberellin in the shoot apex, gibberellic acid affects cell division not only in the sub-apical but also in the apical meristem. Top dry weight was increased by both concentrations of gibberellic acid at the 18 day harvest. Root dry weight, as in experiment 6, was completely unaffected by

TABLE 17. Results of seventh GA_3 application experiment. Differences in top, stolon and tuber growth in SD without and with repeated applications of GA_3 at 2 concentrations, at harvest after 7, 13 or 18 days.

Treatment	7 day harvest					13 day harvest					18 day harvest					
	SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.		SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.		LDC	SDC	SD + 1 μgGA_3	SD + 10 μgGA_3	S.A.	
				p	LSD				p	LSD					p	LSD
Top ht.(cm)	15.5	26.2	32.2	0.001	4.49	22.3	43.9	58.5	0.001	6.18	26.3	21.4	51.0	70.4	0.001	7.43
Node no.	14.8	16.0	14.6	ns		15.0	16.3	16.4	ns		15.5	15.0	17.0	17.0	0.001	0.84
Top dwt.(g)	-	-	-	-		1.27	1.66	1.40	ns		1.34	1.29	1.89	2.02	0.05	0.54
Root dwt.(g)	-	-	-	-		0.37	0.31	0.26	ns		0.37	0.27	0.26	0.30	ns	
Stolon no.	1.5	2.0	2.0	ns		5.0	5.6	4.8	ns		5.3	11.6	4.8	4.0	0.05	3.11
Tuber no.	0	0	0	-		3.4	2.8	0.2	0.005	2.69	0	9.0	3.6	2.0	0.05	3.18
Tub. stolon no.	0	0	0	-		3.2	2.8	0.2	0.005	2.55	0	7.8	2.8	2.0	0.005	2.83
Tuber fwt.(g)	0	0	0	-		-	-	-	-		0	2.22	2.75	0.90	ns	
No. plants tub.	0/5 (0%)	0/5 (0%)	0/5 (0%)	-		5/5 (100%)	5/5 (100%)	1/5 (20%)	-		0/4 (0%)	5/5 (100%)	5/5 (100%)	3/5 (60%)	-	
Replication	5	5	5	-		5	5	5	-		4	5	5	5	-	

For abbreviations, see Section VIII.

treatment.

Stolon number was unaffected at the first two harvests, but was decreased by both concentrations of gibberellic acid at the 18 day harvest. This was quite unexpected, both since gibberellin is found, if anything, to promote stolon development (eg Tizio, 1964 b) and since stolon formation is usually completed long before plants are 35 days old. It seemed likely that this result was caused by the abnormally high stolon numbers in the short day controls, and was due to chance.

No plants had tuberised at the 7 day harvest, but at the 13 and 18 day harvests, gibberellic acid treatment caused a marked inhibition of tuberisation, in terms of number of tubers and tuberising stolons; tuber fresh weight (where recorded, at the 18 day harvest) was unaffected, although it appeared to be reduced by the higher concentration. The inhibition of tuberisation was only found with the higher concentration (10 μg) of gibberellic acid at the 13 day harvest, but at the 18 day harvest it was found with both concentrations. This suggests that lower concentrations require to be applied over a longer period, or for a total of more times, in order to affect tuberisation; it therefore seems that the necessary conditions for retardation of tuberisation are not simply the continuous presence of a certain minimum level of gibberellic acid. It is possible that the greater stolon numbers in the short day controls at the 18 day harvest may have been partly responsible for the effect of gibberellic acid on tuberisation at that harvest, but this was not the case at the 13 day harvest, (where there were no significant differences in stolon number) nor at the 15 day harvest in experiment 6 (where the only stolon number differences

were between the two gibberellin treatments. The results of this experiments therefore confirm those of experiment 6, and suggest that repeated gibberellic acid application causes retardation of tuberisation.

From the results of experiments 6 and 7, it at first appears unlikely that the retardation of tuberisation by gibberellic acid treatment is brought about in the same way as that caused by low intensity long day conditions. While that due to gibberellic acid is accompanied by a very marked increase in top height and generally also in top dry weight, top height and dry weight of plants grown in long days did not generally show the same degree of increase over the short day controls. Plants grown in long days nevertheless showed a more marked inhibition of tuberisation than those treated with gibberellic acid in short days. The situation may, however, perhaps be explained as follows. In long day conditions and with no additional application of gibberellic acid, endogenous gibberellins are produced in the plant and supplied to the tops, where they promote extension growth, and to the stolons, where they inhibit tuberisation. When extra gibberellic acid is supplied to plants grown in short days, enough gibberellin will again be available to inhibit tuberisation of the stolons and to produce normal top growth; there will also, however, probably be some "extra" gibberellin available if repeated applications of fairly high concentration are given. Since the shoot apex is likely to be the most active sink, this extra gibberellin, superfluous to the needs of normal growth, will cause extra elongation. In addition, the contribution of endogenous inhibitors must be taken into account (see Section VII).

Summary of results of experiments with gibberellic acid (see also Table 18)

In almost all experiments, except where treatment was applied to tuber pieces before planting (experiment 5), gibberellic acid at one or more concentrations caused an elongation of the tops. This was accompanied in some cases (experiments, 1, 2, 4, 6 and 7; some harvests) by an increase in top dry weight, but by an increase in node number only after repeated gibberellic acid treatment (final harvests, experiments 6 and 7). The increased top height appeared, therefore, to be brought about mainly by an effect on the sub-apical meristem (as expected from the work of Sachs et al, 1959) although a smaller effect may have been exerted on the apical meristem; the latter effect may, however, have been indirect and due to the availability of more assimilate in the more vigorously growing plants.

The effects on stolon number were occasional, small and variable, and probably mostly unreal. In experiments 1-5, in which gibberellic acid was applied once only, tuber number was either unaffected or increased by treatment, as was tuber fresh weight (Fig 17 and Tables 11-15). In experiments 6 and 7, in which gibberellic acid was applied repeatedly, however, tuberisation appeared to be suppressed by gibberellic acid treatment (as expected from the results of other workers: see Section V (i)). Application of 1 μg gibberellic acid/plant caused a noticeable reduction in the number of tubers and tuberising stolons, and the suppression was even greater and sometimes complete at 10 μg gibberellic acid/plant (Tables 16 and 17). It therefore appears that, while a single application of gibberellic acid is enough to

TABLE 18. **Summary of results of gibberellic acid application experiments (Section V (ii)).**

Harvest date is quoted in days from beginning of experimental period. Effect of gibberellic acid treatment:- ↗ = increase in relevant parameter with all levels of GA_3 treatment used ; ↗10(etc) = increase with 10µg(etc) GA_3/plant; ~ = relevant parameter unaffected by treatment; ↘ = decrease in relevant parameter with all levels of GA_3 treatment used; ↘10(etc) = decrease with 10µg(etc) GA_3/plant. Bracketed values denote <u>apparent</u> effects (where no valid statistical analysis could be made). 1>10 etc. = Value with 1µg GA_3 greater than that with 10µg GA_3 etc. Daylengths are given for experimental period. For other abbreviations, see Section VIII.

Experiment	1		2	3		4				5	6			7		
Concentration of GA (µg/plant)	10		1, 10 100, 1000	0.01, 0.1, 10		1, 10				1000	1, 10			1, 10		
Daylength	LI LD	SD	SD	SD		SD				SD	SD			SD		
						24 day		35 day								
Harvest date (days)	14	14	14	14	20	14	18	7	14	14	6	12	15	7	13	18
Top ht.(cm)	↗	↗	~1, 10; ↗100, 1000	↘0.01; ~1; ↗10	~0.01, 1; ↗10	~1; ↗10	~	~	↘1; ~10	~ (Sprouting ↗·)	~1; ↗10	↗1, 10	↗1, 10	↗1, 10	↗1, 10	↗1, 10
Node no.	~	~	~	~	~	~	~	~	~	~	~	~	↗1, 10	~	~	↗1, 10
Top dwt.(g)	~	↗	~1; ↗10, 100, 1000	~	~	~1; ↗10	~	~1; ↗10	~	~	~	↗1, 10	~1; ↗10	—	~	↗1, 10
Root dwt.(g)	—	—	—	—	—	—	—	—	—	—	~	~	~	—	~	~
Stolon no.	~	~	~	~0.01, 10; ↗1	~	~	~	~	~	~	~	~	10>1	~	~	↘1, 10
Tuber no.	~	~	~	~	~	1>10	~	(~1); (↗10)	(↗1, 10)	~	—	—	(↘1, 10)	—	~1; ↘10	↘1, 10
Tub. stolon no.	—	—	~	~	~	~	~	(~1); (↗10)	↗1; ~10	~	—	—	(↘1, 10)	—	~1; ↘10	↘1, 10
Tuber fwt.(g)	~	~	↗1, 10, 100 ~ 1000	—	—	—	—	—	↗1; ~10	↗	—	—	(↘1, 10)	—	—	~ (↘10)

cause a response in top growth, repeated applications are necessary to bring about an inhibition of tuberisation.

These findings do not enable one to decide whether gibberellic acid exerts its effect on tuberisation directly at the stolon tip, or indirectly through the effects on top growth. This question is further considered in Section V (iii). The results are discussed along with those obtained with other growth-active substances applied to the whole plant or to the stolon tips (Section V (ii) and (iii)) and those concerning the extraction of gibberellin-like substances (Section V (iv)) in Section VII (General Discussion).

Experiment with gibberellic acid and indole acetic acid alone and in combination

The investigation was carried out in the form of a factorial experiment to discover any possible interaction between these two growth promoters in their effects on growth and tuberisation. As in the previous two experiments, repeated applications of the compounds were given; Table 10 gives details of the experiment. Two harvest dates were again used to try to ensure detection of the first signs of tuberisation; the results are presented in Table 19.

At the 12 day harvest, there was no interaction between gibberellic acid and indole acetic acid for top height or node number. The indole acetic acid main effect for top height was found to be not significant, but the gibberellic acid main effect was highly significant, top height being increased, as expected from the results of the previous experiments. Node number was also unaffected after 12 days by indole acetic acid, but was increased

TABLE 19a. Results of GA_3/IAA factorial experiment. Differences in top, stolon and tuber growth in plants grown in SD without and with IAA and GA_3 alone and in combination at harvest after 9 or 12 days.

Treatment	9 day harvest							12 day harvest						
					S.A.							S.A.		
	SDC	SD + GA_3	SD + GA_3 + IAA	SD + IAA	GA_3 M.E.	IAA M.E.	I	SDC	SD + GA_3	SD + GA_3 +IAA	SD + IAA	GA_3 M.E.	IAA M.E.	I
Top ht.(cm)	16.8	47.4	38.3	23.2	-	-	0.001	19.7	49.7	43.7	22.0	0.001	ns	ns
Node no.	15.0	18.0	16.4	16.6	-	-	0.005	16.4	18.0	18.2	16.6	0.005	ns	ns
Top dwt.(g)	1.10	1.61	1.47	1.31	-	-	0.05	1.20	1.68	1.72	1.61	-	-	0.005
Stolon no.	5.4	4.6	4.0	5.2	ns	ns	ns	4.8	4.0	6.6	5.2	ns	ns	ns
Tuber no.	1.8	0.6	0.2	2.4	0.005	ns	ns	4.2	2.0	1.4	2.8	0.001	ns	ns
Tub. stolon no.	1.4	0.6	0.2	1.8	0.05	ns	ns	2.4	1.4	1.2	2.0	0.05	ns	ns
Tuber fwt.(g)	-	-	-	-	-	-	-	0.63	0.17	0.11	0.69	0.005	ns	ns
Replication	5	5	5	5	-	-	-	5	5	5	5	-	-	-

GA_3 M.E. = gibberellic acid main effect
IAA M.E. = indole acetic acid main effect
I = interaction

Values quoted under statistical analysis are values of p.

For abbreviations, see Section VIII.

TABLE 19b. Results of GA_3/IAA factorial experiment (continued).
Breakdown analyses for interactions.

	9 day harvest								12 day harvest							
	Effect of IAA				Effect of GA_3				Effect of IAA				Effect of GA3			
	$+GA_3$		$-GA_3$		+IAA		-IAA		$+GA_3$		$-GA_3$		+IAA		-IAA	
	p	LSD	p	LSD	p	LSD	p	LSD	p	LSD	p	LSD	p	LSD	p	LSD
Top ht.(cm)	ns	10.2	0.05	4.51	0.001	4.84	0.001	3.76	-	-	-	-	-	-	-	-
Node no.	ns	2.57	0.05	1.11	ns	2.39	0.05	1.96	-	-	-	-	-	-	-	-
Top dwt.(g)	ns	0.23	0.005	0.11	ns	0.36	0.005	0.25	ns	0.17	0.005	0.20	ns	0.23	0.001	0.14

For abbreviations, see Section VIII.

by gibberellic acid. Top dry weight showed a significant negative interaction between the two treatments; breakdown analysis (Table 19 b) showed that both compounds alone had a promotive effect on top dry weight, but that neither exerted an effect in the presence of the other.

At the 9 day harvest, significant negative interactions were found with the treatments for all three top growth parameters: top height, node number and top dry weight. The results of breakdown analysis were as follows (Table 19 b). For top height gibberellic acid had a promotive effect both with an without indole acetic acid. The latter exerted a smaller promotive effect when applied alone, but none in the presence of gibberellic acid. For both node number and top dry weight, both gibberellic acid and indole acetic acid exerted a small promotive effect when applied alone, but neither had any effect in the presence of the other. It is possible that in some cases the lack of effect of either growth substance when both were applied together may have been the result of higher variance in the plants given both compounds.

Although the results on top growth are rather difficult to interpret fully, it appears that gibberellic acid acted in its usual way (see above), causing an increase in top height, usually accompanied by an increase in top dry weight, and also in node number after repeated applications. After 12 days, gibberellic acid treatment produced increases in top height and node number which were unaffected by indole acetic acid treatment, and even after 9 days, its effect on top height was unaffected by indole acetic acid. Indole acetic acid also seemed to act in its usual role of a growth promoter, but the effects of its action were very

small, and were not found in the presence of applied gibberellic acid.

Neither growth substance had any effect on stolon number (as would be expected, since stolon formation should have been completed before treatment) or on the diageotropic growth of the stolons, such as was found by Booth (1963) with decapitated plants. An effect on the growth habit, however, may require treatment earlier in the life of the plant, when stolons are still forming, or it may require the elimination of the natural supply of growth substances from the shoot apex. Negatively geotropic leafy shoots as found by Booth, were in fact obtained in the topping experiment in Section III, in which the apex was removed.

In the present experiment, tuber number, number of tuberising stolons and tuber fresh weight (where recorded) were decreased by gibberellic acid, as expected from previous results. Indole acetic acid treatment, however, had no effect on tuberisation. Therefore, while it may cause small differences in the growth of tops when applied to the intact plant, indole acetic acid does not appear to be directly involved, either alone or in combination with gibberellic acid, in the control of tuberisation.

First experiment with 2-chloroethyltrimethylammonium chloride (CCC)

This and the following two experiments were carried out to amplify the information obtained from the above experiments with gibberellic acid, by possibly implicating the plant's endogenous gibberellins in the control of tuberisation. The details of the experiments are given in Table 20 and in Section V (ii) a, and the results are presented in Tables 21 and 22.

TABLE 20. Time schedules and treatments for CCC and ABA application experiments (Section V (ii))

Experiment	Temp. after E (^{o}C day/night)	Age at E (days)	Age at A (days)	Harvest date (days from E)	Replication	G.S. Application		
						Concentration	Solvent	Amount of solution applied
CCC1	20/17	35	15	14	10	0, 10^{-3}M, 10^{-2}M	H_2O	100ml/pot
CCC2	20/17	43	42	14	10	0, 10^{-3}M, 10^{-2}M	H_2O	100ml/pot
CCC3	30/27	35	35	14	10	0, 10^{-3}M, 10^{-2}M	H_2O	100ml/pot
ABA	20/20	35	35, 35 + 1	14	10	10 µg/plant	50:50/ C_2H_5OH: H_2O	20 µl drop

Temp. after E = Temperature during the experimental period
Age at E = Age of plants at beginning of experimental period
Age at A = Age of plants at application of growth substance
G.S. application = growth substance application.
Harvest date is quoted, as in all experiments in the main body of the work, as days from the start of the experimental period.
All experiments used plants treated in both SD and LI LD daylength régimes during the experimental period.

TABLE 21. Results of first CCC application experiment. Differences in top, stolon and tuber growth for plants grown in LILD or SD with or without CCC at 10^{-2} or 10^{-3}M at harvest after 14 days (plants 15 days old at CCC treatment).

Treatment	SD					LILD				
	0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.		0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.	
				p	LSD				p	LSD
Top ht.(cm)	32.8	19.8	15.8	0.001	2.56	32.4	20.9	16.2	0.001	3.23
Node no.	19.6	19.2	18.7	ns		21.0	19.2	16.9	0.005	2.45
Top dwt.(g)	2.09	1.63	1.75	0.005	0.25	2.10	1.42	1.47	0.001	0.29
Stolon no.	9.0	7.7	6.9	0.05	1.15	7.3	8.3	7.2	ns	
Tuber no.	4.5	5.7	5.2	ns		0.2	0.1	1.1	-	
Tub. stolon no.	3.1	3.7	3.3	ns		0.2	0.1	0.7	-	
Tuber fwt.(g)	0.90	1.50	2.40	ns		0.03	0.07	0.50	-	
No. plants tub.	9/10 (90%)	9/9 (1 rotted) (100%)	6/7 (3 rotted) (85.7%)	-		2/10 (20%)	1/9 (1 rotted) (11.1%)	4/9 (1 rotted) (44.4%)	-	
Replication	10	10	10	-		10	10	10		

For abbreviations, see Section VIII.

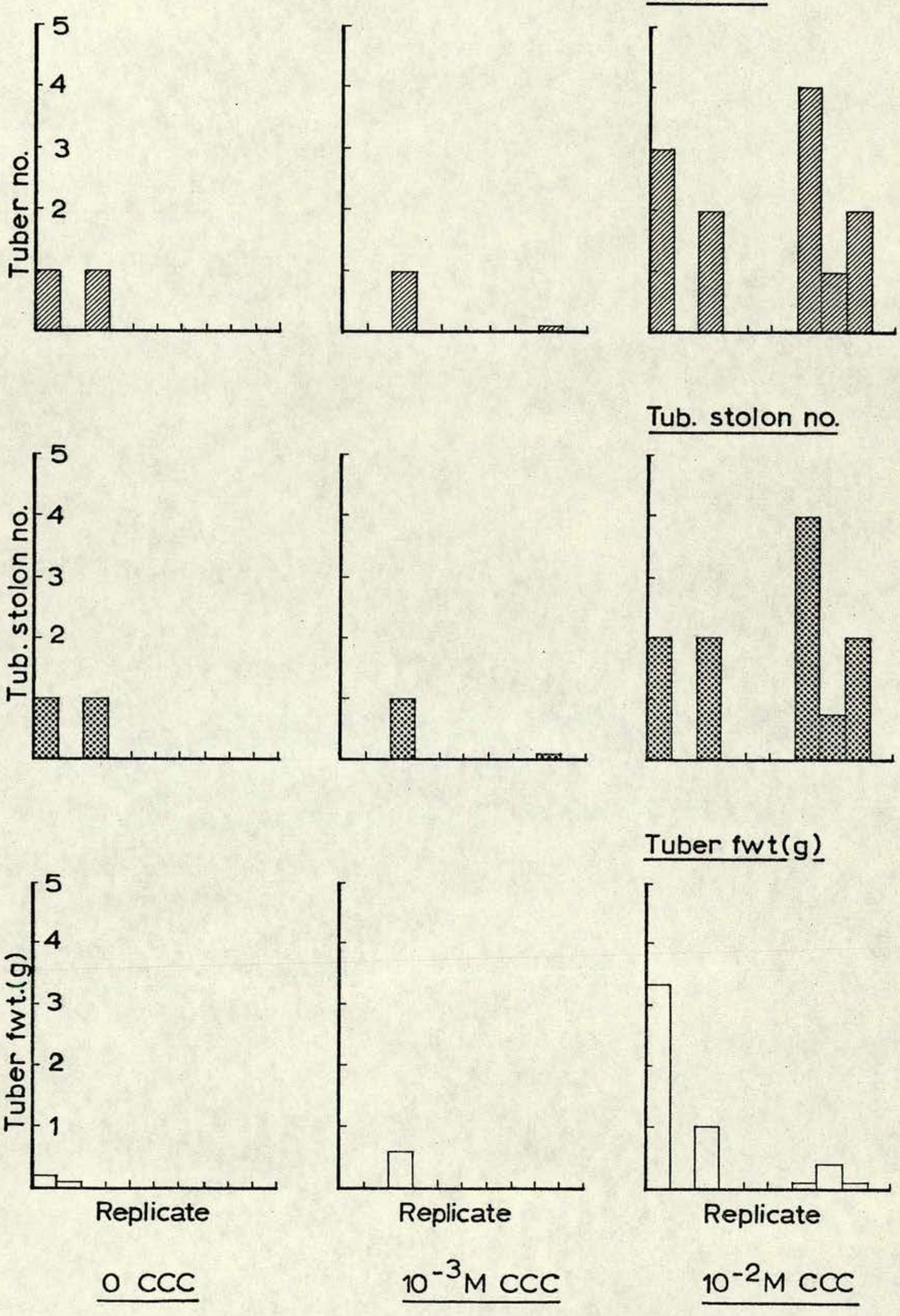
In the first experiment, in which plants were 15 days old when treated, CCC at both concentrations (10^{-3}M and 10^{-2}M) caused a marked decrease in top height, in both the daylength regimes used during the experimental period (Table 21). This result was as expected from the results of other workers both in the potato (eg Dyson and Humphries, 1966; Humphries and Dyson, 1967) and in a great many other plant species eg wheat (Tolbert, 1960), Bryophyllum (Zeevaart and Lang, 1963), sugar beet (Humphries and French, 1965). CCC treatment also decreased top dry weight in agreement with the results of Dyson and Humphries (1966) and Humphries and Dyson (1967) for potato and those of other workers with other plant species (eg Cathey and Stuart, 1961), although exceptions have been found (eg Humphries, 1963). Node number was decreased by 10^{-2}M CCC in long days (although not in short days); no such effect was found by Humphries and Dyson (1967) for the main stem, although they found a decrease in the rate of leaf production on lateral stems. CCC was also found to produce thicker stems in the present experiment, in agreement with findings for other plant species eg wheat (Tolbert, 1960) and tomatoes (Wittwer and Tolbert, 1960). These results for top growth, which are in agreement with the vast literature on the effects of CCC, suggest that CCC was acting in its normal way, inhibiting gibberellin synthesis (see Section V (i)) and bringing about opposite effects on growth from those obtained with gibberellic acid (see above). It would therefore be expected that CCC treatment would also bring about an opposite effect to that of gibberellic acid on tuberisation.

There was an effect of CCC on stolon number (decreased) in short

days, but none in long days. There was an apparent increase in tuberisation, in terms of tuber number, number of tuberising stolons and tuber fresh weight, with 10^{-2}M CCC treatment in long days, but there were too many zeroes in the data to allow a valid statistical analysis to be performed (Table 21 and Fig 19). No significant effects on tuberisation were found in the plants grown in short days, although there appeared to be an increase in tuber fresh weight with CCC; this may have been found to be not significant due to high variability in the data.

The lack of a marked promotive effect (although a slight one was recorded) of CCC on tuberisation was unexpected and not in agreement with the results of other workers eg Dyson (1965) and Dyson and Humphries (1966), who found with the variety Majestic that CCC hastened tuber formation, although it later slowed tuber growth. It was thought possible that CCC might have been applied too early in the growth of the plant (day 15) so that by the time tuber initiation began, the effect of CCC on metabolism was no longer being felt (ie biosynthesis of endogenous gibberellins was proceeding in a normal way, not influenced by applied CCC), and tuberisation was therefore not greatly affected by CCC treatment. This is perhaps unlikely, however, in view of the fact that Dyson and Humphries (1966) found tuberisation to be retarded with CCC treatment given at emergence from the soil, which was considerably earlier in the life of the plant than the application in the present experiment. It seems more likely that CCC applied at 15 days from planting, at which time stolon production would have been taking place, had some effect on this process. This would be expected if stolon production and growth is dependent on a gibberellic acid/indole acetic acid interaction, as proposed by

FIGURE 19. CCC application experiment 1. Histograms showing raw data for tuber number, number of tuberising stolons (Tub. stolon No.) and tuber fresh weight in plants grown in low intensity long days (LI LD) and treated or untreated with CCC.



Tuber no.
Tub. stolon no.
Tuber fwt(g)
Tuber no.
Tub. stolon no.
Tuber fwt.(g)
1
2
3
4
5
Replicate
Replicate
Replicate
0 CCC
10⁻³M CCC
10⁻²M CCC

Booth (1963). Also, Humphries and Dyson (1967) found stolon growth to be inhibited by CCC although less so in Up-to-Date than in some other varieties. In the present experiment, a significant decrease in stolon number was found with CCC treatment in short days, and this effect may have masked a hastening of tuber formation. There was no decrease in stolon number in the plants from long days, and these plants did in fact appear to show a promotion of tuberisation with CCC. These plants might be expected to have had a higher level of endogenous gibberellins than those grown in short days (see Section V (i) and (iv)) so that the effect of CCC would be expected to be rather small.

Second experiment with CCC

Because of the possibility of the early application of CCC affecting stolon production and this effect masking effects on tuberisation in the first experiment, a second experiment was carried out in which the plants used were older at the time of treatment with CCC, which was given immediately before transfer to differential daylength conditions. The plants were 43 days old at the time of the latter operation, because they had been rather slow to sprout; Table 20 gives details of the experiment, which was in other respects similar to the first one, and the results are given in Table 22.

As in the first experiment, CCC caused a marked reduction in top height in both daylength regimes. Node number and top dry weight were, however, unaffected, in contrast to the results of the previous experiment. This may have been because of the shorter time between CCC treatment and harvest in the present experiment, causing these trends to be less marked by the harvest

TABLE 22. Results of second CCC application experiment. Differences in top, stolon and tuber growth for plants grown in LILD and SD with or without CCC at 10^{-2} or 10^{-3} M, at harvest after 14 days (plants 42 days old at CCC treatment).

Treatment	SD					LILD				
	0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.		0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.	
				p	LSD				p	LSD
Top ht.(cm)	29.5	20.9	18.9	0.001	3.50	32.2	21.9	17.6	0.001	1.62
Node no.	18.7	19.0	18.9	ns		20.4	20.8	19.3	ns	
Top dwt.(g)	2.29	2.04	1.89	ns		2.09	2.00	1.93	ns	
Stolon no.	8.4	8.6	7.0	ns		8.7	8.4	8.3	ns	
Tuber no.	3.7	5.4	7.8	0.005	2.44	0.2	1.2	4.7	0.001	2.42
Tub. stolon no.	2.7	3.0	3.7	ns		0.2	1.0	3.2	0.001	1.45
Tuber fwt.(g)	1.7	3.3	6.4	0.005	2.17	0.6	1.6	3.5	0.005	1.90
No. plants tub.	10/10 (100%)	10/10 (100%)	10/10 (100%)	-		2/10 (20%)	7/10 (70%)	10/10 (100%)	-	
Replication	10	10	10	-		10	10	10	-	

For abbreviations, see SectionVIII.

date. Top dry weight appeared to be decreased by CCC, but this effect cannot have been great enough to overcome the high variability present in the data.

Stolon number was unaffected by treatment; this was to be expected, as most stolon formation should have already occurred by the time of application of CCC. Tuberisation was significantly increased by 10^{-2}M CCC treatment in both daylength regimes. 10^{-3}M CCC also appeared to cause a smaller promotive effect, but this was not found to be significant, probably because of high variability. All plants from short days had tuberised by harvest, but of those from long days, a greater percentage of CCC-treated plants had tuberised than of the controls.

From these results it appears that, while CCC applied to young plants (experiment 1) has only a slight effect on tuberisation (probably because an inhibitory effect on stolon production and growth), when applied to older plants, it causes a substantial promotion of tuberisation. These results are in agreement with those of Dyson and Humphries mentioned above, except that tuber weight was found to be increased; such an effect was not found by these workers, probably because their harvests were mostly carried out some considerable time after the first CCC treatment.

The effects of CCC on tuberisation found in the present experiment are what would be expected if endogenous gibberellins acted to inhibit or delay tuberisation, since application of CCC to plants just before the expected onset of tuberisation should prevent or reduce gibberellin synthesis at this time.

Third experiment with CCC

This experiment was conducted to determine whether the promotive effect of CCC on tuberisation found in the second experiment would still result at high temperatures (30°C day/27°C night) which are less favourable to tuberisation than the low temperatures used in the experiments above. Details of the experiment are given in Table 20 and the results in Table 23.

There was a large decrease in top height with CCC in both day-length regimes, as in the previous experiments; CCC treatment had no effect on node number in short days, and a small, probably unreal, effect in long days (the value with 10^{-3}M CCC was greater than that with 10^{-2}M, but neither of these values differed significantly from that for the controls). Top dry weight was unaffected by CCC. These results are similar to those of experiment 2, in which CCC treatment was given, as in the present experiment, at the beginning of the differential day-length period.

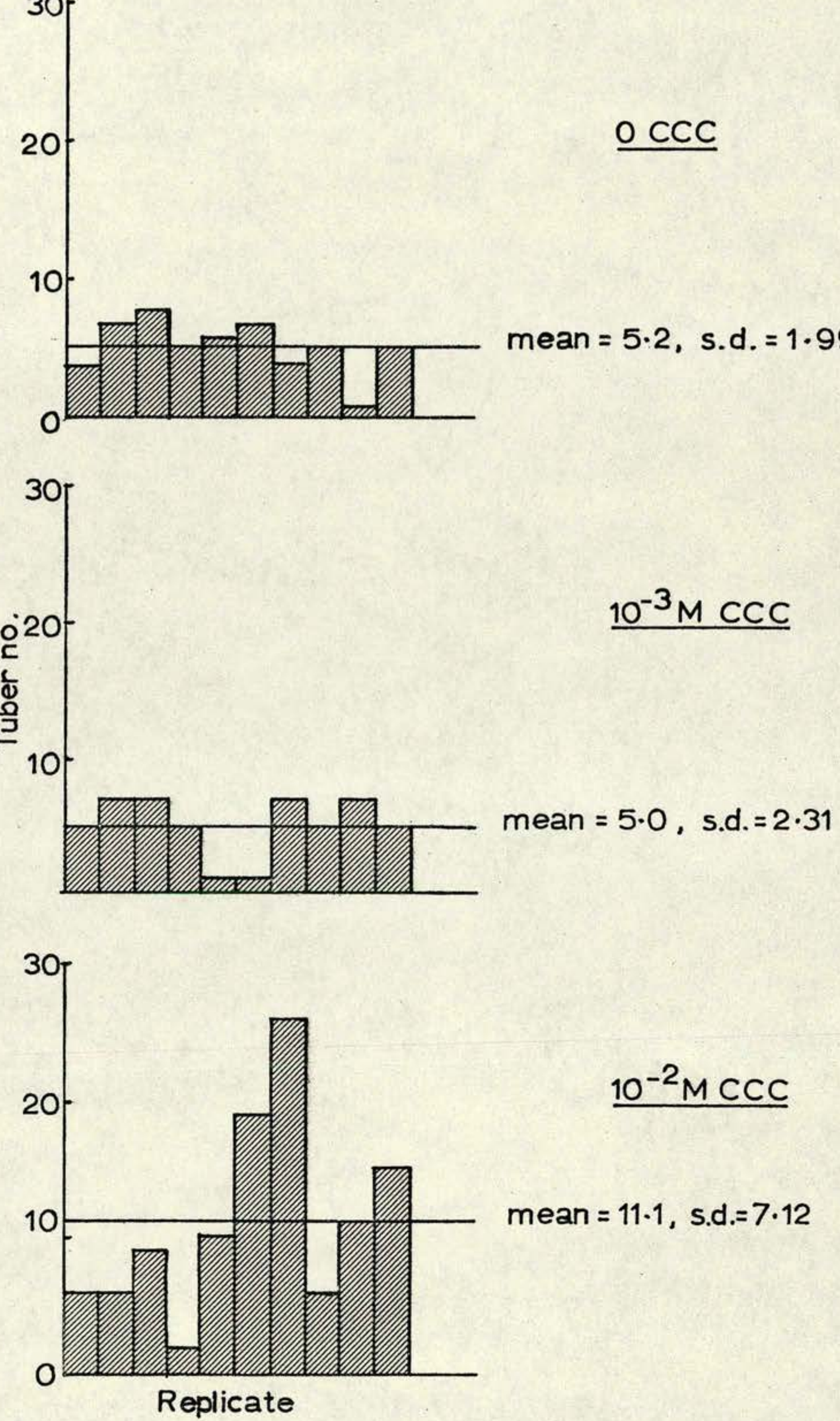
Stolon number was unaffected by CCC in short days, but 10^{-2}M CCC caused a slight decrease in long days. No tuberisation occurred in long days; this was not surprising, since tuberisation, although it did occur, was very little advanced in the plants from long days in the first two experiments, in which temperatures were favourable to tuberisation. All plants from short days (with the exception of one which had rotted) had tuberised. There was an apparent increase in tuberisation with 10^{-2}M CCC, in terms of tuber number, number of tuberising stolons and tuber fresh weight (Table 23 and Fig 20), but the first two of these parameters were found to show no significant differences. In

TABLE 23. Results of third (high temperature) CCC application experiment. Differences in top, stolon and tuber growth for plants grown in LILD and SD with or without CCC at 10^{-2} or 10^{-3}M at harvest after 14 days (plants 35 days old at CCC treatment).

Treatment	SD					LILD				
	0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.		0 CCC	10^{-3}MCCC	10^{-2}MCCC	S.A.	
				p	LSD				p	LSD
Top ht.(cm)	37.8	29.3	16.3	0.001	4.25	53.3	39.8	22.0	0.001	1.07
Node no.	17.7	17.2	16.5	ns		17.8	18.9	16.9	0.05	1.33
Top dwt.(g)	1.25	1.13	1.17	ns		1.31	1.20	0.99	ns	
Stolon no.	6.7	6.2	6.6	ns		4.7	4.1	3.6	0.05	0.96
Tuber no.	5.2	5.0	11.1	ns		0	0	0	-	
Tub. stolon no.	4.4	4.0	6.1	ns		0	0	0	-	
Tuber fwt.(g)	0.87	1.2	2.7	0.05	1.36	0	0	0	-	
Non-tub. stolons										
With starch	1.0	1.6	0.2	0.05	0.31	0.3	1.0	0.55	ns	
No starch	1.2	0.3	0.2	ns		3.3	3.1	3.2	ns	
% with starch	45.5	81	50	-		8.3	24	16	-	
No. plants tub.	10/10 (100%)	10/10 (100%)	9/9 (1 rotted) (100%)	-		0/10 (0%)	0/10 (0%)	0/10 (0%)	-	
Replication	10	10	10	-		10	10	10	-	

For abbreviations, see Section VIII.

FIGURE 20. CCC application experiment 3. Histograms showing raw data for tuber number in plants grown in short days (SD) and treated or untreated with CCC.


30
20
10
0
0 CCC
mean = 5·2, s.d. = 1·99
30
20
10
Tuber no.
10^{-3}M CCC
mean = 5·0 , s.d. = 2·31
30
20
10
0
10^{-2}M CCC
mean = 11·1, s.d.= 7·12
Replicate

the case of tuber number at least, this was probably because of the high variability in the data; tuber number in plants given 10^{-2}M CCC ranged from 2 to 26, s d 7.12 .

In the present experiment, in addition to tuberisation data, starch deposition in stolons which had not tuberised visibly was also examined, since it was found possible to detect stages of tuberisation earlier than visible swelling in this way (see Section VI). Only white stolons were examined, since green stolons rarely tuberise. In plants from short days, the number of untuberised stolons with starch deposits increased significantly with 10^{-3}M CCC treatment, suggesting that a larger number of stolons were about to tuberise in these plants (Table 23). The number of such stolons fell again with 10^{-2}M CCC, but this was almost certainly because the number of visibly tuberised stolons was higher in this treatment than in the controls or the plants given 10^{-3}M CCC (Fig 20). When stolons pass from the stage of starch deposition to the stage of visible swelling, the number of non-visibly tuberised stolons with starch deposition obviously falls and that of visibly tuberised stolons rises. These results are perhaps better expressed as the percentage of non-visibly tuberised stolons containing starch (Table 23). In the plants from long days, there was also an apparent increase with CCC treatment in the percentage of non-visibly tuberised stolons containing starch. Although these changes were not significant, probably because of the high variability of the data, they nevertheless tend in the same direction as those from plants kept in short days, and suggest that in the plants kept in long days also, CCC may be exerting some promotive effect on tuberisation.

It appears from these results that tuberisation is accelerated at high as well as at low temperatures by CCC treatment, although the effect was not massive enough to overcome the inhibiting effect on tuberisation of long days and high temperatures together. Because the level of tuberisation in short days was quite appreciable, even in the controls it was not possible to establish definitely whether CCC could relieve the effect of high temperatures on tuberisation. It is possible that the effect of high temperatures on tuberisation would only be noticeably felt in short days if the plants were grown in these conditions throughout the growing period as well as the experimental period, as was the case with the plants in the first preliminary experiment (Section II).

Summary of results of experiments with CCC

While the results with exogenous gibberellic acid (above) suggest the involvement of g ibberellins as a delaying influence in the control of tuberisation, to demonstrate that endogenous gibberellins are actually involved it is necessary to demonstrate changes in their level correlated with the progress of tuberisation (see Section V (iv)) and also that manipulation of endogenous gibberellin synthesis has some effect on tuberisation. These experiments have shown that the latter is the case: when synthesis of endogenous gibberellins was reduced by CCC treatment applied at the beginning of the differential daylength period (experiments 2 and 3), when plants were approaching tuberisation, tuberisation was promoted at both low and high temperatures.

A more marked promotion of tuberisation would probably have been found if repeated applications (as in gibberellic acid experiments 6 and 7) had been given. CCC treatment was unable to cause

tuberisation in long days at high temperatures (experiment 3), presumably because both these conditions are inhibitory to tuberisation and even when gibberellin synthesis was reduced for some time, this was not enough to cause tuberisation. This suggests that other factors may also be involved. Rather than conducting an experiment with repeated applications of CCC, however, it was considered more valuable to proceed to stolon feeding experiments with gibberellic acid and CCC (Section V (iii)), since the present experiments with whole plants had demonstrated a promotion of tuberisation, albeit not under the most extreme non-inductive conditions. The results are further discussed in Section VII.

Experiment with abscisic acid

Since only a very small amount of abscisic acid was available, it was applied as a drop to the apex instead of as a foliar spray (as used by El-Antably et al, 1967 and Smith and Rappaport, 1969). Details of the experiment are given in Table 20 and the results in Table 24.

Abscisic acid had no effect on top height, and none on node number or top dry weight in short days, although a reduction in both these parameters was found in long days.

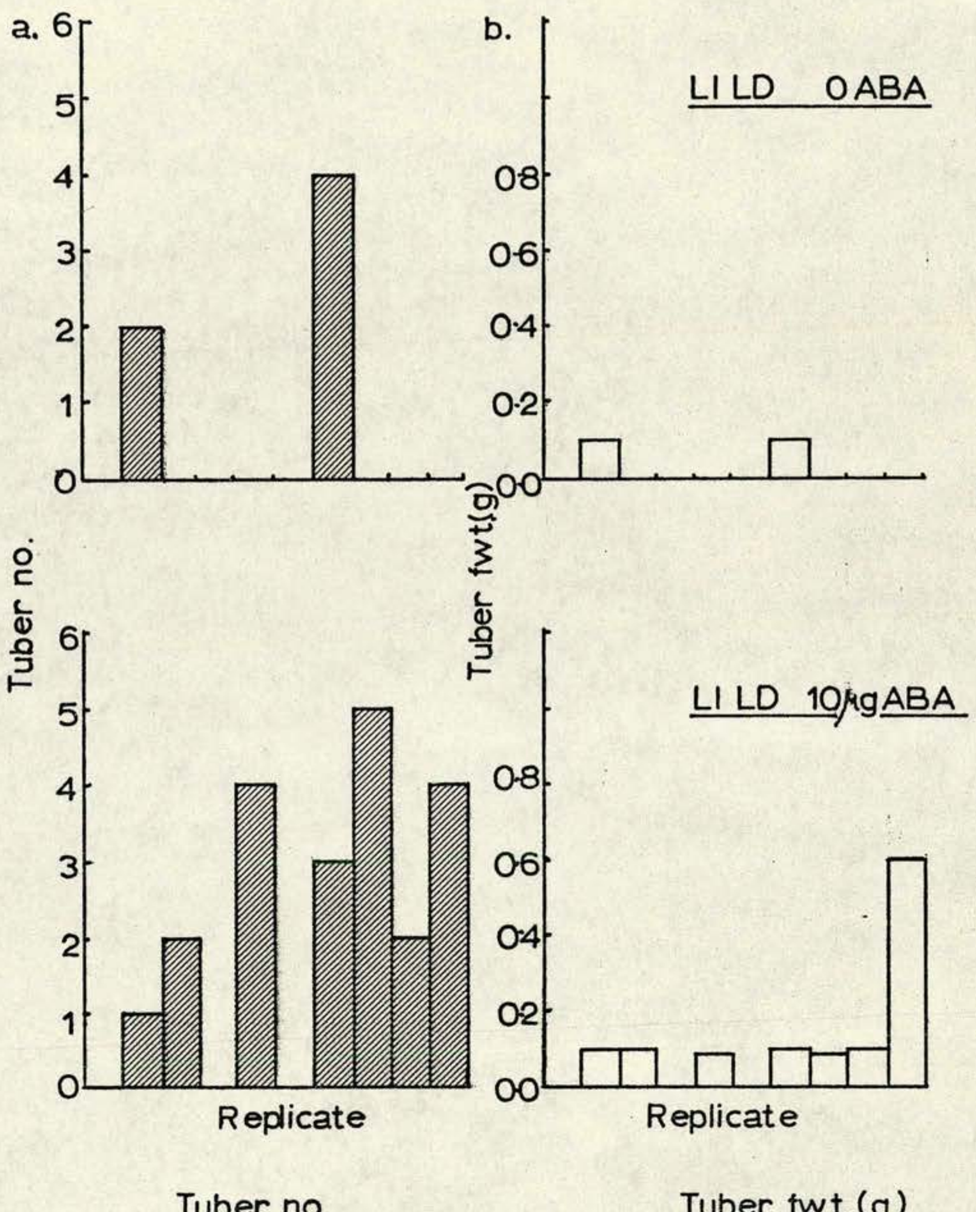
Stolon number was unaffected by treatment. Tuberisation (measured by tuber number, number of tuberising stolons and tuber fresh weight) was unaffected in short days, as were the numbers of non-visibly tuberised stolons with and without starch deposition. In long days, however, tuber number and number of tuberising stolons were significantly increased by abscisic acid (Table 24 and Fig 21 (a)). Tuber fresh weight appeared to be

TABLE 24. Results of ABA application experiment. Differences in top, stolon and tuber growth for plants grown in LILD or SD with or without ABA.

Treatment	SD				LILD			
	No ABA	10μg ABA/plant	S.A.		No ABA	10μg ABA/plant	S.A.	
			p	LSD			p	LSD
Top ht.(cm)	49.2	44.5	ns		60.4	59.6	ns	
Node no.	15.0	13.9	ns		18.1	16.3	0.05	1.57
Top dwt.(g)	3.46	3.33	ns		3.97	3.50	0.05	0.42
Stolon no.	13.5	15.1	ns		14.8	15.1	ns	
Tuber no.	10.0	10.7	ns		0.6	2.1	0.05	1.05
Tub. stolon no.	8.8	8.2	ns		0.6	1.7	0.05	0.95
Tuber fwt.(g)	0.99	1.22	ns		0.02	0.12	ns	
Non-tub. stolons								
With starch	0.2	0.7	ns		0	0.1	-	
No starch	0.6	2.8	ns		14.7	13.3	ns	
% with starch	25	20	-		0	0.7	-	
No. plants tub.	10/10 (100%)	10/10 (100%)	-		2/10 (20%)	7/10 (70%)	-	
Replication	10	10	-		10	10	-	

For abbreviations, see Section VIII.

<u>FIGURE 21.</u> Abscisic acid application experiment. Histograms showing raw data for tuber number and tuber fresh weight in plants grown in low intensity long days (LI LD) and treated or untreated with abscisic acid.


a.
b.
LI LD 0 ABA
LI LD 10µg ABA
Tuber no.
Tuber fwt.(g)
Replicate
Replicate
6
5
4
3
2
1
0
0·8
0·6
0·4
0·2
0·0
Tuber no.
Tuber fwt.(g)

increased, but the difference was not significant, and was probably due to the single very high value in one of the treated plants (Fig 21 (b)). There was again no effect on starch deposition in non-visibly tuberised stolons. A much higher percentage of treated plants than of long day controls had tuberised.

It appears from these results that abscisic acid exerts a promotive effect on tuberisation in plants grown in long days, in agreement with the results of El-Antably et al (1967), and in conflict with those of Smith and Rappaport (1969). Although no effect of treatment was found in plants kept in short days, this may have been because tuberisation was well advanced by the harvest date and an effect may have thus been missed. It is also possible that in short day conditions, the plants were already producing sufficient growth inhibitor or anti-gibberellin, if such compounds are involved, for a maximal rate of tuberisation to be taking place by harvest, the additional abscisic acid being superfluous.

The decrease in node number and top dry weight in plants from long days may indicate an indirect effect on tuberisation, mediated by effects on top growth. Whether this is the case or not, however, the results suggest a promotive effect of abscisic acid on tuberisation when applied at low concentration under long day conditions. The results also tend to overcome some of the objections of Smith and Rappaport (1969) to the experiments of El-Antably et al (1967) on the grounds of the very large doses of abscisic acid required to induce tuber formation, and of the abnormal stunted appearance and early leaf senescence in treated

plants (see Section VII); in the present experiment, a fairly low concentration was applied on only two occasions, and the treated plants had a perfectly normal appearance. The implications of these results are discussed in Section VII.

(iii) Group B: Stolon feeding experiments

a. Introduction and general methods

The experiments in Group A, described in Section V (ii), showed that gibberellic acid, CCC and abscisic acid all exerted some effect on tuberisation when applied to the intact plant. It was not clear from these results, however, whether the various growth-active substances exerted their effect indirectly (by changing the growth pattern of the whole plant) or specifically at the site of tuber formation, the sub-apical region of the stolon.

In the present experiments, growth-active substances were applied directly to the stolon tip. It was thought that the results thus obtained would be more specific with respect to tuberisation than those from the experiments in Group A. In the latter, the the growth substances were required to be translocated to the stolon tips in order to exert their effect directly at the site of tuber-formation, or to exert their effect indirectly through the growth of the plant as a whole.

A number of workers have observed tuberisation in excised pieces of potato stem grown by tissue culture methods (eg Mes and Menge, 1954; Chapman, 1955, 1958) and others (McCorquodale and Moorby, 1968; Palmer and Smith, 1969 a and b, 1970) have applied these techniques to the investigation of various growth-active substances on stolon tips. It was felt that it might be more valuable to treat individual stolon tips _in vivo_ (ie while still attached to the parent plant) rather than _in vitro_ (ie stolon tips excised from the parent plant and grown on a culture medium which was required to provide everything needed by the stolon tips for

growth and tuberisation). Accordingly, a stolon feeding technique was established in which the terminal 2-3 cm of stolons were placed in small bottles containing the appropriate treatment solution (see Section II (ii) d). Treated stolons on the same plant always received the same treatment as each other.

Using this method, two experiments were carried out with gibberellic acid, GA_3, one using CCC, one using abscisic acid, ABA, (all as specified in Section V (ii)) and two using a cytokinin, 6-furfurylaminopurine, Kinetin, KIN (Hopkin and Williams). Kinetin was chosen because it was readily available and had been found by Palmer and Smith (1969 a) to be more effective in promoting tuberisation in their system than the other synthetic cytokinins which they used. Although the naturally-occurring compounds are often quantitatively more active than kinetin, there appear to be no qualitative differences in activity between the natural and synthetic cytokinins (Helgeson, 1968) so that results from kinetin studies should give a good idea of the effects of endogenous cytokinins. Environmental conditions were as usual (Section II), except that in the experiments with CCC, abscisic acid and kinetin, the temperature during the experimental period was maintained at 20°C due to the requirements of other users. Details of the experiments are given in Table 25.

b. Preparation of solutions of growth-active substances

Gibberellic and abscisic acid solutions were prepared by dissolving the solid in a very small amount of ethanol and then adding the appropriate amount of water to give the required stock solution. CCC and kinetin were made up in completely aqueous

TABLE 25. Time schedules and treatments for stolon feeding experiments (Section V (iii))

Experiment	Growth Substance	Concentration	Volume in Bottle (ml)	Age at E (days)	Final Harvest (days from E)	Temperature after E (days) (^{o}C day/night)	Daylength after E	Replication	
								No. of plants/ treatment	No. of stolons treated/plant
1st GA_3	GA_3	1,10 ppm (2.9×10^{-6}M, 2.9×10^{-5}M)	1.5	42	23	20/17	SD (controls + GA_3 treatments)	8	2
							LILD (controls)	8	2
2nd GA_3	GA_3	1,10 ppm (2.9×10^{-6}M, 2.9×10^{-5}M)	2.0	35, 56	27	20/17	SD (controls + GA_3 treatments)	6, 35 day (5, controls)	2
								7, 56 day	2
							LILD (controls)	3	2
1st CCC	CCC	10^{-4}M, 10^{-2}M	2.0	35	23	20/20	SD	10	2
2nd CCC	CCC	10^{-4}M, 10^{-2}M	2.0	35	28	20/20	SD (all treatments)	10	2
							LILD (all treatments)	10	2
ABA	ABA	0.5, 1.0 ppm (1.9×10^{-6}M, 3.8×10^{-6}M)	2.0	35	23	20/20	SD (all treatments)	10	2
							LILD (all treatments)	10	2
1st KIN	KIN	10^{-6}M, 10^{-5}M, 10^{-4}M	2.0	35	24	20/20	SD (controls + KIN treatments	10	2 (some broken)
							LILD (controls)	10	2
2nd KIN	KIN	10^{-5}M, 10^{-4}M	2.0	35	16 (SD plants); 23 (LILD plants)	20/20	SD (all treatments	10	2 or 1(1 plant/ treatment)
							LILD (all treatments)	10	2

Age at E = Age of plants at beginning of experimental period (transfer to differential daylength régimes and stolon treatment begun). Final harvest date is given in days from the beginning of the experimental period, as usual. Daylength régimes for the experimental period are given (Daylength after E). SD = short days; LILD = low intensity long days.

solutions. CCC dissolved readily, but kinetin required special treatment. A stock solution (10^{-4}M) of kinetin was prepared and autoclaved to dissolve the powder; dilutions were then made to give 10^{-5}M and 10^{-6}M solutions. It was found that kinetin remained in solution even at a concentration of 10^{-4}M after standing at room temperature for several weeks.

c. The experiments; results and discussion

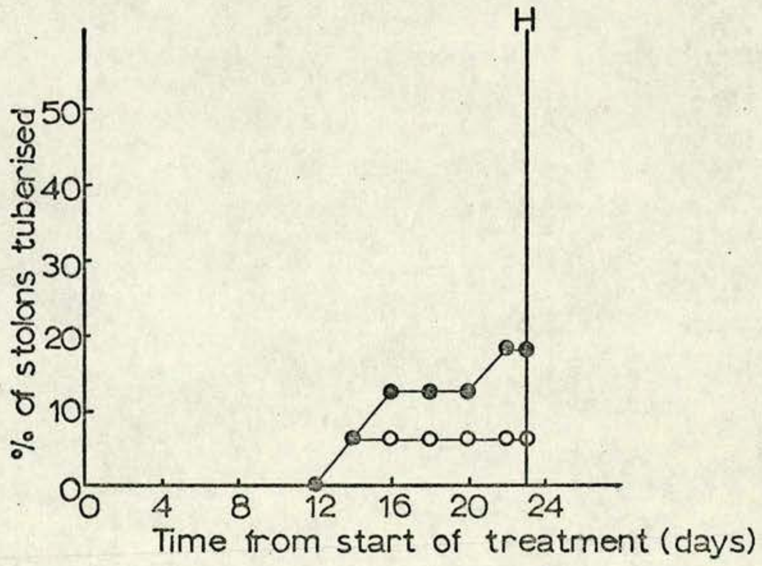
First feeding experiment with gibberellic acid

Details of the experiment are given in Table 25. The results are presented in Figs 22 and 23. Gibberellic acid treatment had no effect on top height (Fig 23 (b)). The progress of tuber formation is shown in Fig 22, in which the lower curve shows the number of stolons tuberised at the tip (apex of stolon) only, and the upper curve the total number tuberised (either at the tip or at a side bud : some branched stolons which had not yet tuberised at the tip did bear tubers at the side buds). By the final harvest after 23 days, none of the gibberellic acid treated stolons had tuberised; the only treated stolons to tuberise were short day water controls (Figs 22 and 23 (a)). Rotting of stolons was accelerated by the higher concentration (10 ppm) of gibberellic acid, but this did not appear to be due to the pH of the solution, as both this and the water control solutions gave readings in the region of pH 6. The difference in tuberisation between the water controls and the stolons given 1 ppm gibberellic acid could not, however, be accounted for by rotting, since the level of rot was slightly greater in the former than in the latter.

The water control stolons appeared normal, although there was some enlargement of the lenticels. Stolons treated with

FIGURE 22. Results of gibberellic acid stolon feeding experiment 1. Graphs of tuberisation in treated stolons (%) with time.

There was no tuberisation in stolons given gibberellic acid treatment.



●—● SD, no GA_3, total tuberisation

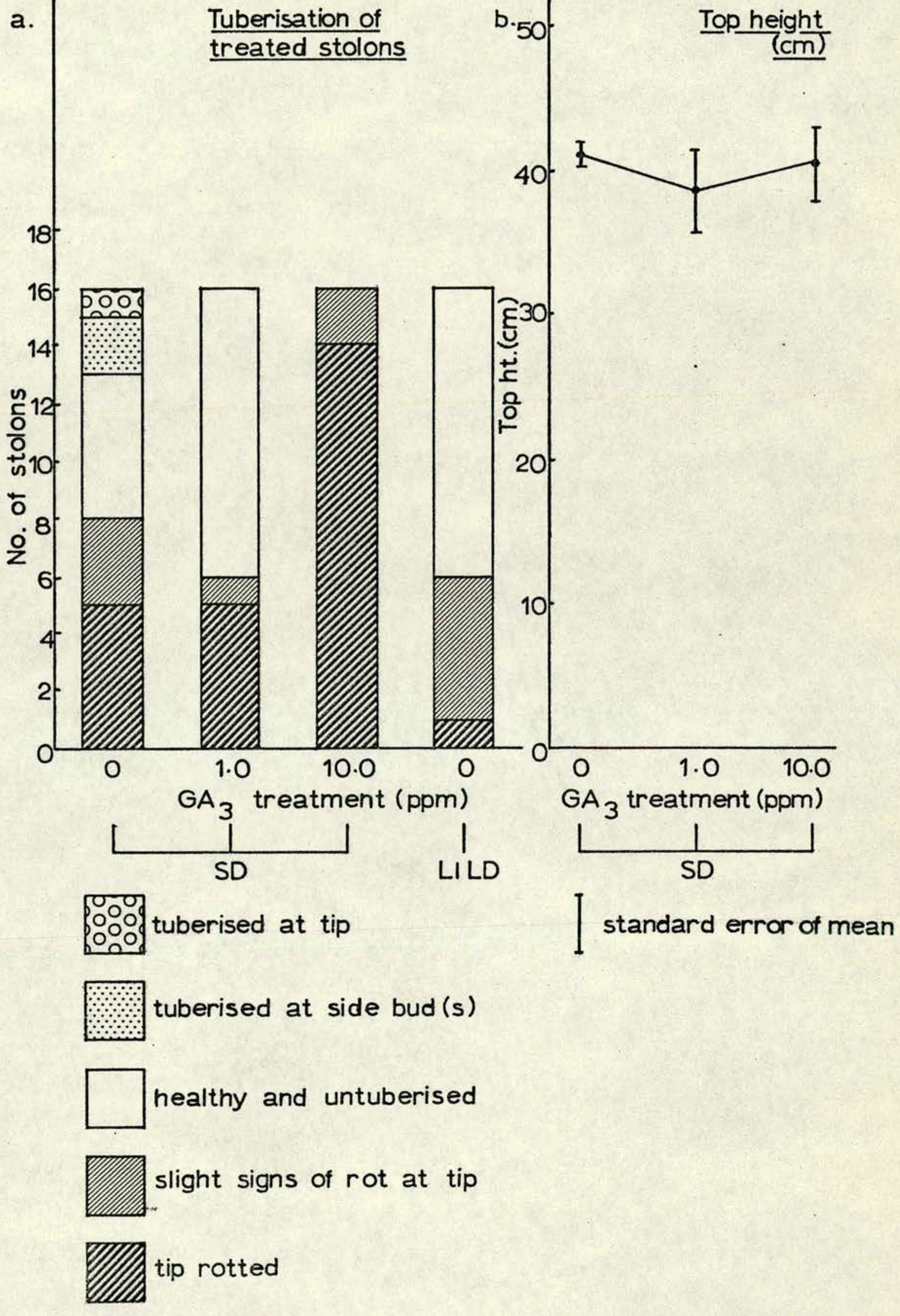
○—○ SD, no GA_3, tip only tuberised

H= Histogram for this date shown in Fig.23

FIGURE 23. Results of gibberellic acid stolon feeding experiment 1.

a. Histogram showing tuberisation in treated stolons at harvest after 23 days.

b. Top height of parent plants with and without gibberellic acid stolon feeding treatment, at harvest after 23 days.


a.
Tuberisation of treated stolons
No. of stolons
0
2
4
6
8
10
12
14
16
18
0
1·0
10·0
0
GA3 treatment (ppm)
SD
LI LD
b.
Top height (cm)
Top ht. (cm)
0
10
20
30
40
50
0
1·0
10·0
GA3 treatment (ppm)
SD
tuberised at tip
tuberised at side bud (s)
healthy and untuberised
slight signs of rot at tip
tip rotted
standard error of mean

gibberellic acid, and especially their tips, had a very long thin appearance compared to the controls, because of considerable internode extension; side branches, where present, also elongated in this way. This was as expected (see Section V (i) and (ii)) since stolons are simply stems with reduced leaf expansion and diageotropic growth.

The results therefore show an effect of gibberellic acid on growth of the stolons, causing them to elongate considerably and inhibiting tuber formation.

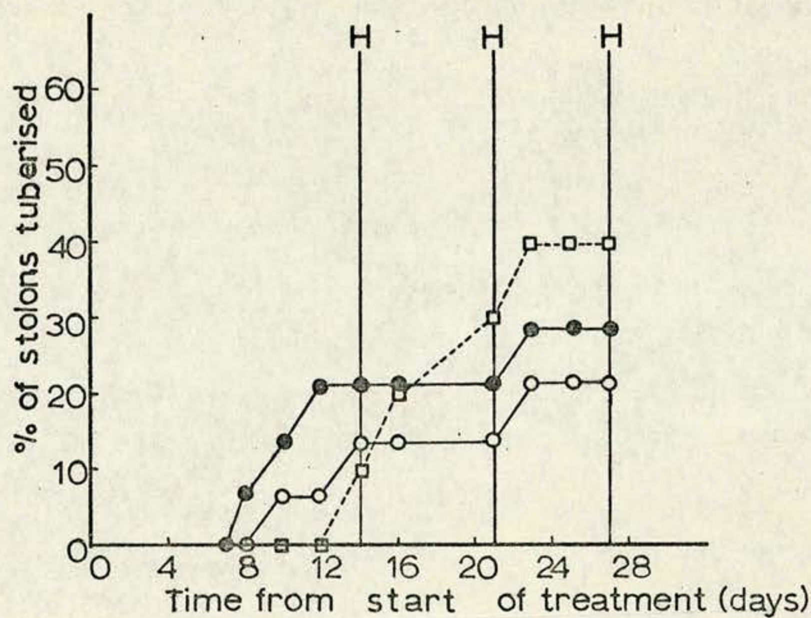
Second feeding experiment with gibberellic acid

This experiment was similar to the first and was carried out to check the results obtained. Plants of two ages (35 and 56 days old) were used, as it was thought that the older plants would tuberise earlier and that it might therefore be possible to detect any tuberisation at the higher gibberellic acid concentration before rotting began. A slightly greater volume of treatment solution was used than in the first experiment to ensure that the stolon tips were completely covered, no matter in which direction they grew. Details of the experiment are given in Table 25 and the results in Figs 24 and 25 and Table 26.

The gibberellic acid treatment had almost no effect on top growth, stolon number, or tuberisation of untreated stolons (Table 26); the only effect detected was a slight decrease in top dry weight in the 35 day old plants given 1 ppm gibberellic acid, but this was not thought to be a real effect. An as large or larger supply of gibberellic acid was theoretically available to the plants in this and the previous experiment as was supplied to

<u>FIGURE 24</u>. Results of gibberellic acid stolon feeding experiment 2. Graphs of tuberisation in treated stolons (%) with time.

There was no tuberisation in stolons on plants grown in low intensity long days, or on stolons treated with gibberellic acid.

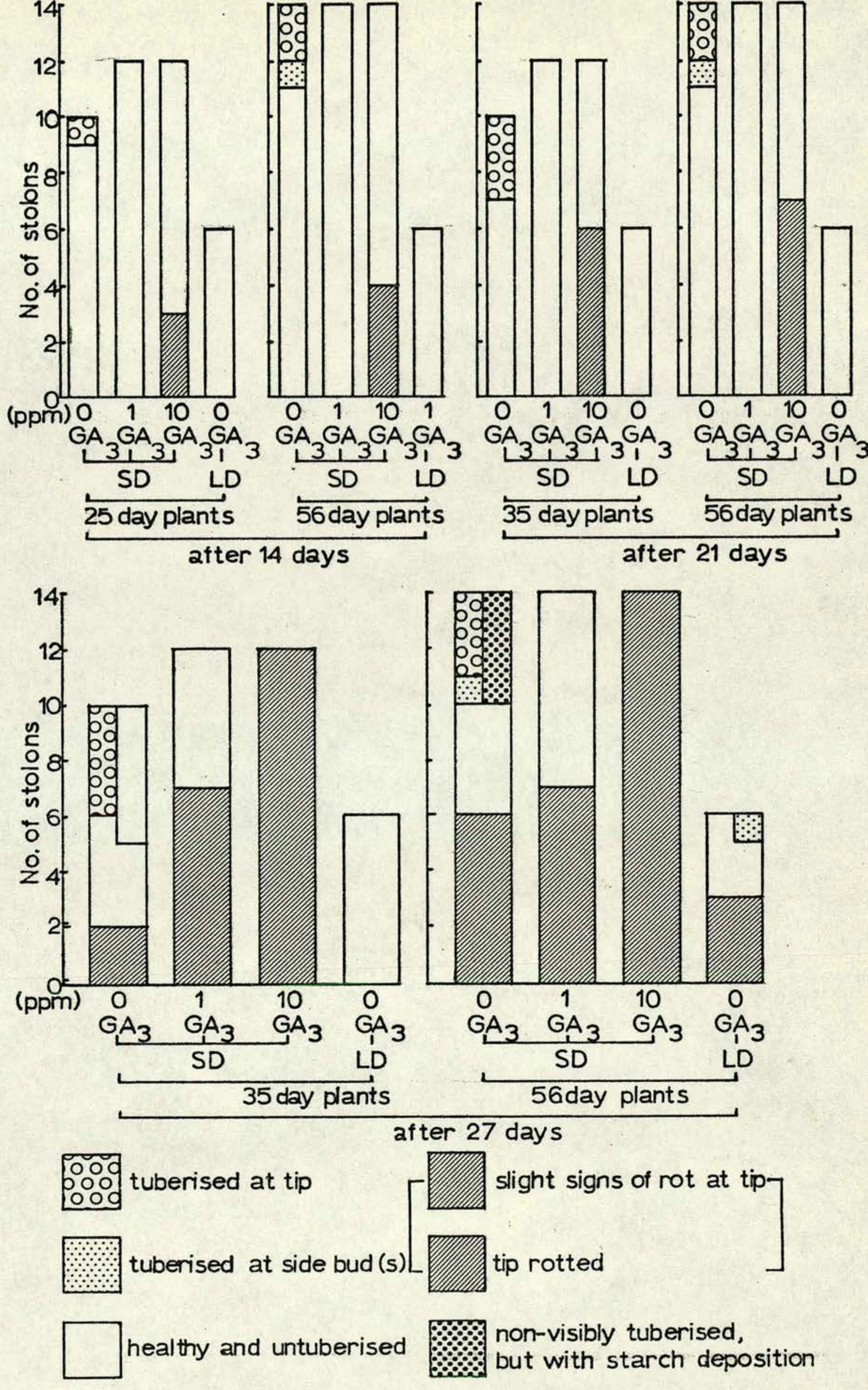


●—● total tuberisation]
○—○ tip only tuberised] SD, no GA_3, 56 day plants

□----□ tip only tuberised, 35 day plants, SD, no GA_3

H = histograms for these dates shown in Fig 25

<u>FIGURE 25</u>. Results of gibberellic acid stolon feeding experiment 2. Histograms showing tuberisation in treated stolons after 14, 21 and 27 days.



No. of stolons
(ppm)
0
1
10
GA3
SD
LD
25 day plants
56 day plants
after 14 days
35 day plants
56 day plants
after 21 days
35 day plants
56 day plants
after 27 days
tuberised at tip
tuberised at side bud (s)
healthy and untuberised
slight signs of rot at tip
tip rotted
non-visibly tuberised, but with starch deposition

TABLE 26. Results of second GA_3 stolon feeding experiment. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 27 days.

Treatment	35 day plants						56 day plants					
	LDC	SDC	SD + 1ppm GA_3	SD + 10 ppm GA_3	S.A.		LDC	SDC	SD + 1ppm GA_3	SD + 10 ppm GA_3	S.A.	
					p	LSD					p	LSD
Top ht.(cm)	45.1	28.9	25.8	23.3	ns		54.7	40.2	40.9	38.5	ns	
Node no.	24.5	19.3	17.9	19.0	ns		24.0	20.0	19.8	20.3	ns	
Top dwt.(g)	2.13	1.96	1.57	2.12	0.05	0.37	2.06	2.51	2.28	2.24	ns	
Stolon no.	6.0	7.2	4.8	4.7	ns		5.0	6.1	7.9	6.6	ns	
Tuber no.	0	7.3	3.7	5.8	ns		0	4.4	4.6	6.1	ns	
Tub. stolon no.	0	4.7	3.0	3.5	ns		0	4.4	3.8	3.8	ns	
Replication	3	6	6	6	-		3	7	7	7	-	

For abbreviations, see Section VIII.

to those given gibberellic acid in the experiments in Group A, and the effect on stolon growth showed that the compound was absorbed. The fact that no effect on top growth was found, therefore, suggests that gibberellic acid was not translocated to the main body of the plant from the treated stolons, and therefore that any effect on tuberisation was being exerted at the stolon tip itself, and not mediated by a response of the tops; the latter mode of action remained a possibility from the results of the gibberellic acid experiments in Group A (Section V (ii)).

As in the previous experiments, gibberellic acid treatment appeared to inhibit tuberisation of treated stolons (Figs 24 and 25). Fig 24 shows the progress of tuberisation with time in the treated stolons of the short day controls, beginning at day 8 in the 56 day old plants with a swollen side bud, and followed at day 10 by the first swollen tip, again on a 56 day old plant. No side buds tuberised in the 35 day old short day controls. The level of tuberisation in these plants was finally higher than that in the 56 day old plants (although it began earlier,as expected, in the latter) probably due to the higher level of rot in the 56 day old short day controls (Fig 25). When tuberisation began in the short day controls of the 35 day old plants (at day 14), there were no signs of tuberisation on any of the gibberellic acid treated stolons, and a little rot had begun with the highest level (10 ppm) of gibberellic acid. By 21 days (see Fig 25) there was still no tuberisation in gibberellic acid treated stolons although there was no rotting in those given the lower concentration (1 ppm). By 27 days (final harvest), signs of rot had begun in the latter and also

in the short day controls; there was still no tuberisation in any gibberellic acid treated stolons.

Starch deposition was examined at the final harvest, after 27 days (see Section VI). Starch deposition was well advanced, as expected, in the visibly tuberised stolons of the short day controls, and also in one non-visibly tuberised stolon in these, and in the long day controls (Fig 25), but no starch had yet been laid down in any of the 1 ppm gibberellic acid treated stolons; it was not possible to test the badly rotted stolons given 10 ppm gibberellic acid. It therefore seems that even the first stages of tuberisation had not occurred in the gibberellic acid treated stolons, even after almost 4 weeks; these stolons again showed the elongated appearance noted in experiment 1.

These results again show a delaying effect of gibberellic acid treatment on tuberisation when applied directly to stolon tips, and again the effect does not appear to be mediated by an effect on top growth. The results are in agreement with those of McCorquodale and Moorby (1968) with stolon tip cultured *in vitro* (see Section V (i)); these workers also concluded that gibberellic acid exerts its effect on tuberisation at the stolon tip itself.

It remains unclear, however, whether the gibberellins causing delay in tuberisation come entirely from other parts of the plants, or whether some gibberellin synthesis, enough to affect tuberisation, occurs in the stolon itself.

First feeding experiment with CCC

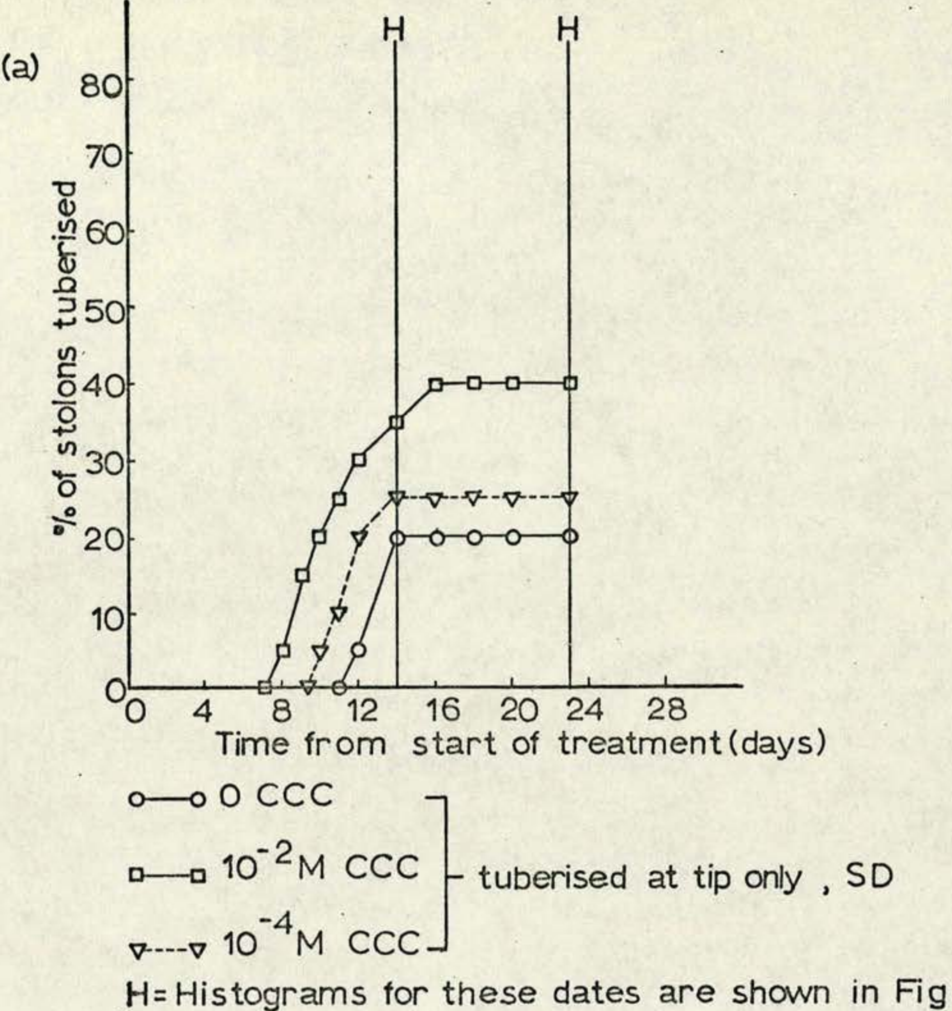
The purpose of this investigation was to discover whether inhibition of gibberellin synthesis in the stolon had any effect on tuberisation. The CCC experiments in Section V (ii) were incapable of resolving this question because, since CCC was applied as a soil drench, it could have been absorbed by both roots and stolons. If CCC applied to certain stolons only has no effect on top growth or the tuberisation of untreated stolons, but at the same time causes a promotion of tuberisation in the treated stolons, this would suggest that gibberellin synthesis in the individual stolons plays some part in the control of tuberisation. If, on the other hand, no effect of CCC is observed either on top growth or on tuberisation of treated stolons, this result, taken together with the promotive effect of CCC in the experiments of Group A (Section V (i)), would suggest that no appreciable gibberellin synthesis takes place in the stolon tip, and that the gibberellins concerned in the delay of tuberisation are all formed elsewhere in the plant and transported to the stolons, where they exert their effect.

Details of the present experiment are given in Table 25 and the results are presented in Figs 26 and 27 and Table 27. CCC treatment had no effect on top growth (Table 27) as measured by top height, node number and top dry weight (in contrast to the experiments of Group A, where these parameters were reduced by CCC). This may have been because the total supply of CCC to the plants in the present experiment was small compared to that used in the experiments in Group A. Tuberisation of untreated stolons was also unaffected (Table 27).

FIGURE 26. Results of CCC stolon feeding experiment 1. Graphs of tuberisation in treated stolons (%) with time.

a. stolons tuberised at tip only

b. total tuberisation: stolons tuberised at tip or at side bud(s).


(a)
H
H
80
70
60
50
40
30
20
10
0
% of stolons tuberised
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 CCC
10^{-2}M CCC
10^{-4}M CCC
tuberised at tip only, SD
H = Histograms for these dates are shown in Fig 27

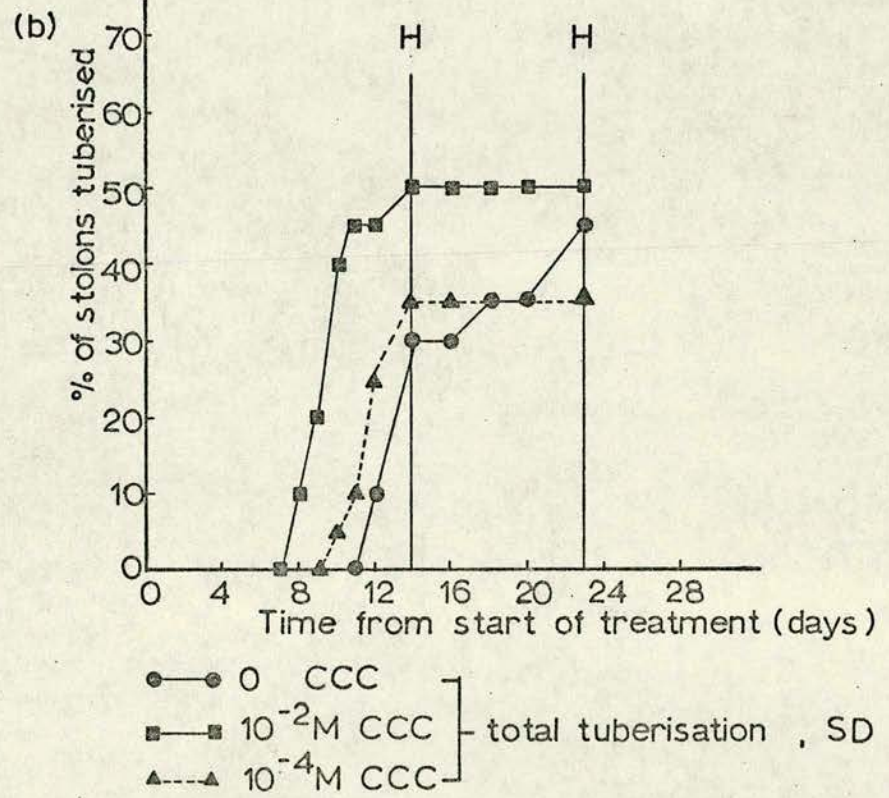
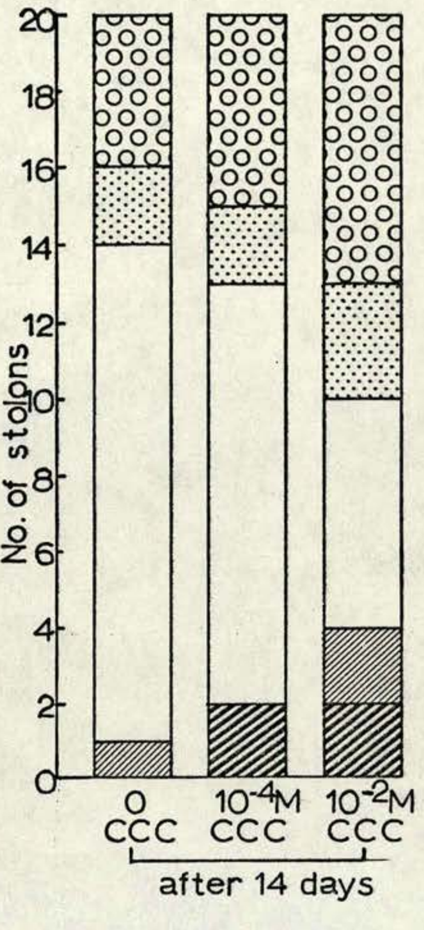
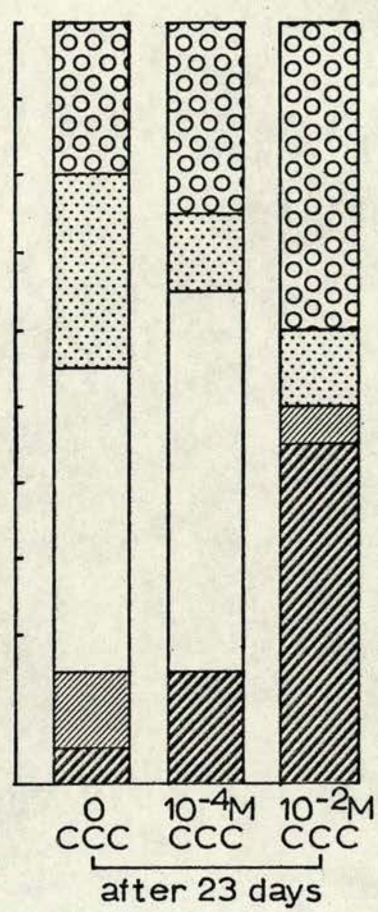

(b)
H
H
70
60
50
40
30
20
10
0
% of stolons tuberised
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 CCC
10^{-2}M CCC
10^{-4}M CCC
total tuberisation, SD

FIGURE 27. Results of CCC stolon feeding experiment 1. Histograms showing tuberisation in treated stolons after 14 and 23 days.





tuberised at tip

tuberised at side bud (s)

healthy and untuberised

slight signs of rot at tip

tip rotted

TABLE 27. Results of first CCC stolon feeding experiment. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 23 days.

Treatment	SDC	SD+10^{-4}MCCC	SD+10^{-2}MCCC	S.A.	
				p	LSD
Top ht.(cm)	51.8	47.9	49.5	ns	
Node No.	19.8	19.4	20.9	ns	
Top dwt.(g)	3.11	2.86	3.12	ns	
Tuber No.	6.9	7.0	7.8	ns	
Tuber fwt.	20.1	17.9	22.9	ns	
Replication	10	10	10	-	

For abbreviations, see Section VIII.

CCC treatment did, however, appear to cause a promotion of tuberisation in treated stolons. The progress of tuberisation at the tip alone and of total tuberisation (at either a side bud or the tip) for the various treatments is shown in Fig 26 (a) and (b) respectively. There is little difference between the two types of curve, although higher tuberisation is shown in the latter group (b). This probably only means that in some stolons (those which during the experiment had developed side branches) tuberisation took place at a side bud before doing so at the tip (as expected from the results of the study on tuberisation in different types of stolons described in Section III).

Both sets of curves showed that CCC brought about a hastening of tuberisation, the effect being small although fairly consistent at 10^{-4}M and more pronounced at 10^{-2}M. The only inconsistency in the effect of CCC was found between 20 and 23 days in the curves shown in Fig 26 (b), where there was a higher level of tuberisation in the water controls than in the stolons given 10^{-4}M CCC. It appears that this result was due to a higher number of water controls beginning to tuberise at a side bud after day 16. This may have occurred because of the higher number of stolons remaining both untuberised and without signs of rot up to this time in this group of stolons compared to the other treatments. Generally, however, the increases in tuberisation with CCC were found despite the higher level of rotting in CCC treated stolons than in water controls (see Fig 27).

Since CCC was found to cause no effect on top growth (which would have been expected if CCC were being transported there from the treated stolons and inhibiting gibberellin synthesis in the shoot

apex and young leaves), and since there was no effect of CCC on untreated stolons, it appears that the effect of CCC on tuberisation in treated stolons was a local one exerted on the treated stolon tips themselves. While these results show that gibberellin synthesis in the stolon tip contributes to the control of tuberisation, it is likely that synthesis in many parts of the plant is normally involved. When the level of gibberellin in the stolon tip falls, it appears that tuberisation is favoured (see also Section V (ii) and (iv)). In the present experiment, there will have been a period after the start of CCC treatment when the gibberellin present in the tip before treatment had been used up by extension growth of the stolon, but no replacement supplies will have reached the tip from the rest of the plant. During this time, it is envisaged that conditions may have been suitable (perhaps due to the lowering of a gibberellin/growth inhibitor ratio - see Section VII) for tuber initiation.

Second feeding experiment with CCC

This experiment was similar to the previous one, except that stolons on plants grown in long days during the experimental period were also given CCC treatment to determine whether a promotion of tuberisation would result in such stolons also. Details of the experiment are given in Table 25 and the results are shown in Figs 28 and 29 and in Table 28.

As in the previous experiment, CCC treatment had no effect on top growth or tuberisation of untreated stolons (Table 28). Also as before, CCC caused a promotion of tuberisation in treated stolons on plants grown in short days. Graphs for tuberisation at the tip alone and total tuberisation were again similar (Fig 28);

FIGURE 28. Results of CCC stolon feeding experiment 2. Graphs of tuberisation in treated stolons (%) with time.

a. Stolons tuberised at tip only.

b. total tuberisation: stolons tuberised at tip or at side bud(s).

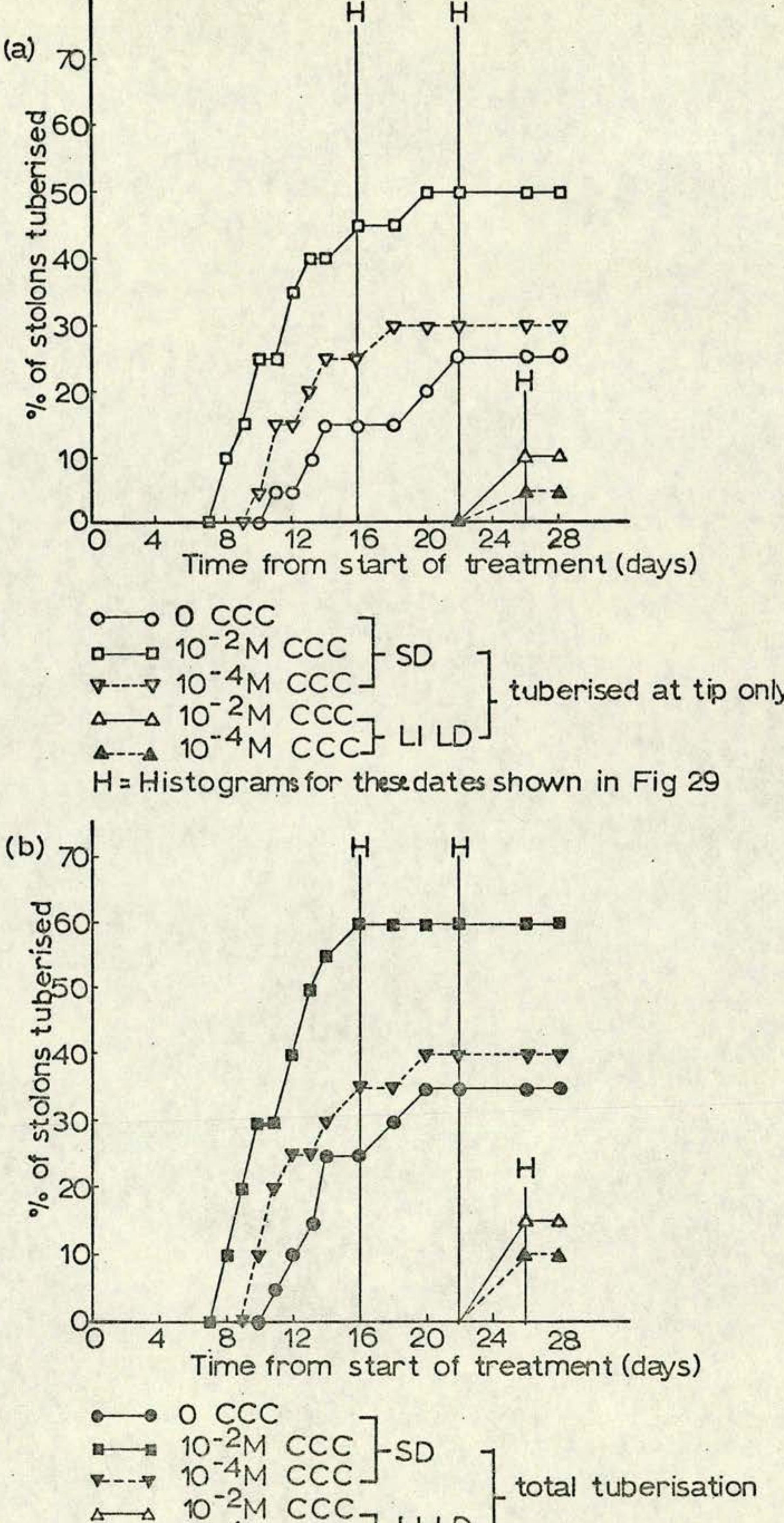
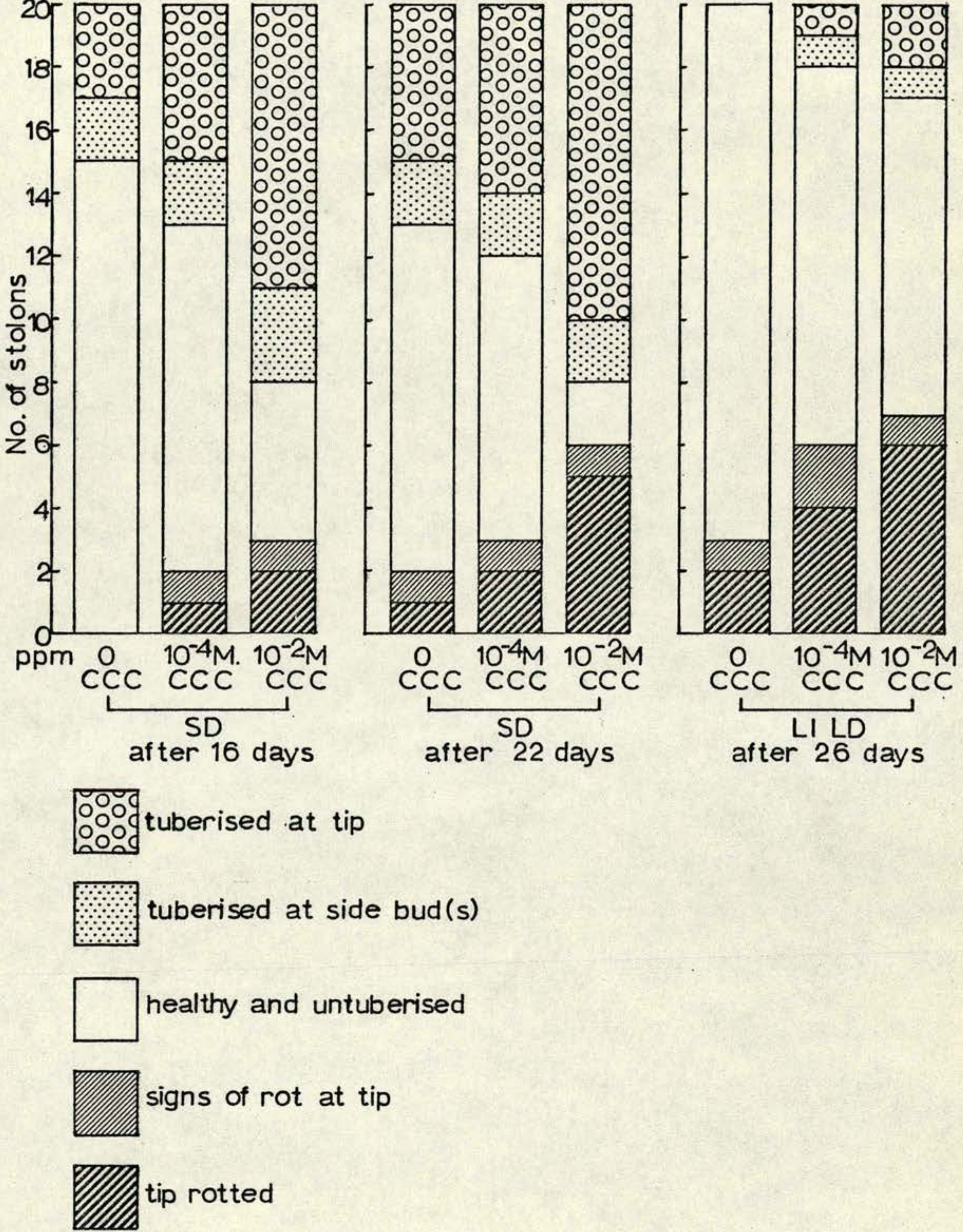

(a)
H
H
H
% of stolons tuberised
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 CCC
10^-2M CCC
10^-4M CCC
SD
10^-2M CCC
10^-4M CCC
LI LD
tuberised at tip only
H = Histograms for these dates shown in Fig 29
(b)
H
H
H
% of stolons tuberised
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 CCC
10^-2M CCC
10^-4M CCC
SD
10^-2M CCC
10^-4M CCC
LI LD
total tuberisation

FIGURE 29. Results of CCC stolon feeding experiment 2. Histograms showing tuberisation in treated stolons after 16, 22 and 26 days.



No. of stolons
20
18
16
14
12
10
8
6
4
2
0
ppm
0
CCC
10⁻⁴M.
CCC
10⁻²M
CCC
SD
after 16 days
0
CCC
10⁻⁴M
CCC
10⁻²M
CCC
SD
after 22 days
0
CCC
10⁻⁴M
CCC
10⁻²M
CCC
LI LD
after 26 days
tuberised at tip
tuberised at side bud(s)
healthy and untuberised
signs of rot at tip
tip rotted

TABLE 28. Results of second CCC stolon feeding experiment. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 28 days.

Treatment	LI LD Plants					SD plants				
	OCCC	10^{-4}MCCC	10^{-2}MCCC	S.A.		OCCC	10^{-4}MCCC	10^{-2}MCCC	S.A.	
				p	LSD				p	LSD
Top ht.(cm)	48.9	45.1	48.1	ns		34.2	36.1	36.9	ns	
Node No.	17.8	18.0	18.5	ns		16.8	16.2	16.6	ns	
Top dwt.(g)	2.51	2.43	2.49	ns		2.15	2.24	2.30	ns	
Stolon No.	5.6	4.4	4.7	ns		4.8	3.9	4.7	ns	
Tuber No.	0	0.2	0	ns		3.3	2.8	2.8	ns	
Tuber fwt.(g)	0	0.02	0	ns		9.3	8.6	9.0	ns	
Replication	10	10	10	-		10	10	10	-	

For abbreviations, see Section VIII.

few side buds tuberised compared to the number of tuberised tips, perhaps because stolons had few branches. Again as before, there was a small but consistent promotion with 10^{-4}M and a larger one with 10^{-2}M CCC. Although rotting was not great, the level was somewhat higher with CCC as in the first experiment, especially at the higher concentration (Fig 29). In the case of the plants grown in long days, tuberisation of treated stolons was also promoted by CCC, although the numbers of tuberised tips were very small, even after 28 days (Figs 28 and 29).

These results show good agreement with and extend those of the first experiment, pointing to a direct effect of CCC on gibberellin synthesis in the stolon bringing about a promotion of tuberisation, even in non-inductive long days.

McCorquodale and Moorby (1968) did not find this result with their stolon tip cultures. With low concentrations of CCC, very little effect was observed, and at higher concentration (10^{-2}M) they found an inhibition of tuberisation, which they attributed to a toxicity effect. They concluded that the effect of CCC on whole plants was exerted not in the stolons but on the rest of the plant, reducing the supply of gibberellin to the stolons from elsewhere in the plant. Palmer and Smith (1969 b) also found that CCC inhibited stolon growth but did not cause initiation of tubers in the absence of kinetin; kinetin, however, is probably necessary for tuberisation to occur (see below and Section VII), so that these results are not really comparable with those of the present experiments,

or with those of McCorquodale and Moorby, who included kinetin (1 x 10^{-3} ppm), among other compounds, in their basal growing medium.

It is more difficult to explain the differences between the results of the present experiments and those of McCorquodale and Moorby. It is possible, however, that the gibberellin level in their material may have been very low due to environmental or culture conditions, so that inhibition of gibberellin synthesis by CCC had little effect on tuberisation. The stolons used in the present experiments may have been growing more rapidly, since they were in a more natural condition than excised stolons. This more rapid growth would probably be associated with greater gibberellin synthesis, and the contribution of this gibberellin in delaying tuberisation in relation to that of gibberellin which had been sent from other parts of the plant might be expected to be greater; inhibition of gibberellin synthesis would therefore have more effect in this system.

Whatever the explanation for the above differences, it appears from the results of the present experiments that some gibberellin synthesis does take place in the stolon tip and that this gibberellin, probably acting together with gibberellin from elsewhere in the plant, exerts a delaying effect on tuberisation.

Feeding experiment with abscisic acid

Having demonstrated an involvement of gibberellic acid and CCC directly at the stolon tip similar to that found when these compounds were applied to the whole plant (Section V (ii)), it was desirable to discover whether such a parallel effect would

also be found with abscisic acid. Details of the experiment are given in Table 25, and the results in Figs 30 and 31 and Table 29.

There was no effect of abscisic acid on top height or node number, except for a small increase in top height (at 1 ppm) which was not thought to be a real effect, and which may have been due to two of the plants in the treatment being especially tall. There was a reduction in top dry weight with 0.5 ppm abscisic acid, but not with 1 ppm. Any effects on top growth were therefore small and inconsistent and probably unreal. Tuberisation of untreated stolons was also unaffected by abscisic acid.

In the treated stolons in short days abscisic acid appeared to have very little effect on tuberisation at the lower concentration, and an inhibitory effect at the higher concentration. After 16 days, stolons given 1 ppm abscisic acid showed more rotting than the controls, but the inhibitory effect of abscisic acid on tuberisation was visible after 12 days, at which time rotting was slightly less among the abscisic acid treated stolons than among the controls (Fig 31). The inhibition could not, therefore, have been merely due to a greater number of rotted stolons among those treated with abscisic acid.

Tuberisation at the tip alone (Fig 30 (a)) and total tuberisation (tip and side buds, Fig 30 (b)) showed much the same sort of progress in stolons from short days. In both cases, there was little difference between the curves for the water controls and for 0.5 ppm abscisic acid. It appears however that, although the lower concentration (0.5 ppm) had no effect on tuberisation

FIGURE 30. Results of abscisic acid stolon feeding experiment. Graphs of tuberisation in treated stolons (%) with time.

a. stolons tuberised at tip only

b. total tuberisation: stolons tuberised at tip or at side bud(s).

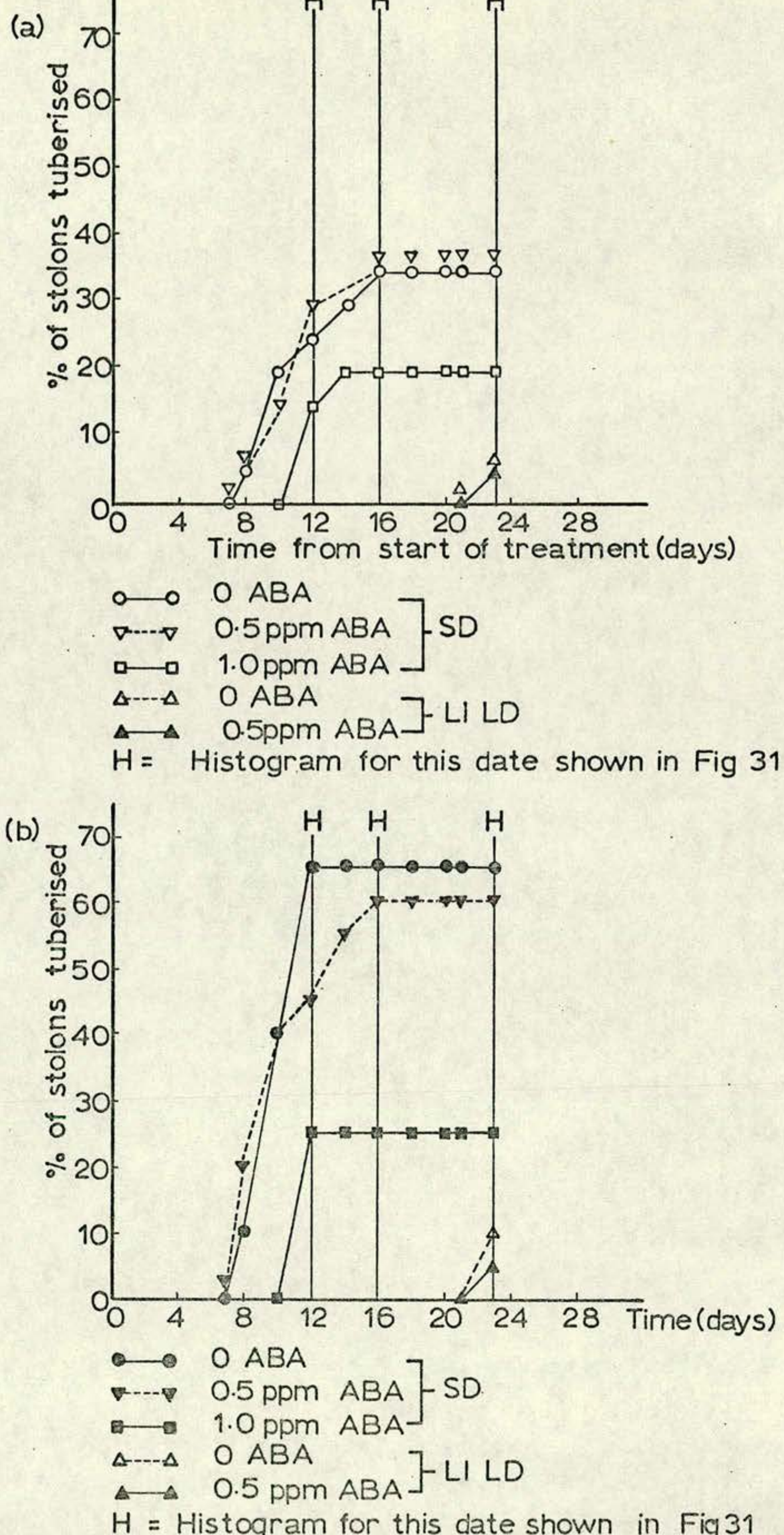
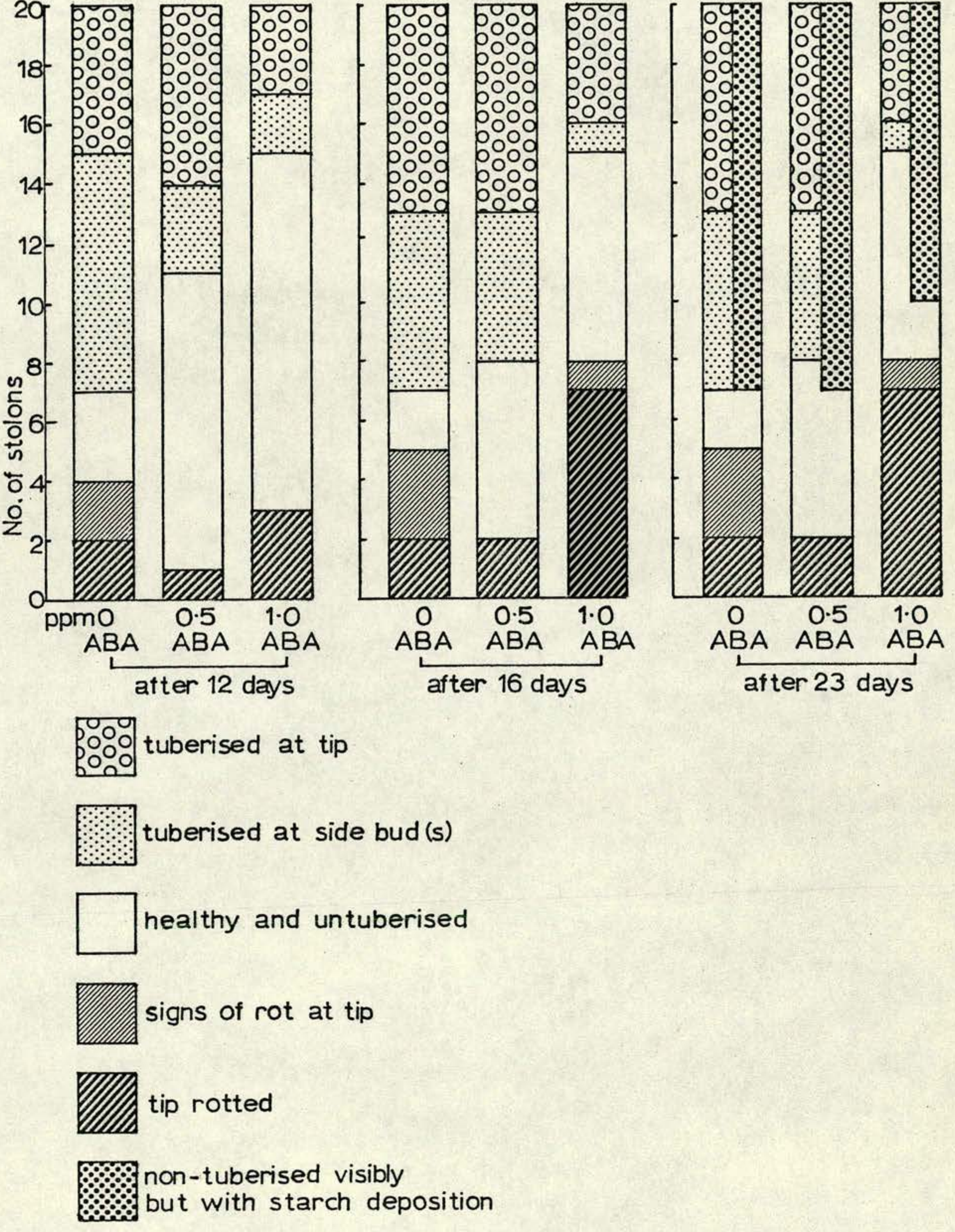

(a)
H
H
H
% of stolons tuberised
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 ABA
0·5 ppm ABA
1·0 ppm ABA
SD
0 ABA
0·5ppm ABA
LI LD
H = Histogram for this date shown in Fig 31
(b)
H
H
H
% of stolons tuberised
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
Time (days)
0 ABA
0·5 ppm ABA
1·0 ppm ABA
SD
0 ABA
0·5 ppm ABA
LI LD
H = Histogram for this date shown in Fig 31

FIGURE 31. Results of abscisic acid stolon feeding experiment. Histograms showing tuberisation in treated stolons after 12, 16 and 23 days.



No. of stolons
0
2
4
6
8
10
12
14
16
18
20
ppm 0 ABA
0·5 ABA
1·0 ABA
after 12 days
0 ABA
0·5 ABA
1·0 ABA
after 16 days
0 ABA
0·5 ABA
1·0 ABA
after 23 days
tuberised at tip
tuberised at side bud(s)
healthy and untuberised
signs of rot at tip
tip rotted
non-tuberised visibly
but with starch deposition

TABLE 29. Results of ABA stolon feeding experiment.. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 23 days.

Treatment	SDC	SD+0.5 ppm ABA	SD+1.0 ppm ABA	SA.	
				p	LSD
Top ht.(cm)	54.6	54.3	58.9	0.05	3.29
Node No.	20.7	20.3	20.1	ns	
Top dwt.(g)	3.15	2.53	3.01	0.01	0.32
Tuber No.	6.6	5.1	5.4	ns	
Tuber fwt.(g)	18.8	15.8	18.3	ns	
Replication	10	10	10	-	

For abbreviations, see Section VIII

at the tip, it did reduce tuberisation at side buds after 9 days (Fig 30 (b)). It is possible that a difference in the rate of cell division, or in some other factor, between tips and side buds, results in abscisic acid being more effective in inhibiting tuberisation at side buds than at tips. Both sets of curves however (Fig 30 (a) and (b)) show an inhibition of tuberisation with 1 ppm abscisic acid; this is more marked in the total tuberisation curves (Fig 30 (b)), showing that the effect is felt on tuberisation both at the tip and at side buds; once again, the effect appears to be slightly more marked on side buds than on the tip.

At the final harvest after 23 days, when starch deposition in treated stolons was recorded, there appeared to be less inhibitory effect of 1 ppm abscisic acid on starch deposition than on the appearance of visible tubers in stolons from short days, especially when the higher level of rotting in the stolons given 1 ppm abscisic acid is considered (Fig 31). This suggests that the inhibitory effect of the higher concentration of abscisic acid on tuberisation was due principally to an effect on some part of the process other than starch deposition, perhaps cell division (see Section VII).

Very little tuberisation had taken place in the stolons on plants from long days, but there was again no evidence for a promotion of tuberisation by abscisic acid. Two tubers were found in the controls, one with 0.5 ppm and none with 1 ppm abscisic acid (Figs 30 and 31).

These results are in general agreement with those of McCorquodale

and Moorby (1968) with stolon tip cultures _in vitro_; they found, using concentrations of abscisic acid up to 1 ppm in the growth medium, that low concentrations had little effect and that 1 ppm inhibited tuberisation. Abscisic acid stopped stolon growth, although the stolons appeared normal and resumed growth when returned to the basal medium. The results are also in agreement with those of Smith and Rappaport (1969) from an experiment in which stolon tips of plants grown in solution culture under long days were wrapped in cotton saturated with a solution of abscisic acid (1 ppm) on alternate days for 3 weeks. They found no promotive effect on treated stolons or on untreated stolons on the same plant. The only difference between the results of the present experiment (and also those of McCorquodale and Moorby) and those of Smith and Rappaport is that the latter found no _inhibition_ of tuberisation with abscisic acid treatment,

Palmer and Smith (1969 b) have also found, with stolon tips _in vitro_, that abscisic acid at concentrations from 7.5×10^{-4} mM to 7.5×10^{-2} mM, and in the absence of kinetin, inhibited stolon elongation but no tubers were formed. In the presence of 1.6×10^{-2} mM kinetin, the effect of abscisic acid on stolon elongation and tuberisation was less marked; concentrations of 7.5×10^{-4} and 7.5×10^{-3} mM failed to inhibit "the kinetin-induced tuber formation", whereas a concentration of 3.0×10^{-2} mM markedly inhibited tuber initiation.

The medium used by McCorquodale and Moorby (1968), as mentioned above, contained kinetin as well as abscisic acid, so that their results are comparable with those of Palmer and Smith (1969 b) with kinetin and abscisic acid together, as are those of the

present experiment if it is presumed, as seems likely, that the attached stolons received from the parent plant whatever cytokinins they required throughout the experimental period.

Thus in all the systems used, abscisic acid had either no effect or an inhibitory effect on stolon growth and tuber initiation when applied directly to stolons. It appears, therefore, that the promotion of tuberisation by abscisic acid, if this does in fact normally occur, does not take place directly at the stolon tip, and must be brought about by some effect exerted on the tops. The possible mode of action of abscisic acid and its possible interations with gibberellins and cytokinins are discussed in Section VII.

First feeding experiment with kinetin

Details of the experiment are given in Table 25 and the results are presented in Figs 32 and 33 and Table 30. Kinetin had no effect on either top growth of tuberisation of untreated stolons (Table 30). In the treated stolons, there was very little effect on the total number of stolons tuberised at either the tip or a side bud (Figs 33 and 32 (b)). 10^{-6}M kinetin had very little effect on tuberisation at the tip (Fig 32 (a)), but the higher concentrations (10^{-4} and 10^{-5}M) had an inhibitory effect, almost from the beginning of tuber initiation. This appears to be the opposite situation from that found with abscisic acid treatment, with which the inhibition of tuberisation was more marked at the side buds than at the tip. There was very little effect of any concentration of kinetin treatment on rotting of the stolons (Fig 33).

FIGURE 32. Results of kinetin stolon feeding experiment 1.
Graphs of tuberisation in treated stolons (%) with time.
a. stolons tuberised at tip only.
b. total tuberisation: stolons tuberised at tip or at side bud(s).

(a)

% of stolons tuberised

Time from start of treatment (days)

○—○ 0 KIN
▽----▽ 10^{-6}M KIN
□—□ 10^{-5}M KIN
△—△ 10^{-4}M KIN

SD, tuberised at tip only

H = Histogram for this date shown in Fig 33

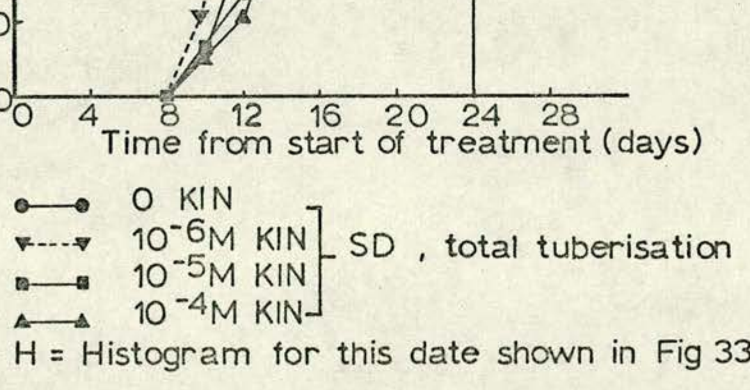
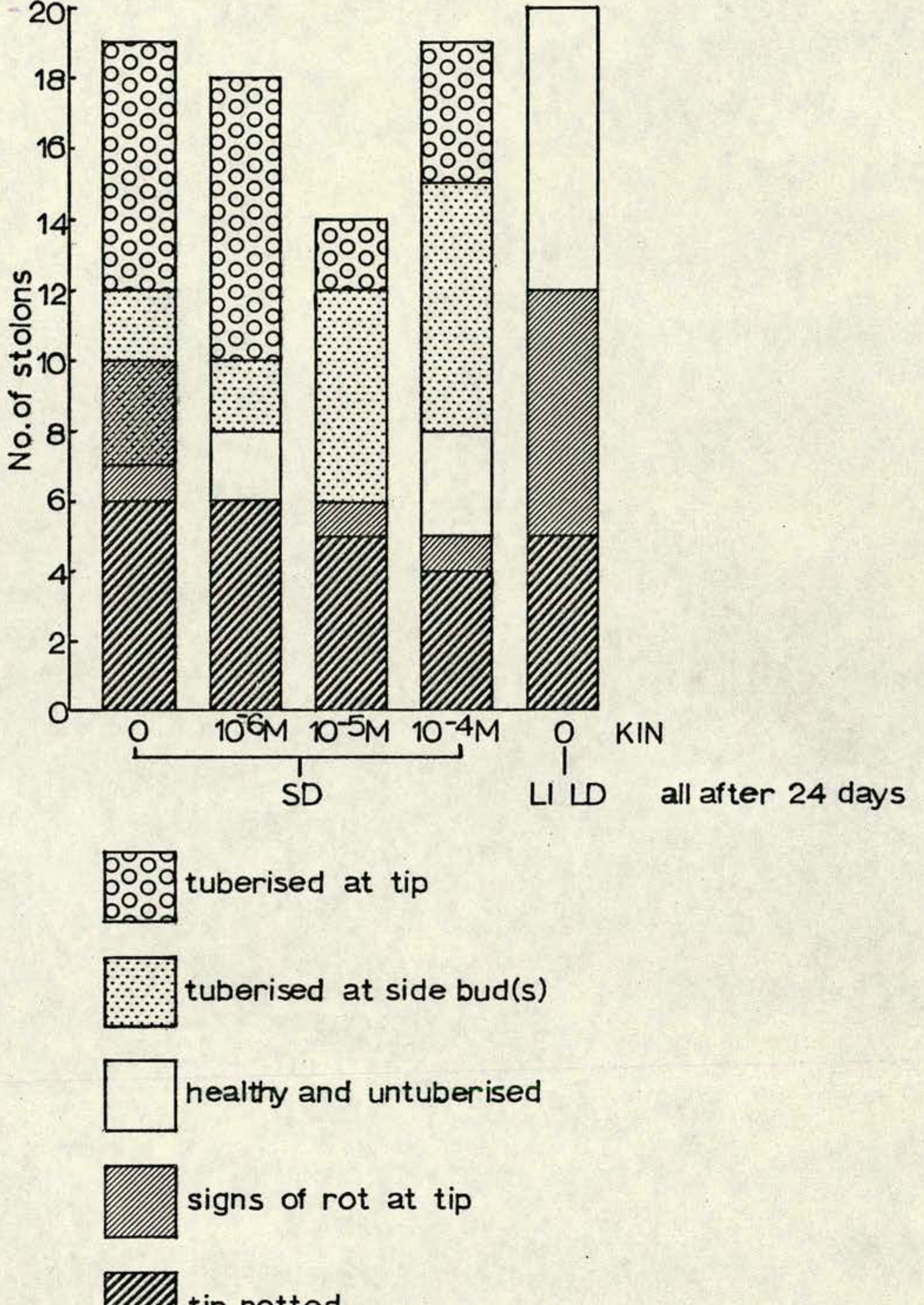


FIGURE 33. Results of kinetin stolon feeding experiment 1. Histograms showing tuberisation in treated stolons after 24 days.



No. of stolons
20
18
16
14
12
10
8
6
4
2
0
0
10⁻⁶M
10⁻⁵M
10⁻⁴M
0
KIN
SD
LI LD
all after 24 days
tuberised at tip
tuberised at side bud(s)
healthy and untuberised
signs of rot at tip
tip rotted

TABLE 30. Results of first KIN stolon feeding experiment. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 24 days.

Treatment	LDC	SD				S.A.	
		0 KIN	10^{-6}M KIN	10^{-5}M KIN	10^{-4}M KIN	p	LSD
Top ht.(cm)	67.4	38.9	38.1	35.1	34.2	ns	
Node No.	22.8	16.2	16.6	15.8	16.0	ns	
Top dwt.(g)	2.60	1.90	1.86	1.67	1.77	ns	
Stolon No.	2.1	3.0	3.7	4.7	3.6	ns	
Tuber No.	0	2.6	2.8	3.3	2.8	ns	
Tub. stolon No.	0	2.3	2.5	3.0	2.6	ns	
Tuber fwt.(g)	0	9.0	8.6	9.2	9.3	ns	
Replication	10	10	10	10	10	-	

For abbreviations see Section VIII.

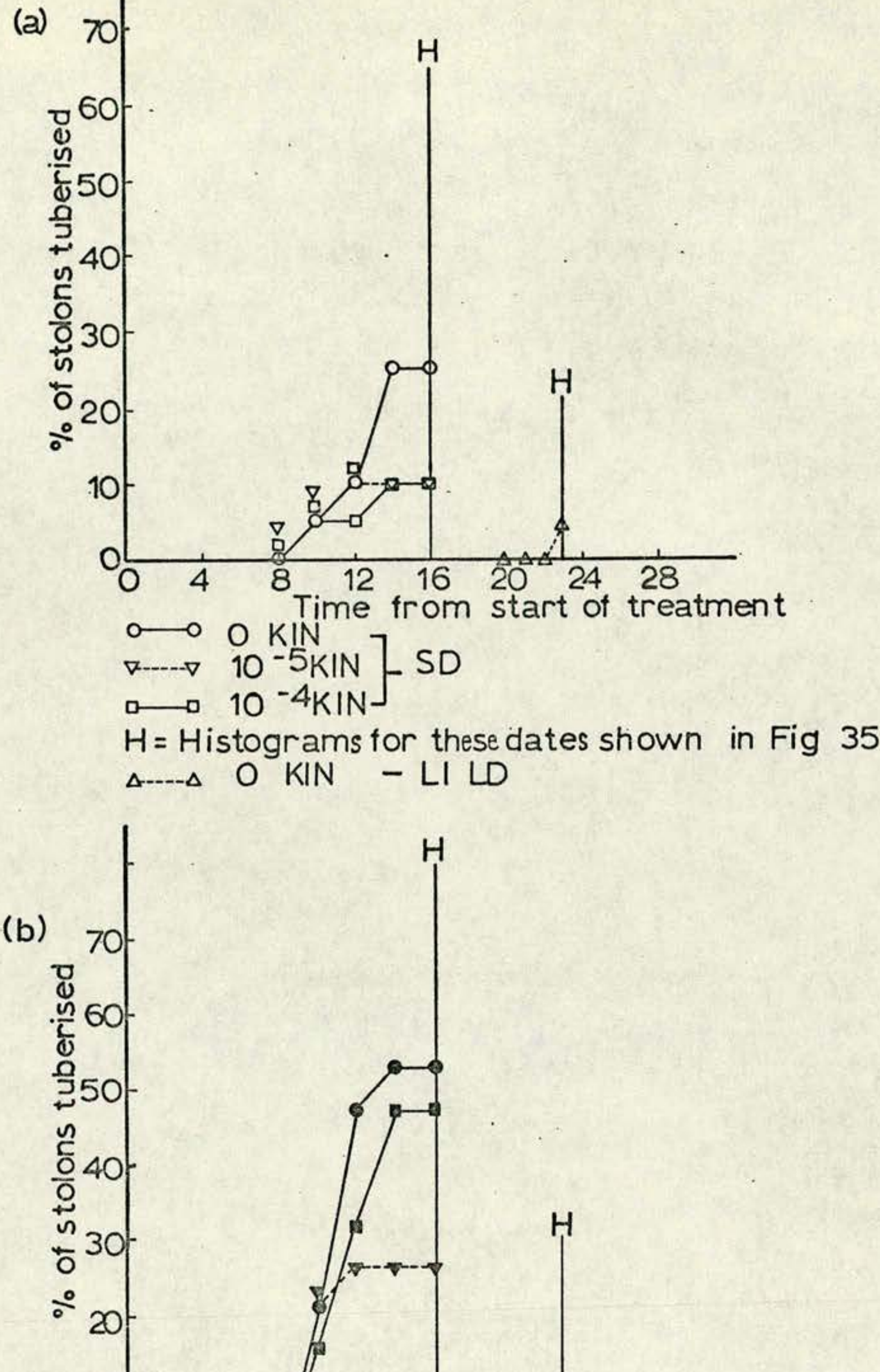
Second feeding experiment with kinetin

In this experiment, plants grown in long days as well as plants grown in short days during the experimental period were treated, to determine whether promotion of tuberisation could be detected in long days if it could not be detected in short days (see above). The details of the experiment are given in Table 25 and the results in Figs 34 and 35 and Table 31.

For the plants grown in short days, there was no effect of kinetin treatment on growth of tops or tuberisation of untreated stolons, as in the first experiment. At the time of the final harvest of the plants from short days, there was very little effect of kinetin treatment on total tuberisation of treated stolons (see Fig 35).

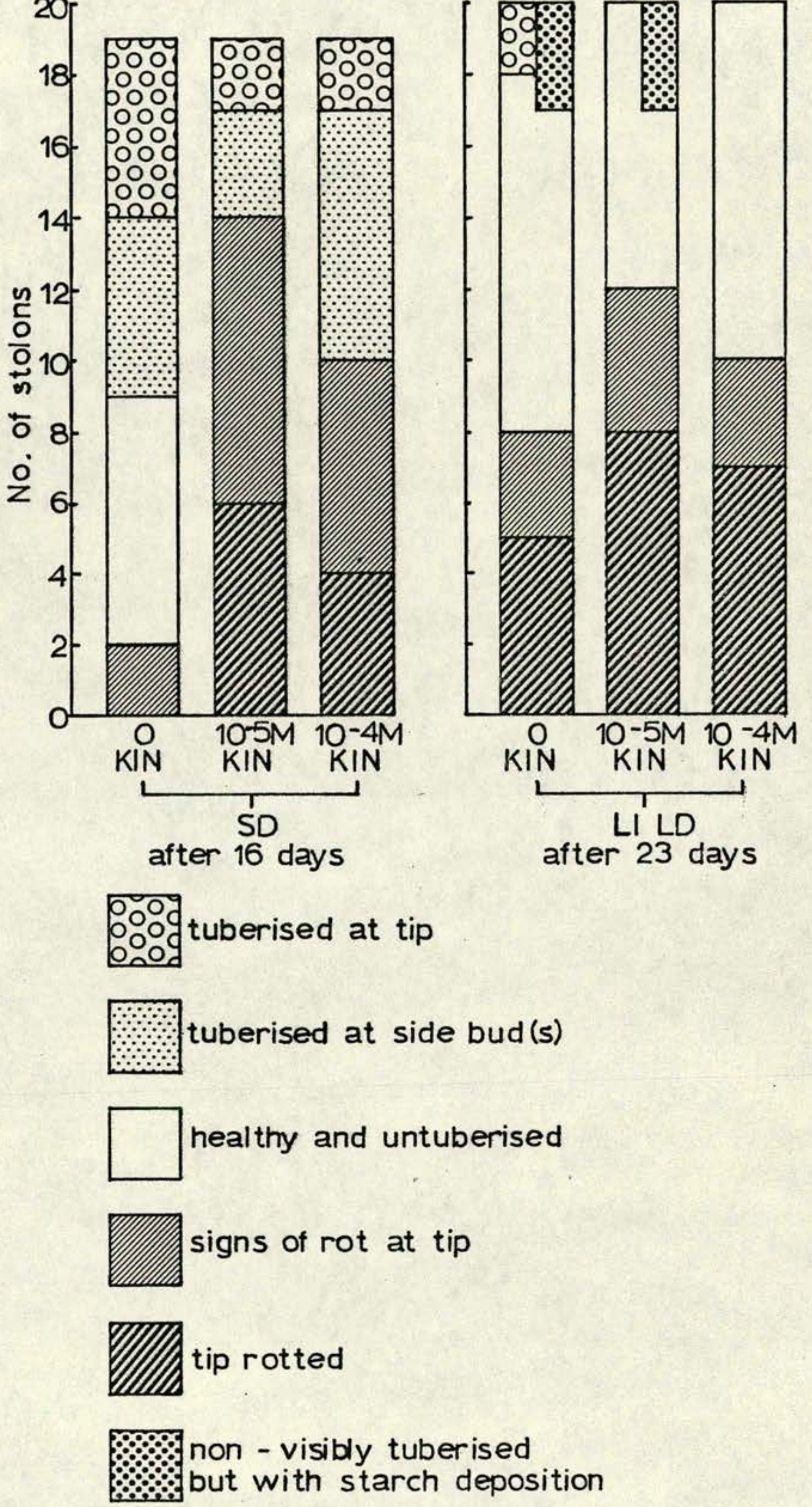
Fig 34 shows an inhibition of both total tuberisation (Fig 34 (b)) and tuberisation at the tip alone (Fig 34 (a)), especially, in the case of total tuberisation, with 10^{-5}M kinetin. This effect may have been due to a higher level of rotting in these stolons (see Fig 35); that this was the case is supported by the fact that few new tubers were formed after 10 days with 10^{-5}M kinetin treatment, although tubers continued to be formed for a longer period in the other treatments. Except for this anomalous result for total tuberisation with 10^{-5}M kinetin, the results tend in the same direction as those of the previous experiment, total tuberisation being little affected, and tuberisation at the tip alone being inhibited by 10^{-5} and 10^{-4}M kinetin. Tuberisation appeared to have been "diverted" from the tip to the to the side buds. This may possibly have been caused by the accumulation of much higher levelsof kinetin at the tip than at

FIGURE 34. Results of kinetin stolon feeding experiment 2.
Graphs of tuberisation in treated stolons (%) with time.
a. stolons tuberised at tip only
b. total tuberisation: stolons tuberised at tip or at side bud(s).



(a)
% of stolons tuberised
70
60
50
40
30
20
10
0
H
H
0
4
8
12
16
20
24
28
Time from start of treatment
0 KIN
10 -5KIN
10 -4KIN
SD
H = Histograms for these dates shown in Fig 35
0 KIN - LI LD
(b)
% of stolons tuberised
70
60
50
40
30
20
10
0
H
H
0
4
8
12
16
20
24
28
Time from start of treatment (days)
0 KIN
10 -5KIN
10 -4KIN
SD
0 KIN — LI LD
H = Histograms for these dates shown in Fig 35

FIGURE 35. Results of kinetin stolon feeding experiment 2. Histograms showing tuberisation in treated stolons after 16 days (short day, SD, plants) and after 23 days (low intensity long day, LI LD, plants).



No. of stolons
20
18
16
14
12
10
8
6
4
2
0
0
KIN
10-5M
KIN
10-4M
KIN
SD
after 16 days
0
KIN
10-5M
KIN
10-4M
KIN
LI LD
after 23 days
tuberised at tip
tuberised at side bud(s)
healthy and untuberised
signs of rot at tip
tip rotted
non - visibly tuberised
but with starch deposition

TABLE 31. Results of second KIN stolon feeding experiment. Differences in top, stolon and tuber growth on untreated stolons at final harvest after 16 days (SD plants) and 23 days (LI LD plants).

Treatment	SD plants			S.A.		LI LD plants			S.A.	
	0 KIN	10^{-5} MKIN	10^{-4} MKIN	p	LSD	0 KIN	10^{-5} MKIN	10^{-4} MKIN	p	LSD
Top ht.(cm)	37.2	39.0	39.6	ns		-	-	-	-	
Node No.	17.8	18.5	18.7	ns		-	-	-	-	
Top dwt.(g)	2.33	2.48	2.46	ns		3.40	4.47	3.71	ns	
Stolon No.	4.4	4.6	4.6	ns		4.0	3.6	3.0	ns	
Tuber No.	3.6	3.7	3.4	ns		0.2	0	0	-	
Tub.stolon No.	3.4	3.1	2.5	ns		0.2	0	0	-	
Non-tub. stolons										
with starch	0.5	0.6	1.3	ns		0.1	0.5	0.1	-	
no starch	0.6	0.8	0.8	ns		0.4	3.0	2.9	ns	
Tuber fwt.(g)	3.82	2.91	3.99	ns		0.22	0	0	-	
Replication	10	10	10	-		10	10	10	-	

For abbreviations, see Section VIII.

side buds, the former being the more active sink, since it usually grows faster; these high levels, together with the normal supply of endogenous cytokinins, may have resulted in a toxicity effect at the tips.

Both experiments showed that, in short days, kinetin definitely had no promotive effect whatever on tuberisation.

In the case of plants grown in long days in the present experiment, no tuberisation was found in kinetin treated stolons, although 2 tubers were found in the controls, and another non-visibly tuberised tip had laid down starch (Figs 34 and 35). Three non-visibly tuberised tips showed starch deposits in the stolons given 10^{-5}M kinetin at the final harvest of plants from long days (after 23 days), but no starch deposition was found in any of those given 10^{-4}M kinetin. The long day results were therefore rather inconclusive, but once again provided no evidence of a detectable promotion of tuberisation by kinetin treatment such as was found by Palmer and Smith (1969 a, 1970).

It is possible that the difference between the results of the present experiments and those of Palmer and Smith is due to the fact that the results of the latter were obtained in isolated stolon tips grown on an artificial medium, whereas those of the present experiments were obtained with stolons still attached to the parent plant; there are two possible ways in which this could cause differences in results. Firstly, the cytokinin used in the present experiments may have been unable to penetrate the stolons, or to move to the site of action; penetration would be easier in excised stolons because of the broken surface following excision. However, cytokinins have been shown to

penetrate into leaves (eg Möthes, 1960), and requirements for movement would presumably be the same in both systems after penetration. Secondly, and perhaps more likely, the supply of endogenous cytokinins from the parent plant in the present experiment may have made extra cytokinin unnecessary in either daylength regime. It seems likely that cytokinins, among other substances such as carbohydrates, may be required for tuberisation, whether or not they are part of the tuberising stimulus (see Section V (i)). It would therefore be expected that tuberisation of isolated stolon tips *in vitro* would require the inclusion of one or more cytokinins in the growth medium, just as it requires a suitable sugar concentration in the medium (Mes and Menge 1954; Gregory, 1956).

The lack of promotive effect of kinetin in the present experiments suggests that, while cytokinins may be necessary for tuberisation, they are probably not one of the principal factors which control the process. This question is further discussed in Section VII.

(iv) Group C: Extraction and assay of endogenous gibberellin-like substances from stolon tips in various developmental states

a. Introduction

To show that endogenous gibberellins exert a delaying influence on tuberisation, it is necessary to demonstrate that their level in the plant varies in a way which is inversely correlated with the developmental state of the plant with respect to tuberisation, more gibberellin being present in plants without signs of tuberisation. Okazawa (1960) found higher gibberellin levels in leaves under non-inductive conditions (see above) but at the commencement of this work the only investigation carried out on growth substance levels in the stolon tip itself was that of Booth (1963), described above (V (i)). An investigation of the gibberellin content of stolon tips at various stages of development was therefore carried out to see if a relationship could be found between gibberellin level and tuberisation. The developmental state of non-green stolons (tuberised or non-tuberised) was at first estimated by whether visible swelling had or had not taken place in the sub-apical region of the tip; green stolons, which do not normally tuberise, were also included. Later, following the investigations described in Section VI, stolons which had not visibly tuberised were further divided according to the presence or absence of starch deposition.

b. Extraction and assay procedure

Stolon samples for extraction and assay were taken from harvests of other experiments, the samples being separated on the basis of their developmental state, estimated by one of the above methods, ie on visible swelling or starch deposition. Immediately after harvest, the stolon tip (terminal 1 cm) material was divided

into samples each consisting of one developmental category (except in the first comparison, see below). The samples, after being weighed, were freeze-dried overnight to constant weight with a vacuum freeze-drying apparatus, using a mixture of solid carbon dioxide and acetone as the coolant. They were then kept in sealed bottles at -20°C until assay.

Various methods for extraction and assay of gibberellins were investigated. Extractions were carried out using organic solvents, and also aqueous buffers as primary solvents; the latter have several advantages (see Jones, 1968). Extracts were further extracted with ethyl acetate, evaporated down and taken up in methanol.

Extraction and separation by thin layer silica gel chromatography (Zeevaart, 1966, with modifications by Dale and Felippe, 1968) was followed by assay by a lettuce hypocotyl bioassay (Frankland and Wareing, 1960, with minor variations by Dale and Felippe, 1968). No gibberellin activity was detected by the bioassay, which is sensitive to most gibberellins. Investigation showed some of the stages at which loss occurred, and improvements were made at appropriate points, but it was subsequently decided that another more sensitive bioassay procedure, for which such specific extracts of the material were not required, would be more suitable, especially for dealing with small samples of stolon tips. Difficulty caused by the small amounts of gibberellin present in stolons was found by McCorquodale and Moorby (1968), who bioassayed some of their cultured material but were unable to detect any gibberellin activity.

Further trials were therefore carried out using the more sensitive bioassay procedure, the barley half-seed α-amylase bioassay (Jones and Varner, 1967), which depends upon the fact that gibberellin-induced α-amylase release from barley half-seeds is proportional to the log of the concentration of gibberellin applied. Trials were made using this bioassay with some slight modifications, with a series of known gibberellic acid concentrations (0.05 μg/ml down to 0.00005 μg/ml). Good results were obtained, with sensitivity usually down to 0.0005 μg/ml, and so it was decided to adopt this procedure. The methods used are detailed below.

1. Extraction procedure

On the day before assay, each stolon-tip sample was ground in a chilled mortar with ice-cold sterile tris (hydroxymethyl) aminomethane buffer, pH 7.2, and the brei strained through very fine nylon cloth. The extracts were shaken gently and kept in ice at 1°C overnight (for maximum extraction). After 15-16 hours, the preparations were centrifuged at 0°C (3000 rpm for 20 min). The supernatant, made up to an exact volume (6, 7 or 10 ml) in each case, was used as the final extract for bioassay.

2. Barley seed

Jones and Varner (1967) used *Hordeum vulgare* var. Himalaya, which is a naked variety; nakedness was desirable to avoid the extra procedure of de-husking. Most assays in the present work used *H. nudum*, line Ac 120 (1967 harvest) kindly provided by the Scottish Plant Breeding Station at Pentlandfield, Midlothian.

3. Preparation of assay materials and assay procedure

Day 1: The barley seeds were surface-sterilised (20 min in 20% sodium hypochlorite), rinsed with sterile water, then incubated

in sterile water at 1°C for 16 hours, after which time they were soft enough for cutting, but most seeds had not begun to germinate.

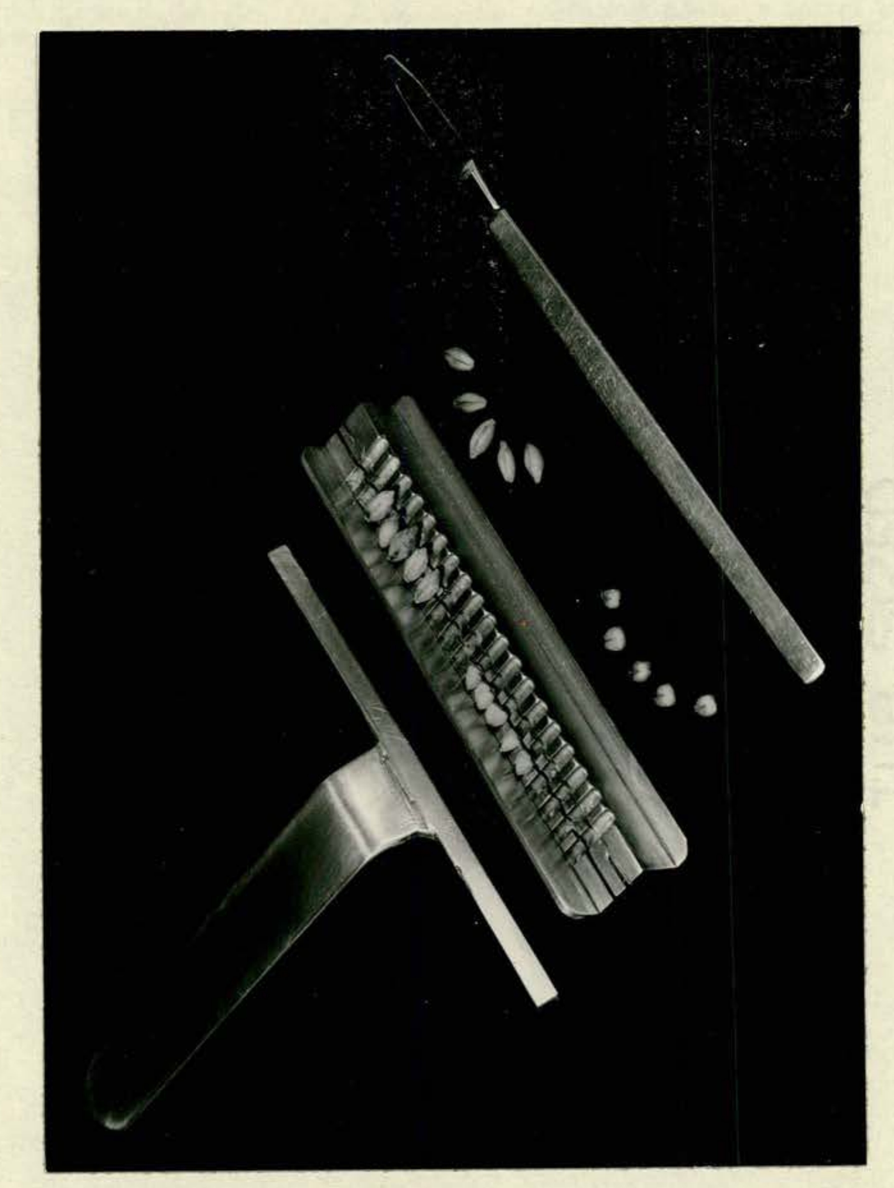
Day 2: The grains were cut in half transversely, retaining the endosperm halves and discarding the embryo halves. No grains were used which were small, discoloured, flattened or which had germinated; any half-grain containing any of the embryo was also discarded. Grains were cut using the apparatus shown in Fig 36. After cutting, the endosperm halves were imbibed for 24 hours at 22.5°C in the dark on moist filter paper in a Petri dish. The entire cutting procedure was carried out under sterile conditions.

Day 3: The incubation of the half-seeds with the extract solutions to be tested was begun, using 25 ml sterile Erlenmeyer flasks, each containing 10 half-grains, 1 ml sterile phosphate buffer (containing 12.00 g $KHPO_4$ and 0.876 g $CaCl_2.6H_2O$ in a final volume of 2 l of buffer, final pH 4.3) and 1 ml of test solution. After preparation, again in sterile conditions, the stoppered flasks were placed in a water bath at 25°C (± 1°C) and shaken at a rate of 40-60 agitations/min for 24 hours. A range of gibberellic acid controls was also included, 1 ml of gibberellic acid solution (made up with sterile water) at concentrations of 0,0.00005, 0.0005, 0.005 and 0.05 μg/ml being added instead of the test solution. Controls were also set up without half-seeds for each test solution, to check on the amount of amylase in the extract. In most cases, diluted extract samples were also assayed. Each treatment was replicated twice.

Day 4: The liquid in each flask was decanted and washings (3 ml sterile water) added to it; it was then spun for 10 min

FIGURE 36. Apparatus used for cutting barley seed for barley half-seed α-amylase bioassay (Section V (iv)).

The apparatus consisted of a brass cutting bed with depressions to hold the seeds and grooves to control the line of the cutting scalpel, and a brass bar (top left) with a handle for holding the seeds in place during cutting. Also shown are some cut and some uncut seeds.



at top speed in a bench centrifuge to bring down large debris, and the supernatant (which was fairly clear and the pigmentation usually slight) quickly decanted.

A large number of test tubes was set up, each containing 1 ml of a solution of soluble starch, freshly made up by adding 375 mg of BDH Analar soluble potato starch to 250 ml boiling phosphate buffer (M/15 PO_4, pH 7.0) and allowing it to cool. Soluble starch was used instead of native starch as specified by Jones and Varner (1967) as it was found that native starch caused variation in the colour with iodine from one occasion to another, whereas soluble starch gave a consistent blue colour. The test tubes were equilibrated (5 min at 30°C) before using for the assay. The assay systems were set up at regular timed intervals by adding 1 ml extract solution to the starch solution, and the reaction stopped after a timed incubation period at 30°C with 1 ml iodine/potassium iodide solution (diluted 1 in 10 with water from a stock solution made by dissolving 6g KI and 600 mg solid iodine in 100 ml water. Water was used to make up the iodine reagent instead of the 0.05N HCL of Jones and Varner's method because the latter again caused varying colours with starch; this problem was avoided when water was used). The first few incubations gave a time schedule for the whole experiment; the incubation times were varied, depending on the level of gibberellin-like activity in the extracts, from 15 sec to 5 min.

The optical density (OD) of the blue solution from the assay, added to 25 ml water, was determined using an SP 600 spectrophotometer, reading at 620 nm. Graphs were plotted of the

change in optical density with time for each sample tested, and for the controls, using the mean values for each pair of duplicate flasks. The gross gradient thus obtained for each sample was corrected for the amount of amylase present in the extract itself by subtracting from it the gradient of the appropriate amylase control; this gave the corrected gradient for the sample.

A calibration curve was constructed by plotting the gradients of the graphs obtained in this way for the gibberellic acid controls against gibberellic acid concentration on a log scale. From this curve, the amount of gibberellin-like substances in any given sample could be found from the gibberellic acid equivalent of the corrected gradient for the sample. The value thus obtained was adjusted for the volume in which the original stolon tip sample was suspended before assay, and for the fresh weight of the original sample, to give the gibberellin-like activity in μg gibberellic acid (GA_3) equivalents per gramme fresh weight of the original sample. An example of the calculation for a sample of stolon tip material from plants grown during the experimental period in long days in the first comparison (C1) is given below.

Gross gradient of OD change for sample = 0.065

Amylase control gradient = 0.025

Corrected gradient for sample = 0.065-0.025 = 0.040

GA_3 equivalent of corrected gradient (obtained from GA_3 control Calibration curve) = 0.00040

Fresh weight of original sample = 0.5523g

Total volume in which sample was

suspended = 10 ml

Volume of sample used in assay = 1 ml

Coefficient of gibberellin activity

in sample $= 0.00040 \times \frac{10}{0.5523} =$

$= 0.0072\ \mu g\ GA_3$ equivalents/

g fwt of original sample

Examples of graphs of change in optical density with time for the samples and controls and calibration curves for the comparisons carried out are shown in Figs 37-43.

c. Comparisons of stolon tip material in different developmental states

Using the method described above, six comparisons of stolon tip material were carried out. Full details of the samples used are given in Table 32, and the results are given in Table 33 and 34.

The first comparison (C1) used total stolon tip samples (ie all the tips on the plants) from plants 49 days old, grown during the experimental period in short days or low intensity long days. The tips from the plants grown in short days included some which were tuberised visibly, but none of the tips from the plants grown in long days had tuberised visibly. More gibberellin-like activity was found in stolon tips from the plants grown in long days than in those from the plants grown in short days, in which no gibberellin-like activity was detected.

This is a similar result to that found by Okazawa (1960) for extracts of leaf blades of potato plants grown in long or short days. Booth (1963) has pointed out that Okazawa's

FIGURE 37. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No. 1). Calibration curve in which is plotted gradient of graph of change in optical density (O.D.) of final assay solution after incubation with time of incubation for control solutions of GA_3 (Fig 38) against GA_3 concentration.

The O.D. of the final assay solution depended upon the amount of starch remaining in it after incubation; the more starch remained, the deeper was the blue colour with iodine, and the higher the O.D. at 620 nm.

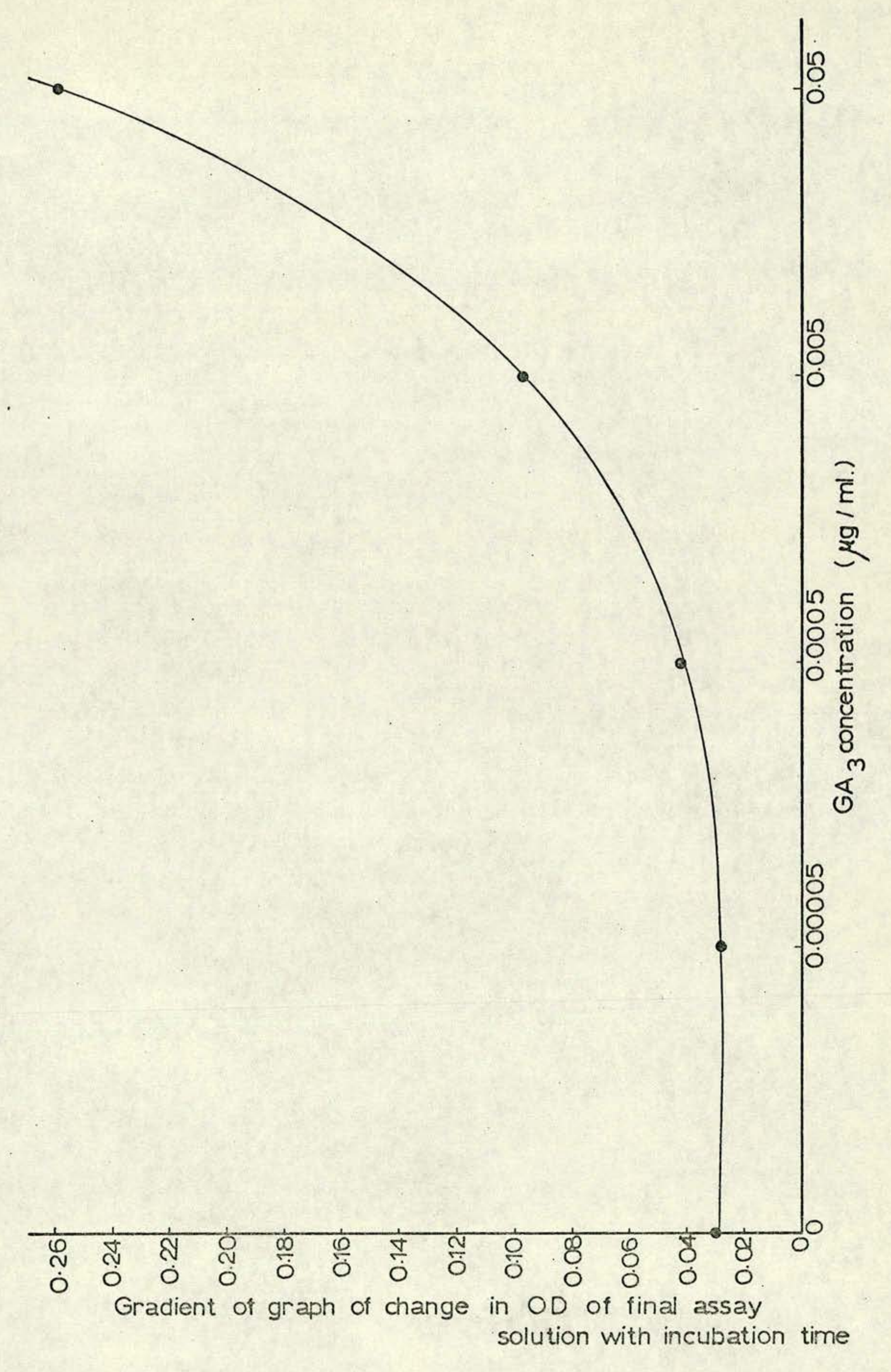
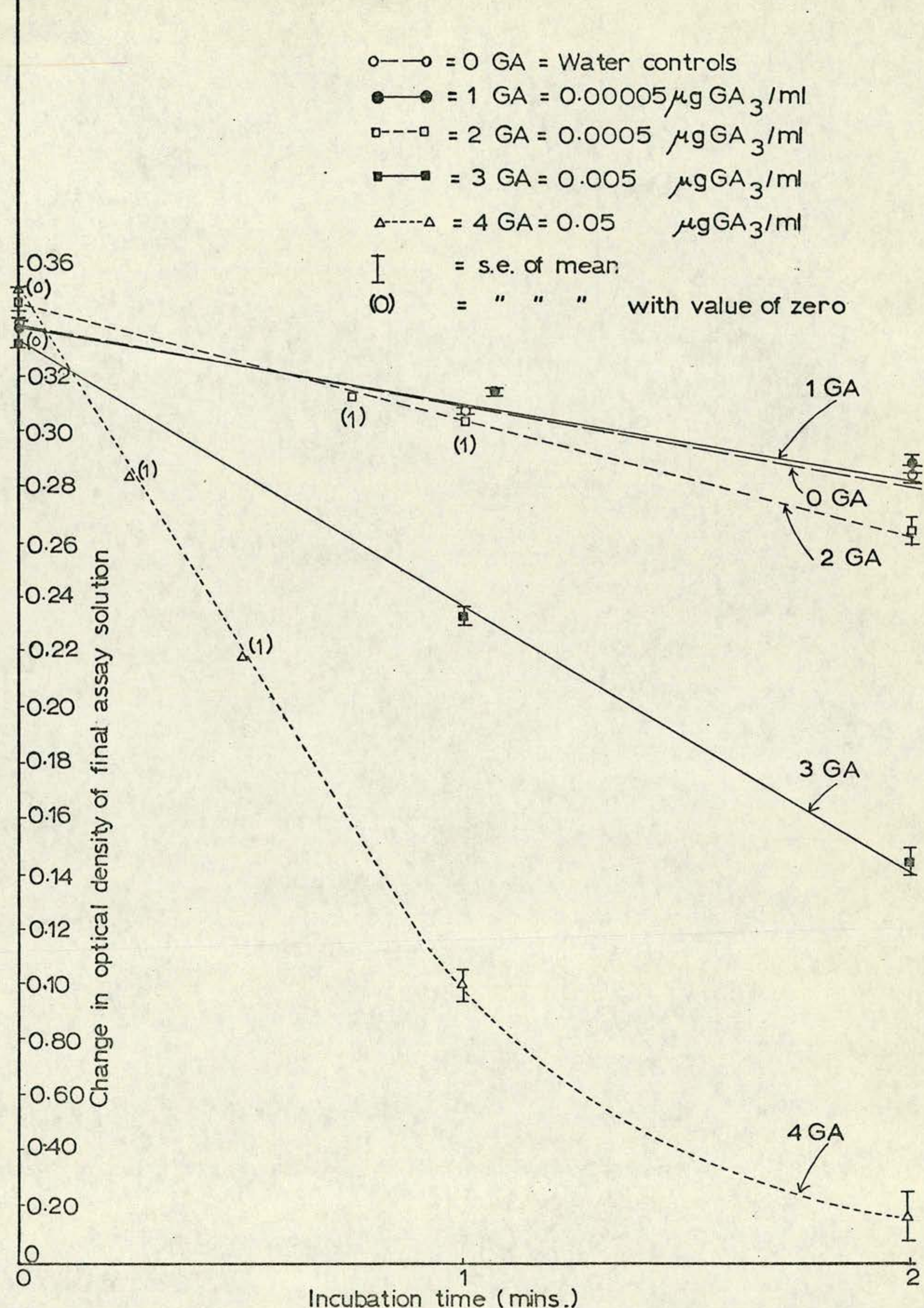

0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
Gradient of graph of change in OD of final assay solution with incubation time
0
0.00005
0.0005
0.005
0.05
GA3 concentration (μg / ml.)

FIGURE 38. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No 1). Graphs showing change in optical density (O.D.) of final assay solution after incubation with time of incubation for control solutions of GA_3.

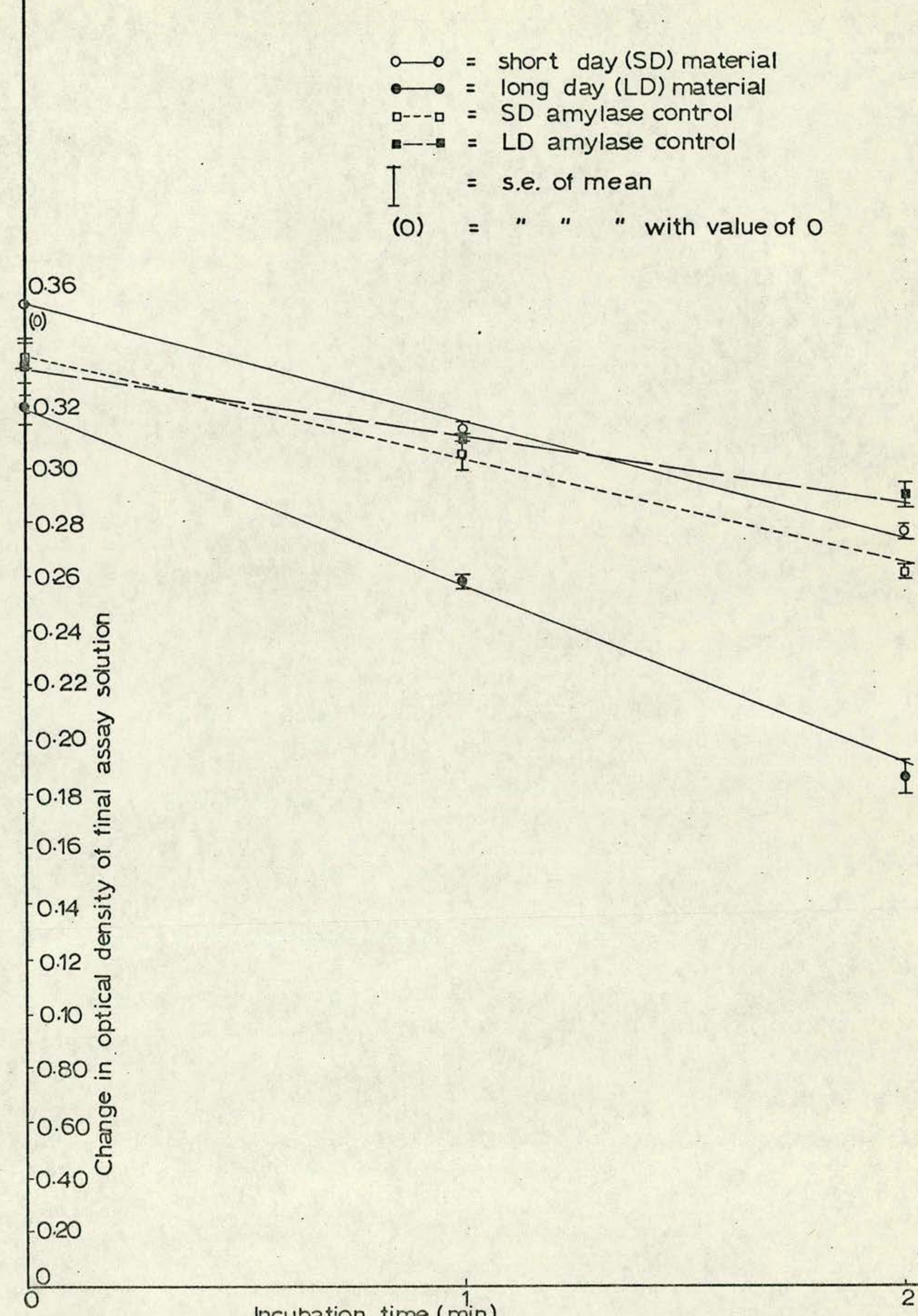
Points are means of two values unless otherwise indicated. Standard errors of means are shown.



= 0 GA = Water controls
= 1 GA = 0.00005 μg GA3/ml
= 2 GA = 0.0005 μg GA3/ml
= 3 GA = 0.005 μg GA3/ml
= 4 GA = 0.05 μg GA3/ml
= s.e. of mean
(0) = " " " with value of zero
1 GA
0 GA
2 GA
3 GA
4 GA
(0)
(1)
0.36
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.80
0.60
0.40
0.20
0
Change in optical density of final assay solution
0
1
2
Incubation time (mins.)

FIGURE 39. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No 1). Graphs showing change in optical density (O.D.) of final assay solution after incubation with time of incubation for test samples and amylase control solutions.

Points are means of two values unless otherwise indicated. Standard errors of means are shown.



o—o = short day (SD) material
●—● = long day (LD) material
□---□ = SD amylase control
■—■ = LD amylase control
= s.e. of mean
(0) = " " " with value of 0
(0)
0·36
0·32
0·30
0·28
0·26
0·24
0·22
0·20
0·18
0·16
0·14
0·12
0·10
0·80
0·60
0·40
0·20
0
Change in optical density of final assay solution
0
1
2
Incubation time (min)

FIGURE 40. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No. 2). Calibration curve in which is plotted gradient of graph of change in optical density (O.D.) of final assay solution after incubation with time of incubation for control solutions of GA_3 (Fig 41) against GA_3 concentration.

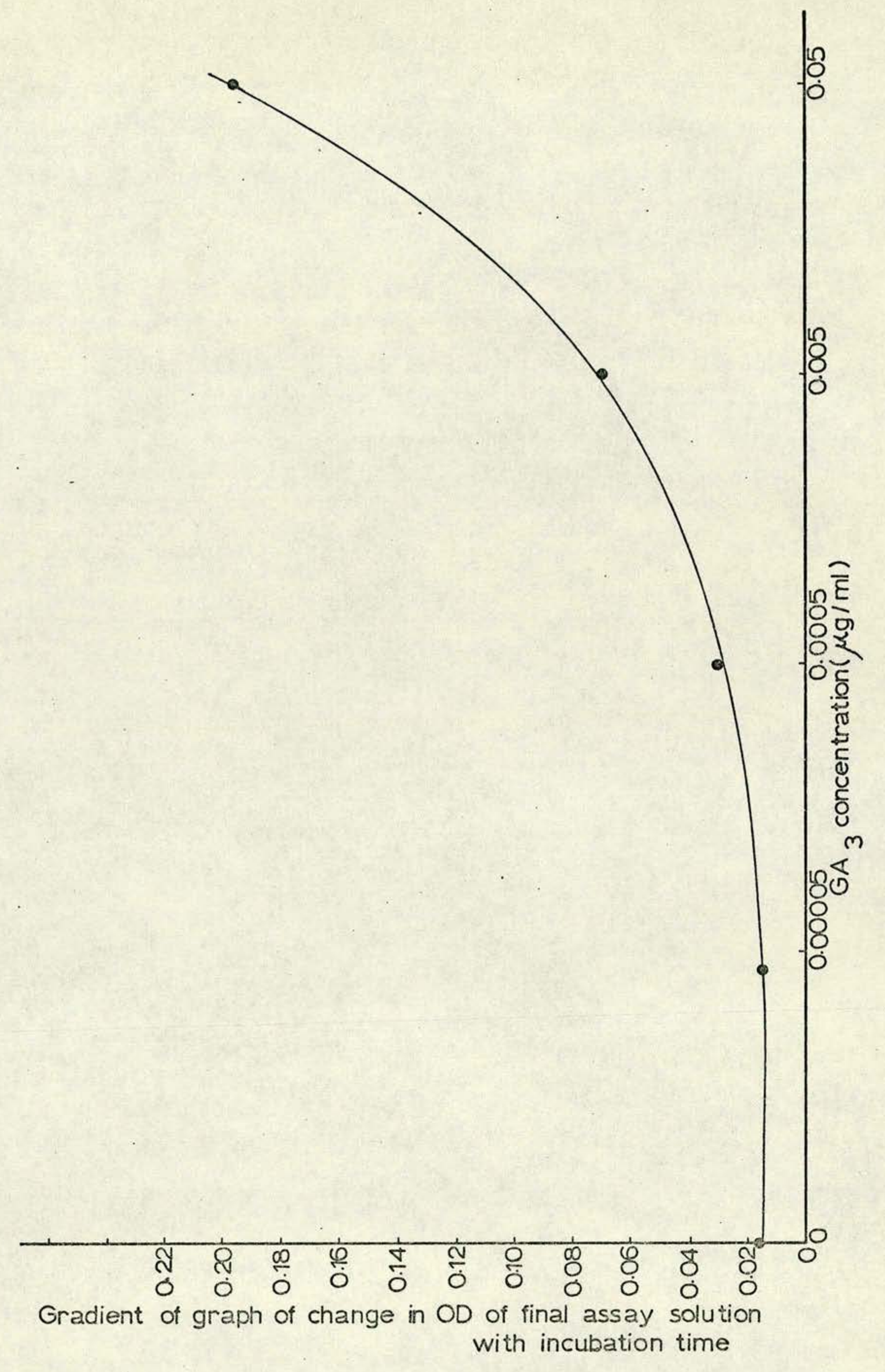
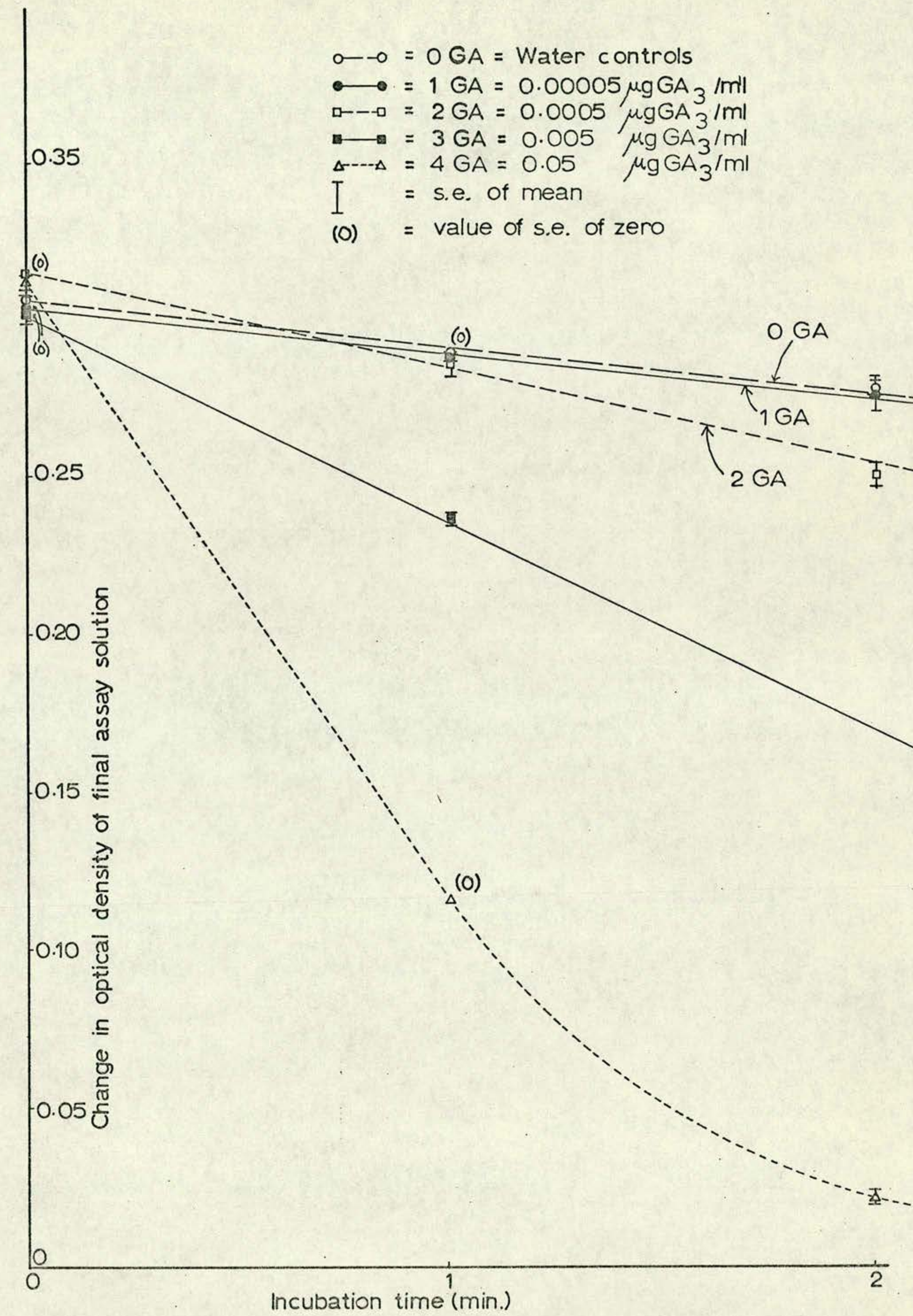

Gradient of graph of change in OD of final assay solution
with incubation time
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0
0.00005
0.0005
0.005
0.05
GA 3 concentration(μg/ml)

FIGURE 41. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No. 2). Graphs showing change in optical density (O.D.) of final assay solution after incubation with time of incubation for control solutions of GA_3.

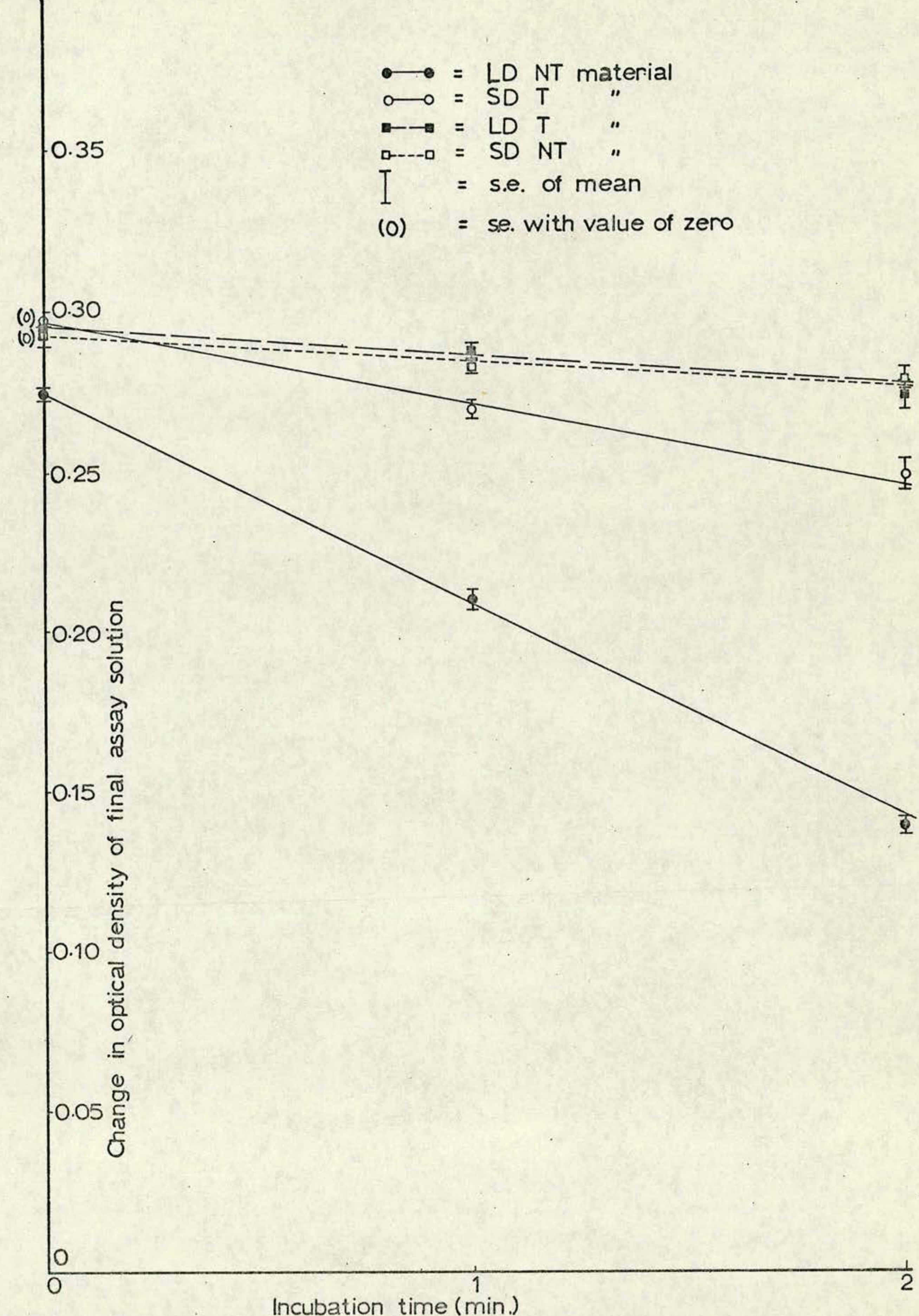
Points are means of two values unless otherwise indicated. Standard errors of means are shown.



= 0 GA = Water controls
= 1 GA = 0·00005 µg GA3 /ml
= 2 GA = 0·0005 µg GA3 /ml
= 3 GA = 0·005 µg GA3 /ml
= 4 GA = 0·05 µg GA3 /ml
= s.e. of mean
(0) = value of s.e. of zero
0 GA
1 GA
2 GA
(0)
0·35
0·25
0·20
0·15
0·10
0·05
0
Change in optical density of final assay solution
0
1
2
Incubation time (min.)

FIGURE 42. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No. 2). Graphs showing change in optical density (O.D.) of final assay solution after incubation with time of incubation for test samples.

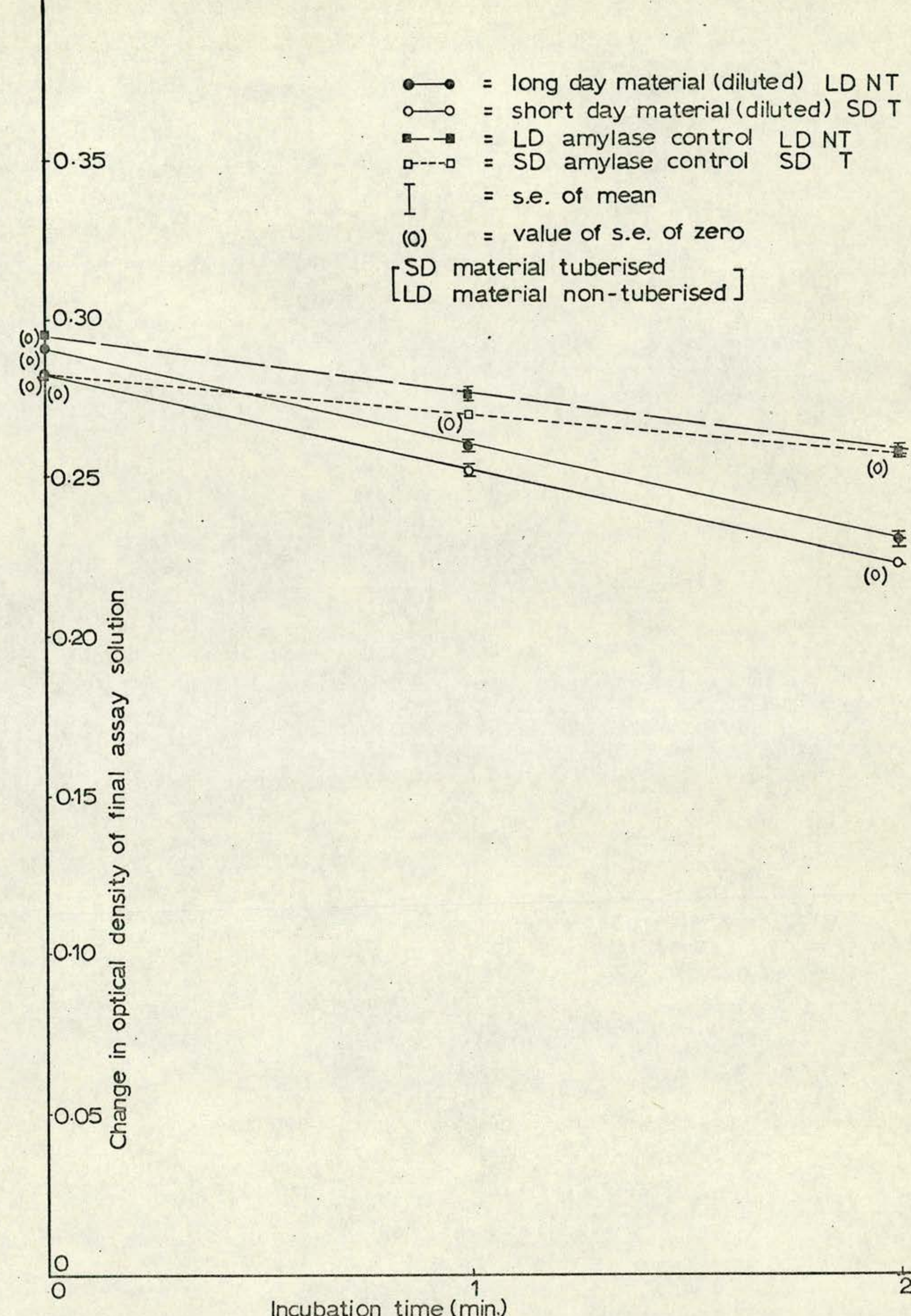
Points are means of two values unless otherwise indicated. Standard errors of means are shown.



= LD NT material
= SD T "
= LD T "
= SD NT "
= s.e. of mean
(0) = se. with value of zero
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
(0)
(0)
Change in optical density of final assay solution
0
1
2
Incubation time (min.)

FIGURE 43. Comparison (using barley half-seed bioassay) of levels of gibberellin-like substances (No. 2). Graphs showing change in optical density (O.D.) of final assay solution after incubation with time of incubation for amylase control solutions and diluted test samples.

Points are means of two values unless otherwise indicated. Standard errors of means are shown.



= long day material (diluted) LD NT
= short day material (diluted) SD T
= LD amylase control LD NT
= SD amylase control SD T
= s.e. of mean
(0) = value of s.e. of zero
SD material tuberised
LD material non-tuberised
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
(0)
Change in optical density of final assay solution
0
1
2
Incubation time (min.)

TABLE 32. Samples of stolon-tip material used in comparisons of levels of gibberellin-like substances using barley half-seed α-amylase bioassay (Section V (iv)).

Comparison	Growing conditions of plants	Special conditions	Developmental category of stolon tips w.r.t. tuberisation	Fresh wt.(g) of material in stolon tip sample
C1	35 HI LD+14 LI LD	-	[total stolon samples, not divided into developmental categories]	0.55
	35 HI LD+14 SD			0.58
C2	49 LI LD	-	NT	0.71
	"	-	T	0.79
	49 SD	-	NT	0.69
	"	-	T	0.86
C3	35 HI LD+ 15 SD	-	NT	0.78
	"	-	T	0.69
	"	-	G(NT)	0.38
C4	35 HI LD+15 LI LD	+ 10 μg GA_3/plant	NT	0.60
	35 HI LD+15 SD	+ 10 μg GA_3/plant	NT	0.55
	"	+ 100 μg GA_3/plant	NT	0.21
		all applied on 5 dates		
C5	35 HI LD+58 LI LD	Last 51 LI LD in nutrient culture	NT/NS	0.64
	"		NT/S	0.73
	"		T	2.63
	"		G(NT)	0.48
C6	58 HI LD+22 SD	The 22 SD in nutrient culture	NT/NS	0.14
			NT/S (2 samples)	0.56 and 0.39
			T (2 samples)	1.99 and 1.88

NT = no visible tubers
T = visible tubers
NS = no starch deposition
S = starch deposition
G = green

LI LD = low intensity long days
HI LD = high intensity long days
SD = short days

TABLE 33. Coefficients of gibberellin-like activity (μg GA_3 equivalents/g fwt. of original sample), together with the figures leading to their calculation, obtained from comparisons of stolon tip material using the barley half-seed α-amylase bioassay (Section V (iv)). Values for undiluted samples.

Comparison	Stolon samples	Gross (G) gradient of OD change graph	Amylase control (AC) gradient	Corrected gradient (G gradient minus AC gradient)	GA_3 equivalent (for corrected gradient value) from calibration curve	Correction factors		Final coefficient of gibberellin-like activity (μg/g fwt. of original sample)
						For fwt. (x)	For volume (x)	
C1	LD SD	0.065 0.044	0.025 0.038	0.040 0.006	0.00040 0	1/0.5523 (1/0.5781)	10 (10)	0.0072 0
C2	LD NT LD T SD NT SD T	0.067 0.009 0.009 0.027	0.019 - - 0.013	0.045 0.009 0.009 0.014	0.00069 0 0 0	1/0.7139 (1/0.7907) (1/0.6948) (1/0.8640)	6 (6) (6) (6)	0.0058 0 0 0
C3	SD NT SD T SD G	0.014 0.006 0.019	0.006 0.002 0.005	0.008 0.004 0.014	0.00057 0 0.00075	1/0.7823 (1/0.6922) 1/0.3791	7 (7) 7	0.0051 0 0.0131
C4	LD + 10μgGA_3 SD + 10μgGA_3 SD + 100μgGA_3	0.148 0.116 0.112	0 0 0	0.148 0.116 0.112	0.061 0.053 0.052	1/0.6027 1/0.5469 1/0.2070	6 6 6	0.607 0.581 1.507
C5	LD NT/NS LD NT/S LD T LD G	0.072 0.024 0.030 0.071	0 0 0.007 0	0.072 0.024 0.023 0.071	0.00550 0 0 0.00545	1/0.644 (1/0.725) (1/2.625) 1/0.479	7 (7) (7) 7	0.0598 0 0 0.0797
C6	SD NT/NS SD NT/S(1) SD NT/S(2) SD T(1) SD T(2)	0.038 0.037 0.030 0.080 0.056	0.005 0 0 0.016 0	0.033 0.037 0.030 0.064 0.056	0.0050 0.0053 0.0035 0.0073 0.0065	1/0.14 1/0.56 1/0.39 1/1.99 1/1.88	7 7 7 7 7	0.2500 0.0663) mean = 0.0628) 0.0646 0.0257) mean = 0.0242) 0.0249

Key on next page

TABLE 33. (Key)

LD = long day

SD = short day

NT = no visible tubers

T = visible tubers

NS = no starch deposition

S = starch deposition

G = green

TABLE 34. (key)

LD = long day

SD = short day

NT = no visible tubers

T = visible tubers

NS = no starch deposition

S = starch deposition

G = green

* = evidence for presence of growth inhibitor(s)

- = no evidence for presence of growth inhibitor(s)

TABLE 34. Coefficients of gibberellin-like activity (μg GA_3 equivalents/g fwt. of original sample), together with the figures leading to their calculation, obtained from comparisons of stolon tip material using the barley half-seed α-amylase bioassay (Section V (iv)). Values for diluted samples.

Comparison	Dilution	Stolon samples	Gross (G) gradient of OD change graph	Amylase control (AC) gradient	Corrected gradient (G gradient minus AC gradient)	GA_3 equivalent (for corrected gradient value) from calibration curve	Correction factors		Final coefficient of gibberellin-like activity (μg/g fwt. of original sample)
							For fwt. (x)	For volume (x)	
C1	No dilutions made								
C2	1 in 10	LD NT	0.030	0.010	0.020	0.000067	1/0.7139	60	0.0056 -
		LD T	No dilution made						
		SD NT	No dilution made						
		SD T	0.030	0.007	0.023	0.000080	1/0.8640	60	0.0056 *
C3	1 in 2	SD NT	0.013	0.003	0.010	0.00063	1/1.0920	14	0.0081 *
		SD T	0.012	0.001	0.011	0.00066	1/0.6922	14	0.0085 *
		SD G	0.003	0.003	0	0	(1/0.3791)	(14)	0 -
C4	1 in 2	LD + 10μgGA_3	0.098	0	0.098	0.028	1/0.6027	12	0.5576 -
		SD + 10μgGA_3	0.083	0	0.083	0.0084	1/0.5469	12	0.738 *
		SD + 100μgGA_3	0.105	0	0.105	0.045	1/0.2070	12	2.608 *
C5	1 in 10	LD NT/NS	0.034	0	0.034	0.00054	1/0.644	70	0.0587 -
		LD NT/S	0.022	0	0.022	0	(1/0.725)	(70)	0 -
		LD T	0.044	0.001	0.043	0.00071	1/2.625	70	0.0189 *
		LD G	0.030	0	0.030	0.00050	1/0.479	70	0.0731 -
C6	1 in 10	SD NT/NS	0.015	0.001	0.014	0.00050	1/0.14	70	0.2500 -
		SD NT/S(1)	0.015	0	0.015	0.00054	1/0.56	70	0.0675 -
		SD NT/S(2)	0.010	0	0.010	0	(1/0.39)	(70)	0 -
		SD T(1)	0.024	0.002	0.022	0.00082	1/1.99	70	0.0305 *
		SD T(2)	0.020	0	0.020	0.00075	1/1.88	70	0.0279 *

Key on previous page

results, which demonstrate higher gibberellin levels under long days than under short days, can equally well be used to show a significantly higher growth inhibitor content of plants in short days, and he has himself (see above) demonstrated the appearance of a growth inhibitor in tuberising stolons. Okazawa also found that gibberellic acid would overcome the inhibitory effects in bioassay of a growth inhibitor present in the leaf blades. The release of α-amylase from barley half-seeds is thought to be specific to gibberellins; neither auxins nor cytokinins have any effect (Jones and Varner, 1967). Chrispeels and Varner (1966), however, have shown that abscisic acid can inhibit the effect of gibberellic acid in inducing α-amylase synthesis, although on a molecular basis, approximately ten times as much abscisic acid is required to inhibit the gibberellin-induced response.

In view of these facts, it is probably more correct to say that the first comparison of stolon tip material showed that the balance of endogenous gibberellins to endogenous growth inhibitors was higher in stolon tips in long days than in short days. Later comparisons included dilutions of the samples, to detect the presence of naturally occurring growth inhibitors in the extracts. This is possible because, when an extract containing both gibberellin and growth inhibitor is diluted, sensitivity to growth inhibitor activity will decrease much more rapidly and will be lost long before it is lost to an equivalent amount of gibberellin activity. If the growth inhibitor activity present in the undiluted extract is no longer detected by the assay system

after the extract has been diluted, there will be a resulting apparent increase in gibberellin activity (on the basis of equivalents of GA_3 activity/g fwt of original tissue) in a diluted extract when compared to that in an undiluted extract. Jones and Varner (1967) therefore recommend the use of diluted samples to detect the presence of inhibitors, and Smith and Rappaport (1969, see below), also use the criterion of apparently-increased gibberellin activity observed after dilution to demonstrate the presence of inhibitors.

It was also decided that in future comparisons, the material would be divided more specifically into various stages of tuberisation, rather than simply on the basis of the growing conditions of the parent plants.

In the second comparison (C2), the material was obtained from plants which had been grown for 49 days in low intensity long days or in short days. By this time, some tubers had formed on both categories of plant, and the stolons from both categories were divided into those showing visible signs of tuberisation and those showing no such signs. No gibberellin-like activity was detected in either of the samples from plants grown in short days, nor in the sample of tuberised tips from plants grown in long days. It is probable that no activity was found in the untuberised tips from plants grown in short days because these tips were on the point of tuberising (having received 49 short day cycles). Some gibberellin-like activity was, however, found in the stolons showing no visible signs of tuberisation obtained from plants grown in long days. The 1:10 dilutions showed evidence for the presence of a growth inhibitor or inhibitors

in the tuberised stolon tips from plants grown in short days ($0.56 \times 10^{-2} \mu g$ GA_3 equivs/g fwt cf no detectable activity in the undiluted extract), but not in the non-tuberised tips from the plants grown in long days (in which activity was very close to that found in the undiluted extract). Dilutions were not made of the other two extracts in this comparison, because of the time required for setting up and carrying out the assay, and the necessity to quickly estimate the final starch-iodine colour before fading occurred. In later comparisons, assay of more samples was made possible by the use of a "Zipette" automatic pipette.

The next comparison (C3) used stolon tip samples from plants given 15 short day cycles, again separated on the basis of visible signs of tuberisation; a sample was also included of tips of green stolons, which do not normally tuberise. The highest levels of gibberellin-like activity were found in the green stolon tips, less than half the amount being found in the non-tuberised stolons, and no activity at all in the tips showing visible swelling. Dilutions (1:2) showed some growth inhibitor activity in both non-green samples, the amount being greater in tips which had visibly tuberised than in those which had not ($0.85 \times 10^{-2} \mu g$ GA_3 equivs/g fwt cf zero, and 0.81×10^{-2} cf $0.51 \times 10^{-2} \mu g$ GA_3 equivs/g fwt respectively). No evidence for growth inhibitor activity was found in green tips. It might have been expected that the final coefficient of activity for the diluted sample of green stolon tip material would have had roughly the same value (or greater) as that for the undiluted sample, but in fact the detection of the gibberellic acid calibration curve

only reached about 0.0004 μg GA_3 equivalents in this comparison, and the GA_3 equivalent for the corrected gradient for the diluted sample would have been only about 0.00036, and so this would have been undetectable.

The results of this and the previous comparison suggest that when stolon tips tuberise, there is an accompanying reduction in the balance of naturally occurring gibberellins to naturally occurring growth inhibitors. Evidence for the presence of a growth inhibitor was found in both the second and third comparisons in visibly tuberised stolon tips from plants grown in short days. The other sample in which growth inhibitor activity was detected was the sample of tips which had not visibly tuberised in the third comparison. It is possible that these tips had progressed some way along the path towards tuberisation, since the parent plants received 15 short day cycles. The level of gibberellin-like activity in these tips was still appreciable, however, notwithstanding the appearance of a growth inhibitor. This lends support to the idea that it is the balance between endogenous gibberellins and growth inhibitors which controls tuberisation.

The levels of gibberellin-like activity found in the long day (Comparison 1) and non-tuberised (Comparisons 2 and 3) samples were all of the same order of magnitude (the one exception to this being in C2 as discussed above), and short day (Comparison 1) and tuberised (Comparisons 2 and 3) samples all showed no activity.

During the progress of this work, Smith and Rappaport (1969) reported findings in agreement with these for the variety Red Pontiac, with samples consisting of tubers and of stolons

without visible signs of tuberisation. It appears that the whole stolon, not only the tip, was included in the untuberised samples, but this is not clear. Smith and Rappaport showed, using the dwarf pea and maize mutant bio-assays, that non-tuberised stolons contained high levels of endogenous gibberellin activity and developing tubers low activity. Assay of diluted extracts showed that low gibberellin-like activity may have been due to the presence of a growth inhibitor.

The levels of gibberellin activity found by these workers were somewhat higher than those found in the first three comparisons described here, but were of the same order as those found in the fifth and sixth comparisons. This is probably because they used material grown under field conditions, whereas the present experiments used material grown in controlled environment conditions from small tuber pieces which shows less vigorous growth. The material used in the fifth and sixth comparisons was grown during part of its development in nutrient culture, which produced more vigorous plants than those grown completely in sand in pots, and this may be an explanation for the higher overall gibberellin levels found in these comparisons.

If gibberellic acid applied to the apex of the shoot exerts its effect by travelling to the stolon tip, the gibberellin levels in the stolon tips of plants so treated should be higher than those in untreated plants. The next comparison (C4) investigated this point using stolon tip material from plants which had received repeated apical applications of solutions containing 10 or 100 μg gibberellic acid per plant (see Table 32). The highest

gibberellin-like activity was found in the sample from plants grown in short days and given 100 μg gibberellic acid per plant, and lower values in the samples from plants grown in short or long days and given 10 μg gibberellic acid per plant. All values obtained were a great deal higher (x 10 - x 100) than those found in any of the other comparisons, and this suggests that gibberellin applied to the shoot apex does in fact exert its effect by directly or indirectly increasing the gibberellin content at the stolon tips of the treated plant, especially as the highest gibberellin-like level was found with the largest apical application of gibberellic acid. Dilution indicated the presence of a growth inhibitor in the stolon tips from plants grown in short days (2.61 cf 1.51 μg GA_3 equivs/g fwt in those given 100 μg GA_3/plant and 0.74 cf 0.58 μg GA_3 equivs/g fwt in those given 10 μg GA_3/plant), but not in tips from plants grown in long days; in the latter, the estimate of the coefficient of gibberellin-like activity for the diluted sample was close to that for the undiluted sample. It thus appears that applied gibberellin does not act by preventing the production of a growth inhibitor, again supporting the idea of control by a balance between endogenous gibberellins and growth inhibitors.

At this point the possibility was considered of dividing stolon tips into categories giving a more accurate assessment of their stage of development towards tuberisation; the histological study described in Section VI was accordingly carried out. While it was found, as expected, that visibly swollen stolon tips had laid down large amounts of starch throughout their tissues, a consistent progression of starch deposition in various tissues

was also found prior to visible swelling. Following this study, stolon tips were divided into three categories: visibly tuberised, not visibly tuberised but with starch deposition, and not visibly tuberised and with no starch deposition (see Section VI for criteria for these categories).

In the fifth comparison (C5), the material came from plants grown in sand for 35 days in high intensity long days and then transferred to low intensity long days for 58 days, the last 51 of these being in nutrient culture (see Section II (ii) d). By harvest, tuberisation had begun in these plants, and the stolon tips were divided into the categories described in the previous paragraph, plus a fourth, green stolons. Green stolons again had the highest levels of gibberellin-like activity, and there was no evidence of growth inhibitor activity in the extract. A somewhat lower level of gibberellin-like activity was found in the stolons with no visible signs of tuberisation or starch deposition, and none at all in either the visibly tuberised stolons or those showing no visible signs of tuberisation but which had laid down starch. Dilution showed growth inhibitor activity only in the visibly tuberised stolons (1.89 x 10^{-3} μg GA_3 equivs/g fwt cf zero in the undiluted extract).

The sixth comparison (C6) was similar to the fifth in the categories used, but without green stolons. The parent plants were grown for 58 days in high intensity long days in sand, and then transferred for 22 short days to nutrient culture. The highest levels of gibberellin-like activity were found in stolon tips showing no visible signs of tuberisation and no starch deposits, intermediate levels in those which had not visibly tuberised but which had laid down starch and the lowest values in visibly

tuberised tips. The only extracts with evidence of growth inhibitor activity were those of visibly tuberised tips. The zero value for the coefficient of gibberellin activity in one of the diluted samples of stolons not visibly tuberised but with starch deposits was probably found for the same reason as that suggested in the case of the diluted sample of green stolon tips in Comparison 3; the expected value of the GA_3 equivalent would be about 0.00035, and the limit of detection from the gibberellic acid control calibration curve in this comparison was around the same value.

As mentioned above, the coefficients of gibberellin activity in the last two comparisons (C5 and C6) were much higher than those in the first three comparisons (C1-C3), although of the same order of magnitude as each other, probably because the plants were grown in nutrient culture and were more vigorous than those grown in pots. The results of comparisons 5 and 6, however, confirm those from comparisons 1-3, in that tuberisation appears to be correlated with a lowering of the ratio of endogenous gibberellins to endogenous growth inhibitors; the results further show that the value of this ratio falls before visible tuberisation begins, the fall being correlated with the deposition of starch in the tissues of the tip prior to visible swelling.

The results of Booth (1963) and of Smith and Rappaport (1969) are consistent with these findings, but no other worker has yet tried to demonstrate a correlation between the very first stages of tuberisation (starch deposition) and the levels of endogenous growth substances.

d. Summary

Assay of aqueous extracts of stolon tip material using the barley half-seed α-amylase bioassay of Jones and Varner (1967) for gibberellins gave the following results:-

1. Green stolons, which do not generally tuberise, show the highest levels of gibberellin-like activity.
2. The stolons of plants grown in long days have higher overall levels of gibberellin-like activity than those of plants grown in short days.
3. Stolon tips which show no visible signs of tuberisation have higher gibberellin-like levels than visibly tuberised tips.
4. Of stolon tips showing no visible signs of tuberisation, those which have begun to deposit starch have lower levels of gibberellin-like activity than those in which no starch has been laid down.
5. Application of gibberellic acid to the apex of the parent plant, which treatment delays tuberisation, leads to an increase in the level of gibberellin-like activity in the stolon tips.

It therefore appears that tuberisation is associated with low levels of gibberellin-like activity in the stolon tip. The first stages of tuberisation,ie the deposition of starch in the sub-apical region,are correlated with a fall in the gibberellin level, and this fall continues as tuberisation progresses, the lowest levels of activity being found in tips with visible signs of tuberisation.

Extracts from visibly tuberised stolon tips, or from tips which might be expected to be about to tuberise, mostly showed evidence for the presence of a growth inhibitor or inhibitors (see Table 34);

an increase in the growth inhibitor level therefore seems to accompany the fall in gibberellin level.

These studies provide support for the theory of control of tuberisation by changes in the balance of endogenous gibberellins to endogenous growth inhibitors. The results of Comparison 4 suggest that applied gibberellic acid does not exert its effect by preventing the production of endogenous growth inhibitors, but by increasing the gibberellin level at the stolon tip; the changes in the levels of these two types of substance may therefore be to some extent independent. It appears that daylength may control the levels of both endogenous gibberellins and growth inhibitors, short days lowering the level of the former and probably raising the level of the latter and thus causing hastened tuber initiation.

SECTION VI INVESTIGATION OF STARCH DEPOSITION IN STOLON TIPS IMMEDIATELY PRIOR TO AND INCLUDING THE BEGINNING OF VISIBLE SWELLING

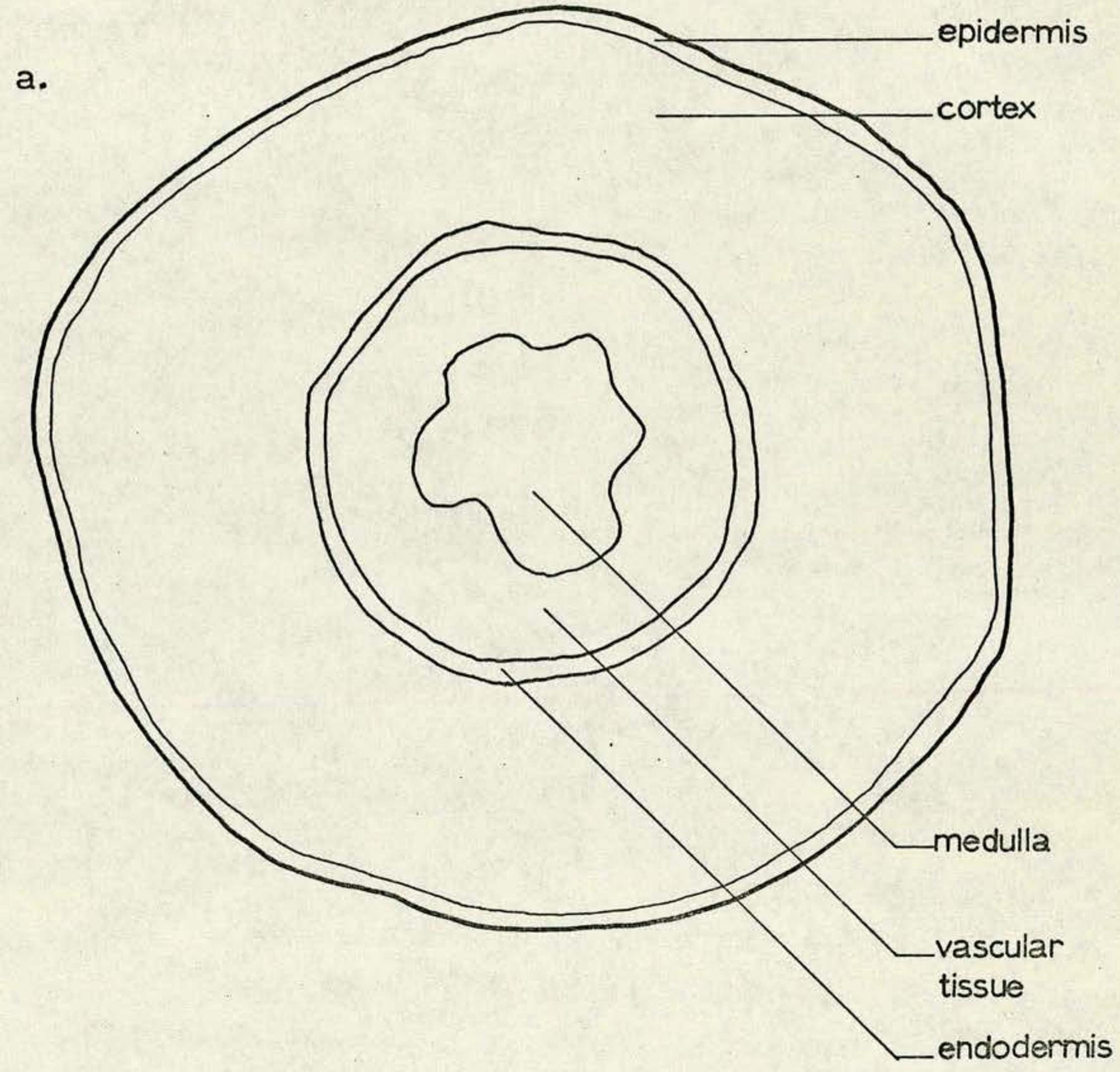
Lovell and Booth (1967) carried out experiments in which plants treated with gibberellic acid and untreated controls were harvested at intervals and the growth of shoots, stolons and tubers measured and sugar and starch contents of the various parts determined. They found that the appearance of starch in stolons correlated closely with tuber initiation in both groups of plants; this was not the case with sugar level. It therefore appeared that a more accurate estimation of the developmental state of stolon tips might be obtained by observation of their starch status. A series of stolon tips was therefore examined histologically for starch deposition. The series included green stolon tips (which do not tuberise), tips which would not be expected to be on the point of tuberisation (from young plants grown in long days), tips which would be expected to be about to tuberise, but which showed no visible swelling (from plants grown in short days and on which several stolons had already tuberised) and visibly tuberised stolon tips.

Transverse sections were cut from the region approximately 0.5 cm behind the tip of a large number of such types of stolons. Hand sections were found to be quite adequate for determining the level of starch deposition after staining with iodine (the stock solution used in Section V (iv)). For photographing the various stages of starch deposition (see figures in this section), sections were cut using a Cryostat (SLEE Freezing Microtome). Staining was with iodine and light green and photographs were taken soon after staining to avoid fading.

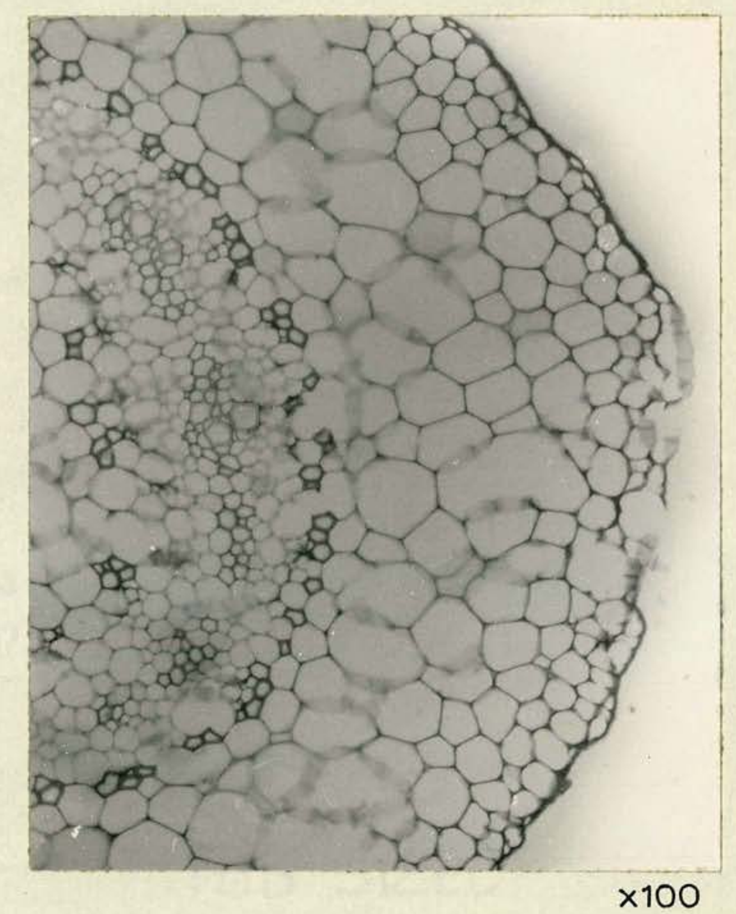
It was found that, by histological examination, developmental stages of tuberisation earlier than visible swelling could be detected. A very few stolons contained no starch grains whatever (see Figs 44 a and b), but this condition was a very unusual one among the stolon tips examined.

Almost all tips, whether in plants about to tuberise (grown in short days) or not (young plants from long days) were found to contain a narrow ring of cells, usually one or two cells across radially, with starch grains clearly visible forming a starch sheath in the region of the "endodermis" (Figs 45 a-f). Fig 45 b shows the very beginnings of this deposition of starch, activity having begun in about 6 cells. Due to the impossibility of focussing simultaneously on different planes of a rather thick section, the details of cell walls are not completely clear, but it can be seen that starch deposition is limited to a few locations situated in a ring at the position of the endodermis. A few starch grains have been moved from their original positions due to breakage of cells and loss of cell contents during sectioning. Such grains are indicated in all the figures by arrows. Figs 45 c and d show parts of an almost complete ring of endodermal cells containing starch grains. In the cells where the process has begun, large numbers of starch grains may be seen (marked "S" in Fig 45 d), although there are still a few cells in the ring in which no grains are present. No starch deposition has taken place in any of the other tissues. Figs 45 e and f show the final condition of the development of the ring of starch-containing cells in the endodermal region, in which all the cells have begun to lay down starch. After this stage, starch

FIGURE 44. a. Distribution diagram of transverse section of stolon containing no starch deposition in any tissue.
b. Part of transverse section of stolon showing no starch deposition in any tissue.


a.
epidermis
cortex
medulla
vascular tissue
endodermis

b.



×100

FIGURE 45. a. Distribution diagram of transverse section of stolon showing the beginning of starch deposition in the "endodermis", immediately outside the stele.

b. Part of transverse section of stolon showing the very first stages of starch deposition in the "endodermis".

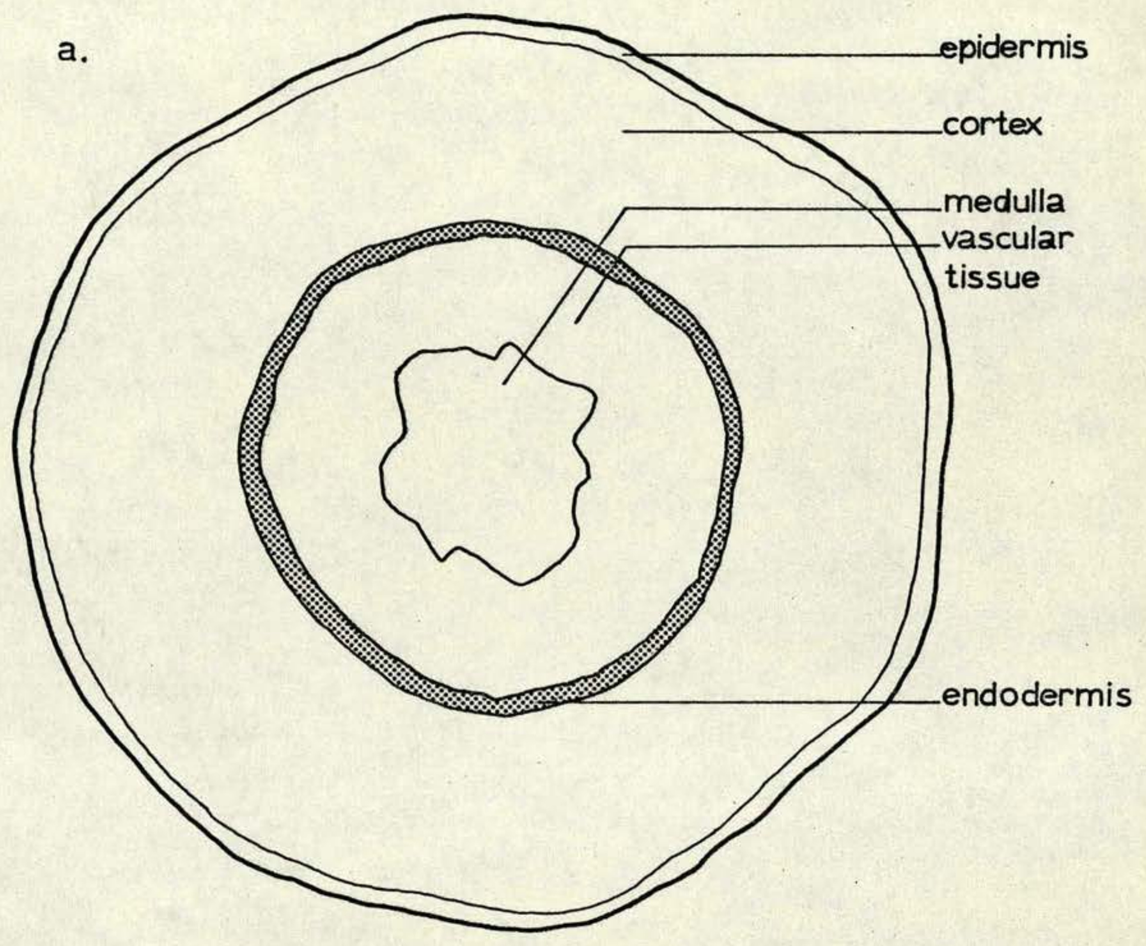
c. Part of an almost complete ring of "endodermal" cells containing starch grains.

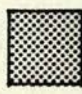
d. High power detail of another part of the almost complete ring of starch-containing "endodermal" cells shown in (c). S = cells containing large numbers of starch grains.

e. Part of complete ring of starch-containing "endodermal" cells.

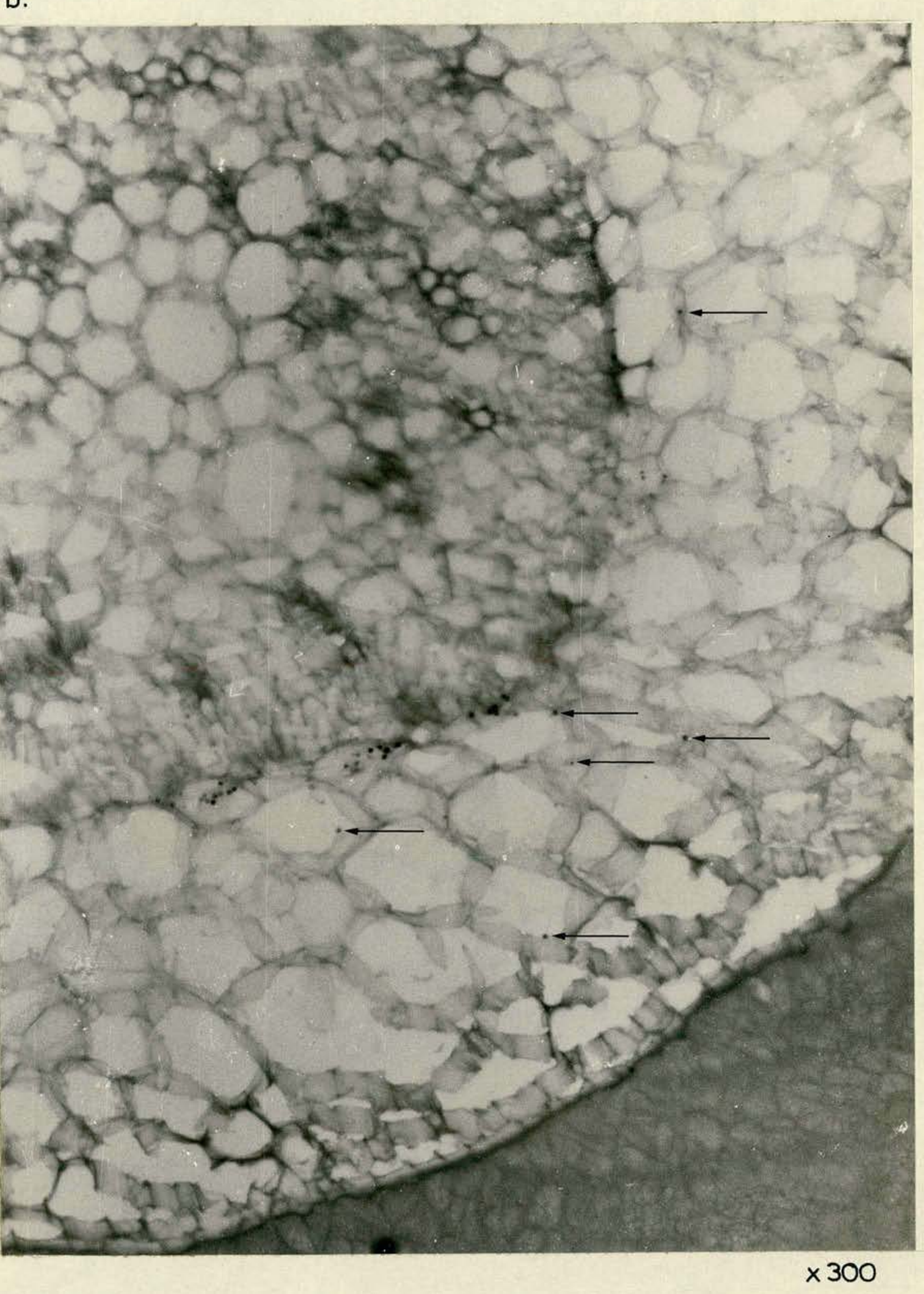
f. High power detail of part of complete ring of starch-containing"endodermal" cells.

Starch grains which have been moved to artificial positions during preparation are shown by arrows.



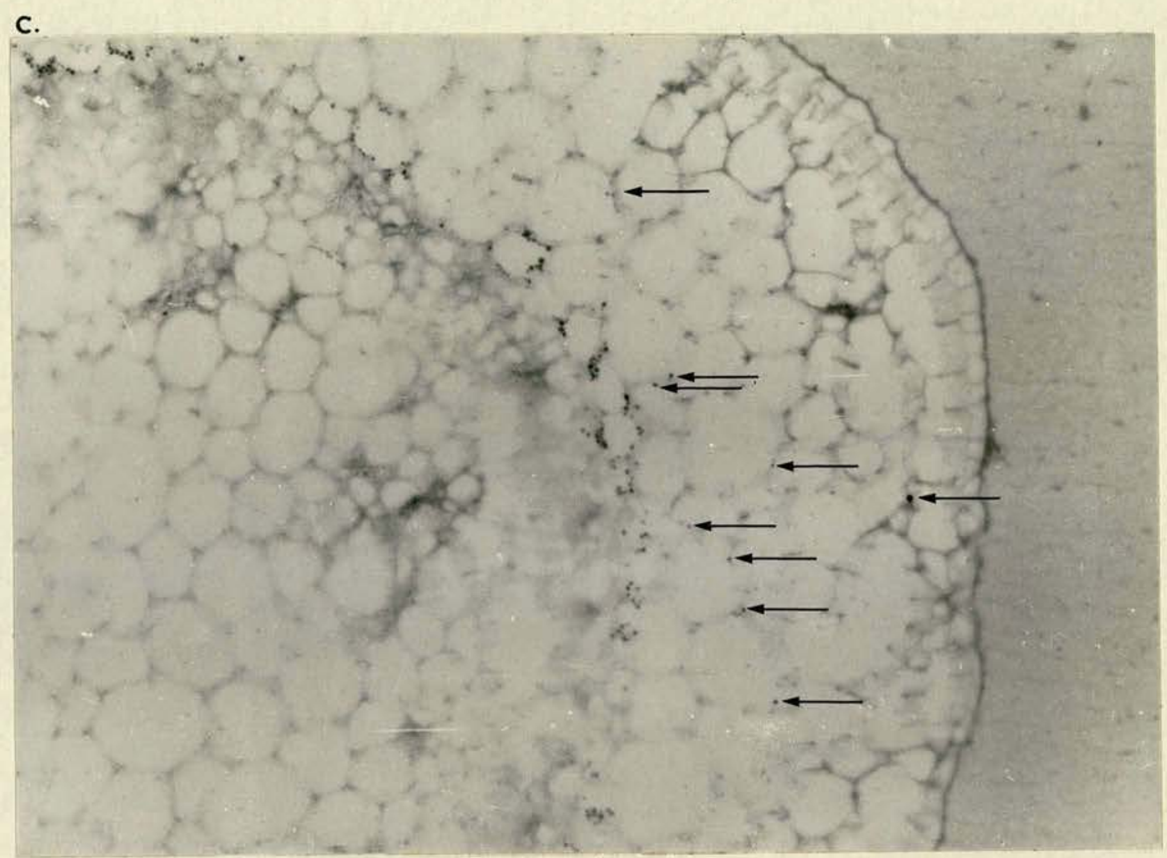

starch deposition

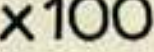
b.



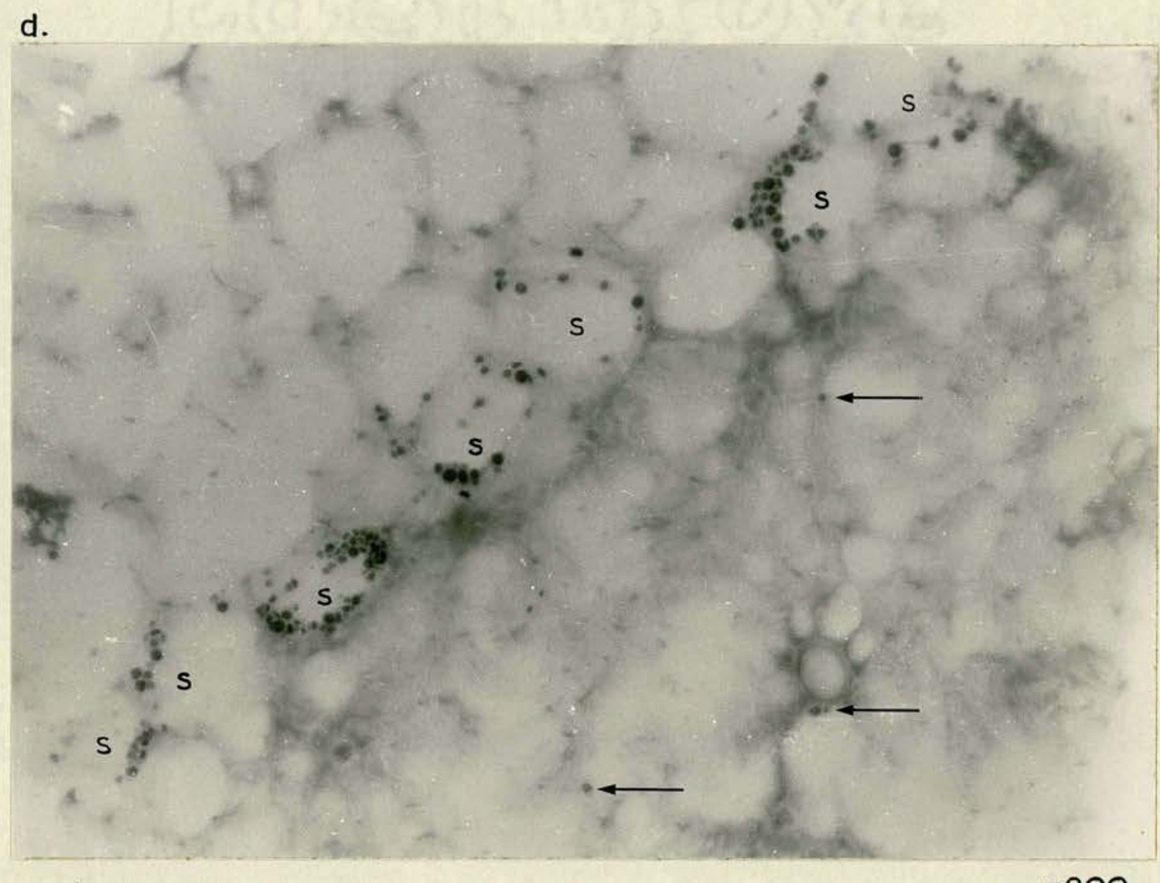
x 300

c.



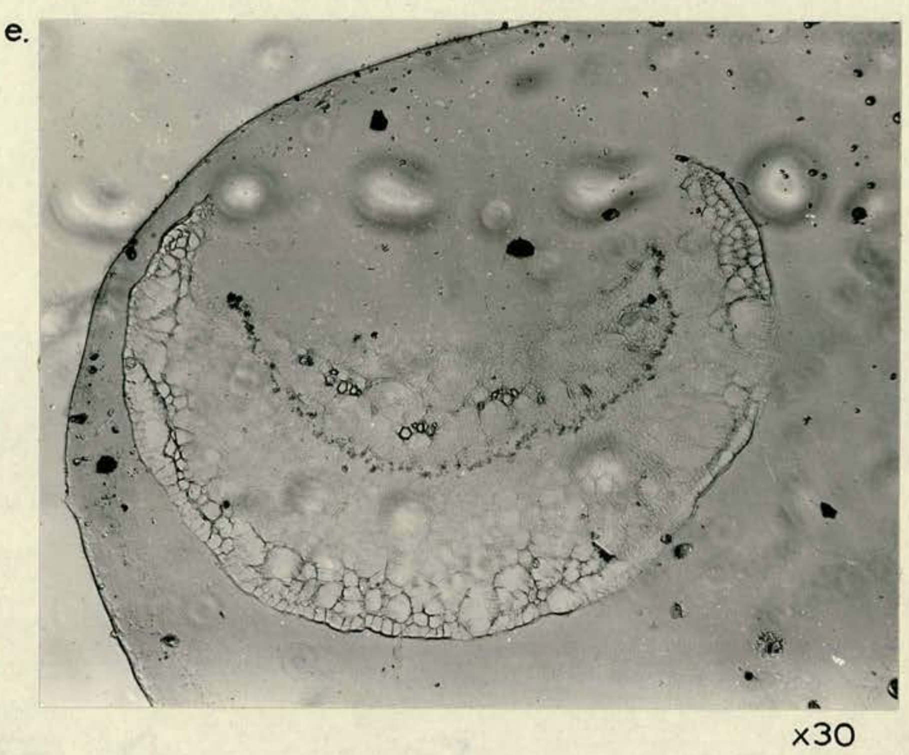

x100

d.



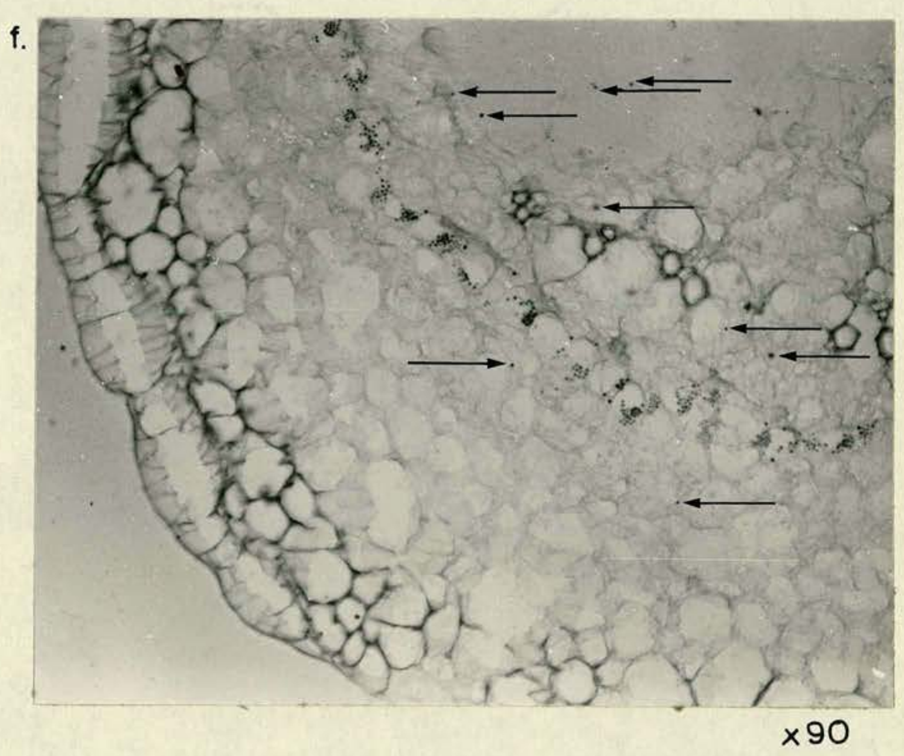
x200

e.



x30

f.



x90

deposition begins in other tissues of the stolon tip. For the purposes of classification in other parts of the work, stolons in the condition described above were considered to have no starch, as this condition appeared to be the normal basic one in non-visibly tuberised material.

Starch deposition first appears (after the establishment of the ring of starch-containing cells in the endodermis) in the cortex, and in the inner cortical cells before the outer ones. Fig 46 shows the stage in which the cells of the inner cortex begin to lay down starch.

This stage is followed by starch deposition in the outer cortex (as in Fig 47 a, which shows starch deposition in the endodermis and throughout the cortex). Sections were never found showing starch deposition in the outer but not in the inner parts of the cortex. Figs 47 b, c and d show deposition throughout the cortex.

After starch deposition has taken place throughout the cortex, it begins in the medulla. Sections were never found with deposition in the medulla and not in the cortex. The first signs of deposition in the medulla may be seen in Fig 47 b, in which 4 cells in the centre of this tissue have begun to produce starch grains; these cells are identified in the small accompanying diagram. A larger number of medullary cells (17 as opposed to 4), again in the centre of the tissue, have begun to lay down starch in the section shown in Fig 47 c. Fig 48 a shows the next stage, in which starch deposition increases in the medulla, being already well established in the endodermal and cortical tissues. Fig 48 b shows starch deposition throughout the cortex,

FIGURE 46. Distribution diagram of transverse section of stolon showing starch deposition beginning to spread out from the "endodermis" into the inner cortex.

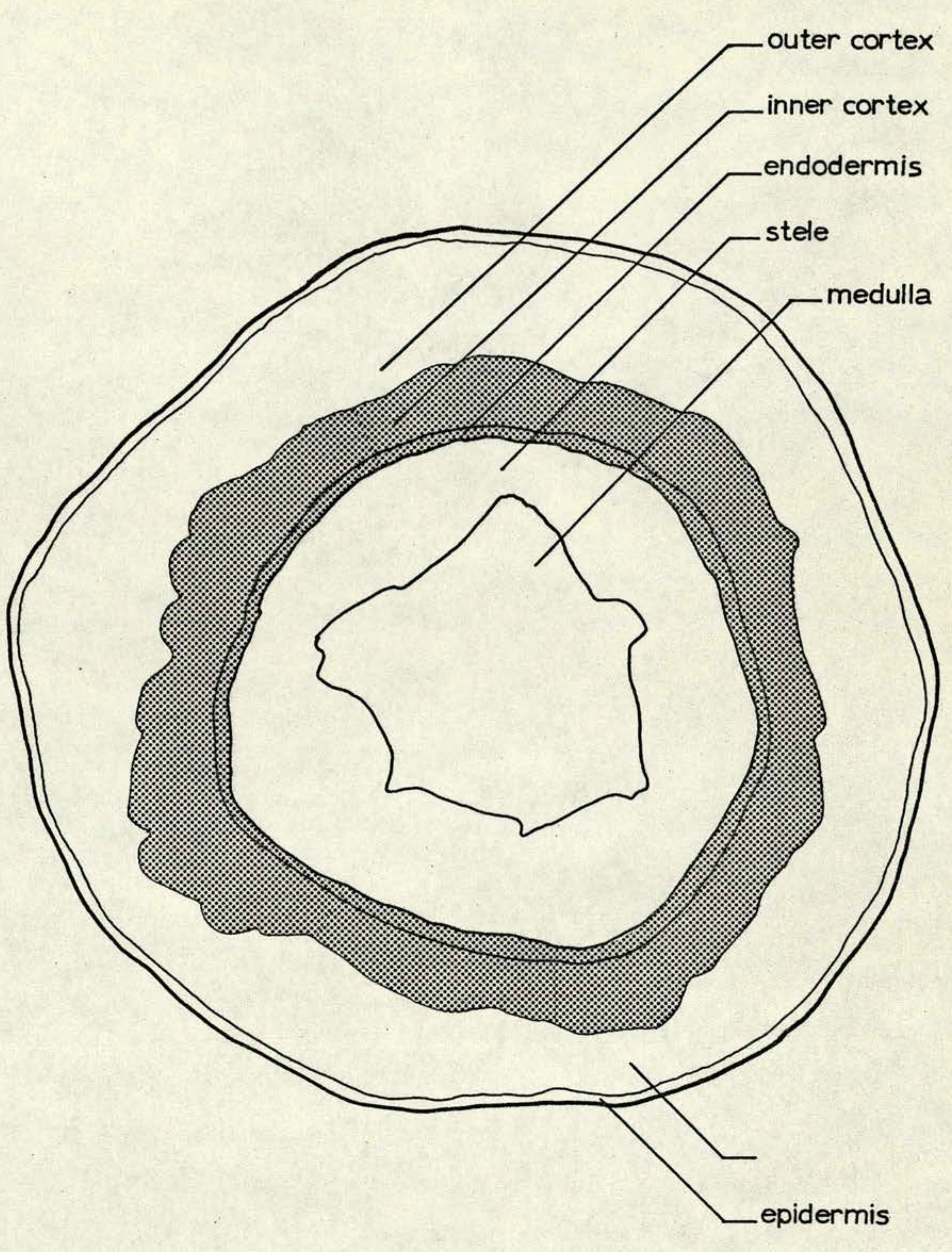

outer cortex
inner cortex
endodermis
stele
medulla
epidermis
starch deposition

FIGURE 47. a. Distribution diagram of transverse section of stolon showing starch deposition throughout endodermis and cortex. (At this stage a few starch grains may also be found in the medulla).
b. Starch deposition throughout "endodermis" and cortex. The first signs of starch deposition may be detected in the medulla (see small diagram).
c. Starch deposition throughout "endodermis" and cortex. More cells in the medulla have begun to lay down starch.
d. High power detail of stolon showing starch deposition throughout cortex.

Displaced starch grains are shown by arrows.

a.

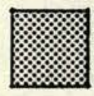
epidermis

cortex

endodermis

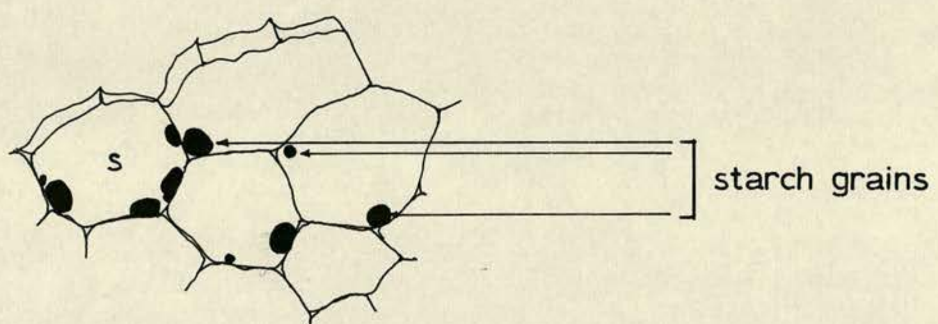
stele

medulla

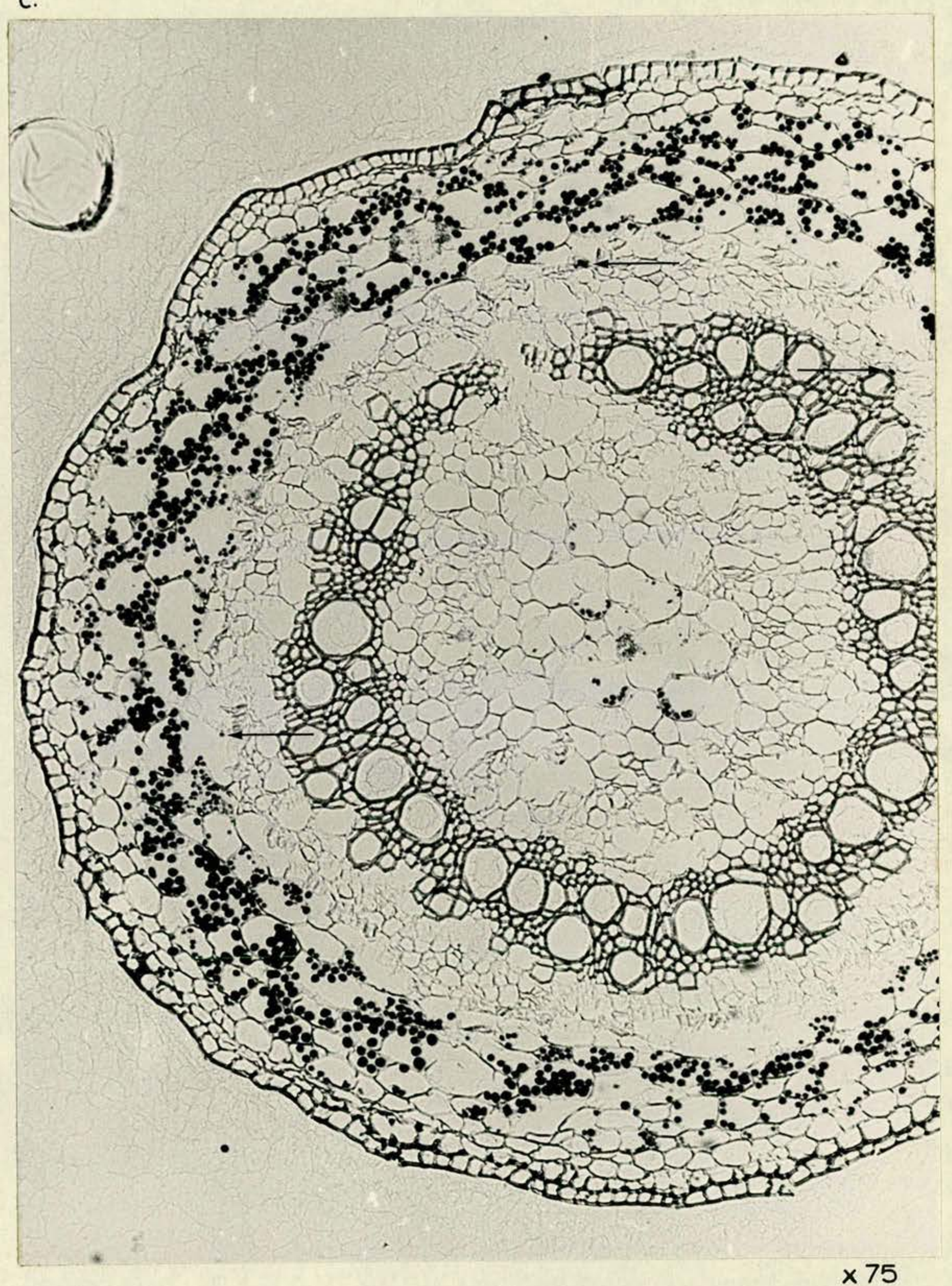
 starch synthesis

b.

x75

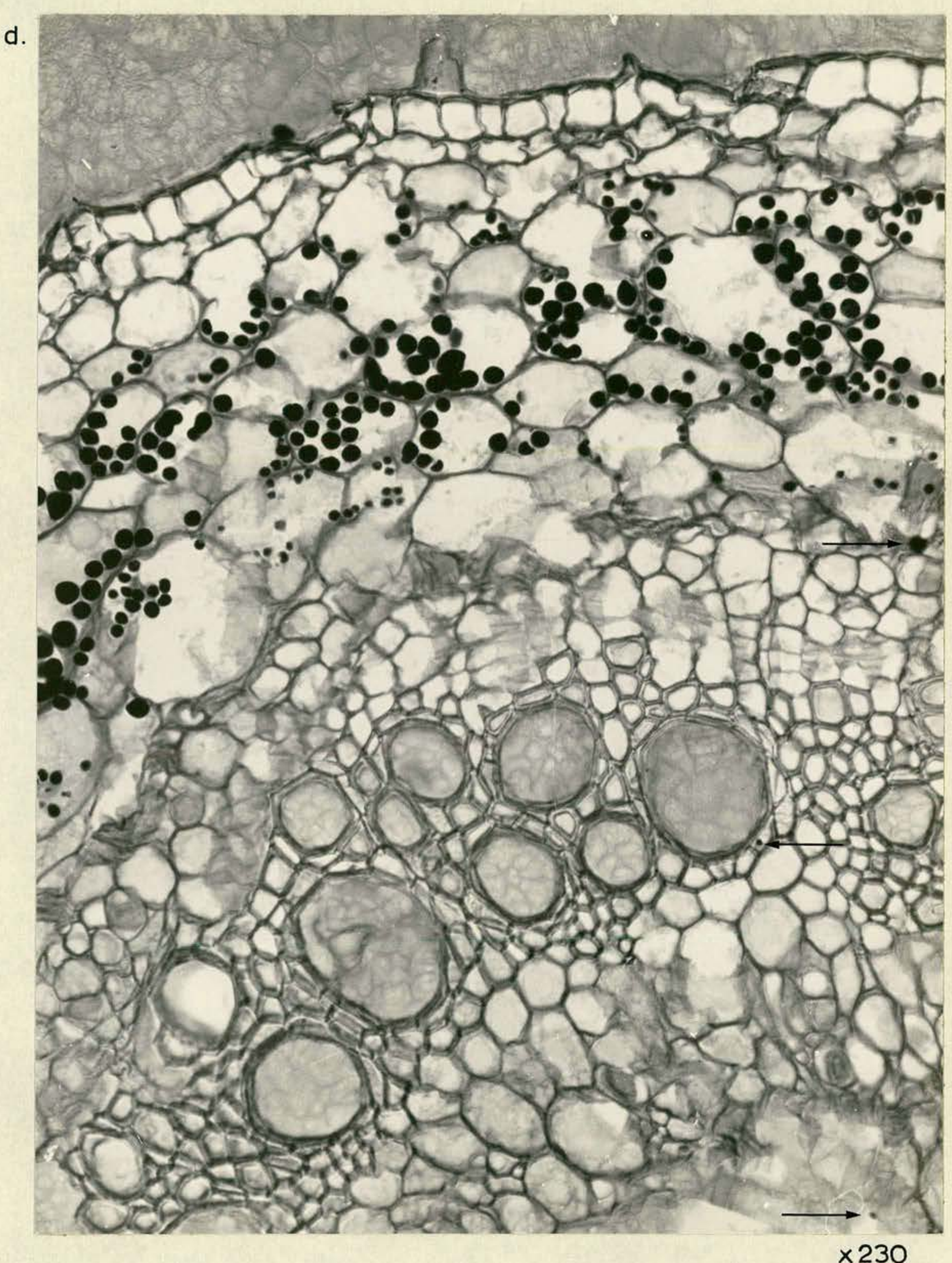


c.



x 75

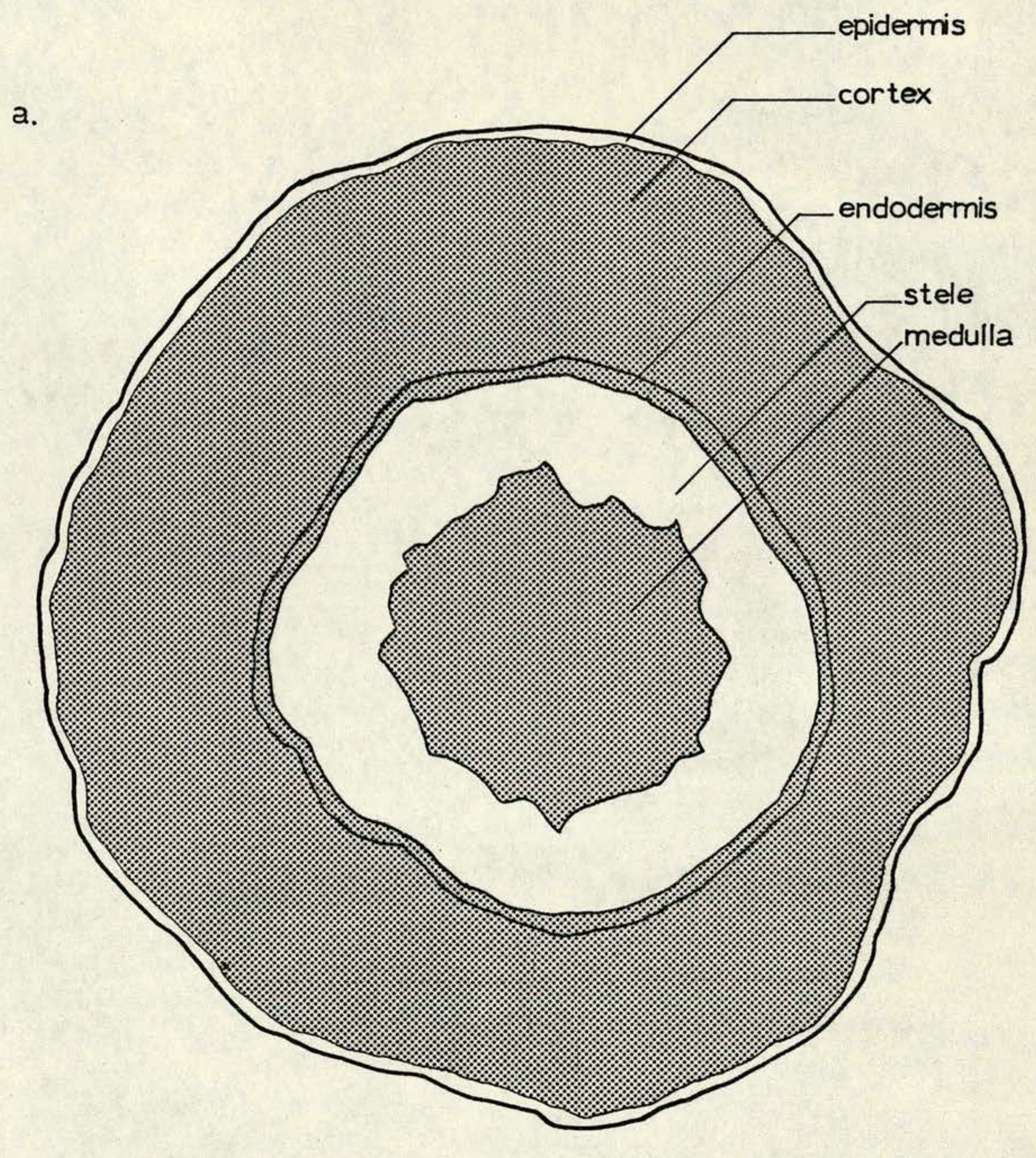
d.



x230

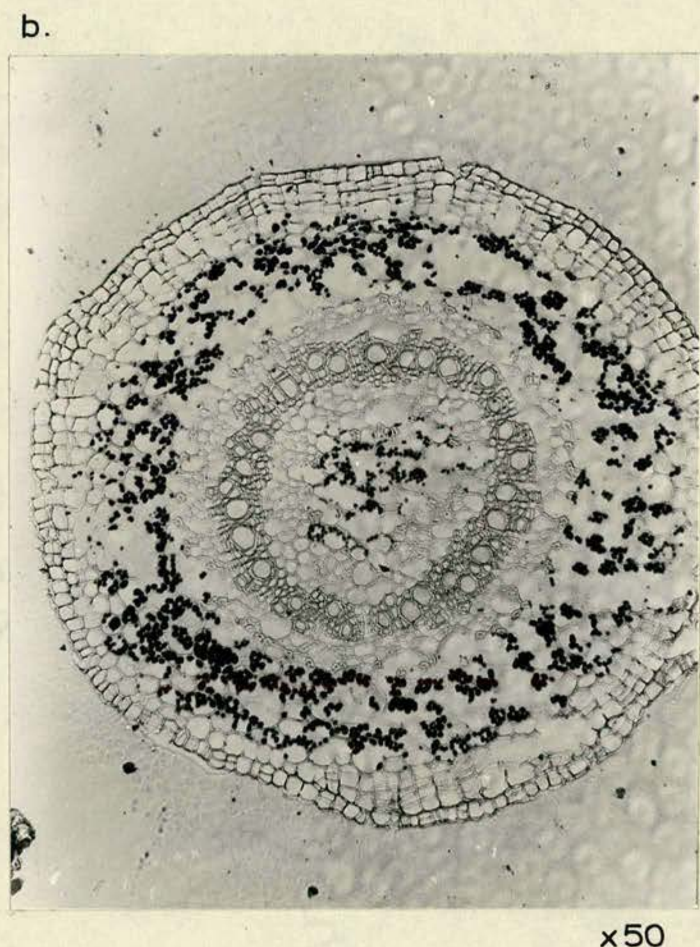
FIGURE 48. a. Distribution diagram of transverse section of stolon showing starch deposition far advanced in cortex and now also taking place in medulla.
b. Stolon showing starch deposition throughout cortex and some deposition in the medulla.
c. Starch deposition throughout cortex, and now having extended also to almost every medullary cell.
d. High power detail of part of stolon shown in (c).

Displaced starch grains are shown by arrows.



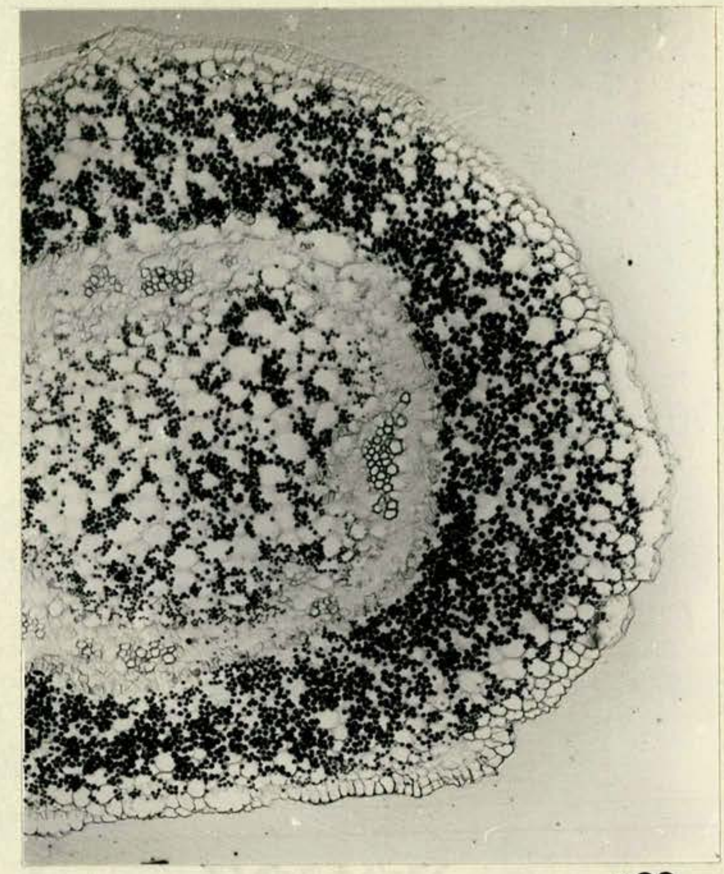
starch deposition

b.



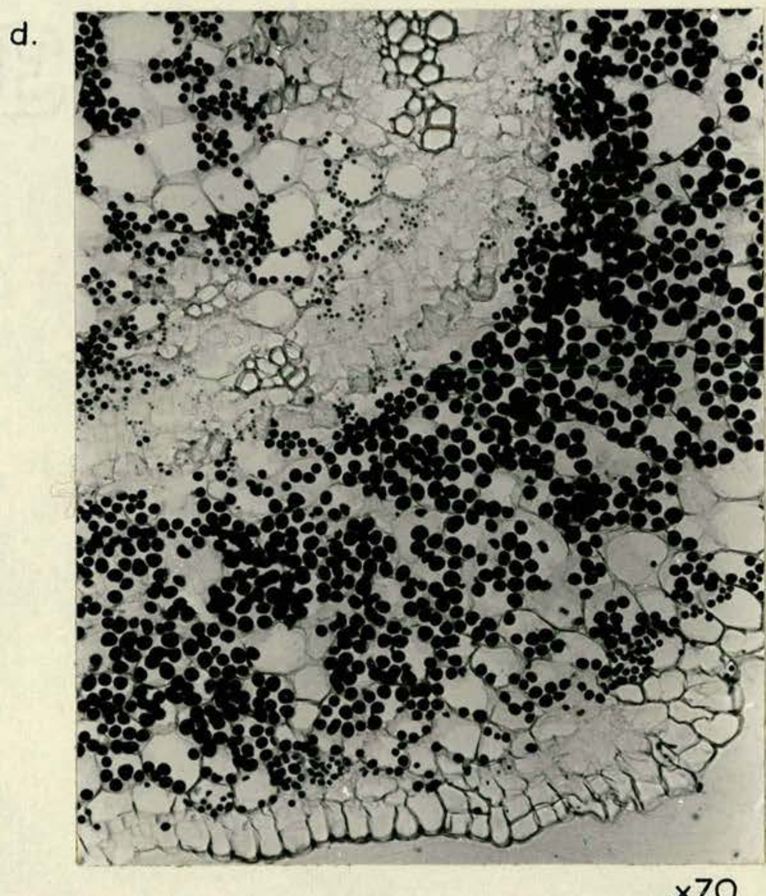
x50

c.



x30

d.



x70

and some deposition in the medulla, and Figs 48 c and d show a greater amount of deposition in the medulla, almost every cell containing some starch.

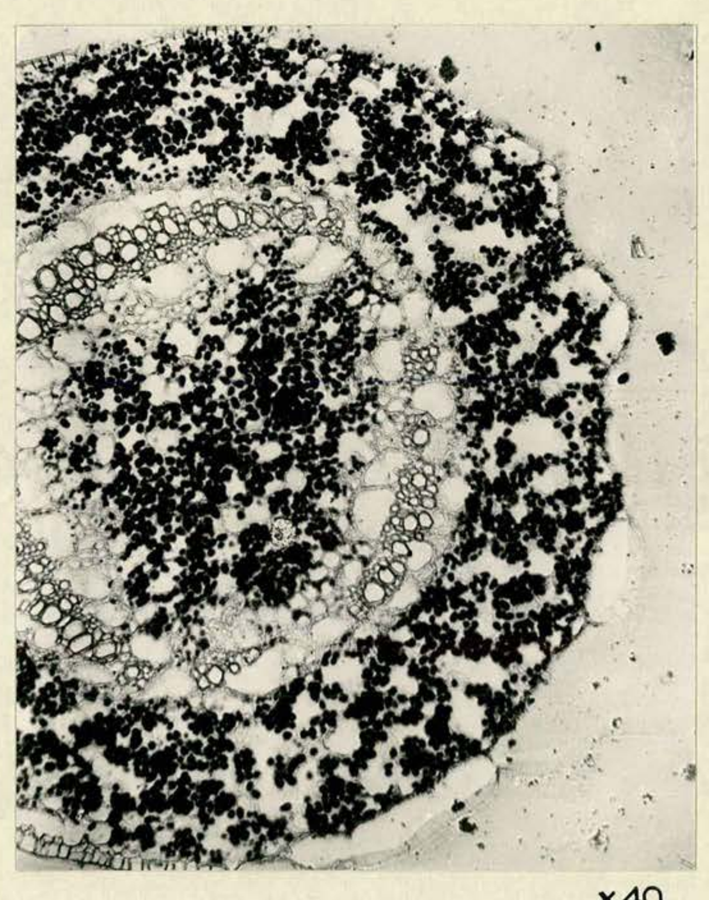
Fig 49 shows the final stage before visible swelling takes place, in which massive starch deposition has occurred in cortex and medulla; this is also the state of deposition in the very young visibly-swollen tuber.

The pattern of starch deposition described above may perhaps reflect the location of the various tissues with respect to the phloem, which might be expected to supply metabolites and also perhaps the hormonal tuberising stimulus (see Section VII). Phloem in the potato is bicollaterally disposed, but there is more external than internal phloem, so that endodermis and cortex might be expected to receive larger amounts of such substances than the medulla, resulting in earlier starch formation there.

Starch deposition was not generally found in the tissues of the stele before the onset of visible swelling, and these tissues could be seen quite clearly in hand sections, after iodine staining, as a light-coloured ring.

It was usually the case that green stolons, which never tuberise under normal conditions, contained no starch grains; exceptions to this were found in which starch deposition had taken place throughout endodermis, cortex and medulla, but this was never correlated with visible swelling. Non-green stolons were found in all stages of the sequence described above. On branched stolons which had some visibly tuberised parts, other parts showing no visible signs of tuberisation were generally found to

FIGURE 49. Stolon demonstrating the very heavy starch deposition immediately before visible swelling of the tip. Note massive starch deposition throughout cortex and medulla.



×40

have a high level of starch deposition. Parts of the main stolon adjacent to visibly tuberised side buds were also usually full of starch.

When visible swelling commences, cell divisions begin to take place in all directions in medulla and cortex (see Fig 50, especially 50 c). Fig 50 b shows a high-power transect through a transverse section of a young tuber. Starch deposition can be seen in all the tissues, including those of the stele. The section shown in Fig 50 was not stained specifically for starch, but the Haematoxylin and Orange G stain used enables the starch grains to be seen as grey oval structures.

Summary

Histological examination of stolon tips from plants grown in different daylength régimes showed that developmental stages of tuberisation earlier than visible swelling could be detected. A consistent sequence of starch deposition in the various tissues was found. Deposition began in the cells of the endodermis, extended to the inner and then the outer cortex and then to the medulla, first in the centre and later throughout this tissue. When visible swelling commenced, starch was also laid down in the tissues of the stele, which were devoid of it until this stage.

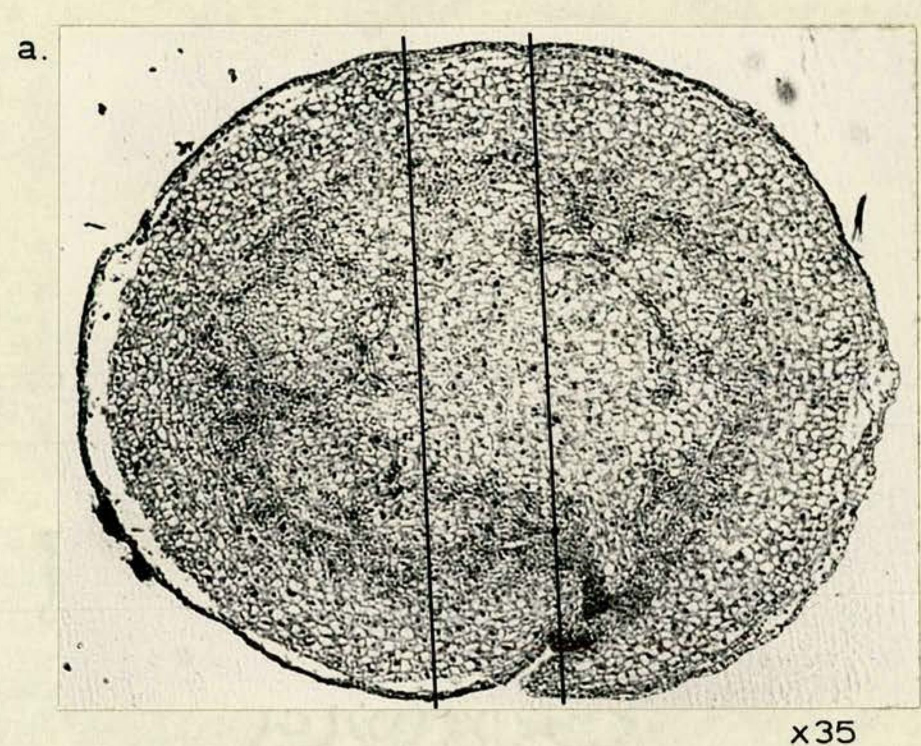
FIGURE 50 a. Transverse section of young tuber (position of high power transect, shown in (b), marked by parallel lines).

b. High power transect of transverse section of young tuber shown in (a).

c. High power detail of part of young tuber showing "random" directions of cell division (arrows).

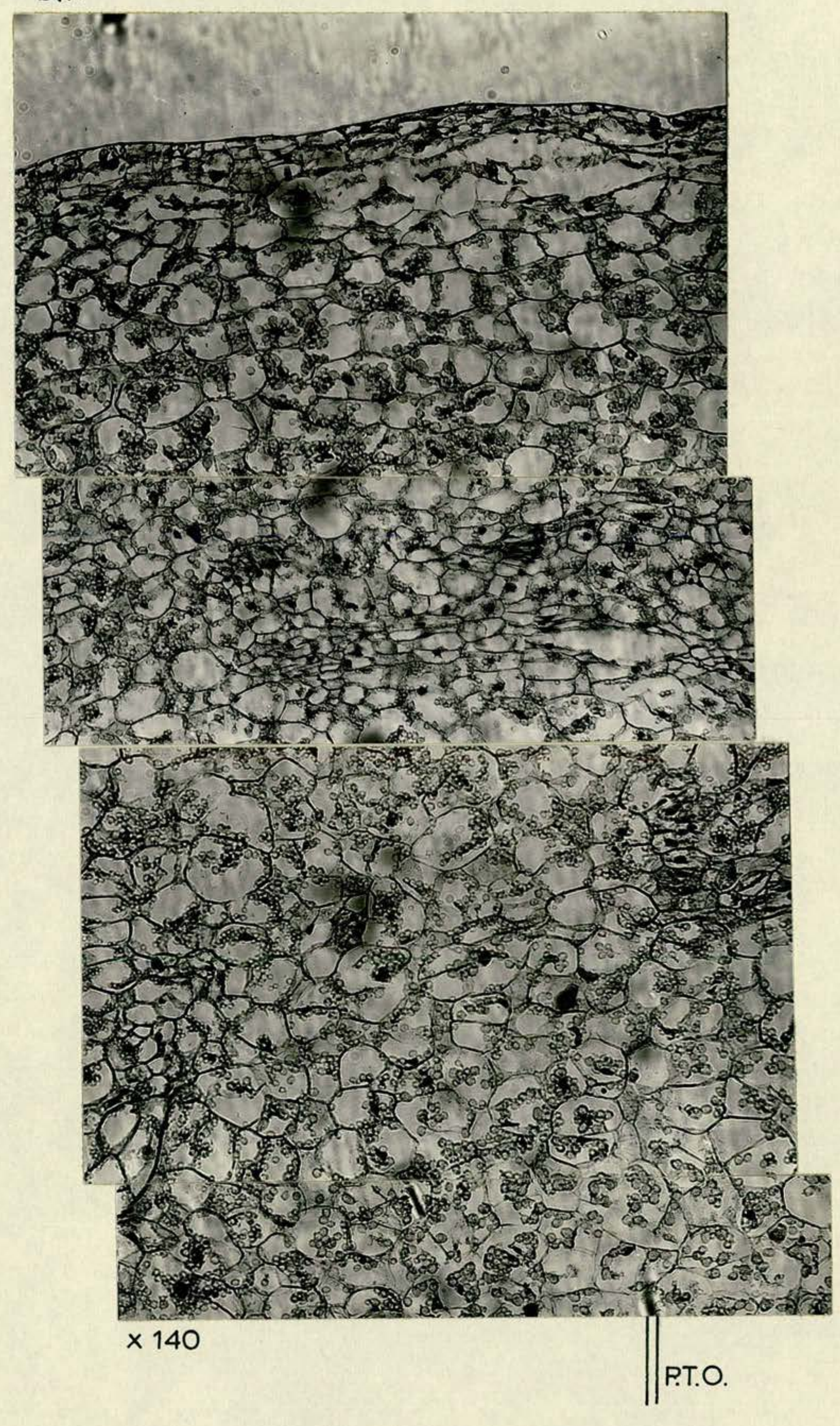
D = products of recent cell divisions - note new cell walls orientated in many different directions.

a.



x35

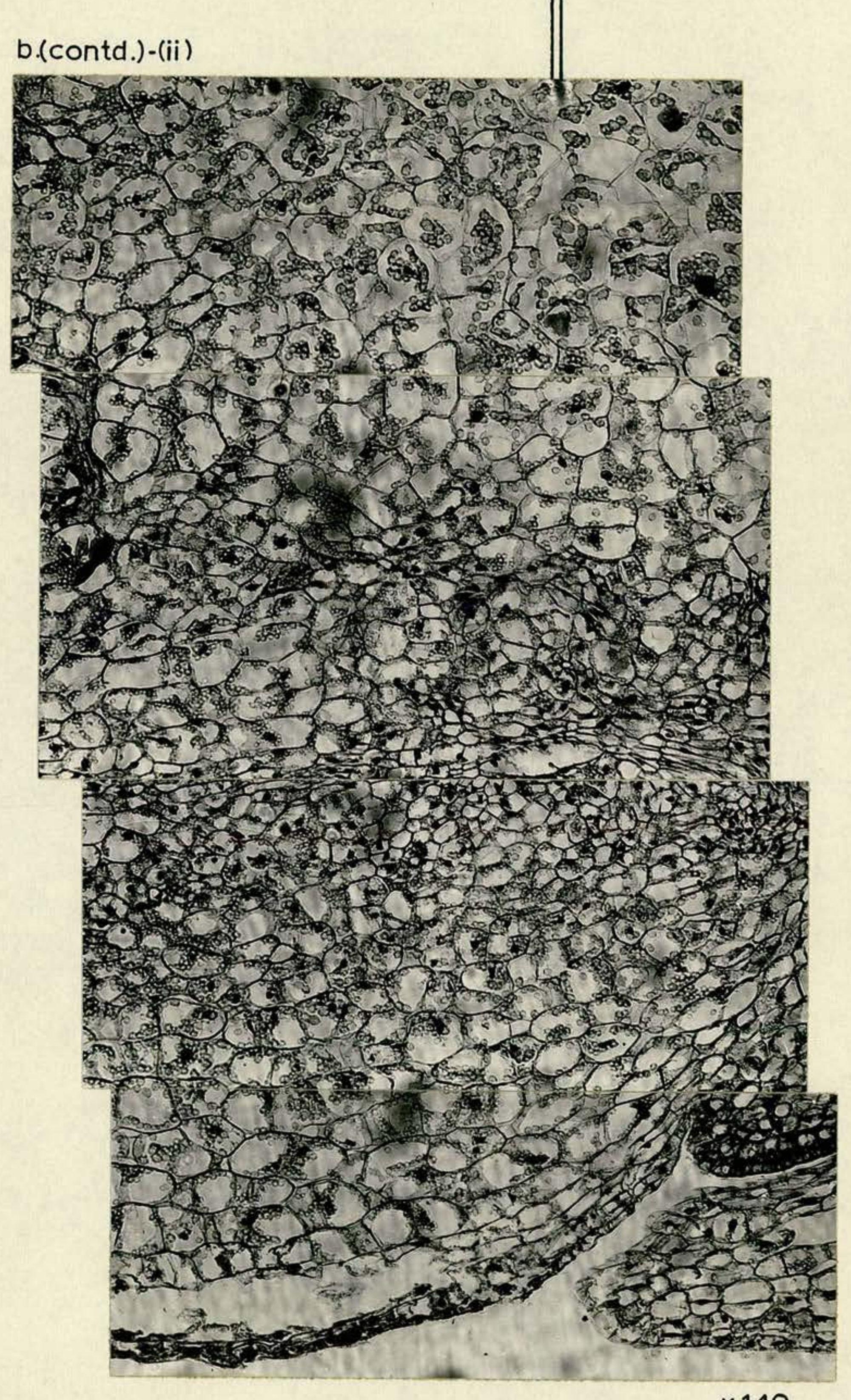
b(i)



x 140

P.T.O.

b.(contd.)-(ii)



x140

c.

x 280

SECTION VII GENERAL DISCUSSION

Synopsis of the argument and development of this section:

(i) Discussion of the results of the light break experiments described in Section IV, leading to the conclusion that it is likely that tuberisation is controlled by a stimulus of a hormonal nature.

(ii) Summary of the consideration of the growth substances which might possibly be involved as part of the tuberising stimulus (as discussed in Section V (i)).

(iii) Discussion of the results of the experiments involving the effect of growth-active substances on tuberisation, when these substances were applied to the whole plant and to individual stolon tips (Section V (ii) and (iii)), and also of the results of the extraction studies on stolon tips (Section V (iv)), together with the results of other workers, divided as follows:-

a. Discussion of the possible role of auxin in the control of tuberisation.

b. Discussion of the possible role of gibberellins.

c. Discussion of the possible involvement of growth inhibitors, in particular abscisic acid.

d. Discussion of the idea (supported by the results already described) of a control of tuberisation by daylength mediated by a change in the balance of gibberellins and naturally occurring growth inhibitors similar to that which has been proposed for the control of dormancy in the buds of woody species.

e. Discussion of the possible contribution of cytokinins.

(iv) Suggested scheme of action for the effects of daylength and growth substances (exogenous and endogenous) on tuberisation.

(v) Summary.

(i) Conclusions from light break experiments

It is generally agreed (see Section I) that in cultivated varieties of Solanum tuberosum L. the initiation of tuberisation is hastened by growing the plants in short day conditions and delayed by growing the plants in long day conditions (see eg Gregory, 1965). The response to daylength is quantitative, not qualitative. That this is the case in the variety Up-to-Date, which was used for the present work, was established in the Preliminary Experiments described in Section III. One of these experiments III(iv)) also showed, as found by Gregory (1956), that the greater the number of short day cycles given, the more tuberisation was promoted (see Fig 14).

The light break experiments described in Section IV examined the photoperiodic basis of this response of tuberisation to daylength. The first experiment showed that a white light break treatment in the middle of the long night of plants grown in short days was able to partially inhibit tuberisation, although no accompanying effect on the growth of the tops was detected (Table 6). Tuber number, number of tuberising stolons, tuber fresh weight and percentage of plants tuberised were all reduced by light break treatment, although not so greatly as in the low intensity long day control plants, in which all these parameters had a value of zero. These results show that the effect of daylength on tuberisation is a direct and truly photoperiodic one, not mediated by

different amounts of overall top growth. This conclusion is in agreement with that of Mokronosov and Lundina (1959) from light break treatment in S.demissum, and of Slater (1963) from his experiments with different amounts of total daily radiation at different daylengths, and with light break treatments given to plants of S.tuberosum grown in short days (see Section IV (i)). Slater concluded from the results of the first experiment that the photoperiodic effect found on tuberisation occurs independently of differences in total daily radiation. In the second of his experiments, the results with Arran Pilot are in complete agreement with those obtained in the first light break experiment (Section IV) in that a quantitative inhibitory effect on tuberisation, unaccompanied by detectable effects on top growth, was obtained with light break treatment.

The second and third light break experiments in Section IV again showed that tuberisation could be partially inhibited in plants grown in short days by light breaks given in the middle of the long night period. This effect was found with white and red treatments, and was sometimes, but not always, associated with a slight decrease in top height; no differences were found in top dry weight (Tables 7 and 8). These results confirm those of the first experiment, namely that the light break treatment caused reduction in tuberisation even when there was no effect of the growth of the tops (at least in terms of the parameters measured). and that the effect on tuberisation was therefore a genuine direct photoperiodic effect. It was unfortunately found impossible to demonstrate a reversal of the effect of a red light break by subsequent exposure to far-red light but, as

discussed in detail in Section IV, it was felt that this was probably due to the inadequacy of the techniques used. Especially, it was felt that the unavoidable time spent in changing the filter plates between the red and subsequent far-red treatments probably enabled Pfr to complete its inhibitory act before it could be re-converted to Pr by the far-red exposure.

The fourth and fifth light break experiments showed that a blue light break was also effective in partially inhibiting tuberisation, again with no detectable effect on the growth of the tops (Table 9). The implications of these results have already been fully discussed. To summarise:- It is possible that the inhibitory effect of the blue light break on tuberisation may be due to a photoreceptor such as that proposed by Mohr (1959, 1964) and still evoked by Esashi (1969) instead of or as well as phytochrome; it seems unlikely, however, that such a photoreceptor only is involved, since tuberisation is also inhibited by a red light break, which Mohr (1969) now considers to be exclusively phytochrome-mediated. It appears most likely, since similar effects can be achieved by light breaks in the red and in the blue regions of the spectrum, and since phytochrome is known to absorb light in both these regions (Siegelman and Butler, 1965; Hillman, 1967), that the inhibition of tuberisation by light break treatment is mediated by phytochrome. The only inconsistency with this explanation is the failure to reverse the red effect by far-red, which would have conclusively demonstrated the involvement of phytochrome, but, as mentioned before, this was probably caused by practical difficulties.

In any case, although it was not found possible to conclusively demonstrate phytochrome involvement, the light break experiments

described in Section IV did show, in agreement with those of Slater (1963) and Mokronosov and Lundina (1959), that the effect of daylength on tuberisation is a genuinely photoperiodic one.

The question which must be asked at this point is: Can this demonstration of a genuinely photoperiodic response of tuberisation to daylength be used to differentiate between the two theories of tuberisation: 1. tuberisation controlled by a specific tuberising stimulus, probably hormonal in nature and 2. tuberisation promoted by an increased supply, and inhibited by a decreased supply, of photosynthate from the tops to the stolons?

To consider first the second theory, a decreased supply of photosynthate supplied to the stolons, which would cause inhibition of tuberisation, could come about in two different ways. It could occur by a decrease in the total supply of photosynthate available in the plant (although this idea has been largely discounted: see Section I). It is not likely that this could be brought about by a very brief light break treatment, since both treated and untreated plants were subjected to the same amount of photosynthetic light during the short day period. A decreased carbohydrate supply to the stolons could also result from a change in the distribution of the available photosynthate within the plant, less being sent to the stolons and more to the tops, as proposed by workers such as Borah and Milthorpe (1962) - see Section I. It is possible that this is what happens when light break treatments are given to plants grown in short days. If this were the case, however, one would expect to find a difference at harvest in top dry weight. Such a difference was

not found in any of the light break experiments (see Section IV, Tables 6, 7 a, 8, 9). It is conceivable that the decreased amounts being sent to the stolons were so small that, although these were sufficient to partially inhibit tuberisation, they were insufficient to bring about an increase in the growth and dry weight of the tops.

While a change in the distribution of carbohydrate from stolons to tops brought about by the light break treatment cannot be entirely discounted, it nevertheless seems much more likely that the explanation of the effect lies in the operation of a hormonal tuberising stimulus, the formation of which is controlled by photoperiod. This mechanism does not require the correlation of any differences in top growth and dry weight with tuberisation in its early stages, and this explanation is therefore consistent with the results of the light break experiments of Section IV.

The results of other workers (eg Gregory, 1956; Chapman, 1958; Madec and Perennec, 1959; Madec, 1963; Okazawa and Chapman, 1962; Lovell and Booth, 1967; see Section I) have also provided a great deal of support for the involvement of a specific hormonal stimulus in tuberisation, probably made up of one or a combination of growth substances the level(s) of which may be altered by different environmental conditions, including day-length.

(ii) Summary of consideration of growth substances possibly involved in tuberisation

Many substances have been proposed as being implicated in the control of tuberisation, either as the whole or a part of the tuberising stimulus, or as antagonists of its action. As

discussed in detail in Section V (i), those most likely to be involved are those which might be expected, on the basis of their known properties, to exert a controlling action on one or more of the changes known to take place at the stolon tip prior to and at the time of tuber initiation. Proposed substances must also satisfy the criteria of site(s) of formation and mobility within the plant also discussed in Section V (i). From a consideration of these questions, it appears that the substances most likely to be involved in the control of tuberisation are endogenous auxins, gibberellins, cytokinins and growth inhibitors.

(iii) Discussion of the results of experiments with growth-active substances

a. The possible role of auxin

It was found (GA_3/1AA experiment, Section V (ii)) that although indole acetic acid sometimes caused small differences in the growth of the tops when applied to the intact plant, it had no effect on tuberisation on the basis of tuber number, number of tuberising stolons and tuber fresh weight (Table 19). The results of other workers using auxins (see Section V (i)) are somewhat conflicting. Support for their involvement in tuberisation has come from the work of Borah (1959), van Schreven (1956) and Tizio (1964a) who considered the effect to be promotive, and that of Gausman et al (1958), who considered the effect to be inhibitory. On the other hand, the work of Dostál (1945) with Ficaria and Ito and Kato (1951) with potato suggests that auxin is not specifically involved in the control of tuberisation. The GA_3/1AA experiment (Section V (ii)) lends support to the views of this second group of workers, and suggests that auxin is not involved in the control of tuberisation, at

least in S.tuberosum, variety Up-to-Date.

b. The possible role of gibberellin

Positive results have, however, been found in the course of the present studies for the participation in the control of tuberisation of the gibberellins. In the experiments in which gibberellic acid was applied to the growing point of the tops (gibberellic acid application experiments 1-7 and GA_3/1AA experiment, Section V (ii)), it was found that the effect on tuberisation was dependent upon whether one or several applications of gibberellic acid were given to the plants. Although one application (as given in experiments 1-5) was found sufficient to greatly increase the growth of the tops, it was not found to prevent or delay the initiation of tubers (Tables 11-15 and Fig 17). For the latter effect to be achieved, it was found necessary to repeat the applications of gibberellic acid on several occasions (as in experiments 6 and 7 and the GA_3/1AA experiment). Tuberisation in terms of tuber number, number of tuberising stolons and tuber fresh weight, was found to be markedly reduced by such repeated applications of gibberellic acid (see Tables 16, 17, 19). There are two possible explanations for the difference in the effect on tuberisation found with single and with repeated applications of gibberellic acid. Either there is a need for the maintenance of a continuously high level of gibberellin in the plant in order to prevent the initiation of tubers, or there is a need for enough gibberellic acid to saturate the ability of the tops (or more particularly of the sub-apical meristem) to use gibberellic acid in extension growth. In support of the latter possibility is the likelihood that, when applied to the tops, gibberellic acid would be drawn to the strongest sink,

usually the stem apex. The applied gibberellic acid would be especially likely to be used in the sub-apical meristem of the tops since it was adjacent to this region that it was applied. A single application might therefore be expected to be used up by top growth, but, with repeated applications, it would be likely that some gibberellic acid would also be available for transport to the stolons where it could inhibit the formation of tubers. This could partly explain the situation found under normal growth conditions in different daylengths. It is possible that in long days, enough endogenous gibberellin is produced to result in normal top growth and also the suppression of tubers, whereas in short days, in which lower levels of gibberellin are produced, (Okazawa, 1960), most of the available gibberellin is used by the growth of the tops. Tops are generally, if any difference is found, taller under long day conditions. If this is the case, it is only part of the explanation (see below).

There is not, however, a certain level of top height response above which gibberellin is automatically sent to the stolons and below which none is sent, all the gibberellin being used in top growth. That this is the case is shown by the fact (see, for example, Table 17, 18 day harvest) that a less than maximal top height response can be accompanied by a partial inhibitory effect on tuberisation. The inhibitory effect on tuberisation, is nevertheless, generally greater in the plants given higher levels of gibberellic acid treatment and exhibiting a greater top height response. This shows that the distribution effect of applied gibberellic acid is perhaps more complex than that suggested above, and may also suggest that the other possible mechanism of

action of repeated gibberellin application, namely the maintenance, over an extended period of time, of a high gibberellin level in the plant, may also be important.

It is strange in view of the above results that Dyson and Humphries (1966) were able to record a delay in tuber formation with a single application of gibberellic acid, given by soaking the seed pieces prior to planting for 1 hour in a 50 mg/l solution of gibberellic acid, a similar treatment to that given in experiment 5.

The results of the gibberellic acid application experiments are nevertheless in general agreement with those found by other workers (Rappaport, Lippert and Timm, 1957; Okazawa, 1959; Dyson and Humphries 1963, 1966; Lovell and Booth, 1967; Tizio 1964 b, 1966). It therefore seems clear that gibberellic acid, when applied to whole plants at a suitable concentration, causes retardation of tuberisation.

Evidence that endogenous gibberellins are actually involved in this way in the control of tuberisation in the normal plant was obtained from the result of further experiments in which the inhibitor of gibberellin biosynthesis, CCC was applied as a soil drench to whole plants. It was found that if CCC were applied just before the differential daylength period, when the plants were soon to be about to produce tubers, treatment brought about a promotion of tuberisation in terms of tuber number, number of tuberising stolons and tuber fresh weight, the effect being more marked in long day conditions (Table 22). When applied earlier in the life of the plant, however, when stolons were forming, the effect of CCC on tuberisation was not so noticeable (Table 21).

This was probably, in the case of plants grown in short days during the experimental period, because CCC tended to reduce stolon number (as would be expected on the basis of the work of Booth, 1963, who showed that gibberellic acid is promotive of stolon outgrowth). In the case of the plants grown in long days, there was no effect of CCC on stolon number, and it appeared possible that CCC brought about a promotion of tuberisation in these plants (Table 21 and Fig 19), although there were too many zeroes in the data to allow a valid statistical analysis to be performed. The fact that CCC relieved the partial inhibition of long days on tuberisation as well as enhancing the promotive effect of short days, provides more evidence for the idea that it is by raising the level of endogenous gibberellins that long days exert, at least in part, their inhibition.

The promotive effect of CCC on tuber formation was obtained at both low (Table 22) and at high temperatures (Table 23) but CCC treatment was unable to overcome the combination of unfavourable conditions of high temperatures and long days, in which no tuberisation at all was found (Table 23). This result may perhaps indicate that high temperature exerts its effect on tuberisation in a different way from that in which long days exert their effect, that is, not on the level of endogenous gibberellins.

It could be argued that CCC treatment promotes tuberisation because of the reduction in top growth brought about by lowering the level of synthesis of endogenous gibberellins. (The results of all three CCC application experiments showed that top height was markedly reduced, the effect being more pronounced at the

higher CCC concentration; this was also the case with the promotive effect of CCC treatment on tuberisation - see Tables 21, 22, 23). There was, however, no significant effect of CCC treatment on top dry weight by the time of harvest in experiments 2 and 3 (although such an effect was found when younger plants were treated, as in experiment 1). Therefore, although it remains a possibility that the effect of CCC on tuberisation is mediated by its effect on the growth of the tops, it seems more likely that it is due directly to a decrease in the level of endogenous gibberellins, which have an effect which is inhibitory of tuberisation.

The results of these experiments are in agreement with those of other workers (Dyson and Humphries 1963, 1966; Dyson, 1965; Humphries and Dyson, 1967; see Section V (ii)), with respect to the effects on top growth and on tuberisation.

The gibberellic acid and CCC application experiments (Section V (ii)), together with published data, suggest strongly that the level of endogenous gibberellins exerts an effect on tuberisation in the potato plant, low levels of these substances being more conducive to tuberisation than high levels.

It is not clear from the results of these experiments, however, how and where in the plant the effect of gibberellin level on tuberisation is exerted. Some light is thrown on this question by the experiments with gibberellic acid and CCC described in Section V (iii), in which these substances were fed directly to the stolon tips, while the stolons were still attached to the parent plant.

The stolon feeding experiments showed that gibberellic acid applied to the stolon tip directly caused a marked delay in tuberisation at concentration of both 1 and 10 ppm (Figs 22-25). Also, no effect of gibberellic acid treatment was noted on either top growth or the growth and tuberisation of stolons on the same plants but not subjected to gibberellic acid treatment (Table 26 and Fig 23 (b)); this suggests that the effect on tuberisation observed in the treated stolons was a local one brought about directly at the treated stolon tips and not mediated in any way by growth of the rest of the plant. These results are in agreement with those of McCorquodale and Moorby (1968) with excised stolon tips grown in vitro on agar to which was added gibberellic acid at concentrations of 0.1 and 1 ppm. McCorquodale and Moorby have also interpreted their results to mean that gibberellic acid exerts its inhibiting effect on tuberisation directly at the stolon tip. These experiments therefore provide good evidence for the idea that tuberisation is controlled by hormonal stimuli, and in particular that one of the growth substances involved in this control is gibberellin, which acts in a direction inhibitory to tuberisation.

Further experiments of the stolon feeding type were carried out using CCC in the bottles applied to the stolons instead of gibberellic acid (see Section V (iii)). These studies showed (Figs 26-29 and Tables 27 and 28) a marked promotion of tuberisation by CCC treatment, again without any effect on tops or untreated stolons. These results suggest that some gibberellin synthesis, enough to affect the progress of tuberisation, normally occurs at the stolon tip, and that when this is unable

to occur due to the presence of CCC, tuberisation at the stolon tip is hastened. As pointed out in the account of the CCC stolon feeding experiments (Section V (iii)), this does not mean that gibberellin supplied from the tops to the stolons is not involved in the control of tuberisation; the gibberellic acid application experiments described in Section V (ii) showed that such gibberellin also had an effect. It does demonstrate, however, the likelihood that gibberellin synthesised in the stolon tips has some contribution to make. The temporary reduction in the gibberellin level at the stolon tip, brought about by the inhibition of local synthesis by CCC, is thought to be enough to cause promotion of tuberisation before more gibberellin arrives at the stolon tips from the tops.

These results disagree with those of McCorquodale and Moorby (1968) with cultured stolon tips in vitro. They found no promotion of tuberisation when CCC was included in the growth medium, and deduced that gibberellin synthesis in the stolon is not important for the control of tuberisation. Possible reasons for this discrepancy between the two sets of results have been given in the account of the experiments in Section V (iii); briefly, it was thought to occur because of different levels of endogenous gibberellins in the two types of material, grown under different conditions.

The role of gibberellins in tuberisation (as an inhibitory influence) is further supported by the work of Okazawa (1960) on extraction of endogenous gibberellin-like substances from potato plant tops under different environmental conditions (see Section V (i)). While these findings indicate a correlation of low

gibberellin levels throughout the plant with conditions which are promotive of tuberisation, the extraction studies described in Section V (iv) of the present work are more directly relevant to an evaluation of the influence of gibberellin on the stolon tip itself. It was found (see Table 33) that green stolons (which do not normally tuberise) had the highest levels of gibberellin-like substances of all the stolons examined, and that total stolon tip samples from plants grown in long days, which tend to inhibit tuberisation, have higher levels than similar samples from plants grown in short days, which tend to promote tuberisation. Also, stolons showing visible signs of tuberisation were found to have lower levels of gibberellin-like substances than stolons showing no such visible signs of tuberisation. These results have been confirmed by Smith and Rappaport (1969) in the variety of S. tuberosum Red Pontiac; they showed that stolons without visible tubers contained considerably more gibberellin-like activity than did the young tubers harvested at the same time.

The studies described in Section V (iv) have further shown differences in the levels of gibberellin-like substances in stolons in stages of tuberisation prior to visible swelling. Lovell and Booth (1967) found that starch deposition occurred in the stolon tips prior to tuber initiation; this has been confirmed and the sequence of deposition in the various tissues of the stolon tip determined (Section VI). Stolons on the point of tuberisation, then, are found to possess deposits of starch in the cortex or in the cortex and medulla. Extractions of stolon tips which had not visibly tuberised, and which were in

different developmental states as shown by the presence or absence of starch deposition, were also carried out. It was found that those tips with no starch deposition had higher levels of gibberellin-like substances than those in which starch deposition had begun, the latter being nearer to visible tuberisation. The fact that the rise in gibberellin-like activity occurs <u>before</u> the appearance of the visible swelling is good evidence that endogenous gibberellins have some role in controlling the process. It was also found that stolons from plants which had received repeated apical applications of gibberellic acid had higher levels of gibberellin-like substances; this seems to indicate that gibberellic acid applied to the tops does indeed, directly or indirectly, raise the level of gibberellin in the stolon tips.

These results show that the level of gibberellin-like substances in stolon tips appears to be inversely correlated with tuberisation, or the nearness of untuberised tips to the onset of visible tuberisation.

From a consideration of the results of all the experiments using gibberellic acid and CCC, and those of the extraction studies of stolon tips, it therefore appears that gibberellin level in the stolons of the potato plant is inversely correlated with the progress of tuberisation, and that tuberisation may be brought about by reducing the level of gibberellins at the stolon tips, either by cutting down the supply from the tops, or by inhibiting synthesis at the tip itself. If gibberellin level in the tops is boosted beyond that which can be readily used in the growth of the shoot apex itself, tuberisation may be retarded by the export

of the extra gibberellin to the stolons, where it inhibits tuberisation.

Further extractions of stolon tip material which might prove informative would be extractions from plants which had been treated with CCC or from stolon tips treated directly with CCC; it would be expected on the basis of the above arguments that gibberellin levels in such stolon tips would be found to be lower than controls which had received no CCC treatment. Time did not allow these experiments to be done.

Another point of interest relevant here is that the order in which different types of stolons have been found to tuberise (see Section III (vii)) is that which would be expected if gibberellins acted to inhibit tuberisation. The tips of short unbranched stolons tuberised before the tips of long, branched stolons. The work of Lovell and Booth (1969) has demonstrated that stolon growth shows an initial lag phase, and the later formed the stolon, the longer the lag phase. Their work also suggests that the short, unbranched stolons are those which have been initiated last, and these stolons would therefore be growing more slowly than the others at the time of tuber initiation. They will therefore be less active sinks than the longer branched stolons which were initiated earlier, and which are in their phase of rapid growth at the beginning of tuberisation in the plant. The short stolons will therefore receive less gibberellin from the tops, and in addition, since they are growing more slowly, they will also be producing less gibberellin themselves. The gibberellin level in these short stolons will therefore be lower

than in long branched stolons, and tuberisation in them will be more readily brought about as a result.

The earliest tuberisation of all was found at side branches of long branched stolons. Lovell and Booth (1969) found that such side branches grew much more slowly than the main axis; it would be expected that the apical bud in such a stolon would be the main sink for gibberellin entering the stolon, so that very little would reach the side branches. The latter would therefore have very low levels of gibberellin and would hence be expected to tuberise early. That the order of tuberisation in different types of stolon tip was that expected from the above predictions, is therefore consistent with the hypothesis that gibberellin acts as an inhibitor of tuber initiation.

The results of the topping experiment (see Section III), in which removal of the apex and very young leaves led to promotion of tuberisation, also lends support to an inhibitory role of gibberellins, since these compounds have been shown to be produced in stem apices and young leaves (Jones and Phillips, 1966).

c. <u>The possible involvement of growth inhibitors</u>

It appears, however, that endogenous gibberellin is not the only factor to be involved in the control of tuberisation. There is also a growing body of evidence to suggest that endogenous growth inhibitors are also involved.

The results of the extraction of gibberellin-like substances from stolon tips (Section V (iv)) show evidence for the presence of a growth inhibitor or inhibitors in some of the extracts (see

Table 34). Most of the extracts showing evidence for growth-inhibitor activity were those from visibly-tuberised stolon tips or from tips which might be expected to be about to tuberise. These results are in agreement with those of Okazawa (1960) with tops and Booth (1963) and Smith and Rappaport (1969) with stolons. These workers have shown that the level of a growth inhibitor or inhibitors rises at the onset of tuberisation. It has been suggested that this inhibitor is abscisic acid (El Antably et al, 1967). A role of abscisic acid in promoting tuberisation is attractive if, as appears to be the case (see above), gibberellins are involved as tuber-inhibiting substances, in view of the negative interactions found between abscisic acid and gibberellins in many different physiological processes (see Section V (i)). Evidence for the presence of abscisic acid in potato tissues has been presented in Sections V (i).

Also relevant to a possible role of abscisic acid in tuberisation are the studies with synthetic abscisic acid which have shown a number of responses, all connected with the initiation or maintenance of dormancy or the cessation of extension growth, the situation which is found in the stolon tip at tuber initiation (eg Eagles and Wareing (1964), El-Antably et al (1967), Aspinall, Paleg and Addicott (1967)).

In one of the present studies (Section V (ii)), abscisic acid treatment of the apical growing point was found to have no effect on the top or stolon growth or tuberisation of plants grown in short days during the differential daylength (experimental) period (Table 24). In plants grown in long days

during this period, however, although there was no effect on top height, node number and top dry weight were slightly decreased by treatment; an increase was found in tuber number, number of tuberising stolons and the number of plants tuberised (Table 24 and Fig 21).

This result is in agreement with that of El-Antably et al (1967) who found (see Section V (i)) a promotive effect of abscisic acid on tuberisation when applied as a foliar spray to whole plants grown in long days.

Different results, however, were found by Smith and Rappaport (1969), who conducted similar experiments to those of El-Antably et al with potatoes of S. tuberosum, variety White Rose, grown under long day conditions. They were unable to induce tuber formation by daily applications of solutions of 1 mg/l abscisic acid as a spray applied to the leaves. The work of Claver (1970), using sprouts of the variety of S. tuberosum Katadhin, confirms the findings of Smith and Rappaport. Claver found that the growth of the sprouts was inhibited by abscisic acid treatment, inhibition being proportional to the concentration used; abscisic acid was also found to inhibit the formation of tubers, although this effect was smaller in "old" sprouts, which had been left attached to the mother tuber for 4 weeks before use than in "young" sprouts, which had been separated from the mother tuber promptly after sprouting.

Smith and Rappaport (1969) have commented that they find the results of El-Antably et al (1967) unconvincing because

of the requirement for very large doses of abscisic acid to induce tuber formation, the low percentage of tubers formed on treated plants, the formation of tubers on control plants and the stunted appearance and early leaf senescence in the treated plants. The last two effects were noted by the investigators and led them to question whether the effect of abscisic acid was a direct one. Smith and Rappaport also point out that in extracts from young tubers of the variety Red Pontiac, chromatographed according to the method of Mitchell (1958), they observed that the bulk of inhibitor activity migrated to the Rf range 0.4-0.5. Since the Rf of abscisic acid in Mitchell's developing solvent is 0.69, they consider this to be further evidence against abscisic acid being the inhibitor responsible for tuber formation.

Although there was a small effect of abscisic acid treatment on top growth in the present work in the plants kept in long days (Table 24), the plants certainly did not appear abnormal or stunted; their appearance was quite normal. Nor was there any need for massive doses of abscisic acid to bring about the promotive effect on tuberisation which was observed. It is, however, possible that the effect of abscisic acid on tuberisation was not a direct one, and was mediated by an effect on the growth of the tops, perhaps due to a reduction in the level of endogenous gibberellins.

The results of the abscisic acid stolon feeding experiment (Section V (iii)) suggest that this was in fact the case.

The only effects of abscisic acid on top growth or on the untreated stolons (see Table 29) were very small and probably unreal. In the treated stolons, abscisic acid appeared to have very little effect on tuberisation at the lower concentration, and an inhibitory effect at the higher concentration (Figs 30 and 31). There was certainly no evidence to suggest that abscisic acid, when applied to the stolon tip, promotes tuberisation.

The results of this experiment are in agreement with those obtained by McCorquodale and Moorby (1968) with cultured stolon tips in vitro, in which abscisic acid was supplied in the culture medium at concentrations up to 1 ppm. They found that abscisic acid stopped growth of the stolons, although their appearance was quite normal, and on being returned to the basal medium, their growth was resumed. Abscisic acid was found to have very little effect on tuberisation at concentrations lower than 1 ppm, whereas an inhibition of tuberisation was found at the highest concentration (1 ppm). Smith and Rappaport (1969) have confirmed these results, finding no effect of abscisic acid on tuberisation in an experiment in which stolon tips were treated with a solution of 1 mg/l abscisic acid, and in another in which the compound was incorporated into the culture medium of excised stolon tips grown in vitro.

These findings are also confirmed by those of Palmer and Smith (1969 b) who, in addition to finding no promotion of tuberisation with abscisic acid, found instead an inhibition in stolons

cultured _in vitro_ (see Section V (iii)).

From the results of all these experiments, therefore, it seems unlikely that abscisic acid has a direct promotive effect on tuberisation at the stolon tip.

Very little is known about how abscisic acid causes its growth inhibiting effect on plants, although various suggestions have been put forward. Wareing, Good and Manuel (1968) have proposed that abscisic acid may act in some cases as an inhibitor of gibberellin biosynthesis. It has also been proposed (Thomas, Wareing and Robinson, 1965; Chrispeels and Varner, 1967) that the inhibitor may act as a specific gibberellin antagonist _in vivo_, although in other tests, the two substances appear to act independently (Robinson and Wareing, 1964; Milborrow, 1966). Abscisic acid has also been shown to interact with hormones other than the gibberellins (eg Aspinall et al, 1967; van Overbeek, Loeffler and Mason, 1967). There is also evidence to suggest that abscisic acid has some role in the control of nucleic acid and protein synthesis, perhaps by an effect on RNA synthesis (Villiers, 1968; Wareing et al, 1968) or DNA synthesis (van Overbeek et al, 1967). If this were the case, it would not be surprising to find interactions between abscisic acid and growth promotive hormones; such interactions could occur at many different points between the site of hormone action and the ultimate effects.

The mode of action of abscisic acid as an inhibitor of gibberellin

biosynthesis would be consistent with the result found in the experiment in which abscisic acid was applied to the apex of whole plants, in which treatment only promoted tuberisation significantly in long days. It is possible that gibberellin levels were generally too high in these plants to allow tuberisation to begin (see Section V (iv)), and that this was able to take place when the levels were reduced. In short days, however, the level of gibberellins in the plants may be lowered sufficiently by the effect of the environmental conditions alone to allow tuberisation to commence, so that no effect of abscisic acid would be detected.

If it is correct that abscisic acid exerts its effect by inhibiting gibberellin synthesis, it might be expected that a promotion of tuberisation would also be obtained with abscisic acid application directly to the stolon tips.(as was found with CCC, Section V (iii)), but this was not the case. It is possible, however, that this was not found because of an inhibitory action of abscisic acid on cell division; evidence for such an action is provided by the fact that in all the stolon studies described above, abscisic acid caused cessation of stolon elongation. This effect is the opposite of what was found with gibberellic acid (marked promotion of elongation), presumably because of its action on cell division in the sub-apical meristem. The action of abscisic acid at the stolon tip, therefore, may be twofold, operating on both gibberellin metabolism and on cell division. At the lower concentration, the effect on gibberellin metabolism may have been small enough to be easily overcome by gibberellins arriving from the tops, and the effect on cell

division may have been small enough not to completely prevent tuberisation; the result of these two effects would be that the progress of tuberisation would proceed fairly normally. At the higher concentration, however, when the effect of abscisic acid on gibberellin metabolism might be expected to result in a promotion of tuberisation, it is possible that the concomitant increased inhibitory effect on cell division resulted in a marked inhibition of growth of the stolon or, in stolons which would otherwise develop tubers, an inhibition of tuber formation. That this may be the case is supported by the high percentage of starch deposition in stolons without visible signs of tuberisation at the high abscisic acid concentration in the stolon feeding experiment (see Section V (iii), Fig 31).

It seems unlikely, in view of the results of the experiments described above, no matter what possible effects it may have at the stolon tip, that abscisic acid is directly involved in the control of tuberisation in the normal plant. This conclusion is supported by the results of Smith and Rappaport (1969) on the chromatographic behaviour of the inhibitor extracted by them from tuberising stolons (see above).

d. Possible daylength control of the balance between gibberellins and growth inhibitors

Although abscisic acid does not appear to be involved, however, there is good evidence to suggest that an inhibitor of some kind is involved in the control of tuberisation. The appearance of this inhibitor is usually correlated with the onset of tuberisation or conditions which promote it (ie short days) and with lowered levels of endogenous gibberellins (Okazawa, 1960;

Booth, 1963; Smith and Rappaport, 1969 and studies in Section V (iv)), and is therefore expected to act in a way opposite to the gibberellins in the control of tuberisation.

Such a mutual control of tuberisation by gibberellins (acting to inhibit or delay tuberisation) and a natural growth inhibitor or inhibitors (acting to promote or hasten tuberisation) would be consistent with the results of the topping experiment (Section III) and the results obtained by Okazawa and Chapman on pruning of potato plants with forked stems, if gibberellins are considered to be produced in the stem apex and very young leaves (Jones and Phillips, 1966) and endogenous growth inhibitors in the young mature leaves (Wareing, 1954; Waxman, 1957).

Such a control would also be consistent with the findings of workers who have investigated bud dormancy in woody species; such a parallel would be understandable, since the control of tuberisation may be thought of as another aspect of the control of dormancy.

Interaction of gibberellins and abscisic acid has been found in the control of dormancy in _Betula_ buds (Eagles and Wareing, 1964), and reciprocal changes in the levels of endogenous inhibitors and gibberellins apparently correlated with dormancy or release from dormancy, occur in many woody species (eg Eagles and Wareing, 1954; Digby and Wareing, 1966). Wareing (1969) considers that the evidence suggests that dormancy in buds is regulated by a balance between endogenous gibberellins and growth

inhibitors such as abscisic acid.

In this connection, it is also interesting that Perennec (1966) has found that induction of tuberisation in potato by short days is always accompanied after transfer to long day conditions by an inhibition of the growth of buds on the aerial parts, and that the strength of this inhibition increases with the length of exposure to short day cyles, becoming total and permanent when the irreversible induction of tuberisation is attained. The inhibition can be relieved by the removal, before transfer to long days, of all or some of the leaves which have received the induction by short days. Perennec has also found that the stimulating effect of gibberellic acid on stem elongation diminishes as the inhibition exerted by the leaves on the buds increases. He considers that these facts suggest the presence, in the leaves subjected to short days, of a factor inhibiting to buds, of which the action appears to be antagonistic to that of the gibberellins, and that its properties imply that it must form a part or the whole of the tuberisation stimulus.

It has been proposed (Galston and Davies, 1969) that under natural conditions, the balance of the levels of these two types of compound may result from a divergent phytochrome-controlled isoprenoid pathway, which produces predominantly gibberellic acid in long photoperiods and predominantly abscisic acid, or some other inhibitor or inhibitors, in short photoperiods, although this is disputed by Mohr (1972). From the evidence presented above, it seems likely that this type of control, brought about

by the above, or some other similar type of mechanism, may be involved in tuberisation in the potato. Long days, which are less inductive of tuberisation, may cause the formation of high levels of gibberellins and low levels of growth inhibitors (which probably do not include abscisic acid) and short days, which are more inductive of tuberisation, may cause the formation of low levels of gibberellins and high levels of growth inhibitors.

It would appear from Comparison 4 in the extraction studies described in Section V (iv) that exogenously-applied gibberellic acid does not act by lowering the level of endogenous growth inhibitors; plants treated with exogenous gibberellic acid have stolons which have very high levels of gibberellin-like substances, and which may also have quite high levels of growth-inhibitors (see Table 34).

It therefore seems most likely that the effect of daylength is to alter the balance of gibberellins and endogenous growth inhibitor(s), the effect being exerted separately on each type of growth substance, and that it is the nature of this balance at the stolon tip which determines whether or not it will tuberise. High gibberellin/growth inhibitor levels delay or prevent tuberisation and low gibberellin/growth inhibitor levels promote it. Any factor which lowers the value of this ratio will tend to promote tuberisation.

The likelihood of the participation of growth inhibitors as well as gibberellins in the control of tuberisation necessitates some modification of the explanation proposed above for the relation

between top height, tuberisation and daylength.

One possibility is that in normal long day conditions, enough gibberellin is produced for the growth of the tops and also to suppress tuberisation. The levels of growth inhibitors in such plants are low, and this also leads to an inhibition of tuberisation. In plants grown in short days, however, the levels of growth inhibitors are higher, tending to promote tuberisation. Enough gibberellin is again produced for top growth but, since less total gibberellin is produced, less is left to be sent to the stolons, so that the delaying influence of gibberellins is less strongly felt. Under these conditions, tuberisation will be promoted by short days (because of the lower gibberellin/growth inhibitor ratio) and delayed by long days. Also, even though the same amount of gibberellin may be required and used for top growth in short days or long days, tops of plants grown in long days will tend, if anything, to be taller than those grown in short days because of the higher growth inhibitor levels in the latter.

Another possibility would be that the same amounts of gibberellin are produced in both short days and long days, but that a greater proportion of them is diverted to the stolons in long days. The tops, however, could still make as great or greater growth in long days as in short days because of the lower levels of growth inhibitors in long days. This seems less likely to be the case, however, in view of Okazawa's (1960) results on the different levels of gibberellin-like substances in tops of plants grown under short or long day conditions, in which he found lower levels in short days.

e. The possible contribution of cytokinins

The remaining factor to be considered is the possible participation of the cytokinins in the control of tuberisation.

The results of the stolon feeding experiments using kinetin (Section V (iii)) showed no effect on the growth of the tops or untreated stolons (Tables 30 and 31), and very little effect on treated stolons (Figs 32-35). There was a slight inhibitory effect on tuberisation of treated stolons with concentrations of 10^{-4} and 10^{-5}M at the stolon tips, but this appeared to be compensated for to a large extent by tuberisation at side buds, especially in the first experiment, so that very little effect on total tuberisation was found. There was no effect of the lowest concentration 10^{-6}M (experiment 1). There was certainly no evidence for any promotive effect of kinetin on tuberisation in the system used.

These results do not agree with those of Palmer and Smith (Palmer and Smith 1969 a and b, 1970, Smith and Palmer, 1970), who consider that they have demonstrated a requirement for cytokinins in the tuberisation of isolated stolons grown in vitro (see Section V (i)). Possible reasons for the difference between the present results and those of Palmer and Smith are given in Section V (iii), the most likely of which appears to be that it is caused by the different systems used in the two groups of experiments. It is considered that in the stolon feeding system used in the present work, sufficient cytokinins to allow normal tuber formation to occur are supplied from the parent plant, to which the stolons are still attached, in long day as well as in short day conditions (since no promotion of

tuberisation occurred even under long days). In excised stolon tips, as used by Palmer and Smith, however, it is considered that cytokinins must be supplied in the culture medium to allow tuberisation to occur, perhaps because they are necessary to establish a sink for metabolites, or to allow normal cell division, because there is no cytokinin supply from the parent plant.

It might be expected that if cytokinins promoted tuberisation, they would be found to act in a way opposite to the gibberellins (which appear to delay or inhibit tuberisation) in other systems also. With a few exceptions, however (eg the studies on growth of Wittwer and Dedolph, 1963), the effects of the two groups of compounds have been found to be parallel (see Letham, 1967), eg the work on flowering of Michniewicz and Kamienska (1964, 1965) with cytokinin, and of Lang and Reinhard (1961) with gibberellic acid. It is, however, possible that gibberellins and cytokinins may act on different stages of the tuberisation process.

Palmer and Smith (1969 b) have proposed that "in potato the importance of abscisic acid and other endogenous growth inhibitors may be to inhibit the activity of gibberellins and arrest stolon elongation, allowing the tuber inducing hormones, cytokinins, to exert their effect." It is possible to re-phrase this: "In potato the balance of abscisic acid and other endogenous growth inhibitors and gibberellins may determine the type of growth at the stolon tip. When this is arrested, tuberisation will take place, provided that an adequate supply of factors for cell division and enlargement (including cytokinins and photosynthate) is available." On the basis of the present work, it is thought

most likely that the latter alternative is the correct one, although it is possible, but perhaps unlikely for the reasons given in the account of the second stolon feeding experiment with kinetin, that the lack of effect in the present studies was due to difficulties in penetration or transport of the applied cytokinins.

(iv) Suggested scheme of action for daylength and growth substances

a. How many growth substances are involved?

As discussed in Section V (i), from the literature concerning their effects in other systems, all three groups of growth substances mentioned above (gibberellins, growth inhibitors and cytokinins) would appear to be capable of exerting a control on one or more of the microscopically visible changes which occur at the tuberising stolon tip, although the present work suggests that only the first two groups are in fact directly involved. It is possible that some or all of the processes occurring in the tuberising tip may be controlled separately by one or several hormones, or that the initiation of one of the processes by one or more hormones automatically leads to the sequence of events observed.

From what is known about the hormonal control of other physiological processes in plants (see, for example, Galston and Davies, 1969), it would seem more likely that more than one growth substance is involved; work on dormancy in buds in particular would suggest that tuberisation is likely to be controlled by a balance of growth promoting and growth inhibiting hormones, and the work described here appears to confirm that this is probably the case, gibberellins being the

growth promoting hormones involved and the growth inhibitor(s) being inhibitor(s) other than abscisic acid. The identity of the growth inhibitor involved is completely unknown, but it is perhaps relevant that a new class of inhibitors, the batatasins, have very recently been isolated from yam bulbils by Hashimoto, Hasegawa and Kawarada (1972).

It appears likely from the work of Lang (1956, 1960) and Sachs et al (1959, 1960) on sub-apical meristems that the balance of these two types of growth substance may act to promote or inhibit cell division in the sub-apical meristem of the stolon. Cell elongation may also be controlled by this balance, although there is less evidence on this point.

It also seems likely, for example from the work of Chrispeels and Varner (1966, 1967) that this balance may also control the level of starch synthesis in the stolon, the endogenous growth inhibitor acting in the same sort of way with gibberellin as abscisic acid has been observed to do.

As mentioned above, it is possible that the control of tuberisation is carried out by such a balance of gibberellins and inhibitors alone, or that this balance merely stops extension growth of the stolon, allowing some other stimulus, perhaps a cytokinin, to exert its effect; further work is need to clarify this point.

It is envisaged that when these changes are brought about at the stolon tips by the combined actions of the growth substances involved, the characteristics of the stolon tip sinks also change, and this change then alters the pattern of

translocation of various substances within the plant. This changed pattern of translocation is, however, not involved in the initiation of tuberisation, and comes about entirely as a result of tuber initiation.

b. Suggested scheme of action

A suggested scheme to explain and summarise the effects on tuberisation of the various factors investigated in the course of the present work is presented in Fig 51; the action of the various factors is interpreted in terms of their proposed effects on the balance of gibberellins and naturally occurring growth inhibitors both in the plant as a whole, and at the stolon tips in particular.

(v) Summary

In the potato, S. tuberosum, tuberisation appears to be controlled by a hormonal stimulus, and not merely by secondary effects of differences in the growth of the tops caused by different environmental conditions.

While carbohydrates and perhaps cytokinins are required for tuberisation, among other compounds, it appears from the results of the present studies that neither of these classes of compound is the true tuberising stimulus.

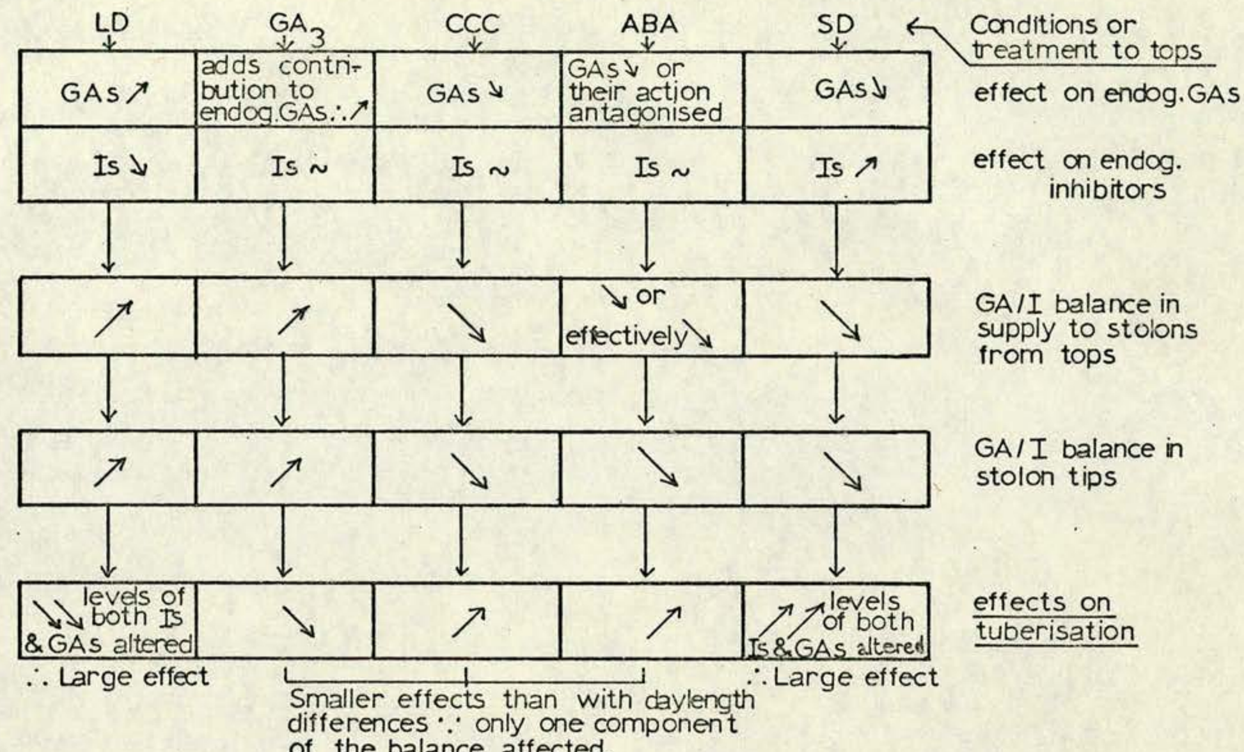
It seems more likely that the stimulus consists of a balance between endogenous gibberellins and endogenous growth inhibitors (although probably not abscisic acid). Tuberisation appears to be brought about by environmental conditions or treatments which cause a lowering of this ratio of gibberellins to endogenous growth inhibitors at the stolon tips (examples being short

FIGURE 51. Suggested scheme of action for effects of daylength and growth-active substances on tuberisation.

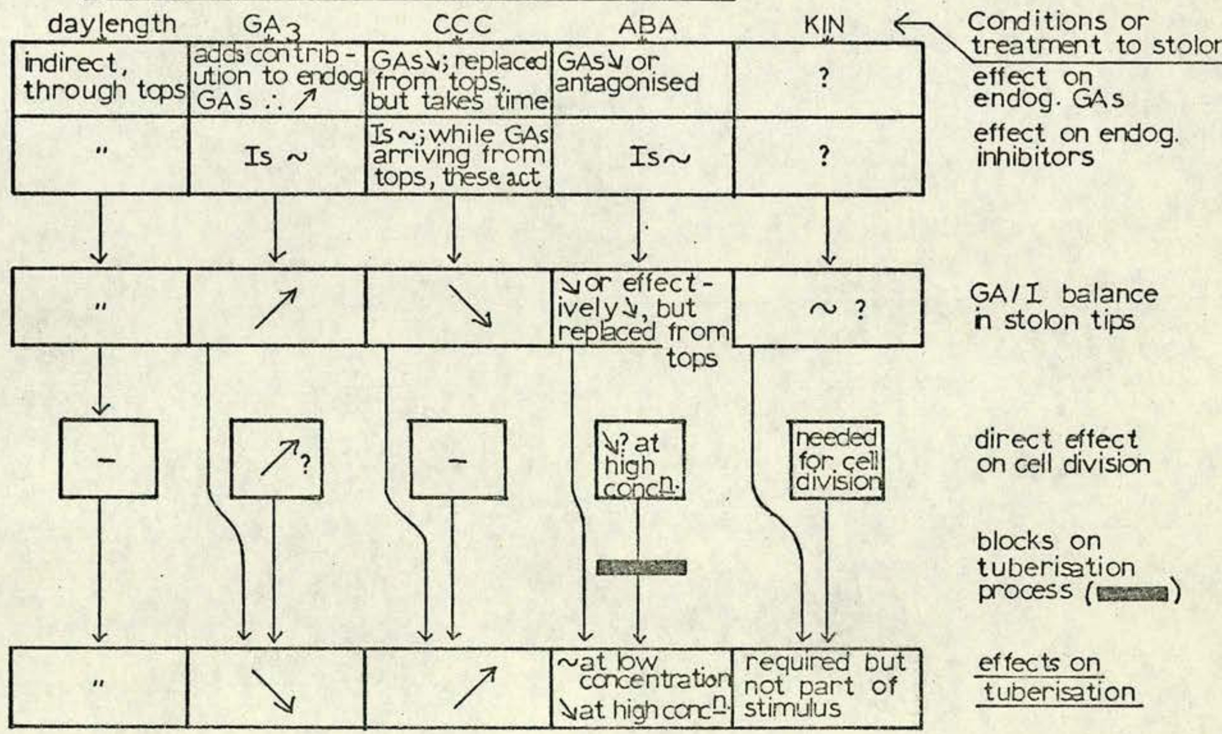
The action of the various factors is interpreted in terms of their proposed effects on the balance of gibberellins on naturally-occurring growth inhibitors both in the plant as a whole, and at the stolon tips in particular.

LD = long days; SD = short days
↗ = level raised; ↘ = level lowered;
∼ = level unaffected
GAs = endogenous gibberellins;
Is = endogenous inhibitors.

SCHEME FOR EFFECTS ON TOPS



SCHEME FOR EFFECTS ON STOLONS DIRECTLY



GAs = endogenous gibberellins

Is = endogenous inhibitors

↗ = level increased; ↘ = level decreased; ~ = level unaffected

photoperiods, treatment of the tops with growth inhibitors such as CCC or abscisic acid, and treatment of the stolon tips themselves with CCC); it has also been proposed that the mother tuber exerts an influence promotive of tuberisation, perhaps because it adds to the supply of endogenous growth inhibitors.

The hypothesis proposed above is supported by studies, in the present work and by other workers, in which various growth-active substances have been applied to whole plants, to individual stolon tips still attached to the parent plant, or to isolated stolon tips *in vitro*. It is also supported by the measurements which have been made of the levels of endogenous gibberellin-like substances and growth inhibitors in tops and stolon tips subjected to different environmental conditions, or in different developmental states with respect to tuberisation. Although the control of tuberisation, by daylength in particular, appears to be mediated by a change in the level of the endogenous gibberellin/growth inhibitor ratio, the requirements found generally for carbohydrates and by some workers for cytokinins are probably for the enlargement of the tuber once it has been initiated, although the role of the cytokinins is far from clear.

SECTION VIII APPENDIX

(i) Analysis to determine whether there was any effect of the source of tuber material on the parameters measured at harvest

Figs 52-55 shows graphs in which various parameters (top dry weight, stolon number, tuber number and tuber fresh weight) are plotted against age of the plant. The purpose of these was to attempt to determine whether there was any effect of the source of the tuber material on these parameters, and therefore if there was any variation between experiments which was caused by the source of material. Each experiment, as mentioned in Section II, used material from a single source, so that such variation, if it did exist, was never involved within experiments. Attempts have been made on the graphs to delimit areas containing all the points obtained with one source of material (solid and broken lines). It can be seen from the graphs of top dry weight and stolon number (Figs 52 and 53) that some separation of points based on source of material is possible for these parameters, ie the points obtained from material from one source tend to occur in one area of the graph, suggesting that each batch of material had its own characteristic growth rate, and its own characteristic amount of stolon production. In the case of tuber number and fresh weight, however (Figs 54 and 55), no such separation appeared possible, thus indicating that the tuberisation response of plants is largely independent of the source of the tuber material from which they are grown.

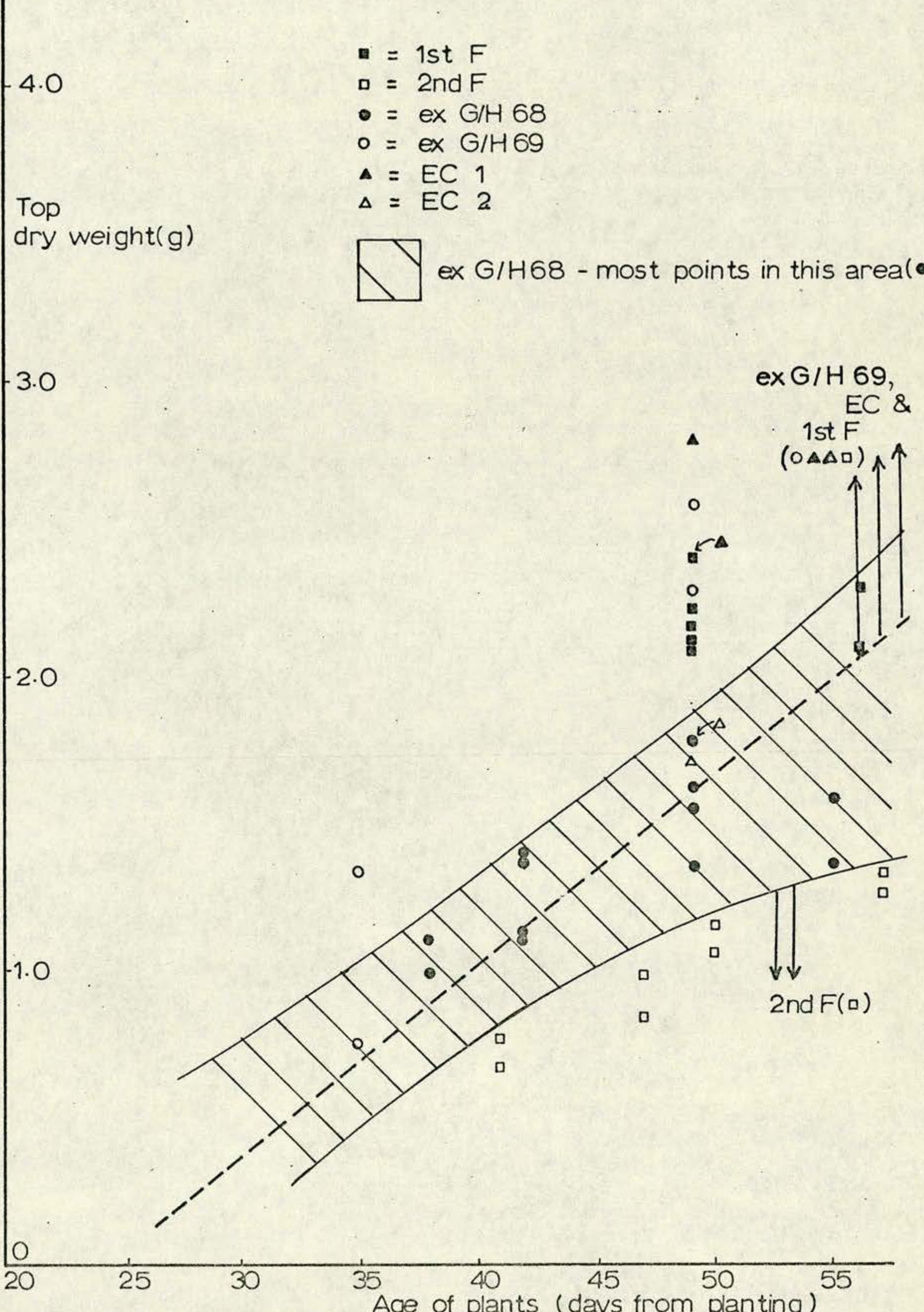
This study, therefore, demonstrates that, while top and stolon growth are to some extent affected by the source of the tuber material, tuberisation is apparently largely independent of this.

FIGURE 52. Results of analysis to determine effect of source of tuber material on parameters measured at harvest (Section VIII (i)). Graph of top dry weight against age of plants from planting for plants grown from material from different sources.

1st F	=	first supply of material obtained from Buchan Potato Growers Ltd, Fraserburgh.
2nd F	=	second supply of material from Fraserburgh.
ex G/H68	=	material from plants grown in the glass house at the Botany Department, Edinburgh and harvested in 1968.
ex G/H69	=	material similarly grown and harvested in 1969.
EC 1	=	first supply of material obtained from the A.S.S. Station, East Craigs, Edinburgh.
EC2	=	second supply from East Craigs.

Points are means of several values; replication level varies with experiment from which points were obtained.

(These abbreviations apply to Tables 52-55).



■ = 1st F
□ = 2nd F
● = ex G/H 68
○ = ex G/H 69
▲ = EC 1
△ = EC 2
ex G/H68 - most points in this area(●)
Top dry weight(g)
4.0
3.0
2.0
1.0
0
ex G/H 69, EC & 1st F (○▲△□)
2nd F(□)
20
25
30
35
40
45
50
55
Age of plants (days from planting)

FIGURE 53. Results of analysis to determine effect of source of tuber material on parameters measured at harvest (Section VIII (i)). Graph of stolon number against age of plants from planting for plants grown from material from different sources.

For abbreviations, see legend to Fig. 52.

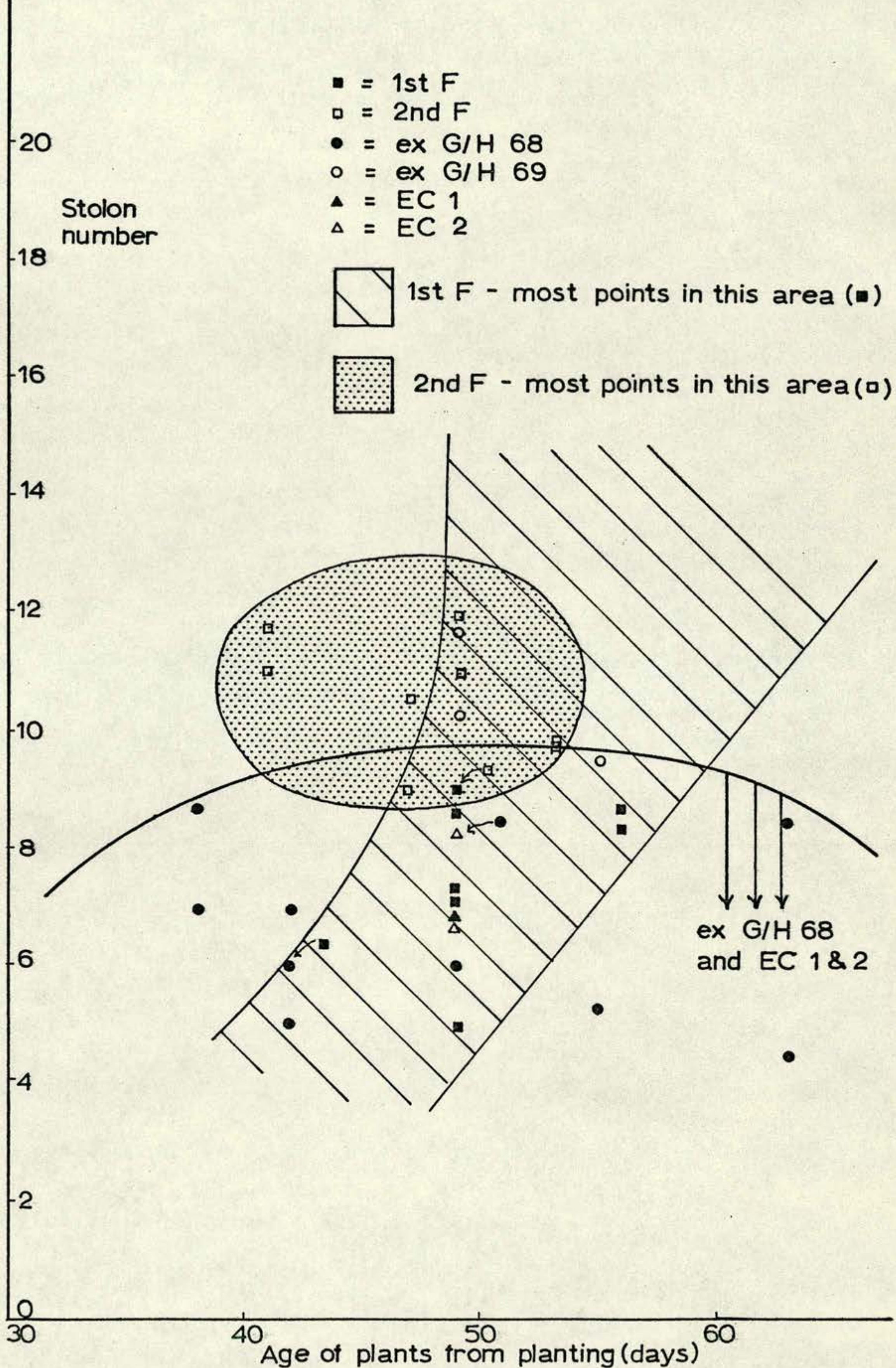

■ = 1st F
□ = 2nd F
● = ex G/H 68
○ = ex G/H 69
▲ = EC 1
△ = EC 2
1st F - most points in this area (■)
2nd F - most points in this area (□)
Stolon number
20
18
16
14
12
10
8
6
4
2
0
30
40
50
60
Age of plants from planting (days)
ex G/H 68 and EC 1 & 2

FIGURE 54. Results of analysis to determine effect of source of tuber material on parameters measured at harvest (Section VIII (i)). Graph of tuber number against age of plants from planting for plants grown from material from different sources.

For abbreviations, see legend to Fig. 52.

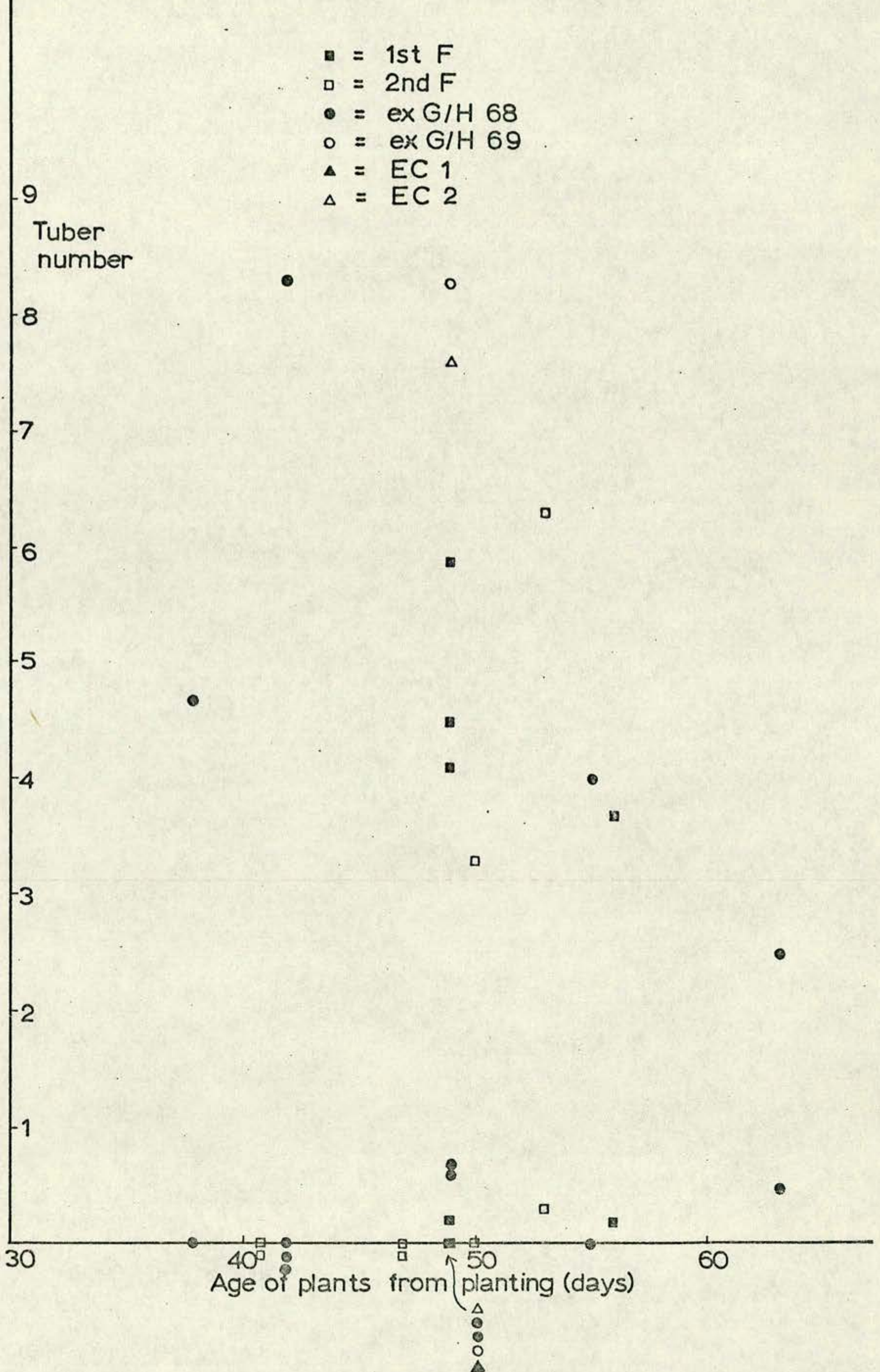
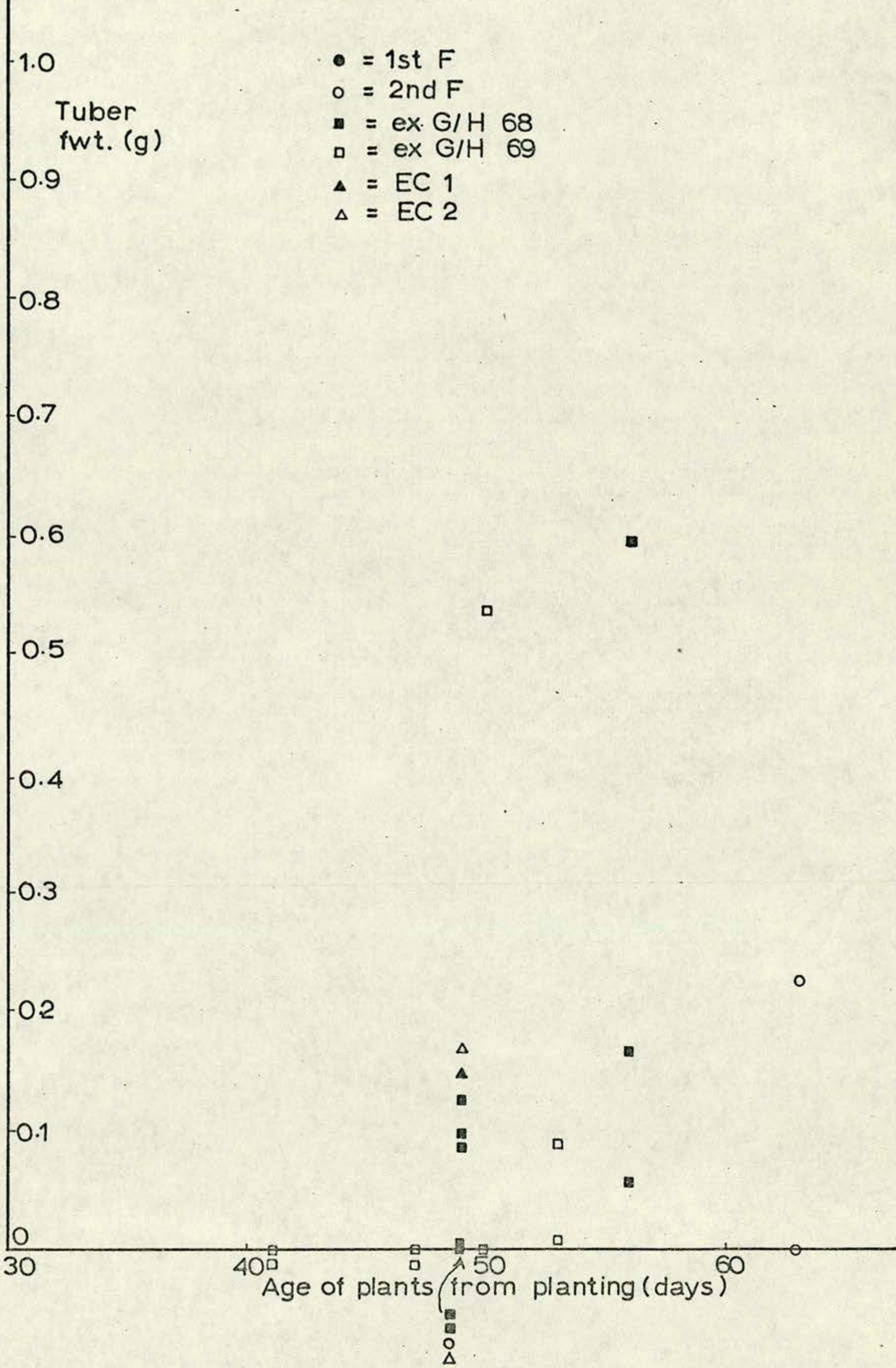

■ = 1st F
□ = 2nd F
● = ex G/H 68
○ = ex G/H 69
▲ = EC 1
△ = EC 2
Tuber number
9
8
7
6
5
4
3
2
1
30
40
50
60
Age of plants from planting (days)

FIGURE 55. Results of analysis to determine effect of source of tuber material on parameters measured at harvest (Section VIII (i)). Graph of tuber fresh weight against age of plants from planting for plants grown from material from different sources.

For abbreviations, see legend to Fig. 52.



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Tuber
fwt. (g)
● = 1st F
o = 2nd F
■ = ex G/H 68
□ = ex G/H 69
▲ = EC 1
△ = EC 2
30
40
50
60
Age of plants from planting (days)

The results of separate experiments, as far as tuberisation is concerned, would thus not be expected to show differences caused by the source of material.

(ii) Nutrient solution

The make up of the nutrient solution used throughout the experiments is given in Table 35 (below).

TABLE 35 Composition of Hoagland's No 1 solution (after Hoagland and Arnon (1938).

Major nutrients (g/l of administered solution):-

KH_2PO_4	0.1361
KNO_3	0.5056
$Ca(NO_3)_2$	1.805
$MgSO_4$	0.4930
+ FeEDTA	

Micronutrients (mg/l of administered solution:-

H_3BO_3	28.6
$MnCL_2.4H_2O$	18.1
$ZnSO_4.7H_2O$	2.2
$CuSO_4.5H_2O$	0.8
$H_2MoO_4.H_2O$	0.9

(iii) Key to abbreviations

The following is a key to the abbreviations used in all the Tables in which harvest data are presented.

Abbreviations used throughout Tables of results for parameters measured at harvest

Top ht.(cm)	=	Height of tops (cm)
Node no.	=	Number of nodes on stems of tops
Top dwt.(g)	=	Dry weight of tops (g)
Root dwt.(g)	=	Dry weight of roots (g)
Stolon no.	=	Total number of stolons produced
Tuber no.	=	Number of tubers (visibly swollen stolon tips and side buds)
Tub. stolon no.	=	Number of stolons bearing visible tuber(s)
Tuber fwt.(g)	=	Total fresh weight (g) of tubers
Non-tub. stolons	=	Stolons which have not visibly tuberised
With starch	=	Number of non-visibly tuberised stolons which have starch deposits in their tissues.
No starch	=	Number of non-visibly tuberised stolons which have no signs of starch deposition (see Section VI) in their tissues
% with starch	=	Percentage of non-visibly tuberised stolons which have starch deposits
No. plants tub.	=	Number of plants tuberised, expressed as a ratio of the total number of plants in the treatment ie 2/3 = two plants tuberised out of three, and as a percentage (bracketed values)

Replication	=	Level of replication of the treatment. All values in the columns above are means of this number of original values (except for number of plants tuberised)
SD	=	Short days
LD (LILD)	=	Long days (low intensity long days)
SDC	=	Short day controls
LDC	=	Long day controls
S.A.	=	Results of statistical analysis. Level of significance of variance ratio from the analysis of variance (p) and least significant difference (LSD) are quoted. Analyses did not usually include the data for the long day controls; where these data were included the appropriate values of p and LSD are underlined in the Table; ns = not significant.

Harvest dates are quoted as the number of days from the beginning of the differential daylength (experimental) period.

SECTION IX LITERATURE CITED

Addicott, F.T., J.L. Lyon, K. Ohkuma, W.E. Thiessen, H.R. Carns, O.E. Smith, J.W. Cornforth, B.V. Milborrow, G. Ryback and P.F. Wareing (1968) Science, N.Y. 159, 1493.

Arney, S.E. and P. Mancinelli (1966) New Phytol. 65 : 161-175.

Arora, N., F. Skoog and O.N. Allen (1959) Am.J.Bot. 46 : 610.

Arthur, J.M., J.D. Guthrie and J.M. Newell (1930) Am.J.Bot. 17 : 416.

Artschwager, E. (1924) J. agric. Res. 27 : 809.

Aspinall, D., L.G. Paleg, F.T. Addicott (1967) Aust. J. Biol. Sci. 20 : 869.

Barnes, M.F., E.N. Light and A. Lang (1969) Planta 88 : 172.

Bates, G.H. (1943) Nature, Lond. 152 : 135.

Beaumont, J.H. and J.G. Weaver (1931) Proc. Am. Soc. hort. Sci. 28 : 285.

Bennett-Clark, T.A. and N.P. Kefford (1953) Nature, Lond. 171 : 645.

Bernard, N. (1902) Revue gén. Bot. 14 : 1, 58, 101, 139, 170, 219, 269.

Blommaert, K.L.J. (1954) Nature, Lond. 174 : 970.

Blumenthal-Goldschmidt, S. and L. Rappaport (1965) Pl. Cell Physiol., Tokyo 6 : 601.

Bodlaender, K.B.A. (1958) Jaarb. Inst. biol. scheik. Onderz. Landb-Gewass. : 45.

Bodlaender, K.B.A. (1960) Jaarb. Inst. biol. scheik.Onderz. LandbGewass : 69.

Bodlaender, K.B.A. (1963) in The Growth of the Potato, Proc. 10th Easter School in agric. Sci, Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe Eds., Butterworths, Lond. pp 199-210.

Boo, L. (1961) Physiologia Pl. 14 : 676.

Booth, A. (1963) in The Growth of the Potato, Proc. 10th Easter School in agric. Sci., Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe Eds., Butterworths, Lond. pp 99-113.

Booth, A. and P.F. Wareing (1958) Nature, Lond. 182, 406.

Boothby, D. and S.T.C. Wright (1962) Nature, Lond. 196, 389.

Borah, M.N. (1959) The effect of light intensity, length of day and temperature on growth and tuber formation in the potato. Ph.D. thesis, Univ. of Nottingham.

Borah, M.N. and F.L. Milthorpe (1959) Rep. Univ. Nottingham Sch. Agric. 41.

Borah, M.N. and F.L. Milthorpe (1962) Indian J. Pl. Physiol. 5: 53

Borthwick, H.A. (1964) Am. Nat. 98 : 347.

Borthwick, H.A. and R.J. Downs (1964) Bot. Gaz. 125 : 227.

Bottomley, W., N.P. Kefford, J.A. Zwar and P.L. Goldacre (1963) Aust. J. biol. Sci. 16 : 395.

Brian, P.W. and H.G. Hemming (1957) Naturwissenschaften 44 : 594.

Brian, P.W. and H.G. Hemming (1958) Ann. Bot., N.S. 22 : 1.

Brian, P.W., H.G. Hemming and M. Radley (1955) Physiologia Pl. 8 : 899.

Bushnell, J. (1925) Tech. Bull. Minn. agric. Exp. Stn. No. 34.

Burg, S.P. and E.A. Burg (1965) Science, N.Y. 148 : 1190.

Burt, R.L. (1961) Some effects of temperature on the growth and development of the potato. Ph.D. thesis, Univ. of Nottingham.

Burt, R.L. (1964a) Eur. Potato J. 7 : 197.

Burt, R.L. (1964b) Aust. J. biol. Sci. 17 : 867.

Burton, W.G. (1952) New Phytol. 51 : 154.

Burton, W.G. (1963) in The Growth of the Potato, Proc. 10th Easter School in agric. Sci., Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe, Eds., Butterworths, London., pp 17-40.

Burton, W.G. (1966) in The Potato: A Survey of Its History and of Factors Influencing its Yield, Nutritive Value, Quality and Storage, Veenman, H. and N.V. Zonen, Wageningen.

Butler, W.L., S.B. Hendricks and H.W. Siegelman (1964) Photochem.& Photobiol. 3 : 521.

Catchpole, A.H. and J. Hillman (1969) Nature, Lond. 223 : 1387.

Cathey, H.M. and N.W. Stuart (1961) Bot. Gaz. 123 : 51.

Chapman, H.W. (1955) Am. Potato J. 32 : 207.

Chapman, H.W. (1958) Physiologia Pl. 11 : 215.

Claver, F.K. (1960) Phyton, B. Aires 15, 29.

Claver, F.K. (1966) Phyton, B. Aires 23, 5.

Claver, F.K. (1970) Phyton, B. Aires 27, 25.

Cleland, R. (1964) Physiologia Pl. 17,126.

Clor, M.A. (1967) Nature, Lond. 214, 1263.

Courduroux, C. J. (1966). Thèse, Univ. de Clermont Ferrand.

Chrispeels, M.J. and J.E. Varner (1966) Nature, Lond. 212 : 1066.

Chrispeels, M.J. and J.E. Varner (1967) Pl. Physiol., Lancaster 42 : 1008.

Cumming, B.G., S.B. Hendricks and H.A. Borthwick (1965) Can. J. Bot. 43 : 825.

Dale, J.E. and G.M. Felippe (1968) Planta 80 : 288.

Davis, G.E. (1941) Am. Potato J. 18 : 266.

Denisen, E.L. (1953) Proc. Am. Soc. hort. Sci. 62 : 411.

de Vries : see V

Digby J. and P.F. Wareing (1966). Ann. Bot., N.S. : 30 : 607.

Doposcheg-Uhlár, J. (1911) Flora, Jena 102, 24.

Doroschenko, A.E., G.D. Karpechenko and E. Nesterov (1960) Trudȳ prikl. Bot. Genet. Selek. 23, No. 2 ; 31.

Dostál, R. (1945) Bull. int. Acad. tchèque, Cl. Sci. math. nat. Méd. 46, No. 1.

Downs, R.J. and H.W. Siegelman (1963) Pl. Physiol., Lancaster 38 : 25.

Driver, C.M. and J.G. Hawkes (1943) Techn. Commun., imp. Bur. Pl. Breed. Genet. No. 36. Lond. : Imp. Agric. Bur.

Dyson, P.W. (1965) J. Sci. Fd Agric. 16 : 542.

Dyson, P.W. and E.C. Humphries (1963) Rothamsted Exp. Stn. Rep. p 89.

Dyson, P.W. and E.C. Humphries (1966) Ann. appl. Biol. 58 : 171.

Eagles, C.F. and P.F. Wareing (1964) Physiologia Pl. 17 : 697.

Edelman, J. (1963) in discussion at end of chapter by Madec, P., in The Growth of the Potato, Proc. 10th Easter School in agric. Sci., Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe Eds., Butterworths, Lond.

El-Antably, H.M.M., P.F. Wareing and J. Hillman (1967) Planta 73, 74.

Esashi, Y. (1969) Pl. Cell Physiol., Tokyo, 10 : 583.

Frankland, B. and P.F. Wareing (1960) Nature, Lond. 185 : 255.

Fredericq, H. (1964) Pl. Physiol., Lancaster 39 : 812.

Galston, A.W. and P.J. Davies (1969) Science, N.Y. 163 : 1288.

Garner, W.W. and H.A. Allard (1923) J. agric. Res. 23 : 871.

Gausman, H.W., E.G. Corbett and R.A. Struchtemeyer (1958) Am. Potato J. 35 : 742.

Goldsmith, M.H.M. (1969) in Physiology of Plant Growth and Development, Wilkins, M.B., Ed., McGraw-Hill, Lond., pp 127-162.

Gregory, L.E. (1954) Some factors controlling tuber formation in the potato plant. Doct. dissert., Univ. of Calif., Los Angeles.

Gregory, L.E. (1956) Am. J. Bot. 43 : 281.

Gregory, L.E. (1965) Encyclopedia of Plant Physiology, Ruhland, W., Ed. 15/1 : 1328.

Gunning, B.E.S. and W.K. Barkley (1963) Nature, Lond. 199 : 262.

Haber, A.H. and H.J. Luippold (1960) Pl. Physiol., Lancaster 35 : 168.

Hanke, H.J., K.M. Hartmann and H. Mohr (1969) Planta 86 : 235.

Harada, H. (1960) Annls. Physiol. vég., Paris 2 : 249.

Hartmann, K.M. (1966) Photochem.& Photobiol. 5 : 349.

Hartmann, K.M. (1967a) Z. Naturf. 22b : 1172.

Hartmann, K.M. (1967b) Book of Abstracts, European Photobiology Symposium, Hvar (Jugoslavia) pp 29-32.

Hartmann, K.M. (1967c) Naturwissenschaften 54 : 544.

Harvey, R.B., A. Reichenberg, B. Lehner and P.C. Hamm (1944) Pl. Physiol., Lancaster 19 : 186.

Hashimoto, T. (1961) Bot. Mag., Tokyo 74 : 110.

Hashimoto, T., K. Hasegawa and A. Kawarada (1972) Planta 108, 369.

Headford, D.W.R. (1962) Eur. . Potato J. 5 : 14.

Helgeson, J.P. (1968) Science, N.Y. 161 : 974.

Hemberg, T. (1949) Physiologia Pl. 2 : 24.

Hemberg, T. (1952) Physiologia Pl. 5 : 115.

Hendricks, S.B. (1959) in Photoperiodism and Related Phenomena in Plants and Animals, Withrow, R.B. Ed., AAAS, Washington, pp 423-438.

van Hiele, F.J.H. (1955) Pootaardappelhandel 9, 1.

Hillman, W.S. (1967) A. Rev. Pl. Physiol. 18, 301.

* Humphries, E.C. (1963) Ann. Bot., N.S., 27 : 517-532.

Humphries, E.C. and P.W. Dyson (1967) Ann. appl. Biol. 60 : 333.

Humphries, E.C. and S.A.W. French (1960) Ann. appl. Biol. 48 : 177.

Humphries, E.C. and S.A.W. French (1965) Ann. appl. Biol. 55 : 159.

Ito, H. and T. Kato (1951) Tohoku J. agric. Res. 2 : 1.

Jacobs, W.P. (1952) Am. J. Bot. 39 : 301.

Jones, R.L. (1968) Planta 81 : 97.

Jones, R.L. and I.D.J. Phillips (1966) Pl. Physiol., Lancaster 41 : 1381.

Jones, R.L. and J.E. Varner (1967) Planta 72 : 155.

Kasperbauer, M.J., H.A. Borthwick and S.B. Hendricks (1963) Bot. Gaz. 124 : 444.

Kato, T. (1958) Science, N.Y. 128 : 1008.

Katsumi, M. (1962) Physiologia Pl. 15 : 115.

Kende, H., H. Ninnemann and A. Lang (1963) Naturwissenschaften 50 : 599.

Kluge, M., E. Reinhard and H. Ziegler (1964) Naturwissenschaften 51 : 145.
Kopetz, L.M. (1937) Züchter 9 : 181
Kopetz, L.M. and O. Steineck (1954) Züchter 24 : 69.

Krijthe, N. (1948) Versl. Landbouwk. Onderz. Ned. 54 : 1.

Krijthe, N. (1955) Neth. J. agric. Sci. 3 : 291.

Krug, H. (1960) Eur. Potato J. 3 : 47-49, 107.

Krug, H. (1963) in The Growth of the Potato , Proc. 10th Easter School in agric. Sci, Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe Eds., Butterworths, London pp 221-229.

Kuraishi, S. (1959) Scient. Pap. Coll. gen. Educ. Tokyo 9 : 67.

Kuraishi, S. and F.S. Okumura (1956) Bot. Mag., Tokyo 69 : 300.

Lang, A. (1956) Naturwissenschaften 12 : 284.

Lang, A. (1960) Planta 54 : 498.

Lang, A. and E. Reinhard (1961) Adv. Chem. Ser. 28 : 71.

Letham, D.S. (1963) N.Z. Jl Bot. 1 : 336.

Letham, D.S. (1967) A. Rev. Pl. Physiol. 18 : 349.

Linschitz, H., V. Kasche, W.L. Butler and H.W. Siegelman (1966) J. biol. Chem. 241 : 3395.

Linschitz, H. and V.Kasche (1967) Proc. natn. Acad. Sci. U.S.A. 58 : 1059.

Lippert, I.F., L. Rappaport and H. Timm (1958) Pl. Physiol., Lancaster 33 : 132.

Lockhart, J.A. (1956) Naturwissenschaften 12 : 284.

Lockhart, J.A. (1960) Pl. Physiol., Lancaster 35 : 129.

Lovell, P.H. and A. Booth (1967) New Phytol. 66 : 525.

Lovell, P.H. and A. Booth (1969) New Phytol. 68 : 1175.

Madec, P. (1961) Annls Physiol. vég. 3 : 209.

Madec, P. (1963) in The Growth of the Potato, Proc. 10th Easter School in agric. Sci., Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe Eds., Butterworths, Lond., pp. 121-131.

Madec, P. and P. Perennec (1959) Eur. Potato J. 2 : 22.

Madec, P. and P. Perennec (1962) Annls Physiol. vég. 4 : 5.

Madison, M. and L. Rappaport (1968) Pl. Cell Physiol., Tokyo 9 : 147.

Magrou, J. (1938) C. r. Séanc. Soc. Biol. 127 : 793.

Magrou, J. (1939) C. r. Séanc. Soc. Biol. 130 : 1163.

Mann, J.D., K.H. Yung, W.B. Storey, M. Pu and J. Conley (1967) Pl. Cell Physiol., Tokyo 8 : 613.

McClelland, T.B. (1928) J. agric. Res. 37 : 603.

McComb, A.J. (1964) Ann. Bot., N.S. 28 : 669.

McCorquodale, I.A. and J. Moorby (1968) paper presented to S.E.B. Conference, Glasgow, July 1968.

Mes, M.G. and I. Menge (1954) Physiologia Pl. 7 : 637.

Michniewicz, M. and Kamienska, A. (1964) Naturwissenschaften 51 : 295.

Michniewicz, M. and Kamienska, A. (1965) Naturwissenschaften 52 : 623.

Michniewicz, M. and A. Lang (1962) Planta 58 : 549.

Milborrow, B.V. (1966) Planta 70 : 155.

Milborrow, B.V. (1967) Planta 76 : 93.

Milthorpe, F.L. (1962) TagBer. dt. Akad. LandwWiss. Berl. 48 : 11.

Miller, C.O. (1956) Pl. Physiol., Lancaster 31 : 318.

Miller, C.O. (1961) A. Rev. Pl. Physiol. 12 : 395.

Mitchell, L.C. (1958) J. Ass. off. agric. Chem. 41 : 182.

Mohr, H. (1959) Planta 53 : 219.

Mohr, H. (1964) Biol. Rev. 39 : 87.

Mohr, H. (1969) in Physiology of Plant Growth and Development, Wilkins, M.B., Ed., McGraw-Hill, Lond. pp. 509-556.

Mohr, H. (1972) in Lectures in Photomorphogenesis, Springer-Verlag, Berl. and N.Y.

Mokronosov, A.T. and T.N. Lundina (1959) Dokl. Akad. Nauk SSSR 127 : 924.

Molliard, M. (1915) C. r. hebd. Séanc. Acad. Sci., Paris 161 : 531.

Molliard, M. (1920) C. r. Séanc. Soc. Biol. 83 : 1257.

Möthes, K. (1960) Naturwissenschaften 47 : 337.

Möthes, K. (1964) in Régulateurs naturels de la Croissance Végétale (Colloque International, Gif-sur-Yvette, 1963). Centre National de la Recherche Scientifique, Paris, pp 131-140.

Möthes, K. and L. Engelbrecht (1959) Mber. dt. Akad. Wiss. Berl. 1 : 367.

Murray, D. (1968) Light and leaf growth of Phaseolus. Ph.D. thesis, University of Edinburgh.

Ninnemann, H., J.A.D. Zeevaart, H. Kende and A. Lang (1964). Planta 61: 229.

Nösberger, J. and E.C. Humphries (1965) Ann. Bot., N.S. 29 : 579.

Oortwijn Botjes, J.G. (1927) Tijdschr. PlZiekt. 33 : 1.

Osborne, D.J. and M.K. Black (1964) Nature, Lond. 201 : 97.

Osborne, D.J. and C.C. McCready (1965) Nature, Lond. 206 : 679.

van Overbeek, J., J.E. Loeffler and M.I.R. Mason (1967) Science, N.Y. 156 : 1497.

Okazawa, Y. (1955) Proc. Crop Sci. Soc. Japan 23 : 247.

Okazawa, Y. (1959) Proc. Crop Sci. Soc. Japan 28 : 129.

Okazawa, Y. (1960) Proc. Crop Sci. Soc. Japan 29 : 121.

Okazawa, Y. (1969) Proc. Crop Sci. Soc. Japan 38 : 25.

Okazawa, Y. (1970) Proc. Crop Sci. Soc. Japan 39 : 171.

Okazawa, Y. and H.W. Chapman (1962) Physiologia Pl. 15 : 413.

Okazawa, Y. and H.W. Chapman (1963) Physiologia Pl. 16 : 623.

Paleg, L.G. (1960) Pl. Physiol., Lancaster 35 : 293.

Palmer, C.E. and O.E. Smith (1969a) Nature, Lond. 221: 279.

Palmer, C.E. and O.E. Smith (1969b) Pl. Cell Physiol., Tokyo 10: 657.

Palmer, C.E. and O.E. Smith (1970) Pl. Cell Physiol., Tokyo 11: 303.

Perennec, P. (1966) Bull. Soc. fr. Physiol. vég. 12 : 175.

Penot, M. (1963) C. r. hebd. Séanc. Acad. Sci., Paris 256 : 488.

Penot, M. (1964) C. r. hebd. Séanc. Acad. Sci., Paris, 258 : 2881.

Phillips, I.D.J. and R.L. Jones (1964) Planta 63 : 269.

Plaisted, P.H. (1957) Pl. Physiol., Lancaster 32 : 445.

Pohjakallio, O. (1951) Acta Agric. scand. 1 : 153.

Pohjakallio, O. (1953) Physiologia Pl. 6 : 140.

Pratt, L.H. and W.R. Briggs (1966) Pl. Physiol., Lancaster 41 : 467.

Racca, R.W. and Tizio, R. (1968) Eur. Potato J. 11 : 213.

Rappaport, L., L. F. Lippert and H. Timm (1957) Am. Potato J. 34 : 254.

Razumov, V.I. (1960) Bot. Zh. SSSR 45 : 939.

Robinson, P.M. and P.F. Wareing (1964) Physiologia Pl. 17 : 314.

Sachs, R.M., C.F. Bretz and A. Lang (1959) Am.J. Bot 46 : 376.

Sachs, R.M., A. Lang, C.F. Bretz and J. Roach (1960) Am.J. Bot. 47 : 260.

Sachs, T. and K.V. Thimann (1964) Nature, Lond. 201: 939.

Sadler, E.M. (1961) Factors influencing the development of sprouts of the potato. Ph.D. thesis, Univ. of Nottingham.

Salisbury, F.B. (1963). The Flowering Process, Pergamon Press, Oxford.

Salisbury, F.B. and J. Bonner (1956) Pl. Physiol., Lancaster 31 : 141

van Schreven, D.A. (1949) Tijdschr. PlZiekt. 55 : 290.

van Schreven, D.A. (1956) Pl. Soil 8 : 49, 56, 76.

Schacht, H. (1856) Bericht über Kartoffelpflanzen und deren Krankheiten, Berl.

Schick, R. (1931) Züchter 3 : 365.

Scott, T.K. and W.R. Briggs (1960) Am.J.Bot. 47 : 492.

Scott, R.A. and J.L. Liverman (1956) Pl. Physiol., Lancaster 31 : 321.

Seth, A. and P.F. Wareing (1964) Life Sci. 3 : 1483.

Siegelman, H.W. and W.L. Butler (1965) A. Rev.Pl. Physiol. 16 : 383.

Simpson, G.M. (1966) Can. J. Bot. 44:115.

Skoog, F. (1938) Am. J. Bot. 25 : 361.

Slater, J.W. (1963) in The Growth of the Potato. Proc 10th Easter School in agric. Sci., Univ. of Nottingham, Ivins, J.D. and F.L. Milthorpe, Eds., Butterworths, Lond., pp 114-120.

Smith, O.E. and J.L. Lyon (1966) Proc. 18th Ann. Cotton Improv. Conf., Memphis, Tenn. pp 287-301.

Smith, O.E. and C.E. Palmer (1970) Physiologia Pl. 23 : 599.

Smith, O.E. and L. Rappaport (1969) Am. Potato J. 46 : 185.

Snow, R. (1935) New Phytol. 34 : 347.

Söding, H. (1936) Ber. dt. bot. Ges. 54 : 291.

Sondheimer, E. and E.C. Galson (1966) Pl. Physiol., Lancaster 41 : 1397.

Sprent, J.I. (1968) Planta 81 : 80.

Spruit, C.J.P. (1966) Meded. LandbHoogesch. Wageningen 66 : 1.

Steineck, O. (1955) Bodenkultur 8 : 254.

Steineck, O. (1956a) Z. PflZücht, 36 : 196.

Steineck, O. (1956b) Bodenkultur 8 : 374.

Steineck, O. (1958) Z. PflZücht, 39 : 403.

Stelzner, G. and M. Torka (1940) Züchter 12 : 233.

Takimoto, A. and K.C. Hamner (1965) Pl. Physiol., Lancaster 40: 852.

Thimann, K.V. (1963) A. Rev. Pl. Physiol. 14 : 1.

Thimann, K.V. (1969) in The Physiology of Plant Growth and Development, Wilkins, M.B., Ed., McGraw-Hill, Lond. pp 3-45.

Thomas, T.H., P.F. Wareing and P.M. Robinson (1965) Nature, Lond. 205 : 1270.

Tizio, R. (1964a) C. r. hebd. Séanc. Acad. Sci., Paris 258 : 6503.

Tizio, R. (1964b) C. r. hebd. Séanc. Acad. Sci., Paris 259 : 1187.

Tizio, R. (1966) C. r. hebd. Séanc. Acad. Sci., Paris 262 : 767.

Tizio, R. (1969) Eur. Potato J. 12 : 3.

Tolbert, N.E. (1960) Pl. Physiol., Lancaster 35 : 380.

van Hiele, van Overbeek, van Schreven, see H, O, S respectively.

Vanderhoef, L. N. and J.L. Key (1968) Pl. Cell Physiol., Tokyo 9 : 343.

Varga, M. and L. Ferenczy (1957) Acta bot., Szeged 3 : 111.

Varner, J.E. and G. Ram Chandra (1964) Proc. natn. Acad. Sci. U.S.A. 52 : 100.

Varner, J.E., G. Ram Chandra and M.J. Chrispeels (1965) J. cell. comp. Physiol. 66 Suppl. : 55.

Villiers, T.A. (1968) Planta 82 : 342.

Vöchting, H. (1887) Biblthca bot. No. 4.

Vöchting, H. (1900) Jb. wiss. Bot. 34 : 1.

de Vries, H. (1878) Landw. Jbr 7 : 217, 591.

Wang, D. (1961) Can. J. Bot. 39 : 1595.

Wareing, P.F. (1954) Physiologia Pl. 7 : 261.

Wareing, P.F. (1969) in Physiology of Plant Growth and Development, Wilkins, M.B., Ed., McGraw-Hill, Lond. pp 605-644.

Wareing, P.F., J. Good and J. Manuel (1968) in Biochemistry and Physiology of Plant Growth Substances (Proc. 6th Inter. Conf. on Plant Growth Substances), Wightman, F. and Setterfield, G., Eds., Runge, Ottawa, pp 1561-1579.

Wareing, P.F., C.F. Eagles and P.M. Robinson (1964) in Régulateurs naturels de la Croissance Végétale (Colloque International, Gif-sur-Yvette, 1963) Centre National de la Recherche Scientifique, Paris pp 377.-386.

Wassink, E.C. and J.A.J. Stolwijk (1953) Meded. LandbHoogesch. Wageningen 53 No. 5 ; 99.

Waxman, S. (1957) The development of woody plants as affected by photoperiodic treatments. Ph.D. thesis, Cornell Univ., Ithaca, N.Y.

Weatherley, P.E. (1950) New Phytol. 49 : 81.

Wellensiek, S.J. (1923) Meded. LandbHoogesch. Wageningen 27, No. 3.

Wellensiek, S.J. (1924) Tijdschr. PlZiekt. 30 : 177.

Wellensiek, S.J. (1929) Meded. LandbHoogesch. Wageningen 33 : 6.

Went, F.W. (1928) Recl Trav. bot. . neerl. 25 : 1.

Went, F.W. (1959) Am. J. Bot. 46 : 277.

Werner, H.O. (1934) Res. Bull. Neb. agric. Exp. Stn No. 75.

Werner, H.O. (1940) J. agric. Res. 61 : 761.

Winkler, E. (1961) Flora, Jena 151 : 621.

Wittwer, S.H. and R.R. Dedolph (1963) Am. J. Bot. 50 : 330.

Wittwer, S.H. and N.E. Tolbert (1960) Am. J. Bot. 47 : 560.

Zeevaart, J.A.D. (1966) Pl. Physiol., Lancaster 41 : 856.

Zeevaart, J.A.D. and A. Lang (1962) Planta 58 : 531.

Zeevaart, J.A.D. and A. Lang (1963) Planta 59 : 509.

Zimmerman, P.W. and A.E. Hitchcock (1936) Am. J. Bot. 23 : 690.

Zweig, G., S. Yamaguchi and G.W. Mason (1961) Adv. Chem. Ser. 28 : 112.

* Hoagland, D.R. and D.I. Arnon (1938) Univ. Calif. agric. Exp. Stn. Circ. 347.

Hofstra, G., G.J.A. Ryle and R.L. Williams (1969) Aust. J. biol. Sci. 22 : 333.

ABSTRACT OF THESIS

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Title of Thesis: Studies on tuberisation in the potato, Solanum tuberosum L.

SUMMARY

Tuber initiation in a late maincrop variety of cultivated potato (Solanum tuberosum L.) was studied. The two principal areas of investigation were firstly, whether the response of tuberisation to daylength is direct and truly photoperiodic or whether it is mediated by the growth of the tops (haulms) and secondly, the hormonal nature of the hypothetical tuberising stimulus proposed by several workers; studies were also carried out on the histology of the stolon tip immediately prior to the appearance of visible swelling.

Experiments in which plants grown in inductive short days were subjected to light break treatment showed that such treatment causes partial inhibition of tuberisation and that the effect of daylength is a genuinely photoperiodic one. The most likely explanation of the effect of light break treatment was thought to be control of tuberisation by a hormonal stimulus, the formation of which is regulated by photoperiod, among other factors.

Studies on the nature of the proposed hormonal stimulus provided good evidence for the involvement of endogenous gibberellins, as an influence acting to inhibit or delay tuberisation: tuberisation was partially inhibited by repeated applications of gibberellic acid and promoted when the synthesis of endogenous gibberellins was inhibited by CCC, when these compounds were applied either to the plant as a whole or directly to the site of tuberisation, the stolon tip. The level of endogenous gibberellin-like substances in the stolon tip was found to be inversely correlated with inductive daylength conditions and with the degree of advancement of the developmental state of the stolon tip along the path towards tuberisation, the level of gibberellin-like substances falling as the tip began to tuberise. This correlation extended to the period immediately prior to the onset of visible swelling, in which starch deposition takes place in the tissues of the tip; this deposition occurred in a consistent sequence in the various tissues. It seemed unlikely from the results of the present studies that abscisic acid forms part of the tuberising stimulus (although endogenous growth inhibitor(s) appeared in the stolon tip at tuberisation), although it may act indirectly through effects on overall growth. There was also no evidence to support the suggestion that cytokinins promote tuberisation.

The present work provides support for the theory that the tuberising stimulus consists of a balance between endogenous gibberellins and growth inhibitors (the identity of the latter being unknown). Tuberisation appears to be promoted by environmental conditions or treatments which cause a lowering of the ratio of endogenous gibberellins to growth inhibitors at the stolon tips, although other factors may also be involved.

Use other side if necessary.