

THE CONTROL OF VEGETATIVE SHOOT GROWTH
IN CITRUS

by

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To

Mabel, Harrie

Georgia

and

Shanta

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SUMMARY

Vegetative buds of citrus were found to consist of an apex and approximately eight leaf primordia enclosed within up to five prophylls. Secondary buds usually existed in the axils of the prophylls.

A study of the ontogeny of vegetative buds revealed that the axillary bud develops rapidly during the first five days of shoot growth, from an apical dome with perhaps one prophyll to the structure described above. After a period of dormancy the young shoot emerges. At that time there is rapid development of leaf initials. Stem elongation also commences at bud burst and proceeds as a wave of internode extension from the base to the tip. As the stem elongates the leaves begin to expand but their expansion continues beyond the duration of stem elongation. Shoot growth finally ends with the abortion of the tip of the shoot and maturation of the leaves. The entire process is described in a summary encompassing 10 stages of development.

Bud burst and shoot growth was studied using budded plants of Valencia sweet orange on Troyer citrange rootstocks. It was concluded that citrus buds undergo different stages of dormancy and the effects of various growth substances applied to the bud on bud burst and shoot growth depend upon the stage of dormancy: High concentrations of 6-benzyl amino purine (BAP) induced dormant buds to burst

but had no effect on buds which were induced to grow whereas alpha-naphthalene acetic acid (NAA) promoted bursting in buds induced to grow, i.e. non-dormant buds. Gibberellic acid (GA_3) inhibited bud burst and subsequent shoot growth when applied to dormant buds. However, GA_3 applied to the young shoot after bud burst increased shoot length, leaf number, internode length and shoot dry weight. In contrast, removing a 2 mm ring of bark from around the stem (ringing) of Valencia orange seedlings reduced shoot length, mean internode length, leaf area and dry weight of the new shoot.

Using these treatments it was established that different factors control the activity of different meristems, that citrus leaves normally photosynthesise well below their capacity to do so, that within reasonable limits carbohydrate supply (including current photosynthate) does not influence shoot growth and that bidirectional phloem translocation of unidentified substances is essential for normal shoot growth. There were indications that mixtures of growth substances supplied below a ring and transposition of bark from below to above a ring could partially overcome the effects of ringing, whereas inorganic phosphate or asparagine supplied above the ring had no effect.

Declaration

I hereby declare that the thesis here presented is my own work, that it contains no material previously published, except where due reference is made in the text, and that no part of it has been submitted for any other degree.

(Dyanand Raj Kumar)

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1. INTRODUCTION

Citrus species constitute the world's second most important group of fruits. In 1974 the production of citrus (43.8 million metric tons) was surpassed only by that of grapes (63 million metric tons) and exceeded the production of bananas (35.8 million tons) and apples (21.4 million tons) (FAO Production Yearbook, 1975). Despite its commercial importance there is a surprising dearth of basic information about the growth of the citrus plant.

In commercial production, citrus plants are generally propagated by budding the desired scion onto seedling rootstocks. The cultivars of both scion and rootstock are carefully chosen for their individual characteristics, as well as the suitability of their combination. Even so, certain stock/scion combinations are difficult to produce or take an uneconomically long time to attain saleable size. This may be due to the implanted bud surviving but failing to burst or bursting late in the growing season, or to the young shoot producing only minimal growth, for instance as little as one leaf borne on a stem of less than 2 cm.

These problems led to an interest in the control of shoot growth in citrus. This interest was heightened by the realisation that vegetative growth in citrus is intimately linked to reproductive growth — shoots which bear fruit in one season give rise to purely

vegetative shoots the following season. In addition, it became clear that very little definitive work had been done on vegetative growth in this evergreen plant and consequently, little is known about its physiology. As a result, this investigation into various aspects of vegetative shoot growth in citrus was initiated.

1.1. Growth of the seedling

In a botanical sense, a plant is a seedling from the time it emerges from the seed until it forms green leaves and becomes independent of food stored in the cotyledons (Schneider, 1968). However, throughout this work the term seedling will be used in the sense of a plant grown entirely from seed, as opposed to budded plants, in which mature buds are grafted onto seedlings (buddlings) or to rooted cuttings.

Citrus seeds are polyembryonic and generally have a true zygotic embryo plus one or more nucellar embryos (Frost, 1943; Schneider, 1968). These together with their cotyledons, are enclosed in a thin inner seed coat or tegmen and a tough, woody outer seed coat or testa. On germination, the radicle(s) emerges through a break in the micropylar end of the testa and rapidly elongates to form a thick, fleshy taproot surmounted by a short hypocotyl bearing the cotyledons. The epicotyl grows out from between the cotyledons and usually bears two opposite, simple leaves, the first foliar leaves. The next one or two leaves initiated on the apex may be simple

and the subsequent one or two intermediate between simple leaves and the typical unifoliate compound leaves of the mature plant (Hayward and Long, 1942; Frost, 1943; Schneider, 1968).

Under South Australian conditions seedlings usually take 12 to 18 months to grow to a height of around 30 to 35 cm, at which time they are transplanted from seedbeds into rows in the nursery. The following spring or summer, from 3 to 9 months later, these seedlings are budded. Approximately one year after budding the scion has grown to a size such that the plants are ready to be transplanted into an orchard. The duration of each phase varies with different cultivars but it generally takes 2 to 3 years to produce a budling which is acceptable for planting (Blain, 1976; Moss, 1975; I.Tolley, personal communication).

1.2. Morphology of vegetative buds of citrus

The young seedling develops through the activity of the apical bud. The shoot apex consists of an apical meristem surrounded by leaf promordia and successively older leaves. In a resting condition, e.g. at the end of a growth flush or under stress conditions, the apical meristem and leaf primordia are enclosed in two or three prophylls which may or may not develop into leaves during the following flush of growth. Initially growth is monopodial though this growth pattern exists only for a relatively short part of the life of a citrus

tree. Growth in the mature plant is sympodial, the young shoots arising from buds in the axils of leaves, usually near the distal ends of existing shoots.

Axillary buds in many species of citrus, including sweet orange (C. sinensis [L] Osbeck) and lemon (C. limon [L] Burm. f) are multiple and consist of three to five individual buds. Each bud within this complex of multiple buds consists of an apical meristem surrounded by successively older leaf primordia. The most developed bud often has 5 to 6 leaf primordia surrounding the apex although up to 8 have been reported. The entire complex of buds is covered by 2 to 5 prophylls (Bartholomew and Reed, 1943; Schneider, 1968; Scott et al., 1948).

1.3. Development within the bud and growth of the vegetative shoot

In spite of an exhaustive search of the literature, no publication was found which described the morphological changes which occur during development within the vegetative bud of citrus although there is a considerable body of literature on the development and changes within floral buds (e.g. Abbott, 1935; Nir et al., 1972).

Similarly, no detailed description of the growth of an individual shoot could be found. Schroeder (1951) states that "upon commencement of a growth flush the shoot lengthens by elongation of the internodes behind the tip"! Bui-Huy-Dap and Nguyen-Hun-Thuoc (1962) observed the growth of individual shoots of 5 citrus

species more closely and have presented graphs of shoot length with time. These showed a linear increase in shoot length for each species followed by an abrupt arrest of shoot extension and a plateau of shoot length. The rate and duration of the linear growth phase varied between species. The authors stress that shoot extension growth stops before the shoot tip abscises. The interval between these two events varied from 2 to 49 days and was characteristic of the species.

The development of the procambia and the vascular system in the young shoot is described in detail by Schneider (1968) and Scott et al. (1948).

1.4. Dormancy in citrus

There has been some debate about the existence of true dormancy or rest in citrus and this has been confounded by the diversity of nomenclature used. Throughout this work the system of terminology proposed by Romberger (1963) will be used. His definitions are :

Dormancy — A general term for all instances in which a tissue predisposed to elongate (or grow in some other manner) does not do so.

Quiescence — Dormancy imposed by the external environment.

Correlative inhibition — A type of physiological dormancy maintained by agents or conditions originating within the plant, but not within the dormant organ itself.

Rest — A type of physiological dormancy maintained by agents or conditions within the organ itself.

Citrus plants grow by a series of flushes in which axillary buds burst and the emerging shoots lengthen. After stem extension growth has ended, the apical part of the shoot aborts and abscises. No further flush occurs until the previous one has ended. There are 2 to 5 flushes of shoot growth within one year and these can be thought of as alternating periods of shoot growth and dormancy (Ahmed, 1964; Bui-Huy-Dap and Nguyen-Hun-Thuoc, 1962; Cooper, 1965; Cooper et al., 1969; Moss 1975; Sauer, 1951; Schroeder, 1951).

Whether citrus buds go through a stage of rest is not fully resolved. Cooper and Peynado (1959) state that "dormancy of the type in which buds do not elongate unless first exposed to low temperatures apparently does not exist in citrus". Their evidence comes mainly from the responses of whole plants to changes in temperature and to treatments with plant growth substances (Cooper and Peynado, 1958, 1959; Cooper et al., 1969). Surkova (1962) agrees that citrus does not have a clearly expressed rest period during winter. He compared 5 species of citrus with 2 related species, Nagami kumquat (Fortunella margarita Sw.) and trifoliate orange (Poncirus trifoliata Raf.) which are more frost resistant. Unfortunately details of the methods used

and of the results obtained are not clearly given. The results, as interpreted by the author, characterize citrus as a plant with "incomplete winter dormancy (rest) and a relatively weak resistance to freezing".

When buds taken at various times of the year from the spring flush of Shamouti orange trees were grown on a modified Murashige and Skoog solid medium under favourable environmental conditions, the time required for buds to burst ranged from 2 to 22 days and varied with the time of year that the buds were excised (Altman and Goren, 1974a). Newly formed buds sampled in April, and 10 month old buds sampled during winter (December - January) required the longest time to burst. It was concluded that there is an innate dormancy in citrus buds which is not dependent upon the state of growth of other organs on the tree, i.e. rest. However, in the truly resting buds of deciduous species no metabolic activity is found, and buds on detached shoots do not burst even after more than 50 days under favourable environmental conditions (e.g. Semin and Madis, 1964; Williams, 1976). Although the difference is quantitative, its magnitude suggests that the mechanism of dormancy may be different.

Using 2 year old Poncirus trifoliata seedlings, Stathakopoulos and Erickson (1966b) found that bud burst was not so much related to the duration or extent of chilling as to the daily average degree-hours above 15.5°C (60°F). In those experiments the longest time

taken for buds to burst was 25 days. It was concluded that there is no chilling requirement for bud burst in trifoliate orange plants, i.e. the buds do not undergo rest.

Defoliation of mature citrus shoots induces axillary buds along the shoot to burst which suggests that the axillary buds are in a state of correlative inhibition by the leaves (Cooper et al., 1969; Stathakopoulos and Erickson, 1967).

The available evidence suggests that citrus buds undergo periods of 1) quiescence and of 2) correlative inhibition, but not of rest as defined by having a chilling requirement. Since by definition, these two stages are imposed 1) by the external environment and 2) by agents or conditions within the plant respectively, they will be further discussed under these headings in relation to both bud burst and shoot growth.

1.5. Factors affecting bud burst and shoot growth in citrus

1.5.1. Environmental factors

As is pointed out by Reuther (1973) the precise effects of any environmental factor on a growth process either in the field or under controlled environment conditions are very difficult to ascertain due to the interrelation of each parameter with several others. For this reason, and also because much of the published work is either qualitative or quantified in very broad terms (e.g. mean canopy dimensions) the effect of

environmental factors are discussed in general outline here, with closer scrutiny only where data are available.

1.5.1.1. Temperature

Citrus trees grow more quickly under tropical conditions than under sub-tropical conditions (Bain, 1949; Reuther, 1973). Motial (1965) and Krishnamurthi et al., (1960) found that there were 5 flushes of growth per year under Indian conditions while under conditions of longer winters Sauer (1951) reported 3 growth flushes per year. Rooted Washington Navel cuttings produced 3 growth cycles when grown for 34 weeks at day/night temperatures of $24^{\circ}/19^{\circ}\text{C}$ and 5 flushes at $30^{\circ}/25^{\circ}\text{C}$ (Lenz, 1969a). The temperature regimes had no effect on the number of shoots which grew in the first flush, but in the second and third flushes the cuttings under $24^{\circ}/19^{\circ}\text{C}$ produced more shoots than those under $30^{\circ}/25^{\circ}\text{C}$. Assuming that there is a positive correlation between the number of buds which burst and the number of shoots which grew, the results indicate that the lower temperature regime increased bud burst in the second two flushes. However, when the total number of shoots which grew over the entire 34 week period are compared, more shoots grew in 8 hour or 16 hour days under the $30^{\circ}/25^{\circ}\text{C}$ regime (but fewer in 12 hour days) than under the $24^{\circ}/19^{\circ}\text{C}$ regime. The results indicate that temperature may affect the number of buds which burst in a flush as well as the interval between flushes, and that a temperature effect can be influenced by other environmental

factors such as, daylength.

The data presented by Reuther (1973) for 3 locations in the U.S.A. and by Sauer (1951) for Mildura, Australia show that the spring flush produces more new shoots than the combined total of all the other flushes in a year, and that there is no correlation between temperature and the incidence of new shoots throughout the year. Since considerably fewer shoots grow during summer than in the spring flush, even though temperatures are higher, it would appear that in the field, other factors, including internal plant factors, influence bud burst to a greater degree than temperature.

On the other hand, there are temperature limits outside of which bud burst will not occur. Webber (1943) states that although different species and cultivars may vary slightly, 12.8°C^* is approximately the temperature above which citrus plants begin to grow.

The evidence suggests that low soil temperatures inhibit bud burst : Under favourable day/night air temperatures ($26.7^{\circ}/21.1^{\circ}\text{C}$) no buds burst with soil temperatures of 12.2°C whereas large numbers burst with soil temperatures of 23.3°C (Stathakopoulos and Erickson,

*Temperatures given in degrees C to one decimal place are not meant to indicate that level of accuracy but are conversions of figures given in the literature in degrees F.

1966b). Mendel (1969) reported that in the coastal plain of Israel buds burst when the soil temperature rises above 12°C , irrespective of the air temperature, and Kozyrev (1973) found that the most effective factor in the start of growth in spring was a soil temperature of at least 10°C at a depth of 30 cms for about 5 days.

In relation to the upper extreme Reuther (1973) cites examples where citrus plants were held at a constant temperature of 38°C or a diurnal regime of $40^{\circ}/30^{\circ}\text{C}$ and produced "reasonably vigorous" to "rapid shoot growth". Since some of these plants were held under these conditions for extended periods (up to 3 years at 38°C) and grew reasonably well, clearly bud burst was not totally inhibited. It would appear that the upper temperature limit to bud burst is not known.

Studies using seedlings in temperature-controlled chambers indicate that at a constant temperature of 12° to 13°C there is virtually no shoot elongation, at 15°C there is retarded growth with small leaves, at between 25° and 31°C there is maximum shoot growth (as measured by stem elongation or fresh weight increase) and at 35°C growth is decidedly inhibited (Mendel, 1969; Reuther, 1973; Webber 1943).

In field trials in Delhi, Krishnamurthi et al., (1960) found that extension growth started when the mean weekly air temperature was 13.6°C and maximum total extension growth occurred when the mean weekly temperature ranged between 18.0° and 24.1°C . Although there was some

growth during the period when the temperature varied between 24.1° and 36.0°C , this growth was slow. Unfortunately, these data, like many others relating to field measurements, are vague and give no detailed information on the effects of temperature on individual bud burst and shoot growth.

The mean height and dry weight per plant of Washington Navel cuttings grown in a phytotron for 34 weeks at day/night air temperatures of $30^{\circ}/25^{\circ}\text{C}$ was greater than at $24^{\circ}/19^{\circ}\text{C}$ (Lenz, 1969a). Moss (1973) used rooted Late Valencia cuttings to investigate the effect of 2 temperature regimes on regrowth after pruning. He found that under $18^{\circ}/13^{\circ}\text{C}$ day/night air temperatures, the first growth flush after pruning was smaller, and was comprised of fewer shoots, fewer leaves and a shorter total shoot length than under $27^{\circ}/22^{\circ}\text{C}$. In an experiment to determine the effects of night temperatures on growth, dormancy and cold hardiness of 24 citrus cultivars and 6 related species, Young and Peynado (1962) subjected 9 month-old seedlings to an 8 hour day temperature of 21.1°C and sequentially reduced the night temperature from 15.5°C through 13.9°C , 12.2°C and 10°C to 8.9°C . The plants were kept at each of the upper four night temperatures for 8 weeks, and at the lowest for 4 weeks. The more cold-hardy cultivars stopped growing earlier, i.e. at higher night air temperatures. However, even at 8.9°C , five cultivars still showed some extension growth.

Table 1. Relationship of soil or root temperature to growth

<u>Reference</u>	<u>Plant material</u>	<u>Experimental conditions</u>	<u>Root temperatures (°C) for root growth</u>			<u>Root temperatures (°C) for shoot growth</u>		
			<u>Minimum</u>	<u>Optimum</u>	<u>Maximum</u>	<u>Minimum</u>	<u>Optimum</u>	<u>Maximum</u>
Cary, 1969; 1970; 1971; 1972	<u>C. sinensis</u> cv Washington Navel and Late Valencia**	Constant root temperature in glass house	25-30° better than 19°C			25° better than 19°C		
Girton, 1927 (see Reuther, 1973)	<u>C. aurantium</u> , sour orange*	Controlled temperature compartments	12-13	25-26	37-38	12-13	25-31	37-38
Halma, 1935	<u>C. limon</u> cv Eureka ** <u>C. paradisi</u> cv Marsh** <u>C. sinensis</u> cv Valencia**	Soil in heated frames in the open	-	-	-	Lemon: 16-27°C through winter and spring better than 12-22° or 3-20°C Grapefruit and orange : 12-22° better than 3-20° or 16-27°C.		
Krishnamurthi et al., 1960	<u>C. sinensis</u> cv Hamlin/ <u>C. limon</u> cv Jatti khatti† <u>C. reticulata</u> cv Nagpur Santra/ <u>C. limon</u> cv Jamberi† <u>C. paradisi</u> cv Marsh Seedless/ <u>C. limon</u> , rough lemon† <u>C. aurantifolia</u> , sweet lime**	Field, New Delhi	16	28-35	37	-	-	-
Labanauskas et al., 1958	<u>C. sinensis</u> cv Koethen* <u>C. aurantium</u> , sour orange* <u>C. reticulata</u> cv Cleopatra* <u>C. limon</u> , rough lemon*	Constant root temperature in glass house	14	28-35	-	14	21-35	-
Lenz, 1969a	<u>C. sinensis</u> cv Washington Navel**	Controlled environment compartments	30°/25°C day/night better than 24°/19°C					
Stathakopoulos 1966	<u>Poncirus trifoliata</u> * <u>C. sinensis</u> cv Washington Navel/ <u>P. trifoliata</u> † <u>C. sinensis</u> cv Washington Navel/ <u>C. sinensis</u> cv Koethen†	Constant root temperature in glasshouse	22° better than 31° which better than 12°C			31° better than 22° which better than 12°C.		

* Seedlings
† Budded plants
** Rooted cuttings

It is clear that root or soil temperature influences shoot and root growth (Table 1). Although the details vary with the genotype and experimental conditions and techniques used, the general impression is that there is no root or shoot growth in citrus at soil temperatures of less than 12°C , optimum root growth around 25°C , optimum shoot growth around 30°C and severely restricted, if any growth above 37°C .

There is some evidence on the effects of temperature on specific physiological processes which may help to elucidate the effects of temperature on growth. Low soil temperatures ($10^{\circ} - 15^{\circ}\text{C}$) reduce water uptake by citrus roots (Cameron, 1940; Reuther, 1973; Tamai, 1961). Similarly, nitrogen uptake is reduced at low root temperatures but increases rapidly ($Q_{10} =$ approx 2) as root temperatures increase from 9° to 30°C (Wallace, 1953).

At temperatures which induce bud dormancy (12° to 13°C) there is a reduction of cambial activity in the stem and an increase in soluble sugars and a reduction in starch levels in the shoots. These changes are associated with greater frost-resistance in citrus (Dugger and Palmer, 1969; Randhawa and Soni, 1969; Sharples and Burkhart, 1954; Surkova, 1962; Young, 1961; Young and Peynado, 1965).

The rate of apparent photosynthesis (AP) increased rapidly with temperature up to 20°C and more slowly as the temperature increased to 25°C or 30°C .

Increasing the temperature above 30°C reduced AP, whereas dark respiration increased almost linearly ($Q_{10} = 2.0$) as temperature increased from 20° to 35°C (Khairi and Hall, 1976a; Kriedemann, 1968; Possingham and Kriedemann, 1969; Thompson et al., 1965).

Thus the temperature of the environment influences the number of buds which burst, the length of the interval between flushes of shoot growth, the duration of shoot growth and the extent of both root and shoot growth. Although the details vary with genotype and method of propagation of the plant material as well as with the environment in which the experiments are carried out (field versus controlled environment) and the previous conditioning of the plant material, it seems clear that shoots of citrus (a) will not grow in ambient temperatures of less than 12°C , (b) grow most rapidly and produce greatest increases in dry matter in air temperatures of 25°C to 30°C and at root temperatures of 25° to 35° , and (c) grow poorly, if at all, at air or root temperatures of 40°C and over.

1.5.1.2. Light and Photoperiod

Generally, citrus plants grown in 16-hour photoperiods produce more flushes and more shoots (i.e. increased bud burst) over a given length of time than in 8-hour photoperiods. For example, Red Blush grapefruit trees grown under controlled-environment conditions for 32 weeks produced 3 flushes in 16-hour photoperiods and

2 flushes in 8-hour photoperiods. Coincident with the increase in number of flushes was an increase in the number of shoots (Young, 1961). Similar results were obtained by Piringer et al. (1961) who found in a comparison of 8, 10, 12, 14 and 16-hour photoperiods that the latter was the only treatment which consistently reduced the number of days between flushes.

The results of Lenz (1969a) demonstrate that photoperiod has some influence on bud burst but that its effect is confounded by other factors : Under $24^{\circ}/19^{\circ}\text{C}$ day/night temperatures more shoots were produced in the second and third flushes in 12-hour photoperiods than in either 8-or 16-hour photoperiods whereas, under $30^{\circ}/25^{\circ}\text{C}$ the reverse was true.

The literature is almost unanimous about the effects of daylength on shoot growth in citrus : In long photoperiods, growth is greater than in short photoperiods, once other environmental conditions are favourable (e.g. Stathokopoulos, 1966; Warner, 1971; Warner and Upadhyya, 1968). The increase in total stem length under 16-hour photoperiods (Piringer et al., 1961; Young, 1961; Warner and Upadhyya, 1968) resulted from increases in both the number of internodes as well as in internode length (Piringer et al., 1961). Young (1961) also found that the internode length of the first two flushes was longer under long photoperiods although on plants treated for 32 weeks the mean internode length was not affected.

There is an interaction between temperature and daylength on shoot growth as well as on bud burst. Growth of P. trifoliata plants in 8-hour photoperiods under favourable temperature conditions was markedly reduced compared with that in 16-hour photoperiods, but dormancy was not induced; under unfavourable conditions (soil temperature of 12.2°C) plants in 8-hour photoperiods became dormant sooner than those in 16-hour photoperiods (Stathakopoulos, 1966; Stathakopoulos and Erickson, 1966). Lenz (1969a) also found that the mean height per plant and dry weight per plant increased with increasing daylength under either 24°/19°C or 30°/25°C day/night temperatures and that under similar daylengths, plants grew taller and attained greater dry weight at the higher temperature regime. However, as Reuther (1973) postulates, under sub-tropical conditions when natural day length is less than optimal, temperature conditions inhibit growth so increasing daylength under such conditions does not increase growth. Conversely, when temperatures are suitable for growth, the days are naturally longer, i.e. daylength is not limiting.

The quality of light also influences the growth of citrus shoots. When 8 hours of natural daylight was extended by 8 hours of low intensity incandescent light, budded Red Blush grapefruit plants increased shoot length and the total number of branches formed, and the interval between flushes was reduced, compared to plants

grown under 8 hours of natural daylight. Extending the 8 hours of natural light with 8 hours of low intensity fluorescent light resulted in plants with stem-length characteristics of short days (i.e. short shoots) but with the accelerated flushing of long days (Piringer et al., 1961).

Seedlings grown under celluloid covers which transmitted blue plus far-red light or red plus far-red light were taller and had longer internodes than those under covers transmitting only blue light or under white celluloid (which reduced total intensity of transmitted irradiation to the same level as under the coloured covers — 20% of full daylight) (Erner et al., 1972). It seems that far-red light promotes internode elongation but is not the critical factor controlling the interval between flushes. Young (1961) also found that increasing an 8-hour natural daylength by 8 hours of low intensity (800 lux) incandescent light increased shoot growth compared to an 8-hour daylength. However, when the natural daylength period was extended by 8 hours of high intensity (12900 lux) incandescent and fluorescent light there was an almost 5-fold increase in shoot growth and a 6-fold increase in the number of shoots which grew over an 8-week period.

The data suggest that long photoperiods might influence shoot growth in at least 3 different ways :

- 1) reduce the period between flushes, due to longer periods of light either with or without a sizeable far-red component.

- 2) increase internode length, due to longer exposure to far-red light and
- 3) increase overall shoot growth by increasing the duration of photosynthesis.

On the other hand, increasing light intensity reduced the growth rate of sweet lime seedlings (Monselise, 1951, quoted by Mendel, 1969). Similarly, sour orange seedlings grown in full daylight were significantly shorter, with shorter average internode lengths and smaller leaves than those grown under white celluloid which transmitted only 20% of daylight (Erner et al., 1972). However the former had more leaves and higher dry matter than the shaded ones which suggest that the shaded seedlings were etiolated.

There have also been investigations into the effect of light intensity on some physiological parameters of citrus plants. Kriedemann (1968, 1971) published apparent photosynthesis/light intensity curves for orange and lemon leaves of various ages. There was a rapid increase in the rate of apparent photosynthesis with increasing irradiance up to approximately 10^5 ergs second⁻¹ cm⁻² (approx. 26900 lux). At higher light intensities apparent photosynthesis remained constant or fell slightly.

In a study of fruit growth of Satsuma mandarin, Kadoya (1973) varied the light intensity from 62000 lux (unshaded control) to 28000 or 5000 lux by shading the plants with 1 or 3 layers of black cheesecloth and measured

the rate of translocation of ^{14}C into the fruit. The amount of label in the fruit fell with decreasing light intensity. Although the light intensity was well below optimal levels for photosynthesis in the more heavily shaded treatment (compare Kriedemann, 1968), the intensities in the other 2 treatments were optimal and supra-optimal. Even so, there was a greater concentration of label in the fruit of the unshaded control plants than in those shaded by 1 layer of cheesecloth. Three explanations are possible : a) that the reduced light intensity may have reduced the amount of ^{14}C fixed b) that the amount of photosynthate available for translocation was reduced, or that c) the rate of translocation per se was reduced. Of these the latter appears most likely.

Reducing the intensity of daylight above sour orange seedlings increased the chlorophyll content and reduced the ascorbic acid content and the peroxidase activity in the leaves (Erner et al., 1972). Daylength also had an effect on the peroxidase activity in the shoot tips of three citrus cultivars and P. trifoliata, but this, as well as the activities and numbers of esterase isoenzymes and leucine amino-peptidases, varied with genotype as well as with photoperiod (Warner and Upadhy, 1968).

1.5.1.3. Water and Mineral Nutrition

In tropical areas, availability of soil moisture has a profound effect on bud burst. As Cassin

et al., (1969) have stated, precipitation of less than 100 - 150 mm per month can induce dormancy in citrus under tropical conditions and the first effective rain after a drought induces bud burst 20 to 28 days later.

Similarly, shoot extension growth is affected by soil water availability (Cooper et al., 1969). Soil water potentials of less than - 2.5 bars reduced the shoot length and number of leaves of potted Satsuma orange trees (Suzuki and Kaneko, 1970).

Since reviews on mineral nutrition and fertilization as well as details of the effects of deficiencies in citrus already exist (Chapman, 1968; Chapman and Kelly, 1943, Davidson Ben David, 1975; Embleton et al., 1973a, 1973b; Klotz and Fawcett, 1973) no effort is made to discuss these topics here except to point out that deficiency symptoms soon appear if the macro-element or micro-element nutrition of the plant is not adequately maintained.

1.5.2. Endogenous factors

1.5.2.1. Carbohydrates

There is very little data on the effects of levels of carbohydrates on bud burst in citrus. Indirect evidence (e.g. Jones et al., 1974, 1975; Kar and Randhawa, 1968) suggest that the number of buds which burst in spring and the time of their bursting may be related to the concentrations of starch in both the roots and the mature leaves just prior to bud burst.

In studies using $^{14}\text{CO}_2$ growing shoots were found to be strong sinks and imported assimilates, mainly as sugars, from nearby leaves. Expanding leaves did not export any labelled assimilates; only after they had fully expanded and had begun to harden did they contribute assimilates and even then only to nearby sinks such as developing fruits (Kadoya, 1974; Kadoya and Tanaka, 1972; Kriedemann, 1969a, 1969b, 1970; Kubota and Motoyama, 1972a). Other data also demonstrate that the young shoot uses carbohydrate available in older tissues : When 1-year-old shoots of young Satsuma orange trees were pruned, the level of available carbohydrate in the mature tissue after a flush of growth had developed was lower than in the unpruned controls (Muramatsu and Iwagaki, 1970). Although starch never completely disappeared from the xylem of field-grown trees, small but detectable reductions in starch content occurred shortly after new shoot growth began (Cameron and Schroeder, 1945).

The relationship between new shoot growth and carbohydrates in the mature tissue in citrus does not seem to have been quantified — no account was found of an attempt to relate levels of carbohydrate to the amount or extent of new vegetative shoot growth. Nevertheless, it is clear that the growth of the young shoot makes great demands on, and is therefore dependant upon, carbohydrate assimilated and/or stored in the mature tissue.

1.5.2.2. Amino acids and Protein

Although the contents of amino acids and proteins in buds and shoots are more likely a consequence of growth than the cause of it, these compounds are considered to be of such importance in growth and development that a review of the literature on them in citrus is included.

It has been established that nitrate nitrogen is seldom found in citrus trees, that free amino acids make up less than 5% of the total nitrogen content of the plant and that the composition of the amino acid pool varies with genotype and mineral nutrition, including micro-element nutrition (Labanauskas and Handy, 1971; Stewart, 1962; Stewart and Wheaton, 1967; Ved Parkash et al., 1964).

In a comparison between floral buds and buds in which flowering was prevented by repeated applications of gibberellic acid, Monselise and Hubermann (1973) found an apparent increase in protein concentration in buds and mature leaves during flower differentiation. They also found differences in the patterns of salt-extractable proteins of buds at evocation time and during floral histogenesis and concluded that proteins in floral buds differ from those in buds where flowering is prevented by gibberellic acid.

There was a marked decrease in the rate of protein synthesis in excised buds during bud burst and early growth of vegetative shoots in vitro (Altman and

Goren, 1974a).

A type of pummelo (C. grandis) stem callus which produced shoots had higher concentrations of nitrogen, amino acids and proteins than another type which did not undergo any differentiation (Chaturvedi et al., 1974a). Moreover undifferentiated callus of the former type contained considerably lower levels of free amino acids than did callus of a similar type which had differentiated into buds (Chaturvedi et al., 1974b). These results suggest that bud morphogenesis in citrus is accompanied by increased levels of free amino acids in the tissue but this has not been verified in tissues from growing plants.

1.5.2.3. Plant growth substances

The mode of action of any single plant growth substance has not yet been clearly defined. As a result, uncertainties exist in conclusions drawn from measurements of endogenous levels or from the effects of externally applied plant growth substances. Nevertheless, in the elucidation of the roles which these compounds play, data on endogenous levels and on effects of exogenous applications do contribute to an understanding of the overall picture; indeed, if a chemical is to be accepted as the one controlling a particular process, application of the pure compound should substitute for the organ or tissue which is the normal source of the chemical (Jacobs, 1959).

Therefore data obtained using exogenous applications of plant growth substances will be included where they help to establish or elucidate some role of these substances in citrus shoot growth.

Auxins: In 1963 Khalifah, Lewis and Coggins reported the presence in young orange fruits of a growth-promoting compound which co-chromatographed with indole-3-acetic acid (IAA) but which differed from IAA in its fluorescent and electrophoretic characteristics. They called the compound "citrus auxin". Subsequently a number of papers were published by the same and other groups of workers in which references were made to "citrus auxin" (e.g. Lewis et al., 1965; Bakhshi, 1967; Hanks and Feldman, 1968). However, in 1971 a group of 7 authors reappraised the existence and identity of auxins in citrus and concluded that young fruitlets contained IAA and indoleacetamide (Goldschmidt et al., 1971a). They discounted the presence of an auxin distinct from indolic auxins and suggested that the concept and term "citrus auxin" were not justified and should be avoided.

IAA-like compounds have been found in actively growing shoots (Goldschmidt and Monselise, 1968; Goldschmidt et al., 1971b), leaves (Goren et al., 1971; Rao and Narasimham, 1974) and shoots from plants infected with Radopholus similis (Hanks and Feldman, 1968) as well as in flowers and fruits of citrus (Goldschmidt, 1968; Goren and Goldschmidt, 1970).

Auxin-like activity was lowest in stems of healthy Valencia orange trees at the time of new growth in March and August. In contrast, no consistent pattern was found in stems of trees infected with Radopholus similis (Feldman et al., 1966).

Using a technique in which bud explants were grown on agar medium, Altman and Goren (1974a) found a pattern of bud burst in buds excised at different dates during the year from the spring flush. In this pattern the periods during which buds took the least time to burst corresponded to the periods of spring and summer flushes in vivo. Additions of IAA (at 10^{-5} or 10^{-6} M) to the medium at the time of the summer flush increased the time taken for the buds to burst, i.e. retarded bud burst. At other times, when buds seemed inhibited or dormant, IAA had no effect on the time required for bursting.

This retardation of bud burst by auxin is supported by other studies :

When alpha-naphthalene acetic acid (NAA) was applied in lanolin to the leaf scars of defoliated trifoliate orange seedlings there was an increasing inhibition of bud burst with increasing concentrations of NAA of 0.1% and 1.0%. Defoliated controls showed the same extent of bud burst as 0.01% NAA and non-defoliated controls the same as 1.0% NAA (Stathakopoulos and Erickson, 1967).

Similarly, a 1% aqueous solution of the ethyl ester of NAA sprayed onto the trunks and scaffold branches of pruned lemon trees significantly reduced the number of new shoots counted 11 months later (Boswell et al., 1976).

The same concentration of auxin (1.0% NAA), applied exogenously, reduced shoot extension growth and hastened the abscission of the shoot tip (Boswell et al., 1976; Stathakopoulos and Erickson, 1967).

The role of auxin in bud formation is not as clear. Callus tissue predisposed to differentiate only formed buds when 0.25 ppm 6-benzylaminopurine (BAP) and 0.1 ppm NAA were added to the culture medium (Chaturvedi et al., 1974a; 1974b). However, when 5 or 20 ppm indole-3-butyric acid (IBA) in agar cylinders were mounted onto the ends of freshly decapitated epicotyls of 2-leaved seedlings, bud formation was greatly reduced compared to the controls in which the agar contained only water (Salomon, 1976). The differences in these 2 responses may be due to the concentrations of auxin used — a similar range of concentrations induced effects varying from non-inhibition to strong inhibition of bud burst and shoot growth (Stathakopoulos and Erickson, 1967).

Thus, although auxins exist in citrus shoots and may be necessary for shoot bud differentiation, the data from endogenous levels and exogenous applications indicate that high concentrations retard bud burst, reduce

shoot growth and cause premature abscission of the shoot tip.

Cytokinins: No report could be found regarding the identification of any cytokinin in citrus nor was any species of citrus or its relatives included in Skene's (1975) table of plants in whose xylem sap cytokinin-like activity had been reported. This probably means that no work has been published on endogenous cytokinins of citrus rather than that they are absent.*

As with other plants, callus obtained from citrus tissue grows more rapidly in the presence of cytokinin (kinetin) than without it (Altman and Goren, 1974b).

Cytokinin promoted the differentiation of pummelo stem callus into shoot buds (Chaturvedi et al., 1974a; 1974b) and increased the number of buds which differentiated in decapitated citrus seedlings (Salomon, 1976). When BAP (10^{-5} M) or kinetin was added to the culture medium used for growing excised buds, the explants produced up to 6 adventitious shoots instead of the normal two. Further, BAP was able to reduce the time required for dormant buds (excised between flushes) to burst but not for buds taken from the tree at the time of flushing (Altman and Goren, 1974a). When BAP (10-500 ppm) was applied to leafy or defoliated Marsh grapefruit shoots in winter, buds burst earlier and there was more growing buds per shoot at the time of the spring flush than on

* See footnote next page

control shoots. The number of new shoots per tree in the spring flush of Temple orange trees was also increased by 25 or 50 ppm BAP (Cooper et al., 1969).

Although exogenous cytokinin promotes bud formation and burst, there is little evidence of its effect on shoot growth in citrus. Kinetin applied to the stem of 1-leafed cuttings increased the rate of $^{14}\text{CO}_2$ fixation in the mature leaf, and this effect was heightened if callus was present at the base of the cutting (Hoffmann and Lenz, 1975). This may have been due to increased metabolic activity in the axillary bud and/or basal callus, or to an effect of kinetin on photosynthesis per se — the study did not differentiate between these two nor were there any observations on the effect of kinetin on shoot growth.

Kinetin (100 ppm) applied to either leafy or defoliated growing shoots of Marsh grapefruit trees in the field had no effect on the duration of growth nor on the abortion of shoot apices. It also had no effect on the length of leafy new shoots but reduced the length of shoots whose leaves were removed as they grew (Cooper et al., 1969).

So, though cytokinin activity has not as yet been reported within vegetative citrus tissue*, applications of cytokinin promote bud formation and burst but seem to have no effect on normal vegetative shoot growth.

* Note added in press: Bausher and Cooper (1976) have recently reported 0.2 to 10 ng zeatin equivalents of cytokinin-like activity per ml root exudate of three citrus species.

Gibberellin: Gibberellin-like substances have been obtained from various tissues of citrus. In 1961, Sumiki and Kawarada reported the first positive identification of a gibberellin from citrus shoots — GA_1 . Subsequently, Khalifah et al., (1965) isolated three GA-like compounds from young fruits of Washington Navel and Eureka lemons; two of these compounds were similar to GA_1 and GA_9 but the third was not similar to any of the 9 gibberellins known at that time. A GA-like compound was also extracted from flushing citrus stems by Goldschmidt and Monselise (1968a, 1968b) but was not identified.

A variety of GA-like compounds, the levels of activity of which varied throughout the year, were found in the leaves and bark of field grown trees. When the total activity of water-soluble GA-like compounds was measured throughout the year peaks of activity were found in the new lateral shoots and in the bark of trunk and branches at the time of flushing (Goren et al., 1971; Wallerstein et al., 1973). In a comparison between 3 types of actively growing lemon shoots, the highest GA-like activity was found in purely vegetative shoots and the least in shoots which bore only flowers; shoots bearing flowers as well as leaves showed an intermediate level of activity (Goldschmidt and Monselise, 1972).

Exogenously applied GA promotes bud burst : In decapitated 2-leaved seedlings, 5 or 20 ppm GA_3 reduced the formation of buds at the cut surface of the epicotyl,

but promoted the growth of shoots from buds which did form (Salomon, 1976). When GA_3 , either as the acid or the potassium salt (KGA_3), was sprayed onto the mature foliage of trees in the field or onto potted plants in growth chambers bud burst was generally improved and hastened. This occurred whether the trees in the field were treated in winter or in summer. Moreover, KGA_3 induced buds on actively elongating, defoliated shoots to burst while buds on the defoliated controls were inhibited (Cooper and Peynado, 1958; Cooper et al., 1969; Siddiqui, 1959).

Gibberellic acid also reduced the time required for excised buds grown in vitro to burst in summer when the time required on basal medium was minimal and the buds were ready to burst (Altman and Goren, 1974a). However GA_3 did not hasten burst in dormant buds excised from trees in winter or spring. This may have been due to the concentration of GA_3 used being sufficient to cause induced buds to burst but not to overcome inhibition in dormant buds.

Gibberellin also influences differentiation within the bud, and hence, the type of shoot which is formed (Goldschmidt and Monselise, 1972; Sauer, 1951). If GA is applied to buds before the pre-differentiation stage (see Abbott, 1935) or shortly afterwards, flower bud formation is retarded, arrested or even reversed (Goldschmidt and Monselise, 1972; Hirose, 1968; Monselise,

1973; Monselise and Goren, 1969; Monselise and Halevy, 1964; Moss, 1975; Moss and Bellamy, 1972; Nir et al., 1972).

Gibberellin applied as a single droplet to the shoot apex, or as a spray or paste to other aerial parts of the plant, increased shoot length in a wide range of citrus genotypes and over a wide range of citrus plants, from very young seedlings to trees in the field. The majority of the evidence suggests that application of GA increases shoot fresh weight (Bhambota and Kaul, 1966; Randhawa and Iwata, 1965; Randhawa and Singh, 1959), shoot dry weight (Lenz and Karnatz, 1975; Monselise and Halevy, 1962; Randhawa and Iwata, 1965), leaf number (Bhambota and Kaul, 1966; Jovanovic, et al., 1968; Thomas et al., 1963). The relationship between leaf area and gibberellin application varies: in some studies, applying GA increased leaf area (Randhawa and Singh, 1959; Thomas et al., 1963) and in others it reduced leaf area (Ahmed and Khan, 1964; Bhambota and Kaul, 1966; Monselise and Halevy, 1962). Although individual shoots on GA-treated trees displayed the usual extended shoot length and retained their apices after extension growth had ended, total shoot elongation on these trees was less than that of the unsprayed controls (Cooper and Peynado, 1958).

Gibberellin-stimulated shoot growth is characterized by elongated internodes, long narrow "willow-like" leaves often with elongated petioles and

no second abscission zone, long thorns and, sometimes, attached apices after maturity (Cooper and Peynado, 1958; Cooper et al., 1969; Randhawa and Iwata, 1965; Salomon, 1976; Thomas et al., 1963; Thorpe and Hield, 1970; Ting, 1962). The elongated internodes have larger cells in the cortex and pith i.e. the increased length is due to greater cell expansion rather than to greater cell division (Ahmed and Khan, 1964; Bhambota and Kaul, 1966; Thomas et al., 1963). In contrast, GA treatment reduced root growth whether applied to the foliage or to the roots (Bhambota and Kaul, 1966; Lenz and Karnatz 1975; Monselise and Halevy, 1962; Nishiura and Iba, 1964; Saito and Yamamoto, 1965). When GA was added to the culture solution the reduced root growth was accompanied by fungal growth on the roots (Randhawa and Iwata, 1965; Ting, 1962).

From a comparison of the total dry weight of seedlings with their leaf area and total chlorophyll content Monselise and Halevy (1962) concluded that GA increased the synthetic efficiency of leaves. Similarly, Lenz and Karnatz (1975) found that GA_3 increased photosynthetic efficiency and this was paralleled by an increase in transpiration rate (though a better water-use efficiency). This suggested that the higher rate of photosynthesis was controlled by stomatal movement. Gibberellic acid also increased cambial growth and stem diameter, and this may have enhanced translocation (Lenz and Karnatz, 1975).

The accumulation of GA-like substances in the bark and leaves above a ring and the reduction of GA-like activity in roots after ringing (Goren et al., 1971; Wallerstein et al., 1973) indicate that GA is phloem-translocated, but there is no evidence to suggest that it is translocated exclusively in the phloem.

In summary, the activity of gibberellins (GA₁, and GA₉ and other GA-like compounds) apparently increases in citrus around the time of flushing and is greater in growing vegetative than in growing floral shoots. Applications of GA retard flower formation, promote bud burst and shoot growth and retard root growth. The evidence suggests that GA is translocated in the phloem of citrus plants.

Inhibitors: Abscissic acid-like inhibitors have been found in the leaves and bark and in growing shoots of citrus (Goldschmidt and Monselise, 1968a, 1968b; Goren et al., 1971). The concentration of ABA-like inhibitors was lowest in mature spring flush leaves when the growth of the new flush was most intense (Goren et al., 1971). However, in a comparison of such inhibitors in stems of healthy trees and of trees suffering from spreading decline, Feldman et al., (1966) found that there was more ABA-like inhibitor at or shortly after flushes of growth in healthy trees; infected trees contained more inhibitor throughout almost the entire

year but the level fell through winter and was lowest at the time of the spring flush. As Hanks and Feldman (1968) suggest, "dormancy in citrus trees may not be principally controlled by growth inhibitors alone".

On the other hand, 20 ppm ABA considerably reduced bud formation on epicotyls of decapitated seedlings (Salomon, 1976) and concentrations of greater than 100 ppm delayed bud burst in the field and under controlled environment conditions (Cooper et al., 1969; Young and Cooper, 1969). In studies with excised buds, ABA completely inhibited the bursting of buds taken from the tree in summer (June) but this inhibition disappeared when the buds were transferred to basal medium without ABA (Altman and Goren, 1974a).

The results of bioassays of chromatograms from various citrus tissues (Goldschmidt and Monselise, 1968a, 1968b; Goren et al., 1971) leave little doubt that other growth inhibitors exist in this group of plants. Some of these have been identified :

Crystals of hesperidin were obtained in extracts from stems of trees infected with exocortis or spreading decline (Feldman et al., 1966; Hanks and Feldman, 1970). The concentration of hesperidin was always greater in diseased trees than in healthy trees and increased through winter but fell in spring when the new flush was growing. When applied to 1-year-old seedlings hesperidin delayed bud burst for 7 weeks (Feldman et al., 1966; Hanks and Feldman, 1968).

Growth-inhibiting coumarins have been isolated from the leaves (Khastagir, 1947) and roots (Tomer et al., 1969) of citrus. One of these, seselin, retarded radicle growth in a number of seedling germination systems and this was associated with increased peroxidase and IAA-oxidase activity (Goren and Tomer, 1971).

Other growth-inhibitory phenolic compounds also occur in citrus stems and buds (Feldman et al., 1966). Buds with both light and heavy infestations of the citrus bud mite (Aceria sheldoni) contained more growth-inhibiting phenolic compounds than uninfested buds (Ishaaya and Sternlicht, 1969). Whether this increase was due to a response of the plant to the infestation or to injection of these compounds into the plant by the mite was not investigated.

It is clear that there is an array of growth-inhibitors in citrus tissues and increases in endogenous levels of some of these have been correlated with retarded growth and disease symptoms. The evidence that endogenous levels of abscisic acid control bud burst or shoot growth is not convincing even though exogenous applications of ABA inhibit bud burst.

1.5.3. Location of the bud

The location of a bud on the shoot, in relation to both gravity and its distance from the apex of the shoot and the root influences bud burst and shoot growth. Using a range of citrus plant material Halma (1926) showed that

buds nearer the apex of mature shoots burst earlier and grow into longer shoots than buds located further towards the base of the mature shoot. He also demonstrated that only the dorsal buds on horizontal shoots burst and grow. The work of Soni and Randhawa (1975) and Suzuki et al., (1973) support the conclusion that shoots (called terminal shoots) arising from buds located at the distal ends of mature shoots are larger than those from more proximally located buds (called lateral shoots).

It appears that lateral buds and shoots are more inhibited than terminal buds and shoots and that this inhibition is mediated by endogenous factors but probably is reinforced by environmental factors.

1.6. Some problems associated with experimental work on tree crops

Due primarily to the perennial nature of the plant, research with tree crops often entails the solving of problems distinct from those found in annual plants. The most significant of these is the rate of growth which determines the time factor in both the most trivial investigation and in manipulations aimed at gaining familiarity with the material and the establishment of a workable experimental system. Propagation and the subsequent growth of tree plant material to the stage at which it can be used experimentally is time-consuming; it becomes necessary to establish and maintain large populations of plants in advance of experimentation to avoid lengthy delays between experiments.

These large populations require large areas of glasshouse or controlled-environment space. Unless identical environmental conditions can be maintained over extensive areas, populations having different histories of early growth may have to be used. This contribution to the variability in the material is added to the inherent variability of tree plant material which tends to be high compared with populations of annual species. Variability is further increased by interactions between the effects of different climatic conditions, of different groups of experimental material and of different stages of development.

In this study several types of experimental plant material were used in efforts to investigate and overcome some of these difficulties; some specific problems encountered with different kinds of plant material are discussed below.

Rooted cuttings: Success in the propagation and growth of rooted cuttings is reported to vary considerably between individual research workers and between locations (Nelson, 1976). Despite this, it was thought that rooted cuttings would be an expedient form of plant material as they should be of similar genotype and consist of mature, not juvenile, plant material; moreover they can be grown in small containers under uniform environmental conditions.

However, according to the literature, the propagation of citrus cuttings is slow: It took 2½ months for roots to develop on Washington Navel cuttings (Shafrir and Mendel, 1970). Only 5% of leafy cuttings and 48% of defoliated cuttings had rooted after 2 months in a study by Fatta del Bosco and De Michele (1968). De Fossard and Lenz (1966) observed well-developed roots in 70 to 80% of cuttings 6 weeks after planting, and 3 months later these plants had produced 2 growth flushes. Similarly under their most favourable conditions, Gates et al., (1961a) obtained shoot growth in 33% of cuttings 3 months after removal from the tree. These reports suggest that the

propagation of cuttings is low-yielding as well as time-consuming.

The success of propagation of rooted cuttings also varies with the time of year (Bouزيد, 1975).

Cuttings taken in summer produce the most roots and are most successful (De Fossard and Lenz, 1966; Fatta del Bosco and De Michele, 1968; Gates et al., 1961a; Lenz, 1964). Under South Australian conditions greatest success in striking is obtained with cuttings taken in January (P. Kriedemann, personal communication, 1976).

Other factors which affect the rooting of cuttings and/or their subsequent growth are the position on the parent tree from which the cuttings is taken (Lenz, 1964), the phosphorus status of the tree (Gates et al., 1961a), the age of the cutting (Bouزيد, 1975; Shafrir and Mendel 1970; Singh 1962), the part of the stem from which the cutting is taken (Bouزيد, 1975), the time of day it is taken (De Fossard and Lenz, 1966; Gates et al., 1961a), and the nature and temperature of the rooting medium (Bouزيد, 1975; De Fossard and Lenz, 1966; Shafrir and Mendel, 1970; Shafrir, 1970). Clearly, rooted cuttings can be very variable material unless strict precautions are taken to ensure that as many of the above factors as possible are kept constant. Even so, there are certain to be differences between batches of cuttings even if only one batch is taken from the parent tree each year, due to uncontrollable variations in the climatic and cultural

environment surrounding trees in the field. In addition, as only cuttings taken during a short period in summer root and grow satisfactorily, large populations have to be established at that time in order to provide experimental material for the entire year.

Budlings: Plants propagated by budding (budlings) are at least two years old before they are ready for sale (Blain, 1976; Moss, 1975) and consequently the established practise is to propagate them either in the field or in large containers. Field-grown budlings are perforce grown in variable edaphic and climatic conditions, with the risk of pest and disease infestations, etc. In comparison, container-grown budlings are easier to select and manipulate and can be grown under more uniform soil and climatic conditions. However, the large size of the containers required (Platt and Opitz, 1973) often precludes the use of such material in controlled-environment conditions.

Some characteristics of budded plants per se also produce problems. Stock/scion incompatibility is a widespread phenomenon in horticulture (Nelson, 1968) and exists in citrus (Bevington, 1976). The possible inherent, but unknown, variation within the implanted bud (Shamel, 1943) is a source of concern for the commercial nurseryman as well as for researchers working with budded material. It is difficult, at present to determine if

and when a particular implanted bud will burst, and how it will grow after it has burst (Moss, 1975; I.Tolley, personal communication).

It is also possible that many of the factors relating to the parent tree which cause variation in the growth of rooted cuttings may influence the growth and development of budlings, e.g. the position on the parent tree from which the bud has come, the phosphorus status of the tree, age of the budwood, etc.

In order to obtain statistically valid data in the light of this inherent variability it is necessary to replicate treatments many times, thereby increasing the number of individuals needed and limiting the number of experimental treatments which can be compared.

Budlings may be obtained at an advanced stage from professional nurserymen, thereby saving the time of the researcher, but citrus is only budded, and can only be supplied at restricted periods of the year.

Seedlings: Perhaps the most convenient experimental material for some aspects of citrus research is the seedling. However, they are not without problems. Even under optimal conditions citrus seeds require 3 to 4 weeks for germination (Monselise and Kadman-Zahavi, 1965) and a further 2 months before leaves beyond the first foliage leaves emerge (Schneider, 1968). Moreover, under sub-tropical conditions, seeds are available from a tree only

once in the year and may rapidly lose viability depending on the method of extraction and the conditions of storage (Monselise and Kadman-Zahavi, 1965; Platt and Opitz, 1973; I.Tolley, personal communication).

To maintain a supply of seedlings, therefore, it is necessary to have either a regular propagation programme and try to retain seed viability throughout the year, or to sow as many seeds as practicable and grow the seedlings throughout the year. In the first strategy seedlings of a uniform maturity can be supplied throughout the year provided the problems of seed viability are solved. In the second, the seedlings used will be of different stages of maturity when comparing experiments carried out at different times.

Perhaps the greatest advantage in using seedlings is the uniformity they afford. Although there is as yet no test which accurately distinguishes between zygotic and nucellar seedlings of polyembryonic varieties (Frost and Soost, 1968) roguing out of the visibly larger- and -smaller-than-average seedlings in a population sown at the same time produces a crop of seedlings which grow uniformly and which are probably largely nucellar (I.Hosein, I.Tolley, personal communications).

In summary, the main practical problems in tree crop experimentation revolve around the time scale of any operation or investigation, the variability of material, the large populations which must be constantly

maintained and consequently the large areas necessary for these plants and the effects of the previous cultural and environmental history of the plant material on its subsequent growth.

1.7. The nature and scope of the present work

The experimental section of this report is divided into three parts: The first concerns the morphology of dormant buds and the growth of vegetative shoots of citrus. The second describes efforts to promote bud burst and shoot growth of implanted buds in commercial budlings in citrus. In the third, treatments which alter the extension growth of axillary buds of citrus seedlings are examined and their effects on some aspects of the physiology of the plant are assessed.

2. EXPERIMENTAL

2.1. A study of the morphology of buds and some characteristics of vegetative shoot growth in citrus

This work was undertaken during the initial stages of the programme with the aim of developing an overall understanding of the material. Rooted cuttings were used as the experimental material.

2.1.1. Development of the bud

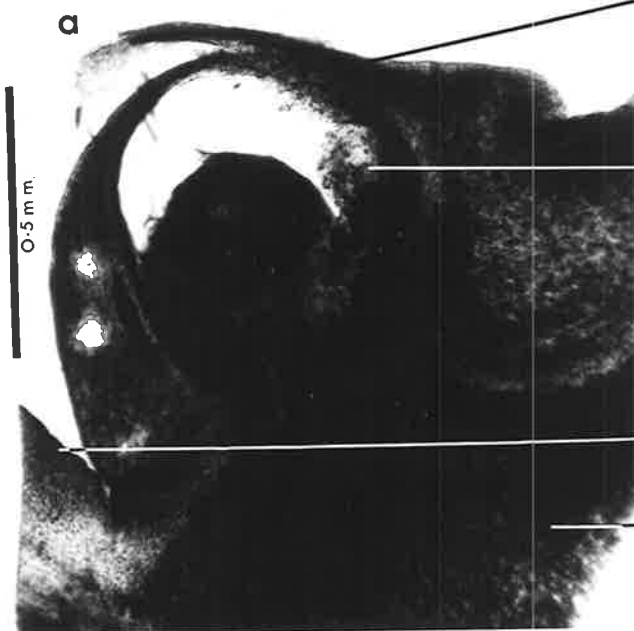
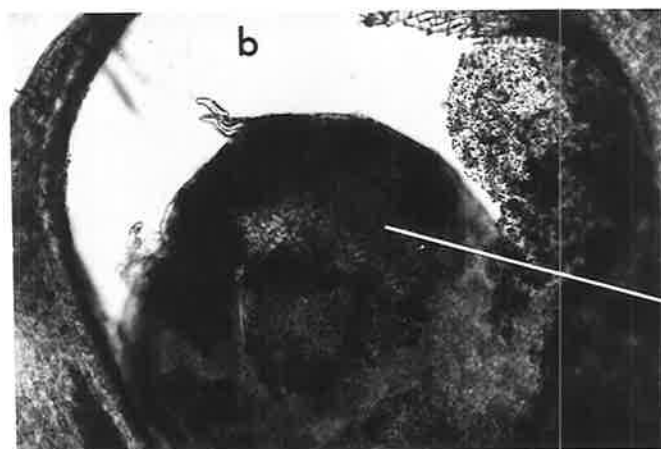
Introduction: Descriptions of axillary buds of citrus (Bartholomew and Reed, 1943; Schneider, 1968; Scott et al., 1948) refer to an apical meristem and leaf primordia enclosed in prophylls. Bud primordia within the axils of the primordial leaves are not mentioned (except the presence of accessory buds in the axils of prophylls (Schneider, 1968)), nor is there any account of the further development of the vegetative axillary bud. In contrast, the ontogeny of the axillary buds of many species of angiosperms and gymnosperms have been described in detail (Garrison, 1949, 1955; Goebel, 1905; also see references cited by Romberger, 1963). It would seem axiomatic that a knowledge of the origin of the shoot should precede an understanding of its subsequent growth and this investigation was undertaken to fill the evident gap in published information.

Methods and Materials: Axillary buds on shoots of various ages on rooted cuttings of Citrus sinensis (L.)

Osbeck cv Washington Navel sweet orange were dissected under a dissecting microscope.

The plants were obtained from cuttings taken at dawn from trees which had been thoroughly watered previously. Each cutting was approximately 15 cm long and had up to 7 leaves. The lower leaves were removed and the stem bases dipped in talc containing 0.1% IBA (Seradex No. 1). The cuttings were placed in either sand or Perlite in a mist propagator with bottom heat. Humidity was maintained close to 100% with a mist generator controlled by an electronic leaf. The propagator was located in a glass-house maintained at a temperature between 20°C and 30°C. After the cuttings had rooted they were transplanted into a 1 to 1 mixture of washed, coarse, Waikerie sand and John Innes potting compost (Lawrence, 1950) in 10cm diameter pots. As they outgrew the containers the cuttings were transplanted (complete with potting mix) into larger containers (12.5 cm diameter) containing John Innes potting compost. The potted cuttings were grown in growth cabinets at 30°C with 16 hours of light per day generated by banks of fluorescent tubes supplemented with incandescent bulbs. The plants were supplied regularly with a solution of John Innes "feeder" (Lawrence 1950) and were grown under these conditions for at least 6 months before shoots were taken for dissection.

Plants were examined twice each week and new shoots were labelled as they appeared. All the buds on



c

Primary bud

Foliage leaf
primordium of
primary bud

Prophyll of
primary bud

Secondary bud

Tertiary bud

Prophyll of
secondary bud

Petiole

Thorn

Stem

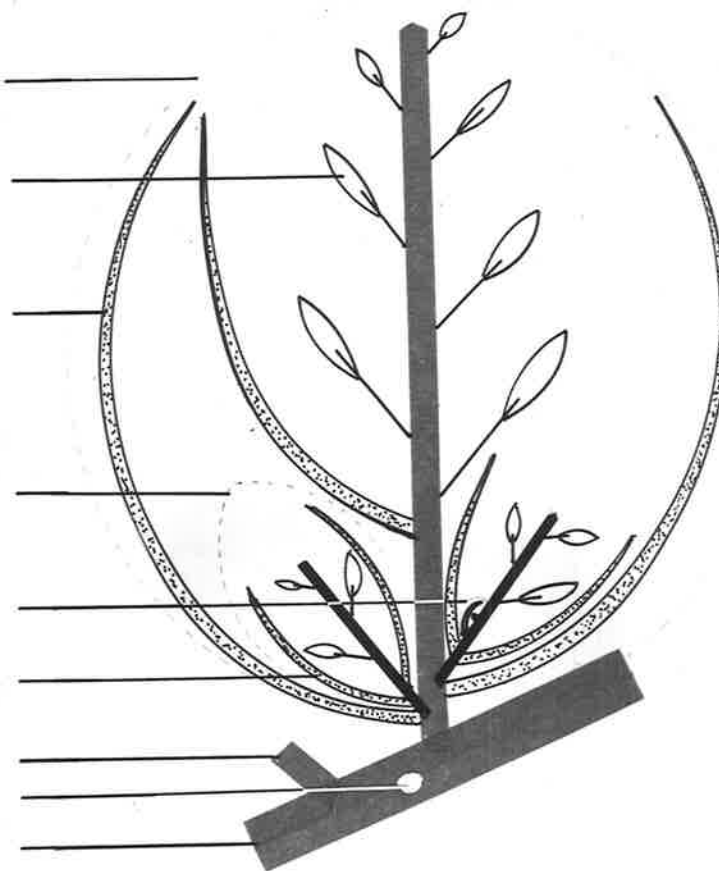


Figure 1. The axillary bud complex of Washington Navel sweet orange.

- a) Section of bud showing prophylls and leaf primordia of primary bud, and secondary bud in axil of prophyll.
- b) Enlargement of central part of a) showing secondary bud and leaf primordia of primary bud.
- c) Expanded diagrammatic representation of an axillary bud.

19 shoots were dissected and the contents of each bud recorded. Although the outer prophylls are quite different from the primordial leaves and leaf primordia, they are here considered as primordial leaves as it was difficult to determine conclusively whether a particular structure would develop into a foliage leaf or into a prophyll. The shoots varied between 1 and 48 days of age when the buds were harvested.

All the plants became infested with 2-spotted mite (Tetranychus urticae) despite treatments involving natural predators and various pesticides. It was found that the infestation could be kept within tolerable limits by spraying the foliage with tap water daily.

Results and discussion: Throughout this section the term bud refers to any structure consisting of some or all of the following: an apical meristem or dome, leaf primordia, primordial leaves and overlying prophylls.

In accord with the nomenclature used by Garrison (1949, 1955) the mature or main bud is called the primary bud and the bud or bud primordia in the axils of primordial leaves within a large mature bud are referred to as secondary buds (Figure 1). Therefore, the dormant mature axillary buds of citrus consist of a primary bud with secondary buds in the axils of the outer prophylls. The older secondary buds are often enclosed within their own prophylls. The internodes between the prophylls and leaf primordia of the primary bud are not elongated so

the primary and secondary buds seem to arise from the same axillary meristem and lie close together. The entire structure is less than 15 mm wide and can be seen with the naked eye. However the leaf primordia are very small and magnification is necessary during dissection.

Secondary buds are referred to as "accessory buds" by Schneider (1968) but differ from accessory buds as defined by Garrison (1955). The accessory buds of Juglans cinerea for example, arise from the same meristems as the primary buds and are not axillary buds within the primary buds (Garrison, 1955). Although no cytological studies were carried out to clarify the origin of these buds in citrus, the fact that they exist in the axils of the prophylls of the primary bud and not completely outside it strongly suggests that they are secondary buds.

Apart from this and the lack of bud primordia in the axils of the foliage leaves of the primary buds, the mature axillary buds of sweet orange seem to resemble closely those of Juglans cinerea — the primary citrus bud has 3 to 5 prophylls and up to 8 foliage leaves whereas secondary buds are smaller and tend to have 2 to 3 prophylls and up to 5 leaf primordia.

Frequently a tertiary bud is found in the axil of the oldest prophyll of the secondary bud and consists of 1 to 3 leaf primordia covering a dome-like apex.

Table 2. Composition of axillary buds of rooted cuttings of Washington Navel

Age of shoot (days)	No. shoots dissected	Average no. leaf primordia* in primary bud				Average no. buds per node				Average no. nodes on :-	
		Basal	Proximal $\frac{1}{3}$	Middle $\frac{1}{3}$	Distal $\frac{1}{3}$	Basal	Proximal $\frac{1}{3}$	Middle $\frac{1}{3}$	Distal $\frac{1}{3}$	Maturing shoot	Aborting tip
1	5	0.5	0.5	0.5	0	0.6	0.6	1	0	†	†
5	1	4	5	4	3.5	3	3	3	2	5	6
9	1	7	7.5	6.5	6.5	2	2	2.5	2	7	-
10	1	8	10	8.7	6.8	3	3	3	2	11	7
17	1	10	8.3	9.3	9.3	3	3	3	2.7	10	-
24	4	3	7.2	8.8	8.4	1.2	2.3	2.6	2.4	8.2	-
32	2	8.5	10.1	10.4	8.8	3	3.7	3.2	3.2	11.5	8
40	2	7	9.2	10.8	12.3**	3	2.5	2.5	2.5	7	-
44	1	7	10	11.5	19**	3	3	3	4	5	-
48	1	0***	9.7	11	10.7	2***	3.7	3	3	11	-

* Includes prophylls, primordial foliage leaves and leaf primordia.

** One bud on distal $\frac{1}{3}$ had just burst and its leaves are included.

*** "Blind bud", i.e. 2 small protuberances at node, no typical bud organisation.

† It was not possible to tell how many of the primordial leaves on 1-day-old shoots were going to grow into mature leaves and how many would abort with the shoot tip.

Blanks under the "aborting tip" column indicate the tip had abscised before the sample was taken.

Throughout this study, during which more than 300 buds were dissected, no secondary buds were ever found in the axils of primordial foliage leaves within the dormant primary bud, not even in swollen buds where the outer 2 prophylls were spread apart and the buds seemed about to burst. In Syringa, Goebel (1905) observed that axillary buds were only laid down as the primary bud unfolded. However, Garrison (1949) showed that the bud primordia were present in the axils of primordial leaves while still within the primary bud, but became obvious only after bursting of the primary bud. The pattern in Washington Navel sweet orange may well be similar though the sequence of events must be more rapid: in sweet orange the secondary buds appear as the primary buds burst whereas in Syringa the secondary bud in the axil of the oldest leaf increases considerably in volume, producing a visible bud primordium during the 2 weeks following the bursting of the primary bud.

The secondary bud in the axils of the oldest primordial leaves of the bursting primary bud consists of a dome which is sometimes concealed by an overlying leaf primordium. During the next 5 days, under growth cabinet conditions, about 4 new leaf primordia arise and this rate continues up to 10 days after (primary) bud burst when the axillary bud has approximately 8 primordial leaves (Table 2). Thereafter the rate of proliferation falls drastically and very few, if any, new leaf primordia

are initiated for the remainder of the bud's dormant period.

The ontogeny of the buds in the axils of the primordial prophylls is even more remarkable. Within 5 days after bud burst the single dome in the axil of the young leaf on the young shoot is transformed into a complex of multiple buds: primary, secondary and sometimes even tertiary. This indicates that there is not only rapid proliferation of leaf initials in the young axillary bud but there is also rapid morphogenesis of buds in the axils of the primordial prophylls.

A similar spurt of activity in the primary bud must also occur as the buds burst. Even in buds about to burst the primary bud consists of 7 to 8 leaves enclosed in up to 5 prophylls. Yet 1 day after bud burst the young shoot has 12 to 15 leaves, not including the prophylls.

Therefore the ontogeny of an axillary citrus bud is characterized by 2 periods of rapid development separated by a varying period of dormancy : 1) the appearance of the (primary) bud primordium and differentiation of leaves and secondary buds from this bud primordium early in the growth of the parent shoot, and 2) proliferation of leaf primordia at the time of bud burst.

Since the location of a bud along a shoot influences its subsequent growth (Halma, 1926; Soni and

Randhawa, 1975; Suzuki et al., 1973) buds were dissected along each shoot to assess whether there was any relation between bud location and stage of development. As the number of nodes on individual shoots varied widely the data is classified into 4 groups - the basal bud, referring to the most proximal bud, which seldom grows and is often a "blind bud" i.e. a vestigial bud which, for example, is never used in commercial budding (I.Tolley, personal communication), buds of the proximal third, of the middle third, and of the distal third of the stem. This classification is supported by observations (see following section) that the pattern of growth and maturity of the shoot is from the base to the tip and can be conveniently examined on this basis.

At the time when a new shoot emerges from between its prophylls no buds can be seen in the axils of the leaves of the distal third, while leaves of the proximal and middle thirds have bud primordia in their axils (Table 2). Five days later, all leaves destined to mature subtend multiple axillary buds. There are fewer secondary buds within the axillary buds of the distal third than within the axillary buds of the middle or proximal thirds. This difference exists for at least the first 10 days (Table 2) but may not be significant as only one shoot was dissected on each of the 5th, 9th and 10th days. Similarly there is a tendency for buds of the distal third to have fewer leaf primordia during

this period. On shoots older than 10 days there is no difference in the average number of buds per node nor in the average number of leaf primordia per bud along the shoot except for the primary bud of the basal node which has fewer leaf primordia than those at the other nodes along the shoot. Thus the propensity of distal buds to burst is not manifested in an increase in leaf or bud primordia within the dormant bud.

There is usually a thorn or thorn rudiment associated with each axil. This sometimes consists of 2 to 3 prophylls enclosing up to 3 leaf primordia and thus resembles the accessory bud of Juglans cinerea (Garrison, 1955).

Whether these accessory buds arise from the same meristem as the axillary buds, as in the case of Juglans, cannot be determined from this study, nor is it clear when and from what tissue is the axillary bud initiated (see discussions by Garrison, 1959 and Romberger, 1963).

It appears that there is a rapid differentiation in the apical bud and axillary meristems within the bud around the time of bud burst and for a short period afterwards, and that the time and rate of differentiation varies along the stem. These questions warrant further and more detailed study which would be greatly facilitated if plant material were available in which time of bud burst could be accurately predicted.

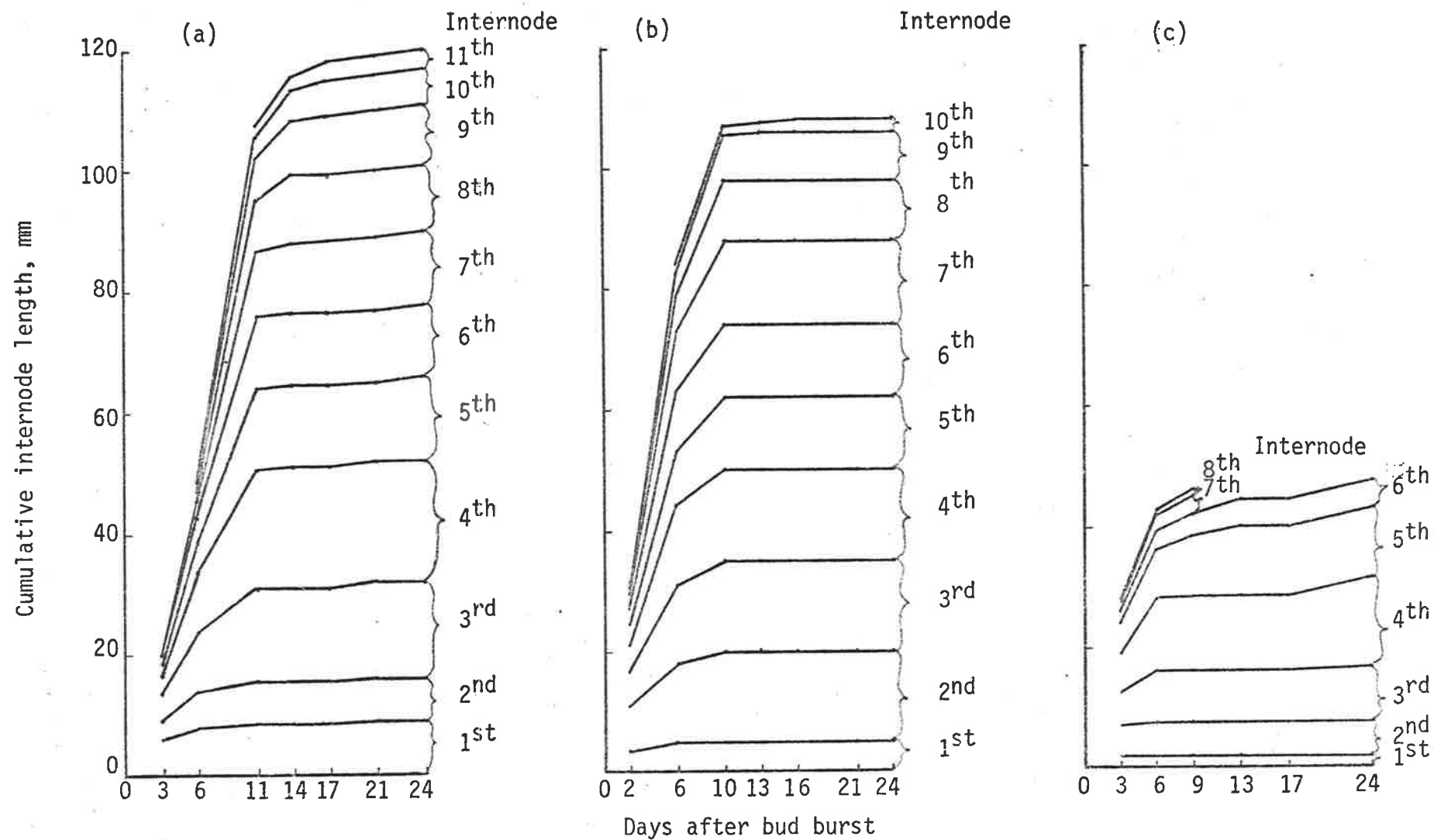


Figure 2 Growth pattern of 3 shoots, showing lengths of individual internodes.

2.1.2. Growth of the shoot and abortion of the tip

Methods: The plant material, and the methods of propagation and rearing were as described earlier (2.1.1. Methods). The plants were examined twice per week and new shoots labelled as they appeared. On the observed shoots the length of every leaf greater than 5 mm and of the internode below it were recorded twice per week for the following 3 weeks. Attempts to measure leaves shorter than 5 mm and the internode below them resulted in damage to the young parts and were abandoned.

Results and discussion : Although the environment was kept constant and the plants were evenly watered and fertilized, there was considerable variation in the growth of individual shoots, both on a single plant and between different plants. There were differences in the times at which buds burst, as well as in the final lengths of the shoots and in the numbers of leaves per shoot. Some indication of the range is given in Figures 2a and 2c. The pattern of growth of an average healthy shoot is shown in Figure 2b. The plants had been growing under non-inductive conditions (Moss, 1976a) for more than 6 months, and care was taken to see that no water-stress was inflicted on them, yet some buds produced floral shoots; these shoots were not included in the assessment of shoot growth.

In general, the stem elongated rapidly for approximately 10 days after bud burst, then elongation

stopped. This was true of short shoots as well as long shoots (Figure 2). A similar rate, duration and extent of shoot growth was found in C. sinensis under field conditions in Viet Nam (Bui-Huy-Dap and Nguyen-hun-Thuoc, 1962).

The most basal internode elongated first and was almost fully elongated within 3 days of shoot emergence. By this time the internodes of the proximal third of the stem had also commenced to elongate, whilst those of the middle and distal thirds had scarcely grown. Within 6 days of bud burst the proximal and middle thirds had greatly elongated while the most distal internodes remained unexpanded. During the following 4 to 6 days the proximal internodes elongated slightly whereas the internodes in the middle and distal portions of the stem grew rapidly. The pattern of growth over the whole stem was very similar to that of Liquidambar styraciflua (Lam and Brown, 1974) and to that of individual internodes of Helianthus (Garrison, 1973) in that there was a wave of elongation from the base to the tip, but it differed from the growth pattern of the stem of Helianthus (Garrison, 1973) in that consecutive internodes elongated in rapid succession and the whole extension process was of much shorter duration.

In the fully extended shoot, the internodes of the base and tip were shorter than those in the middle, i.e. the variation in internode length along the stem

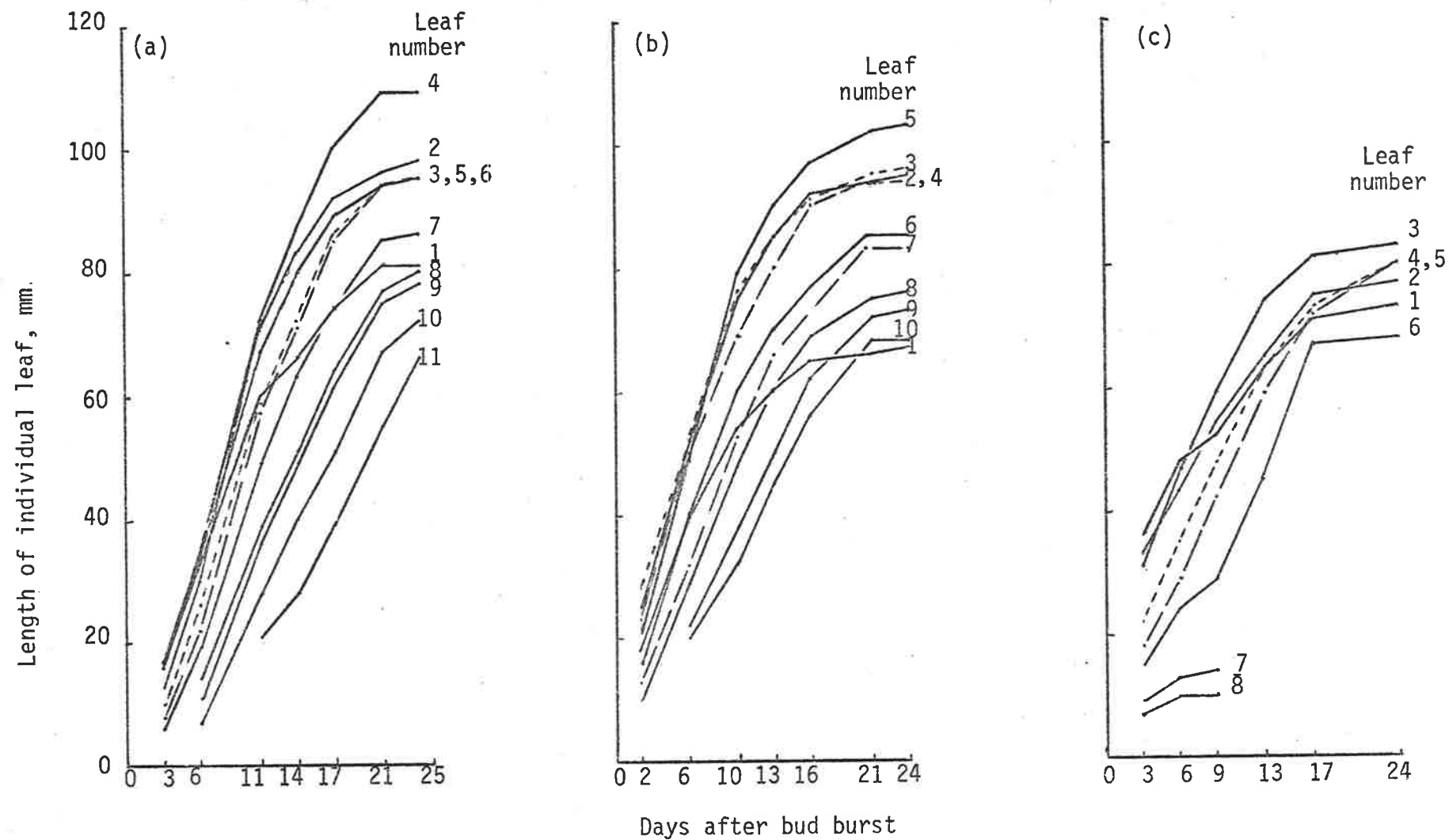


Figure 3 Elongation of the individual leaves of the 3 shoots of Figure 2
Leaves numbered acropetally, i.e. most basal leaf = no. 1.

conformed to the bell-shaped curve typical of trees (Lam and Brown 1974).

The lower and middle leaves were longer than 5 mm within 3 days of bud burst whereas the upper leaves did not achieve this length until around 6 days after shoot emergence (Figure 3). In this context the bud prophylls are not included in the leaves as the internodes between them did not elongate and they never formed part of the extended shoot.

As the shoot grew the internodes expanded, the leaves grew slowly, reflexed away from the stem and then began to expand rapidly. At the time of arrest of extension growth of the stem the leaves were expanding rapidly, and this expansion lasted for at least 3 weeks. Under field conditions in California, Valencia orange leaves grew more slowly and over a much longer period and never attained the lengths found in this study (Scott et al., 1948). This may have been due to climatic, cultural or genotypic factors, or to the location of the leaves on the shoots. It is clear from Figure 3 that at maturity the lower-middle leaves of the shoot were largest even though they started elongating rapidly at the same time as the leaves above and below them. In general, each leaf of the distal half of the shoot was shorter than the leaf just basipetal to it. This pattern was established early in the growth cycle and was maintained as the shoot grew. However, the most basal leaf

a



b



Figures 4a and 4b. Citrus shoots at different stages of development.

4a) Young shoot at stage 7, just before arrest of stem elongation.

Arrow indicates last leaf which will expand.

4b) Older shoot, stage 10.

Arrow indicates aborting tip.



Figure 4c. A lopped budling in the field.

The young shoot has emerged and is at stage 5.

Aluminium guard around stock inhibits shoot growth from lower buds (suckers) and protects stem from sand blasting.



Figure 4d. Examples of variability of shoot growth in budded plants.

Left: Scion has made rapid continuous growth in the first flush; it is at stage 6 to 7.

Right: First flush produced a short shoot with about eight leaves. Second flush has started and is growing rapidly; it is at stage 5.

was usually among the shortest on the shoot, because the duration of its linear growth phase was shorter than the rest.

Within 10 days after shoot emergence it was usually clear which leaves would elongate and which would abort with the tip; the latter leaves were shorter than 5 mm at that time (Figure 4a). In fact, the region of abscission was indicated in this way even by 5 days after bud burst; the leaves and internodes distal to it generally did not elongate. Occasionally the lower 1 or 2 leaves on the aborting tip would expand slightly, but the rate of their growth never approximated that of leaves destined to mature (e.g. leaves 7 and 8, Figure 3c).

The aborting tips of shoots of rooted cuttings in growth cabinets were often dead and dry by the time they abscised, and were unsatisfactory for a study of the morphology of the abscising apex. The very variable occurrence of bud burst and shoot growth under these conditions also provided few apices for study at any one time. Consequently, material used in the study of abscising tips was obtained from young trees of Washington Navel scions on Troyer citrange (Poncirus trifoliata [L] Raf. x Citrus sinensis [L] Osbeck, see Hodgson, 1967) rootstock grown in John Innes potting compost in 22-litre tins. The plants had been growing in the tins, located in the open, for the previous 17 months. The plants were divided into 2 groups: group 1 consisted of leafy, healthy-looking

Table 3. Data on the abscising apices of budded Washington

Navel plants

	<u>Group 1</u>	<u>Group 2</u>
	(7 plants)	(6 plants)
<u>No. of leaf primordia</u>		
No. apices dissected	45	40*
Range per apex	7 to 14	7 to 16
Mean no. per apex	10.8	10.6
Stand. error of mean	0.21	0.36
<u>Length of oldest leaf**</u>		
No. leaves measured	29	30
Range of lengths, mm	1.9 to 18.0	1.1 to 16.0
Mean of lengths, mm	5.17	5.89
Stand. error of mean	0.634	0.610
<u>Length of oldest internode</u>		
No. internodes measured	45	42
Range of lengths, mm	0.0 to 4.2	0.0 to 5.5
Mean of lengths, mm	0.92	0.74
Stand. error of mean	0.155	0.157

* Two buds were lost during dissection after their oldest leaves and internodes were measured but before the number of leaf primordia were determined. Data from them are not included in this section.

** Frequently the oldest leaf abscised before the apex did, and was not present at sampling, hence the reduced numbers.

The oldest leaf and oldest internode are usually the longest on the abscising tip.

plants; the plants of group 2 had suffered severe defoliation of their older leaves although the new flush was green and healthy-looking. The abscising apices were collected during December 1974 at the end of elongation of the first summer flush. At the time, the second summer flush had just started and buds on mature wood had begun to burst. The abscising apices were light green or yellow in colour, and their leaves and stems were turgid. The apices were collected from one plant at a time, kept in water, and dissected under a dissecting microscope, equipped with a micrometer eyepiece, on the same day. The results are presented in Table 3.

The number of leaf primordia on the abscising apices was remarkably constant, as demonstrated by the small standard errors of the means, and did not differ between groups. The limited data obtained from rooted cuttings under growth cabinet conditions (Table 2) indicate that the average number of leaf primordia on abscising apices (approx. 7) under those conditions was decidedly fewer than on apices from potted plants growing in the open (Table 3). This difference may be due to cultural and environmental factors or to the nature of the plant material.

Certainly the number of leaf primordia on the aborting tip seems characteristic of shoots growing under a given set of conditions and is reminiscent of the characteristic interval between arrest of shoot elongation

and abscission of the shoot tip of different species of citrus (Bui-Huy-Dap and Nguyen-hun-Thuoc, 1962).

The lengths of the oldest leaf and oldest internode of the abscising tip were more variable than the number of leaf primordia. However, they bear out the observations made on the shoots of rooted cuttings, that the leaves and internodes of the abscising tip do not elongate greatly, in contrast to the rest of the shoot (Figure 4b). Even though the budded plants were much larger and more vigorous than the rooted cuttings, the average size of the oldest leaf on the aborting tip was less than 6 mm and the oldest internode was, on average, less than 1 mm long.

The observations on bud morphology, shoot growth and tip abortion produced a picture of shoot growth which can be conveniently divided into stages as described overleaf.

<u>Stage</u>	<u>Description</u>
1	Implanted or dormant bud.
2	Swollen bud, ready to burst.
3	Bud burst or shoot emergence - leaves visible, emerging through prophylls.
4	Most basal internode elongating; small but clearly distinct shoot which can no longer be considered a bud; leaves upright, lying close to stem.
5	Lower 2-3 internodes elongating, lower leaves assuming a position perpendicular to stem.
6	Middle part of shoot elongating, middle leaves becoming perpendicular to stem, lower and middle leaves elongating.
7	Uppermost 2-3 internodes elongating, upper leaves spreading out; abscission zone becoming apparent.
8	Arrest of stem elongation; all leaves open, upper ones expanding rapidly; abscission zone clearly discernible.
9	Abscission of shoot tip.
10	Maturation of foliage leaves.

2.1.3. Differences between flushes on rooted cuttings

Introduction: In preliminary tests using rooted cuttings of Washington Navel orange great variability was found in the material. Despite efforts to overcome the variability, large differences were found between shoots, even on the same plant. Gates et al. (1961a) had found differences in leaf number and dry weight of shoots of successive flushes on similar cuttings so it was decided to determine whether there were any differences between flushes in the plants being used.

Methods: Rooted Washington Navel cuttings similar to those described in Section 2.1.1. were grown for 9 months in two growth cabinets. The conditions within the cabinets were as described in Section 2.1.1. except that one cabinet was maintained at 30°C while the other was kept at 25°C.

At the time the measurements of the mature flushes were made the plants were producing their 6th flush under these conditions. Each shoot had been labelled as it emerged so the number of flushes along any branch was easily ascertained. The number of leaves and the shoot length of the 3rd and 4th flush of 3 branches on each of 13 plants from each cabinet were recorded. Average internode length was found by dividing the shoot length of the flush by the number of leaves; the shoot tip usually abscised at the uppermost node of the mature shoot leaving no internode above the uppermost leaf.

Table 4. Effect of temperature and flush on shoot growth

	Mean shoot length, mm		No. leaves per shoot		Mean internode length, mm	
	25°C	30°C	25°C	30°C	25°C	30°C
3rd flush	153.3	174.7	17.1	19.1	26.3	26.9
4th flush	208.9	222.0	25.4	24.1	24.5	26.1
p=0.05	55.5		3.3		N S	
LSD						
p=0.01	75.2		4.5		N S	

N S = No significant difference

The data was analysed as a split plot design, with temperature (or, more correctly, growth cabinet) as the main plots and flush as the sub-plots.

Results and discussion: There was no significant effect of temperature on any parameter; however average shoot length tended to be greater for the 30° than for the 25°C cabinet (Table 4). There was a significant effect of flush on shoot length and on number of leaves per shoot - the later flush (the 4th flush) consisted of longer shoots with more leaves per shoot. This was particularly true at 25°C. Neither temperature nor flush had a significant effect on mean internode length.

The cuttings used by Gates et al. (1961a) must have been more vigorous. The mean leaf numbers of the 1st to 4th flush were 8, 22, 64 and 100 respectively. The authors commented on the high variability of their material and quoted the standard error for each mean (which increased with successive flushes) as evidence of this fact but did not analyse these differences further. They also found that the dry weight of the 2nd flush was more than twice that of the 1st, and that this difference was largely due to the increase in dry weight of the laminae of the 2nd flush. The total leaf area of the 2nd flush was much greater than that of the 1st flush although the reverse was true of the dry weight per unit area.

This increase in leaf area with later flushes must be due, at least in part, to the increase in leaf number, as shown in their data and in Table 4, and emphasises that the growth of a young shoot under these conditions is influenced by the amount of mature tissue on which it is borne.

The results of this study provide some insight into several aspects of the growth of an individual citrus shoot and indicate that there were at least three stages in its growth and development which were particularly important in determining its final size. These were:

- 1) The period of axillary bud formation
- 2) The period of rapid leaf proliferation
- 3) The arrest of elongation growth

The first and second of these seemed to occur at and immediately after shoot emergence, whereas the third became apparent within 10 days of bud burst.

However although this study provided an explanation for some of the variability in shoot growth of rooted cuttings, it did not provide a means of overcoming the variability. Consequently efforts were made to find more suitable plant material.

2.2. A study of the effects of plant growth substances on bud burst and shoot growth in citrus

The failure of scion cultivars to make commercially satisfactory growth when budded or grafted to certain rootstocks is a well-known phenomenon among horticultural plants (Nelson, 1968). In citrus this incompatibility is particularly true of Eureka lemon (C. limon) on trifoliate orange (Poncirus trifoliata) or its hybrids, Troyer and Carrizo citrange rootstocks (Bevington, 1976; Tolley, 1973). In addition, the growth of scions of almost all cultivars of mandarins and of many cultivars of sweet orange on Troyer citrange rootstock is variable and generally not as good as on rough lemon or sweet or sour orange rootstocks. Because of certain other characteristics Troyer citrange is a highly desirable rootstock (Stannard, 1976; I.Tolley, personal communication) and it is important that the bud burst and shoot growth defects be overcome.

Retarded bud burst or shoot growth of the scion may be due to: a) dormancy within the implanted bud*, (b) physiological or anatomical incompatibility between the stock and the bud (scion), (c) dormancy or inhibition of shoot growth induced by the stock, especially apical dormancy; or to a combination of any or all of these.

*The excised or implanted bud referred to in this section consists of the entire structure enclosed within the prophylls together with the thorn or thorn rudiment, the base of the subtending leaf and the surrounding shield of bark and wood (Platt and Opitz, 1973).

These factors in turn may be influenced or controlled by plant growth substances.

Although bud burst in citrus is retarded and shoot growth reduced by exogenous applications of auxin (see Introduction, section 1.5.2.3.) evidence exists which indicates that auxin may be involved in promoting shoot growth. In Norway spruce (Piceetum myrtillosum) the total content of IAA, the amount of free IAA and the amount of protein-bound IAA were positively correlated with the rate and extent of shoot growth (Merkis and Skuodene, 1974). Tucker and Mansfield (1973) found that auxin levels in the buds of Xanthium strumarium increased following decapitation and then fell as the buds emerged. They suggested that auxin was utilized for growth. However from a review of the literature on auxins in buds and stems of trees (Romberger, 1963) it is clear that no universal relationship has been established between this plant hormone and the growth of buds and shoots. Nevertheless, up to 1000 ppm IAA or IBA have been found to improve bud-take in bud grafting of grapefruit (Citrus paradisi Macf.) on Karna Khatta (Citrus karna Raf.) rootstock (Maiti et al., 1959). Therefore auxin was included in this study of the hormonal control of shoot growth in citrus.

Although little work has been done on cytokinin with respect to shoot growth in citrus, the evidence from other plants indicates that it may be an important factor. Bud growth in Pisum sativum was stimulated by BAP, and

this stimulation preceded or was accompanied by cell division (Nagao and Rubinstein, 1975, 1976). From studies using inhibitors of bud development in the same species Lee et al. (1974) deduced that lateral bud development depended on the synthesis of a cytokinin which occurred within the bud itself. Hewitt and Wareing (1974) found that cytokinin levels in trees of 3 different genera increased prior to bud burst and that maximum levels in the sap and vegetative buds were obtained before or during bud break. In a mechanism of dormancy-release in Populus balsamifera buds proposed by Bachelard and Wightman (1974), cytokinin produced in the roots is translocated to the buds and activates cell division of the apical meristems.

Ethylene or ethylene-releasing chemicals increase lateral bud development in many species (Abeles, 1973). Injections of 10 μ l of 125 ppm 2-chloroethylphosphonic acid (CEPA) into the base of bud scales of apple hastened bud burst and increased the number of buds which burst (Williams, 1976). In citrus 500 or 1000 ppm CEPA induced leaf abscission in the field (Gallasch, 1974), and defoliation is known to promote bud burst in this crop (Mc Carty et al., 1965; Stathakopoulos and Erickson, 1967). Thus ethylene may directly or indirectly promote bud burst.

Gibberellins have promoted seed germination (Burns and Coggins, 1969) and seedling growth (Krishnamurthi, 1960) of citrus and have hastened bud burst and shoot growth (see Introduction, section 1.5.2.3.). However, there does

not seem to be any report of GA being used to increase bud burst or shoot growth of the implanted scions of budlings.

In the present study, budlings of Valencia orange (Citrus sinensis [L] Osbeck) on Troyer citrange (C. sinensis x Poncirus trifoliata) were used to investigate the effects of auxins, cytokinins, ethylene and gibberellins, on bud burst and shoot growth of the scions.

For much of the work in this section budlings in a commercial citrus nursery were used (kindly made available by Mr. Ian Tolley, Renmark). Thus large numbers of plants with similar life histories were available permitting an investigation of the problems of delayed bursting and poor growth of implanted buds.

2.2.1. Excised buds treated by soaking in growth substance solutions before budding

Introduction: Terminal buds, i.e. buds in the axils of the most distal leaves, have been found to burst earlier and to produce larger shoots than buds located further down the shoot (Halma, 1926; Soni and Randhawa, 1975; Suzuki et al., 1973), although Teixeira et al. (1971) found that the location of buds had no effect on scion growth. However the buds in the latter study came from the distal five nodes of the budwood sticks and their growth was not compared to that of buds from more proximal locations on the sticks.

The effects of the location of the bud along the budwood stick and of applied growth substances on bud burst were investigated by dipping the excised buds in various solutions before budding.

Methods: Seedlings of Troyer citrange growing in rows in a nursery on Murray sand (a coarse, brown sand) were budded with Valencia orange using the "micro-bud" technique (Platt and Optiz, 1973; Wishart, 1974). The buds were obtained from the second summer flush. Each budwood stick had approximately 10 buds. The budwood sticks were divided into proximal and distal sections, separated by 3 nodes in the middle. Buds from the distal part of the sticks (top buds) were pooled after excision and kept in one jar and those from the proximal part

(bottom buds) were kept in another. The buds were cut from the sticks on 30 January kept in labelled jars in the refrigerator overnight and budded the next day. Before insertion into the rootstocks the buds were soaked for approximately 10 minutes in one of the following aqueous solutions:

Growth substance	ppm	M
GA ₃	10	2.9×10^{-5}
	100	2.9×10^{-4}
BAP	10	4.4×10^{-5}
	100	4.4×10^{-4}
NAA	10	5.4×10^{-5}
	100	5.4×10^{-4}
CEPA	15	10.5×10^{-5}
	150	10.5×10^{-4}

Buds of the control plots were soaked in water. In addition, there was a treatment in which maleic hydrazide (MH) at 1000 ppm was painted onto the rootstock below the scion in order to reduce shoot growth (suckers) of the stock. The buds used in these plots were also soaked in water.

The experiment was laid out as randomized complete blocks with 15 plants per plot and 5 replicates.

There was no sign of bud burst in the scions 30 days after budding but buds of the rootstocks below the scions had burst. The number of plants on which any of these buds had burst were counted to determine the

Table 5. Effect of growth substance and bud position on
no. buds which survived*

Growth Substance	Conc. ppm	<u>Total no. surviving buds</u>					
		162 days		202 days		325 days	
		Top	Bottom	Top	Bottom	Top	Bottom
GA ₃	10	25	29	25	29	24	21
	100	10	4	4	4	4	4
BAP	10	45	44	45	42	44	42
	100	26	14	26	14	26	13
NAA	10	67	42	67	42	67	42
	100	70	38	70	38	70	37
CEPA	15	53	48	53	48	53	43
	150	31	15	30	15	29	13
MH	1000	70	33	70	33	69	33
Control		65	57	65	57	65	57

* Out of 75 buds per treatment

effectiveness of the maleic hydrazide treatment. There was a high incidence of death of the implanted bud, probably due to the effects of the growth substances. The number of surviving buds and the number of them which burst were counted 162, 202 and 325 days after budding. The results are presented for "top" and "bottom" buds, referring to the position of the buds on the budwood stick, not to the location of the implanted bud on the rootstock.

Results and discussion: There was a significant effect ($p < 0.001$) of growth substance on the number of buds which survived at each of the 3 times, using the combined values for top and bottom buds, (Table 5). There was also a significant interaction ($p < 0.001$) between growth substance and bud position on survival at each of the 3 times.

The results show that NAA had little or no effect on bud survival whereas GA_3 , BAP and CEPA were detrimental, especially GA_3 ; higher concentrations increased this deleterious effect. More top buds survived than bottom buds.

No growth substance improved survival or bud-take compared to the control. In contrast, Maiti et al. (1959) found that dipping buds in 1000 ppm auxin increased bud-take to 43% compared with 19% in the control. These percentage bud-take values are very low and no comment is made on this. Thus we are left unaware of which were

Table 6. Effect of growth substance and bud position on
proportion of surviving buds which burst

Growth Substance	Conc. ppm	Percent surviving buds which burst					
		162 days Top	162 days Bottom	202 days Top	202 days Bottom	325 days Top	325 days Bottom
GA ₃	10	30	8	61	49	97	84
	100	72	0	100	67	100	67
BAP	10	10	0	74	57	87	82
	100	64	8	69	45	88	63
NAA	10	13	2	90	50	99	83
	100	0	5	69	52	97	86
CEPA	15	3	0	65	82	93	92
	150	8	4	84	54	97	100
MH	1000	3	4	81	71	97	92
Control		7	8	78	41	89	82

the most important factors in bud survival and consequently which factors auxin may have influenced. In the present experiment 87% of top buds and 76% of bottom buds in the controls survived. The former figure was about average for this nursery using the micro-bud technique. It is possible that in the experiments of Maiti et al., auxin may have affected some factor which was not a problem under the South Australian conditions e.g. rate of callus generation, hence the lack of response to auxin in the latter situation.

The plants were budded at the end of January, in the height of summer. Contrary to the usual situation (Platt and Opitz, 1973) these summer buds did not burst and grow before winter. By 162 days (12 July) and 202 days (21 August) a significant effect of bud position on the proportion of surviving buds which burst was apparent ($p < 0.05$ and 0.01 respectively) but this was not so at 325 days (22 December) as most surviving buds had then burst (Table 6).

There was no significant effect of growth substance on bud burst when the results from top and bottom buds were pooled, but there was a significant interaction ($p < 0.05$) between bud position and growth substance on percentage bud burst at 162 days. Gibberellic acid at 10 or 100 ppm improved the burst/survival ratio of top buds. However, GA_3 was very deleterious to the buds and though 100% of the surviving buds of the 100 ppm

GA treatment (top buds) burst, only 4 buds (out of 75) survived.

At 162 days, 100 ppm BAP also produced a high proportion of bud burst in the surviving top buds and was not as detrimental to bud survival as GA_3 .

Auxin, especially at the lower concentration (10 ppm NAA), improved the percentage bud burst in the following spring and summer (at 202 and 325 days).

Soaking the buds in CEPA was the only treatment which appeared to improve bud burst of the bottom buds, but this effect also was only apparent at 202 and 325 days.

The results indicate that more top buds survived treatment with growth substances than bottom buds and that top buds burst earlier than bottom buds. The bursting of top buds was hastened by 100 ppm GA_3 or 100 ppm BAP whereas NAA improved percent bud burst of top buds and CEPA of bottom buds later in the year. However it became clear that, in general, dipping the buds in solutions of growth substances before budding was detrimental to bud survival.

2.2.2. Buds treated with growth substances after
budding

2.2.2.1. Growth substances applied in agar or wick-fed
into budded plants in the field

Methods: A second experiment was laid down using Valencia orange buds on Troyer citrange rootstocks grown on Murray Sand. The plants were budded on 3 January using conventional buds and budding techniques (Platt and Opitz, 1973).

Based on the results of the previous experiment, only top buds were used. The tapes were removed from around the buds and the rootstocks were lopped (Platt and Opitz, 1973) on 26th January. The treatments were applied on the following day. The experiment involved 9 growth substance treatments and a control, applied in 2 ways: in aqueous solution fed by a wick or in 3% agar.

In wick-feeding, 500 μ l of solution was put into 1 ml stoppered plastic centrifuge tube in the bottom of which a cotton thread was anchored in paraffin wax. The thread extended through the lid of the tube and was inserted into the plant between the wood and the bark using a sewing needle. The top of the tube was kept as close as possible to the point of insertion of the thread into the plant to minimise evaporation of the solution from the thread. The excess thread was cut off flush with the stem at its point of emergence. In this experiment the thread was inserted into the tissue of the rootstock just below the implanted scion.

The agar solutions were made by adding 3% (w/v) of Bacto-agar to the warmed aqueous solution of growth substance and stirring thoroughly. The warm solution (500 μ l) was put into a cup made by adhesive tape attached to the stem of the rootstock so that the solution was in contact with the bud. On cooling a gel formed which adhered to the bud. The gel dried out, forming a scale, within 3 days of application.

The growth substance treatments were 500 μ l of:

10^{-5} M GA₃
 10^{-6} M GA₃
 10^{-5} M BAP
 10^{-6} M BAP
 2×10^{-6} M NAA
 10^{-5} M GA₃ + 10^{-6} M BAP
 10^{-6} M GA₃ + 10^{-6} M BAP
 125 ppm (87×10^{-5} M) CEPA
 250 ppm (174×10^{-5} M) CEPA as
 foliar spray to lopped rootstocks
 H₂O Control

The experimental design was a split plot with the 2 methods of application as the main plots and the 10 growth substance treatments as the sub-plots. It was laid out as randomized complete blocks with 6 replicates of the main plots (i.e. 12 replicates of sub-plots) and 40 plants per main plot or 4 plants per

Table 7. Effect of growth substances and the method of
their application on bud burst of the implanted
buds

Treatment		% Bud Burst			
		Days after treatment			
		8	15	22	29
<u>Main Plots</u>					
Agar		49.6	77.0	81.4	81.4
Wick-fed		47.1	69.1	77.4	78.2
<u>Sub-Plots</u>					
GA ₃	10 ⁻⁵ M	42.0	70.8	77.1	77.1
GA ₃	10 ⁻⁶ M	54.2	74.5	76.1	80.4
BAP	10 ⁻⁵ M	66.7	91.3	91.3	91.3
BAP	10 ⁻⁶ M	72.9	82.6	82.6	82.6
NAA	2 x 10 ⁻⁶ M	79.2	95.7	95.7	95.7
GA ₃ + BAP	10 ⁻⁵ M } 10 ⁻⁶ M }	39.6	68.8	85.4	89.6
GA ₃ + BAP	10 ⁻⁶ M } 10 ⁻⁶ M }	47.9	66.7	78.7	78.7
CEPA	125 ppm	0	2.1	16.7	16.7
CEPA spray	250 ppm	16.7	81.2	89.6	93.8
Control		64.6	85.4	89.6	89.6

sub-plot. Guard plants were left on either side of the CEPA spray treatments.

Results and discussion: Contrary to the previous experiment almost 100% of the treated buds survived. The improved rate of survival may have been due to the facts that the treatments were applied after the buds had knitted to the stock rather than before, and that they were applied either externally, to the cuticle, or into the vascular system rather than to relatively large areas of cut tissue on the buds. In addition, the large buds used in this experiment were probably more likely to withstand any deleterious effects of the growth substances than the "micro-buds" of the first experiment.

Within 8 days after treatment (i.e. 9 days after lopping or 32 days after budding) more than 60% of the buds in the control plots had burst and 2 weeks later almost 90% had burst (Table 7). The ethylene releasing compound CEPA applied to the bud at 125 ppm inhibited bud burst. Applying 250 ppm CEPA to the foliage delayed bud burst of the scion and did not induce leaf abscission in the rootstock.

Gibberellic acid, either alone or in combination with BAP also delayed bud burst, whereas BAP had no effect. Auxin (NAA) promoted bud burst compared with control, at each assessment date. The method of application of the growth substances had no effect on bud burst.

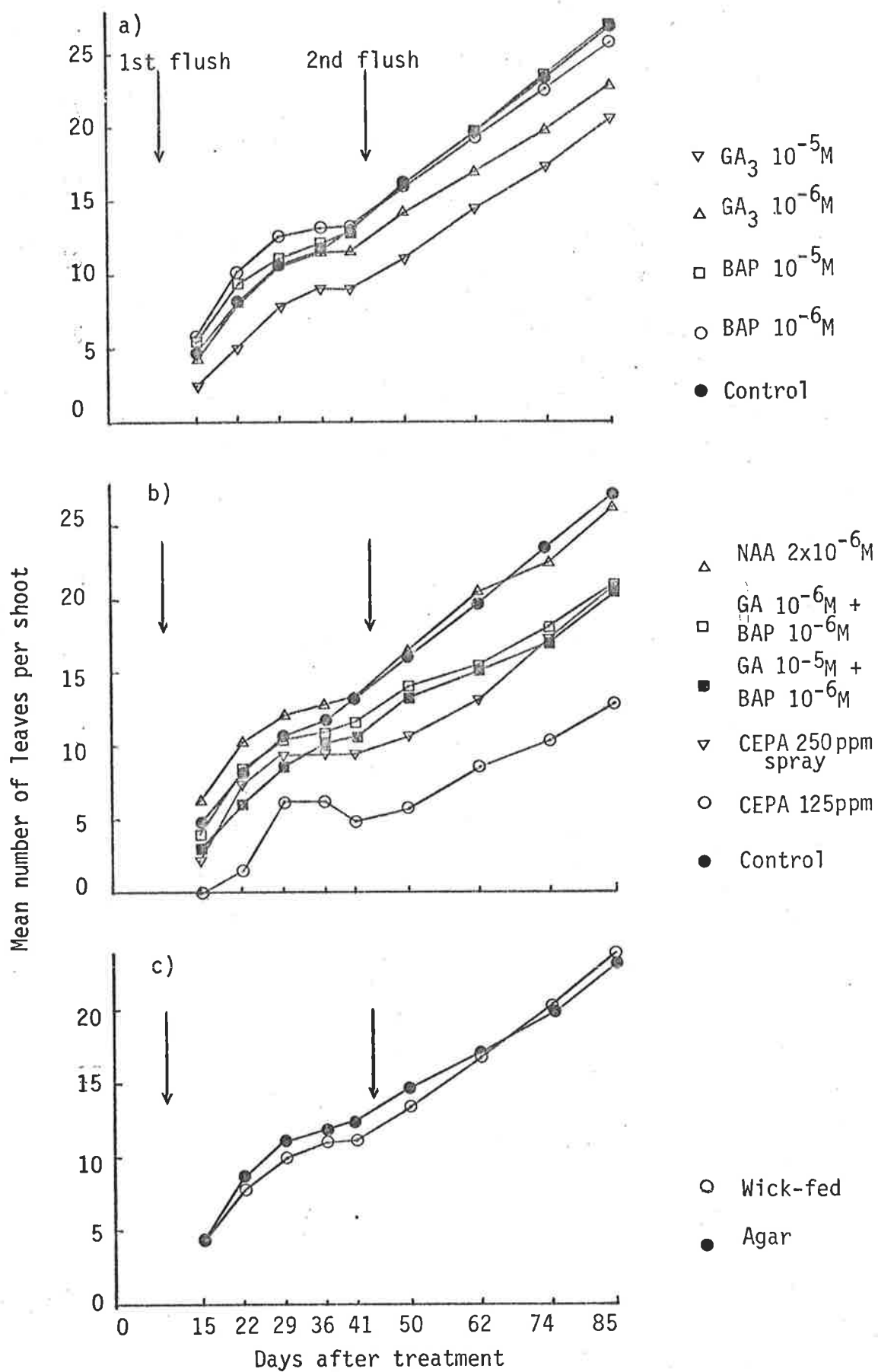


Figure 5. Effect of growth substance and method of application on mean number leaves per shoot of buds which burst.

Both BAP and NAA promoted leaf production in the first but not in the second flush compared to the control. (Figures 5a and 5b)* whereas CEPA reduced leaf production, especially when applied directly to the bud (Figure 5b). GA_3 at $10^{-5}M$ reduced leaf number of the second flush while $10^{-6}M$ GA_3 reduced leaf number of both flushes (Figure 5a). The combinations of GA_3 and BAP also reduced leaf number, especially of the second flush (Figure 5b).

The method of application of the growth substances had little effect on leaf number per shoot (Figure 5c).

The effects of growth substances on shoot length were very similar to the effects on leaf number. Cytokinin and auxin increased shoot length in the first flush but not in the second (Figures 6b and 6a) while

* Figures 5 and 6 include only data from those scions which had burst. Statistical analyses were carried out on all the data for days 15, 22 and 41. To correct the skewed distributions the shoot length data were transformed into $\log_{10}$ and leaf number data into arc sine of the square root. The effects of growth substances were significant to the following extents:

	Leaf number Arc sine of sq. root	Shoot length Log
Day 15	$p < 0.001$	$p < 0.01$
Day 22	$p < 0.001$	$p < 0.001$
Day 41	$p < 0.01$	$p < 0.01$

As the data are presented in untransformed form no LSD's are given.

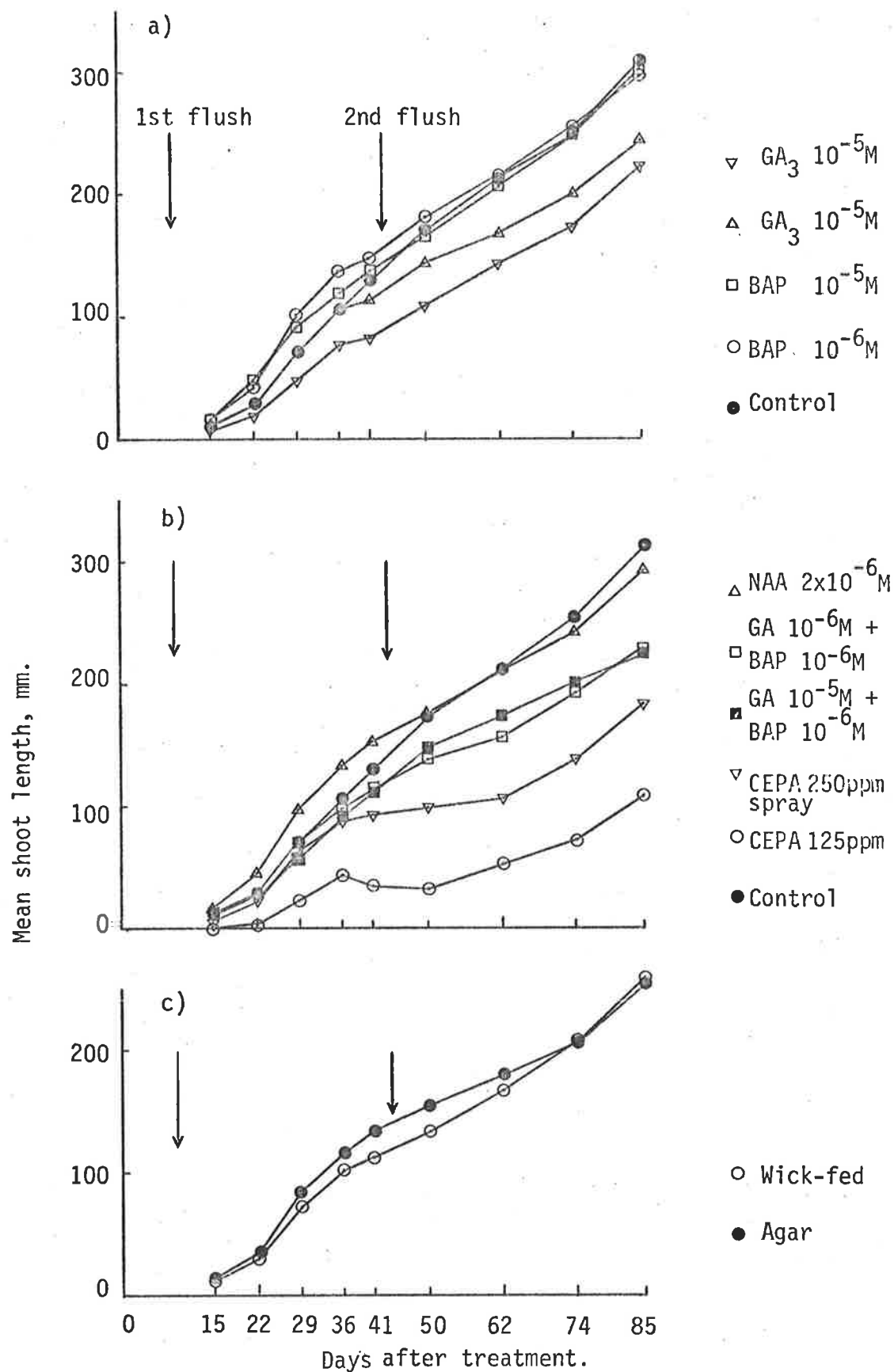


Figure 6. Effect of growth substance and method of application on mean shoot length of buds which burst.

combinations of BAP and GA_3 had no effect on shoot elongation during the first flush but reduced shoot length in the second flush (Figure 6b). Both CEPA and GA_3 reduced shoot length, especially during the second flush, CEPA was particularly inhibitory when applied directly to the bud (Figure 6b).

The agar method of application of growth substances produced slightly greater average shoot lengths than the wick-fed method throughout most of the experiment (Figure 6c).

The effects of the growth substances on leaf number were due mainly to the differences in leaf number by day 15 (Figure 5), whereas the effects on shoot length were due more to differences in rate of elongation after day 15 (Figure 6). The effects were :

- a) CEPA was strongly inhibitory.
- b) GA_3 applied before bud burst tended to delay bud burst and reduce shoot growth.
- c) NAA promoted bud burst and both NAA and BAP promoted growth of the first flush.
- d) Combinations of GA_3 + BAP delayed bud burst and reduced shoot growth, especially in the second flush.

2.2.2.2. A further experiment using growth substances in agar in the field

Introduction: The beneficial effects of auxin and cytokinin in the previous experiment led to further investigation into the effects of various concentrations of auxin and of various combinations of the 2 growth substances. It was also decided to combine GA_3 and NAA as there is a current view that auxin must be present before gibberellin can exert its effect on growth (Brown, 1971). An additional treatment of GA_3 was applied to the shoot tip after bud burst in an effort to avoid the deleterious effect of GA_3 .

Methods: Valencia buds (taken from the distal 20 cm of the budwood sticks) on Troyer citrange rootstock, located in the same nursery as the previous experiment, were used. The budding tapes were removed and the rootstocks lopped on 7 March. The next day the following treatments were applied:

Treatment	1	-	$2 \times 10^{-4} M$ NAA
	2	-	$2 \times 10^{-5} M$ NAA
	3	-	$2 \times 10^{-6} M$ NAA
	4	-	$10^{-5} M$ BAP
	5	-	$10^{-5} M$ BAP + $2 \times 10^{-5} M$ NAA
	6	-	$10^{-5} M$ BAP + $2 \times 10^{-6} M$ NAA
	7	-	$10^{-6} M$ NAP + $2 \times 10^{-5} M$ NAA
	8	-	$10^{-6} M$ GA_3 + $2 \times 10^{-6} M$ NAA
	9	-	35 μg GA_3 applied to shoot tip
	10	-	H ₂ O control

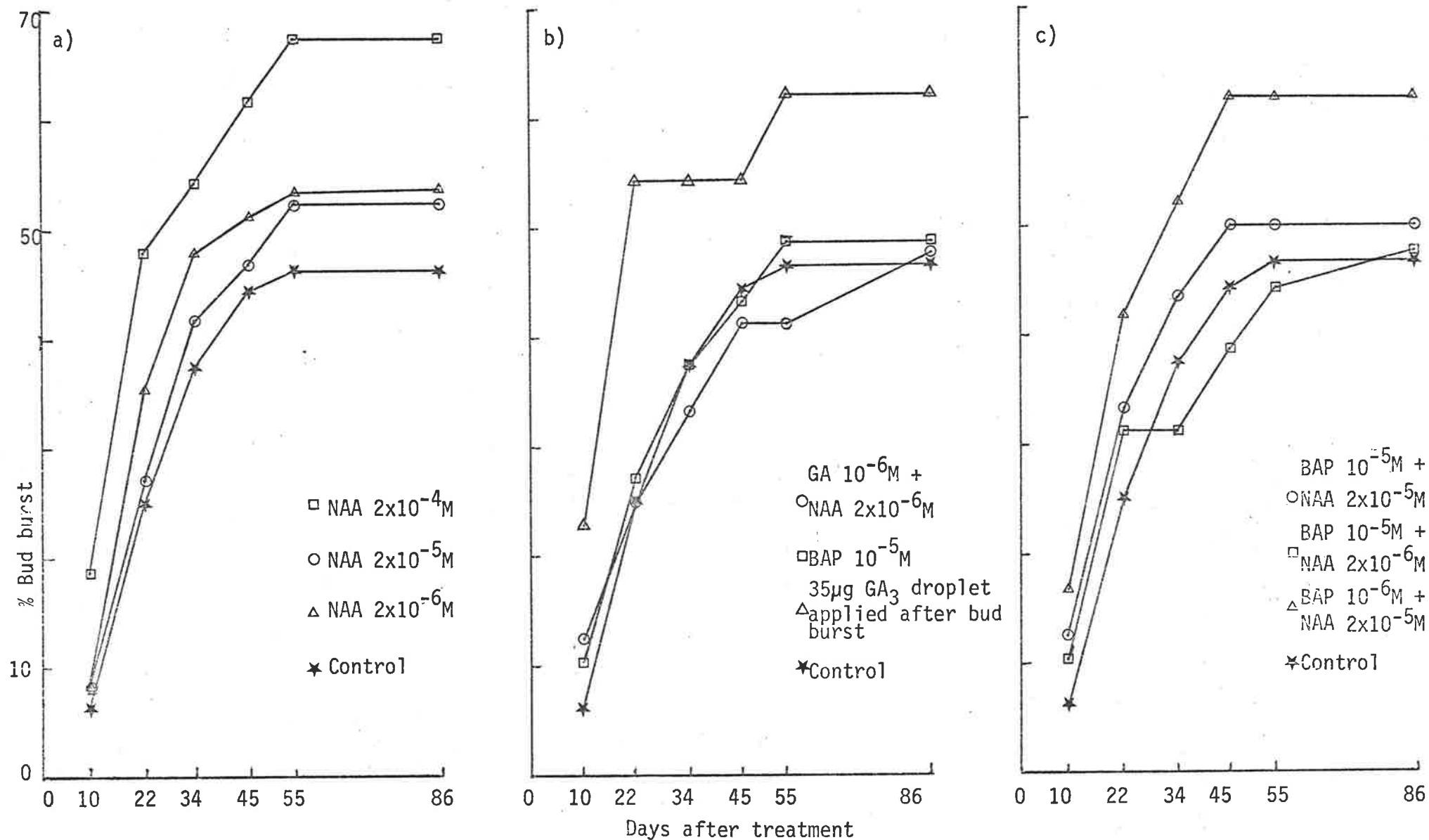


Figure 7: Effect of growth substances on bud burst of plants budded in autumn.

All except treatment 9 were applied in 3% agar gels (500 μ l) to the implanted buds before bud burst (see 2.2.2.1. Methods). Treatment 9 was applied as a 5 μ l droplet of ethanolic solution (plus 0.05% Tween 20) to the young shoot shortly after bud burst.

The experiment was laid out as randomized complete blocks with 4 plants per plot and replicated 12 times.

Results and discussion: Unlike the previous experiment the rate of bud burst was slow and the percent bud burst low; by 86 days after treatment less than 50% of the implanted buds of the control had burst. Since both stock and scion plant material, budding technique, soil type and cultural practices in this experiment were similar to those of the previous experiment, the retarded bud burst is attributed to climatic factors. This experiment was started in early March, i.e. early autumn, at a time of decreasing daylength and temperature. Night temperatures in particular were considerably lower than during the previous experiment. These conditions may have induced dormancy in the buds either before removal from the parent tree or after implantation onto the stock or both.

In all the treatments where auxin alone was applied, percent bud burst was consistently greater than the control (Figure 7a), with 2×10^{-4} M NAA producing the greatest increase. BAP at 10^{-5} M had no effect on bud

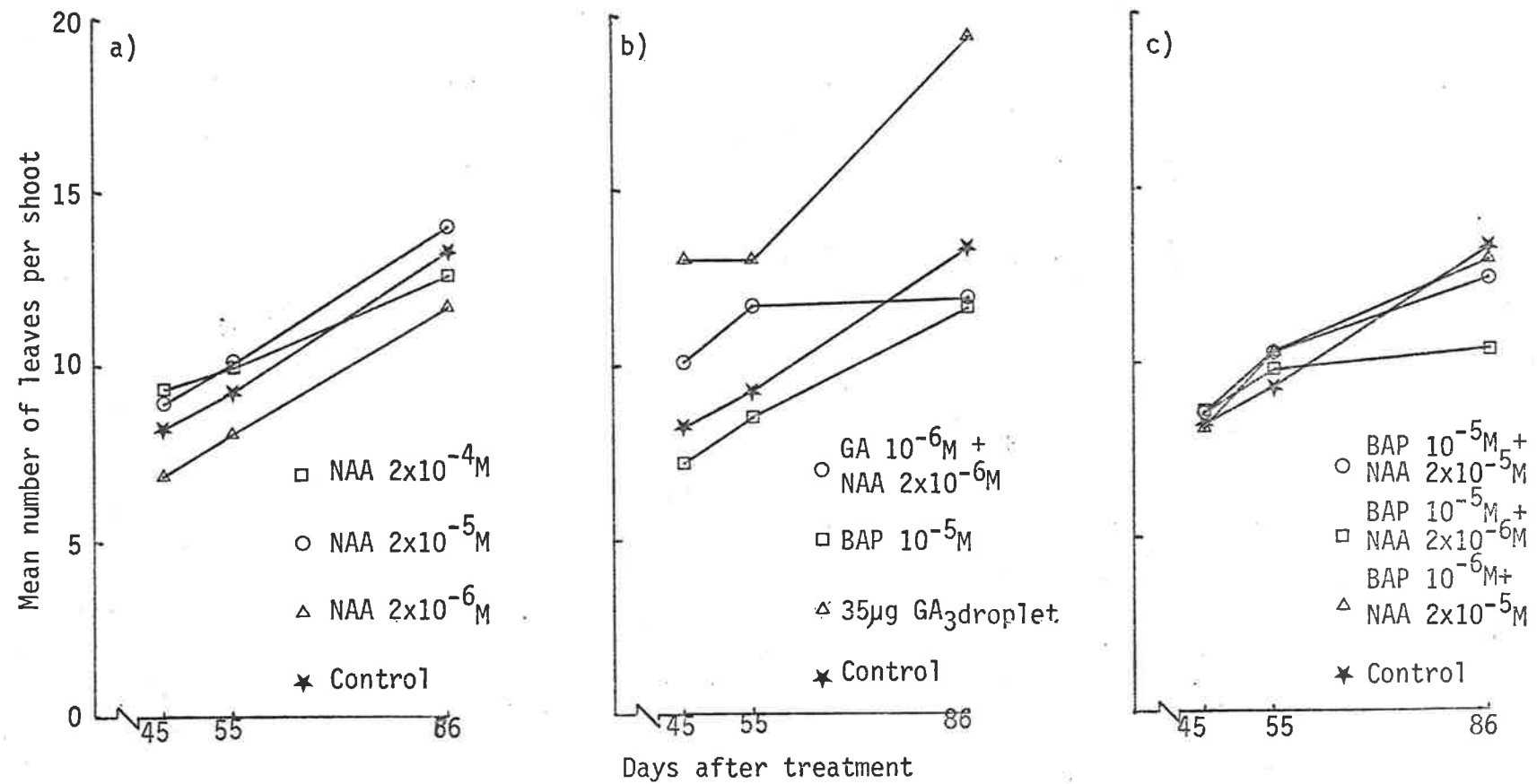


Figure 8: Effect of growth substances on number of leaves per shoot of buds which burst on plants budded in autumn.

burst (Figure 7b) whereas combinations of BAP and NAA had varying effects (Figure 7c); in one instance the combination (10^{-6} M BAP + 2×10^{-5} M NAA) promoted bud burst compared to the control or to a similar level of NAA whereas another (10^{-5} M BAP + 2×10^{-6} M NAA) tended to retard bud burst. It was not possible to assess the significance of this interaction from this experiment.

Due to the retarded rate of bud burst, measurement of shoot length and leaf number of the young shoots began 45 days after treatment and even then, less than 50% of the buds of most treatments had burst. In contrast to the previous experiment the average number of leaves per shoot* was less than 15 for all except GA_3 treatment, even after 86 days of growth (Figure 8). The slower rate of leaf proliferation was probably due to the adverse climatic factors described earlier.

There was no significant effect of treatment on the average number of leaves per shoot, even though a droplet of GA_3 on the shoot tip resulted in an increase of almost 50% over the control by day 86 (Figure 8b).

* As in the previous experiment the average shoot length and number of leaves per shoot were determined by dividing the respective totals by the number of implanted buds which had burst. This produced uneven numbers of plants between treatments and made normal parametric statistical analysis invalid. However, all the data relating to shoot length and leaf number, including that from plants which did not shoot, were analysed after normal distributions were obtained using log transformations.

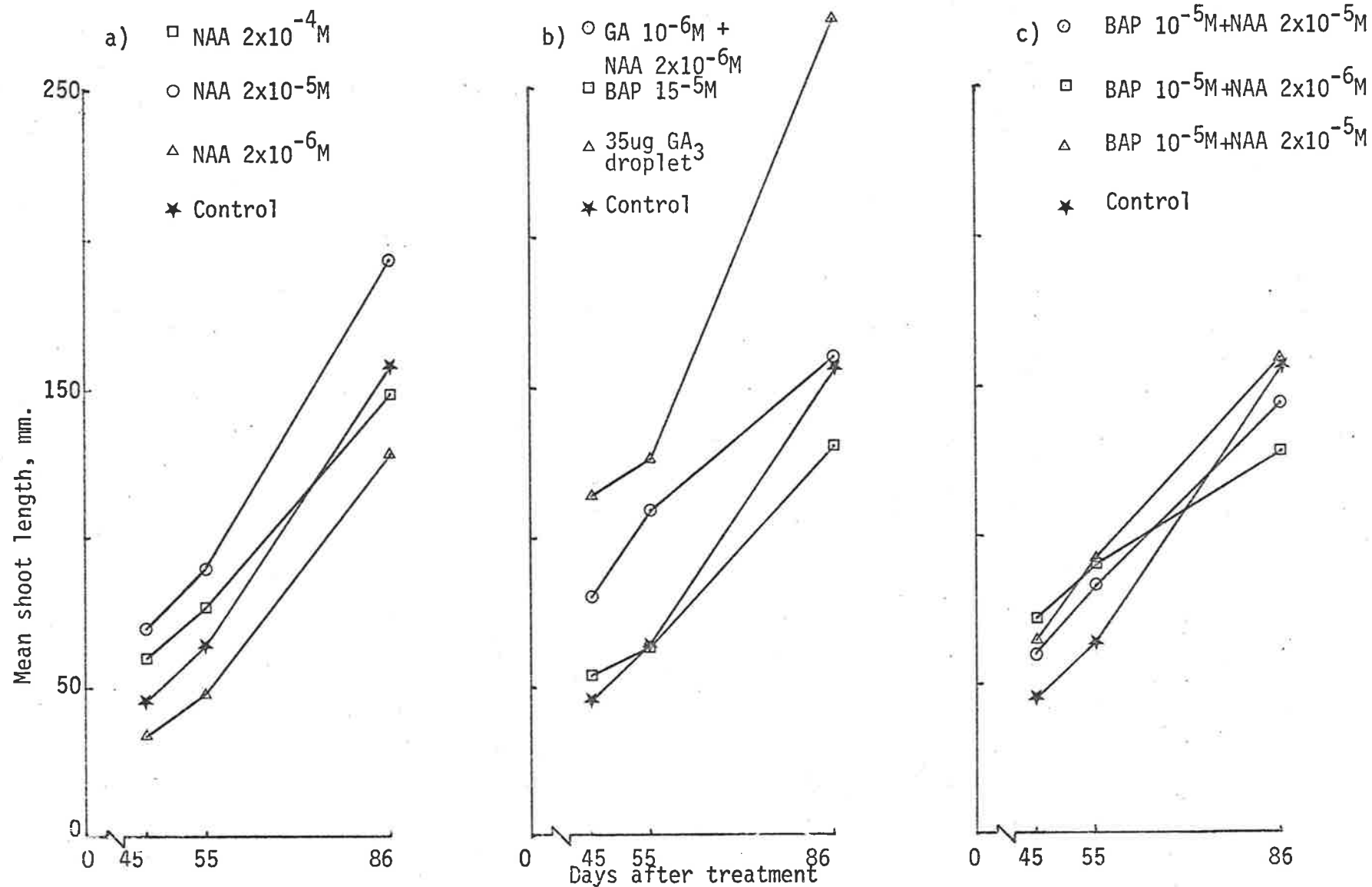


Figure 9. Effect of growth substances on the length of shoots from buds which burst in plants budded in autumn.

As with leaf number, the average length of the shoot was less in this experiment than in the previous one and there was no significant effect of treatment (Figure 9) although the droplet of GA_3 on the growing shoot tip considerably increased the length of the shoot 86 days after treatment (Figure 9b).

Thus the experiment was inconclusive due to the high variance of the data and the inhibited bud burst and shoot growth; this behaviour may be explained by climatic factors.

2.2.2.3. Treatment of budded plants in pots in a glasshouse with growth substances

Introduction: During the winter, experiments were continued using potted plants in a glasshouse. The previous experiments indicated that auxin promoted bud burst and shoot growth, that GA_3 applied before bud burst was inhibitory and that cytokinin had no effect on bud burst but promoted shoot growth. However, the two previous experiments had been carried out during shortening day-lengths. Since Altman and Goren (1974a) had found that excised orange buds responded differently to growth substances at different times of the year (and therefore under different daylengths) and the present experiment was being carried out during increasing daylengths it was decided to include treatments of auxin plus cytokinin combinations. A GA_3 treatment was also included in an attempt to clarify the tendency observed in the previous experiment.

Methods: Seedlings of Troyer citrange were grown in seedbeds on Murray Sand and transplanted into black polythene bags (22 cm in diameter and 25 cm deep) containing sawdust of Murray Red Gum (Eucalyptus camaldulensis, Dehnhardt). Before use, the sawdust had been cured by first drenching with a 0.6% solution of commercial wetter (Teepol), then applying the equivalent of 1 kg N (either as urea or $Ca(NO_3)_2$) per cubic metre

and sprinkle-irrigating to run-off every 3 to 4 weeks for 3 months during the summer.

After the transplanted seedlings had become established in the sawdust they were budded with Valencia sweet orange buds, using conventional techniques. The budded plants, with budding tapes attached, were transferred to a glasshouse at the Waite Institute where, on the following day, they were lopped and the tapes removed.

The following treatments were applied on 8th August, approximately 2 months after the tapes were removed:

$2 \times 10^{-2} \text{M}$	NAA
$2 \times 10^{-3} \text{M}$	NAA
$2 \times 10^{-4} \text{M}$	NAA
$2 \times 10^{-5} \text{M}$	NAA
$2 \times 10^{-6} \text{M}$	NAA
$2 \times 10^{-2} \text{M}$	NAA + 10^{-4}M BAP
$2 \times 10^{-3} \text{M}$	NAA + 10^{-4}M BAP
10^{-2}M	BAP
10^{-4}M	BAP
10^{-6}M	BAP
10^{-3}M	GA_3

Ethanol + 0.05% Tween 20(Control)

Each growth substance, dissolved in ethanol containing 0.05% Tween 20, was applied as a 5 ul droplet to the scion. The experiment was laid out as randomized complete blocks with 1 plant per plot and 6 replicates. At the time the

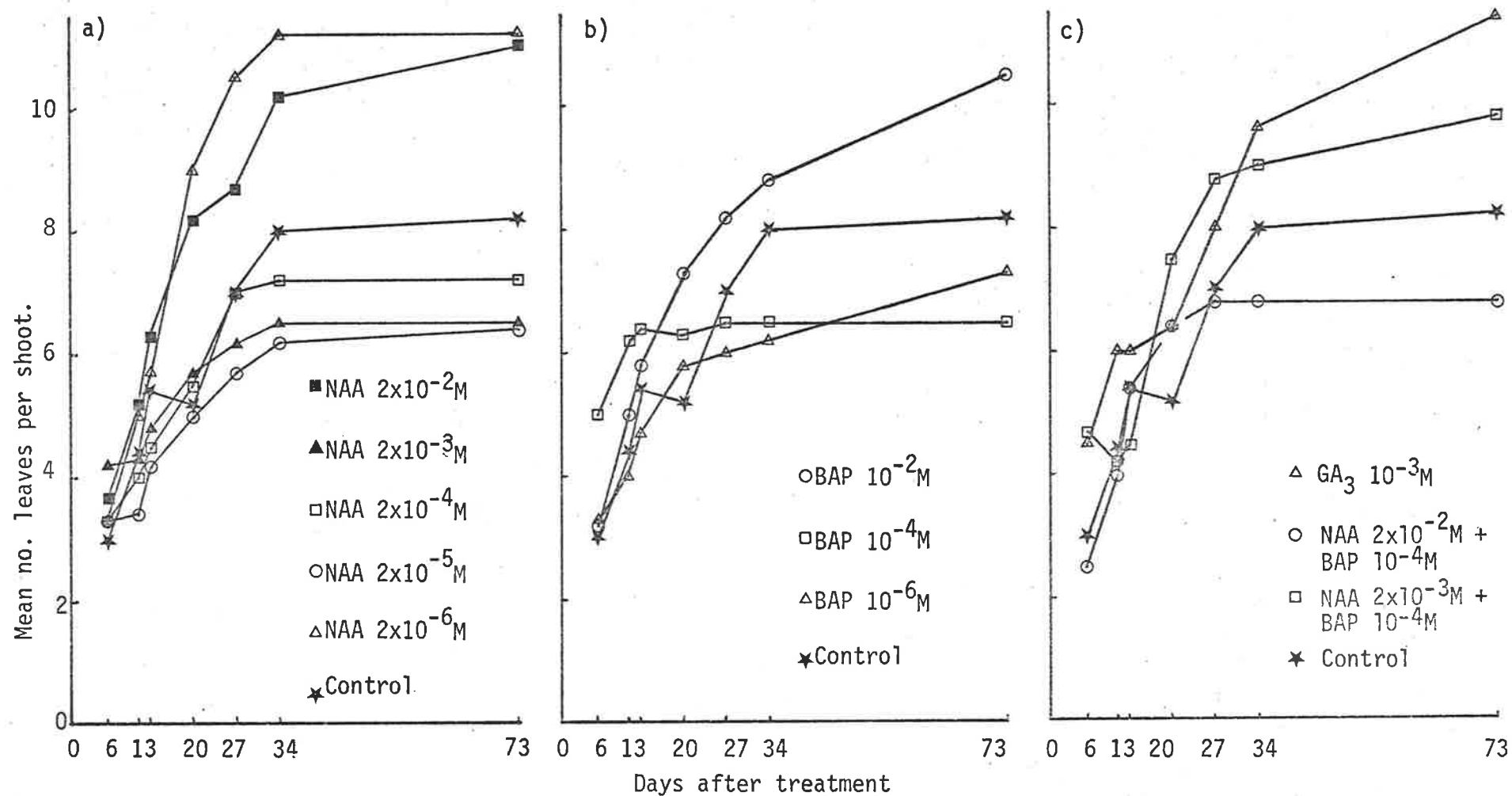


Figure 10: Effect of growth substances on number of leaves of budlings in pots in glasshouses.

treatments were applied the implanted buds on most of the plants had burst, so the plants were grouped into replicates depending upon the stage of growth of the bud. There were 2 replicates with buds at each of stage 2-3, 3-4 and 4-5 (for description of bud stages, see end of section 2.1.2.).

The plants were supplied once a fortnight with a solution of commercial, water soluble, compound fertilizer (Aquasol) supplemented with additional iron chelate. The glasshouse was maintained above 12°C by electric radiators.

Results and discussion: The effect of treatment on bud burst could not be assessed, since most of the plants were treated after bud burst.

Contrary to expectation there was no apparent correlation between the concentration of auxin applied and its effect on either the number of leaves per shoot or the average shoot length (Figures 10a and 11a). Both the highest and lowest concentrations (2×10^{-2} M and 2×10^{-6} M NAA) increased the number of leaves and average shoot length compared to the control, whereas all other concentrations reduced both shoot length and leaf number.

Of the cytokinin treatments only 10^{-2} M BAP produced more leaves per shoot than the control (Figure 10b). Up to 13 days after treatment the average number of leaves of the 10^{-4} M BAP treatment was greater than the

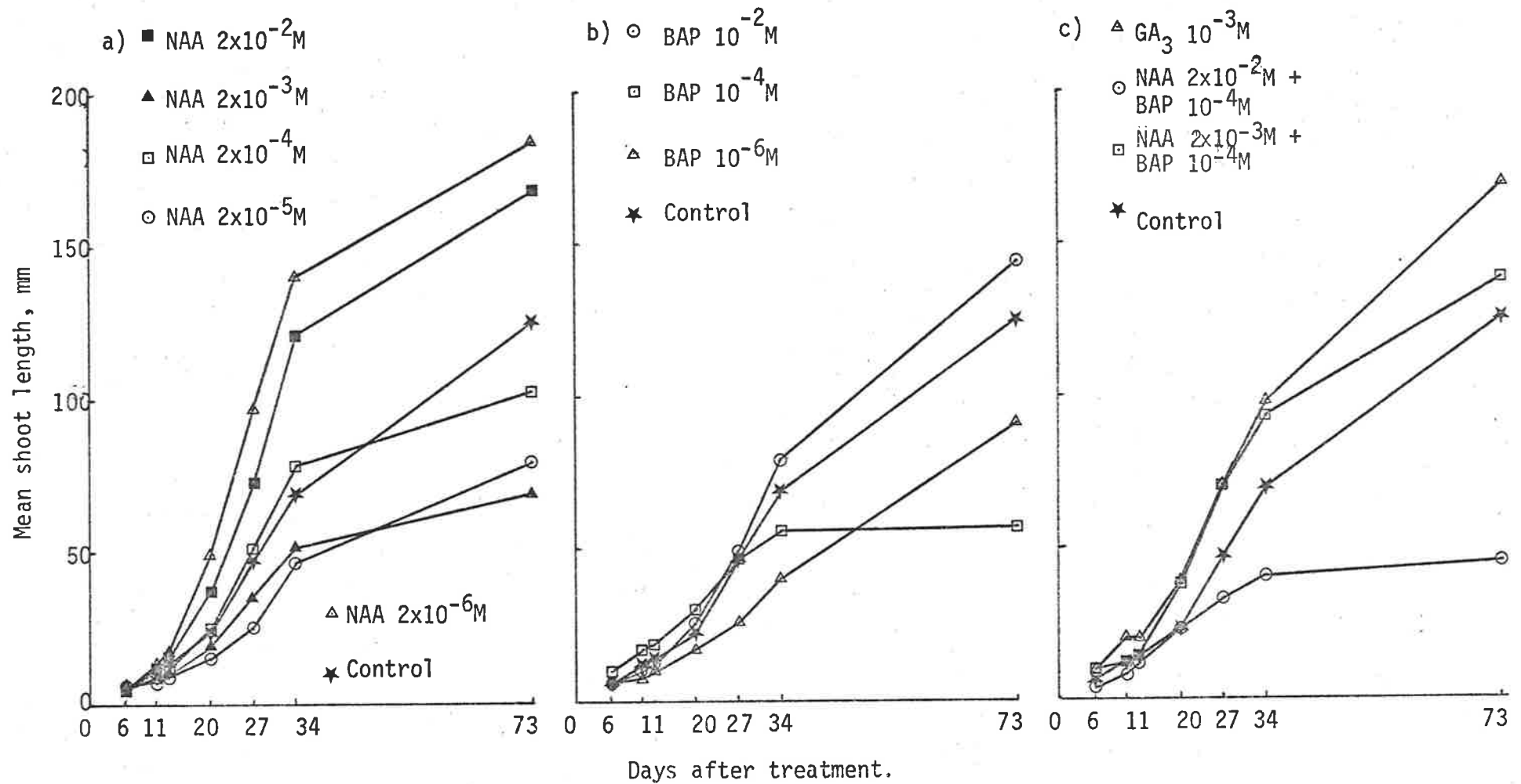


Figure 11. Effect of growth substances on shoot length of budlings in pots in a glasshouse.

control but thereafter no further leaves appeared unlike the other treatments. It appears that 10^{-4}M and 10^{-6}M BAP were inhibitory to shoot growth and reduced both leaf number and shoot length, though these effects were not apparent before day 13. In contrast, the average leaf number of the 10^{-2}M BAP treatment continued to increase after 13 days and this was followed by an increase in shoot length compared to the control (Fig 10b and 11b).

The effects of combinations of NAA and BAP were as diverse as those of NAA alone; the same concentration of NAA ($2 \times 10^{-2}\text{M}$) which increased leaf number and shoot length considerably reduced both parameters when combined with 10^{-4}M BAP (Figures 10c and 11c), whereas $2 \times 10^{-3}\text{M}$ NAA, which on its own reduced leaf number and shoot length, increased both parameters when combined with 10^{-4}M BAP.

A 5 μl droplet of 10^{-3}M GA_3 increased leaf number and shoot length compared to the control throughout the experiment (Figure 10c and 11c). This was contrary to the effects of GA_3 in the first two experiments but similar to the results of the third experiment and may be because most of the scion buds in this experiment had burst at the time of application of the treatments, and the subsequent environmental conditions were conducive to shoot growth. On the other hand it is possible that the solvent used for the growth substances (ethanol in this experiment) may have promoted the entry of GA_3 into the tissue (e.g. by dissolving away the waxy cuticle)

and thereby increased its potency.

The effects of growth substances at day 73 were not significant although there was a three-fold difference in shoot length and a 78% difference in leaf number. The lack of significance, which reflected the high variability of the material, was disappointing considering the efforts which had been made to reduce the variability, viz, the use of plant material of constant genotype grown in uniform soil and climatic conditions and selection at or after bud burst.

2.2.2.4. Effects of photoperiod, auxin and cytokinin on bud burst and shoot growth of decapitated seedlings

Introduction: As the treatments in the last experiment were applied at or after bud burst the effects of growth substances on bud burst during a period of lengthening photoperiod could not be ascertained. In the earlier experiments of this series exogenous applications of auxin promoted bud burst and shoot growth. Those experiments were carried out during periods of decreasing photoperiod. There seemed a possible relationship between photoperiod and the hormonal balance within the plant, moreso since it was found that buds excised from trees in the field during lengthening photoperiods burst more quickly when cytokinin was added to the medium on which they were subsequently cultured (Altman and Goren, 1974a). These considerations led to the hypothesis that under shortening photoperiod the level of auxin within the bud is inadequate and limits bud burst and shoot growth whereas under lengthening photoperiod the endogenous level of cytokinin is limiting.

It had been found that bud burst in budlings was too variable and the size of the plants too great for use in an experiment of this kind. In contrast, axillary buds of decapitated seedlings burst readily and uniformly and such plants can be grown in small pots suitable for use in growth cabinets. Consequently

seedlings were used for this experiment. However, the limited availability of controlled environment space and of plant material permitted the testing of only the first half of the hypothesis.

Methods: Healthy fruits from a selected Valencia sweet orange tree, known to be virus-free, were harvested on 2 May 1975 and the seeds were extracted from these fruits on the same day. The seeds were air-dried, dusted with Thiram 80 (80% tetramethyl thiuram disulphide) and stored at 5°C until 28 May when they were sown in John Innes compost in peat pots, one seed per pot. Before sowing, part of one cotyledon and the tissues external to it were removed with a razor blade. The peat pots were kept at 30°C and illuminated 24 hours per day.

Under these conditions 95% of the cut seeds germinated within 28 days of sowing but only 30% of comparable uncut seeds germinated in the same time. Generally seeds which had been cut germinated within 11 to 13 days and produced uniform seedlings whereas germination of entire seeds was erratic with consequent uneven seedling growth.

Thirty seven days after sowing, each peat pot with its seedlings in situ was planted into a 10 cm diameter plastic pot containing John Innes compost which had been augmented with 11 mls of a granular, slow-release, compound fertilizer (Osmocote, 18-2.6-10 NPK)

per litre of compost. Thereafter the seedlings were grown in a growth room at 27°/24°C day/night temperature and 16 hour photoperiod. The plants were supplied once a fortnight with a solution of Aquasol supplemented with iron chelate.

At 62 days after sowing the plants were divided into groups based on the size of the largest seedling in the pot and half of each group was put into one of two growth cabinets. The temperature within both cabinets was kept at 25°C but one cabinet was set at 8 hour photoperiod (short days) and the other at 16 hour photoperiod (long days). Both fluorescent and incandescent light was supplied and the intensity at plant height was 20000 lux in each cabinet.

Almost every seed produced multiple seedlings but only the most vigorous seedling in each pot was retained, the others were cut off at soil level when the pots were transferred to the growth cabinets.

At 65 days after sowing each seedling was decapitated above the fifth leaf, and 5 µl of one of the following treatments was applied to the cut surface, petiole and axillary bud of the uppermost leaf :

2 x 10⁻²M NAA

2 x 10⁻⁴M NAA

2 x 10⁻⁶M NAA

10⁻²M BAP

10⁻⁴M BAP

10⁻⁶M BAP

Ethanol + 0.05% Tween 20 (Control)

Table 8. Effect of growth substances and photoperiod on
bud burst of decapitated seedlings

Mean no. buds in uppermost axil which burst

	8 hr photoperiod (SD)			16 hr photoperiod (LD)		
	Day 3	Day 6	Day 10	Day 3	Day 6	Day 10
2×10^{-2} M NAA	0.6	3.0	3.6	0.4	3.4	4.2
2×10^{-4}	0.0	2.4	3.0	0.2	3.2	3.4
2×10^{-6}	0.4	2.0	2.4	0.6	2.8	3.4
10^{-2} M BAP	0.4	1.8	2.4	0.6	3.2	3.6
10^{-4}	0.0	2.0	2.8	0.2	2.6	3.0
10^{-6}	0.0	2.2	2.8	0.0	2.6	3.6
Control	0.2	2.0	3.4	0.4	2.8	3.4

All solutions were made up in ethanol containing 0.05% Tween 20.

The experiment was designed as a split plot with randomized complete blocks. Photoperiod (growth cabinet) constituted the first level or main plots and growth substance treatment the second-level or sub plots. There were 5 replicates (single plants) of the growth substance treatments but only 1 replicate of the photoperiod treatments. Plants within each growth cabinet were rotated twice per week.

On each plant the number of buds which burst in the axil of the terminal leaf was counted 3, 6 and 10 days after treatment and all except the most dominant bud in each axil were removed after each count. Shoot length and the number of leaves longer than 5 mm were recorded twice per week.

Results and discussion: The treated buds burst promptly and uniformly. Within 6 days after treatment an average of more than 2 buds had burst per uppermost leaf axil (Table 8). Bud burst was greater under long days than under short days, the difference being most apparent at day 6 (significant at $p < 0.004$). Though the effects of growth substance treatment on bud burst were not significant the most concentrated auxin treatment ($2 \times 10^{-2}M$ NAA) appeared to promote bud burst, in both photoperiods.

Leaf number per shoot was significantly greater under 16 hour photoperiod ($p < 0.001$ on days 13 and 20).

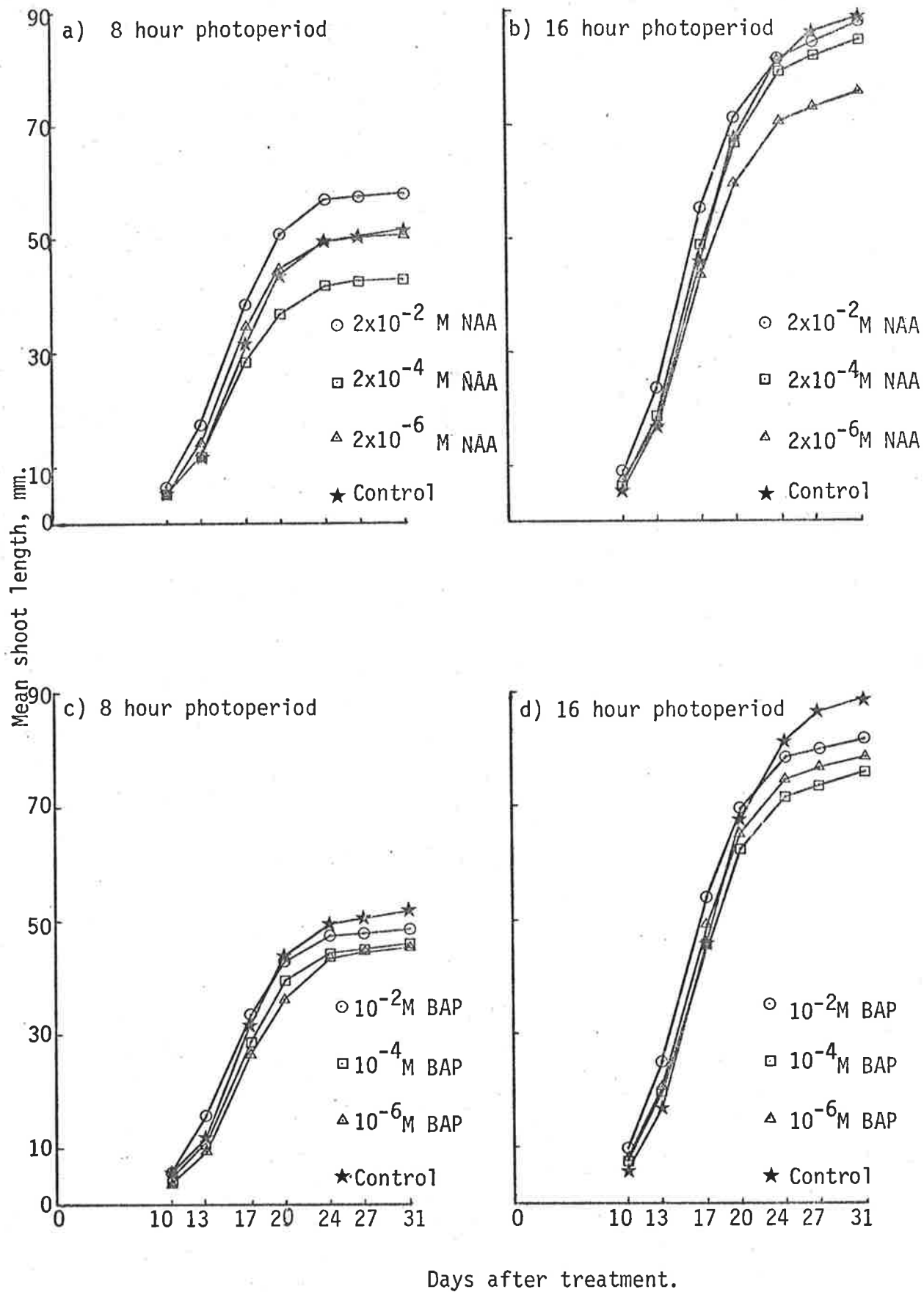


Figure 12 Effects of auxin, cytokinin and photoperiod on mean shoot length.

The most concentrated growth substance treatments increased leaf number in both photoperiods (Table 9). This was particularly true of BAP, especially under 16 hour photoperiod whereas the increase due to 2×10^{-2} M NAA was smaller.

Shoots were much longer under 16 hour photoperiod than under 8 hour photoperiod (significant at $p < 0.02$ at day 31, Figure 12). However there were no significant effects of growth substance on shoot length. Indeed, the only treatment which increased shoot growth was 2×10^{-2} M NAA under 8 hour photoperiod (Figure 12a). The lower concentrations of NAA tended to decrease shoot length as did all levels of BAP used (Figures 12c and 12d).

In summary, 16 hour photoperiod promoted bud burst and shoot growth. In both growth substances only the highest concentration had any promotive effects: NAA at 2×10^{-2} M promoted bud burst and shoot growth (except shoot length under 16 hour photoperiod) whereas 10^{-2} M BAP had no effect on bud burst and shoot length but promoted the number of leaves formed. These effects were apparent with either photoperiod.

The promotive effect of the highest concentration of NAA under 8 hour photoperiod supports the original hypothesis since these plants had previously been under long photoperiod and it could be said that the effect was induced under shortening photoperiod. In further support for this part of the hypothesis shoot

Table 9. Effect of growth substances and photoperiod on the mean number of leaves per shoot

Mean leaf number per shoot at 3 dates after
treatment*

	8 hr photoperiod (SD)			16 hr photoperiod (LD)		
	Day 10	Day 13	Day 20	Day 10	Day 13	Day 20
2×10^{-2} M NAA	3.6	4.8	5.1	4.6	6.2	7.6
2×10^{-4} M	3.0	4.2	5.2	3.0	5.0	7.4
2×10^{-6} M	3.2	4.2	5.0	3.2	5.0	6.6
10^{-2} M BAP	3.8	5.0	5.8	4.2	6.6	8.0
10^{-4} M	2.4	4.0	5.2	3.6	5.0	7.0
10^{-6} M	2.8	3.8	5.0	3.8	5.6	7.4
Control	3.2	4.3	5.4	2.8	5.4	7.6
Probability**	<0.006	N S	N S	<0.015	<0.002	N S

* Leaf numbers were maximal by 20 days after treatment

** Determined by Kruskal Wallis one-way analysis of variance (a non-parametric test), therefore LSD's are not available.

length was not promoted by NAA under the long photoperiod. However bud burst and leaf number were promoted by the highest level of NAA under 16 hour photoperiod, an effect which argues against the hypothesis.

The second part of the hypothesis is not supported by the responses to BAP, though it should be noted that the photoperiod was uniformly long rather than lengthening as stipulated in the hypothesis. BAP did not promote bud burst or shoot length under 16 hour photoperiod (nor under 8 hour), which suggest that levels of cytokinin are not the factor limiting bud burst or shoot elongation. Although the highest concentration of BAP increased leaf number per shoot this effect occurred under both photoperiods.

2.2.3. Discussion: The differences in time required for buds to burst between different experiments was one of the most outstanding features of this series of experiments. In the first experiment 60% of the buds of the control burst by around 200 days after budding (Table 6) while in the second experiment 60% bud burst was obtained in the control within 32 days after budding (Table 7). At the end of the third experiment, 120 days after budding, less than 50% of the control buds had burst (Figure 7). These experiments were performed on budlings growing in the field. In the last experiment of this series decapitated seedlings in growth cabinets were used; bud burst in the control was greater than

100% within 6 days of decapitation and treatment (Table 8). Clearly the buds of the different experiments were in varying states of dormancy.

In the discussion of dormancy in citrus earlier in this thesis (Introduction, section 1.4.) the evidence presented indicated that there is no chilling requirement for bud burst in citrus. This was interpreted as meaning that citrus buds do not undergo rest (physiological dormancy). However the results of Altman and Goren (1974a) show that there is an innate dormancy in citrus buds. It appears that citrus buds may be physiologically dormant but that chilling is not necessary to break this dormancy (Jones et al., 1976). The categorization of "bud dormancy" in this evergreen plant is therefore debatable and non-growing citrus buds are referred to hereafter as being dormant.

Thus the buds in the first and third experiments were very dormant while in the second and fifth experiments the buds were not dormant, i.e. they were "ready to grow" (Jones et al., 1976).

This difference in dormancy influenced the effects of auxin and cytokinin on bud burst: NAA did not hasten bud burst when applied to dormant buds but increased bud burst in spring (Table 6). When applied to the non-dormant buds of the second and fifth experiments NAA promoted bud burst (Tables 7 and 8). These results suggest that auxin does not of itself break dormancy but

promotes the growth of buds which are induced to grow, i.e. buds which are non-dormant. A similar conclusion was reached by Gouwentak (1941, quoted by Romberger, 1963) who contended that auxins are effective as activators only after rest is broken.

Soaking detached dormant buds in solutions of cytokinin hastened bud burst (Table 6), indicating that exogenous cytokinin breaks dormancy in citrus buds. This agrees with the results of Altman and Goren (1974a) that dormant Shamouti orange buds excised one to two months before the natural spring or summer flush were induced to burst by addition of BAP to the culture medium. However BAP had no effect on bud burst of the dormant buds in the third experiment (Figure 7b). This may be due to the low concentration used and its method of application: the concentration was 50-to 500-fold less than that which stimulated the growth of dormant peach (Weinberger, 1969) or apple (Williams and Stahly, 1968) buds, and was applied externally, in contrast to the method of the first experiment of this series and to that of Altman and Goren (1974a). When BAP was applied to non-dormant buds it had no effect on bud burst (Table 7) even if much higher concentrations were used (Table 8). A similar response was obtained by Altman and Goren (1974a). The evidence suggests that exogenous cytokinin induces dormant buds to burst but has no effect on non-dormant buds. This is corroborated by data on endogenous levels

of cytokinin: in the xylem sap and dormant buds of three deciduous tree species the levels of endogenous cytokinin increased at or shortly before bud burst whereas the levels during dormancy were very low (Hewitt and Wareing, 1974).

Contrary to the results of Altman and Goren (1974a) gibberellin reduced bud burst of non-dormant buds (Table 7), and its effects on bud burst of dormant buds (table 6) were equivocal because very few of the buds soaked in GA_3 survived (Table 5). Other studies of the effect of GA_3 on bud burst in citrus have also yielded conflicting results, with GA_3 promoting burst in some instances and having no effect in others (Cooper et al., 1969). On the other hand frequent applications of 25 ppm GA_3 to the foliage of orchard trees in summer promoted bud burst of Eureka lemon trees (Nir et al., 1972). In both the study of Nir et al. (1972) and of Altman and Goren (1974a) relatively large quantities of GA_3 were available to buds as a consequence of the frequent spraying and the quantity of GA_3 -enriched culture medium per bud respectively. In contrast, there was only a very limited supply of exogenous GA_3 to the buds of the second experiment of this study as they were treated with 500 μ l of 10^{-5} or 10^{-6} M GA_3 . Therefore the differences in effect may be due to the quantities of GA_3 available to the buds. However, the inhibitory effect of GA_3 on bud burst cannot be explained.

Contrary to the information quoted by Abeles (1973) and the results of Williams (1976) CEPA did not promote bud burst of top buds (Tables 6 and 7) nor did it induce leaf abscission (Gallasch, 1974). Instead CEPA delayed or inhibited the growth of non-dormant buds (Table 7). This result supports the suggestion of Williams (1976) that ethylene may promote the termination of rest (physiological dormancy, in deciduous species) but that it inhibits later stages of bud development. It is interesting that 15 ppm CEPA was the only treatment which greatly hastened bud burst of bottom buds (Table 6). A similar effect was found in apples (Williams, 1976) and suggests that the type or mechanism of dormancy of bottom buds may differ from that of top buds.

Applications of NAA to non-dormant buds or very young shoots around the time of bud burst increased the number of leaves of the young shoot (Figures 5 and 10 and Table 9), which indicates that auxin promotes leaf proliferation in buds which are ready to grow. However the effects of different concentrations of NAA on leaf number varied widely, (e.g. Figure 10) and this cannot be explained. There was a tendency for auxin to increase shoot length in budded plants (Figures 5 and 11) but not in decapitated seedlings (Figure 12). Sachs and Thimann (1967) postulated that auxin causes shoot elongation only when the internodes have already developed. In the fourth experiment, where the growth substances were applied

to bursting buds and young shoots, the highest and lowest concentrations of NAA promoted shoot length (Figure 11), which tends to support the above hypothesis. However the intermediate concentrations of NAA tended to reduce shoot length which does not concur with the hypothesis. Similarly, in the final experiment NAA applied to buds which were about to burst tended to reduce shoot length (Figure 12). Thus the results do not support the hypothesis of Sachs and Thimann (1967) but do not suggest other hypotheses either.

In the fourth and fifth experiments high levels of exogenous cytokinin (10^{-2} M BAP) promoted leaf proliferation of shoots growing in either short or long photoperiods whereas lower concentrations (10^{-4} M and 10^{-6} M) reduced leaf production (Figure 10 and Table 9). However the results of the second experiment (Figure 5) conflict somewhat with these conclusions.

The increases in leaf number in response to high levels of BAP were not matched by increases in shoot length (Figures 11 and 12). This suggests that 10^{-2} M BAP promoted the initiation of leaf primordia and/or the elongation of such primordia, both of which processes involve cell proliferation (Schneider, 1968; Scott et al., 1948). In contrast, shoot length and therefore internode length were not increased by 10^{-2} M BAP, which indicates that internode elongation is controlled by different factors to leaf initiation.

Gibberellic acid applied to the bud before bud burst reduced the growth of the young shoot and this effect was apparent even in the second flush (Figures 5 and 6). However, when GA_3 was applied at or after bud burst it promoted shoot growth by increasing both leaf number and shoot length (Figures 8 to 11). Apparently exogenous GA_3 inhibits development within the bud, which may be related to activity of the axillary meristem, but promotes the extension of the emerging shoot, presumably by inducing the development of more and longer internodes. The results support the suggestion of Wickson and Thimann (1958) that GA_3 promotes bud elongation only after inhibition has been released.

It is concluded that dormant buds of Valencia sweet orange may be induced to grow by applications of high levels of cytokinin, whereas exogenous auxin may improve the percentage bud burst of buds which are ready to burst. In contrast, gibberellin and ethylene inhibit bud burst. Leaf production is promoted by high levels of cytokinin, by auxin under some circumstances and by gibberellin applied after bud burst, but is retarded by gibberellin applied before bud burst and by ethylene. The response of shoot elongation to the various growth substances is not clear except that shoot length is enhanced by gibberellin applied after bud burst.

The results demonstrate that conclusions about the effects of growth substances on growth and development

of a plant organ must be reserved until all the facets of plant state, environmental conditions and concentrations and methods of application have been considered.

2.3. A study into some aspects of the physiology
of shoot elongation

The growth of a vegetative citrus shoot can be divided conveniently (see end of section 2.1.2.) into bud burst or leaf emergence (stage 3), shoot extension (stages 4-7), the arrest of shoot extension (stage 8), and abortion of the shoot tip (stage 9). The first three of these divisions have profound effects on the ultimate shape, size and appearance of the individual shoot and, consequently, of the tree. Factors involved in bud burst and the effects of these factors on shoot extension were studied in the preceding experimental section. In the work reported in this part the stages of shoot extension and arrest of shoot extension were studied. Methods of promoting or retarding shoot extension growth were explored and the resulting range of growth was used to study some aspects of the physiology of shoot elongation including carbohydrate assimilation and utilisation, the mode of action of exogenous GA_3 and phloem transport.

2.3.1. Manipulation of shoot elongation using plant growth substances and photoperiod

Introduction: To assist investigation of the physiology of shoot elongation growth it was considered desirable to find ways of altering such growth. Treatment with GA_3 is probably the most reliable method of promoting shoot elongation (see literature, section 1.5.2.3.) despite the negative results obtained with GA_3 applied to implanted buds before bud burst (section 2.2.2.1.); when applied after bud burst, GA_3 tended to promote shoot elongation (see previous section, 2.2.).

Absciscic acid (ABA) has been shown to inhibit shoot growth of many plants (reviews by Addicott and Lyon, 1969; Milborrow, 1974) and in citrus, ABA delayed bud burst under some circumstances but not others (Cooper et al., 1969). However because it is such an ubiquitous inhibitor in plants ABA was used in attempts to inhibit or reduce shoot extension growth.

Finally, as confirmed in the last experiment of the previous section, long photoperiods increase shoot growth in citrus (also see section 1.5.1.2.)

Therefore in an effort to obtain a wide range of controlled shoot growth, GA_3 and ABA were applied to young citrus shoots growing under two different photoperiods and the effects on shoot growth were assessed.

Methods: Valencia orange seedlings, grown as described earlier, were used. The 10 week-old seedlings were grouped into blocks depending on their size and decapitated above the sixth to ninth leaf, counting acropetally. All the plants of one block were decapitated above the same leaf. After 12 days at 27°C, 16 hour photoperiod, the uppermost axillary bud on every plant had burst.

A 5 µl droplet of one of the following solutions, made up in ethanol containing 0.05% Tween 20, was applied to each newly-emerged shoot:

10^{-3} M ABA

10^{-3} M GA₃

10^{-2} M GA₃

Ethanol + 0.05 % Tween 20 (Control)

Each block consisted of 8 plants and each growth substance treatment was applied, at random, to 2 plants in each block. Of these, one was put into a growth cabinet at 25°C with 8 hour photoperiod and the other into another growth cabinet at 25°C but with 16 hour photoperiod.

Therefore the experiment was laid out as a split plot design with photoperiod (or growth cabinet) as the main plots and growth substance treatment as the sub plots. There were 4 single-plant replicates of each growth substance in each cabinet.

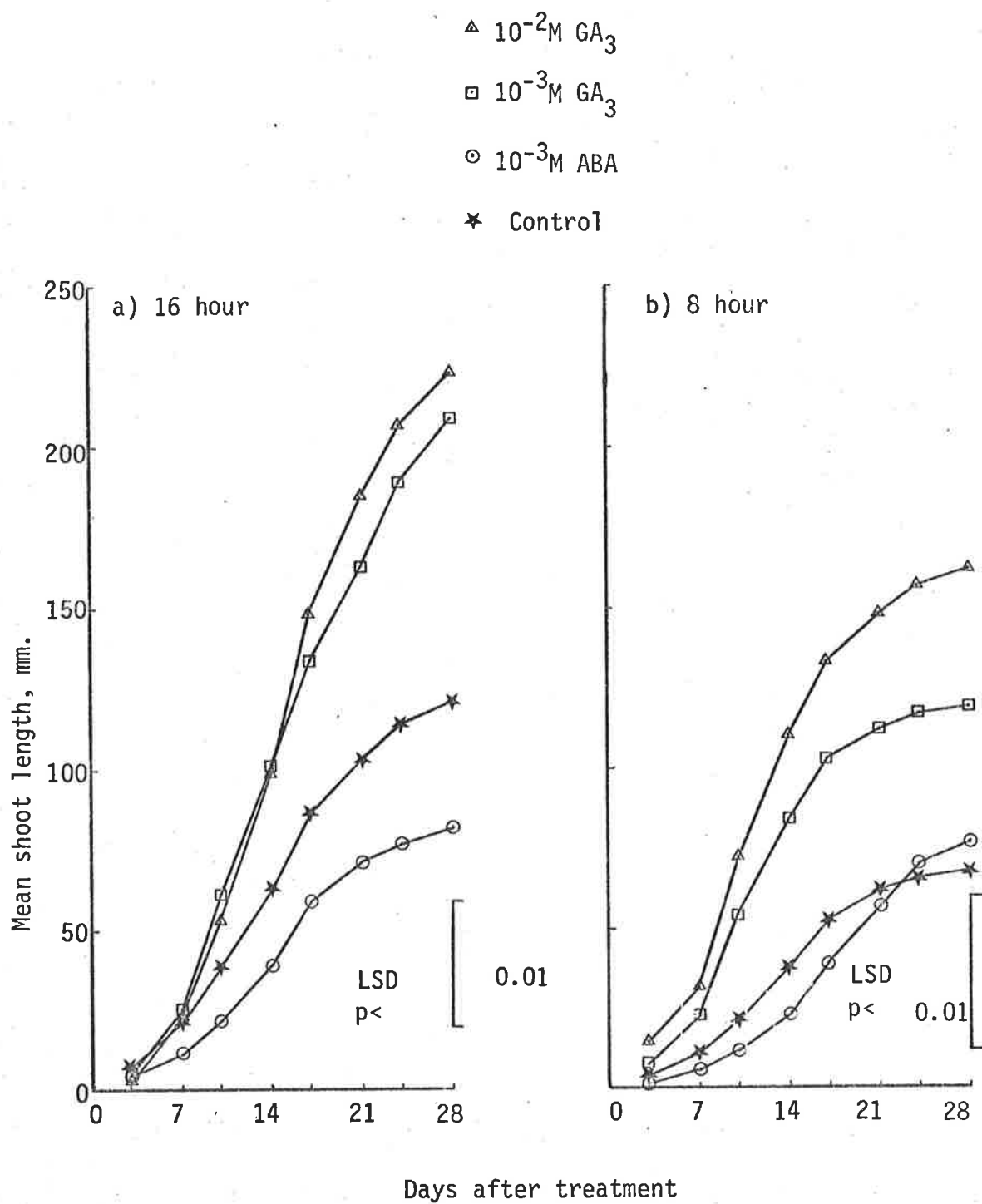


Figure 13. Effect of GA₃ and ABA on shoot length under two photoperiods.

Vertical bar = LSD for data at day 25.

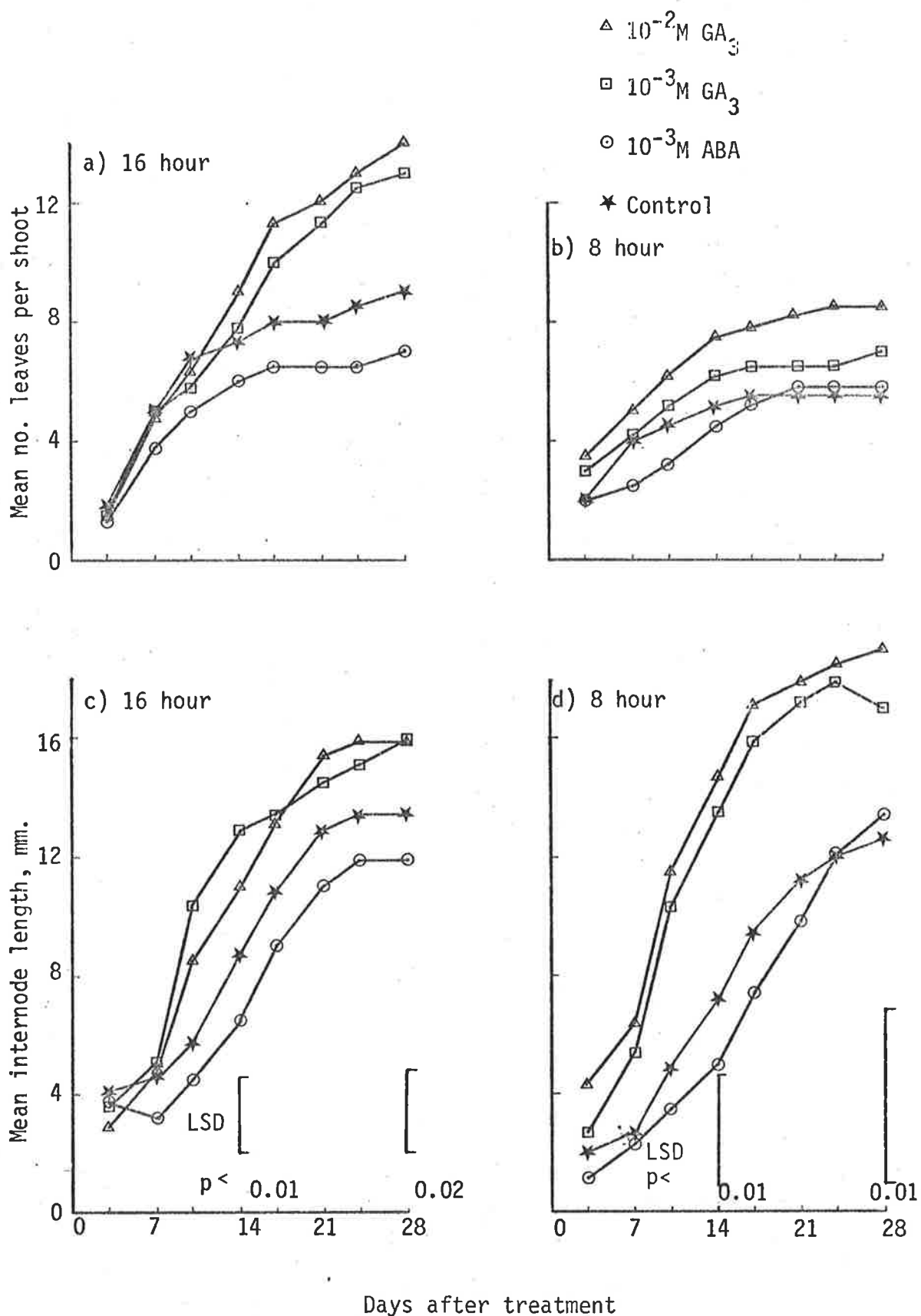


Figure 14. Effect of GA₃ and ABA on leaf number and mean internode length under two photoperiods.

Significant differences in leaf number under 16 hour ($p < 0.0006$) and 8 hour ($p < 0.013$) at day 28. (Determined by Kruskal-Wallis test).

Shoot length and the number of leaves greater than 5 mm long were recorded twice per week for the following 4 weeks.

Results and discussion: Shoot length under 16 hour photoperiod (LD) was significantly greater ($p < 0.01$ on day 28) than under 8 hour photoperiod (SD) (Figure 13). This difference was attributable to the greater number of leaves, and therefore of internodes, which developed under LD (Figures 14a and 14b). There was no difference in internode lengths between the two photoperiod treatments (Figures 14c and 14d).

Gibberellic acid significantly increased shoot length under both photoperiods (Figures 13a and 13b). Under LD there was no difference in shoot length between the two levels of GA_3 (Figure 13a) but both levels produced longer shoots than the control. This increase was largely attributable to an increase in the number of leaves per shoot (Figure 14a) although both levels of GA_3 also increased mean internode length (Figure 14c). Under these conditions GA_3 extended the duration of the rapid leaf proliferation stage but increased the rate rather than the duration of internode elongation, notably at the beginning of the rapid internode elongation stage.

In 8 hour photoperiods (SD) GA_3 significantly increased shoot length (Figure 13b) with the higher

concentration having a greater effect. Both levels of GA_3 increased the mean number of leaves per shoot, the higher concentration producing more leaves. There also were significant increases in mean internode length in SD due to the application of GA_3 (Figure 14d). Therefore the increased shoot lengths as a result of GA_3 in 8 hour photoperiods were due to increases in the number of internodes as well as in mean internode length. The greater shoot length induced by $10^{-2}M$ GA_3 compared to $10^{-3}M$ GA_3 was due primarily to the elongation of a greater number of internodes.

In both LD and SD, GA_3 increased the rate of internode elongation especially during the early stages (between 7 and 10 days after treatment, Figures 14c and 14d). However the effect of GA_3 on leaf proliferation* differed between the two photoperiods : under LD GA_3 extended the duration of the rapid leaf proliferation stage (Figure 14a) whereas under SD GA_3 tended to increase the rate rather than the duration of leaf proliferation (Figure 14b).

Under 16 hour photoperiods, ABA significantly inhibited shoot extension growth (Figure 13a) by reducing both the number of leaves per shoot and the average internode length (Figures 14a and 14c), i.e. ABA inhibited leaf proliferation and internode elongation; These effects of ABA were established in the first 10 days. Under 8

* Leaf proliferation refers to the appearance of leaves greater than 5 mm long subjacent to the apical bud.

hour photoperiod, ABA had no significant effect on shoot length or its components (Figures 13b, 14b and 14c) though there was an initial tendency to inhibit them.

The results indicate that shoot growth is enhanced under 16 hour photoperiods in comparison to under 8 hour photoperiods due to a faster and more prolonged rate of proliferation of leaves. Under 16 hour photoperiods GA_3 increases shoot elongation by extending the duration of leaf (and internode) proliferation and by accelerating internode elongation whereas ABA reduces shoot length by reducing both the number and the average length of internodes. In contrast, under 8 hour photoperiods ABA had very little effect and GA_3 increased shoot length by increasing the rate of both internode proliferation and elongation.

2.3.2. A preliminary investigation into the role of carbohydrate in shoot growth

Introduction: The young actively-growing citrus shoot is a strong sink and competes successfully even against young fruit for assimilates stored and/or assimilated in nearby leaves (Kadoya and Tanaka, 1972; Kriedemann, 1969a). Gibberellin increases the demand by the growing shoot for carbohydrate. However, it is not known which, if any, of the facets of GA- enhanced growth are susceptible to inadequate carbohydrate availability. Therefore it was decided to assess the effects of carbohydrate supply and GA₃ on shoot growth.

In budded citrus plants the tops of the rootstocks are lopped after budding thereby positioning the scion in an apical location while maintaining a source of assimilates to it (Platt and Opitz, 1973). Removing the top of the rootstock just above the implanted bud (topping) should considerably reduce the supply of carbohydrate available to the growing scion.

Consequently budded plants, topped above the implanted bud, were used to assess the effects of exogenous carbohydrate and GA₃ on shoot growth.

Methods: Budded plants similar to those described in section 2.2.2.3. (Methods) were topped about 5 cm above the implanted bud after the bud had burst. In topping, the stem was cut at a sharp angle thereby exposing

as much of the wood and as large a ring of phloem as possible. Before the top was removed the entire stem above the bud was surface - sterilized with 1% sodium hypochlorite and rinsed with autoclaved distilled water.

Tubes containing 3% (w/v) agar gels with or without 2% sucrose were placed over the freshly cut stems immediately after topping. The agar gels contained 0.2% chlorhexidine (Hibitane) and were autoclaved before being put into the tubes. The tubes were sterilized in 2% sodium hypochlorite before being filled. Each tube contained 3 ml of agar.

The treatments consisted of 5 μ l of 10^{-3} M GA₃ (in ethanol + 0.05% Tween 20) or its control, 5 μ l of ethanol + 0.05% Tween 20, applied to the newly emerged shoot, and agar gel containing 2% sucrose or its control (without sucrose) inserted over the top, i.e. the experiment was a 2 x 2 factorial, replicated 11 times and laid out in randomised blocks.

The agar gels were changed twice per week. Each time they were changed the end of the rootstock was cut back to expose fresh tissue, and a strip of bark was peeled back to increase the area of phloem exposed to the agar. In the later changes the epidermis and cortex were also scraped away. At every change the stock was surface - sterilized with 1% sodium hypochlorite.

The plants were grown under glasshouse conditions similar to those described in section 2.2.2.3. (Methods).

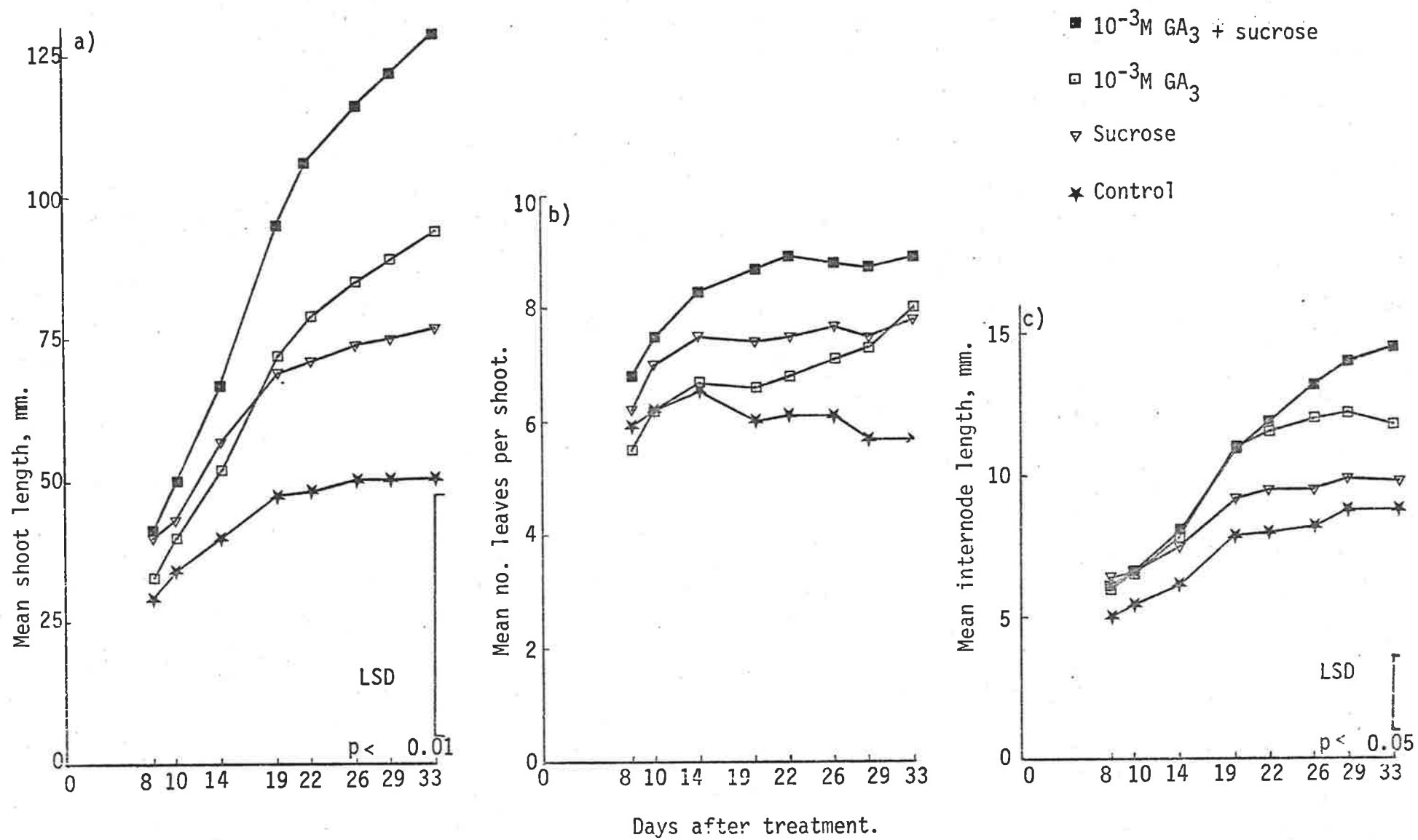


Figure 15. Effect of exogenous GA_3 and sucrose on shoot growth of budded plants. Vertical bars = LSD for data at day 33.

Shoot lengths and the numbers of leaves greater than 5mm long were recorded twice per week.

Results and discussion: As in the previous experiment GA_3 significantly increased mean shoot length. Sucrose also increased shoot length (Figure 15a) and the combination of GA_3 and sucrose considerably promoted the elongation of the shoot.

The differences in mean number of leaves per shoot at day 33 were not significant (Figure 15b). Nevertheless GA_3 appeared to increase leaf number by increasing the duration of leaf proliferation, as occurred in the previous experiment, whereas sucrose tended to increase the early rate of leaf proliferation. The combination of GA_3 and sucrose increased the rate and duration of leaf proliferation compared to the untreated control.

Supplying sucrose exogenously had no effect on internode elongation (Figure 15c) whereas GA_3 increased mean internode length, by increasing the early rate of internode elongation; GA_3 did not extend the period of internode elongation, as also was found in the previous experiment. When GA_3 and sucrose were supplied to the plant both the rate and the duration of internode elongation were increased.

The results indicate that increased carbohydrate supply promotes leaf proliferation but has no effect on internode elongation, whereas the increases in

leaf proliferation and in internode elongation induced by GA_3 are heightened by an increased supply of carbohydrate.

This suggests that increased levels of available carbohydrate promoted leaf initiation during early growth but did not induce the continuation of leaf proliferation as did GA_3 . Moreover, it appears that the carbohydrate supplies within the plant under the conditions of this experiment were adequate for normal internode elongation since increasing the carbohydrate supply did not increase average internode length (although it did tend to increase the number of internodes). In contrast, the endogenous supply of carbohydrate apparently was insufficient to sustain the elongation of the increased number of internodes induced by GA_3 after day 19, i.e. GA_3 increased the demand by the new shoot for carbohydrate.

2.3.3. Source-sink interaction in shoot growth

Introduction: The controversy surrounding whether the accumulation of assimilate in mature photosynthetic tissue reduces the rate of photosynthesis of such tissue, or conversely, whether an increase in the demand for assimilate increases the rate of photosynthesis of mature tissue has not been resolved (see discussion by Geiger, 1976 and review by Neales and Incoll, 1968).

Using single-leaved rooted cuttings of lemon (C. limon) Lenz et al. (1972) found that the rate of photosynthesis of the single mature leaf fell when a ring of bark was removed from around the stem (ringing) just below the mature leaf, and concluded that the developing root system was a strong sink whereas the growing axillary shoot was a relatively weaker sink. However, it is possible that a cincture or ring immediately below the leaf may have separated the phloem of the leaf from that of the axillary shoot as well as of the root thereby isolating both the shoot and the root system from the supply of assimilates synthesised in the mature leaf.

Moreover, it had been shown, using $^{14}\text{CO}_2$, that the young shoot is a powerful sink for both current and stored photosynthate (Kadoya and Tanaka, 1972; Kriedemann, 1969a, 1969b).

Therefore it was of interest to establish more clearly the relationship between the growth of the young shoot and the rate of photosynthesis of the mature leaf.

Axillary shoot growth could be promoted by GA_3 and possibly reduced by applications of ABA, and the assimilate in the mature leaf tissue could be isolated from the root system but kept in confluence with the young shoot by a ring located lower down the stem rather than immediately below the subtending leaf. Moreover, by partially defoliating a non-ringed seedling the amount of assimilate available to the sinks (the growing axillary shoot) could be reduced by reducing the quantity of stored material as well as the photosynthetic area.

An experiment was carried out to assess the effects of varying sink/source ratios, by using the above treatments, on the growth of the young shoot and on the rate of apparent photosynthesis of the mature leaf.

Methods: Valencia sweet orange seedlings, grown under 16 hour photoperiods at $26^{\circ}/21^{\circ}C$ were decapitated above the ninth leaf when the plants were 5 months old. The uppermost axillary bud on each plant burst within five days of decapitation, at which time the treatments were applied.

The experiment was a 3×3 factorial of growth substance treatment x plant manipulation treatment :

Growth substance treatments: 5 μ l droplet of ethanolic solution (containing 0.05% Tween 20) of one of the following was applied to each emerging shoot:

- a) 10^{-3}M GA_3
- b) 10^{-3}M ABA
- c) Control: ethanol + 0.05% Tween 20

Plant manipulation treatment:

- 1) Partial defoliation:

Every second mature leaf removed, starting with the second leaf from the top of the plant. Therefore the leaf subtending the young shoot was left attached.

- 2) Ringing: Ring of bark, 3 mm wide, removed from below lowest node
- 3) Control: Intact plant.

The experiment was replicated 5 times, using single plants as replicates.

Shoot length and the number of leaves longer than 5 mm were recorded for each plant twice per week.

Apparent photosynthesis (AP) (Shibles, 1976) and leaf diffusion resistance (r_1) were measured at decapitation, at the time of treatment and at four times during the growth of the young shoot. The measurements were made on the mature leaf subtending the growing shoot. The plants of one replicate were used.

The rate of AP of single leaves under optimal conditions (Kriedemann, 1968b) was measured by infra-red gas analysis (IRGA) using a differential measuring arrangement.

The details of the system were as follows:

Bottled air was fed through a pressure regulator into two streams, one of which was used as the sample stream and the other as the reference stream. In the sample stream the air passed through a needle valve which controlled the flow-rate, then through distilled water to humidify the gas and finally into the photosynthetic chamber. This chamber was made of Perspex and consisted of two similar halves. Each half consisted of an outer, sealed chamber 15.5 cm deep and 14.5 cm in diameter, which contained water and a smaller inner cuvette (7 cm deep and 5 cm in diameter) fixed in the larger chamber and open at one end. Air from the humidifying vessel flowed into this cuvette through a coiled tube enclosed within the sealed chamber. Water, maintained at constant temperature by passing through a Julabo Paratherm II constant-temperature bath, circulated through the sealed outer chamber thereby maintaining the inner cuvette and the air entering it at a constant temperature. The air inside the cuvette was stirred by an electrically driven fan; there was a shallow layer of water within the inner cuvette to ensure that the air was saturated. The motor of the fan was located outside of the outer chamber and isolated from the air in the cuvette by two seals. The cuvette of the lower half of the photosynthesis chamber had an aperture of 6 cm^2 surrounded by a layer of rubber ("wet suit" rubber) onto which the lower surface

of the leaf was placed. As citrus leaves are hypostomatous (Reed and Hirano, 1931) the upper cuvette, which rested on the upper leaf surface, was sealed off by a Perspex disc. The air flowing out of the lower cuvette passed through a U-tube suspended above liquid nitrogen in a vacuum (Thermos) flask, where the water vapour was deposited as ice. The dry air then passed through a Fisher and Porter flow-meter and into the reference inlet of a Beckman 215B infra-red gas analyser. A Leeds and Northrup Speedomax recorder (10 mV span) was connected to the infra-red gas analyser.

The reference air stream passed through a needle valve and into a U-tube containing dried magnesium perchlorate to remove any moisture. From there it went through another Fisher and Porter flow-meter and into the sample inlet of the analyser.

The water temperature, and consequently the air and cuvette temperature, was maintained at 25°C, and the air flow was 500 ml minute⁻¹. Light was provided by a quartz-iodide lamp mounted above a Perspex tray containing a 5 cm depth of distilled water. The whole assembly could be raised or lowered to vary the light intensity incident upon the leaf. The intensity of the light could also be varied by a rheostat. The lamp was set at a height such that the illuminance at leaf level was 26 000 lux (photosynthetic photon flux density (PPFD) = 700 $\mu\text{E m}^{-2} \text{ sec}^{-1}$) at 240 volts. This was found

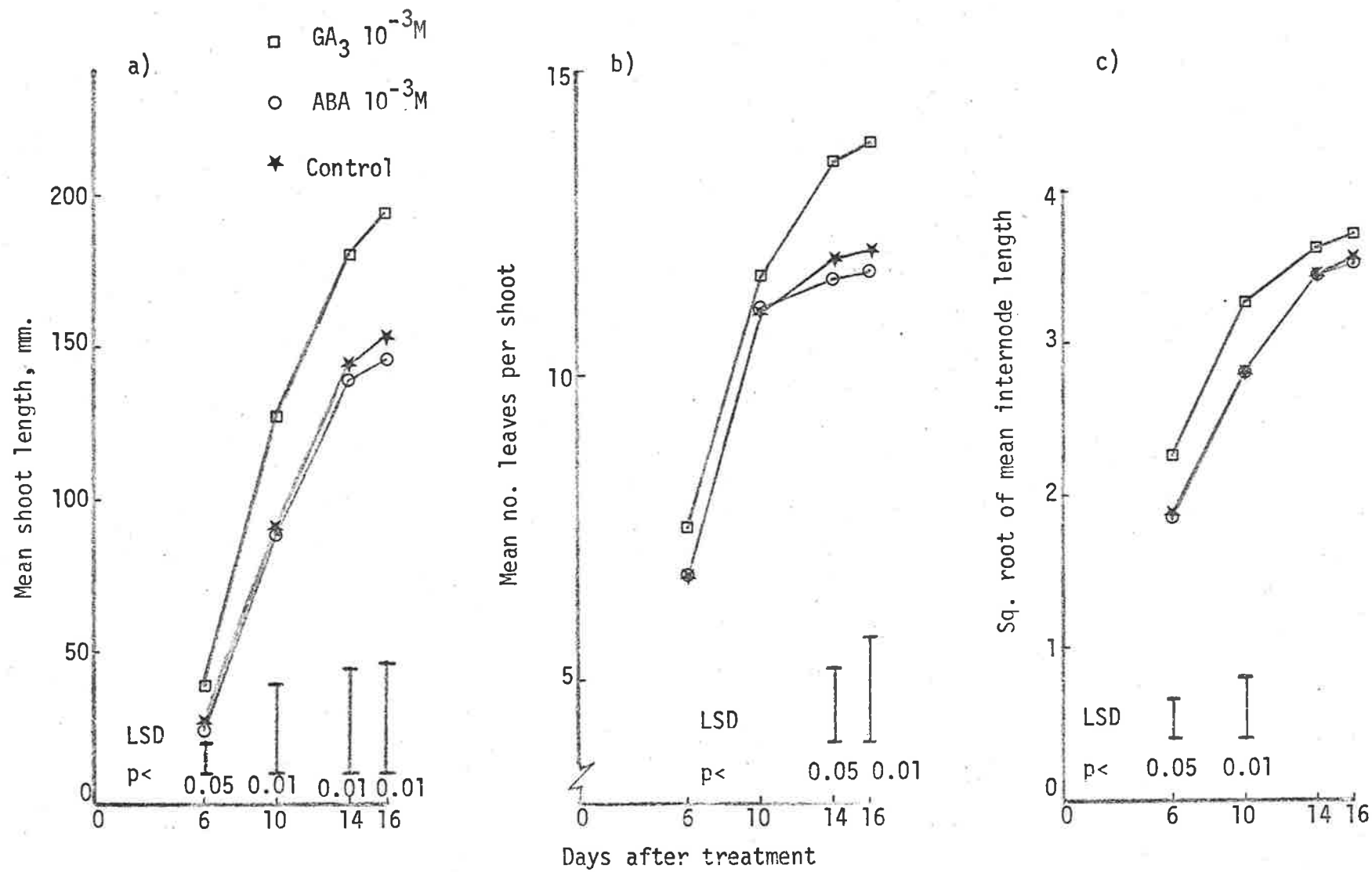


Figure 16. Effect of GA₃ and ABA on shoot growth of decapitated seedlings.

to be the optimum illuminance for photosynthesis and agrees with the results of Kriedemann (1968b). As the operation of the fan in the cuvette eliminated the boundary layer resistance to CO_2 diffusion, and as the light and temperature conditions were optimal, what was in fact measured was the maximum rate of apparent photosynthesis (MAP) at the CO_2 concentration of the bottled air (approximately 330 ppm).

The infra-red gas analyser was calibrated at least once per week. In calibration, standard concentrations of CO_2 in N_2 , achieved by the use of two Wösthoff gas mixing pumps, were compared against N_2 in the analyser.

Leaf diffusion resistance was measured in an aspirated diffusion porometer (Byrne et al., 1970) immediately upon removal of the leaf from the photosynthesis chamber.

Before MAP and r_1 were measured the plants were conditioned to the high light intensity either by slowly moving them into areas of increasing light intensity or by increasing the illuminance using the rheostat. The soil was also kept moist during the entire process. In this way cyclic oscillations in AP and transpiration (Kriedemann, 1968b) were avoided.

Results and discussion: Gibberellic acid significantly increased shoot length from 6 days after application (Figure 16 a). Up to day 10 this increase

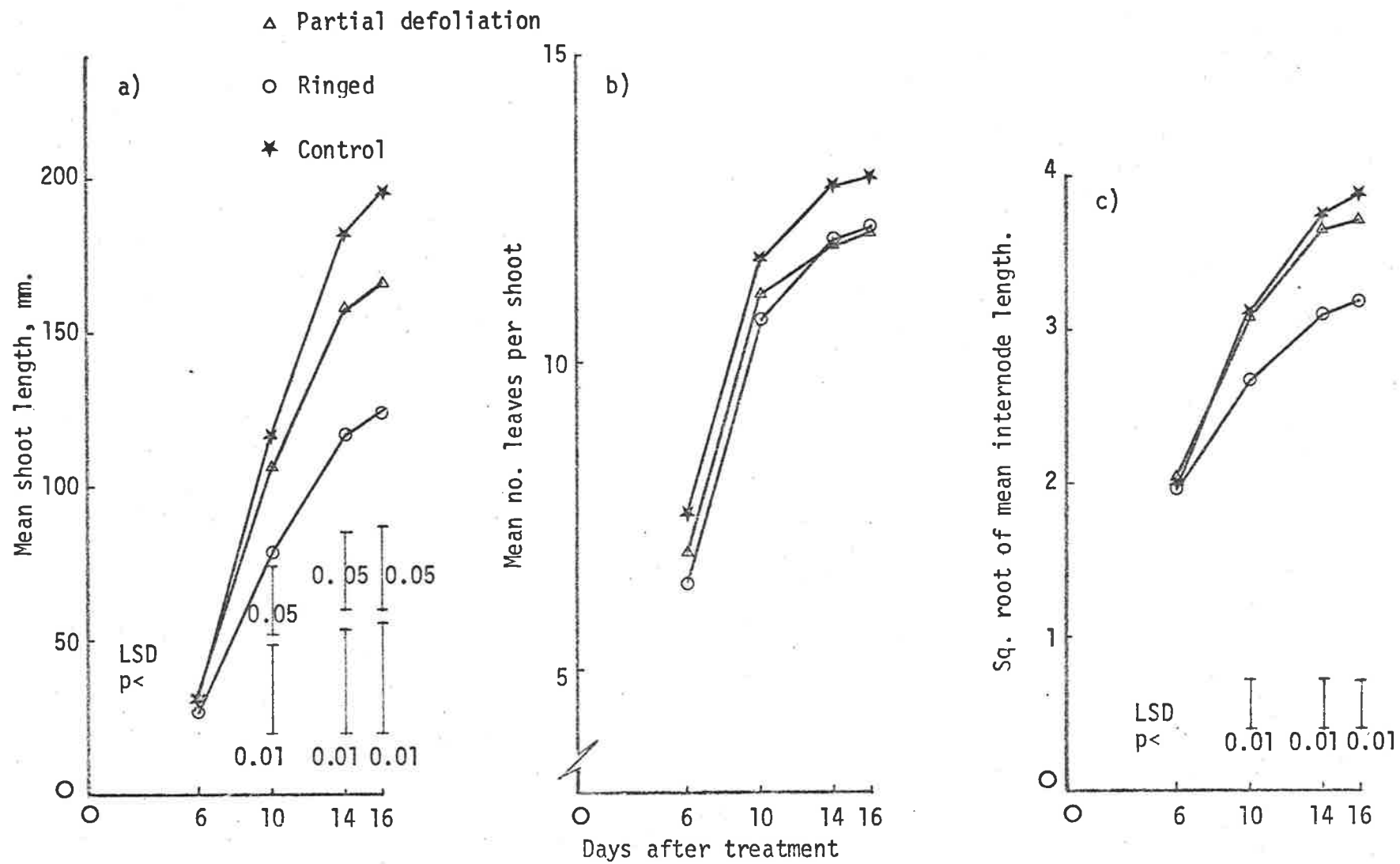


Figure 17. Effect of manipulative treatments on shoot growth of decapitated seedlings.

was due mainly to the significantly greater length of the internodes of GA_3 -treated shoots (Figure 16c), as there were no significant differences in leaf number (Figure 16b). However, after day 10 GA_3 promoted leaf proliferation whereas the number of leaves of the control only increased slightly. During the same period the differences in internode length lessened.

In contrast to earlier results (section 2.3.1.) ABA had no effect on shoot growth, even though the experiment was conducted under 16 hour photoperiods (compare Figures 13a and 14a and 14c). It is difficult to find an explanation for this difference as in both experiments decapitated seedlings, similar ABA solutions and similar methods of application were used.

There were highly significant effects of the manipulative treatments on shoot growth. Ringing considerably reduced the rate of shoot elongation, producing significantly shorter shoots from day 10 (Figure 17a). This was mainly due to the significantly shorter internodes of this treatment (Figure 17c) though it also tended to reduce leaf number (Figure 17b).

Partial defoliation reduced the rate of shoot elongation and resulted in significantly shorter shoots than the control by day 14 (Figure 17a). This reduction was due mainly to reduced internode number (Figure 17b).

There was no significant interaction of treatments on any shoot growth parameter when all the

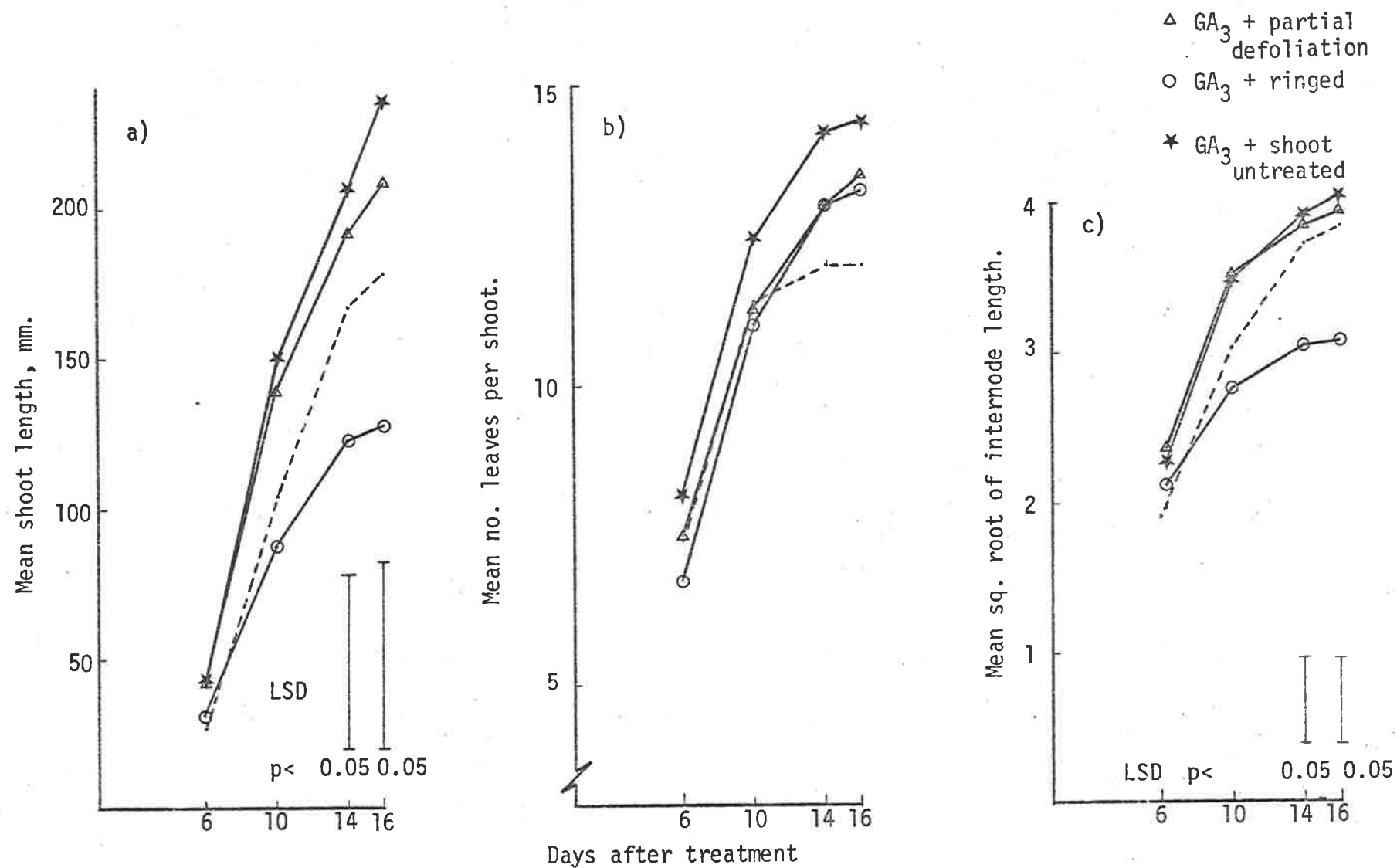


Figure 18. Effect of manipulative treatments on the GA₃-induced promotion of shoot growth.

The broken line indicates the growth of the unringed control to which only ethanol + Tween 20 was applied.

data was considered. However, in a comparison of the effects of the manipulative treatments on the gibberellin effect (Figure 18) ringing reduced the length of the GA_3 -treated shoot to less than that of the unringed, ethanol-treated control, i.e. ringing eliminated the GA_3 effect on shoot length (Figure 18a). Ringing had little effect on the increase in leaf number induced by GA_3 but did significantly reduce the effect of GA_3 on internode length (Figure 18c). In contrast, partial defoliation had no effect on the GA_3 -induced increases in shoot growth.

It seems that GA_3 increases shoot length by promoting leaf proliferation as well as internode elongation, and that ringing inhibits the latter; this suggests that the interruption of phloem translocation reduces one or both of the components of internode length i.e. cell number and cell length.

Gibberellin is considered to promote both cell elongation and division (Jones, 1973). Crozier and Reed (1972) found that removal of the root apices of bean seedlings resulted in the disappearance of GA_1 and the accumulation of GA_{19} in the leaves and apical buds. They postulated that GA_{19} may have been synthesised in the shoot, transported to the roots where it was converted into GA_1 and that GA_1 was subsequently transported back up to the shoot. A similar postulate could explain the loss of the GA_3 effect on shoot growth in response to ringing: GA_3 applied to the young shoot may promote leaf initiation and

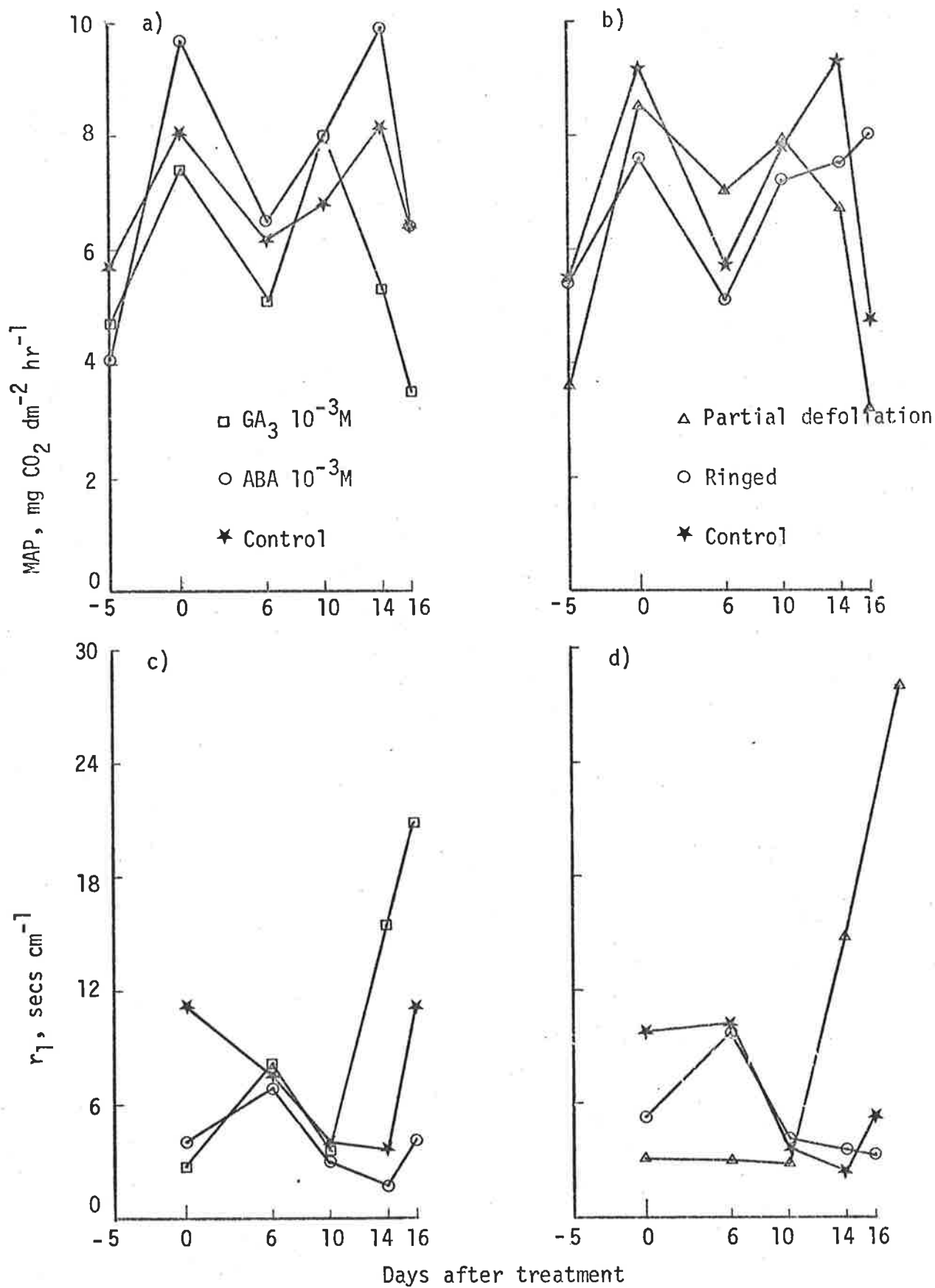


Figure 19. Effect of growth substances and manipulative treatments on maximum rate of apparent photosynthesis (MAP) and leaf diffusion resistance (r_1). Same legend for upper and lower figures.

therefore leaf proliferation, whereas internode elongation may be promoted by another GA which is synthesised in the roots from GA_3 translocated from the shoot. Alternatively GA_3 applied to the young shoot may be translocated to the roots where it may promote the synthesis or release of a metabolite, for example cytokinin which in turn is translocated to the young shoot and promotes internode elongation. A third possibility is that ringing the stem below all of the leaves deprives the roots of an adequate supply of organic nutrients; the resultant poor growth of the roots leads to poor internode elongation due to inadequate mineral nutrient or water supply. These alternatives were considered in the next experiment.

There were large fluctuations in MAP and r_1 from day to day (Figure 19) even though the same leaves on the same plants were used at each measurement. This, together with the fact that only one replicate was used, make the results equivocal. Nevertheless some points are worth making.

The apparent increase in MAP at day 0 (day treatments applied) compared to day -5 (day plants topped) may have been in response to the initiation of shoot growth, and indicates an increase in the ability or potential of the mature leaf to photosynthesise. The second peak in MAP at day 10 or day 14 may be due to the initiation of a flush of root and cambial growth, as it is well known that these follow a flush of shoot growth

(Cameron and Schroeder, 1945; Crider, 1927, quoted by Webber, 1943; Gates et al., 1961a, 1961b; Krishnamurthi et al., 1960; Reed and Mac Dougal, 1937).

Despite the large fluctuations some general effects of treatment on MAP seemed apparent. Of the growth substances, GA_3 tended to reduce MAP while ABA tended to increase it (Figure 19a). This was surprising, as GA_3 increased shoot growth and therefore sink strength, whereas ABA did not. However, it must be emphasised that MAP is a measure of the potential of the mature leaf subtending the young shoot to photosynthesise, not the actual rate of carbon assimilation of the plant under growth cabinet conditions. Therefore the results indicate that GA_3 applied to the young shoot may have inhibited or retarded some part of the photosynthetic pathway and ABA apparently reduced some limiting step of photosynthesis in the mature leaf subtending the young shoot.

The results of leaf diffusion resistance (r_1) (Figure 19c) suggest that ABA increased MAP by reducing r_1 , an effect contrary to the well-established stomatal closure induced by ABA. The effect of GA_3 on r_1 was not clear except at days 14 and 16 when r_1 values were high; on the same days MAP was low, indicating that photosynthetic potential was limited by high leaf resistances. These results suggest that the general reduction in MAP in response to GA_3 was not as a result of increased resistance to CO_2 diffusion, therefore GA_3 may have affected the

photochemical or enzymatic aspects of CO_2 fixation.

Ringling had no effect on r_1 (Figure 19d) despite its tendency to reduce MAP (Figure 19b).

In two partially defoliated plants r_1 increased considerably at days 14 and 16 producing the sharp rises in Figures 19c and d. No explanation can be given for this. Partial defoliation reduced shoot length but had no significant effect on leaf number nor internode length (Figure 17), i.e. approximately one-half of the mature leaf area supported almost the same amount of young shoot growth as the control. Yet the MAP of the partially defoliated plant was no greater than the control and in fact tended to be lower (Figure 19b). Either most of the carbohydrate used in the growth of the young shoot must have come from stored material in the stem and/or roots or the mature leaves normally photosynthesise at rates well below their maximum capacity.

The results indicate that there is no clear-cut relationship between young shoot growth and MAP of the mature leaves. The young shoot depends on carbohydrate from the mature tissue for its early growth. Further studies into the carbohydrate nutrition of the young shoot were carried out.

2.3.4. An investigation into the growth-retarding effects of ringing on GA₃-treated shoots

Introduction: In the previous experiment ringing not only reduced shoot growth but also eliminated the GA₃-induced promotion of shoot growth, mainly by reducing internode elongation. In the discussion three hypotheses about the mechanism of this action were postulated, and this experiment was designed to test those hypotheses.

Method: A 4 x 3 factorial design, involving the following treatments was used:

Ringing: 1 - Ring below all leaves
 2 - Ring above 3 large-sized, mature leaves*
 3 - Control, no ring

Growth
 Substance: a - 5 µl droplet of 10⁻³M GA₃ applied to the leaves and apex of the young shoot
 b - 5 µl droplet of 10⁻³M GA₃ applied to the petiole and stem below a ring (In treatment 3b GA₃ was applied to the stem below all the leaves)

* The first 2-4 foliage leaves of a citrus seedling are small, rounded, sessile leaves, with short internodes between them. Above these the leaves of a Valencia orange seedling are larger, oval to oblong in shape and are borne on winged petioles. The latter are large-sized, mature leaves referred to in treatment 2.

- c - 5 μ l droplets of each of 10^{-3} M GA_3 and 10^{-4} M BAP applied to the young shoot
- d - Control: 5 μ l droplet of ethanol + 0.05% Tween 20 applied to the young shoot

There were 6 single-plant replicates, laid out in randomized complete blocks.

Decapitated Valencia orange seedlings, 3 months old, were used, 10 days after topping. When 7 weeks old, the seedlings had been transplanted from seedboxes into 10 cm diameter pots containing sawdust similar to that described in section 2.2.2.3., Methods. The sawdust was enriched with 60 ml Osmocote (14:61:11.6 N:P:K) slow-release fertilizer per gallon. The seedlings were grown in a growth room with 16 hour photoperiods and 26°/21°C day/night temperatures, and were supplied with Aquasol and additional iron chelate once per fortnight. The experiment was conducted in a growth cabinet at 25°C, 16 hour photoperiod.

Shoot length and leaf number were recorded twice per week. On the same days MAP and r_1 of the mature leaves subtending the young shoots in one replicate were measured using the same equipment and under similar conditions as described in the previous experiment (see 2.3.3. Methods).

By 14 days after treatment many shoots of the ringed treatments started collapsing about half-way along their lengths or dying back from the tip. Although this

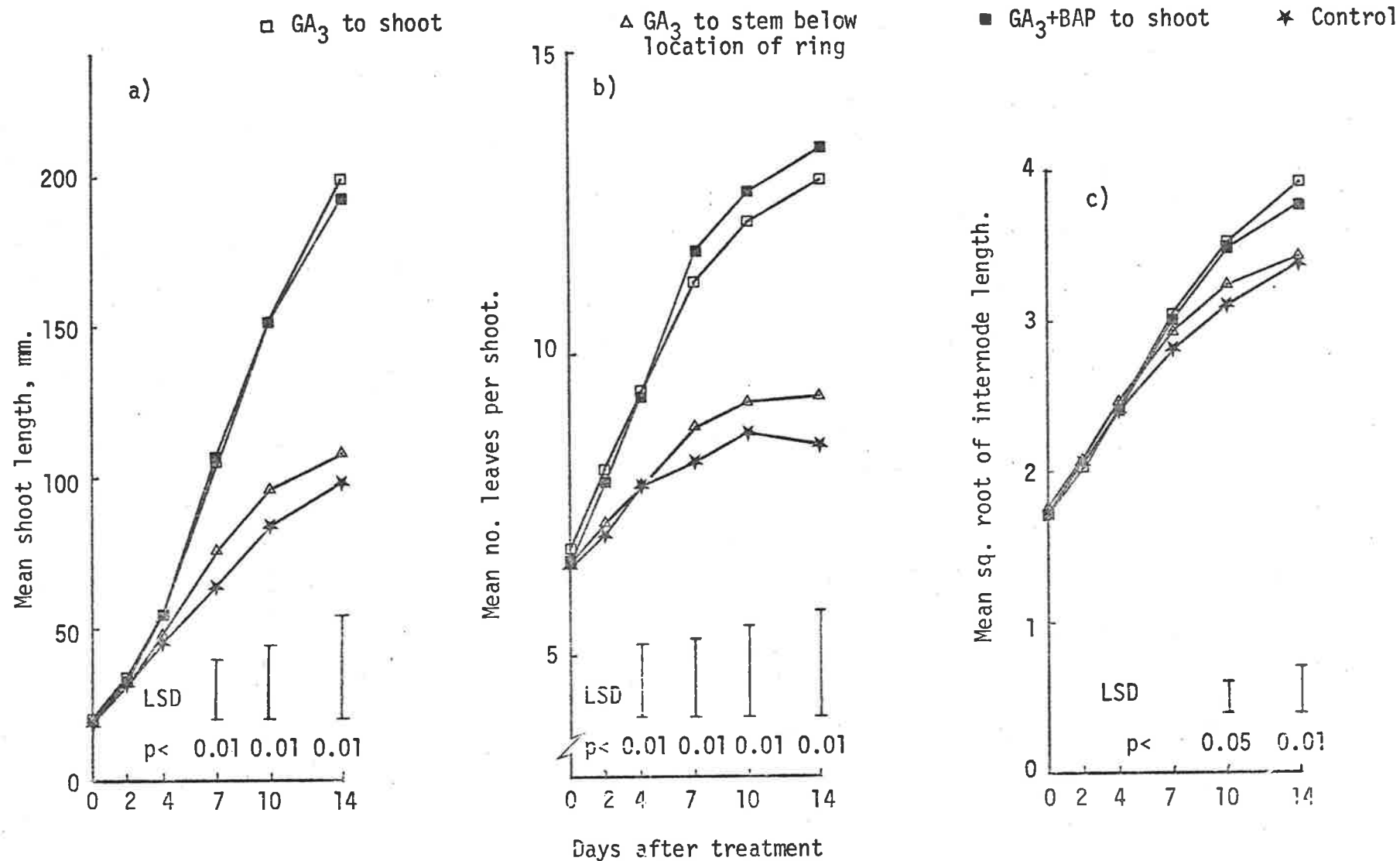


Figure 20. Effect of $GA_3 \pm BAP$, applied at two locations, on shoot growth.

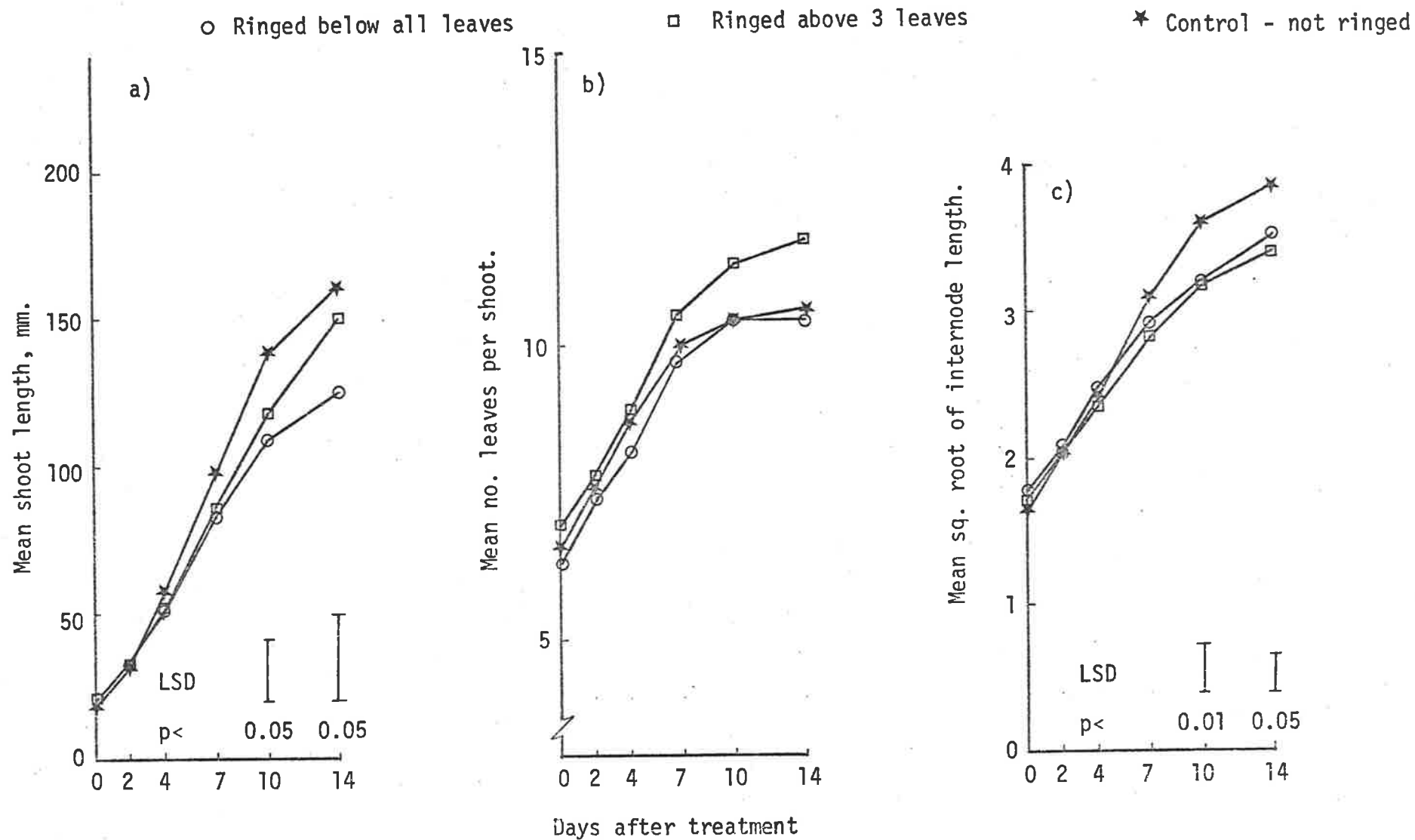


Figure 21. Effect of ringing at two positions on shoot growth.

was found in ringed, control plants it was particularly evident in ringed plants where GA_3 was applied to the young shoot. Therefore only the results up to day 14 are considered.

Results and discussion: As found previously, GA_3 applied to the young shoot significantly increased shoot growth by increasing leaf number and internode length (Figure 20). The simultaneous application of BAP and GA_3 to the young shoot made no difference to the GA_3 effect. When GA_3 was applied to the leaf or bark below the location of a ring (either to the bark on plants ringed below all the leaves or, in plants of treatment 2, to the petiole of the third large-sized, mature leaf and the bark at its node) it had no significant effect on shoot length, leaf number or internode length compared to the control (Figures 20a, b and c resp.). It appears that GA_3 , when applied at this position, did not reach the sites which determine leaf initiation and shoot elongation.

Ringling below all the leaves significantly reduced shoot length and internode length (Figures 21a and 21c) but had no effect on leaf number (Figure 21b), as occurred in the previous experiment. Ringling above 3 mature leaves also significantly reduced internode length (Figure 21c) but tended to increase leaf number (Figure 21b). It is therefore not surprising that this treatment (Figure 21a) had no significant effect on shoot length. Leaving

some leaf area in confluence with the root system had no influence on the main effect of ringing, viz., a reduction of internode length. This indicates that the effect of ringing on shoot growth is not due to the root system being deprived of carbohydrate. On the other hand it is possible that the photosynthetic area below the ring was inadequate for supporting normal root growth, which may be necessary for normal internode elongation. These results suggest that the effect of ringing on shoot growth is not due to root death by "starvation", but the results do not establish whether the ringing effect is due to some malfunction of the root system, due possibly to inadequate supply of carbohydrates and/or other compounds, or is due to the interruption of supply of a compound or compounds from the roots to the shoot, or both.

It has been established in citrus that the root system imports assimilates from mature leaves (Kriedemann, 1969a, 1971) and that ringing interrupts such transport (Goren et al., 1975; Wallerstein et al., 1974). In addition, GA-like compounds accumulated above a ring, with a parallel decrease below the ring (Goren et al., 1971, 1975). These data suggest that the inhibition of shoot growth by ringing is due to a malfunctioning of the root system because of inadequate supplies of carbohydrate and GA.

This interpretation is supported by results of the present experiment on the effect of GA₃ on shoot growth in relation to ringing. Gibberellin applied to the

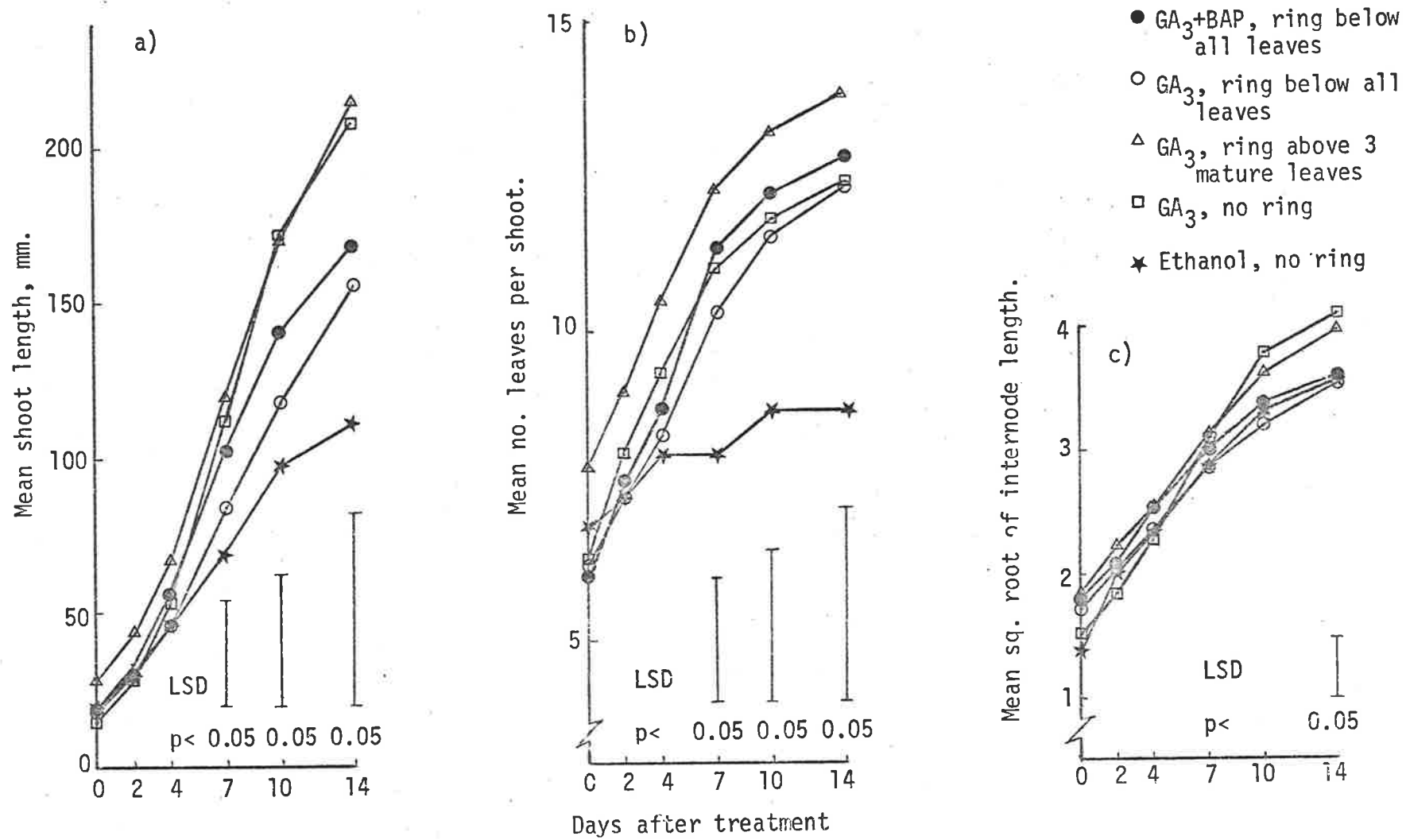


Figure 22. The interaction between GA_3 and ringing on shoot growth.

young shoot on unringed plants increased mean shoot length, leaf number and internode length compared to unringed controls (Figure 22). Ringing below all the leaves reduced the GA_3 effect on shoot length (Figure 22a) by eliminating the GA_3 -induced increase in internode length (Figure 22c). When GA_3 was applied to the shoot and the ring was located above 3 mature leaves however, there was no difference in shoot length, leaf number or internode length from GA_3 -treated unringed plants. It appears that the presence of leaves below the ring allowed the expression of the GA_3 effect on shoot growth, which suggests that a "non-starving" root system is necessary to support the increased growth.

Another hypothesis was that healthy or growing roots supply cytokinin to the young shoot (Altman and Mozes, 1976; also see reviews by Skene, 1975 and Torrey, 1976) and that this is necessary for the manifestation of the GA effect on shoot growth. However, the results of this experiment do not support such a hypothesis, as when $10^{-4}M$ BAP was applied together with GA_3 to the young shoot of plants ringed below all the leaves the subsequent growth of the shoot was not significantly different from that of GA_3 -treated plants ringed in a similar position (Figure 22). A number of explanations for these results are possible:

- a) cytokinin is not the limiting factor which is produced by the roots, b) the BAP which was applied was not absorbed or utilized by the shoot, c) the concentration of BAP which

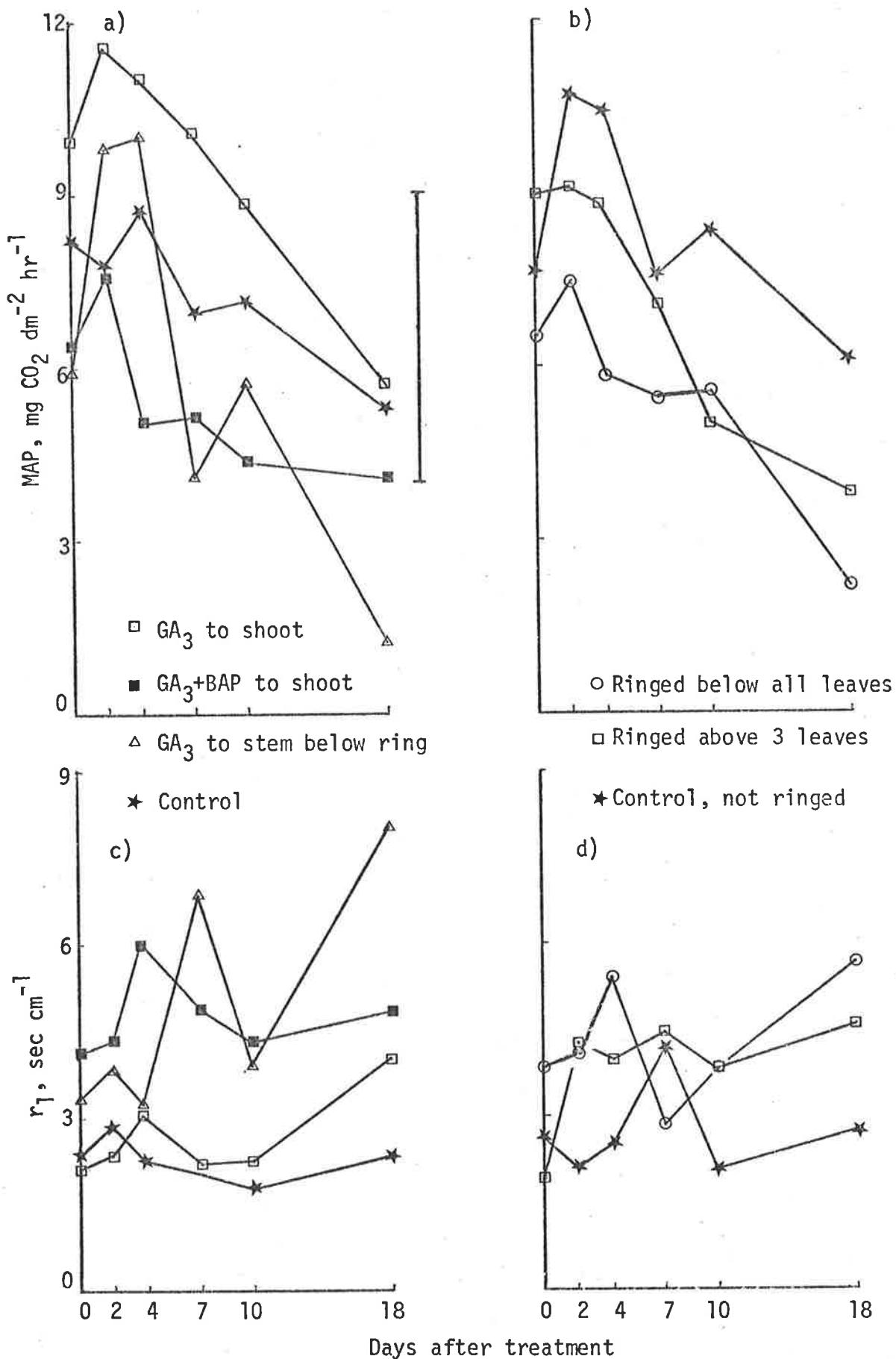


Figure 23. Effects of growth substance and ringing treatments on maximum apparent photosynthesis (MAP) and leaf diffusion resistance (r_l).

Vertical bar = LSD for all data of a).

arrived at the active site was very different from the normal levels, d) the duration of supply of cytokinin was insufficient. So although the evidence suggests that cytokinin is not the important factor coming from the roots, the hypothesis has not been disproven.

The results of this experiment confirm that ringing has no effect on the increase in leaf production induced by GA_3 applied to the shoot. This strongly suggests that GA_3 or its metabolites induces cell division in the shoot tip and that this division is not influenced by ringing, i.e. in contrast to the suggestion of Crozier and Reid (1972) it is not necessary for GA_3 to be transformed into a more potent form in the root in order to increase leaf proliferation in the young shoot.

The curves of MAP showed a general tendency to increase during the first 2 days after treatment and then fall steadily (Figures 23a and 23b). The increase was probably due to the effect of the actively growing shoots which acted as powerful sinks (Kriedemann, 1969a; Lenz, 1975). The fall was correlated with the growth of the young shoot and its increasing ability to supply more of the assimilate needed for its growth (Hino et al., 1974); consequently the demand for carbohydrate from the mature leaves declined, resulting in an accumulation of photosynthate in these leaves, thereby reducing their rate of maximum apparent photosynthesis (Geiger, 1976; Habeshaw, 1973; Neales and Incoll, 1968).

In contrast, r_1 tended to remain constant throughout the experiment (Figures 23c and 23d), which supports the hypothesis that the decline in MAP was due to end-product inhibition or disruption of photosynthesis by the accumulation of starch grains within the chloroplasts (Lenz, 1975).

Gibberellin applied to the shoot increased MAP, compared to the control (Figure 23a). For the reasons stated above this was not unexpected as GA_3 applied to the shoot also increased shoot growth and therefore, sink strength. Moreover, there was almost no difference in leaf resistance between these two treatments (Figure 23c) which suggests that GA_3 , applied to the young shoot, increased the efficiency of the photosynthetic system.

In contrast, when the combination of GA_3 and BAP was applied to the young shoot MAP was reduced (Figure 23a), apparently due to increased r_1 (Figure 23c). This was contrary to expectations as cytokinin enhances import of assimilates into young tissue (Kriedemann, 1968a; Kadoya and Tanaka, 1972) and also increases the rate of CO_2 fixation in mature citrus leaves (Hoffmann and Lenz, 1975). Moreover, application of a combination of GA_3 , IAA and kinetin increased CO_2 fixation by 66 % in lemon cuttings (Hoffmann and Lenz, 1975). The effect of GA_3 + BAP on MAP and r_1 in this experiment cannot be explained.

The response of MAP and r_1 to GA_3 applied to the stem was erratic. Except for the initial rise in MAP in response to early shoot growth, the fluctuations in MAP in this treatment concurred with changes in r_1 (Figures 23a and 23c) and was probably a consequence of varying stomatal resistance. However, it is not apparent why these values varied so widely on different days.

Ringling at either position reduced MAP, the effect of the ring below all the leaves being more pronounced (Figure 23b). This may have been due to the isolation of the sink demands of the root system from the leaves (Lenz et al., 1972). Where the ring was below all the leaves a larger photosynthetic area was available to meet the demands of the young shoot and therefore MAP was reduced. A ring above three leaves, which reduced the leaf area in confluence with the young shoot, did not reduce MAP as much, which suggests that MAP is related to the source: sink ratio. Ringling at either position also increased r_1 but there was no difference between the two ringling treatments on r_1 (Figure 23d), as there was on MAP. This indicates that ringling increases leaf resistance but may limit photosynthesis by influencing some other factor, e.g. end-product inhibition resulting from the reduced growth of the shoot. Therefore the effect of ringling on MAP is probably a consequence rather than the cause of reduced shoot growth.

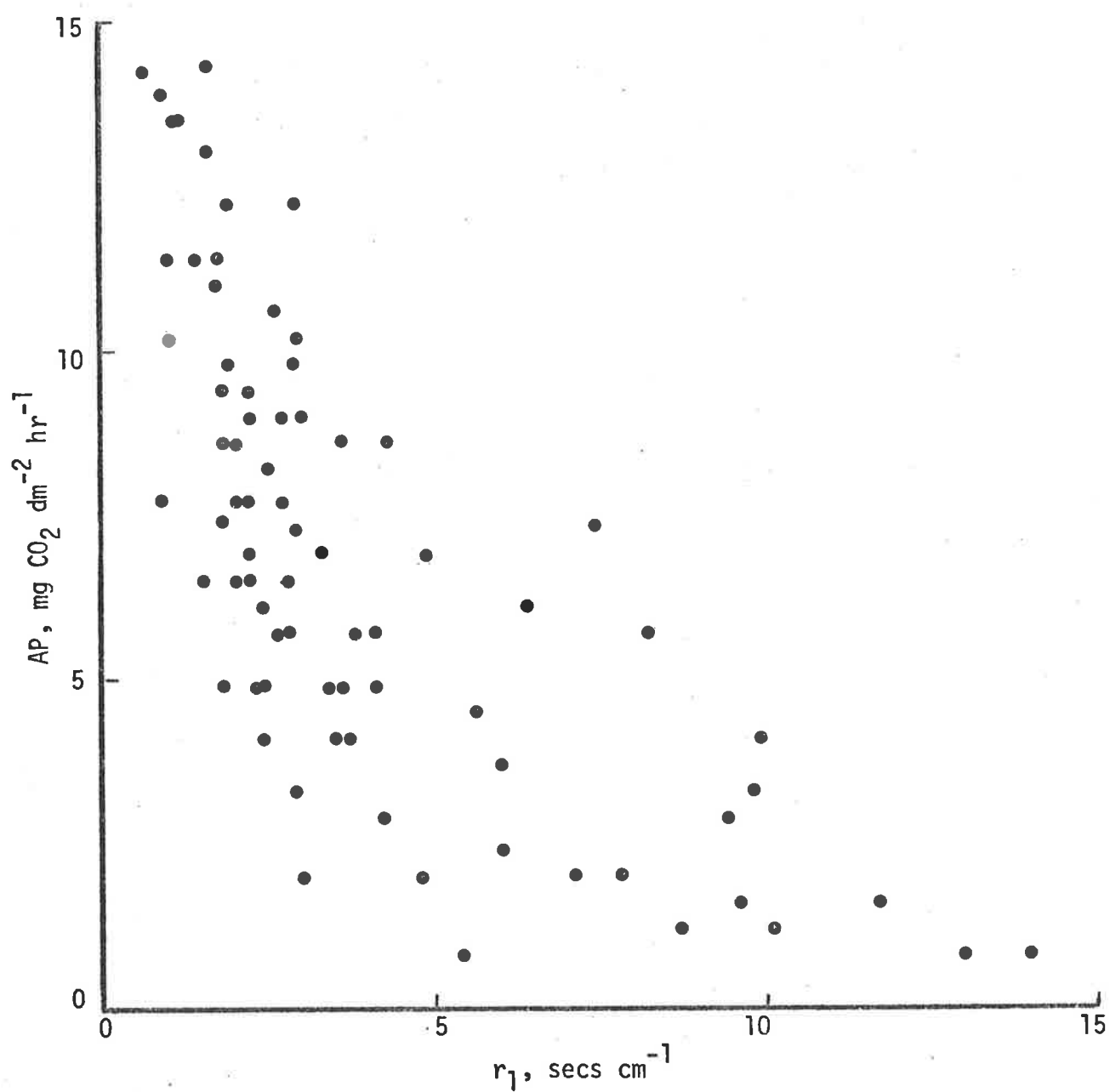


Figure 24. Relationship between rate of apparent photosynthesis and leaf diffusion resistance.

All the data from this experiment were used to establish the relationship between photosynthesis and leaf resistance in mature leaves of Valencia orange seedlings. The results (Figure 24) agree with the data of Kriedemann (1971) and his conclusion that the rate of CO_2 assimilation in orange leaves could be limited by a resistance over and above that encountered by water vapour escaping from the leaf. This is supported by the effects of GA_3 applied to the shoot and of ringing below all the leaves on MAP and r_1 discussed above.

2.3.5. The relationship between shoot growth and carbohydrate supply

Introduction: This experiment was designed to examine various aspects of the relationship between shoot growth and carbohydrate supply. The young shoot is a strong sink for current assimilate which is translocated from nearby leaves as sucrose (Kadoya and Tanaka, 1972; Kriedemann, 1969a). Nevertheless the turnover of ^{14}C labelled assimilate, stored before new shoot growth started, is slow (Kriedemann, 1969b). This suggests that the assimilate necessary for new shoot growth is derived mainly from current photosynthesis, in which case the rate of photosynthesis of the mature leaves should increase with the commencement of new shoot growth in their vicinity.

By reducing the mature leaf area greater demands must be made on the principal source of carbohydrate as a new shoot begins to grow; this should provide an opportunity to identify this source more clearly. In an earlier experiment, reducing the mature leaf area by one half reduced new shoot length (Figure 17) possibly due to an inadequate supply of carbohydrate. The treatment was repeated in this experiment to identify the main source of carbohydrate used by the young shoot and whether the reduction in growth was due to limited assimilate supply.

Gibberellic acid applied to the young shoot increased its growth and this was further promoted by exogenous sucrose (Figure 15); this indicates that endogenous levels of assimilate may limit the expression of the GA_3 promotion of shoot growth. The effects of GA_3 on the potential rate of assimilation and on the level of assimilates were therefore examined.

Shoot growth was reduced by 8 hour photoperiods (section 2.3.1.) which necessarily reduces the duration of photosynthesis. It is possible that the reduction in shoot growth may have been due mainly to an insufficiency of assimilate; therefore the effects of 8 hour photoperiod on photosynthesis and on carbohydrate in the plant were also investigated.

The results of the previous experiment indicated that ringing reduced shoot growth by reducing the demand for assimilates rather than by reducing photosynthesis. A ringing treatment was included to test this hypothesis.

Methods: Valencia orange seedlings, $4\frac{1}{2}$ months old, and grown as described in the previous experiment were divided into 5 replicates according to their size; all of the plants in any one replicate were decapitated above a constant number of leaves, but the number of leaves remaining per plant varied between 11 and 16 in different replicates.

The following treatments were applied seven days after decapitation, when the terminal axillary buds had burst:

Treatment	5 μ l applied to shoot	Manipulative treatment
1	10^{-3} M GA ₃	-
2	Ethanol	8 hour photoperiod
3	Ethanol	Ringed below all leaves: 2 mm ring of bark removed
4	Ethanol	Every other leaf removed (reduced leaf area)
5	Ethanol	- (Control)

The GA₃ solution was made up in ethanol, and all solutions contained 0.05% Tween 20. The experiment was carried out in growth cabinets at 25°C. Illuminance at plant height was 27000 lux, with a Photosynthetic Photon Flux Density (Shibles, 1976) of 410 μ E m⁻² sec⁻¹, supplied by a combination of fluorescent and incandescent sources. The photoperiod was 16 hours unless otherwise stated.

Shoot length and leaf number of the young shoot, MAP of the subtending mature leaf and 80% ethanol-soluble carbohydrate and starch in the mature leaves were measured twice per week. At the end of the experiment the plants were harvested and the following measured :

Sample (ca 40 mg fresh wt) boiled in 15 mls 80% ETOH for 10 mins.
Cooled, volume restored with 80% ETOH
Stored overnight at room temperature

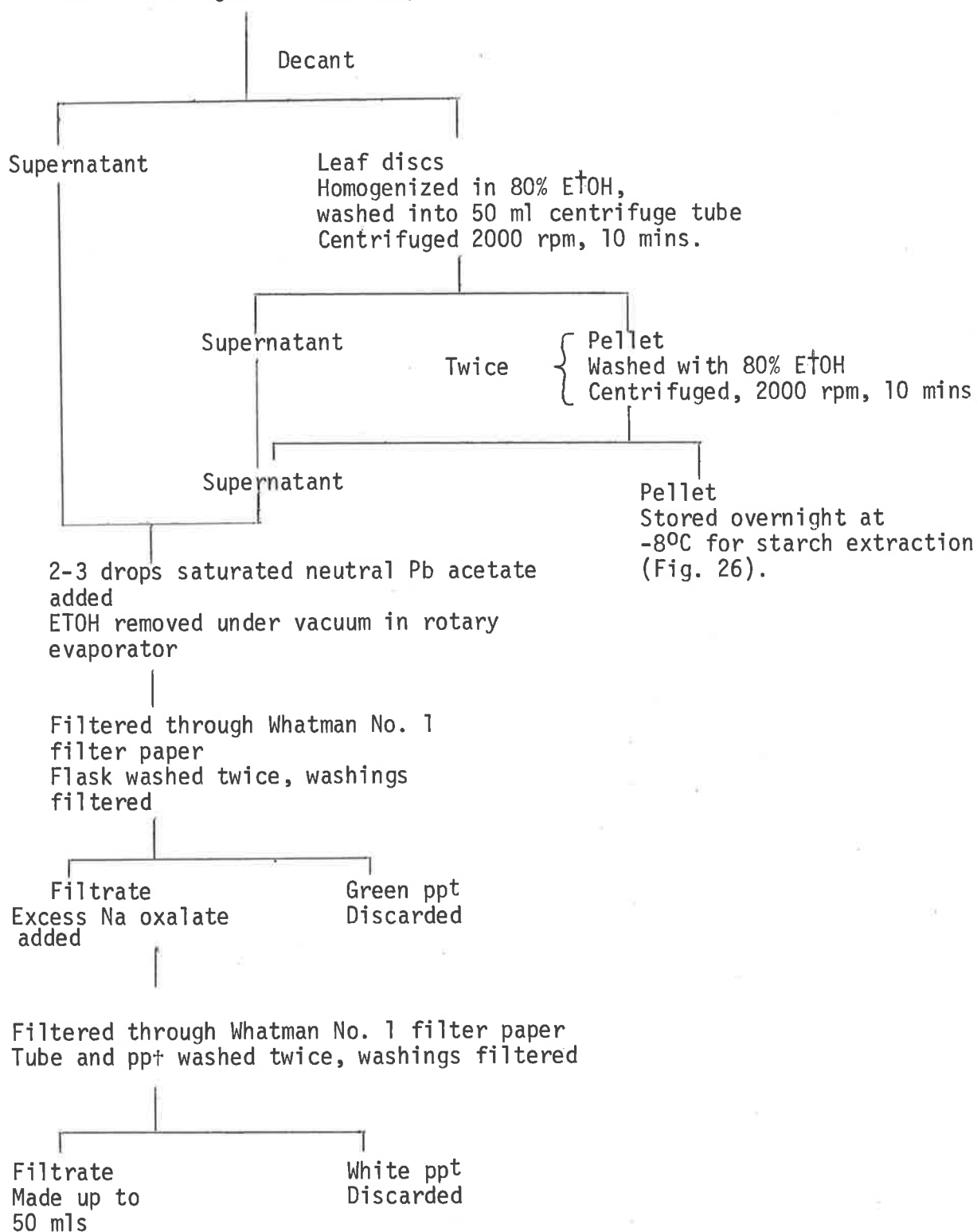


Figure 25. Flow chart for extraction of ethanol-soluble carbohydrate.

2 mls H_2O added to frozen pellet (Fig. 25) and tube loosely covered
Boiled for 15 mins.
Cooled in tap water.

5 mls $HClO_4$ added, left for exactly 10 mins.

Diluted to 10 mls with H_2O and mixed
Centrifuged, 2000 rpm, 5 mins

5 mls supernatant pipetted into 15 ml centrifuge tube containing
1 ml I/KI reagent and 2.5 mls 20% aqueous NaCl
Stirred, allowed to stand for ca 30 mins
Centrifuged, 2500 rpm, 10 mins

Supernatant
Discarded

Pellet (black)
2.5 mls alcoholic NaCl added, stirred
Centrifuge, 2500 rpm, 10 mins.

Supernatant
Discarded

Pellet (black)
1 ml alcoholic NaOH added, stirred
Centrifuge, 2000 rpm, 5 mins.

Pellet (white)

Supernatant
Discarded

2 mls 0.5N aqueous NaCl added
Heated in boiling water bath for
exactly 3 mins, with stirring

Immediately diluted with H_2O
at room temperature.
Made up to 25 mls.

Figure 26. Flow chart for extraction of starch.

dry weight of the new shoots, of the old shoots and of the roots, number of leaf primordia in the apical bud, 80% ethanol-soluble carbohydrate and starch in the new shoot, old shoot and roots.

The conditions and equipment for measuring MAP were the same as described earlier (section 2.3.3. Methods). Measurements were made on all the plants of one replicate before proceeding to another replicate so although the whole process lasted for a day, all the plants within each replicate were measured within two hours. The sequence of replicates and of plants within each replicate was chosen at random each day. Therefore any diurnal variation in MAP was randomly distributed throughout the experiment. MAP was measured in four of the five replicates.

Leaf discs (4 mm diameter) for ethanol-soluble carbohydrate and starch determinations were taken from all the mature leaves, except the leaf for MAP measurements, beginning at 3 pm (i.e. after exposure to 7 hours light) on each occasion. Similar precautions as above were taken to ensure that any differences due to time of sampling were randomized within and between replicates. Starch and 80% ethanol-soluble carbohydrate were extracted as described in Figures 25 and 26. The following reagents were used:

Perchloric acid (HClO_4): 429 gms 72% HClO_4 were weighed into a 2 litre conical flask and made up to around 450 ml by slowly adding distilled water. During this process

the flask was cooled by swirling under a running tap. The solution was made up to 500 ml after it had cooled.

I/KI reagent : 7.5 gms resublimed I_2 and 7.5 gms KI were finely ground in a mortar and a few ml of distilled water added a little at a time. The concentrated solution was decanted into a measuring cylinder leaving a residue in the mortar and the process repeated. After all of the residue had dissolved the solution was made up to 250 ml with distilled water and filtered through Whatman No 1 filter paper with suction.

Alcoholic NaCl: To 350 ml 99% ethanol was added 80 ml distilled water and 50 ml 20% aqueous NaCl. The solution was made up to 500 ml with distilled water.

Alcoholic NaOH: To 350 ml 99% ethanol was added 100ml distilled water and 25 ml 5N NaOH. The solution was made up to 500 ml with distilled water and filtered through Whatman No. 1 filter paper with suction.

Soluble carbohydrate and starch were estimated colorimetrically by anthrone which measures hexoses, pentoses and the sugars of stable glycosides (Yemm and Willis, 1954b). Absorbance at 620 m μ was measured on a Brinkman PC 600 colorimeter.

After harvest the plants were dissected into new shoot, old shoot and roots and dried in a forced-draught oven at 80°C overnight to obtain dry weight.

Table 10. Effect of GA₃ and manipulative treatments on the dry weight of the plant

Mean dry weight in grammes				
	New shoot	Old shoot	Root	Total
GA ₃ to bud	0.75	1.12	0.69	2.56
8 hr photoperiod	0.35	0.98	0.53	1.86
Ringed	0.19	1.19	0.42	1.81
Reduced leaf area	0.58	0.94	0.66	2.18
Control	0.50	0.95	0.65	2.10
p= 0.05	0.23	-	0.12	0.38
LSD				
p= 0.01	0.32	-	0.16	0.53

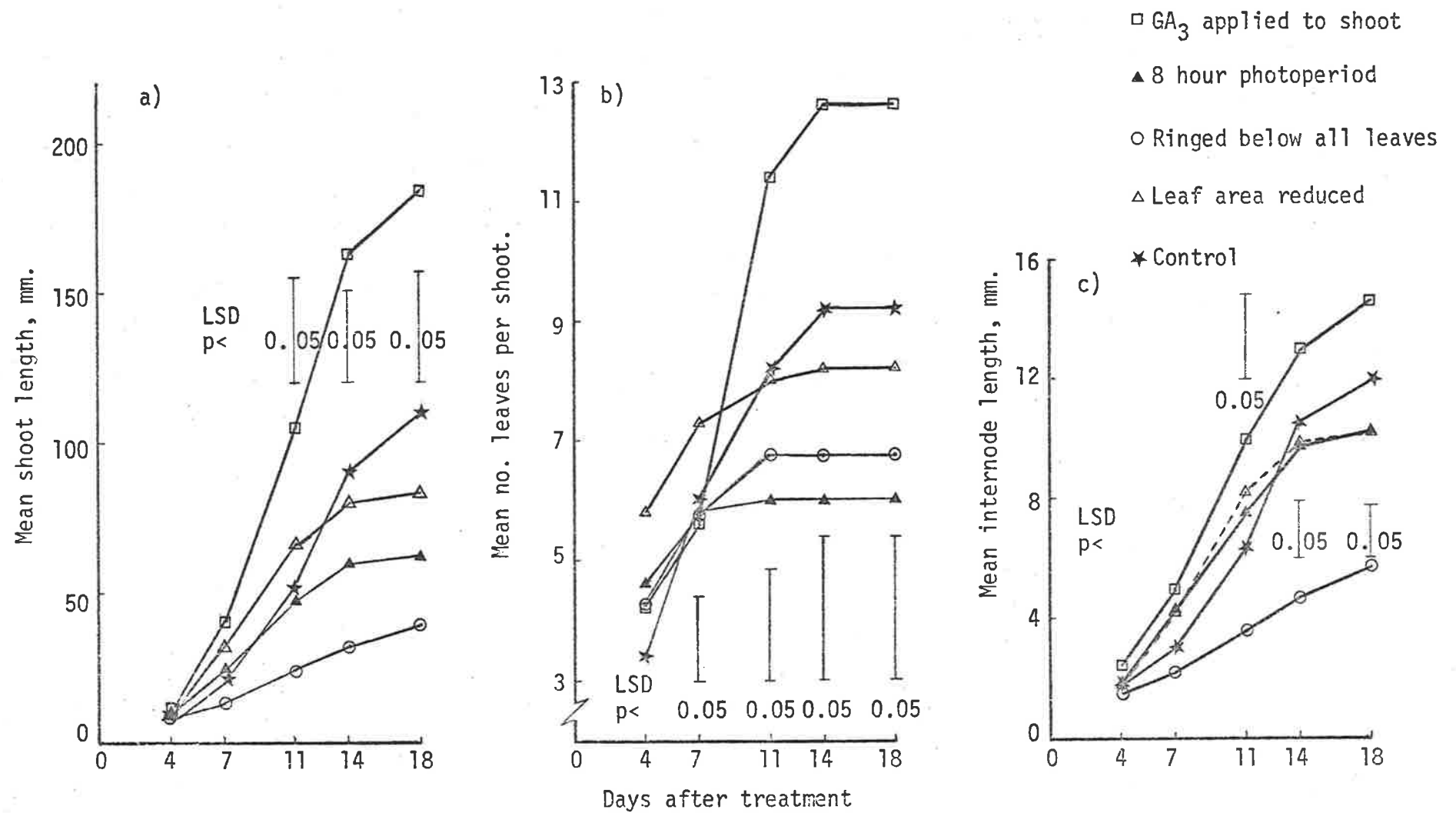


Figure 27. Effect of GA_3 and manipulative treatments on shoot growth.

Results and discussion: The well-established GA_3 promotion of shoot growth was again apparent (Figure 27), as was the reduction in shoot length and internode length induced by ringing (Figures 27a and 27c). However in this experiment ringing also reduced leaf number (Figure 27b). The short photoperiod reduced shoot length due entirely to a reduction in leaf number. In contrast to the other treatments, reducing the leaf area had no significant effect on shoot length, leaf number or internode length compared to the control.

These differences were reflected in the dry weights of the new shoots (Table 10). Gibberellin significantly increased new shoot dry weight and ringing significantly reduced it. Short photoperiods tended to reduce new shoot dry weight compared to the control whereas reduced leaf area slightly increased it.

g J The rate of maximum apparent photosynthesis in the mature leaves increased after decapitation (day -7, Figure 28a) and was greatest at four days after treatment, i.e. during a period of rapid leaf appearance (Figure 27b) and just before the commencement of rapid stem elongation (Figure 27a). This suggests that the photosynthetic mechanism in the mature leaf became potentially more productive with the initiation of new shoot growth and that photosynthetic capacity continued to increase during the early stages of young shoot growth. By the time the lowest leaves on the young shoot had

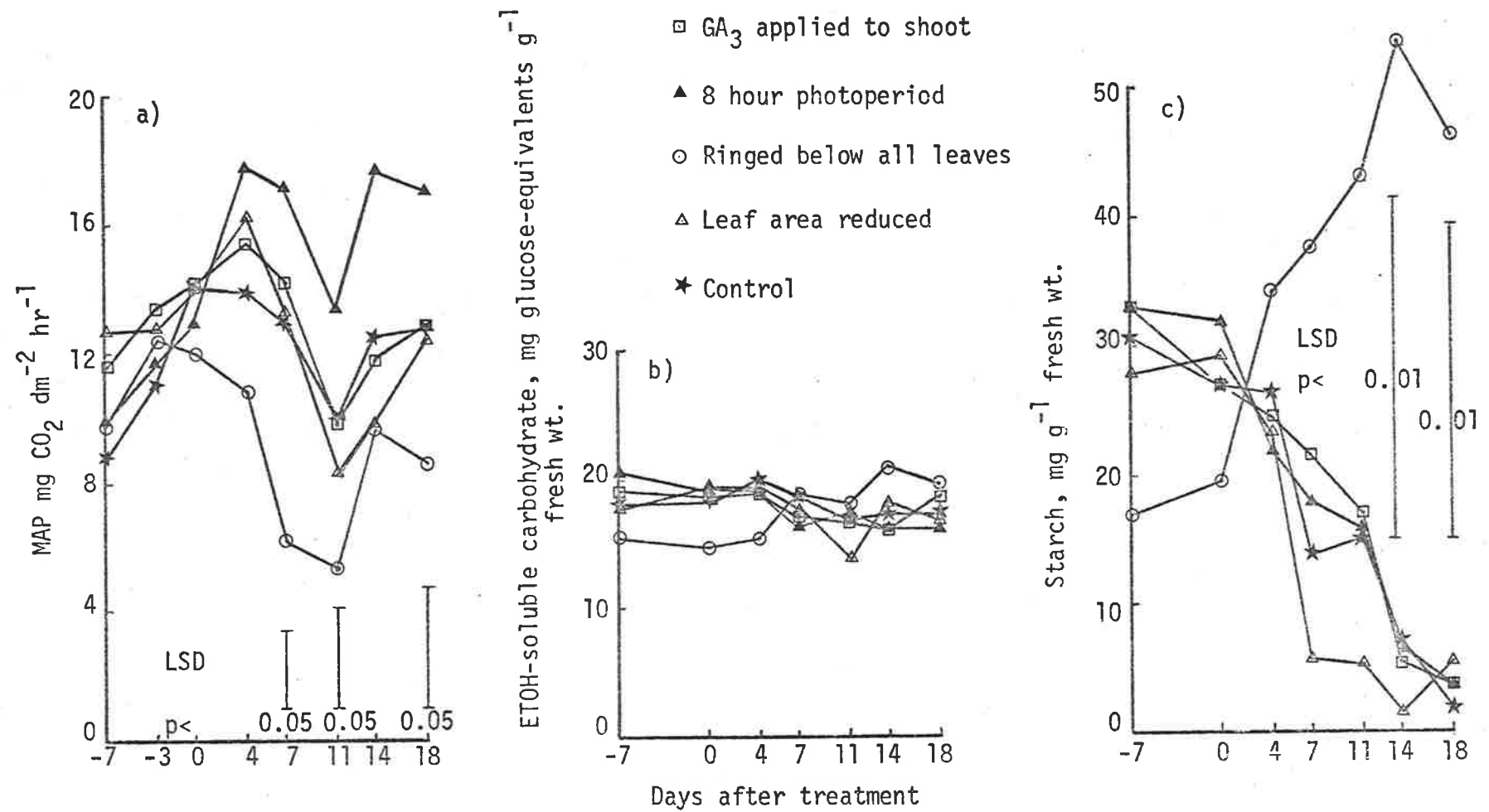


Figure 28. Effect of GA_3 and manipulative treatments on the rate of a) maximum apparent photosynthesis and the levels of b) ethanol-soluble carbohydrate and c) starch in the mature leaves.

expanded however (stage 5 of shoot growth, by day 7) the photosynthetic capacity of the mature leaves had fallen, i.e. photosynthetic capacity increased in response to the appearance of a sink but as sink demand fell, due probably to supply from other sources, photosynthetic capacity of the mature leaves declined.

The trough in MAP at day 11 seems to be a real phenomenon: all replicates showed this decline in MAP even though the treatments were replicated in time and therefore day 11 in different replicates was at different dates; i.e. the reduction in MAP was not due to some malfunction of the equipment or some unfavourable environmental factor on a particular day but appeared to be a characteristic of the mature leaves in this experiment. No explanation can be given for this feature.

Ringling reduced MAP and 8 hour photoperiods increased it whereas both GA_3 applied to the young shoot and a reduction of the mature leaf area had no effect on MAP (Figure 28a).

The quantity of starch in the mature leaf fell drastically following new shoot emergence (Figure 28c) indicating that starch was an important source of carbohydrate for new shoot growth. Contrary to the trend in all other treatments, starch accumulated in the ringed plants markedly after shoot emergence. Where the mature leaf area had been reduced by one half, the starch content of the remaining leaves tended to be less than the control

Table 11. The level of carbohydrates in the plant at the
end of shoot growth (day 18)

	EtOH-soluble			Starch		
	carbohydrate					
	mg g ⁻¹	dry wt		mg g ⁻¹	dry wt	
	New Shoot	Old Stem	Root	New Shoot	Old Stem	Root
GA ₃ to shoot	44.8	24.4	35.4	8.2	20.4	17.4
8 hr photoperiod	37.8	24.2	32.2	6.2	17.2	20.8
Ringed	62.9	35.8	21.3	62.5	72.5	18.3
Reduced leaf area	50.8	27.3	43.4	15.7	23.2	22.4
Control	48.6	21.3	33.7	9.4	28.9	21.6
p = 0.05	-	7.0	9.9	24.2	13.4	-
LSD	N S			N S		
p = 0.01	-	9.6	12.8	33.4	18.6	-

Fresh:dry weight ratios of the control were:

New Shoot 4.8 : 1
Old Shoot 3.5 : 1
Root 5.0 : 1

during the period of rapid shoot growth.

The level of ethanol-soluble carbohydrate in the mature leaves remained remarkably constant throughout the experiment (Figure 28b). This was more striking in view of the large changes with time in MAP and starch content; it indicates that the quantity of ethanol-soluble carbohydrate in the leaf is closely controlled. However the rate of turnover is probably considerable. There was no apparent effect of treatment on ethanol-soluble carbohydrate except a slight increase from day 7 in the ringed plants, further indicating that the levels are tightly controlled.

There were wide differences in the concentrations of ethanol-soluble carbohydrate in various parts of the plant at the end of the experiment (Table 11) : of the parts analysed, new shoots contained the highest levels and old stems the lowest. In contrast, old stems tended to have higher concentrations of starch than roots, while new shoots contained very low levels (Table 11). Ringing produced an accumulation of starch and ethanol-soluble carbohydrate in the stem above the ring and in the new shoot and reduced ethanol-soluble carbohydrate, but had no effect on starch, in the root. Gibberellin had no effect on the distribution or level of starch or ethanol-soluble carbohydrate in the plant at the end of the experiment. Reducing the mature leaf area also had no effect on starch levels throughout the plant but tended

to increase the levels of ethanol-soluble carbohydrate, particularly in the root. The new shoots grown in short photoperiods contained the lowest concentration of ethanol-soluble carbohydrate and starch and the old stem had appreciably less starch than the control. However short photoperiods had no effect on either ethanol-soluble carbohydrate or starch in the root.

The results clarify a number of points concerning shoot growth in citrus. It is apparent that carbohydrate for young shoot growth can be derived from starch in the mature leaves (Figure 28c). The low levels of starch in the old stem and in the root of the control at the end of shoot growth (8 and 4 mg g⁻¹ fresh weight respectively at day 18) indicate that new shoot growth depleted starch reserves throughout the plant. This is not surprising considering that the new shoot comprised 25% of the total dry weight of the plant at day 18 (Control, Table 10). However, as the starch content of the mature stem and the root was not determined before the commencement of new shoot growth it cannot be categorically stated that the starch levels in these tissues did fall as the young shoot grew.

The evidence from the treatment in which leaf area was reduced indicates that current photosynthate is an important source of carbohydrate for the new shoot and that the capacity of the mature leaves to photosynthesise is well in excess of normal requirements.

Reducing the mature leaf area had no significant effect on shoot growth compared to the control (Figure 27 and Table 10). Moreover, the quantity of mature leaf tissue removed for chemical analysis throughout the experiment was of the same order in this treatment as in all others. Consequently the mature leaf area in this treatment was reduced proportionately more as the experiment progressed. Nevertheless, there was no significant difference in MAP, ethanol-soluble carbohydrate or starch between this treatment and the control (Figure 28). Starch levels were lowered somewhat during the second week of growth, but less than would be expected if starch in the mature leaves was the only source of carbohydrate to the young shoot. However the decrease in starch levels in the second week (Figure 28c) could be interpreted as a depletion of the starch reserves in the remaining leaf tissue in response to new shoot growth. This depletion occurred one week earlier than in the control. Nevertheless, young shoot growth was not adversely affected, nor was there any difference in the photosynthetic capacity of the mature leaf (Figure 28a). Moreover, the levels of ethanol-soluble carbohydrate and starch throughout the plant at the end of shoot growth were no less than the controls.

There are three ways in which the growth of the new shoot on partially defoliated plants could have been maintained at normal levels : 1) Mature leaves normally photosynthesise well within their capacity to

do so, thus although MAP did not increase, the actual rate of carbon assimilation during the growth of the young shoot did. 2) The young shoot contributes significantly to its maintenance and growth by its own photosynthesis. If photosynthesis within the new shoot did substitute for inadequate supplies of assimilate from the mature tissue it suggests that new shoots in the control photosynthesised well below their capacity, i.e. new shoot growth is not limited by inadequate supplies of assimilate. 3) The dry matter of the new shoot was derived from material other than ethanol-soluble carbohydrate and starch in the mature tissues. This alternative appears unlikely, especially in the light of the results of Kadoya and Tanaka (1972) and Kriedemann (1969a) referred to earlier.

A comparison of the reduced leaf area treatment with 8 hour photoperiod should yield further information on the relationship between new shoot growth and the supply of assimilates. Unfortunately, in this experiment, the effects due to the shorter duration of photosynthesis cannot be separated from the effects of photoperiod per se as the 8 hour daylength was not lengthened by low intensity light nor was the dark period interrupted by light. Nevertheless the results do shed some light on the role of assimilates in shoot growth of citrus.

Table 12. The number of leaf primordia in the aborting
apical bud at the end of shoot elongation

Treatment	Mean no. leaf primordia
GA ₃ to shoot	6.6
8 hr photoperiod	5.4
Ringed	5.8
Reduced leaf area	5.4
Control	5.4

Difference significant at $p < 0.04$, determined
by Kruskal Wallis non-parametric one way
analysis of variance.

It was hypothesised that the reduced shoot growth under 8 hour photoperiod may be a consequence of inadequate levels of assimilate due to the short duration of photosynthesis. In this experiment new shoot dry weight of plants grown under 8 hour photoperiods was slightly reduced (Table 10). It appeared that new shoot growth under 8 hour photoperiods was sustained by higher rates of photosynthesis as this was the only treatment which significantly increased MAP (Figure 28a). As in the control, starch in the mature leaves of this treatment was depleted at day 18 (Figure 28c) at which time the new shoot contained slightly lower levels of ethanol-soluble carbohydrate and starch than the control (Table 11), but there was significantly less starch in the old stem. The results suggest that the reduced shoot growth under 8 hour photoperiods may in part be a consequence of inadequate availability of assimilates due to the shorter duration of photosynthesis and that under these conditions young shoot growth induces greater photosynthetic capacity in the mature leaf.

Whereas short photoperiods reduced leaf number GA_3 applied to the young shoot significantly increased the number of leaves which expanded (Figure 27b). It also increased the number of leaf primordia in the aborting apical bud (Table 12). Thus it is conclusively established that GA_3 applied to the young shoot increased the production of leaf initials, presumably by inducing

cell division in the apex of the growing shoot.

All parameters of shoot growth were increased by an application of GA_3 to the bud (Figure 27, Table 10 and 12). However this was not matched by an increase in MAP (Figure 28a) nor a more rapid depletion of starch in the mature leaves (Figure 28c) or stem (Table 11); this suggests that either the rate of actual CO_2 assimilation in the mature leaves and/or the young shoot increased or that GA_3 increased leaf area as well as leaf number of the young shoot thereby increasing the amount of CO_2 fixed. The levels of carbohydrates in the young shoot of the GA_3 treated plants were similar to those of the control (Table 11), and, as the evidence from the reduced leaf area treatment indicated, new shoot growth in the control was not limited by inadequate supplies of assimilate. Therefore it appears that GA_3 -induced promotion of shoot growth is not limited by inadequate supplies of assimilate.

In contrast to the effect of GA_3 , ringing reduced almost every parameter of shoot growth (Figure 27) especially shoot dry weight (Table 10) and resulted in a highly significant accumulation of starch in the mature leaves (Figure 28c), stem (Table 11) and new shoot (Table 11). Ringing also increased the level of ethanol-soluble carbohydrate in all organs above the ring, particularly in the old stem (Table 11, Figure 28b). The increase in starch levels in the mature leaf coincided with a decrease

in MAP (Figures 28a and 28c) and strongly suggests end-product inhibition of photosynthesis (Lenz, 1975).

The results of the previous experiment indicated that ringing reduced shoot growth which therefore reduced the demand for assimilates. The data in Figure 27 together with the high levels of ethanol-soluble carbohydrate and starch in the mature leaves (Table 11) confirm this.

Ringing significantly reduced root dry weight (Table 10) and ethanol-soluble carbohydrate in the root (Table 11) but had no effect on starch in the root (Table 11). This suggests that ringing may have retarded root growth by inhibiting the breakdown of starch in the roots as well as isolating the roots from carbohydrate supplied by the shoot.

The general deductions from this experiment are :

- 1) The normal sources of carbohydrates for new shoot growth are starch stored in the mature tissue and current photosynthesis in the mature leaves and in the new tissues themselves.

- 2) Both young and old tissue have the capacity to photosynthesise in excess of their normal requirements.

- 3) Growth of the new shoot is not limited by unavailability of carbohydrate nor do increased levels of carbohydrate either in the new shoot or available to

it increase its growth, i.e. within reasonable limits carbohydrate supply does not influence shoot growth.

2.3.6. An analysis of the ringing effect

Introduction: Ringing below all the leaves reduced shoot growth and produced an accumulation of ethanol-soluble carbohydrate and starch in all organs above the ring but reduced ethanol-soluble carbohydrate in the root while having no effect on starch in the root (Table 11). Moreover ringing appeared to reduce root growth (Table 10). These data indicated that root health or root growth was adversely affected. This in turn could be detrimental to the absorption and/or translocation of mineral elements and water or to the production by, and/or translocation from the roots of organic substances such as amino acids, plant hormones (e.g. cytokinin), vitamins, etc.

To test these possibilities the amount of mature shoot in confluence with the root was altered, by varying the position of the ring, and cytokinin was applied to the mature shoot above the ring.

Methods: Decapitated Valencia orange seedlings growing in Osmocote-enriched sawdust in 10 cm(dia.) pots were used. The seedlings were six months old at the time of the experiment and had been grown in a growth room under 16 hour photoperiod at 26/21°C day/night temperatures.

There were 5 single-plant replicates of the following treatments, arranged in randomized complete

blocks:

- Low ring - ringed below all leaves
- Mid ring - ringed midway along stem, i.e.
 below half the number of leaves
- Mid ring + BAP - ringed below half the leaves,
 500 μ l 10^{-4} M BAP wick-fed above
 the ring
- High ring - ringed above all but 3 leaves,
 i.e. below the third leaf from
 the tip
- Control - Not ringed

In addition one plant per replicate was selected at random for dissection and analysis at day 0 (the time of application of the treatments).

In ringing a complete ring of bark 2 mm wide was removed from around the stem. The wick in the mid ring + BAP treatment was inserted about 1 cm above the ring between the bark and the wood using a sewing needle.

The plants within each replicate were decapitated above a constant number of leaves which varied from 15 to 20 between replicates.

The ringing treatments were applied after the uppermost apical bud had burst, about 12 days after decapitation.

Almost invariably the axillary bud below the ring burst during the experiment; the young shoot was removed as soon as it appeared.

The following parameters were measured twice per week throughout the duration of the experiment:

Shoot length of new shoot

Number of leaves of new shoot longer than 5 mm

Ethanol-soluble carbohydrate and starch in the mature leaves above the ring and similarly below the ring

After harvest at day 18 the following were measured:

Dry weight of : new shoot
old stem
root

Ethanol-soluble carbohydrate and starch in :
new shoot
old shoot
old stem, above ring
old stem, below ring
root

During harvesting it was noticed that new shoots on the ringed plants were lighter coloured and had smaller leaves than the unringed control, and it was decided to measure leaf area of the new shoots. However data from only two replicates could be obtained.

The methods of extraction and determination of starch and ethanol-soluble carbohydrate were the same as described in the previous experiment.

Leaf area was measured by an electronic planimeter (Paton "Electroplan").

Table 13. Effects of ringing at different positions on the growth of the new shoot*

	New Shoot					Old shoot dry wt. g	Root dry wt. g
	Shoot length mm	Leaf no.	Internode length mm	Leaf area ^{**} cm ²	Dry wt. g		
Low ring	125.6	9.8	12.6	71	0.51	3.38	1.26
Mid ring	159.8	11.0	14.6	57	0.66	2.25	1.47
Mid ring + BAP	170.2	13.4	12.7	55	0.68	2.58	1.43
High ring	111.8	9.0	12.2	33	0.37	2.99	1.64
Control-unringed	173.6	10.8	16.2	154	1.14	2.24	1.39
p= 0.05	35.9	2.2	1.7	79	0.25	-	-
LSD						NS	NS
p= 0.01	49.4	3.0	2.4	106 (p= 0.02)	0.35	-	-

* All figures are means of values taken at harvest, 18 days after treatment. At day 0 mean dry weights were:

Old shoot - 2.58 g
Root - 1.47 g

** Mean of two replicates

Table 14. Levels of ethanol-soluble carbohydrate and starch in various parts of the
plant at day 18

	EtOH-soluble carbohydrate mg glucose equiv. g ⁻¹ dry wt.				Starch mg g ⁻¹ dry wt.			
	New Shoot	Old stem above ring	below ring	Root	New shoot	Old Stem above ring	below ring	Root
Low ring	94.3	42.1	21.2	26.9	54.8	69.1	13.2	18.1
Mid ring	88.1	46.4	30.1	56.0	35.7	80.6	41.6	29.5
Mid ring + BAP	82.0	48.7	28.7	50.2	38.5	63.6	38.1	25.2
High ring	77.2	51.7	30.5	56.3	41.2	88.2	49.4	34.9
Control- unringed	77.6		38.0	56.1	21.4		44.8	20.9
Day 0*	-		30.2	-	-		41.4	-
p= 0.05	10.0		7.1	15.6	10.5		10.7	7.8
LSD								
p= 0.01	-		9.5	21.5	14.5		14.4	10.7

* Mean of values from plants harvested at day 0

Results and discussion: New shoot length was reduced by both the high and low rings (Table 13) but, although length was reduced, the effect of the mid ring was not statistically significant.

Ringling at any position had no effect on leaf number of the new shoot but supplying BAP above the mid ring significantly increased leaf number (Table 13).

In contrast, both high and low ringling treatments very significantly reduced the mean internode length of the young shoot, but again the mid ring only produced a non-significant reduction in internode length. However, BAP combined with the mid ring very significantly reduced internode length (Table 13).

Although the leaf areas of the young shoot of only two replicates were measured there were highly significant differences due to treatment. All ringling treatments more than halved the new leaf area compared to the control; the high ring caused the greatest reduction and the low ring caused the least. Supplying BAP above the ring had no effect on the leaf area of the mid-ring treatment.

Dry weight of the new shoot was very significantly reduced by every ringling treatment (Table 14). The high ring produced the greatest reduction and the mid ring with or without BAP the least.

In summary, almost every parameter of new shoot growth was reduced by a ring at any position. The

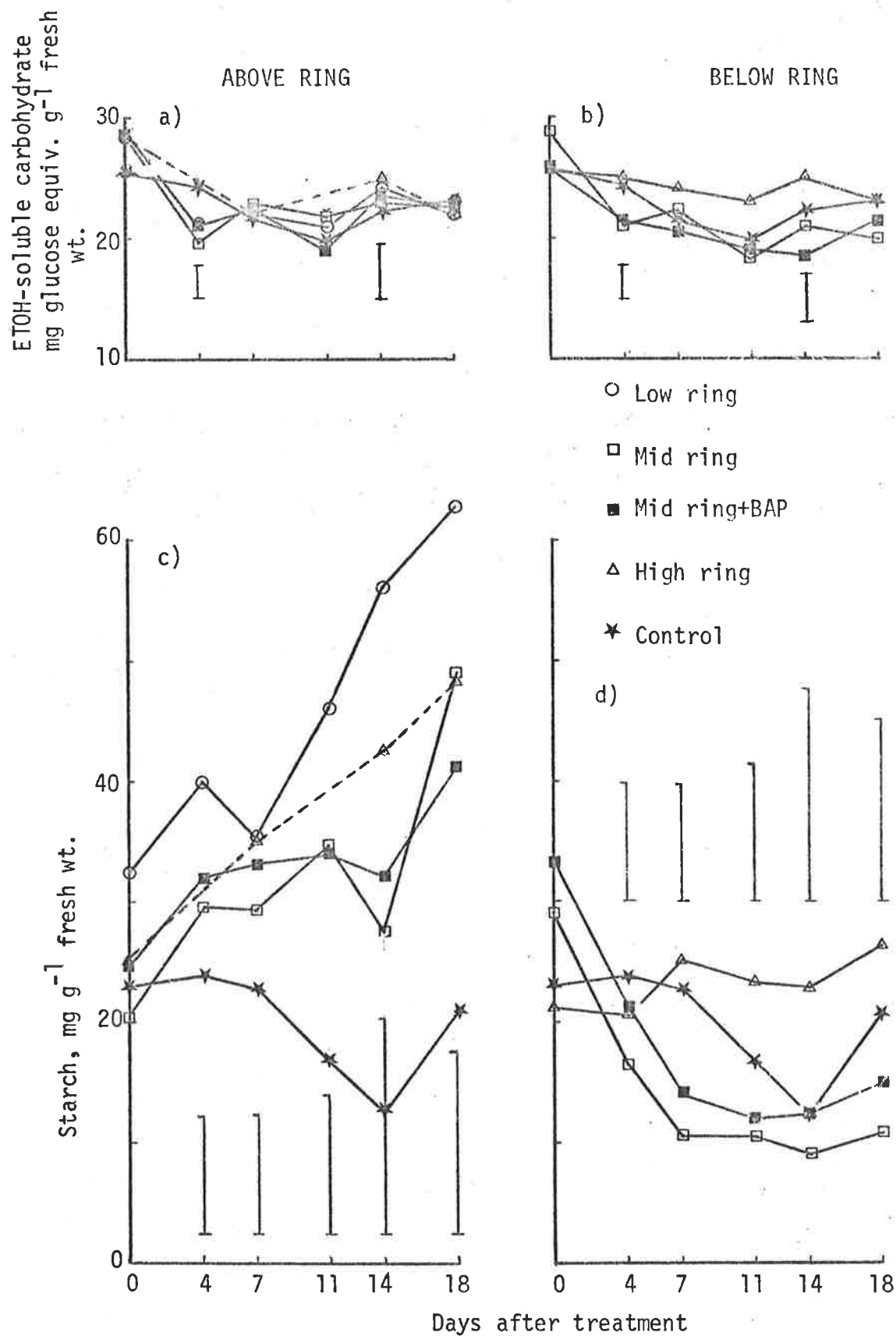


Figure 29. Effect of ringing at different positions on the levels of starch and ethanol-soluble carbohydrate in the mature leaves during new shoot growth. Samples of the control were taken from leaves along the entire mature shoot. Samples from above the ring in the high ring treatment were taken on alternate dates. Vertical bar = LSD $p < 0.05$.

application of cytokinin above a ring at the middle of the stem considerably increased leaf proliferation but had no effect on the other parameters of shoot growth. The most significant effect of ringing was the reduction in dry weight of the new shoot. There was no significant effect of ringing on the dry weight of the old shoot or of the root (Table 14).

As in the previous experiment, the concentrations of ethanol-soluble carbohydrate in the mature leaves remain relatively constant during the growth of the young shoot (Figures 29a and 29b). Apart from a slight increase in the leaves below the ring of the high ring treatment there was no effect of ringing.

In contrast the levels of starch in the mature leaves demonstrated very significant effects of ringing. In the mature leaves above the ring the starch content increased as the new shoot grew in all ringed treatments (Figure 29c). The accumulation of starch was greatest in the low ring treatment.

In the mature leaves below the ring starch levels of the mid ring treatments fell whereas in the high ring treatment the level of starch remained almost constant (Figure 29d). The starch content of the mature leaves of the control remained constant during the first week and fell during the second week.

All ringing treatments caused an accumulation of starch and ethanol-soluble carbohydrate in the new

Table 15. Summary of the effects of position of the
ring compared with unringed control

Measurement	Low ring	Mid ring	High ring
<u>Dimensions</u>			
Shoot length	-	-	--
Leaf number	-	0	-
Internode length	--	-	--
Leaf area	-	-	--
Dry wt. new shoot	--	--	--
Dry wt. old stem	+	0	+
Dry wt. root	-	0	+
<u>Carbohydrate during growth</u>			
Soluble: Above ring	0	0	0
Below ring	NA	0	0
Starch: Above ring	++	+	+
Below ring	NA	-	+
<u>Carbohydrates at harvest</u>			
Starch: New shoot	++	++	++
Above ring	++	++	++
Below ring	--	-	+
Root	-	+	++
Compared with control	(++ Large increase (-- Large decrease	+ Increase - Decrease	0 Nil effect NA Not applicable

shoot and old stem above the ring and a decrease in the old stem below the ring (Table 14).

The effects of ringing treatments on the various parameters measured are summarized in Table 15. The low ring reduced all dimensions of growth measured except dry weight of the old stem. It increased starch in all tissues above the ring and decreased it below.

The high ring had similar effects to the low ring with one difference : carbohydrates (especially starch) and dry weights were increased in tissues below the ring.

The mid ring differed in that leaf number and internode length were not reduced. The effects on root dry weight and starch were intermediate.

The addition of BAP to the mid ring had only one effect: leaf numbers per shoot were increased.

Though ringing had effects (depending on its position) on the levels of soluble carbohydrate and starch in different parts of the plant none of the effects suggest that the reduction in shoot growth was attributable to a limitation of carbohydrate supply. Reduced shoot growth in response to ringing is also not due to insufficient carbohydrate being supplied to the roots, as evidenced by the effect of the high ring treatment.

The results suggest that the ringing effect is due to interrupted phloem translocation (probably acropetally) of shoot growth factors; one of these could

be a cytokinin which influences number of leaves but not leaf area or internode length.

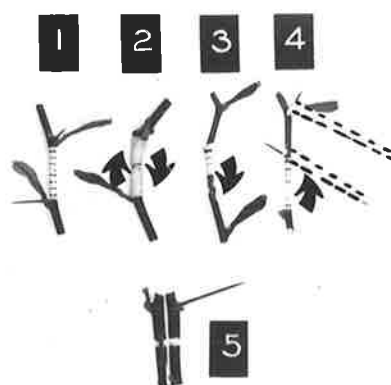
2.3.7. Attempts to overcome the effects of ringing:
Physical transposition of material above and
below the ring

Introduction: The foregoing results have suggested that the effect of ringing in reducing shoot growth may be due to the interruption of phloem translocation. In order to confirm that there is an interruption of the transfer of material through the bark which affects shoot growth, an experiment was carried out using transpositioning of bark by grafting.

Attempts were also made to extract substances by soaking pieces of bark in ethylenediaminetetra-acetic acid, disodium salt (EDTA) (which induces exudation from the phloem, King and Zeevaart, 1974) and wick-feeding this extract.

The effect of ringing could also be due to the effect of the wound per se rather than the interruption of the phloem. An incomplete ring treatment was used to test this hypothesis.

Methods: This experiment was carried out in a growth cabinet under 16 hour photoperiod at 25°C. Valencia seedlings, grown in John Innes compost enriched with Osmocote were ringed at the mid ring position 12 days after decapitation. The following treatments began three days later:



1 2 3 4 5

Figure 30. Ringed seedling.

The narrow bands across the exposed wood are rings of bark left at each time.

Inset: Internodes, showing different treatments:

- 1) Removal, ringed, control.
- 2) a, b interchange, grafted.
- 3) a to b, grafted
- 4) b to a EDTA. The tubes are outlined by dotted lines; the dark areas to the right are bark pieces in the bottom of the tubes. Two tubes are attached as the contents of the first had not been absorbed before the second was applied.
- 5) Incomplete ring, internode bisected to show both sides, one with ring alone and the other at left with a narrow bridge.

Arrows indicate direction of transposition.

Grafting:

Band of bark from above ring placed below ring (a to b) (Figure 30, 3)

Band of bark from below ring placed above ring (b to a)

Band of bark from above ring interchanged with band from below ring (a, b interchange) (Figure 30, 2)

Band of bark from above and below original ring removed and discarded (removal) (Figure 30, 1)

("Above" means distal to, "Below" means proximal to)

EDTA:

Four similar treatments as above except that the bands of bark were finely chopped and put into 10^{-4} M EDTA in 1 ml centrifuge tubes. The solutions (containing the diced bark) were wick-fed above or below the ring as appropriate (Figure 30, 4). Where the bands were discarded EDTA was wick-fed

Incomplete ring -- a 2 mm wide strip of bark was left bridging the ring (Figure 30, 5)

Control -- not ringed

Table 16. Effects of transposition of bark on shoot and root growth of ringed plants

Treatment	New shoot					Root dry wt. g
	Shoot length mm	Leaf number	Internode length mm	Shoot dry wt. g	Leaf area cm ²	
Not ringed	162	13	12.8	1.91	256	4.17
Incomplete ring	161	12	13.2	1.84	241	5.06
<u>Grafting</u>						
a to b	144	13	11.3	1.12	116	4.34
b to a	140	13	10.7	1.66	189	4.62
a, b inter-change	159	12	12.9	1.49	170	4.50
a and b removal	115	11	10.1	1.35	143	3.84
<u>EDTA +</u>						
a to b	140	12	11.6	1.23	137	3.57
b to a	142	12	11.7	1.34	148	4.21
a, b inter-change	101	11	9.6	1.07	125	4.76
a and b removal	125	12	9.7	1.30	126	4.12
LSD p= 0.05	NS	NS	NS	NS	90	NS

In the grafting and EDTA treatments, bands of bark were removed twice per week. The original ring and each band were 2 mm wide; bands were removed starting from immediately above and below the original ring and moving outwards (Figure 30).

In grafting the band of bark was held in position with insulating tape (Figure 30,2). Some of the bands united with the stem tissue at the new location (Figure 30,3).

The plants were 10½ months old at ringing. There were 5 replicates arranged in randomized blocks.

Eighteen days after the central ring was removed the plants were harvested and shoot length, leaf number, leaf area and dry weight of the new shoots and dry weight of the roots were measured.

Results and discussion: Leaf area was the only parameter significantly affected by treatment (Table 16).

All of the grafting and EDTA treatments reduced shoot growth, whereas the incomplete ring did not (Table 16). Thus the wound inflicted during ringing is itself unlikely to be the cause of inhibited shoot growth.

In both grafting and EDTA treatments, transposing segments from below to above the ring tended to promote leaf area and shoot dry weight compared to the "a and b removed" treatment; the effect was greater in

the grafting than in the EDTA treatment. The "a, b interchange" also tended to promote leaf area and shoot dry weight in the grafting but not in the EDTA treatments.

The evidence is equivocal but the data suggests that phloem translocation is essential for normal shoot growth and that acropetal translocation is more important than basipetal translocation.

2.3.8. Attempts to overcome the effects of ringing:
The application of growth substances and
other compounds

Introduction: It has been concluded that the effect of ringing is due to the interruption of the translocation through the phloem of regulating substances. Cytokinin and gibberellin by themselves are unlikely to be the responsible agents since they restore only part of the normal shoot growth. Hence it was considered necessary to test mixtures of growth regulators and apply them at different sites.

Amino acids and amides synthesised in the root are translocated in the phloem predominantly as asparagine/aspartate and glutamine/glutamate (Ziegler, 1975). The reduction in shoot growth may be attributable to a deficiency of amino acids due to ringing; to test this hypothesis asparagine was supplied above the ring.

Ringing may also interrupt the translocation of factors from the young shoot to the root and this may in turn reduce shoot growth. By allowing an axillary shoot to grow below the ring, factors from the shoot can be supplied to the root.

Alternatively, it is conceivable that carbohydrate could have been limiting growth despite the fact that starch accumulated in tissues above a ring. For example, if starch degradation was inhibited as a result of ringing, the supply of an essential carbohydrate pool

may have decreased. The starch content of photosynthesising tissue can be manipulated by altering the supply of inorganic phosphate (P_i) available to the system (Chen-She *et al.*, 1975; Herold *et al.*, 1976; Jensen, 1976) and this hypothesis was explored by supplying P_i above a ring.

Methods: Decapitated Valencia orange seedlings, 10½ months old, were ringed at the mid ring position 12 days after decapitation and the following treatments applied:

NAA	above the ring
GA ₃	above the ring
NAA + GA ₃ + BAP	above the ring
H ₂ O	above the ring
NAA	below the ring
GA ₃	below the ring
NAA + GA ₃ + BAP	below the ring
H ₂ O	below the ring
H ₂ O	unringed
GA ₃	unringed
KH ₂ PO ₄	above the ring
Asparagine	above the ring
H ₂ O	above the ring and young shoot allowed to grow below the ring

Table 17. Effect of growth substances and other compounds
on shoot and root growth of ringed plants

Treatment	New Shoot					
	Shoot length mm	Leaf no.	Inter- node length mm	New shoot dry wt. g	Leaf area cm ²	Root dry wt. g
H ₂ O, unringed	217	14	15.6	1.28	149	3.15
GA ₃ , unringed	224	18	12.6	1.05	117	3.69
<u>Supplied above ring:</u>						
NAA	148	16	9.0	0.85	78	3.20
GA ₃	150	15	10.2	0.76	71	2.68
NAA + GA ₃ + BAP	150	16	9.9	0.70	76	2.52
H ₂ O above ring, young shoot allowed to grow below ring	158	16	9.9	0.66	65	2.73
H ₂ O (Control)	171	15	11.3	0.75	67	2.69
KH ₂ PO ₄	160	14	11.0	0.76	71	3.30
Asparagine	148	13	10.8	0.77	69	4.00
<u>Supplied below ring</u>						
NAA	120	15	8.6	0.45	42	2.99
GA ₃	141	16	8.9	0.55	50	3.05
NAA + GA ₃ + BAP	168	16	10.7	1.00	111	2.66
H ₂ O (Control)	146	16	9.2	0.72	61	3.19
LSD p = 0.05	NS	NS	NS	NS	56	NS

The concentrations of the (aqueous) solutions were:

NAA	$2 \times 10^{-5} \text{M}$
GA ₃	10^{-5}M
BAP	10^{-5}M
KH ₂ PO ₄	10^{-3}M
Asparagine	2% (w/v)

All solutions were wick-fed into the plants and the supply of solution was renewed twice per week. In the unringed plants the wicks were inserted between the bark and wood in the middle of the old shoot.

There were 4 replicates. The size and vigour of the plants between replicates varied widely due to the limited availability of material.

The plants were grown in Osmocote-enriched sawdust in a growth room under 16 hour photoperiod and 26°/21°C.

Shoot length, leaf number, leaf area and dry weight of the new terminal shoot and root dry weight were measured 14 days after ringing.

Results and discussion: The results are presented in Table 17. As in the previous experiment leaf area of the new shoot was the only parameter which was significantly affected.

In contrast to the earlier results GA₃ had little effect on shoot growth in unringed plants. As

concluded in an earlier experiment GA_3 apparently did not reach its site of action when applied to the mature stem.

Only the combination of NAA, GA_3 and BAP applied below the ring tended to alleviate the effect of ringing on new shoot growth, especially leaf area. On the other hand this treatment tended to reduce root dry weight which suggests that the beneficial effect on shoot growth was not a consequence of improved root growth. An alternative explanation is that these growth substances are transformed into more potent forms in the root and are then translocated in the xylem to the young shoot (Altman and Mozes, 1976; Crozier and Reid, 1972).

In unringed plants these compounds, if they are translocated basally in the phloem, must come from the leaves and stem of the old and/or new shoot. However, leaving mature leaves and stem, with or without a young shoot, in confluence with the roots did not overcome the ringing effect. Alternatively, ringing could inhibit the production of these hormones in the root. If this is so it is difficult to understand how the presence of a young shoot below the ring had no promotive effect.

The results of the asparagine treatment tend to disprove the hypothesis that the effect of ringing is due to the interruption of the acropetal translocation of amino acids. In general, however, the inability of exogenous compounds to restore shoot growth of ringed

plants in this experiment has not resolved the source of the ringing effect.

2.3.9. The nature of the differences in internode length induced by GA₃ and by ringing

Introduction: From the results of the earlier experiments it is apparent that GA₃ increases internode length and ringing reduces it. In a previous experiment (2.3.5) it was established that the increase in leaf number induced by GA₃ was due to an increase in the number of leaf initials which were formed. In this experiment attempts were made to determine whether the differences in internode length, which were induced by GA₃ and by ringing, are due to differences in cell size or in cell number.

Methods: Plants were ringed or the young shoots were treated with GA₃ or ethanol as described earlier (2.3.5) and the new shoots were removed at the end of shoot elongation and prepared for examination in a scanning electron microscope (SEM) by the following procedure:

The leaves were removed and each stem was cut into four pieces before being fixed for two days in the following fixative :

5 ml 40% formaldehyde
 10 ml water
 10 ml 25% glutaraldehyde
 25 ml 0.2 M pH 6.8 sodium cacodylate buffer
 (made by combining 50 ml of 8.5% (w/v)
 aqueous solution of Na(CH₃)₂ As O₂.3H₂O
 with 9.3 ml of 0.4 M HCl and making up
 to 200 ml with water).

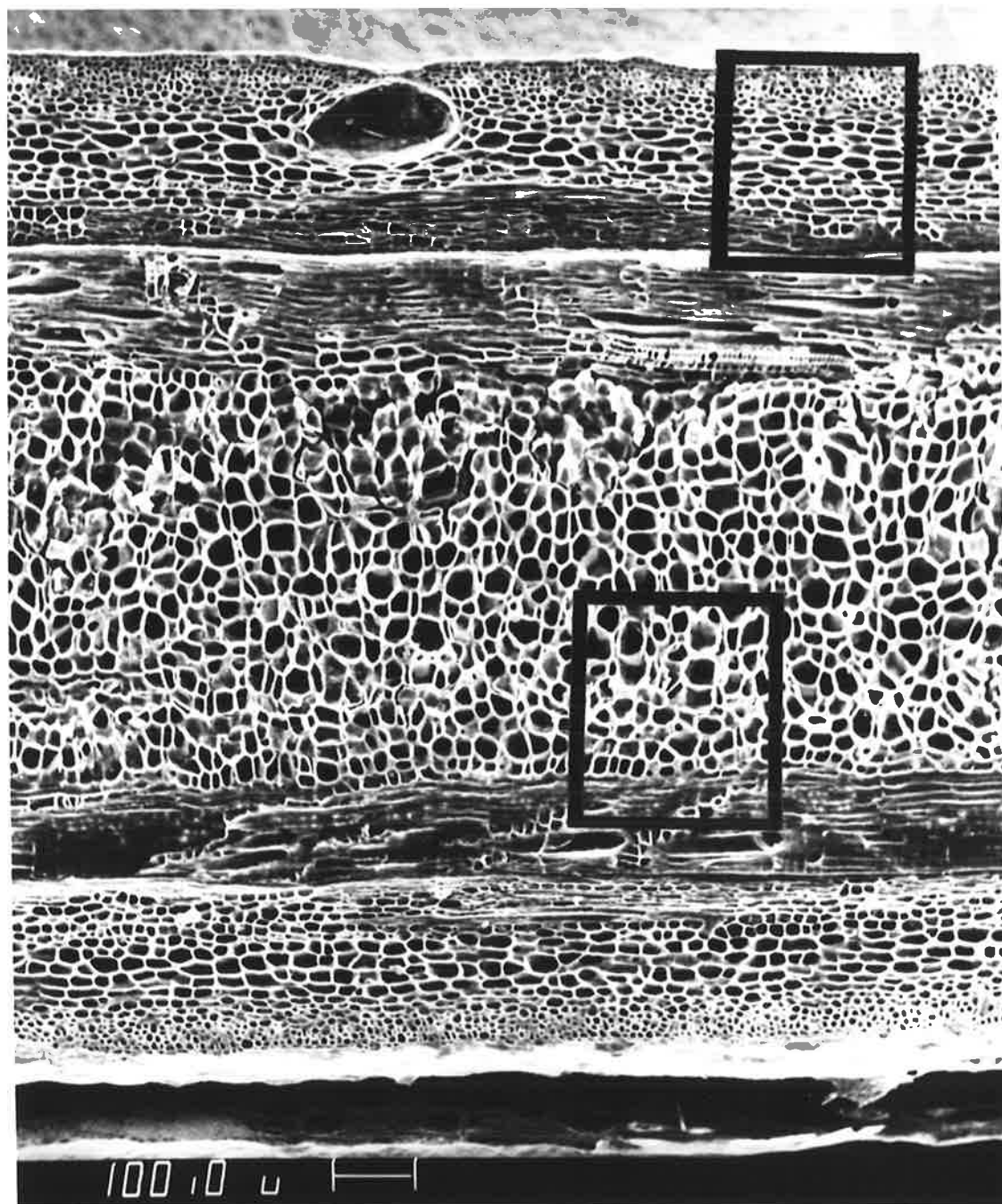


Figure 31. Scanning electron micrograph of bisected internode.

Boxes indicate areas used in cell length determinations of parenchyma of pith and cortex.

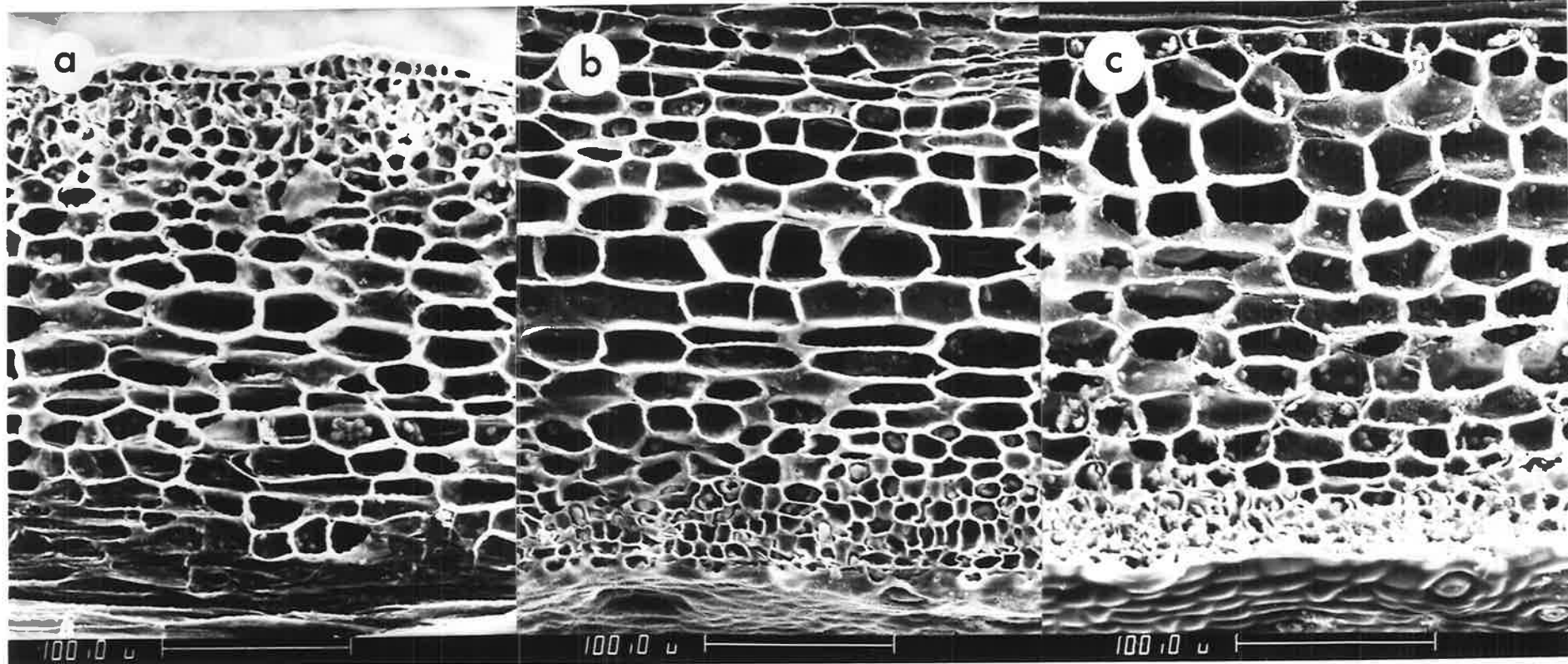


Figure 32. Comparison between the cortex of different treatments.

- a) Control
- b) GA₃-treated
- c) Ringed

Note the epidermis in a) is at the top of the photograph.

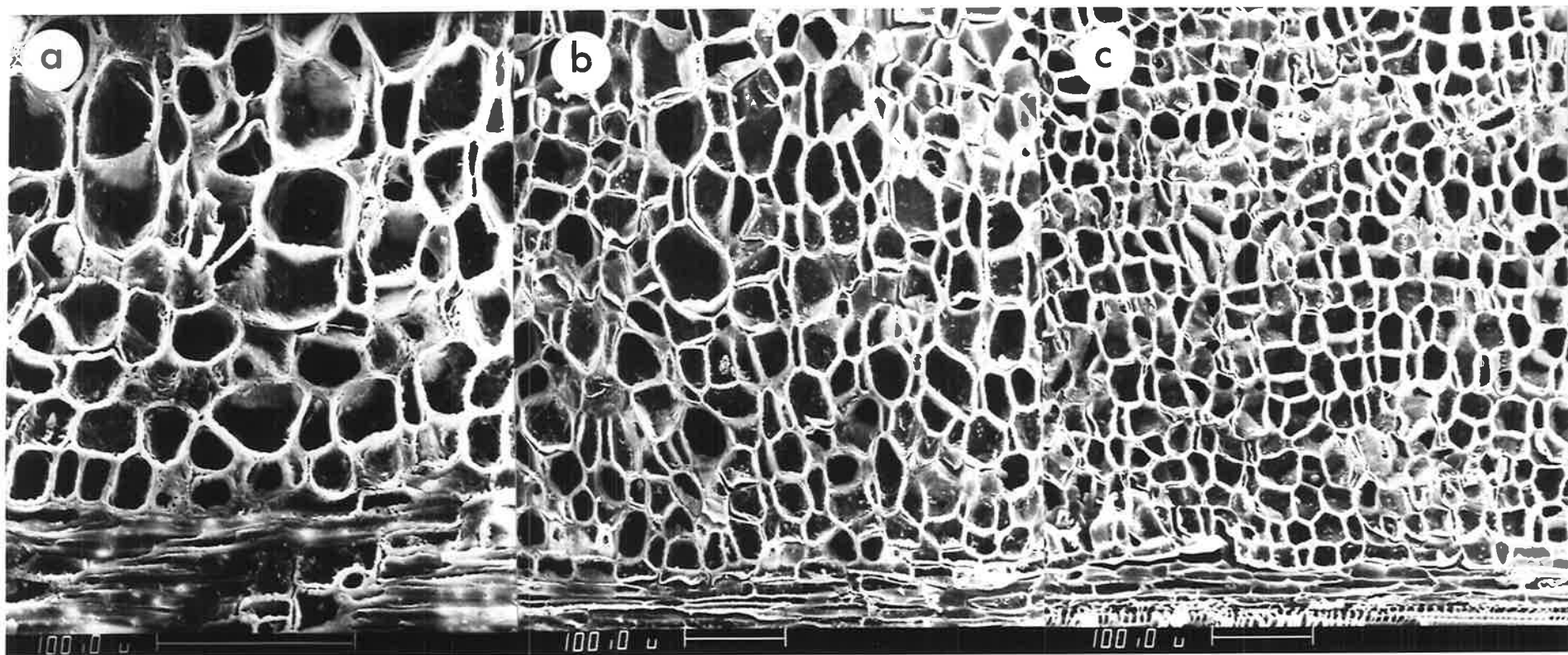


Figure 33. Comparison between the pith of different treatments.

- a) Control
- b) GA₃-treated
- c) Ringed

The fourth internode from the base of each stem was chosen for examination as they had the characteristic appearance of the various treatments. Each (fourth) internode was bisected longitudinally and the halves were dehydrated through the following stages :

40%	Ethanol in water
70%	Ethanol in water
90%	Ethanol in water
100%	Ethanol in water
50/50	Amyl acetate/Ethanol
100%	Amyl acetate

The samples were mounted on aluminium rivets using a commercial contact adhesive (Bonza), were then firstly critical point dried, using liquid CO₂ as the evaporant, and the samples coated under vacuum with carbon, then with gold and platinum. The specimens were examined using a Siemens ETGA Scanning Electron Microscope set at 20 Kv accelerating voltage.

The lengths of cells from 5 rows of the cortex and from 4 rows of the pith of each treatment were determined from the micrographs.

Results and discussion: The overall appearance of the control internode is shown in Figure 31. The micrographs of the cortex of the three treatments are shown in Figure 32, and of the pith in Figure 33.

The mean cell lengths of the cortex and pith of the internode from the GA₃-treated shoot were slightly

Table 18. Effect of GA₃ and ringing on the length of
parenchymatous cells of the internode

	C O R T E X			P I T H		
	Control	GA ₃	Ringed	Control	GA ₃	Ringed
No. cells measured	66	62	67	40	62	73
Range of cell length, μm	7-47	5-81	2-51	7-49	7-48	10-48
Mean cell length, μm	19.8	21.1	14.3	25.7	29.1	25.5
Standard error of mean	1.48	2.26	1.35	1.49	1.48	1.05

greater than those of the control (Table 18). However GA_3 induced an increase of 25% in mean internode length of similar shoots (Figure 27c). Moreover the internodes selected (fourth from the base) exhibited pronounced effects of the treatment, i.e. the GA_3 treated internode was clearly longer than the control. Nevertheless average cell length was only slightly greater which indicates that the increase in internode length was due mainly to an increase in cell number.

Ringling had no effect on mean cell length of the pith but apparently reduced mean cell length of the cortex. The reduced value for the cortex may be partly due to the fact that two of the five rows of cells were measured at lower magnification as the outlines of the cells were not clearly discernible in the micrograph taken at higher magnification (Figure 32). This value may therefore be less accurate than the others. Even so, the mean cell length of the ringed plant was 72% that of the control whereas the average internode length of similar ringed plants were 48% of that of the control (Figure 27c). It appears that the shorter internodes of ringed plants compared to the control are due to fewer rather than shorter cells.

The data suggest that internode length is determined more by cell division than by cell elongation and that GA_3 promotes and ringling inhibits such division.

This is in direct contrast to the results of Ahmed and Khan (1964), Bhambota and Kaul (1966) and Thomal et al., (1963) all of whom reported increases in cell length rather than cell number in the cortex and pith of GA- treated citrus seedlings. No explanation can be given for the opposing results.

2.3.10. Discussion

Shoot length is determined by cell division in the apical meristem, which determines leaf or node number, by cell division in the rib meristem, which determines internode length and by elongation of internode cells (Brown, 1971; Sachs, 1965). Evidence from this series of experiments indicates that in citrus cell division in these two meristems is controlled by different factors. Applied gibberellin increased internode length and leaf number; ringing eliminated the former effect of gibberellin but not the latter. Ringing had a similar effect on shoots not treated with GA_3 . Conversely, leaf number, but not internode length, was increased by long photoperiods and by cytokinin applied above a low ring. In the latter case, leaf area was not increased despite the increase in leaf number which further suggests that cell division in the apical meristem is controlled by different factors to that in the rib and leaf meristems of the young shoot. The differing effects of GA_3 and BAP on internode elongation but not on leaf proliferation indicate that shoot growth depends on one or more sequences of growth substance requirements acting at different meristems (Sachs, 1965).

The data from the ringing treatments indicate that shoot growth is promoted by substances translocated from the root. In ringed lemon plants the presence of roots (originating from callus) above the ring promoted

renewed shoot growth whereas the presence of callus lacking roots in the same position did not (Vinokur, 1953). Smith and Wareing (1964) suggested that roots synthesised substances essential for shoot growth which can be translocated acropetally in the xylem. However the effects of rings at different positions along the stem, and of an incomplete ring, indicate that phloem translocation is necessary for normal shoot growth. Moreover, bidirectional phloem translocation seems necessary.

The active compounds have not been identified. Ziegler (1975) describes more than 10 different groups of substances translocated in the phloem. The results of the final experiments of the present series suggest that translocation in both directions is important: a role for basipetal translocation of growth substances was suggested by the reactions to localised treatments (Table 17) and for acropetal translocation by the effects of the transposing of bark from below to above a ring (Table 16).

The evidence indicate that the interruption of carbohydrate translocation is not the limiting factor, despite the fact that carbohydrates form the bulk of the substances transported in the phloem (Ziegler, 1975; Zimmermann, 1971). Instead, it appears that, even when the young shoot is connected to very restricted sources of assimilate, carbohydrate nutrition of the shoot or root is not the limiting factor in shoot growth.

The nature of the compounds limiting shoot growth whose translocation is interrupted by a bark girdle is unknown. The literature suggests that ringing may influence the distribution of auxin (Cooper, 1936), gibberellins and inhibitors (Goren et al., 1971; Wallerstein et al., 1973) in citrus. However ringing had no effect on gibberellin or cytokinin activity in the root sap of Eucalyptus regnans seedlings (Wilson and Bachelard, 1975). Stoltz (1965) found that ringing Hibiscus rosa-sinensis influenced the concentration of three of the four rooting cofactors (characterized by Hess, 1962) in the bark above the ring; auxin decreased above the girdle and the concentration of amino acids increased. In citrus Stewart and Wheaton (1967) found changes in the identity and quantity of free amino acids and in the rate and extent of C^{14} incorporation (from $C^{14}O_2$) into free amino acids above a ring, and concluded that ringing causes changes in nitrogen metabolism in citrus. But as Stoltz and Hess (1966a) indicate, the changes in amino acids could be a consequence of senescence of tissues distal to the ring rather than an effect of ringing per se.

It must be concluded that there is little, if any, definitive information on the identity or quantity of substances which limit shoot growth and whose translocation is interrupted by ringing.

3. CONCLUDING REMARKS

The above-ground part of a citrus tree is the sum of the growth of a large number of individual shoots. The tree displays phasic growth because of the synchronised behaviour of its shoots. The most evident expression of this is the series of well-defined flushes of growth seen on most citrus trees.

The growth of an individual shoot is thus phasic. During axillary bud formation, which occurs within five days after emergence of the young shoot, there is a short period of rapid development of the primordial structures of the bud. A period of dormancy follows, which ends with another phase of rapid development as the number of leaf primordia suddenly increases and shoot elongation commences, resulting in shoot emergence. This phase continues into shoot elongation. The final phases of shoot growth are leaf expansion and abortion of the shoot tip.

Phasic growth involves the activity of different meristems at various times, e.g. bud formation depends on the activity of the apical meristem whereas at the end of dormancy (whether rest, quiescence or correlative inhibition) both apical and rib meristems become active. Stem elongation is apparently due primarily to activity of the rib meristem and to cell expansion.

It is apparent that different factors regulate different meristems at any one time and the same meristem at different times. For example, dormant citrus buds were

induced to burst by exogenous cytokinin, which on the other hand had no effect on buds which were ready to burst. In the latter, exogenous auxin promoted burst whereas it had no effect on dormant buds. The apical meristem was induced to produce more leaves by exogenous cytokinin and fewer leaves by shortened photoperiod. Gibberellin applied before bud burst inhibited burst presumably by inhibiting activity in the apical and rib meristems. However, when applied after bud burst, GA promoted the activity of both. It appears that the same meristem has a sequence of growth substance requirements at successive stages of development.

Carbohydrates are required for the various phases of shoot elongation and leaf expansion, but the evidence suggests that, within reasonable limits, carbohydrate supply is not a controlling factor. In contrast, these phases are regulated by substances translocated in the phloem. It appears that basipetal translocation (of growth substances?) and acropetal translocation of substances which have not been identified but which accumulate in the bark below a ring are necessary for normal shoot growth.

A number of interesting questions remain unanswered, e.g. what is the nature of the phloem translocated substances, on which meristem and at which stages do they act, where, at what rates and along what pathways are they synthesised and metabolised and

what are the factors controlling these processes?

Answers to any or all of these questions would provide valuable insight into how a citrus tree grows.

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