ASPECTS OF GROWTH CONTROL IN KIWIFRUIT AND BLACKCURRANT

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ABSTRACT

This study emphasized the interplay of several types of growth regulator in the control of growth and development in the kiwifruit plant. Hence different parts of the plant are seen to contribute to this control, the plant appearing as a fully-integrated system with the shoots and roots interacting with each other.

The gibberellins were active in the promotion of stem elongation, and it is considered stem growth is dependent on the photosynthate supply, and that this is mediated by the growth regulators. A leaf had a dominant effect on internode growth, and therefore shoot length, at a very early stage of its development - but the effect of an individual leaf was very localised. The plant roots were not clearly shown to be essential for breaking dormancy, bud burst, or early shoot growth. However it was found the roots could be supplying gibberellins as one of the factors required to maintain normal shoot growth. Cytokinins for early bud growth could have been supplied in the bleeding sap either from the roots or the stem tissue. Reservations are expressed about the need for high spring sap flows for plant growth. High sap flows may be a result of a combination of circumstances at the time, and not a necessary pre-requisite to growth.

SADH and maleic hydrazide are effective shoot growth retardants, and may find commercial acceptance, except their apparent effect in enhancing post-harvest fruit respiration must receive further study.

Bud dormancy was greatest before leaf fall, and dormancy was broken by about 700 hours below $7.2^{o}C$, although warm temperatures were just as effective in overcoming this dormant condition. Winter dormancy appeared to be basi-

cally the same type of condition as correlative inhibition, and it is suggested that each is, in large part a result of an inability to utilise cytokinins or other growth promoters.

It is considered that the juvenile-like condition in blackcurrant is distinct from true juvenility, and the former did not appear to be the result of proximity to gibberellins from the roots. Further, it could not be shown for true juvenility that it was likely to be due to the production of inhibitory levels of gibberellin by the roots.

Part 1.

INTRODUCTION.

A-1 SHOOT GROWTH.

Shoot elongation is an important form of growth in herbaceous and woody plants, some of which grow in a single annual flush of growth, and others in several flushes during the period of suitable growing conditions. The characteristics of shoot growth and the factors affecting it are reviewed in Kozlowski (1964) and Sachs (1965), and they show the variability in shoot elongation and the complexity of its control mechanisms. Information is still required on the relation of shoot growth to other plant processes, and precisely how seasonal growth patterns are controlled.

In fruit trees adequate annual shoot growth, especially in the early years, is essential to maximise yields, but there also comes a point at which the amount or rate of shoot growth is detrimental to flower initiation, fruit set, or fruit quality.

The kiwifruit vine is known for its rampant shoot growth, although vigour varies with the variety (Davison 1971) and growing conditions. While some buds produce a determinate shoot (Brundell 1975a) other indeterminate shoots may readily reach over 200 cms in length. Shoots also readily twine around other shoots they come in contact with, which means the vines quickly become a tangled mass unless frequent summer pruning is carried out, in addition to winter pruning. Uncontrolled growth is conducive to fungal infection, and leads to a reduction in fruit storage life,but the increasing cost of labour for

pruning makes alternative growth control practices attractive.

It is clear many factors affect the time and the extent of shoot growth, including environmental factors and also internal characteristics of the plant both in the stem and other parts of the plant. Since stem elongation is integrated with other aspects of plant growth and development, we should expect activity in the tissues responsible for stem growth to be co-ordinated with, and affected by other plant parts. Thus studies of stem growth have shown a role for the leaves (Sachs 1965) and also the roots (Buttrose and Mullins 1968, Smith and Wareing 1964, Went 1943); for photosynthates, both reserves and from current photosynthesis (Hansen 1971, Priestley 1960); and a role for endogenous growth regulators (Carr et al. 1964, Luckwill 1970).

1.1 Leaf Effects.

While the stem apical meristem proper is the site of the initial cells of the stem, the sub-apical region is the site of formation of most of the cells that make up the mature stem, and so it is the sub-apical meristem that plays a large role in determining plant height (Sachs 1965). Thus, factors affecting cell division in this region would affect stem length. In rosette plants GA-induced stem elongation is accompanied by an enormous increase in sub-apical meristem activity (Sachs et al. 1957), although GA-like substances may also control sub-apical meristem activity in caulescent plants as GA restores cell division and elongation to normal or greater in retardant-treated plants (Sachs and Kofranek 1963). Tammes (quoted by Sachs 1965) found the removal of a few leaves caused inhibition of stem elongation in woody species, but through an effect on cell elongation rather than cell division. The question of whether GA

acts by stimulating cell division or cell elongation is also considered by Arney and Mancinelli (1966), who suggest the main effect of GA^+ is to increase mitotic activity in apical and sub-apical meristems, and any cell elongation effect is a consequence of such activity.

When the shoot tip (including its enclosing leaves) of a plant is removed there is a reduction in the extension of all its internodes especially the more immature ones (Lockard 1956); in Ginkgo shoot elongation does not occur unless there is a continued production of leaves beyond those present in the winter bud (Critchfield 1970); and in the apple tree removing unexpanded immature leaves reduces internode length and shoot length (Kato and Ito 1962).

A number of studies have concentrated on the contribution of young leaves to the growth of individual internodes, and Poll (1973) has shown a marked correlation between shoot length and mean internode length. It has similarly been noted in the kiwifruit shoot (Brundell 1973) that the basal internodes, produced at a time of slow growth rate, are shorter than later ones. Brundell (1973) also found internode length along a shoot was closely related to leaf size, internodes remaining small between leaves that were small.

+

Abbreviations:

ABA Abscisic acid
BAP Benzylaminopurine
CCC Cycocel
GA_3 Gibberellic acid
GA Gibberellin
MH Maleic hydrazide
NAA Naphthalene acetic acid
SADH Succinamic acid 2-2-dimethyl hydrazide

The region of the stem tip is important in the control of internode extension, and it appears the important tissues are the small expanding leaves rather than the apical dome or leaf primordia (Jones and Phillips 1966). Work with bean led to the hypothesis that a GA-precursor is produced in the young leaves and transported to the stem where it is converted to a GA-like material that acts to promote internode elongation (Lockhart 1964). Removal of young leaves from apple shoots retards internode elongation, and it is considered GA-like substances produced by immature leaves contributes to this control (Grauslund 1972, Kato and Ito 1962). In Avena the growth regulator GA_3 is specific in stimulating growth of excised stem segments, and this is a major endogenous gibberellin in this plant. In a careful study of native GA's in Avena Kaufman et al.(1976) concluded the upper two leaves and also the infloresence and nodes (perhaps accumulated there from the leaves) can serve as important sources of the native GA's required for the elongation of the next-to-last internode. A model of shoot growth as an autocatalytic process based on growth regulators produced by young leaves in the shoot tip was proposed by Abbott (1970).

A leaf and its adjacent internode appear to form a localised physiological growing unit, and in apple and plum shoots a young leaf promotes the extension of two internodes above it (Fulford et al. 1968). While this effect was not so clearcut in all varieties, it was always true that the leaf influence extended acropetally rather than basipetally, and it was further shown this effect could be simulated by a localised exogenous GA application. When a group of leaves was removed from near the tip of an apple shoot, there was a large reduction in internode length due to a reduction in their growth rate, the shortest internode always being immediately above the last removed leaf (Barlow and Hancock 1956); although if leaves within the tip bud were removed, then just below the defoliated region there was an increase in

internode length. The young leaves while included in the tip of the apple shoot, thus markedly affect the final length of the internodes between them, and the greater the number of such leaves removed in succession, the greater the reduction in internode length becomes. Then as young leaves are no longer removed there is a gradual recovery of internode length. Removing unexpanded leaves also leads to increased internode number (Kato and Ito 1962). Leaves a short distance below the tip bud have very little influence on elongation of the internodes subtending the tip (Barlow and Hancock 1955), but when a large number of the lower leaves are removed, there is a marked reduction in the extension of many of the upper young internodes, presumably due to the reduced supply of photosynthates. Leaf removal led to an increased rate of dry matter production by the remaining leaves, and a considerable reduction in root growth (Maggs 1965), and when leaves near a shoot tip are removed, the size of an adjacent leaf is increased (Barlow and Hancock 1955). Such compensatory growth following defoliation means the plant rapidly restores its root/shoot ratio (Wareing 1970).

1.2 Root Effects.

When grape vines were maintained with a constant reduced root volume, shoot growth was reduced, and shoot length increments bore a close relation to the level of root pruning. It was considered this could only be interpreted to indicate the roots are the source of a growth substance required for normal shoot growth (Buttrose and Mullins 1968). This idea follows from early observations such as those of Went (1938), and many others have found a role of the roots in the behaviour of the top of the plant (e.g. Smith and Wareing 1964b, Wareing and Nasr 1961). Cooper et al. (1972) suggest a growth regulator originating in the roots controls the spring mobilisation

of reserves that result in their increased concentration in the sap just prior to blossoming.

Waterlogging reduces stem growth, and in the tomato it is considered the reduction in GA coming from the roots (and cytokinins) is an important factor limiting stem growth (Reid and Crozier 1971, Reid and Railton 1974), and Carr et al. (1964) believed the amounts of GA transported from the root to the shoot of their balsam plants was adequate to account for the shoot's growth. In the absence of roots, the growth of uninhibited buds on stem cuttings has been greatly promoted by GA_3 (Smith and Wareing 1964b, Wickson and Thimann 1958), but in both cases the response was short-lived, and for some unexplained reason growth ceased after a certain maximum increment.

While there is some evidence for GA biosynthesis occurring in the roots (e.g. Jones and Phillips 1966), this is not considered unequivocal by Crozier and Reid (1971) although they found plant roots have an important role in GA interconversion.

Cytokinins synthesised in the roots move up the plant in the xylem exudate (Kende 1965, Skene 1975), and cytokinin-like materials in root exudate from apple trees have promoted the growth of isolated apple shoots (Jones 1973). Exogenous cytokinins promote inflorescence development in unrooted grape cutting (Mullins 1967), suggesting these substances in some way substitute for the presence of roots. Miginiac (1971) and Woolley and Wareing (1972) considered plant roots can regulate shoot meristem function, through cytokinins.

The results of Luckwill and Whyte (1968) with apple cultivars indicated the cessation of shoot growth corresponded approximately to the time cytokinins disappeared from the sap.

1.3 Carbohydrate Reserves.

In deciduous plants bud burst in spring is followed by a period of growth, and initially there is no foliage to support this growth. There is also a period of competition between the growing shoot apices, and the developing flowers and fruitlets for the metabolites being produced (Quinlan and Preston 1968), and so it is necessary to determine the source of carbohydrates used in growth, and their distribution through the plant.

Shoot growth has been found positively correlated with bud size, and also with the environment of the year in which the bud was formed; and in many temperate zone woody plants shoot growth depends primarily on carbohydrate reserves, not on current photosynthates (Kozlowski 1964). In a study over 25 years of apple trees grown on a range of rootstocks, a significant negative correlation was established between yield and the following season's shoot growth, but not between yield and shoot growth in the same year (Rogers and Booth 1964). They suggested a heavy crop reduced the level of stored reserves (not specified), and hence next year's growth. Biennial varieties of apple make more shoot growth in their "on" year. Since cropping has a large effect on root growth, reductions in root growth due to a heavy crop could result in reduced shoot growth the following year.

Work with young apple trees has shown soluble sugars, starch, and hemicellulose in the wood and bark support vegetative growth in the spring. It has not been possible to encourage the use of more than 33% of such extractable material in growth, and still keep the plants healthy (Priestley 1970), but clearly a portion of the reserve carbohydrates play an important role in growth. Studies with pecan trees following assimilation of $^{14}CO_2$ showed translocation from stored reserves is evident at bud break, and it appeared they were not used for substantial growth of tissue other than those associated with

elongation (Lockwood and Sparks 1978). Following the relatively rapid depletion of reserves as the first leaves expand, there is a replenishment of reserves before shoot extension becomes active (Priestley 1960), and quantitatively the greater part by far of the total new growth in apple fruits and shoots is based on materials from current photosynthesis (Hansen 1971). Similar changes in the carbohydrate reserves of rooted grape cuttings have been recorded (Buttrose 1966), and in cassava shoot growth made by cuttings is very closely related to the total carbohydrate content of the cuttings (Wholey and Cock 1975). When hardwood cuttings of apple are rooted under conditions favouring carbohydrate loss, it is found the subsequent level of establishment in the field is low (Cheffins et al. 1974). In stem cuttings of the kiwifruit defoliation enhanced flower bud development, presumably by diverting more of the limited reserves to this sink, and over a 26-day period after bud burst, the expanding leaves did not appear to make any important contribution of photosynthates to shoot growth, but rather they appeared as the major metabolic sink in the rapidly developing kiwifruit shoot (Brundell 1975c).

A young expanding leaf imports assimilates, largely from the uppermost leaves below it, and when it reaches 30-50% of its final size it begins to export assimilates (Thrower 1962, Wardlaw 1968). In the soybean when a leaf is 8% of its final size and growing rapidly (typically the outermost leaf of the apical group) it has a rapidly increasing import of C-assimilate (Thrower 1962). As indicated earlier it is at about this stage that a leaf is playing a very significant role in determining the final length of the internode above it.

In woody perennials nitrogen reserves are also important, and there may be a high correlation between the level of storage N and new spring shoot growth (Tromp 1970). Hydrolysis of wood protein occurs before bud break (Oland 1959), and the level of amino acids in grape roots,

canes and buds rises just before bud burst (Kliewer 1967). In grape cuttings both soluble and insoluble N is available for the growth of new roots and shoots, and is even used preferentially over fertiliser-N (Obbink et al. 1973). Vacuum-extracted stem sap of apple shows N concentration increases just before bud burst, and peaks at full bloom (Bollard 1953, Cooper et al. 1972), and this is believed to result from mobilisation of reserves rather than a movement of recently-absorbed nutrients (Bollard 1953).

Exogenous gibberellins stimulate stem elongation of many plants, an effect no doubt of the many changes that treatment has induced. In barley seeds, GA causes the de novo synthesis of α-amylase which results in the hydrolysis of starch, and when wheat is treated with GA_3 the coleoptiles elongate and starch reserves disappear (Boothby and Wright 1962). Perhaps a similar mechanism occurs in GA-stimulated growth of other plants. Grochowska (1973) showed a high starch content in plant tissue is an indicator of low amounts of auxin and gibberellin in those tissues. Working with seedlings, Nanda and Purohit (1965) found GA_3 reduced starch in proportion to the amount of extension growth induced, and showed an increased activity of hydrolytic enzyme in tissue treated with GA_3. Paleg (1965) has shown the corollary between GA treatment of tissue, and the loss of stored starch from these tissues, and there are many examples of changes in enzyme development in elongating tissues with GA_3 treatment- just as changes in DNA and RNA synthesis have been correlated with GA-stimulated growth. These effects are discussed by Jones R.L. (1973), who considers it is not clear whether these GA_3-stimulated changes are the cause of the increased stem growth rate, or if they are simply involved in the maintenance of the higher rate of cell elongation.

In the grape vine after winter rest, starch disappears to support new growth, but GA_3 alone or in combination with BAP was ineffective in promoting the release of sugars from 1 year old canes (Skene 1971). It was

concluded GA_3 did not have a direct effect on the enzymes concerned with starch degradation, and it was noted that IAA was effective in promoting the release of sugars from wood. The largest effect on sugar release was associated with stimulation of cambial activity (Skene 1971).

When apple seedlings or trees were supplied with NAA the starch disappeared from the tissues, but GA had only a very slight effect, and when the shoots were supplied with GA and NAA together, starch loss was enhanced (Pieniazek and Saniewski 1968). The same effect was found in further work, and these authors also consider the degradation of stored carbohydrates in the spring is hormonally-controlled by an interaction of endogenous auxins and gibberellins, the process being initiated by auxins produced by the bursting buds (Saniewski and Pieniazek 1972).

Currently there is interest in the area of hormone-directed transport of metabolites, and Luckwill (1970) bases his model of shoot growth control on a mobilising role for auxin produced in the apex. Gibberellin may have an indirect effect by regulating auxin production, although its synergistic action with auxin was noted above. Seth and Wareing (1967) revealed that the attraction of nutrients towards the source of auxin is greatly enhanced when it is applied together with GA and cytokinin. Treatment of leaves or roots of intact grape plants with cytokinin modifies the pattern of movement of photosynthate, amino acids, and organic acids (Shindy and Weaver 1970, Shindy et al. 1973). Mullins (1970) concluded hormones simply exert a sink effect on the phloem transport system; and in the grape, when only one of two shoots is sprayed with GA_3 there is a marked transfer of assimilate into the sprayed shoot- and so it could contribute to its increased growth (Quinlan and Weaver 1970). In their proposal of SADH action on apple plants (Hoad and Monselise 1976), an induced fall in GA levels in the stem tip contributes to a reduction in its "sink-strength" and

thus reduced translocation to the area - which causes a retardation of internode elongation.

1.4 Growth Regulators.

Some reference has already been made to the involvement of these substances in growth control, but other observations should also be noted.

(a) Gibberellins -

In sunflower plants there is a positive correlation between the quantity of diffusible-GA from an internode and its rate of extension growth (Jones and Phillips 1966), and the reduced stem growth in the flooded tomato plant has been related to the reduced export of gibberellins from the roots (Reid and Crozier 1971). Barendse et al. (1968) consider that early growth of pea seedlings is at least partly regulated by GA's released from a bound form in the seeds, and Crozier et al. (1971b) considered the likely involvement of specific endogenous GA's in the growth of bean seedlings. A greater amount of GA-like substances is found in the transpiration stream of vigorous apple trees (Ibrahim and Dana 1971), and gibberellins have been widely found in the xylem sap of the apple (Luckwill and Whyte 1968) and other plants. Gibberellin converts dwarf beans into pole beans (Wittwer and Bukovac 1957 quoted in Cleland 1969), and in some genetic dwarfs of corn and rice, dwarfism is related to the absence of, or reduced levels of endogenous GA-like substances (Jones R.L. 1973). When a young apple tree is sprayed with GA_3 internode length and shoot length is increased (Luckwill 1968), and this type of response has been used as the basis of a bioassay with grape shoots (Weaver and McCune 1959) and lettuce seedlings (Frankland and Wareing 1960). But when seedlings were treated with different levels of the growth retardant AMO-1618 there was no obvious correlation between stem elongation and the level of extracted GA-activity (Crozier et al. 1973).

It seems generally accepted that gibberellins are primarily responsible for internode elongation (Fulford et al. 1968, Phillips 1972), and it is known they can simulate the effect of a young leaf blade on internode elongation (Fulford et al. 1968). As the different gibberellins vary in the amount of stem elongation they induce (Crozier et al. 1970, Kaufman 1967) one may expect the gibberellins important in stem growth to vary with the plant. While GA_8 appeared to be important in the growth of Phaseolus seedlings, this effect is likely to be restricted to the early stages of germination, and in 12-day old seedlings GA's 1, 4, and 19 appeared to be the main stem-growth promoting GA's (Crozier et al. 1971b). Barlow (1967) reported a negligible effect of GA_3 on the growth of apple trees, but they reacted strongly to GA_4 and less markedly to GA_7. In Picea Dunberg (1976) found a rapid rise in GA and IAA activity during the period of rapid shoot elongation, and in this plant GA-like substances are a prerequisite for elongation. Qualitative changes suggested a GA-interconverson pathway, towards more polar compounds was operating.

Some of the effects produced by auxins are similar to those caused by gibberellins, suggesting the actions of the two substances are connected - however, the two materials may also produce opposite effects. While auxin level does increase in many tissues in the presence of GA, at least some GA responses are independent of the presence of auxin, and the general conclusion appears to be that GA acts with auxin, rather than through auxin.

Many studies (e.g. Kaufman 1965, Ockerse 1967) have considered the relationship of GA to auxin in stem growth; in stem sections, and intact pea plants GA_3 induced extension growth only in the presence of auxin (Brian and Hemming 1958), and they act synergistically promoting in stem growth (Grunwald and Lockard 1973, Jankiewicz et al. 1973). The interactions between auxin and gibberellins are not clear, but evidence continues to appear that the

two work together in causing elongation. GA appears not to work through an auxin-mediated mechanism, nor in ways that have been proposed in various other hypotheses for their interaction, but to interact with auxin in bringing about growth (Kefford 1962).

(b) Auxins, Cytokinins, Inhibitors -

Native auxins are widely found in plants, and at particular stages of development may play a major role (e.g. Goldschmidt 1976). Critchfield (1970) believed his work provided good circumstantial evidence of a dominant role for auxin in controlling shoot extension. There have been attempts to relate the effect of light on stem growth to changes in auxin metabolism (Fletcher and Zalik 1964), but in comparison with GA, auxin often has little effect on stem growth (Jankiewicz et al. 1971, Phillips 1971a).

A difference in effect of IAA and GA on tissue differentiation in elongating apple internodes is recorded by Jankiewicz et al. (1973), and indicates a need for both types of material in growth. In decapitated apple shoots, auxin or BAP did not stimulate elongation more than GA_3 alone, but it was only in the presence of all three that the shoot tip was anatomically like a normal non-decapitated one (Moraszczyk et al. 1974).

Now in contrast to early views about the role of auxin in plants, the old precept of "no auxin, no growth" appears too simple, and Mer (1969) considers much of the work relating auxin level to growth level is questionable. Observations noted so far indicate an inter-relationship between endogenous hormones, and the work of Shein and Jackson (1972) supports the idea of stem growth depending on the local hormone balance - which is modified by a wide range of internal and external factors. Wright (1968) has shown sequential roles for the various groups of growth regulators in the growth of the wheat coleoptile and first leaf. Gibberellins and cytokinins may act sequentially on shoot growth, as suggested by the results

of Ali and Fletcher (1971) who found an application of BAP to an inhibited lateral bud, followed 48 hours later by GA_3 was more effective in promoting shoot growth than when the two hormones were applied together. The existence of such inter-relationships that integrate the whole plant is also reflected in the models of shoot growth of Luckwill (1968) and Jankiewicz (1972, Jankiewicz et al. 1973).

Large quantities of solutes may be transported to the branches of a tree in the xylem sap in the spring (Luckwill and Whyte 1968), and we may expect cytokinins originating in the roots to be an important factor in the control of plant growth (Skene 1975). Both endogenous and synthetic cytokinins promote elongation of isolated apple shoots (Jones 1967, 1973), and Luckwill and Whyte (1968) correlated the fall in sap cytokinin level with the end of shoot extension growth.

The supply of kinetin to plant roots may reduce dry weight accumulation and elongation of the tops (Wittwer and Dedolph 1963), while IAA and/or kinetin under some conditions reduces GA_3-induced elongation in the main stem of dwarf beans (Jackson and Field 1972, Shein and Jackson 1971).

It is also worth noting that a tissue's hormonal content at any one time may not be very meaningful, and what is more important is the rate of hormone turnover in the tissue (Jones R.L. 1973, Zeevaart 1971). There is evidence to suggest root-cytokinins play a role in controlling shoot growth and that they do this in part, via an effect on GA metabolism, and Railton's (1974) results show how a cytokinin can markedly affect GA turnover in plants. Further, when tomato plants are flooded, they have reduced GA and cytokinin levels, and then an exogenous supply of cytokinin raises the GA level and partially relieves their dwarfness (Reid and Railton 1974).

Plant tissues also contain large amounts of inhibitors - not only ABA but also neutral non-polar ones. It has been widely believed ABA is important in regulating shoot growth since a correlation has been found between the increased level of this hormone and the cessation of growth (Phillips and Wareing 1958), and exogenous ABA application may reduce growth. However there is now some doubt about the suggestion (Eagles and Wareing 1964) that ABA is responsible for the cessation of shoot elongation and formation of terminal buds under short days. Thus the highest levels of ABA have been found in the small expanding leaves and upper stem of rapidly-growing shoots of the apple (Powell and Seeley 1970) and poplar (Eliasson 1969). Studies with seedlings of Malus and Betula indicate ABA does not play a primary role in the control of shoot elongation, as short days did not lead to increased amounts of ABA in the shoots (Powell 1976). Further evidence in support of this proposal was obtained by Singha and Powell (1978). Low levels of ABA have been detected in the xylem sap of Actinidia chinensis during the early spring (Davidson and Young 1973). In the control of the rate of stem elongation, and the cessation of growth and development of rest, ABA appears to be just one of a group of factors that are important.

To return to the idea of hormone balance one may note the work of Atkin et al. (1973) with maize. They found as the temperature of the root media was lowered, shoot growth became more restricted, with a decline in GA and cytokinins in the bleeding sap, although inhibitor levels rose - and so it was suggested the change in shoot growth was related to an alteration in the balance of growth regulators exported from the roots to the shoots.

(c) Growth retardants -

Several chemicals have been synthesised that have marked effects on growth and development of plants, and in some instances their use has become commercially feasible.

Chemical control of plant height and shoot growth of many herbaceous and woody plants is now possible, and the materials used may also have other useful effects on crop development. SADH has a range of effects on fruit crops, including a marked reduction in vegetative growth - an effect it has been shown to have on the kiwifruit vine (Davison 1971). Many studies have been made of its mode of action, especially in relation to gibberellin metabolism, since SADH treatment may reduce GA levels in stem tips (Hoad and Monselise 1976; Ryugo and Sansavini 1972), and gibberellic acid can negate or reverse the effect of retardants on plant growth. However, Greenhalgh and Edgerton (1967) showed SADH should not be considered as an "antigibberellin", and some of its effects may result from its interference in other metabolic processes.

The growth retardants CCC and AMO 1618 also affect GA biosynthesis or action (Carr and Reid 1968, Lang 1970, Reid and Carr 1967, Reid and Crozier 1970) although a fall in GA activity after treatment with retardant is not always recorded (Grausland 1972). There is evidence these retardants also have effects other than on endogenous gibberellins, and when grapes were treated with CCC it acted directly on the root meristem to increase cytokinin production (Skene 1970). The data of Crozier et al. (1973) established that some of the effects of AMO-1618 on the growth of Phaseolus seedlings are mediated by factors other than inhibition of GA biosynthesis.

Maleic hydrazide (6- Hydroxy -3(2H)- pyridazinone) is a chemical highly mobile in the plant but which unlike SADH and CCC inhibits normal apical meristem functions (division) and therefore affects both vegetative and reproductive development. It has been widely used to stop sprouting of onions in storage, although it has been used to control shoot growth in apricot (Uriu et al. 1973) and ornamentals (Sachs et al. 1970). This material also breaks apical dominance, so plants become bushy, and newly emerging leaves may be malformed (Sachs et al. 1970, Wu and

Overcash 1968). Many theories of the mode of action of this material have been proposed, but it seems likely it acts through an effect on the nucleus and not by competing with, or inhibiting the synthesis of, some simple metabolite or hormone (Nooden 1969).

B-1 SAP FLOW AND SAP HORMONES.

Translocation within a plant offers the possibility of materials being re-distributed from areas of production or storage to areas of utilisation, and permits an interdependence of the various parts of the plant throughout its seasonal cycle of development. In addition to the widely recognised upward flow of sap in the xylem, there is also an outward flow in the phloem. The composition of this sap flow is diverse, including carbohydrates, amino acids, mineral elements and growth regulators. Not only is it possible that sap flow rate varies through the year, but its composition may also vary, even diurnally (Carr and Pate 1967). Because the behaviour of each part of a plant is affected by, or dependent on, other parts of a plant, considerable work has been done on the substances being transferred within a plant and factors affecting this transfer. Some of the important areas here include:-

(i) Differential mineral element transfer as an explanation of rootstock effect (Jones 1971).

(ii) Sap content of growth regulators in relation to rootstock effect (Ibrahim and Dana 1971).

(iii) Transfer of carbohydrates and mineral elements in relation to fruit quality and storage disorders (Faust et al. 1969, Tromp 1975).

(iv) Mobilisation and transfer of nitrogenous compounds inrelation to spring shoot growth (Tromp 1970).

(v) The effect of flooding on sap gibberellin levels as a mechanism by which stress modifies plant growth (Reid et al. 1969).

(vi) Sap-transported growth regulators in relation to dormancy release (Bachelard and Wightman 1974, Browning 1973) and apical dominance (Woolley and Wareing 1972b).

(vii) Sap growth regulators in relation to fruit set (Skene 1968).

(viii) The role of gibberellins produced in the roots in maintaining a juvenile-like condition in shoots (Schwabe and Al-Doori 1973).

(ix) The redistribution of cytokinins in phloem sap, to plant organs for storage (van Staden 1976).

Clearly, the varying flow of materials through a plant's vascular system is involved in a wide range of plant processes, but in many instances it is still not clear what role is played by the substances known to be present in the sap. A full understanding of growth and development requires this information.

In addition to being the vehicle for moving materials to distant parts of a plant, for analytical work plant sap has the advantage of being relatively pure and free of the many other substances that make purification of woody tissue difficult. When a growing plant is decapitated, xylem root exudate may be collected, or bleeding sap may be collected from an auger boring into the branch of a tree. Some plants produce copious quantities of sap if cut at certain times, including the grape, the kiwifruit vine, and the sugar maple - the latter producing a valuble commercial product, maple syrup. In addition, sap may be collected from shoot tissue by centrifugation or vacuum extraction - the composition of the latter apparently being typical of exuded sap (Jones 1971).

1.1 Sap Flow

In the early spring, sap flow from fresh cuts can be high, and for this reason it is usual to avoid early spring pruning in grape and kiwifruit vines. In South Australia, sap flow in *Vitis vinifera* starts just prior to the start of bud burst and continues for about 3 weeks - when bud burst is complete and new shoots are elongating (Skene 1967).

The sap flow from field grapes in the spring recorded by Skene (1967) in Australia was variable, although during September the flow rates were highest at the early stages of bud burst. Collecting sap from three out of the eight canes on a vine yielded 1-1.2 litres a day at the peak, falling to 400-600cc a day for later harvests, and finally ceasing if cuts were made at the end of bud burst. In 60-day old sunflower seedlings the sap flow rate declined only slightly over the four days following decapitation (Sitton et al. 1967).

Water uptake by plants is, to a large extent affected by transpiration, and water transport is not primarily controlled by metabolic functions within the plant as the amount of water used in metabolism is quantitatively unimportant in relation to the amount transported. The magnitude of water uptake is also determined by the resistance in the plant tissues, and this has been discussed by Kuiper (1964), with particular reference to the effect of root temperature. The relationship between water uptake and bleeding sap flow rate is not known, and since peak sap flows occur before the development of a transpiring leaf surface, the start of plant growth metabolic activity may be very important in determining sap flows. Anderson et al. (1970) described root pressure exudation from excised primary roots of maize by a standing gradient osmotic flow model.

Because of its economic importance, sap flow in the sugar maple has been extensively studied, and both flow rate and composition have been found to vary greatly. Trees in Ithaca, New York produce sap from autumn to early spring with highest flow rates in March, and sugar content greatest in February and/or March (Morrow 1955). Major reasons for the variation in flow are: weather, heredity, exposure, microorganism activity, tree size and vigour, crown size and openness, and the number and depth of tap hole (Morrow 1963). More recently it has been found vacuum pumping can substantially increase sap yield

(Morrow 1972) by reducing friction in the collection tubing. For uniform sap flow between individual trees it is essential they be selected for uniformity, but weather conditions will affect the actual flow. There are indications weather forecasts are valuable in determining the start and length of the sap flow, and a return to cold temperatures can markedly shorten the season's flow (Morrow 1955).

B-2 SAP HORMONES.

It is clear that hormones are transported throughout an intact plant, no doubt to control specific processes, although it may also be for the purpose of storage, or to undergo conversion to other active forms (Crozier and Reid 1971). GA-like substances and cytokinins have been detected in the xylem and phloem sap of a number of plants, and an interchange also appears to occur between these two streams (Lang 1970). Bowen and Wareing (1969) have discussed three possible functions for the GA's circulating in the stem, namely to support cambial function, storage and inactivation.

2.1 Gibberellin-like Substances.

A number of gibberellins and cytokinins being transported in plants have been identified, but the extraction method may have some effect on the substances being removed. In several instances with woody tissue, polar GA-like activity is found which does not partition into acid ethyl acetate but is transferable into butanol (Goldschmidt 1976), and in Ulmus glabra and Acer platanoides most of the GA's present in bleeding sap were in a bound conjugated form (Sembdner et al. 1964). Such GA- glucosides are often inactive in bioassays, and may therefore

be overlooked unless proper extraction techniques are used. Their presence in sap (Sembdner et al. 1974) and seeds (Barendse et al. 1968) suggests they are a specific form of gibberellin for transport and storage.

Gibberellin-like activity in plant sap or tissues is usually determined by bioassay, following extraction and purification with organic solvents, and the use of suitable columns or chromatography. Because of the small quantities of active material being studied and the difficulties of purification, it is not usual to determine precisely which gibberellins of the many known, are present in each extract. The significance to the rest of the plant of the gibberellins rising in the plant sap is not usually clear, and it is probably essential that the identity and metabolism of endogenous gibberellins be determined if progress is to be made in this area. Many GA interconversions can occur in higher plants (Nitsch 1970) and the importance of the roots as a site for this process was shown by Crozier and Reid (1971). Since bioassays vary in their sensitivity to different gibberellins, work employing a limited number of tests may present a misleading or incomplete picture of endogenous gibberellin activity. However, it is clear that plant roots do supply to the shoots of plants some factor affecting its growth, and that gibberellins are synthesised in the root tips and transported in the xylem sap to the shoots (see Crozier and Reid 1971). As has been indicated earlier, while root-GA's may have an effect on stem elongation, they are not the only root factor involved in shoot development. Furthermore the role of the roots in GA metabolism may be more as a site for the interconversion of shoot-synthesised GA's rather than as a major site of GA-biosynthesis

The level of GA-like substances in plant xylem sap is low:

Sunflower	0.02 - 0.65 µg GA_3 equivalents/litre (Kende and Sitton 1967)
Sunflower, about	1.0 µg/litre (Phillips and Jones 1964)
Maize, maximum	0.02 µg/l (Atkin et al. 1973)
Apple, about	0.6 µg/l (Robitaille 1970)
Apple	0.01 - 0.02 µg/litre (Luckwill and Whyte 1968)

Analysis are usually performed on root exudate collected in the first 24 or 48 hours after decapitation, but Sitton et al. (1967) considered there was no loss in activity over a four-day period - although it is not clear if this resulted from continuing synthesis, or the release of stored gibberellins.

2.2 Cytokinins

Substances with cytokinin activity have been detected in the sap of a wide range of plants (e.g. Carr and Burrows 1966, Skene 1975), but a more direct proof that roots can synthesise cytokinins is that these compounds accumulate in excised roots cultured in aseptic conditions (van Staden and Smith 1978). There appears to be a number of aspects of plant development in which root cytokinins are involved. Thus, in apple trees, an increase in xylem cytokinins was correlated with bud burst and extension growth (Luckwill and Whyte 1968), shoot senescence was associated with a fall in cytokinins in sunflower root exudate (Sitton et al. 1967), and changes occur in relation to flowering (Beever and Woolhouse 1974). In the bleeding sap of apple, most of the activity is due to a material with the properties of zeatin riboside, although zeatin ribotide may also have been present (Jones 1973). In other instances also, zeatin riboside

is the major translocational form of cytokinin (Alvim et al. 1976, Davey and van Staden 1976, Hewett and Wareing 1973a), although zeatin may also be an important constituent (Davey and van Staden 1976). There may be additional types of cytokinin transported to the shoot (Kende 1965, Radin and Loomis 1971). In the leaves of poplar there is a decrease in the amount of zeatin riboside in the autumn, and this is replaced by an accumulation of a cytokinin glucoside (Hewett and Wareing 1973b), which material can be detected in the phloem sap (van Staden 1976). Using GLC-MS techniques Horgan et al.(1973) Purse et al.(1976) identified ribosyl-trans zeatin, trans zeatin, and dihydro zeatin in sycamore bleeding sap, in addition to other unidentified cytokinins. Hall (1973) indicated spring maple sap contains isopentenyl adenoside.

Various factors are known to affect the synthesis of cytokinins by roots, and their concentration in sap, including nutrition, pH, temperature, senescence, stress, photoperiod, variety and season. While cytokinin activity can increase in buds independently of the root system, the cytokinins rising in the spring sap may have a role in emergence from winter rest, and also in the beginning of shoot growth.

In apple sap, cytokinin activity rises sharply to a peak at full bloom, and falls quickly to negligible levels as shoot extension ceases (Luckwill and Whyte 1968). Also using vacuum-extracted sap from stems, Alvim et al. (1976) found in willow an abrupt peak in cytokinin concentration at the time of floral bud burst i.e. when their dormancy is broken, and then a rapid decline as shoot extension begins. No account was taken of actual sap flow rates, and therefore of the amounts of solute being transported to the tops of the plants. However Purse et al. (1976) noted the fluctuations in individual active fractions in sycamore sap over 3 months around bud burst, but no clear or marked pattern in cytokinin concentration was

evident.

The level of activity in sap is indicated by various authors, including:-

Sunflower, peaks of	9-13 µg/litre,	kinetin equivalents (Kende 1965)
Apple, peaks of	280 µg/litre,	kinetin eq. (Luckwill and Whyte 1968)
Grape	1-9.6 µg/litre,	kinetin eq. (Skene and Kerridge 1967)
Grape	5-26 µg/litre,	kinetin eq. (Skene and Antcliff 1972)

An effect of root temperature on the export of cytokinin from maize was recorded by Atkin et al. (1973) and in the grape it has a marked qualitative effect on sap-cytokinins (Skene and Kerridge 1967). The application of the growth retardant CCC to the roots of the grape plant increased cytokinin activity in the sap (Skene 1970). The concentration and the amounts of cytokinin transported in the sap declined from the first and second day respectively after decapitation in Vitis (Skene 1970) and sunflower (Carr and Reid 1968), and qualitative changes also occurred during the first three days.

C-1 BUD DORMANCY.

The phenomenon of dormancy is widespread, and exhibited by such different organs as seeds, buds and tubers, although the control mechanisms involved in each of them appear at least homologous. Dormancy occurs in many deciduous fruit trees, and forest trees, although citrus trees do not have such a rest period but continue growing in a series of flushes, between which the lateral buds are inhibited by correlative inhibition of the leaves.

In woody plants there is a period of active shoot growth in the summer, followed by a period of imperceptible growth, which marks the beginning of the development of a dormant condition in the buds. At this stage a number of treatments will result in a resumption of active growth, but this is not true of the following stage of dormancy which may include leaf fall, cessation of growth, and winter rest of the buds. The formation of dormant buds is promoted by short days in many woody plants, apparently due to a direct inhibitory effect transmitted from the leaves (Wareing 1954). Until this dormancy is broken, the bud is unable to grow under conditions of temperature, nutrition and photoperiod normally suitable for growth. However, the time of dormancy is not a period of inert existence for the buds, but one when a number of changes occur (Perry 1971).

Doorenbos (1953) has distinguished between winter dormancy, a nonsystemic inhibitory system present within the dormant tissue, and summer dormancy of buds which finds its cause within the plant but not in the buds, and is systemic. At that time he considered them distinct physiological processes, but subsequently Tinklin and Schwabe (1970) suggested their control system may differ only quantitatively and not qualitatively.

For buds to be released from dormancy they normally must be subjected to conditions different from those inducing dormancy - and for many woody species, winter

chilling is effective. Erez et al. (1966) suggested that bud opening in the spring may be dependent on both a chilling and a light stimulus. Such a plant remains dormant until it has been subjected to a certain amount of chilling, although growth frequently doesn't resume when this point is reached. The buds are then in a state of quiescence until temperatures suitable for growth occur - and changes in the buds in late winter are the result rather than the cause of emergence from dormancy. Abbott (1962) has demonstrated the date of bud break is more strongly influenced by the time when winter chilling occurs than by its intensity.

The chilling requirement for a cultivar varies from year to year, and the rest period in apple and pear trees is modified by rainfall (Westwood and Bjornstad 1978). It was found that bud burst was accelerated in incompletely - chilled buds that were either exposed to winter rain or immersed in water - possibly due to the leaching of a water - soluble inhibitor from the buds.

Dormancy is usually considered broken when buds start to grow within 14 days of being placed in environmental conditions suitable for growth, and after rest is completed it has been found to require progressively less time than this for bud burst (Seeley 1968 quoted in Walker and Seeley 1973). Grape vines behave differently in that there is no chilling requirement to overcome dormancy, and although chilling does reduce the time taken for bud burst to occur, the intensity of dormancy is markedly reduced before the coldest winter period begins (Antcliff and May 1961). Brundell (1976) has shown the kiwi fruit vine has no essential chilling requirement for the termination of rest, although chilling enhances bud break.

A number of chemical treatments have also been found effective to some degree in breaking dormancy in deciduous fruits (Doorenbos 1953), and some of these have been developed for commercial use (Erez and Lavee 1974).

Thiourea has been found a very effective rest-breaking agent for peaches; in addition, sprays of potassium nitrate or a DNOC - mineral oil combination has been found very effective (Erez et al. 1971). Among the growth regulators auxins appear to be ineffective; but the gibberellins can break the dormancy of many seeds, tubers and buds (Phinney and West 1960) including the buds of various trees, including peach (Donoho and Walker 1957) - although they delay bud break of grapes (Weaver et al. 1961). Although there are reports of ethylene breaking dormancy of seeds and bulbs, ethylene - releasing chemicals had no influence on bud break of dormant apple shoots, and ethylene did not appear to have a role in wound-stimulated bud break of dormant apple buds (Paiva and Robitaille 1978). When the cytokinin BAP is applied to dormant apple tree buds there is an increase in the number of buds bursting - although this growth is not sustained (Williams and Billingsley 1970). In work of this type one must be clear as to whether the agent is actually breaking dormancy or simply stimulating growth following the end of dormancy. In pears, GA stimulated bud growth of dormant pears only if the trees had previously received enough chilling to partially break the rest period (Brown et al 1960). The same condition applied to the effectiveness of cytokinin application to stone fruit (Weinberger 1969), although it can be effective on apples (Williams and Stahley 1968). It could well be that cytokinins' involvement in dormancy starts only after other prior changes are induced (Lavee 1973).

1.1 The Development of Dormancy.

Day length and temperature are the most important environmental factors controlling the induction and breaking of dormancy in woody plants, and in many species, short days bring about the formation of resting buds.

In the summer the resting terminal bud of trees, for instance the peach, can be induced by cultural practices to renew growth, but later in the season it becomes increasingly difficult (Walker and Seeley 1973). Similarly Tinklin and Schwabe (1970) working with blackcurrant in England found three periods of differing behaviour of the lateral buds. Bud growth was inhibited initially by an influence from the apex, and then during June and July the leaves became increasingly inhibitory - when the buds can be described as in summer dormancy, and lateral buds are checked by correlative inhibition. In the autumn, when true dormancy has developed, it is the bud scales that provide the bud inhibition - possibly because of the inhibitors accumulated there after, perhaps, formation in the leaves. Dorffling (1976) considers true dormancy to develop progressively from correlative inhibition of the bud, and his data does not support the idea of the inhibitor ABA moving from a production site in the leaves to a site of accumulation in the buds as being the cause of such bud inhibition. Inhibitor levels are frequently found to rise under short days in the autumn, and to decrease with winter chilling and the end of dormancy (Phillips and Wareing 1959, Tinklin and Schwabe 1970). So the leaves and the bud scales can be seen to be involved in the imposition of dormancy - although it may not be only through an accumulation of inhibitors in the bud. Abbott (1970) has suggested dormancy may result from the depletion of leaf-synthesised growth promoters (cytokinin) causing the production of bud scales rather than further leaves.

1.2 Bud Break

Other parts of the plant may be involved in the development of bud dormancy, and in its termination. In Douglas fir seedlings, the initiation of spring shoot growth and bud activity is related to the level of GA

export from the roots (Lavender et al. 1973). In the apple, GA-like activity was detected in the sap only just prior to bud burst (Luckwill and Whyte 1968), and the cytokinins present in the sap at this time may also have a role in the emergence from winter rest. Since it is possible for buds to burst at favourable temperatures, in the absence of roots Skene (1967) has considered it unlikely that substances carried upwards in the sap normally provide the stimulus for bud burst. Further, Hewett and Wareing (1973a) found cytokinin activity in the buds of shoots collected at the end of dormancy can increase independently of a root system. So, while the roots did not appear to be an immediate source of cytokinins for bud growth, it was of interest that in shoots artificially chilled (in the absence of roots), bud and sap-cytokinin levels did not reach the levels present in normal field-chilled material (Hewett and Wareing 1973a). The buds themselves could be a site of cytokinin synthesis, but since in the field vacuum-extracted sap-cytokinin level reached a maximum three weeks before the bud-cytokinin peak, Hewett and Wareing (1973a) considered it more likely these substances were accumulated from the stem. Using maple seed van Staden et al. (1972) found low temperature stratification to induce germination caused endogenous cytokinin activity to increase, which also means this occurred in the absence of roots, which are usually considered a major point of cytokinin synthesis. Work with the grape (Skene 1972) has shown chilling of excised dormant canes increases the cytokinin level of their sap, indicating these substances are available to an emerging bud before a root system is established, or when root supplies are low.

Working with seedlings of Pistacia chinesis, Lee and Hackett (1976) found lateral root formation required the presence of physiologically non-dormant buds, probably through the effects of bud activity on cambial activity. This does not indicate a direct role for active new roots in the termination of dormancy. When mature Shiraz

grape vines were observed, it was found that rapid root growth began ten weeks after bud burst, at which time shoot growth was half-completed (Freeman and Smart 1976).

Clearly a number of growth regulators are associated with dormancy, which is no doubt regulated by a fluctuating balance between them, and thus the metabolic changes that lead to the onset of, or release from dormancy. In contrast bud dormancy has also been discussed less convincingly as being the result of a restriction of normal gaseous exchange by the covering structures (see Wareing 1969).

1.3 Growth Regulators and Dormancy.

Using partially purified extracts, bioassays have frequently been used to assess changes in bud-inhibitor levels, and the pattern has been for inhibitors to increase during autumn and winter, and decrease after the chilling requirement has been met (Phillips and Wareing 1958, Tinklin and Schwabe 1970). Early work concentrated on the β-inhibitor and "dormin", and now with the identification of ABA it is necessary to establish the relationship between both ABA and inhibitor activity and dormancy. The fact that ABA application to many woody plants soon transforms the active shoot's growing tip into a resting bud with typical scales (Wareing and Ryback 1970) suggests an involvement of ABA in bud dormancy, which is supported by other similar observations (see Milborrow 1974). In sap extracted from willow stems, high inhibitor and ABA levels are associated with the onset of dormancy, and growth in the spring was preceded by decreasing levels (Alvim et al. 1976). Without denying a role for other growth regulators, Wright (1975) has made a detailed study of changes in free and bound ABA in beech and blackcurrant buds, the results of which suggest an important role of ABA in dormancy induction and maintenance. It

was found the high autumn levels of ABA declined during the winter (to levels considered adequate to maintain dormancy), apparently by conversion to the bound form, and the level of bound - ABA declined with bud burst and subsequent transport or metabolism. Singha and Powell (1978) postulated that bud break is inhibited by ABA only if it is continuously available at above a certain threshold concentration.

In apricot buds both the onset and the end of rest is correlated with a decrease in inhibitors (Ramsay and Martin 1970), and these workers supported a hormone-balance theory of dormancy control. Like others, they found gibberellins seemed to be involved, and frequently there is a reciprocal variation in the levels of inhibitors and gibberellins associated with dormancy of buds and tubers (Wareing 1969).

So it has been suggested that dormancy ends when endogenous gibberellins increase, and overcome the effect of the inhibitors present, and Browning (1973a) suggested bud ABA may regulate gibberellin supply in the buds. He identified the stimulus for dormancy release of coffee flower buds as gibberellin - produced or released in the buds, rather than supplied by the xylem sap. Since the increase in bud-gibberellins is most rapid after the chilling requirement has been met, changes in GA-like activity may be a secondary, rather than a causal factor in the breaking of dormancy (Larson 1962 quoted in Perry 1971).

Some authors have recently cast doubts on a role for ABA in dormancy. Work with several species could not relate ABA levels to changes in photoperiod and the usual changes in "inhibitor β" (Lenton et al. 1972), leading them to suggest it was the level of growth promoters that changed and resulted in dormancy, and not the level of ABA. Also using gas-liquid chromatography, Mielke and Dennis (1975) found free ABA levels in cherry buds highest in the

autumn, and then declining rapidly. This pattern is very similar to that in beech and black-currant buds (Wright 1975), except in the latter cases the peak usually occurred at an earlier stage of development. In cherry, maximum ABA occurred when 90% of the leaves had abscised, and Mielke and Dennis (1975) consider ABA level is associated with leaf abscission rather than bud dormancy. The correlation in some years between peak ABA and deepest dormancy would perhaps then be coincidental, although ABA levels had returned to very low levels shortly before dormancy was broken. As defoliation prevents the autumn increase in bud-ABA without affecting rest, and bud-ABA levels decline similarly at warm (22°C) or cold (4°C) temperatures, it is considered doubtful that ABA plays a regulating role in winter bud dormancy of sour cherry (Mielke and Dennis 1978). Similarly Emmerson et al. (1978) suggested that if there is an interaction between chilling and ABA, it is of secondary importance in spring bud burst of grape.

Reference has already been made to the work of Hewett and Wareing (1973a), and the model of Abbott (1970), implicating cytokinins in the breaking of dormancy. In Betula and Populus bud break is accompanied by an increase in bud cytokinin (Domanski and Kozlowski 1968). In studies of seed dormancy also these substances are referred to, as in Khan's hypothesis (1971) of their playing only a permissive role in dormancy, and Webb et al. (1973) who suggest endogenous cytokinins are one part of a phasic process of dormancy termination. They also make the point that the state of dormancy may not be determined by the level of hormone present at one time, but by the ability for certain sequential changes to occur within the dormant tissues, and I referred earlier to the possibly meaningless determination of levels of endogenous hormone activity.

In discussing dormancy release in poplar buds, Bachelard and Wightman (1974) suggest this occurs in several distinct phases beginning with an increase in the

GA/Inhibitor ratio, followed by increased translocation and cytokinin production, and finally extension growth. Cytokinin activity was not determined, but their scheme requires the presence of roots to supply it. Others, however have not considered the roots to be the source of cytokinins involved in bud burst (Browning 1973b, Hewett and Wareing 1973a, Skene 1972).

In willow stems the sap-cytokinin activity increased abruptly as the flower buds burst, with another peak when leaf buds burst, with a low activity being present at other times (Alvim et al. 1976). Similarly Hewett and Wareing (1973a) found cytokinin activity in poplar buds and sap rose sharply at the time of bud burst, particularly in material field-chilled rather than artificially chilled. Activity in the sap peaked two weeks before bud burst, and three weeks before a peak in bud-cytokinin activity, and it was observed bud cytokinins rose even when bud burst was induced in the absence of the root system. Irrigation releases coffee flower buds from dormancy, and this is accompanied by a rapid increase in cytokinin activity in stem-sap, followed a day or two later by an increase in bud-cytokinin. Browning (1973b) interprets his data as meaning the cytokinin is released from within the wood itself and not transported from the roots - similar to the views of Hewett and Wareing (1973a).

Working with maple seedlings Dumbroff and Brown (1976) followed root activity and cytokinin activity during the chilling period. When plants were maintained outside, after chilling there was rapid root growth followed closely by a peak in cytokinin activity in the lateral roots and stem. Hormone activity declined just before bud burst, in the usual manner. Dumbroff and Brown (1976) considered that as cytokinins appeared after the transition of the buds from the dormant to the quiescent state i.e. after the chilling requirement has been met, they cannot provide the actual stimulus for dormancy release - although they could well be important for the

subsequent bursting and growth of buds, - somewhat similar to the view of Bachelard and Wightman (1974).

C-2 APICAL DOMINANCE AND CORRELATIVE INHIBITION.

The growth of a lateral bud may be inhibited by a correlative influence of the dominant shoot apex, and this subject of apical dominance is of widespread incidence and can with value be considered at the same time as the phenomenon of bud "dormancy". It has recently been reviewed by Phillips (1975) and discussed by many other authors (see Shein and Jackson 1971, Tucker and Mansfield 1973, Woolley and Wareing 1972b).

In contrast to true dormancy, studies on gravimorphism in fruit trees showed the bud inhibition of normal apical dominance relationships is readily capable of being reversed by changes in orientation (Wareing and Nasr 1961). Work with a number of plants, manipulating the shoots in various ways, has indicated a number of hypotheses that do not explain the clearly defined pattern of bud burst following bending (Smith and Wareing 1964a), and the effects noted were considered more compatible with indirect theories (than theories of direct action) of the role of auxin in apical dominance and correlative inhibition (Wareing and Nasr 1961). These authors found in their plant system, that proximity to the roots conferred some advantage for lateral bud growth, which was not nutritional (Smith and Wareing 1964a, 1964b). Processes occurred in the roots to provide some factor which was apparently capable of passing through the xylem - and while GA_3 could partially substitute for the roots, this substance could be only part of the root-factor complex responsible for bud burst and growth (Smith and Wareing 1964b). But in other studies bud growth followed decapitation in the absence of roots, even when the roots were removed three days earlier - and the initial bud growth rate was inde-

pendent of the presence of roots (Nagao and Rubinstein 1975, Peterson and Fletcher 1975). However, Woolley and Wareing (1972a) indicate a root factor may be stored in stem tissue and subsequently be effective in promoting bud growth. It has been indicated that apical dominance can be modified either by interfering with hormone production in the shoot tip, or by modifying the background hormone balance within the plant (Shein and Jackson 1971), It was shown with gravimorphism in willow, that bud burst was also determined by some mechanism acting between the bud and the immediately adjacent stem tissue (Smith and Wareing 1964a). It is still necessary to determine the mechanism controlling the distribution of the root factor along the shoot, between the various meristems, and determining their behaviour.

Working with decapitated stem cuttings of soybean plants Peterson and Fletcher (1975) found bud growth increased as the length of the stem piece was increased, and treatment with BAP could not replace the effect of the stem, although either carbohydrate or BAP together with stem tissue increased bud growth. However, the stem seemed to supply a factor for bud growth, rather than one to release the buds from their inhibition, and Peterson and Fletcher (1975) are inclined to believe bud growth was inhibited by factors other than just carbohydrate supply. They also indicate a possible auxin involvement, as placing the long stem segment apical to the bud completely inhibited bud growth.

In considering apical dominance and how it inhibits bud development, it is essential to be clear what one is considering. Primarily one is interested in the initial release from inhibition, and how this is controlled, rather than in the subsequent growth of the bud. Some studies of apical dominance have studied the control over lateral shoot growth, and not of bud burst (e.g. Phillips 1971b, Shein and Jackson 1971).

The growing apical bud of a plant or shoot inhibits the development of lateral shoot buds, and decapitation of such a system results in bud growth - otherwise the buds are inhibited by some other means. Other factors are also known to modify apical dominance. Gravity plays a part as orientation of the lateral organ affects bud growth, and instances have been recorded of nutrient supply regulating lateral bud development. Removing some young leaves (but not mature ones) overcomes the inhibition of axillary buds (Shein and Jackson 1971) - the number developing depending on the number of young leaves remaining (Crabbe 1972). Between summer growth flushes the lateral buds of citrus are correlatively inhibited by the leaves, and in Poncirus trifoliata this effect is also produced by NAA (Stathakopoulos and Erickson 1966 quoted in Cooper et al. 1969), and defoliation results in bud break. Similarly in the apple, NAA applied instead of leaves prevents bud burst while normal bud development continues (Fulford 1970). Sprays of KGA and BAP are effective in the absence of the leaves (Cooper et al. 1969). Today it appears emphasis must be placed on the growth regulators in a study of the control of lateral bud development.

2.1 Growth Regulators.

(a) Auxin -

In decapitated plants, the inhibitory effects can be accomplished by applying auxin, which is also synthesised in growing apical buds. This, and the studies of the effects of inhibitors of auxin transport (Phillips 1975) have indicated a primary role for auxin in correlative inhibition.

The primary correlative signal for the inhibition of lateral buds appears to be auxin derived from young growing leaves, and anything else follows from that.

Working with etiolated stems of pea, Wickson and Thimann (1958) found physiological concentrations of auxin completely inhibited bud growth, but the inhibiting action of auxin could be completely antagonised by kinetin. The release of buds from inhibition appeared to result from a fundamental and sudden change, and it was suggested this resulted from an interaction between auxin and a kinetin-like substance within the stem. Thomas (1972) recorded an increased auxin content of the upper lateral buds when the apex was removed, thus creating a new 'sink' towards which subtrates would move. This could include a hormone-directed transport of other growth regulators, and Morris and Winfield (1972) found ^{14}C-labelled kinetin moved to the axillary buds of decapitated plants, but not if the cut stem was pretreated with IAA. So they suggest decapitation resulted in the diversion of cytokinins from within the plant, to the axillary buds, and so caused their outgrowth. Similarly the detailed work of Woolley and Wareing (1972a, 1972c) has indicated a role of auxin in controlling the distribution and metabolism of cytokinins - but auxin activity must still be considered the primary correlative signal.

(b) Gibberellins, Abscisic Acid -

While gibberellin promotes lateral shoot growth after bud inhibition is overcome (Wickson and Thimann 1958), it is not clear what, if any, role gibberellins play in the release of the bud inhibition, or whether they participate in the maintenance of correlative bud inhibition (Phillips 1971b). Working with pea buds Wickson and Thimann (1958) found growth inhibition by IAA was in no way overcome by GA_3, although KGA has some effect in overcoming apical dominance in citrus (Cooper et al. 1969).

The principal site of ABA synthesis seems to be in the mature leaves, and so it is unlikely to act as a correlative signal from the apical bud. But where there is correlative inhibition of an axillary bud by its subtending mature leaf, ABA may be involved, and Dorffling (1964,

quoted in White and Mansfield 1977) correlated levels of extractable "inhibitor β" with the inhibition of lateral bud growth in Acer. However data for Phaseolus vulgaris suggest it is unlikely that ABA acts as a correlative inhibitor in this plant (White and Mansfield 1977). In Xanthium ABA levels are much higher in lateral buds that are not growing, and Tucker and Mansfield (1973) conclude it is synthesised in them in response to apically produced auxin travelling basipetally, and then inhibits bud growth. Tucker (1977) considered auxin exerted its effect through its role in hormone-directed transport, and on the promotion of ABA in the region of the lateral bud. Working with tomato he found ABA was important in the control of bud outgrowth, and considered its formation in the stem (or elsewhere) was induced by auxin.

(c) Cytokinins -

Application of these substances to dormant buds of apple will induce bud burst (Williams and Billingsley 1970), and they will similarly promote lateral bud growth of actively growing apple shoots (Kender and Carpenter 1972). Other reports also have shown the ability of exogenous cytokinins to relieve axillary buds from correlative inhibition (Ali and Fletcher 1970, Sachs and Thimann 1967) - although subsequent growth may require the presence of other growth regulators.

The evidence is that decapitation results in the initiation of new processes within the inhibited buds, and that similar changes follow the direct application of cytokinins to buds. This type of growth regulator apparently plays a fundamental role in apical dominance, and one could assume inhibition of buds by correlative inhibition results from a cyotkinin deficiency. However, this would not make cytokinin the primary correlative signal for bud growth, for that signal must be the factor which determines the cytokinin supply to the buds.

Bud growth could be dependent on the bud's ability to synthesise cytokinins and Sachs and Thimann (1967) suggest this is only initiated when auxin from the apex declines. There is, however, evidence of root-cytokinins being involved in apical dominance and that cytokinins are not synthesised in lateral buds on release from apical dominance (Woolley and Wareing 1972a).

It may be that bud growth is dependent on cytokinin utilisation within the bud rather than its mere presence. It has been argued factors other than cytokinin are limiting bud growth, and these inhibitory factors within the bud must first be mitigated (Nagao and Rubinstein 1975), although they found no increased bud sensitivity to BAP on decapitation.

The third manner in which an effective cytokinin deficiency could occur would be where there is a change in the distribution of root-synthesised cytokinins. Morris and Winfield (1972) suggest the absence of the apex results in cytokinins being transported to the newly-developed centres of growth; similarly Phillips (1968) and Woolley and Wareing (1972c) considered the apex may attract cytokinins, and so divert them from the lateral buds. They found evidence that cytokinins do accumulate in buds before shoot growth occurs, and the auxin is involved in cytokinin distribution (Woolley and Wareing 1972c).

In further work it was found that in addition to influencing cytokinin transport, IAA greatly affects cytokinin metabolism (Woolley and Wareing 1972a). Thus IAA inhibited the accumulation of cytokinin in lateral buds that precedes their growth, and that rather than causing its accumulation at the (apical) site of auxin production, promoted the formation of a cytokinin metabolite in the stem tissues (Woolley and Wareing 1972a).

EXPERIMENTAL

Section 1

Shoot Growth

Using intact plants the first studies recorded the pattern of shoot elongation, and the degree to which the presence of leaves controlled stem growth. Further, the contribution of gibberellins to stem elongation was assessed by applications of gibberellic acid.

Because of the growth behaviour of the kiwifruit vine, particularly of some varieties and in some districts, a full description of the factors controlling stem growth is desirable - both environmental factors and plant characteristics. One possible means of growth control is by chemical regulators, but their use demands a full understanding of their effects on the fruiting plant.

A. Shoot Growth of the Kiwifruit Vine

Initially, some observations were made of shoot elongation in the intact vine, as one aspect of growth control in this plant.

Experiment 1: 1973/4 - In the spring a number of Abbott plants produced from rooted cuttings the previous summer were selected, and pruned to one shoot 8cm long. The plants were grown in plastic bags of sand in the glass-house, and watered frequently with a complete nutrient solution. When the new single shoot was about 30-40cm high, it was trained vertically, and treatments began on 1/10/73 initially with 8 plants in each treatment. At this time each plant had about 10 visible internodes above the first normal leaf.

Treatment 1: Control plants.

2: Decapitated - at a point immediately below the apical bud, and also removing those leaves not yet unfolded from it.

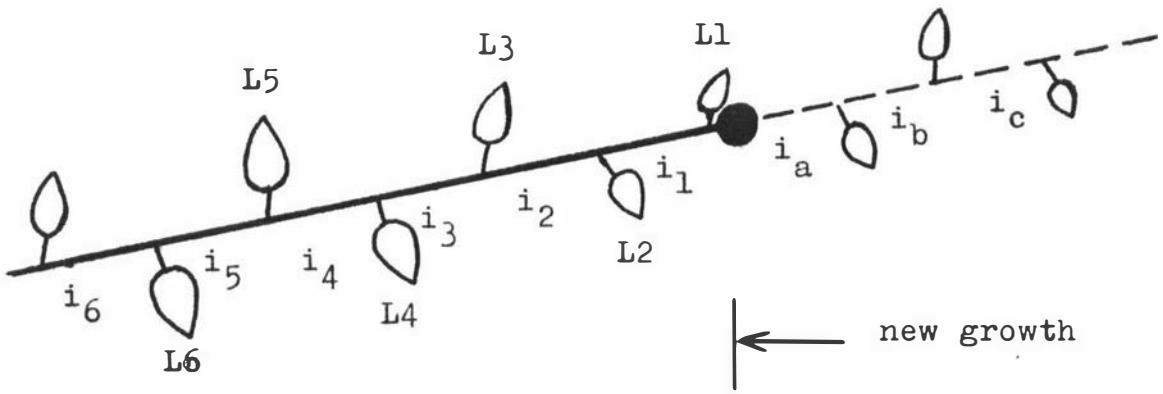
3: Light defoliation (-2L) - the two uppermost unfolded leaf blades (the internode between them being about 3mm long) removed.

4: Moderate defoliation (-4L) - as for (3) but the next two youngest leaves also removed within a couple of days.

5: Severe defoliation - removed all unfolded leaves except the lowest one. Subsequently all leaves were removed as early as possible as they were unfolding from the apical bud.

On all plants the leaf corresponding to that remaining in treatment 5 was marked, and used as the base point for all measurements of shoot length.

At the start of treatment the length of each of the upper six internodes of each plant were measured, and again at intervals until 23/11/73. The length of subsequent internodes was also measured, and the labelling system used was as follows:-



Results

(a) Shoot extension -

Over the 7 weeks following the start of treatment, mean shoot length in the control plants increased linearly and was almost identical with that of the -2L and -4L treatments (Fig.1). Where the plants were continuously defoliated, mean shoot growth rate was lower, and shoot extension over the period of observation, was much less than in the other treatments (Table 1).

However, it will be noted (Fig.11) using plants maintained in a flowing nutrient solution culture, over a shorter period in 1972, that defoliation did not alter the rate of shoot elongation - in the presence or absence of GA_3. In 1972 there was much more variation between plants, whereas in 1973, individual plants corresponded closely with the mean values shown in Fig.1. This, along with the additional 14 days observation this year, makes the 1973 treatment differences more realistic than the earlier observations.

In the month following treatment, the level of extension growth of decapitated plants followed that of the defoliated plants.

It is clear from Table 1 that continuous defoliation resulted in a marked increase in the number of internodes produced, so that finally these plants had an average of 1.97 internodes/10cm stem length, compared with 1.08 in the control plants. Extreme defoliation also resulted in a substantial increase in the size of the one remaining leaf, and this was nearly equalled by the increase in size of the basal leaf of the decapitated plants - although analysis of variance showed there was no significant (p 0.05) difference between treatments in the diameter of this leaf on 23/11/73. However, all treatments resulted in a tendency towards increased size of the basal leaf, over that made by the control plants. A similar effect on internode number and leaf size was record-

Table 1: Mean Response to Pruning Treatments.

Treatment	Initial shoot length (cms) 2/10/73	% increase to 23/11/73	Initial number inter-nodes 2/10/73	Final number inter-nodes 23/11/73	% incr.	Initial basal leaf diam. (cms)	% Increase 23/11/73
1. Control	32.1	709	8.87	28.0++	218	8.3	5.06
2. Decapitate	30.4	-	-	-	-	8.2	9.76
3. -2L	37.0	632	-	-	-	9.1	8.46
4. -4L	35.7	686	-	-	-	9.34	8.14
5. Defoliate	33.8	429	8.71	35.6++	309	8.87	11.6

++ Analysis of variance: significantly different at 1% level. L.S.D. 3.7

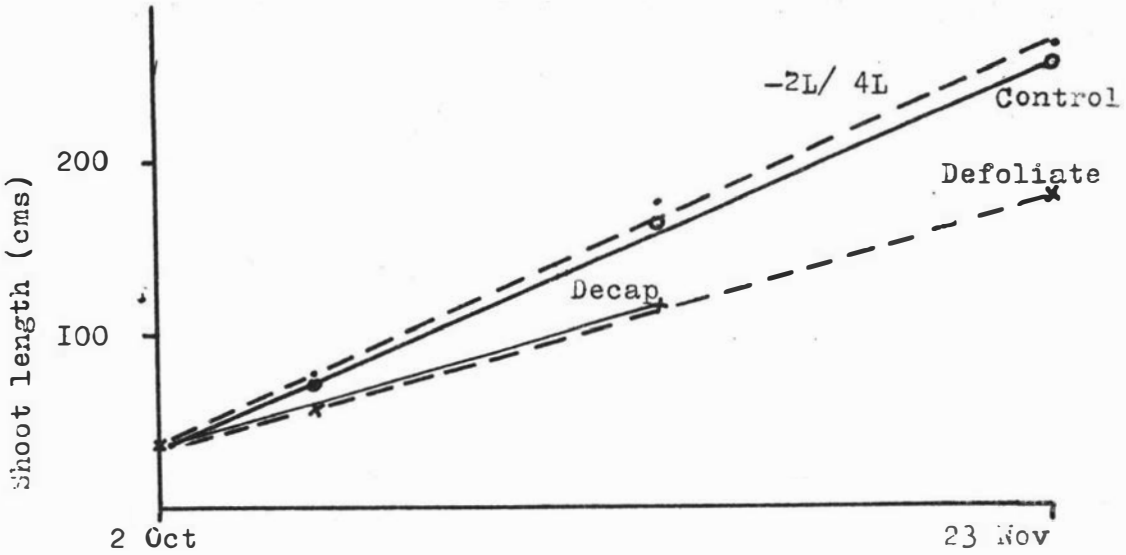
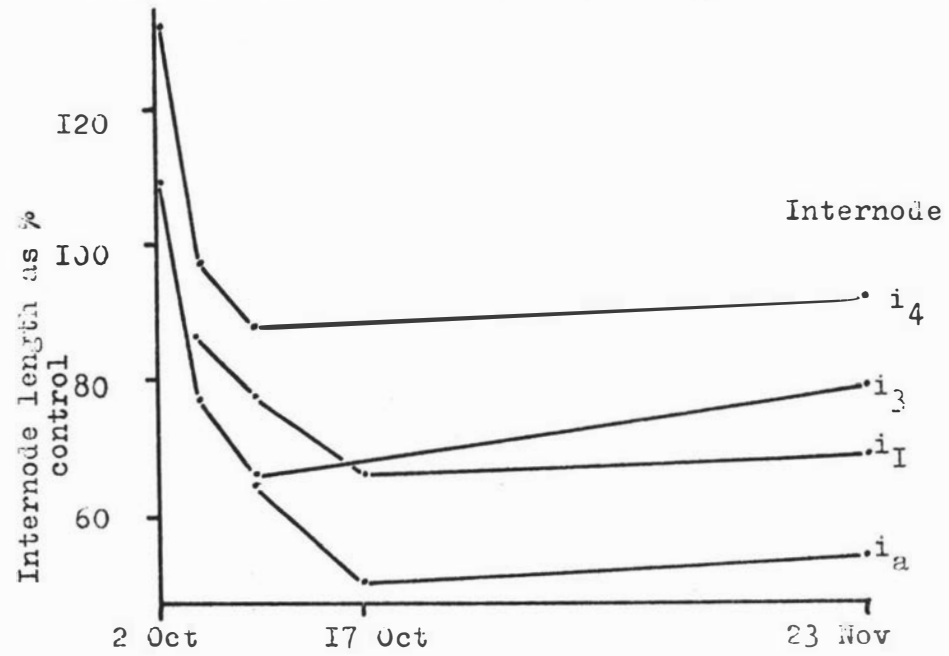
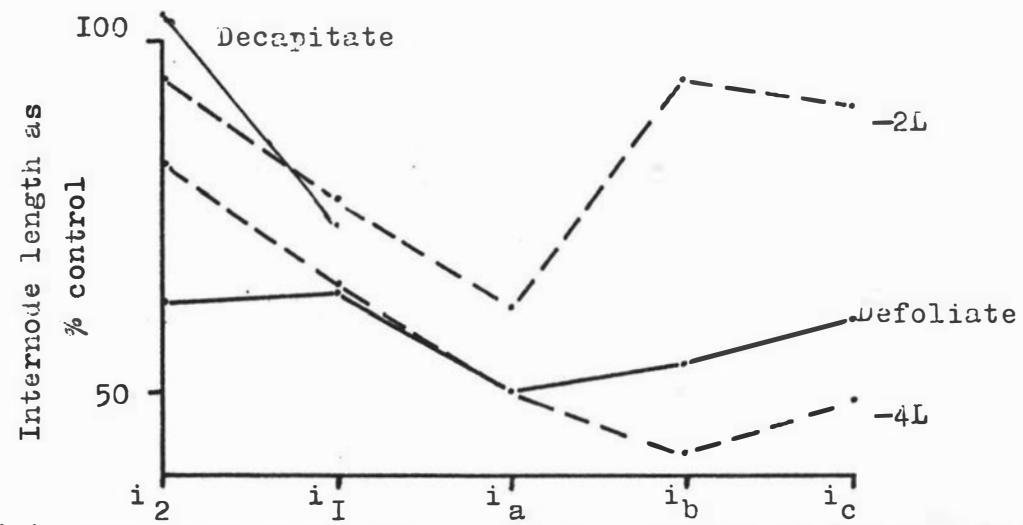


FIG. I. EFFECT OF DEFOLIATION ON SHOOT GROWTH.



(a) Effect of defoliation on internode growth.



(b) Effect of defoliation on internode length after 16 days.

FIG. 2. EFFECT OF DEFOLIATION ON INTERNODE GROWTH.

ed in 1972.

An effect of defoliation on leaf size was also noted with four other plants, two of which had 12 leaves removed in order, as they appeared below the apical bud. The next four leaves were untouched, and at maturity were larger than the equivalent leaves of control plants. The diameter of the first two leaves were 166% of controls, and the next two 127% of controls.

Defoliation stimulated the growth of many axillary buds, but these were removed immediately after bud burst.

(b) Internode elongation -

The effect of treatment on the growth of individual internodes is shown in Fig 2 and 3, expressed relative to the equivalent internode of untreated plants.

Removal of young leaves reduced the growth rate, and the final length of the internode above that leaf. The effect of removing even two small leaves was clearly noticeable two weeks later - and after 7 weeks was noticeable only in the internode above the leaf that was smaller at the time of its removal. Also where four leaves were removed, the reduction in internode length was most marked where its subtending leaf was removed at an earlier stage. Apparently removing a leaf when it is loosely enveloping the growing tip has a greater effect on subsequent internode length than doing so a few days later.

The removal of a small number of leaves had a very localised effect, there being minimal effect on other more distant internodes, although there was a tendency for the first internode below the removed leaf blades to be of increased length.

Each plant had about 10 expanded leaves at the start

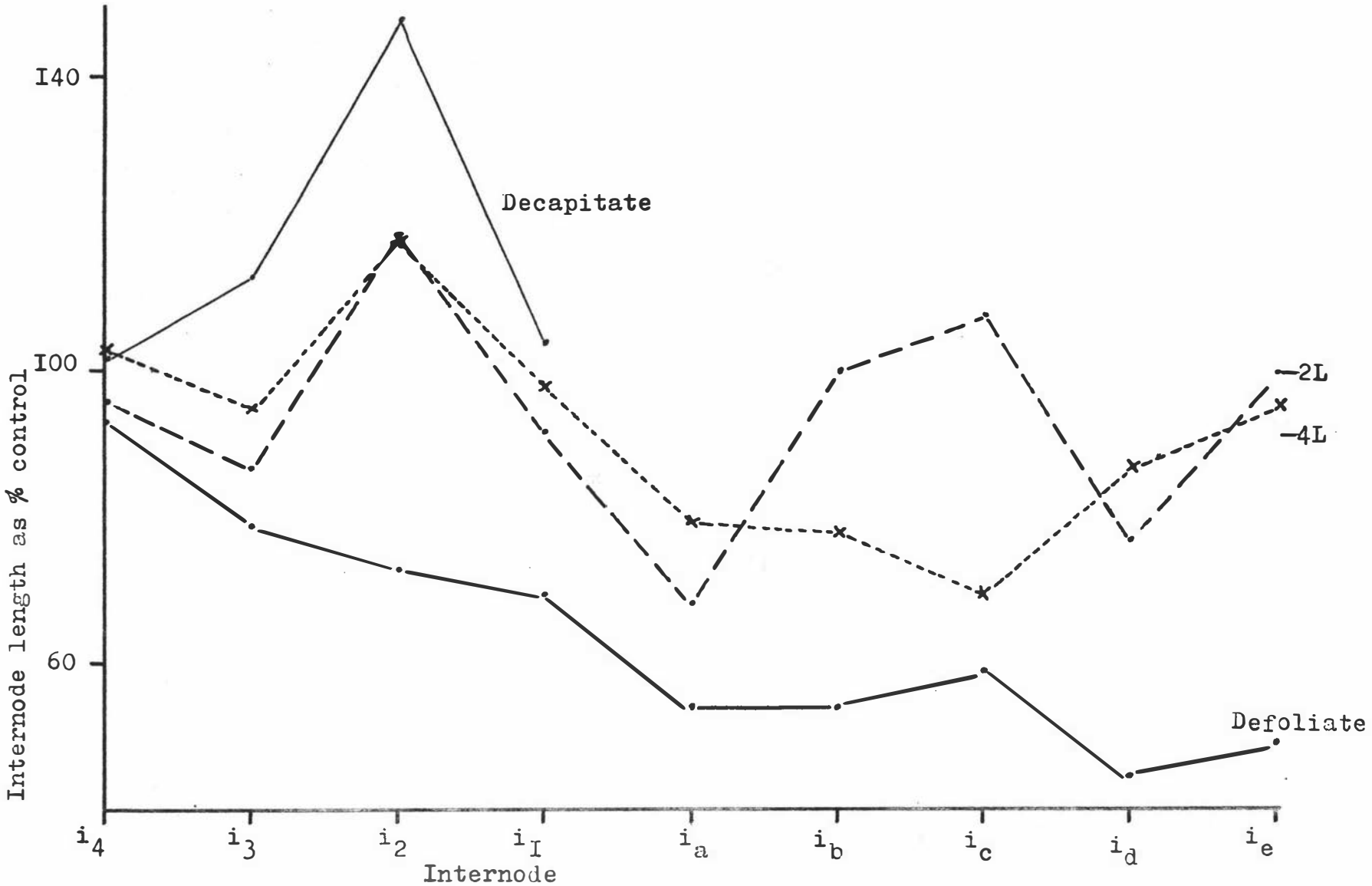


FIG. 3. EFFECT OF DECAPITATION AND DEFOLIATION ON INTERNODE GROWTH AFTER 53 DAYS (23/II/73).

of treatment, and about 12 more expanded leaves at the end of the experiment. However, when all of these leaves were continuously removed, to leave only one basal leaf, the internodes affected were more widespread. Fig. 3 shows that such defoliation results in a series where each succeeding internode becomes a lesser and lesser proportion of the comparable control internode length. Not only were the internodes that were within the apical bud at the start of defoliation affected, but also several lower internodes defoliated while still actively elongating.

When the shoot tip was removed, it resulted in an increase in the final length of one internode. It had no effect on the internode immediately below the tip, but on the next lowest. Later, three further plants were decapitated and compared with matched control plants. After a period of 39 days there was a marked variation between the decapitated plants but usually the length of each of the three upper internodes was increased, and none was reduced. Expressed as a percentage of the controls, means of internode length for internodes one to three were 235, 132, 156% following decapitation.

On an earlier occasion, close-interval measurements were made of internode extension. On 17/10/72 a single strong-growing plant with two similar shoots was selected. It was grown in the glasshouse, and at the start the lowest leaf was almost fully expanded. Five internodes numbered basipetally from number 1 immediately below the apical bud, were each marked in three parts of equal length. At 3-4 day intervals the length of each of these parts was measured.

Results.

Each shoot behaved similarly, and the pattern of growth of each internode is shown in Fig. 4. Typical results for the various parts of each internode are present-

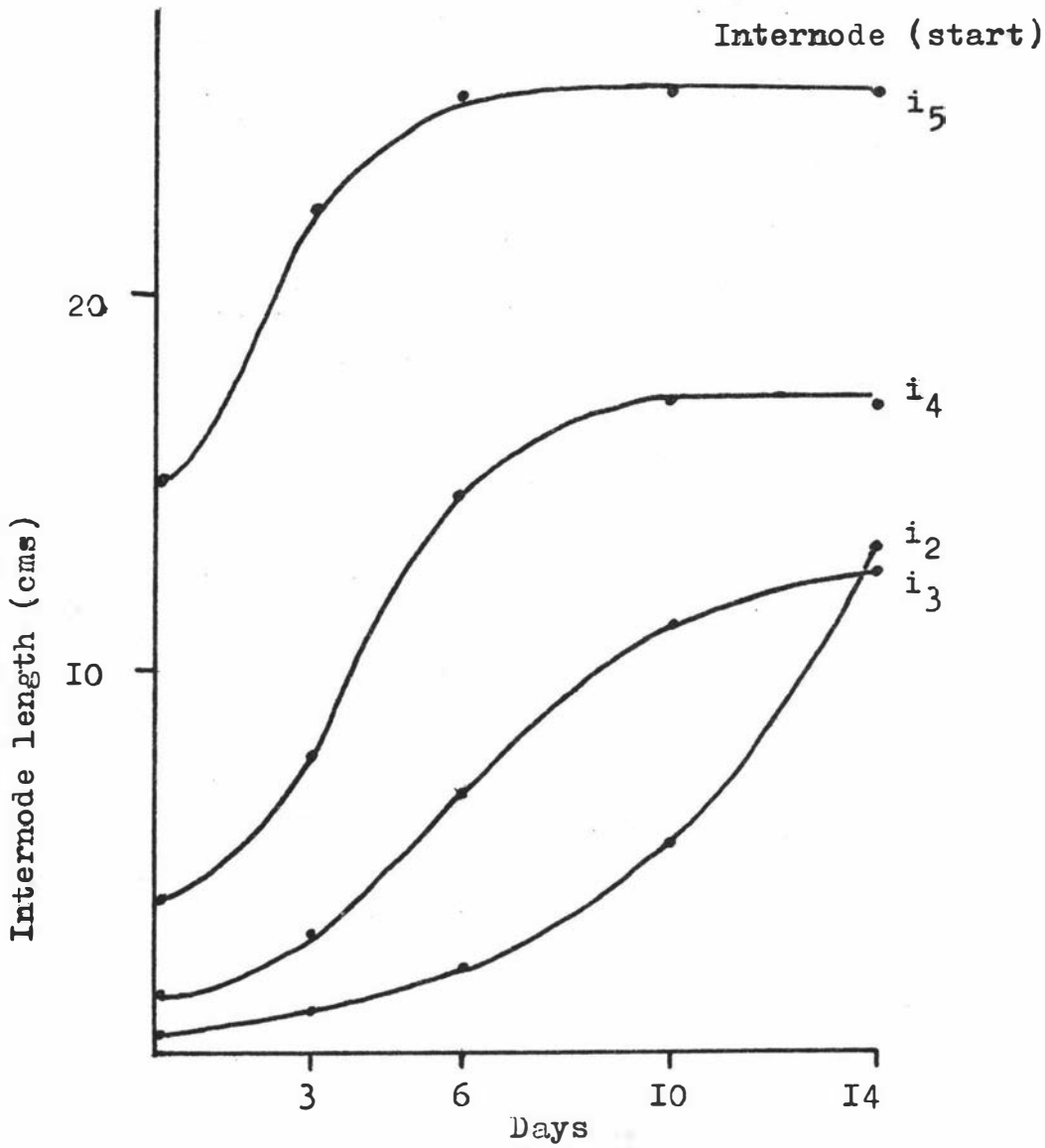


FIG. 4. GROWTH OF KIWIFRUIT PLANT INTERNODES.

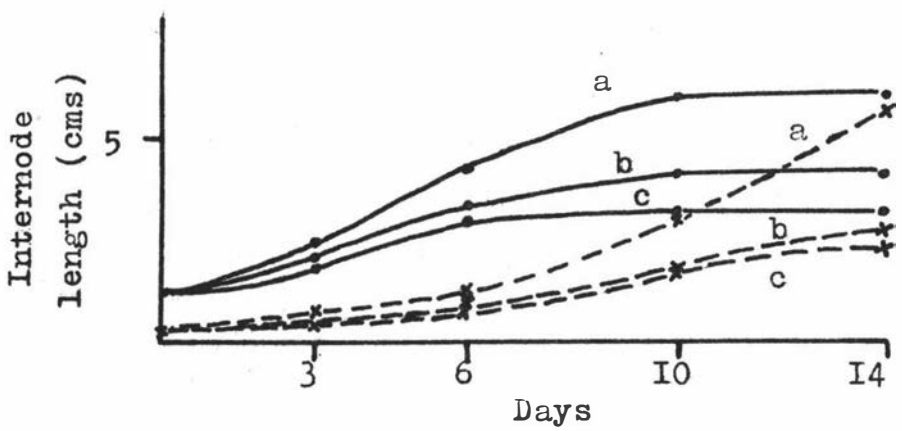


FIG. 5. DISTRIBUTION OF GROWTH WITHIN AN INTERNODE.

i_4

i_2

a Upper one third

b Mid one third

c Lower one third

ed in Fig. 5.

It will be seen that over the two weeks observations, as one moves basipetally the growth pattern of each internode is more advanced. As soon as a given internode became the 7th one below the apical bud, its growth rate began to fall, and when it occupied the 8th position it had ceased elongation.

On 1/11/72 internodes 1 and 2 still had an increasing growth rate. In i_1 this high growth rate was occurring in the upper two-thirds of the internode, that in the lower one-third having been declining for about 4 days. In i_2 only the upper one-third had an increasing growth rate (Fig.5).

In internodes 3 to 5 their high growth rate continued for a time and then levelled off, and as in i_1 and i_2 more growth occurred the more distal the area within the internode.

Experiment 2: 1972 - After stratification, seed of an unknown variety of kiwifruit was germinated on paper moistened with distilled water. At various stages after radicle emergence, 5 - 6 seeds were placed in standard solution of gibberellic acid, and incubated under fluorescent light for 8-13 days.

When stem length was measured, there was clearly an effect of increased elongation with increased GA_3 concentration (Fig. 6). This work was not continued.

Experiment 3: 1973/74 - Healthy, actively growing young Abbott plants in containers in a glasshouse were selected, and sprayed to runoff with an aqueous growth regulator spray and wetting agent.

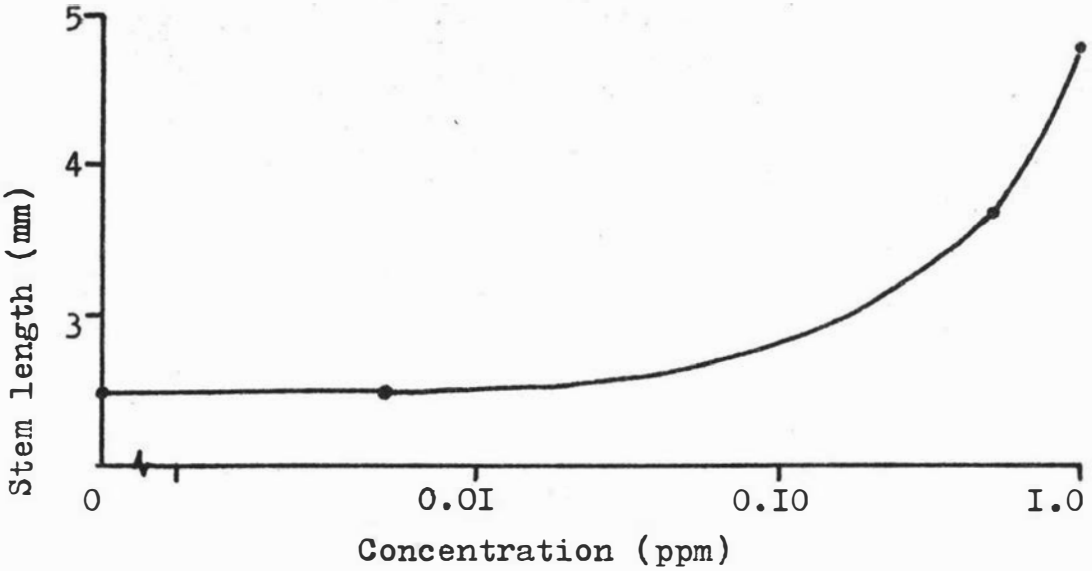


FIG. 6. STANDARD CURVE FOR KIWIFRUIT SEEDLINGS RESPONSE TO GIBBERELLIC ACID (8 days).

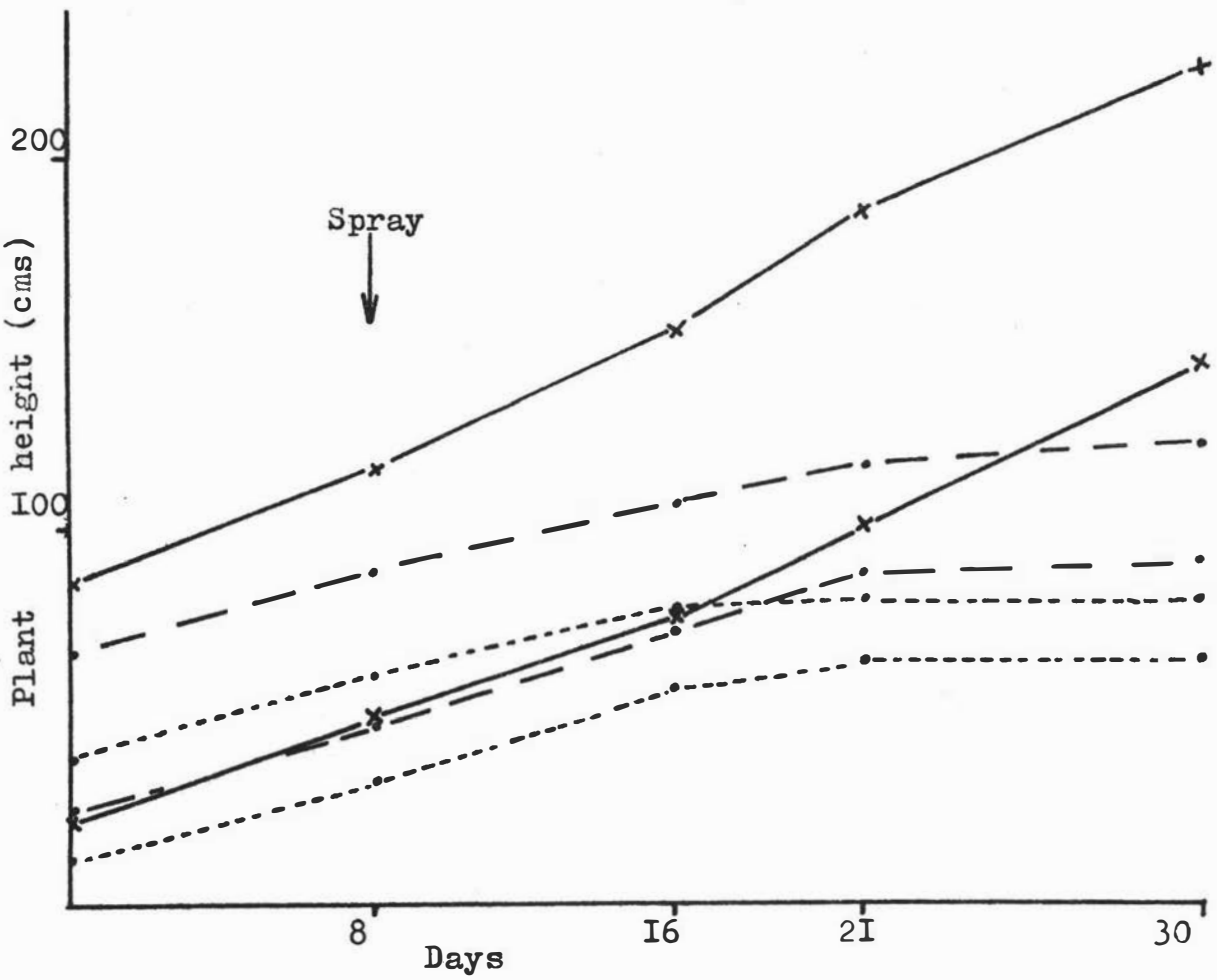


FIG. 7. EFFECT OF GROWTH REGULATOR SPRAYS ON PLANT HEIGHT

×——×Unsprayed •-------•MH spray •— — —•MH/GA spray

Results:

(a) Sprays of either maleic hydrazide (1500ppm), or maleic hydrazide (1500ppm) + GA_3 (200ppm) were applied to three plants.
Both spray treatments resulted in shoot elongation ceasing two weeks after the spray application (Fig.7). The sprays caused a yellow discolouration of the young leaves one week after spraying, and later an inrolling developed in the tip leaves. When extension growth stopped, bud burst occurred in the lower axillary buds. After another month the original shoot tip had died back, and most of the axillary buds had produced a shoot up to 150cms in length, which appeared perfectly normal.

(b) In a comparison with unsprayed control plants, a spray of 200ppm GA_3 to three plants had no effect on shoot growth rate over 3 weeks.

(c) A similar result was obtained with a further group of plants sprayed in early December with either 50 or 500ppm GA_3. Such a single spray did not affect the growth rate of these plants over a 17 day period (Fig. 8).

Experiment 4: 1973/4 - Four plants growing in the glasshouse in containers were watered on one occasion with CCC at 2000ppm. Enough solution was added to cause some loss in drainage.

Results:

Over a 6 week period there was no change in growth rate of the treated plants (Fig. 9). Half of a similar group of plants was watered with CCC at 11a.m., and decapitated 2 hours later, and the bleeding sap collected for two days. In both the first and second day after decapitation, the control plants produced twice as much sap as those treated with CCC.

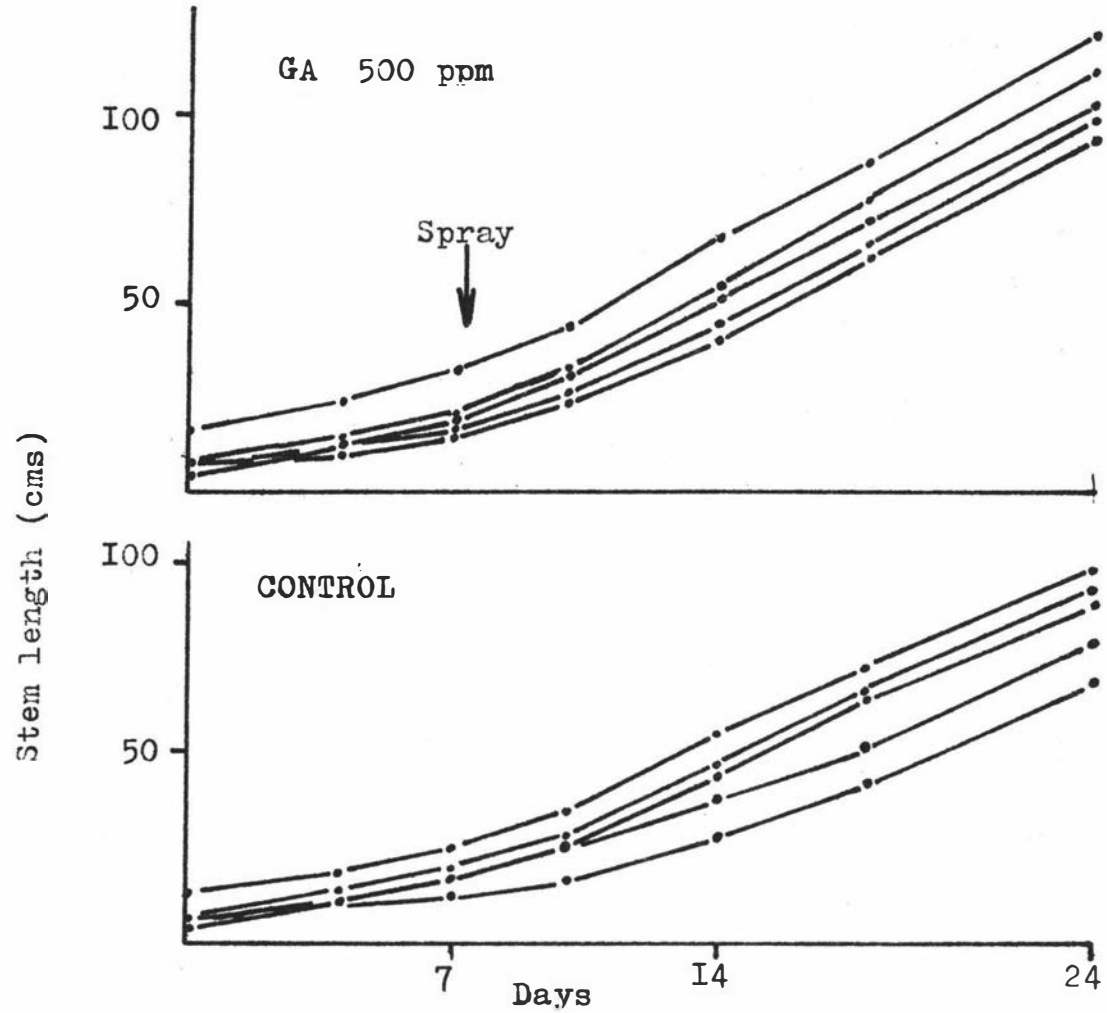


FIG. 8. EFFECT OF GA ON KIWIFRUIT PLANT HEIGHT.

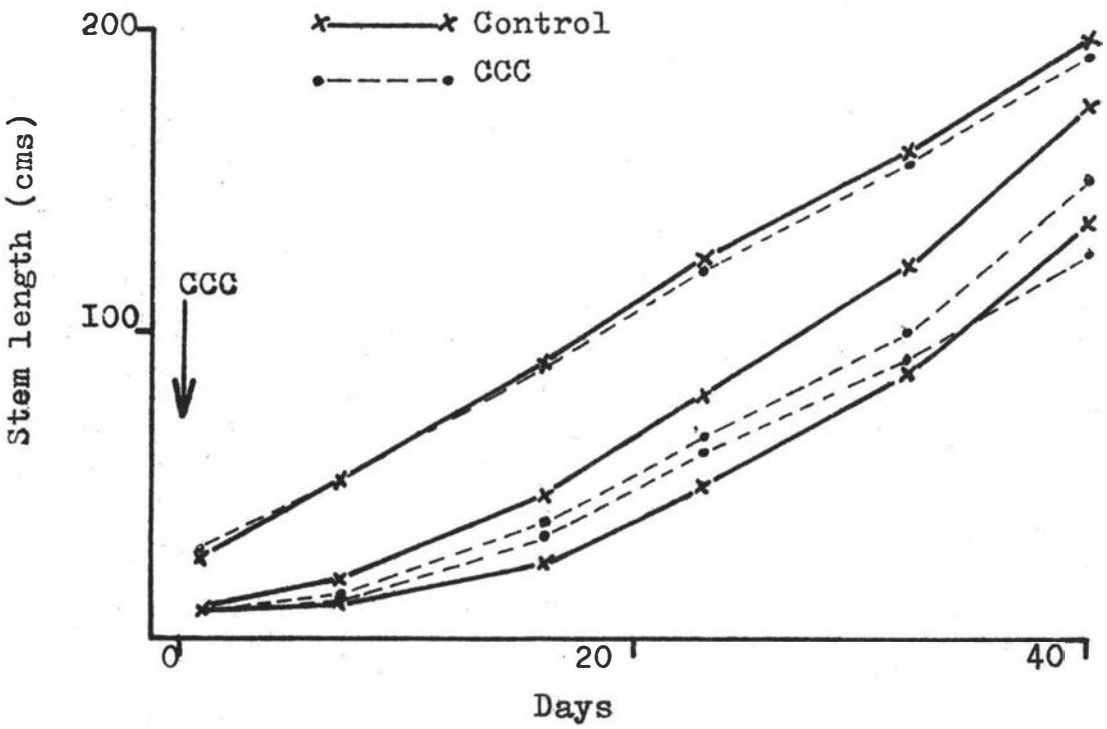


FIG. 9. EFFECT OF CCC ON KIWIFRUIT PLANT HEIGHT.

Experiment 5: 1969/70 - A growth retardant spray was applied to vines on a commercial orchard in Te Puke, and observations of vine growth made by the grower.

One spray of SADH at 2000ppm was applied to runoff to one Hayward and one male vine (4 years old) on 14/12/69, at which time shoots were less than 120cms long, and fruits were about 25% developed. Two similar vines were also sprayed with maleic hydrazide (MH-30) at the same time and concentration.

Observations were then made of shoot growth and fruit quality of sprayed and untreated vines.

Results

(a) Shoot growth -

In both male and female vines sprayed with either MH-30 or SADH, a shortening of shoot internodes was noticeable 10-14 days after spraying, and shoot growth ceased for the season after 3-4 weeks. In the following winter, canes on unsprayed vines were up to 3m long, and ranged from 60-120cms on treated vines. Bud development appeared unaffected.

Vines were also sprayed in November or mid-December 1970. Limited observations indicated a spray of 1700ppm at these times reduced final shoot length over the vine.

(b) Fruit quality -

Fruit which was picked from treated vines in May and June 1970, had a good average weight of 105gm, but only the smaller-sized control fruits (mean 60gm) were available for comparison. Limited measurements of juice quality showed no differences between treatments in total soluble solids, titratable acid or Vitamin C content after ripening at 18.3°C

Maleic hydrazide may affect fruit size. When one-

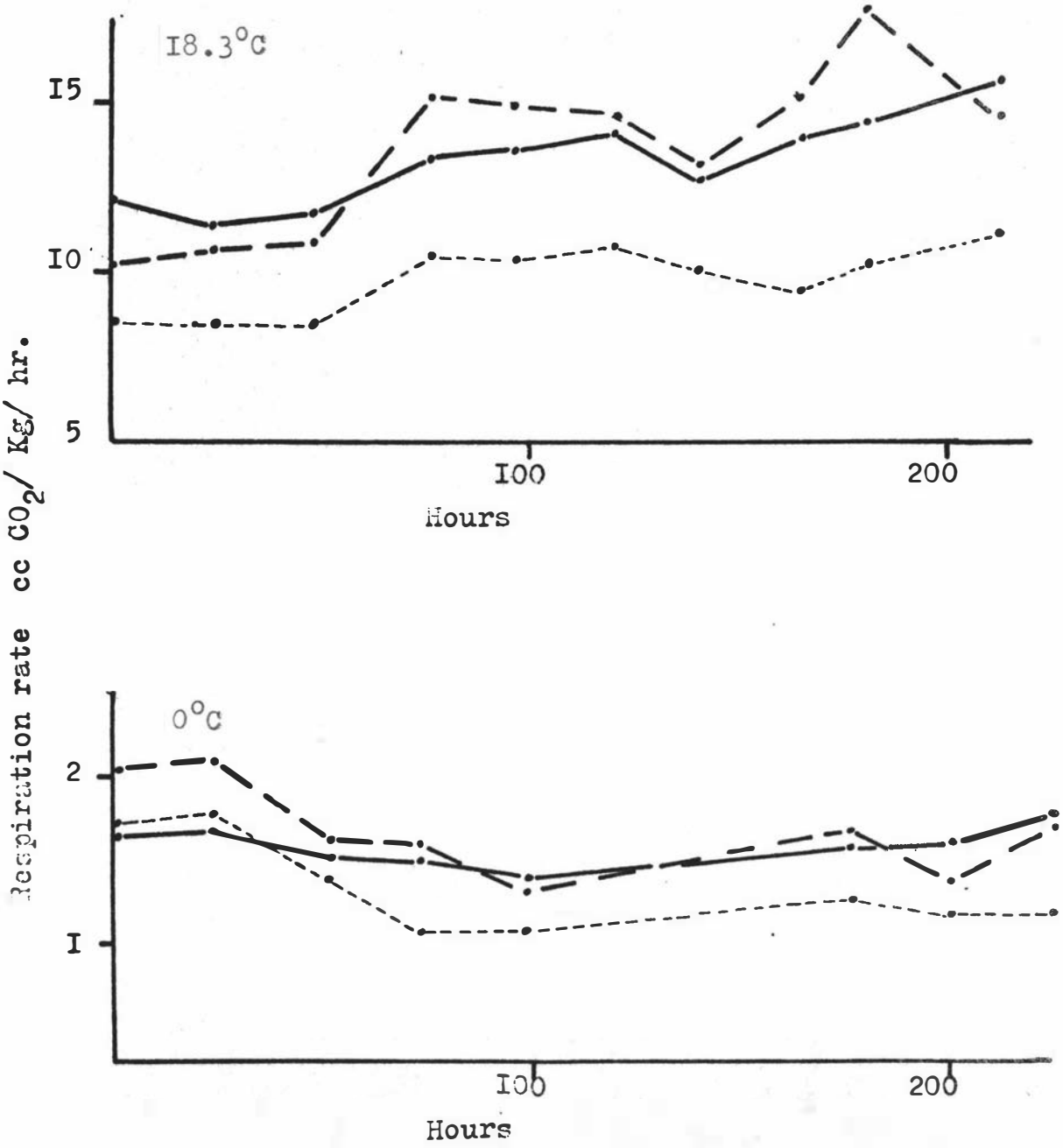


FIG. 10. KIWIFRUIT RESPIRATION AFTER SUMMER SPRAYS.

Unsprayed SADH MH

half of a Monty vine sprayed at full bloom with 1000ppm, the treated half of the vine produced 41% of the fruits, but mean fruit weight was only 52% of the controls (I. Ford pers. comm.).

Measurements of fruit respiration rate were also made with a URAS infra-red gas analyser. Using two replicates of each treatment, of 6-8 fruits each, fruit respiration rate was determined for fruit held at $0^{o}C$, also after dipping in 1000ppm Ethrel[+], and also during ripening at $18.3^{o}C$

On all occasions the pattern of fruit respiration was the same in all treatments (Fig. 10), and the level of respiration was similar. Usually the unsprayed fruits respired at the lowest rate, although mean fruit weight was 61.1gm compared with 102.5gm for the fruits from sprayed vines.

(c) Fruit residues -

Hayward fruits sprayed with 2000ppm SADH on 14/12/69, and untreated fruits were harvested on 16/5/70, and pulped and frozen. Within two months SADH residues were determined by conversion to dimethyl hydrazide, and then reacted with trisodium penta cyanoamine ferroate before absorbance was measured.[++] (Appendix I).

The method gave a mean of 72.7% recovery with this tissue, and when applied to eight sample analyses, represents a mean of 16.95ppm (range 15.6 - 21.8) SADH in fruits at harvest.

[+]48% Ethephon

[++]Residue determinations by R.P. Eade, Ivory Spray Chemicals Ltd., Nelson

B: Explant Shoot Growth

Is is now recognized that growth substances may be synthesised in the roots (Phillips and Jones 1964), and to some extent foliar applications of gibberellins and cytokinins (Railton and Reid 1973, Reid and Crozier 1971) can substitute for the roots. Richards and Rowe (1977) found a direct relationship between the size of the root system and the size of the top it supports, and postulated that the limit on top growth set by the roots involves an internal regulation by the root, especially the production and supply of growth substances.

The effect of gibberellins on shoot growth was studied, and their possible substitution for the roots in controlling elongation. In addition the contribution of cytokinins and auxin to stem growth was considered, and the importance of carbohydrate reserves and mobilisation in supporting growth.

In order to study the control of shoot elongation, observations were made over several seasons of the effects of applying various growth regulators. This was usually done by harvesting 1-node stem cuttings (explants) in the spring at an early growth stage, and immersing most of the one-year old wood in an aqueous solution, in a glasshouse, and then observing changes in shoot elongation.

Experiment 1: 1972/73 - In January 1972, Abbott cuttings were induced to root under mist and then to make limited shoot growth before the winter. On 5 September they were transferred into a glasshouse and grown in shallow troughs in a flowing nutrient solution (Hoagland) for 17 days before treatments were started. Each treatment consisted of eleven single-stem plants, to determine the effect on shoot elongation of removing the leaves or the roots, or their substitution with gibberellin or kinetin. Chemicals in nutrient solution were added from 12 days after the pruning

treatments, and were changed daily.

Treatment 1 - Intact plant
2 - De-root + GA
3 - De-root + 0.4ppm kinetin
4 - De-root + GA + 0.4ppm kinetin
5 - De-root
6 - Defoliate + GA
7 - Defoliation

(GA solution contained 0.4ppm GA_3 + 0.2ppm GA_{4+7}. Defoliation consisted of removing all leaves except the lowest true leaf, and also removing any developing axillary buds).

Results:

The graphs of Fig.11 show the typical growth responses in each of these treatments.

Intact plants grew satisfactorily and showed the usual growth curve, but in all cases where the root system was removed, shoot elongation ceased within three days. No further elongation occurred when nutrient solution or kinetin was supplied (Treatments 5,3). When gibberellin, alone, or in combination with kinetin was added to the basal solution, shoot elongation continued. In both these cases (Treatments 2, 4) no extension growth had occurred for 8 days, yet after 3 days gibberellin induced elongation to recommence, and to continue at a rate similar to that of intact plants. The presence of kinetin did not appear to influence this result. Growth rate declined quickly from about 6 days after ceasing gibberellin additions (on 17 Oct), 3 weeks after GA treatment began.

The results are summarised in Table 2 for those plants that remained alive, but due to the variation between plants statistical analysis was not done.

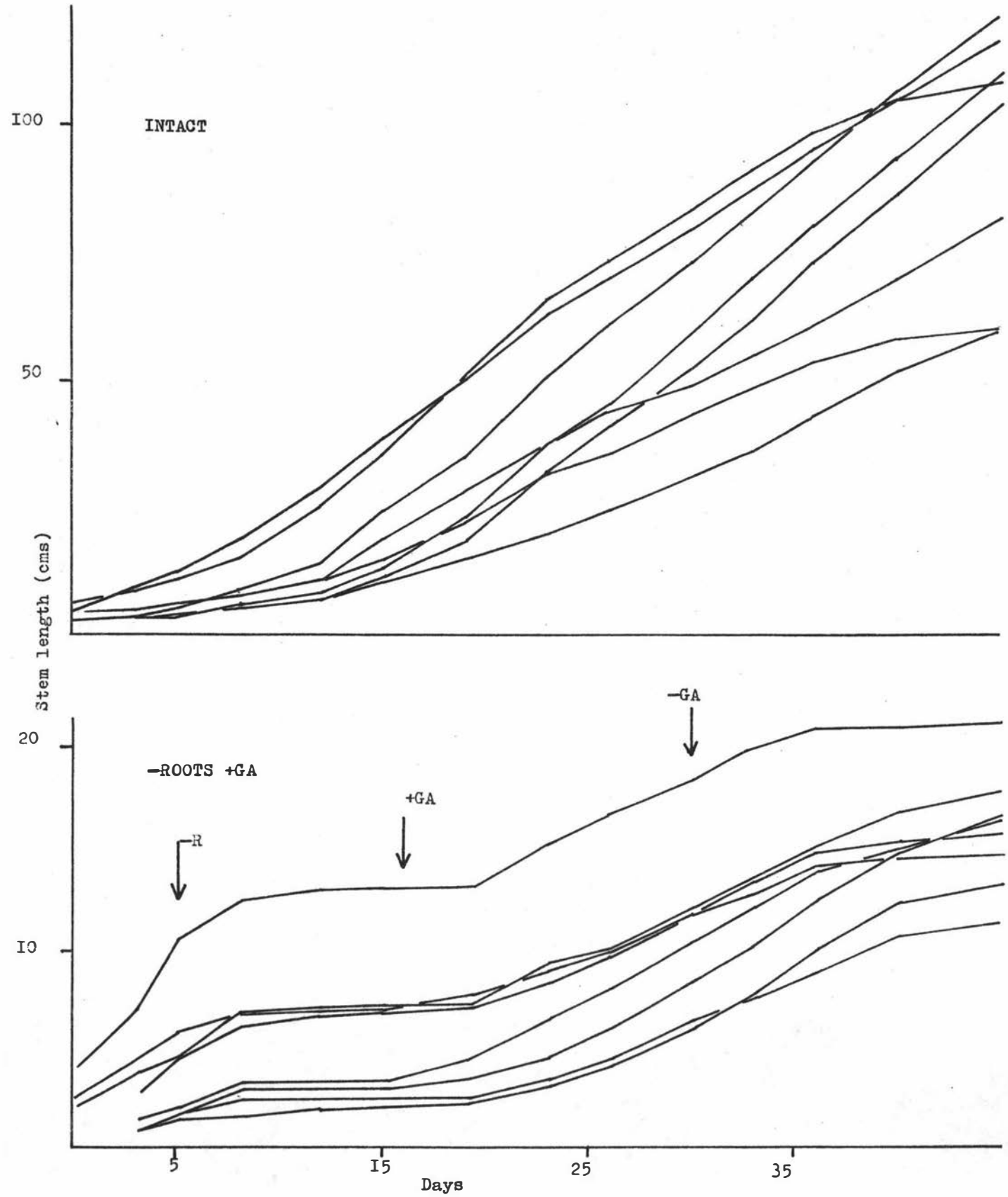


FIG. II. TREATMENT EFFECTS ON STEM ELONGATION KIWIFRUIT

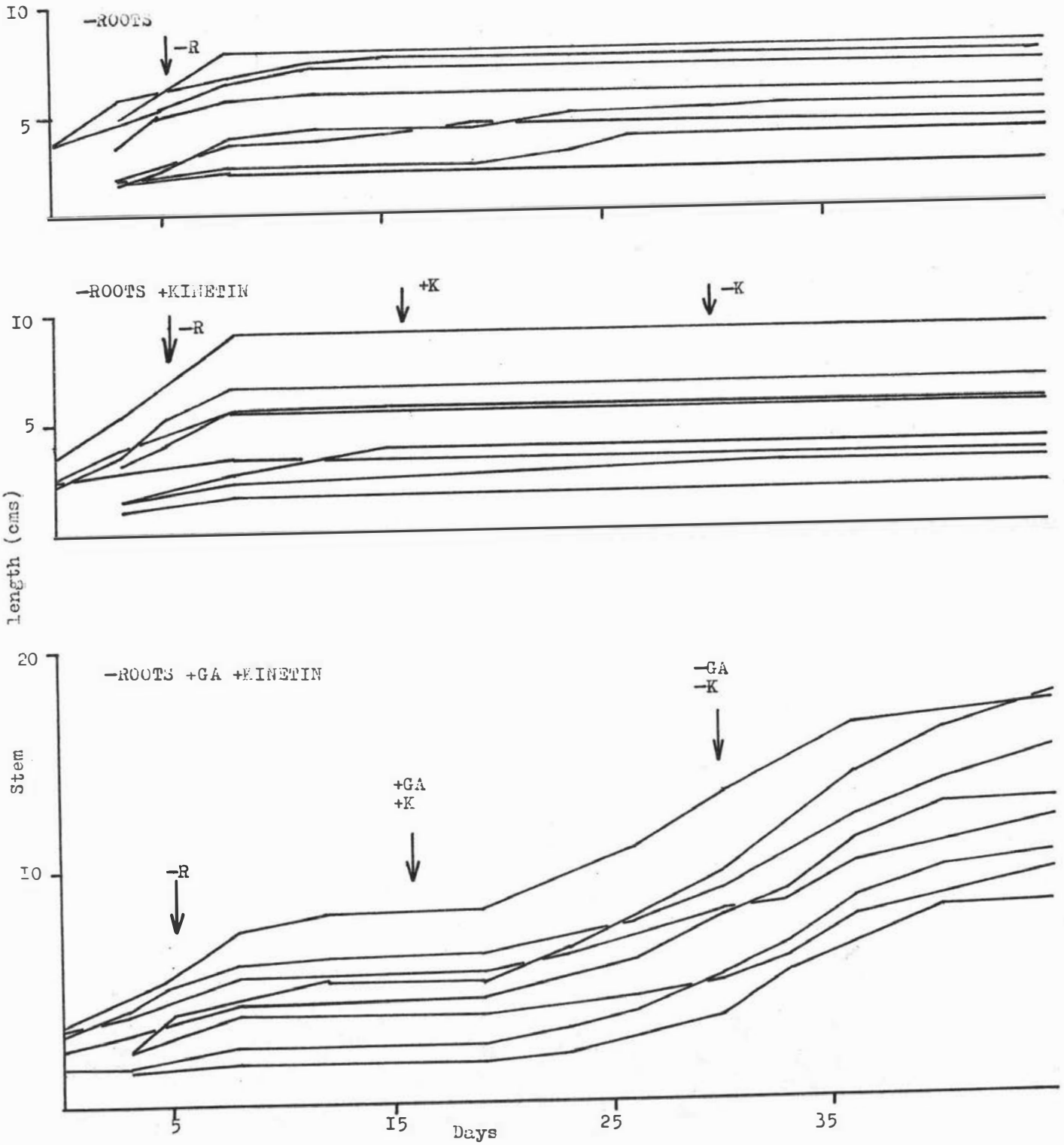
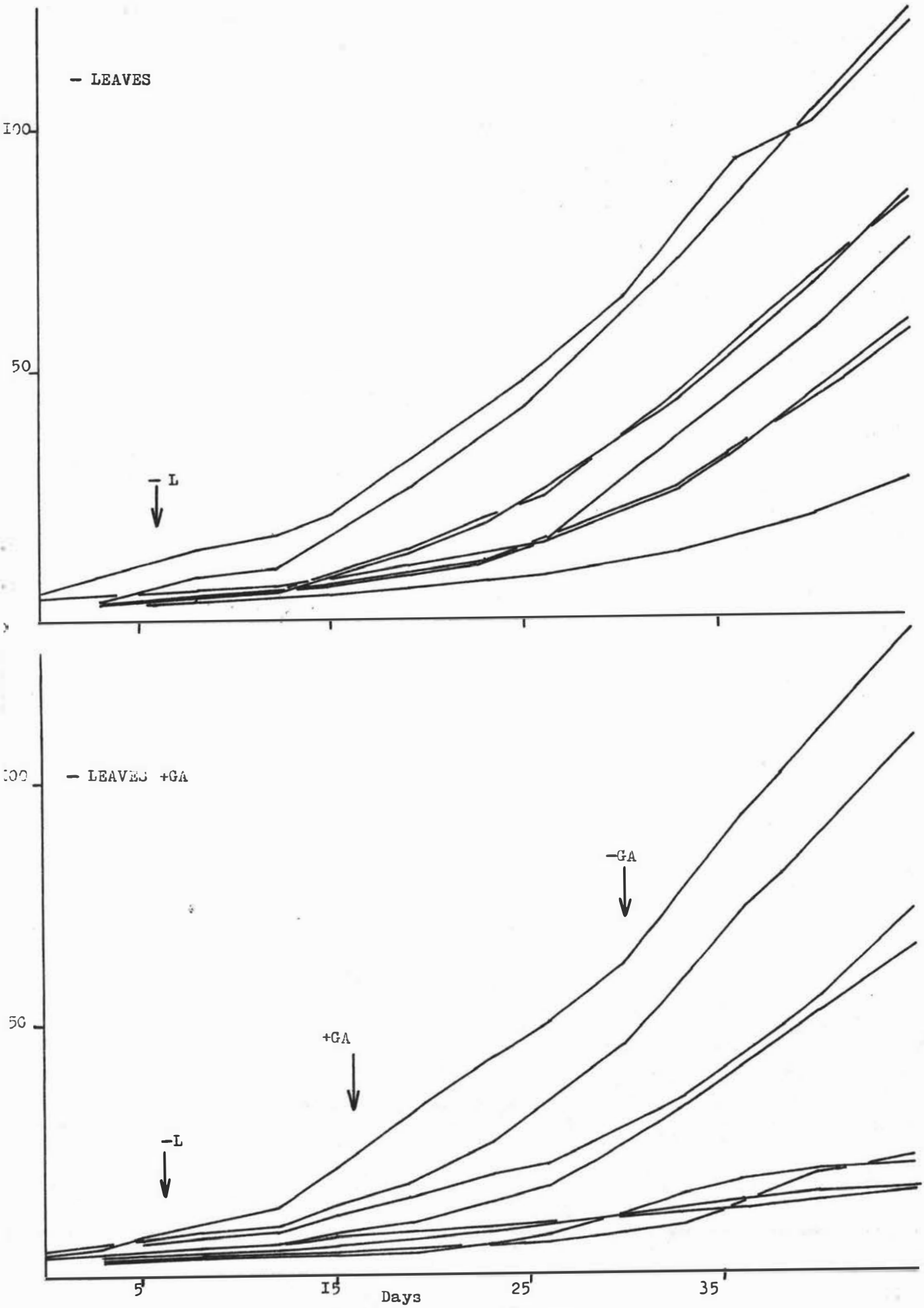


FIG. II. TREATMENT EFFECTS ON STEM ELONGATION KIWIFRUIT



F. II. TREATMENT EFFECTS ON STEM ELONGATION OF KIWIFRUIT.

Table 2: Effect of Pruning and Growth Regulator Treatment on Plant Growth (at 1.11.72)

Treatment	No. Plants.	Mean no. internodes	Mean shoot length (cms)	Mean Diam. base leaf (cms)
1 Intact	8	16.87	94.91	7.64
2 -R + GA	9	8.33	14.70	4.88
3 -R + Kin	11	5.27	4.37	5.19
4 -R + GA/Kin	10	8.00	13.84	4.81
4 -R	9	6.11	5.29	4.28
6 -L + GA	9	18.78	51.67	10.01
7 -L	8	22.62	80.34	11.59

It will be seen from Table 2 that removal of the roots has:-

(a) reduced final shoot length, which has been partially restored by gibberellin.
(b) reduced the size of the oldest leaf, and
(c) reduced internode number, but to a slightly lesser extent where GA was added.

Over the period of observation the relation of final shoot length to original shoot length in de-rooted explants was 1.2 and 3.4 and 3.8 respectively where water, GA or GA + kinetin was added.

Where the leaves only were removed, the plants varied in their behaviour, but in the presence or absence of exogenous GA (Treatment 6, 7) there was no noticeable effect on shoot growth rate or final shoot length. It appears from Table 2 that in these plants with a reasonable growth rate, defoliation:-

(a) increased the number of internodes per plant, and
(b) increased the size of the one remaining leaf.

Experiment 2: 1973/74 - Again small Abbott plants from cuttings rooted the previous summer were used, and explants taken from them soon after shoot growth began the following spring. Eleven days later the hardened shoots were placed in a shaded glasshouse, in vials of aqueous solutions which were changed 3 times a week. Treatments consisted of 11 explants each:

Treatment	1	0.6ppm GA_3
	2	0.6ppm GA_{4+7}
	3	0.4ppm GA_3 + 0.2ppm GA_{4+7}
	4	0.6ppm GA_3 + 10ppm BAP
	5	Water control.

Results:

A number of explants did not survive for the whole period of observation, but any treatment supplying a gibberellin promoted shoot elongation and also did so in the presence of a higher concentration of a cytokinin (Fig. 12, Plate 1).

The rate of shoot growth was clearly increasing from about 8 days after the first GA application, and had virtually ceased after 6 weeks of treatment. But there was considerable variation in final shoot length, between different explants.

Initially mean shoot length was similar in all treatments, and at the final recording it was unchanged in the untreated controls, however it had increased by a factor or 2.3, 2.3, 2.2 and 1.7 in treatments 1 to 4.

Associated with the GA-induced elongation there was an increase in the number of visible internodes - GA increased the mean number (1/10/73 - 10/11/73) by 4, and by only 3 internodes with the application of GA and BAP together.

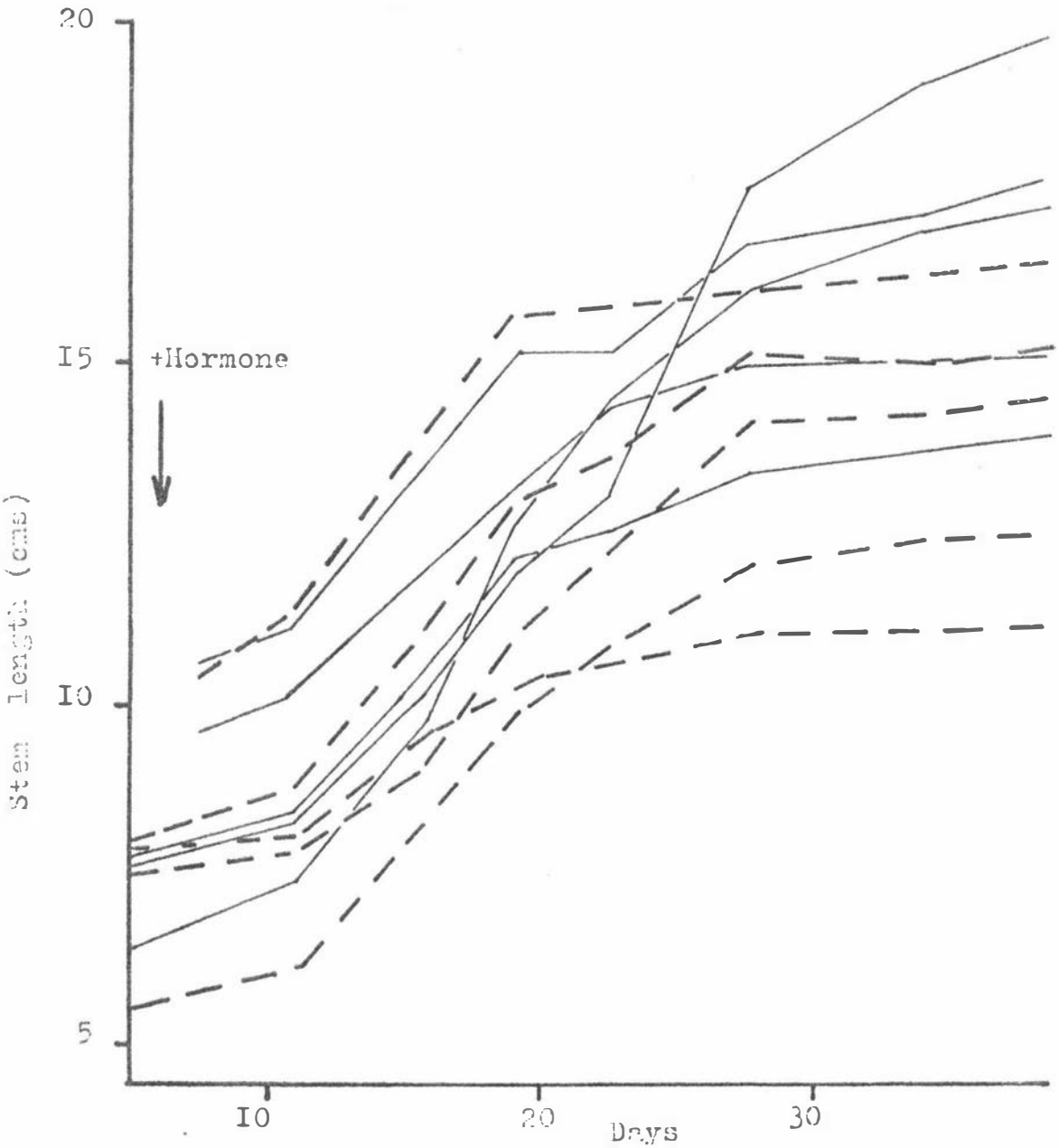


FIG. 12. TYPICAL EFFECT OF GROWTH REGULATORS ON GROWTH OF KIWIFRUIT CUTTINGS.

———— GA_3 — — — GA_3 + BAP

PLATE I: THE EFFECT OF GIBBERELLIN ON SHOOT GROWTH OF A KIWIFRUIT CUTTING - 47 days treatment.

Left - Control, Mid - $GA_{4/7}$ Right - $GA_{3/4/7}$

Arrows show shoot length at start.

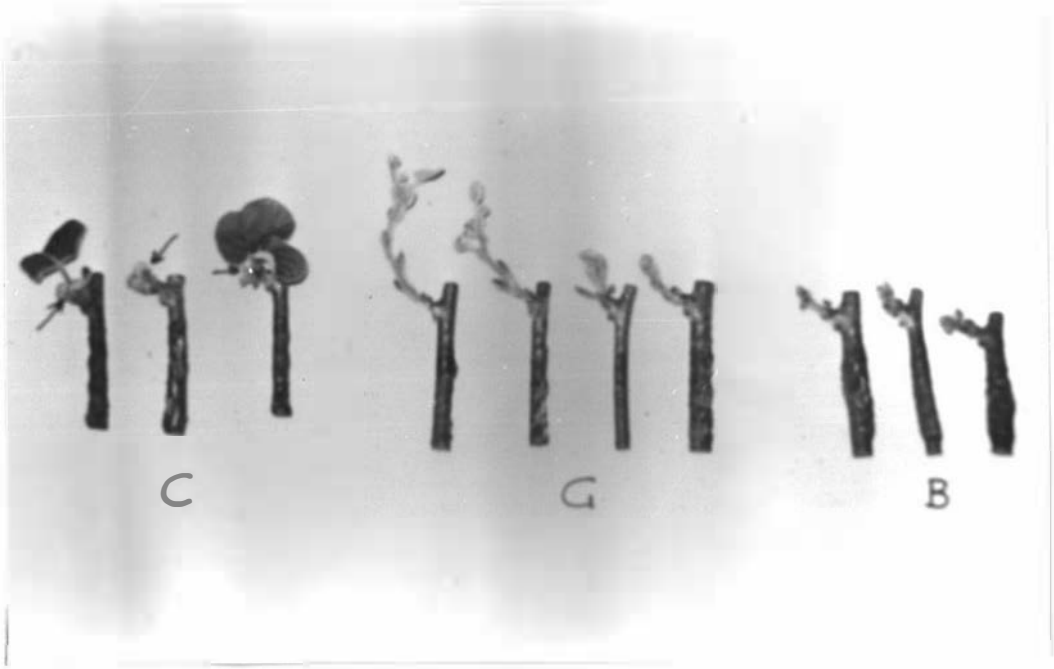


PLATE 2: THE EFFECT OF PRE BUD BURST APPLICATION OF HORMONE ON SHOOT GROWTH, - after 18 days.

C Control, G GA_3 B BAP

Arrows show flower buds.

At the end of the experiment the current season's shoot of each explant was divided into stem and leaves, and the separate oven-dried weights determined (Table 3). For each treatment total dry weight was the same, but, in treatment 1 to 4 leaf weight was 84% of the control, and stem dry weight 129% of the control - although the differences in stem and leaf weights just failed to reach significance ($p = 0.05$).

Table 3: Effect of Growth Regulator Treatment on Explant Mean Dry Weight Distribution

Treatment	Shoot length(cms)		Dry weight (gm)		
	Start	End	Stem	Leaf	Total
1 - GA_3	6.88	15.90	0.376	0.823	1.199
2 - GA_{4+7}	7.14	16.76	0.380	0.843	1.223
3 - GA_{3+4+7}	6.65	14.37	0.336	0.767	1.103
4 - GA_3+BAP	7.64	13.10	0.317	0.735	1.052
5 - Water	7.31	7.47	0.273	0.935	1.208

Following bud burst on an explant the total number of leaves/primordia increases, but no further increase occurs during GA treatment. Winter (August 1976) Abbott buds contained 17 leaf primordia, which rose to 23 (mean) after shoot elongation ceased. After further shoot extension in response to treatment with GA_3, there was no change in the total number of leaves and primordia. In the field long shoots had in excess of 40 leaves in February.

Experiment 3: 1973/74 - Similar chemical applications were also made to one-node explants of the variety Bruno. In this case, wood was collected in the field in late July and early August, before bud break, and treatments began after initial shoot growth had ceased.

As on other occasions gibberellin (GA_3 GA_{4+7} or GA_{3+4+7}) caused a marked increase in shoot length - in the presence or absence of BAP, and BAP alone had no such effect.

When shoot cuttings were used in this manner they were cut to a similar length of about 6cms, although there was some variation in shoot diameter. Records were kept of these Bruno cuttings to examine their contribution to new shoot growth. Data for three separate late-winter harvests of wood from mature vines is shown in Fig. 13. The maximum length of the new shoot is shown plotted against the volume of the original explant determined from length and diameter measurements (assuming a cylindrical shape).

In each case a good linear relationship exists with the larger explants producing the longest shoot before extension growth ceases.

The contribution to shoot growth made by the previous season's wood was also shown with a quantity of Bruno wood harvested shortly before bud burst on 31/8/73. The freeze-dried weight of this wood was 49.8% of the fresh weight. Wood was also held as explants until the new shoot growth had ceased, when the extension growth was removed and the original cutting freeze-dried. It was then 37.2% of its original fresh weight.

This indicated that 1kg (fresh weight) of shoot material harvested close to bud burst, lost 125gm of freeze-dried weight (or 25% of its total freeze-dried weight) in supporting shoot extension. At that point shoot elongation ceased.

Experiment 4: 1975/76 - Observations were also made where growth regulator treatment commenced before bud burst.

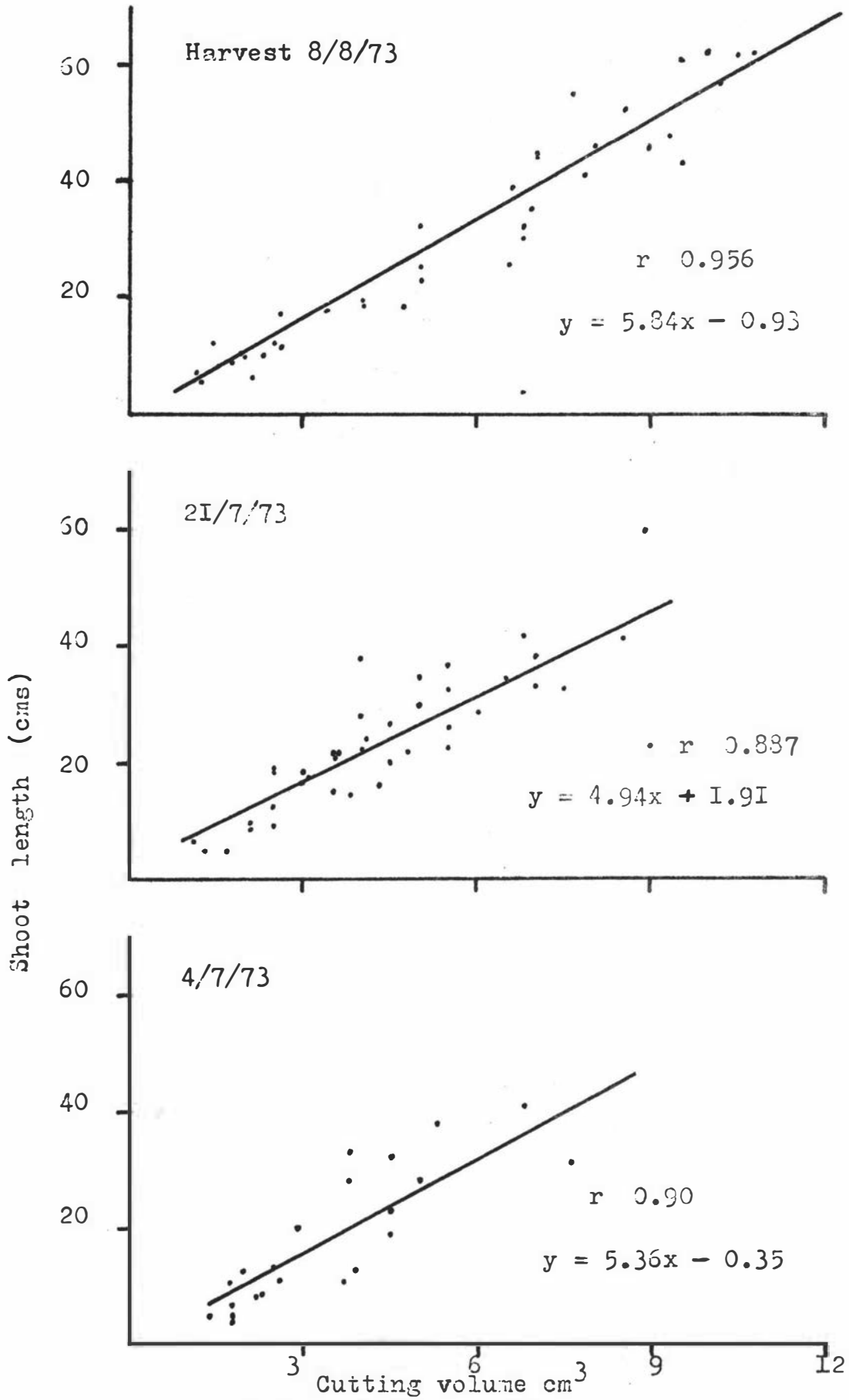


FIG. 13. RELATIONSHIP BETWEEN CUTTING VOLUME AND SUBSEQUENT SHOOT GROWTH

Results:

Using one-year old wood of Bruno collected in the field on 11/8/75, a few days before the first signs of bud burst occurred, early shoot growth was affected by standing immediately in a solution of 50ppm GA_3. So that three weeks later:-

Control: 100% buds burst, their mean stem length was 1.0cm
$+GA_3$: 96% buds burst, their mean stem length was 2.9cm

The effect of hormone treatment being to accelerate the rate of bud growth, and to increase stem length.

A further collection was made on 25/8/75 of Abbott wood at the same stage of development, and one-node cuttings were immediately held under continuous fluorescent light in a heated room as follows:-

Treatment 1 - water
2 - GA_3 50ppm
3 - BAP + GA_3 each 50ppm
4 - BAP 50ppm

After 25 days only 58% of the buds had burst in each treatment, but many of the explants treated with gibberellic acid (Treatment 2, 3) had produced a shoot 7cm long. In contrast, BAP and water explants had produced shoots of very compact growth viz. about 1.5cms. It was also very noticeable that GA_3 had altered the type of development that occurred. Whereas the controls had large, dark green leaves and several prominent flower buds, GA_3 produced very small narrow leaves of pale green colour, and there was no sign of axillary flower bud development. (Plate 2).

In an experiment using Abbott explants, harvested on 5 August, and treated from 31 August:-

(a) 2ppm GA_3 caused a marked resumption of shoot growth
(b) A hormone mixture of 2ppm GA_3, 3ppm diphenylurea, 0.5ppm NAA was phytotoxic, so the effect of such a combination could not be determined.

Experiment 5: 1975/76 - In this instance, the main purpose was to observe the role of auxin in explant shoot growth, using one-node cuttings held in aqueous solution in a cabinet held at21.1- 22.2°Cand a 16-hour day.

In some cases PCIB (p-chlorophenoxyisobutyric acid) was used, but there was considerable difficulty in achieving a suitable concentration as high concentrations rapidly caused wilting and death of new shoot growth.

(a) Dormant Abbott wood was collected on 5/8/75 and held in water as one-node cuttings until shoot elongation had stopped, and treatment began on 31/8/75 with 18 explants per treatment:

Treatment 1 - 10ppm IAA
2 - 2ppm GA_3
3 - 2ppm GA_3 + 10ppm PCIB
4 - 2ppm GA_3 + 50ppm PCIB
5 - Water control

Results:

There was no resumption of shoot elongation on the addition of IAA (Fig.14). Many of the explants did not survive, especially in treatments 2-4. Several explants responded to GA_3 alone, and the effect of including PCIB was not clear, although there were indications that shoot elongation could continue in the presence of this material.

(b) A small quantity of wood of three varieties was taken from the cold room (4°C) on 25 October, where it had been for 3 months, and used in a similar test - with a total of

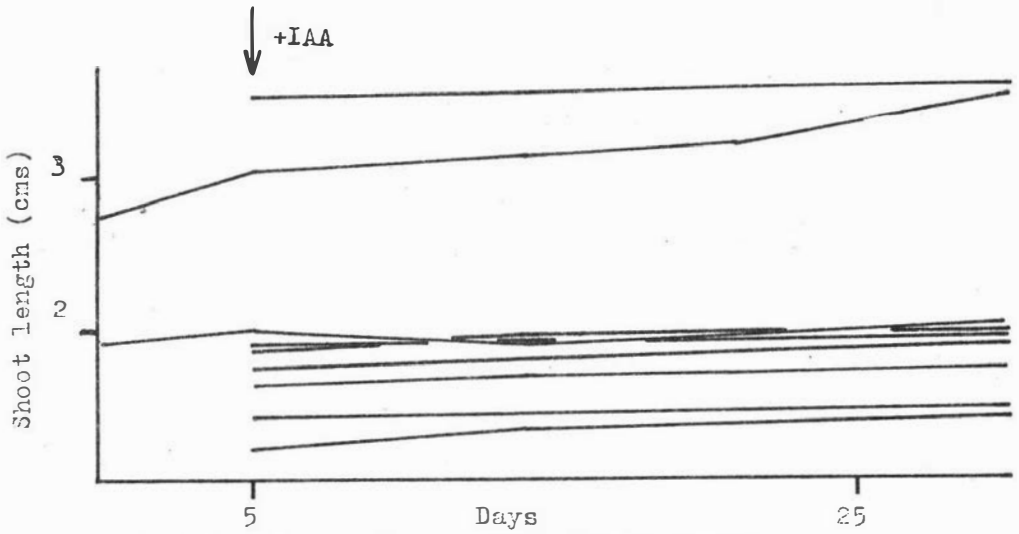


FIG. I4. EFFECT OF IAA ON KIWIFRUIT SHOOT GROWTH

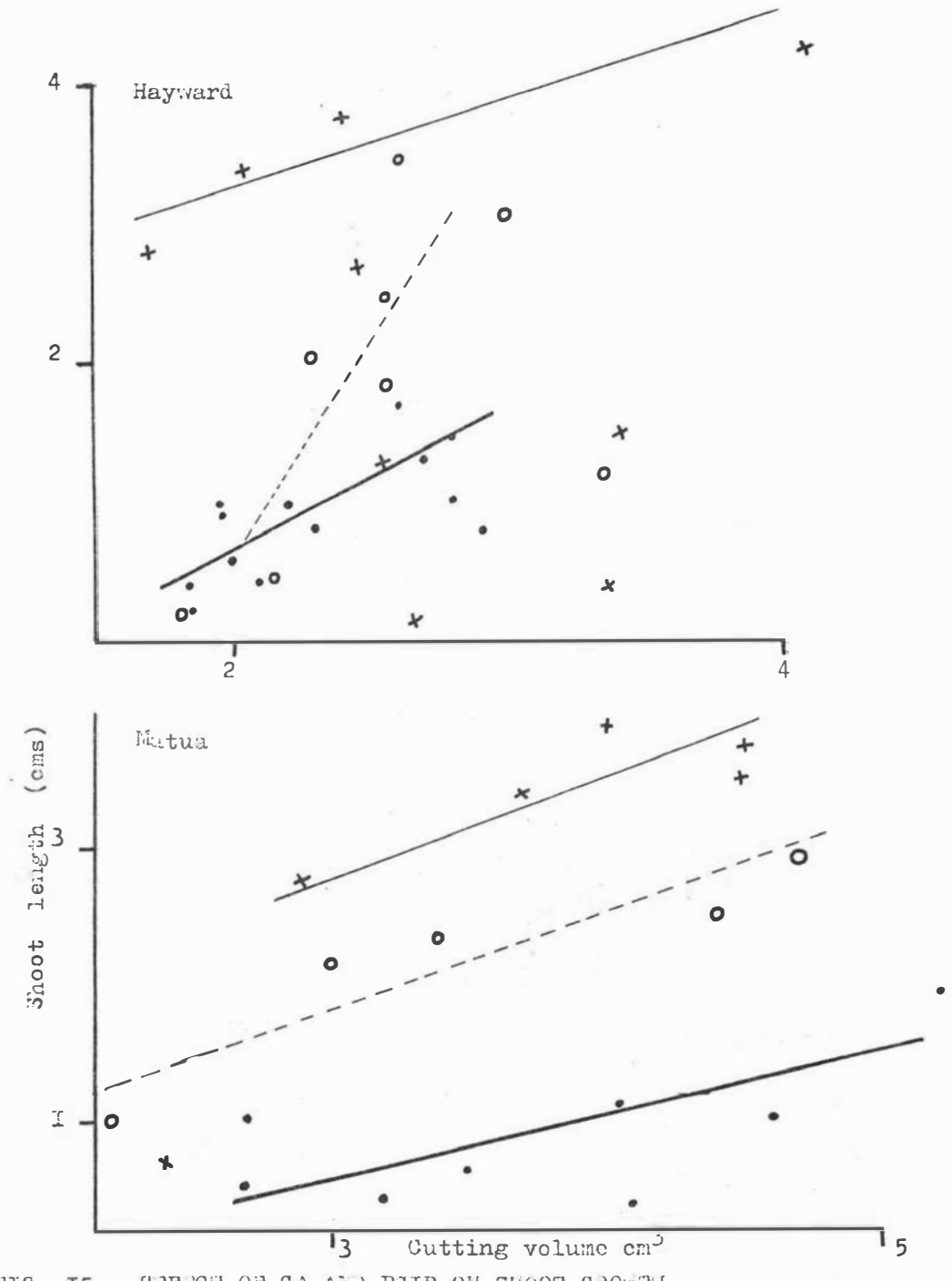


FIG. I5. EFFECT OF GA AND PCIB ON SHOOT GROWTH

34 explants to each treatment:

Treatment 1 - Water control
2 - GA_3 5ppm
3 - 5ppm GA_3 + 0.2ppm PCIB

These explants also were held in a heated lighted cabinet in small jars of solution, and consisted of:

Hayward - 16 explants — Treatment started immediately, pre-bud burst.

Tomuri - 7 explants, Matua - 11 explants — Chemical treatment began 6 days later, when bud burst had been completed

Results:

Almost 100% bud burst was recorded. On 13 November, after 2 or 3 weeks chemical treatment, shoot growth of a very few explants was wilting and dying. Another week later most of the male explant's growth had collapsed, and this was not associated with the presence of PCIB.

The results for two varieties are shown in the form of a scatter diagram (Fig. 15), where the calculated volume of the cuttings is plotted against the maximum shoot length by 18/11/75.

There is some variation within each treatment, but there appears to be a clear trend in response to treatment shown by all varieties, especially with Matua.

The application of gibberellic acid alone, or in combination, increased shoot growth compared with the controls. The effect of PCIB was to reduce the level of growth stimulation brought about by GA_3, without preventing all the response to GA_3. Thus PCIB allowed the explants

to make their normal growth, plus some response to GA_3. Even when PCIB was present from before bud burst (Hayward) bud burst and shoot elongation was not prevented.

Experiment 6: 1975/76 - A preliminary test in 1974 had indicated a fall in starch and reducing-sugar levels in one-year old wood as a result of the shoot growth made by a bud on a stem cutting.

It was decided to further investigate the relationship between explant shoot growth, and changes in the carbohydrate content of the one-node cuttings used.

Methods.

Determinations were made of soluble sugar and polysaccharide reserve levels following methods used by Robinson (1975) - as adapted from Priestley's work (Priestley 1965, Flood and Priestley 1973). Some modifications were made to the technique, including the use of a dinitrosalicylic acid reagent (Miller et al. 1960) for the determination of reducing sugar activity. Samples were compared with a series of glucose standards. Preliminary work indicated the suitability of the method used, which is shown in Fig 16.

Notes for Fig. 16:

(a) For refluxing, the sample was placed in a round-bottomed flask with anti-bumping chips, on a heating mantle, and kept gently simmering. A filter funnel filled with glasswool and glass chips was placed in the top of the flask.

(b) An 8cc aliquot (2 replicates) was mixed with 2cc of dinitrosalicylic acid reagent in a test tube, heated on a boiling water-bath for 8 minutes and made up to 10cc. Absorption was read at 640mu on a Hitachi spectrophotometer.

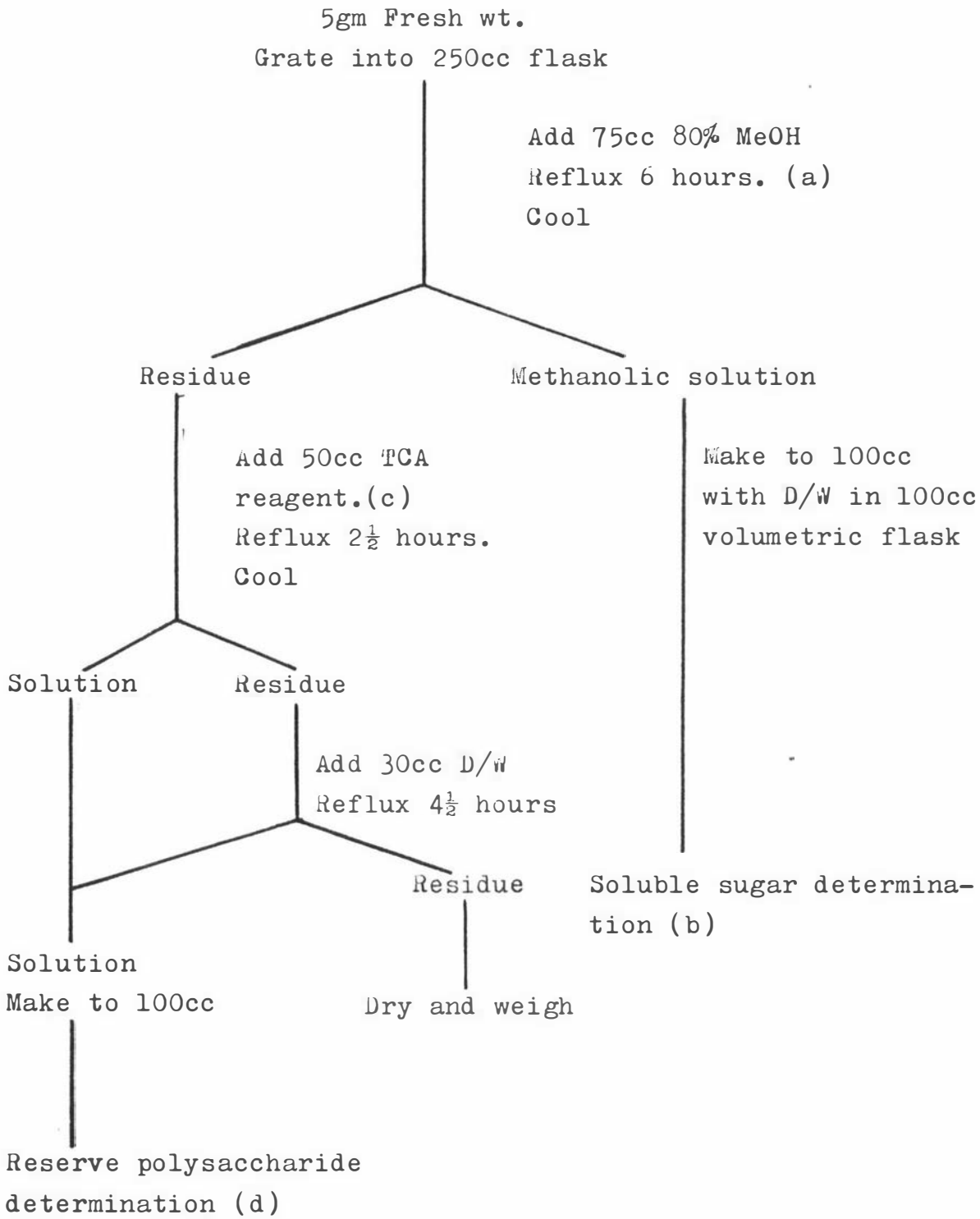


Fig. 16: Extraction of Soluble Sugars and Reserve Polysaccharides.

In a test with acid hydrolysis of this soluble sugar fraction (1N H_2SO_4, heated on water bath 20 mins) there was no significant increase in activity, suggesting sucrose was not an important constituent of this extract.

(c) TCA reagent: 10gm TCA (Trichloracetic acid)
20cc Methanol
- made up to 200cc with distilled water.

(d) Priestley (1965) indicated the TCA/Methanol hydrolysis of starch produces mainly glucose, fructose and arabinose, and the hydrolysate was assessed similarly as in (b) above. Duplicate aliquots of 2cc of solution were added to 2cc dinitrosalicylic acid reagent, and made to 10cc after heating.

Also 2cc of each solution was streaked on Whatman 3MM chromatography paper, and run (ascending) 20cms with diethyl ether to remove the TCA. A paper strip 2.5cm wide each side of the baseline was eluted with 10cc distilled water by holding in a water bath at 100°C for 1 hour. The eluant was then tested as usual.

One each occasion a series of glucose standards was also tested to give a linear standard curve on log-log paper.

For this experiment dormant wood of the variety Abbott was collected in the field on 5/8/75, some of which was placed in a lighted cabinet at 21.1° as one-node cuttings in water. When shoot growth had almost ceased (31 August) some explants were stimulated to make substantial further extension growth by the regular addition of GA_3.

Sampling was as follows:-

Treatment 1 - Dormant wood, harvested 5/8/75
2 - Control. Sampled on 31/8/75 after normal extension growth ceased
3 - Control. Sampled 2/10/75.
4 - Wood sampled on 2/10/75 after GA_3-stimulated growth had occurred.

In each case, only the original one-year old wood (excluding buds) was sampled. There were three samples per treatment, each taken from two different explants. After harvest all samples were weighed and stored in absolute methanol in the deep freeze, until all analyses were done together in mid October.

Results:

These are summarised in Table 4. The dry weight, as a percentage of fresh weight, fell from 27.3% in dormant wood to 22.4% on 31 August in supporting bud growth, and this remained at 23.8% in the two final samplings.

Similarly the measurement of starch reserves show they fell in supporting the initial shoot growth, but that no further significant change occurred when further shoot extension was stimulated by a GA_3 application. Apparently this GA-induced growth was not made at the expense of the carbohydrate reserves in the original cutting, and GA did not increase the proportion of reserve carbohydrate that was mobilised for shoot growth.

Measuring "starch reserves" directly, or after chromatography gave very similar results.

Table 4: Changes in Explant Carbohydrate Levels.
(Mean glucose equivalents gm/100g Dry wt.)

Wood sample	Soluble sugars.	Starch reserves	As % Dormant wood Sugars.	Starch
5 Aug. Dormant	12.06	11.67 a	-	-
31 Aug. Post growth	7.88	5.51 b	65.3	47.2
2 Oct. Post growth	8.33	4.76 b	69.1	40.8
2 Oct. Post GA_3- growth	8.22	5.38 b	68.2	46.1

Analysis of variance - Differences soluble sugars non-significant (5%).
- Differences starch significant (1%), treatment differences determined by Duncan's Multiple Range test (1%).

DISCUSSION

Shoots of the kiwifruit plant show the normal sigmoid growth curve, and although no records of seasonal growth patterns were obtained here, the effect is indicated for plants in Figs.7, 8 and for individual internodes in Fig. 4, and such a growth curve was recorded in the field in limited measurements by Davison (1971). He noted the difference between the weaker growing Hayward, in which shoot growth essentially ceased for non-fruiting shoots at the end of November, but in the stronger growing Monty, growth rate of fruit-bearing shoots declined in late January. The male clone Alpha appears more vigorous than Hayward because it produces a greater proportion of indeterminate shoots during a growing season (Brundell 1975a). This may have a parallel in apple trees, where scion shoot growth on smaller, slower-growing trees (brought about by the presence of a dwarfing rootstock) ceases earlier in the summer than in more invigorated trees, and in the former, growth is restricted to a smaller number of growing points (Avery 1969, Robitaille 1970). Similarly a lateral of the Hayward variety produces a significantly lower percentage (45.5%) of flowering shoots, (ie. bud burst) than do four other more vigorous pistillate varieties (72%) (Brundell 1975d).

It would be of interest to determine the distribution of photosynthates from a shoot, and the leaf contribution to fruit growth. The early development of mature leaves on a determinate shoot would make photosynthates readily available for export to their developing fruitlets (Hansen 1967), a situation seen to occur in the apple tree. By contrast a slower leaf development on elongating indeterminate shoots, and a strong retention in the shoot tip of photosynthates from the apical leaves (Hansen 1967), may place developing fruits on this type of shoot at a relative disadvantage, to be reflected in a reduced potential for large fruit size.

No attempt was made to record or account for the presence of both long (indeterminate) and short (determinate) shoots on a vine (Brundell 1975a), where the termination of growth and abscision of the terminal bud occurs prematurely to form the determinate shoots. Activity in the sub-apical region plays a large part in determining stem length (Sachs 1965), and the development of long-shoots has been found related to the greater rib meristem activity in the sub apical region than is associated with short shoots (Gunckel and Wetmore 1946). This may reflect differences in growth regulator activity, including gibberellins (Sachs and Kofranek 1963), and Critchfield (1970) has provided circumstantial evidence of a dominant and distinct role of auxin in controlling the extension of long shoots. Jankiewicz (1972) has proposed a system in which auxins, gibberellins and cytokinin interact such that there is a differentiation between long shoots and short shoots on a plant. When the dwarf French bean and the sweet pea are treated with gibberellin, apical dominance is strengthened, since instead of aborting, the apical bud continues growing (Brian 1959). One could then enquire whether short and long shoots would respond differently to exogenous GA application.

In a study of the cessation of apical growth and shoot tip abscission in Salix, it was found they were delayed by growth retardants or applying GA_3 to the apex in spring, while ABA and kinetin had no effect (Junttila 1976). This is further evidence that GA may play an important role in the regulation of shoot tip abortion, possibly by ensuring the apex is a strong "sink" able to compete with the developing axillary buds and leaves.

Decapitation

Removal of the tip bud from vigorously growing young plants reduced the rate of growth of the main stem due to the removal of the apical source of growth hormones, along with the loss of the site of cell division and new leaf primordia. Fig. 2, indicates that initially growth in the

uppermost internode was reduced, and although it was not determined here, the literature suggests this was due to the loss of gibberellin-like substances (Fulford et al. 1968, Lockhart 1964). When GA_3 was applied to the decapitated stem of sunflower, pea and bean plants, internode growth was maintained at the same level as control plants, whereas auxin was ineffective in substituting for the apex (Jones and Phillips 1966, Lockhart 1957, 1964, Phillips 1971). So there was here an indication that extension of the distal stem internode was affected by factors originating in the apex - no doubt gibberellins produced by the youngest leaves are an important component of this control.

However 8 weeks after decapitation the only marked effect was on the internode second from the top, which was markedly longer than in the control plants (Fig. 3). Apparently growth factors from within the plant favoured this extra growth, which was also obtained in a subsequent test when decapitation increased the length of each of the upper three internodes. This is in contrast to several other studies where decapitation reduced the length of the upper internodes (Barlow and Hancock 1955, Jones and Phillips 1966, Lockard 1956).

Defoliation

Total shoot length was unaffected by the loss of only two or four young leaves, but a continuing and almost total defoliation resulted in a considerable reduction in stem growth (Fig. 1). However when similar observations were made over a shorter period in the previous year, this effect was not noticeable. So while severe defoliation caused a loss of photosynthetic tissue and of sites of growth regulator production resulting in reduced stem height, any similar initial effect of very light defoliation was negated by subsequent growth.

Severe defoliation resulted in a reduction in the elongation of many internodes, extending from about three

internodes below the apical bud at the start of treatment, up to the last internode measured (Fig. 3). In addition, this treatment greatly increased the total number of internodes produced by these plants (Table 1), each succeeding one being of increasingly reduced size compared with control plants Figs. 2, 3). This effect has also been recorded in apples, where the successive defoliation of an apical leaf reduced the size of the internodes in this region at the end of the season (Barlow and Hancock 1955) The effect in apple also was greater, the greater the number of leaves removed, with the shortest internode being immediately above the last leaf removed - with following internodes showing a gradual recovery. Brundell (1975c) using hardwood cuttings of kiwifruit noted that continuous defoliation increased shoot length and internode number, especially where treatment began early.

While near-total defoliation affected the growth of a large number of internodes, the early removal of either two or four leaves had a similar but more restricted effect. As recorded by Barlow and Hancock (1955) the internode most reduced in size was that one immediately above the last leaf removed, which leaf was also the smallest one at the time of its removal.

It is clear a young leaf plays a major part in determining the final length of an adjacent internode, specifically of the internode above the leaf. Barlow and Hancock (1956) proposed that an "internode factor" Y produced by an immature leaf controlled extension of the two internodes above it. Further, while an expanded leaf has some effect on later internode growth, it is when the leaf is very small and forms part of the apical bud that it is exerting its greatest effect on final internode size - an effect that moves acropetally. The loss of many larger leaves affecting internode growth over a long distance was also noted by Barlow and Hancock (1956), and they postulated the effect was primarily due to a loss of photosynthates. The effect of removing a mature leaf is most likely to be due to reduced availability of

photosynthates, and that of removing immature leaves to changed hormone levels which lower the tissue growth rate (Barlow and Hancock 1955).

There was a tendency for the internode below the lowest leaf removed to be of increased length, and this effect was strong following decapitation (Fig.3). Table 1 also shows that defoliation treatment caused a relatively large but non-significant increase in the size of the basal leaf, and in later observations where twelve leaves were removed at an early stage, there was an increase in size of the next four leaves produced, compared with control plants. The effects of defoliation on stimulating axillary bud burst, increasing leaf production, and increasing leaf size indicates an attempt by the plant to re-establish its root/shoot ratio, effects to be expected when centres of utilisation are removed (Barlow and Hancock 1956). Brouwer (quoted in Richards and Rowe 1977) indicated that in a given situation an equilibrium exists between the top and the root, and that when any external factor disturbs this equilibrium, the plant reacts to reestablish it as soon as possible. In considering such compensatory growth in plants one can accept Wareing's (1970) view that it indicates there is an efficient mechanism for controlling the relative growth of shoot and root, so as to restore the balance typical of those environmental conditions. The emphasis is on there being a root-shoot interaction in this growth response - since metabolites move in both directions between the roots and shoots, it is possible for an interplay between them to operate to maintain this balance in growth. Wareing (1970) then argues from several observations that is is possible some of the effects of defoliation result from a change in the relative supply of root-cytokinins, due to the loss of some of the sites competing for them. One may also have expected these plants to show some reduction in root growth (Maggs 1965), for in apple trees defoliation most heavily penalised the roots, as the dry weight then accumulated in the nearest utilising region which was the stem.

In terms of internode elongation the literature indicates a major role for gibberellin-like substances produced in the apical leaves. These results indicate that at the stage that a leaf is just separating from the apical group it is exerting a stong effect on internode growth, and the work of Thrower (1962) showed this is the stage that a leaf has a rapidly-increasing import of C-assimilate from lower leaves. This may indicate that when an apical leaf is removed, a localised deficiency of assimilates is resposible for reduced cell extension and internode growth, and that this deficiency is brought about by the loss of leaf-synthesised gibberellins, which are known to be active in controlling metabolite distibution (see Hoad and Monselise 1976, Quinlan and Weaver 1970). Brundell (1975c) also has indicated the likelihood of leaf effects on flower bud and shoot development in cuttings being expressed through a hormonal control of metabolite transport.

The further away from the apex an internode becomes, the less effects its subtending leaf has on its size. After a period of rapid elongation, an internode ceases extension growth, and this occurs in small plants when it becomes the eighth internode below the apical bud (Fig. 4). Within an internode it was found (Fig. 5) the growth rate was highest in its upper part and lowest in its proximal part, and elongation ceased first in the lower part of an internode.

Growth regulator effects

The above considerations indicating the likely involvement of gibberellins in shoot growth were supported by the demonstration that the growth of kiwifruit seedlings was increased by GA_3 in a manner similar to that of lettuce seedlings (Frankland and Wareing 1960). Apparently endogenous gibberellin acivity was limiting stem growth in seedlings, although this was not shown to be the case with older plants (Experiment 3). There was no recorded effect

on stem growth of spray of GA_3 at 50, 200, or 500ppm, and the limited observations made did not indicate that any of the inhibitory effect of a maleic hydrazide spray was overcome by a GA_3 spray.

Gibberellins (usually GA_3) have frequently increased shoot growth, and the magnitude of the effect is dependent on the dose, which may be given in several applications. When GA was applied to pears (Modlibowska 1966) and apples (Luckwill 1968) it had no effect on primary shoot growth, but it destroyed the apical dominance and caused the lateral shoots to grow away. Kato and Ito (1962) found foliar sprays of GA_3 promote shoot elongation of apple trees that have not ceased growth, but the effect may sometimes be limited. It has been demonstrated that linear growth on young apple plants was increased only by more concentrated GA_3 sprays, and especially if sprayed as many as 17 or 33 times (Powell et al. 1959).
In conifers, non-polar GAs are very effective in promoting sexual differentiation, but as they are rapidly metabolised within the plant, exogenous applications are effective only when made at high dosages in continuous or frequent treatments (Pharis and Ross 1976). The absence of a response by the kiwifruit was presumably due to inadequate levels of suitable GA being attained within the plant, rather than signifying a non-requirement of GA for shoot extension.

While the growth retardant CCC restricts the shoot growth of many plants it was not shown to restrict growth of kiwifruit plants, and further observations of its effect is required. Its use did restrict the production of bleeding sap, and it is to be expected it would have some effect on growth or development of this plant. While CCC appears to have some effect on plant gibberellins (Reid and Carr 1967, Reid and Crozier 1970), the extent to which this accounts for changes in plant growth is unclear. There is also debate on the mechanism of action of the growth retardant SADH, but there are many reports

indicating a system based on changes in endogenous gibberellins (eg. Ryugo and Sansavini 1972). The suggestion that SADH-induced growth retardation follows from a reduced supply of photosynthates, which is associated with a fall in apical GA levels (Hoad and Monselise 1976) provides an identical explanation to that made earlier to describe the effect of defoliation in reducing internode growth.

Experiment 5 demonstrated that SADH is an effective retardant of kiwifruit shoot growth, and these initial observations suggest this treatment could find a place in mature plantings of vigorous vines. No record is available as to whether a SADH spray affected fruit size, but tests indicated fruit residue levels were low, and quite possibly acceptable. Fruit quality was not shown to be affected, but the fact that respiration rate of treated fruits was consistently higher means that the effect of SADH on fruit quality requires more detailed study. Whereas in the apple a SADH spray reduces the respiration rate of the fruit, such a spray advances maturity of the peach and an early season spray markedly increases the post-harvest respiration rate of this fruit (Looney et al. 1974).

The mode of action of the growth inhibitor maleic hydrazide is uncertain, but appears likely to differ from the retardants, although its application may reduce GA activity, and its effects on growth have been reported to be negated by gibberellin (Brian 1957). As occurs in other plants, maleic hydrazide promptly stopped shoot growth, and caused dieback of the shoot and growth of lateral buds, but at the concentrations used GA did not modify the MH effect.

Using mainly stem cuttings (explants) further observations were made of growth regulator effects on stem growth, particularly the gibberellins.

Defoliation

Intact plants growing in a flowing nutrient solution continued to make good growth which was not apparently affected by continuous defoliation (Fig. 11), a situation somewhat different from that already recorded in another experiment. The reason for the discrepancy is not clear, except that in the presence of leaves, initial growth rate of the stems was generally greater, and none made as little growth as some in the defoliated treatments. As on the earlier occasion, defoliation increased internode number and increased the size of the one remaining leaf.

The continuous supply of a low concentration of GA to the roots of defoliated plants had no effect, which perhaps indicates these exogenous GA's were no substitute for the leaves in the control of internode extension.

Root removal

When the roots of young actively-growing plants were removed, stem elongation ceased almost immediately, and no further growth occurred. Such an effect is not unexpected, and has been attributed to the loss of a major source of the growth substances essential for shoot growth (Carr et al. 1964, Crozier and Reid 1971, Smith and Wareing 1964b). Supplying a low level of kinetin did not stimulate stem growth, and hence was unable to substitute for the roots. However it was very clear that when GA alone, or in combination with kinetin, was applied to the base of de-rooted plants there was a prompt resumption of stem growth (Fig. 11). Treatment with a gibberellin mixture substantially increased the stem length of shoots that had ceased growth, and in this respect replaced the effect of the root system. Three weeks after the first addition of GA, elongation had almost ceased, which may have been due to the withdrawal of this hormone. However it was subsequently

found (Fig. 12) that after a period of GA-induced response, elongation ceased in the continued presence of this hormone, It has been similarly recorded elsewhere that this response to GA is shortlived, and that unaccountably growth ceases after a certain maximum increment (Smith and Wareing 1964b, Wickson and Thimann 1958). In view of later results, it will be suggested growth of explants ceases when the carbohydrate level available for transport in association with the induced active growth centre, becomes limiting.

It was also shown in Experiments 2, 3 and 4 that the gibberellins (GA_3 or GA_{4+7}) were effective in stimulating increased stem growth of shoots that had ceased growth, and in the absence of roots. Especially at a higher concentration (50ppm) GA_3 promoted extension growth, and if applied before bud burst it accelerated the rate of bud growth. The additional extension growth was most noticeable in the internodes within the shoot's apical bud, so that GA resulted in about four more leaves being detached from the apical bud. However exogenous GA seemed to have a limited and localised effect in increasing elongation below the tip, without stimulating growth processes at the apex. Thus during GA-induced growth there was no increase in the total number of leaves and leaf primordia on a detached shoot, whereas in the field there was a continued production of leaf primordia at this stage of a shoot's growth.

In detached apple shoots, cytokinins consistently enhanced primordia production (Jones O.P. 1973), but its effect on kiwifruit shoots was not determined. So while one might suggest that in some respects GA produced an effect (ie. growth) that would otherwise be seen in the presence of a root system, it did not fully reproduce the growth behaviour of an intact plant. To explain a similar situation in willow, Smith and Wareing (1964b) suggested another root-produced factor is also required, which can be produced by active root primordia.

Work with a range of plants indicates that in addition to the young leaves being a source of GA's, roots also can synthesise them and then export them in the xylem (Carr and Reid 1968, Carr et al. 1964, Phillips and Jones 1964, Reid and Carr 1967), and the reduction in stem growth following root pruning can, in part be overcome by exogenous GA_3 application. It appears likely that in the kiwifruit plant also, gibberellins reaching the shoot from the roots contribute to stem elongation, but the literature indicates one should not expect a simple or clear relationship to exist between GA level and stem growth (Crozier et al. 1973, Zeevaart 1971). That the gibberellins GA_3, GA_4, GA_7, caused only a temporary resumption of growth, and a type of growth which showed differences from that in an intact plant, is a sign that factors other than GA originating in the roots are important in stem growth. Absence of the roots means the loss to the shoots of a sink for photosynthates (Humphries and French 1969), and of a source of cytokinins (Sitton et al. 1967), factors which could also affect shoot growth.

In considering the relationship of root GA's to shoot growth in Phaseolus seedlings Crozier and Reid (1971) interpreted their results as showing there is a re-cycling of GA's between the shoots and roots, with the roots merely bringing about an essential interconversion of shoot-GA. The involvement of root GA-metabolism in stem growth control is certain, but elucidation of its precise role will require further detailed examination.

This discussion has indicated factors in addition to gibberellins affect shoot growth, and they could include the growth regulators auxin, abscisic acid, and cytokinins, which materials may interact with each other. Thus ABA will affect GA biosynthesis (Railton and Wareing 1973), BAP markedly alters the rate of GA turnover (Railton 1974), and the importance of hormone balance in the control of shoot growth was stressed by Shein and Jackson (1971, 1972).

Growth of isolated apple shoots is promoted by BAP, and also by a cytokinin extract prepared from apple tree sap (Jones O.P.1967, 1973), suggesting root cytokinins may be essential for shoot growth. In the promotion of growth in tomato seedlings, Aung and Byrne (1976) found a primary influence of GA_{4+7} and BAP on the rate and direction of growth in the apical tissues. Railton and Reid (1973) argued that cytokinins transported to the shoots were of prime importance in maintaining normal growth of tomato plants, since BAP caused a partial recovery of stem growth of waterlogged plants. When a cytokinin was added to the root media of a number of plants it reduced their growth, and had an effect on internode elongation opposite to that of GA_3 (Wittwer and Dedolph 1963). With intact plants it has been found that when applied in combination, kinetin reduces the effect of GA_3 on stem elongation (Shein and Jackson 1972, Wittwer and Dedolph 1963). The same effect was recorded in Avena stem segments by Jones and Kaufman (1971), who found kinetin acts non-competitively with GA_3, and indicated these two types of hormone together would make a good system for the control of stem growth.

The results using stem cutting of kiwifruit show no effect of a low concentration of kinetin alone. Lack of response to applied cytokinin could be due to the marked basipetal movement of these substances, and that its effect on a bud may be recorded only when placed directly in contact with it (Sachs and Thimann 1964). In combination with GA_3 an increased level of cytokinin reduced the GA_3 effect on stem growth. Further tests with increased levels of more suitable cytokinins would assist in determining any effect on explant growth, although Jones O.P. (1973) found some batches of shoots responded to cytokinins far less than others. The application of a single growth regulator may result in abnormal development, and especially where GA was applied from before bud burst the leaves subsequently developing were unusually narrow and pale in colour. When zeatin riboside or other cytokinins

were supplied to isolated apple shoots, the new shoot growth was abnormal, with small poorly developed leaves, and this was attributed to an inadequacy of some other essential factor (Jones O.P. 1967, 1973). Further, it was not surprising to see that a continuous supply of GA_3 from before bud burst suppressed all floral bud development. The gibberellins are potent inhibitors of flowering, and when applied at the time flower induction begins will inhibit flower formation (Griggs and Iwakiri 1961, Junttila 1976, Monselise and Halevy 1964), and in the kiwifruit flower differentiation begins just before bud movement in the spring (Brundell 1975b).

Auxin

Immersing stem cuttings in 10ppm IAA did not promote growth, and such a lack of response could be attributed to a possible lack of absorption, although IAA has not promoted stem elongation in other plants (Grunwald and Lockard 1973 Phillips 1971a, Shein and Jackson 1972). There is some indication that IAA contributes to stem elongation in intact plants (Critchfield 1970, Gunckel and Thimann 1949, Kato and Ito 1962) either alone, or in conjunction with gibberellins (Grunwald and Lockard 1973), and in stem sections IAA promotes elongation (Brian and Hemming 1958, Kaufman 1967) - but usually auxin has less effect on elongation than the gibberellins.

In studies with stem sections, it has been found that the growth response to GA depends on the availability of auxin (Brian and Hemming 1958, Ockerse and Galston 1967), Although when the two are applied together IAA may suppress the GA-promoted growth (Kaufman 1967). Shein and Jackson (1971, 1972) working with bean plants provided some evidence that the growth promotion of GA can be inhibited by auxin, but in studying such growth interactions found a great diversity of results depending on the point of hormone application etc. It seems very dangerous to relate plant growth too closely to responses

found in isolated tissue pieces. If the GA response does require the prsence of auxin, this could explain why stem sections that are excised, and so separated from the site of auxin production, are usually less responsive to GA than in the intact plant.

When the kiwifruit explants were treated with PCIB the results were not as clear cut as they might have been due to the variability of response in the relatively small number of cuttings observed. The results show that PCIB could allow shoots to elongate in response to exogenous GA, although they were less responsive in the presence of the anti-auxin PCIB. In intact plants and stem segments PCIB, a specific auxin competitor, has been found to lower the growth response to GA, and this inhibition has been partly removed by the addition of auxin (Cleland 1964, Kefford 1962, Wright 1968). The evidence for other plants is that GA requires the presence of auxin in order to stimulate growth, and the results for kiwifruit suggests that GA can stimulate extension growth, but that the response is reduced in the presence of a reduced auxin level. Thus while the application of cytokinin or auxin to detached shoots did not cause stem elongation, they can be seen to be involved in the GA-induced response, and so support the idea of growth resulting from the interplay of a number of different types of growth regulator, and hence by implication, for different parts of the plant to be involved in the control of extension growth.

Carbohydrate

In observing the increase in explant shoot length following the application of GA, the amount of the extra growth varied, but overall there seemed to be a fairly constant relationship between the increased growth and the initial length of the cutting. This suggested some characteristic of an explant set a limit on its growth response.

The hardwood stem cuttings that were often used

varied somewhat in size, just as did the extent of the growth that resulted from them. When the initial growth made by Bruno cuttings was compared with the estimated size (volume) of the explant preceding growth, a clear linear relationship occurred (Fig. 13). Hence the amount of new shoot growth was correlated with, and perhaps dependent on, the amount of some factor stored within the dormant stem tissue. Weighing showed the original cutting lost dry matter in supporting new growth, and this amounted to a moderate loss of its own reserves. Priestley (1970) has similarly found that woody tissue loses a certain moderate proportion of its dry weight, up to a certain maximum, in producing new shoot growth, and Kozlowski (1964) has noted the primary importance of reserve carbohydrate for shoot growth of many woody plants. In supporting vegetative growth there is a reduction in the level of sugars, starch, and hemicelluloses in the wood, and their depletion is rapid as the first leaves expand (Buttrose 1966, Priestley 1970). Subsequently these reserves are replenished in the early stages of shoot extension, and Hansen (1971), while showing the importance of reserves in the very earliest phases of spring growth, indicated the probably greater contribution of current photosynthesis to the season's total fruit and shoot growth. Thus for many plants it can be demonstrated that the dormant wood contains reserves that can be mobilised to support new extension growth. In detached stem sections the amount of new growth is limited, whereas in an intact perennial plant with the additional storage capacity of the root system, and long-continued photosynthesis, considerable shoot elongation can occur. Using grape cuttings, Buttrose (1966) found that if they had not developed roots before bud burst, the extent of the new growth was greatly retarded, and this suggests the roots were a source of growth regulators that caused a more effective utilisation of these reserves.

GA-induced stem elongation in kiwifruit explants did not result in an increase in the total dry weight of

the shoot tissue, but there was a change in the distribution of dry weight within the shoot, (Table 3). Hence exogenous GA did not mobilise an increased level of reserves from within the original cutting, but rather transferred dry weight from the leaf tissue to support the extension growth of the stem.

Similarly when the carbohydrate levels in the original cutting were determined (Table 4) it was found starch reserves fell in supporting the initial bud growth, but that no further loss occurred after a period of GA-induced growth. Elsewhere it has been recorded that where exogenous GA increases shoot elongation, there is no increase in the weight of the plants stem tissue (Powell et al. 1959, Shein and Jackson 1971), and where an increase in the dry weight of the plant's top has occurred this is usually associated with a reduction in the root dry weight (Brian 1959). This also indicates an effect of, or role for, GA in changing the distribution of C-assimilates which may then contribute to the resultant increased stem extension growth. Such an effect of GA_3 on the movement of photosynthates in grape plants was demonstrated by Quinlan and Weaver (1970), and Nanda and Purohit (1965) suggested a role for GA in increased extension growth through the mobilisation of starch reserves. Luis and Guardiola (1974) found GA_3 increased transport and mobilisation of reserves, and considered this effect to result from the GA increasing the capacity of the shoot to act as a sink for nutrients.

While in some systems it is clear that gibberellins can directly promote starch hydrolysis by their effect on enzyme synthesis, it is less certain that such an effect occurs in growing plants. Skene (1971) concluded that starch dissolution after winter rest is brought about by an actively-growing cambium, and so any effect of GA would be other than directly in relation to starch degradation. This was supported by the absence of any promotion of sugar release from stem tissue of grapes by GA_3, whereas auxin was very effective in doing so. Similar

observations were made with apple shoots (Pieniazek and Saniewski 1968, Saniewski and Pieniazek 1972), and these authors also believed auxin initiated the degradation of stored carbohydrates, and that this process was subsequently enhanced in the presence of gibberellins. Brian (1959) believed auxin and gibberellin interacted in the control of shoot growth.

It is suggested that in kiwifruit explants, gibberellin interacts with auxin to mobilise carbohydrates that are required for cell extension so that when auxin level falls, the GA is less effective. In the intact plant the roots would contribute to the supply of gibberellins, which is normally adequate, and so there is no response to a GA spray. The GA-response of the explants then ceases at a limit determined by the carbohydrate available for mobilisation, for only so much is available for re-distibution while also maintaining a tissues integrity (Priestley 1970).

Exogenous cytokinins can modify the GA-response, and endogenous cytokinins also are likely to contribute to shoot growth either by maintaining meristematic activity in the apical region, or by exerting their effect through such other hormones as auxins or gibberellins (Luckwill and Whyte 1968, Railton 1974, Railton and Reid 1973). While the gibberellins can affect cell division in the shoot tip (Greulach 1958, Sachs et al. 1959) the cytokinins may have a major role there, and in isolated apple shoots have promoted leaf production (Jones O.P. 1973). In the kiwifruit explants the absence of roots denied the stems such a continuing source of cytokinins, and could account for the absence of further leaf primordia production during the GA-induced stimulation of extension growth.

Section 2

Kiwifruit Vine Sap

A very noticeable characteristic of this plant is the strong sap flow that occurs each spring, which results in heavy bleeding from any fresh wounds. The significance of this flow, and its contents, to the growth of the vine is not clear, but it is usually considered important to avoid excess losses at this time.

At various times in the spring bleeding sap was collected by using secateurs to remove a shoot, and collecting the exudate from the cut surface by placing a rubber tube over the stump and leading the sap through a glass or rubber tube into a clear plastic bag. The bag was changed, usually at one-day intervals, and the collected sap immediately frozen and stored in the deep freeze.

A. Sap Flow

In a preliminary observation in 1972 using established vines of Abbott growing at Levin and trained on a multi-wire system, it was clear substantial quantities of sap could be obtained. On 4 September most buds were emerging, and a small number of one-year old shoots were removed. During daylight of the first day, sap flow was consistently close to 9cc/hour/cut shoot, although by the 9th day it had fallen to 0.5cc.

(a) Sap flow 1973

The same plants were further observed in 1973 although occassional losses occurred through leakage. Shoots removed on 27 or 28 August when only the terminal bud was moving, in some cases had very limited bleeding

in the first 24 hours. Fresh shoots were removed for sap collection on 14 September when many buds were at the open cluster stage; also on 25 September, when most shoots were 2-9cms long (longest ones 25-28cms); and and finally on 8 October some 5 weeks before full bloom, by which time the oldest leaves were almost fully expanded.

1st collection	27 August	Tip buds advanced bud swell
2nd	10 Sept.	Many buds burst
3rd	14 Sept.	Most buds at open cluster
4th	25 Sept.	Shoots mostly 2-9cms length (lower laterals at bud burst)
5th	8 Oct.	Five weeks before full bloom

Results:

Flow rates for each cut shoot, at each harvest date are shown in Fig. 17.

It is clear that while certain patterns are apparent, mean values of sap flow are misleading due to the considerable variation in sap flow rates from shoot to shoot - this variation was smaller two or three days after removing the shoot.

With the earliest harvest daily sap flow increased after cutting, but with subsequent harvests, flow rates fell off to very low values by the third day especially with the final harvest. The 6-week period sampled was virtually the whole of the period the cut shoots bled freely.

Flow rates were lowest in the first harvest ie. immediately before, and at the time of bud burst. They rose to a peak in the 14 and 25 September harvests ie. when the shoots were at the open cluster stage or were up to 9cm long.

In an attempt to relate sap flow to weather conditions, air temperature and rainfall records were studied.

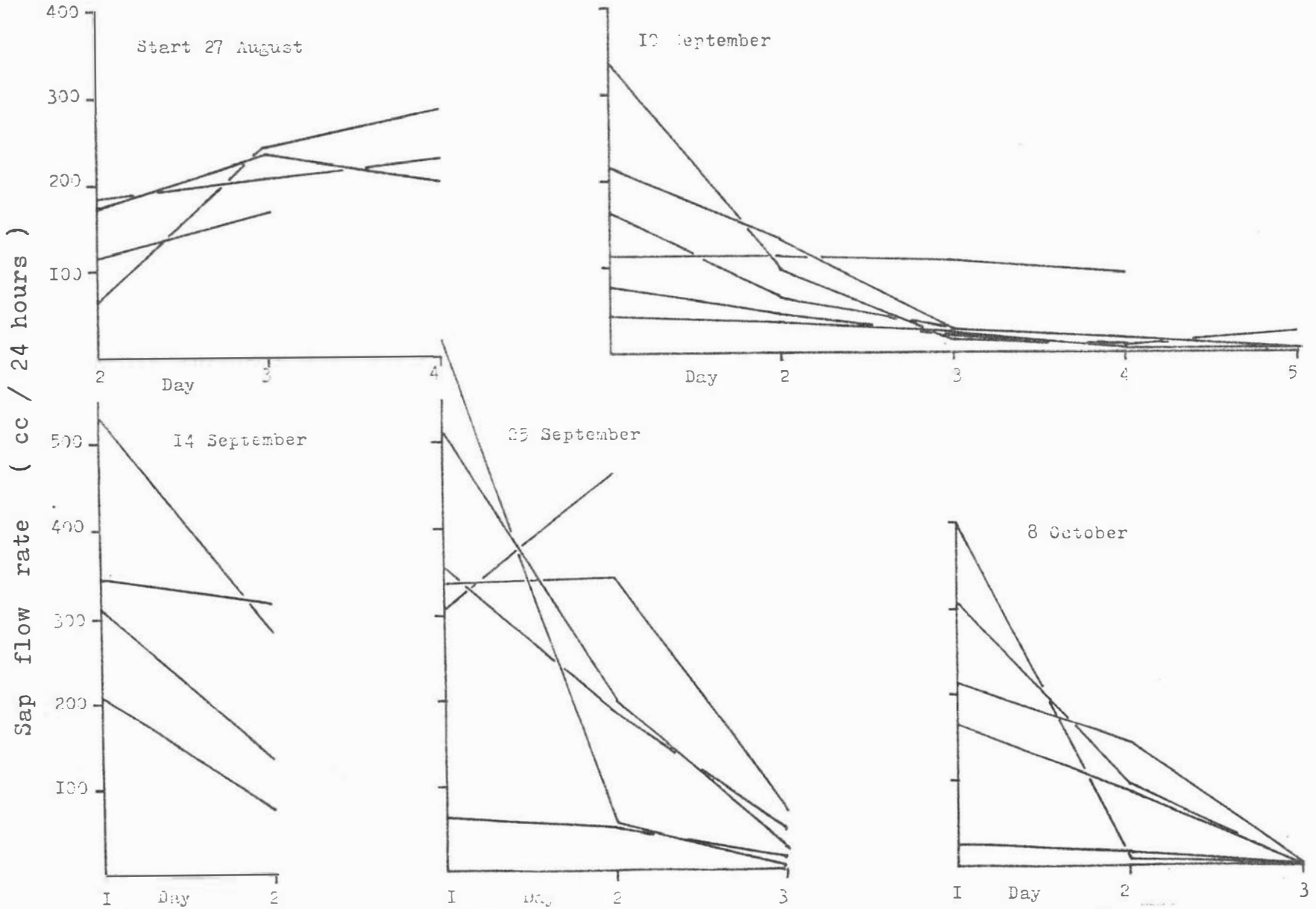


FIG. 17. SAP FLOW FROM INDIVIDUAL SHOOTS CUT ON DAY 0, IN EACH SUCCESSIVE 24 HOUR PERIOD.

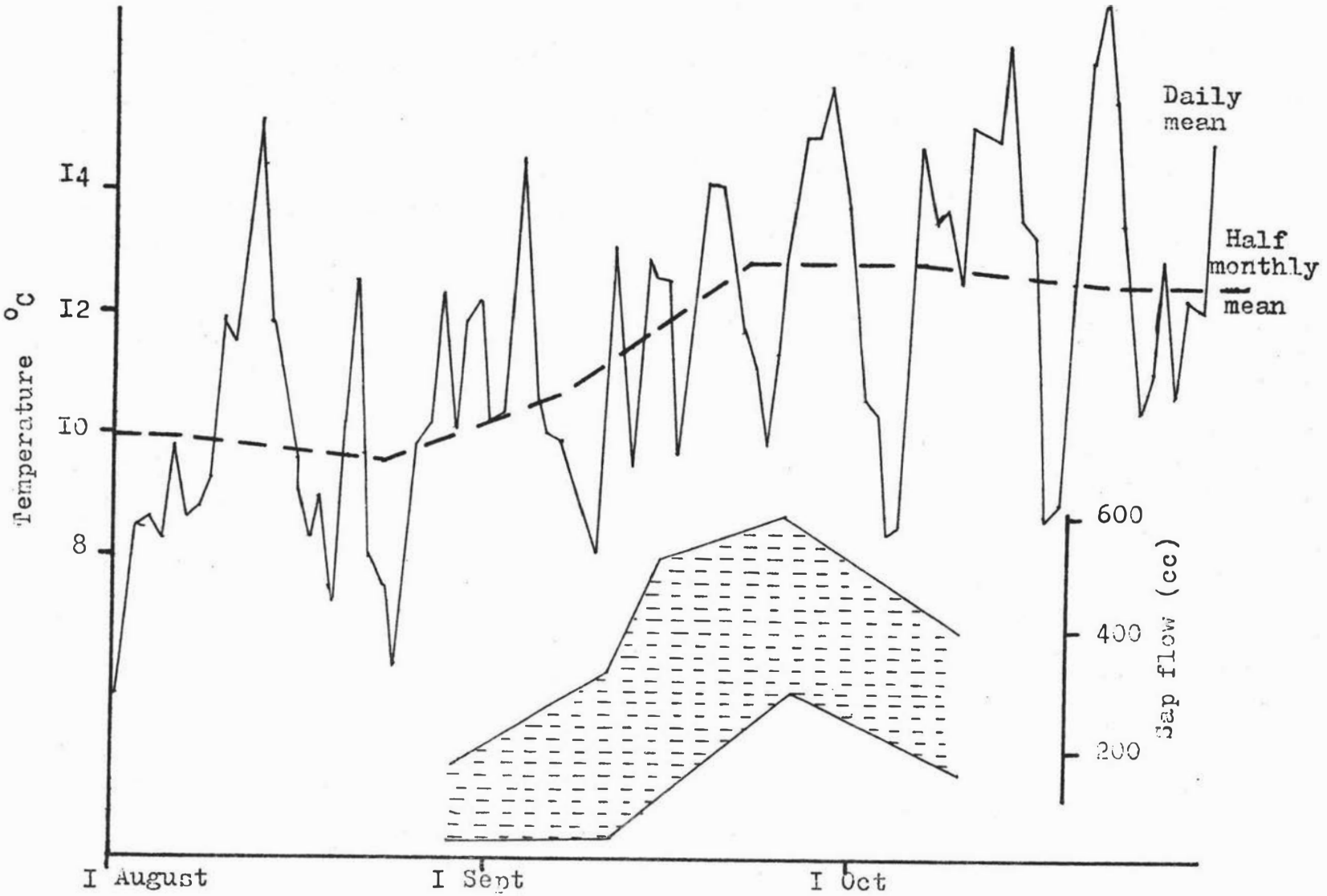


FIG. I8. AIR TEMPERATURES IN RELATION TO THE FIRST DAYS SAP FLOW RATE

Air temperature

Over the period August - October mean daily temperature fluctuated widely, but by taking half-monthly periods it can be seen (Fig. 18) the curve for sap flow closely parallels the half-monthly mean air temperature:

27 August	Harvest 1	10°C	Sap flow		low
10 Sept	2	11°C	"	"	rising
14 Sept	3	12°C	"	"	maximum
25 Sept	4	13°C	"	"	high
8 Oct	5	13°C	"	"	declining

Such a relationship is not surprising as one might expect vine development to be closely related to temperature. In the maple it is suggested that normal temperatures of −3.9°and 5°C' accompany the beginning and end repectively of sap flows, and that seasonal departures from normal are related to seasonal sap production (Morrow 1973).

Rainfall

A period of considerable rain (greater than the 30 year average) appeared to coincide with the greatest sap flow.

During August 84mm of rain fell, and early sap collection were low. During the first day's collection from the third harvest (14/9) there was 16mm of rain, but while there was close to 30mm during the second day of bleeding, this did not prevent flow rates from falling markedly. So while the high rainfall may have contributed to the high sap flow rates in mid and late September, it does not seem likely it played a major part.

Several one-year old Abbott plants in the field had been trained as two-shoot plants and on 25/9/73 14 plants had both shoots cut close to the ground, and 18 had only

one shoot removed for bleeding - the remaining one bearing new shoots of about 6cms length.

Flow rates showed considerable variation between plants, but tended to be highest in those plants furthest advanced in development, and in Day 5 the flow of bleeding sap had ceased from most of the partially-decapitated plants. Although no record was made of shoot size they were all of comparable size, and the difference in sap flow reflects the difference in the amount of wood cut.

cc sap/cut/24 hours

Period		0 - 24	24 - 48	99 - 123hrs
Decapitated	Mean	104	151	86
	Range	26-205	65-311	40-164
+ Shoot	Mean	58	84	15
	Range	17-174	31-192	0-127

(b) Sap flow 1975

In the spring of 1975/6 bleeding xylem sap was collected from 3 year old Abbott vines growing at Massey, and records kept of sap flow. As in the past, sap was collected at about 9.30 each morning and frozen in a plastic bag. On each harvest date each of the two main stems of two plants were cut off, and the sap collected through a rubber tube.

In early August a small sap loss occurred from old cuts on the vines, and shortly after sampling began, usually for 5 days:

No.	Harvest Date	Stage of vine growth
1	12/8/75	No bud movement seen.
2	25/8/75	First 2-3 buds just splitting out of stem.
3	4/9/75	Tip buds at advanced bud burst, most others at advanced swell.
4	12/9/75	Tip buds at tight cluster, most others at advanced bud burst.
5	18/9/75	Tips at open cluster, most other buds at advanced bud burst - tight cluster.

Results:

The flow rate for each cut shoot at each harvest date is shown in Fig. 19, in which it can be seen:
(i) At each harvest, sap flow varies greatly between plants, and between shoots on the one plant. Variation was least at the first harvest.
(ii) Usually flow rates rose to a peak during the third day after decapitation, and then declined. This is also shown in Fig. 20 where the total bleeding sap flow rate in each case is related to a unit size of cut stem - but using actual flow rates produces the same pattern.
(iii) Sap flow rose to a peak at the second harvest, and then declined as development progressed, (Fig. 20).

When other vines were cut 30/9/75, many did not bleed, but vines of other later-developing varieties cut at this time bled heavily.

The pattern of sap flow here varied from that recorded in 1973. In the spring of 1975/6 peak flow rates occurred earlier in the season at an earlier stage of development, and at the final harvest had fallen to low values - the stage which in 1973 gave the highest flow rates. This difference is probably related to the sampling method used, and the large variation in development over the vines used in 1973. Sap collections then were taken from the upper tier of main laterals, the shoots on which

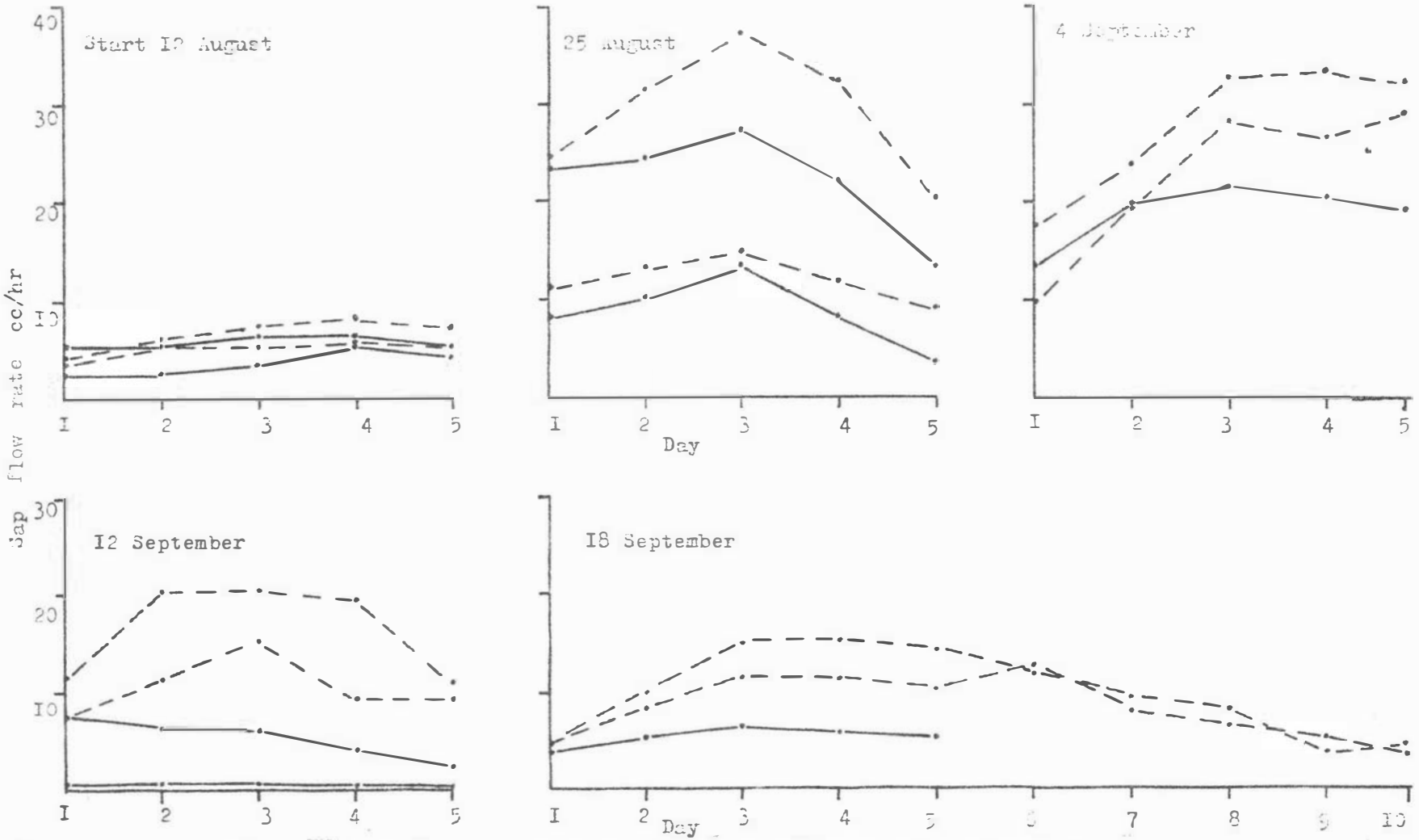


FIG. 19. DAILY SAP FLOW FROM EACH MAIN STEM OF DECAPITATED KIWIFRUIT PLANTS, CUT ON DAY 0.

•————•First plant. •— — —• Second plant.

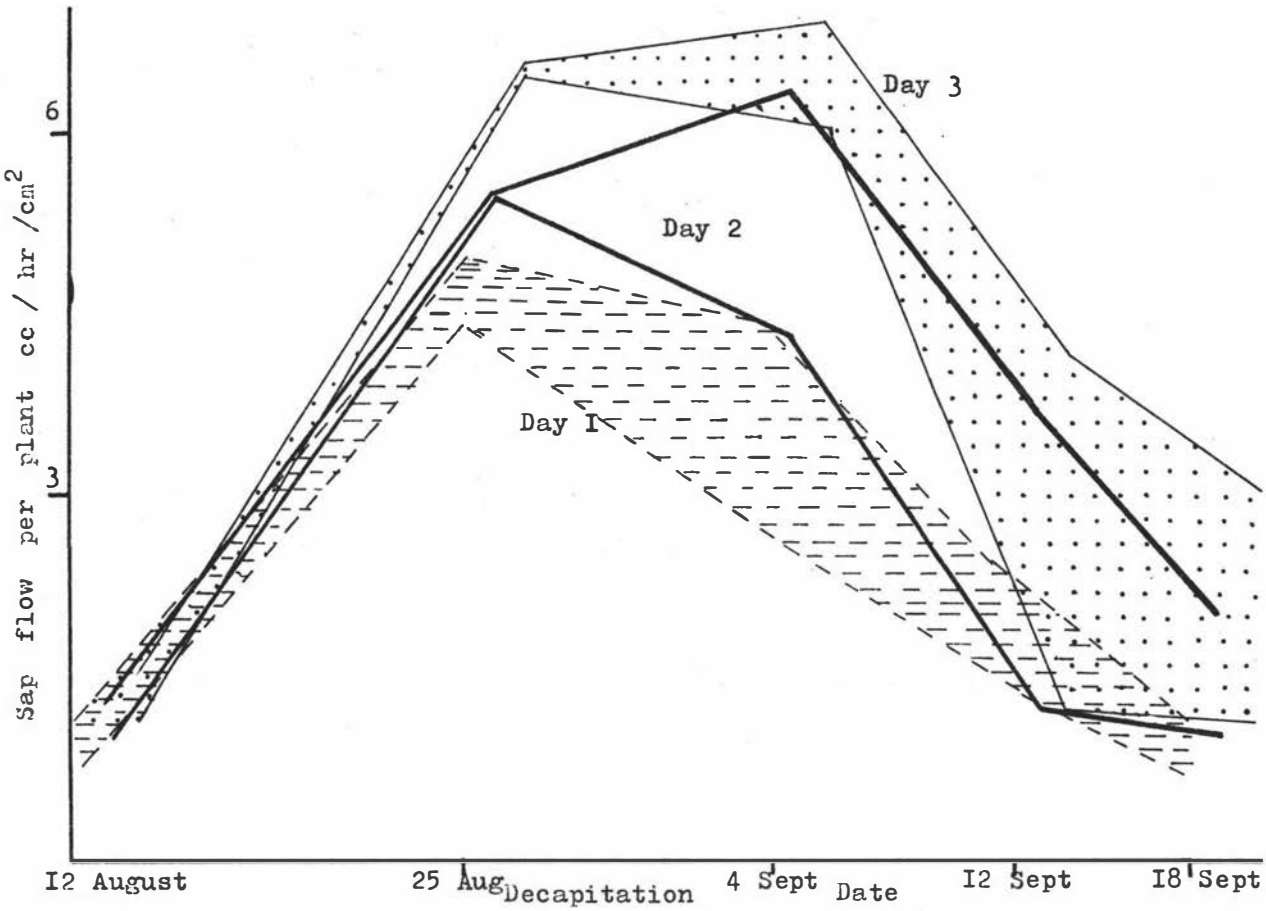


FIG. 20. RATE OF SAP FLOW AT EACH CUTTING DATE, FOR THREE DAYS AFTER DECAPITATION.

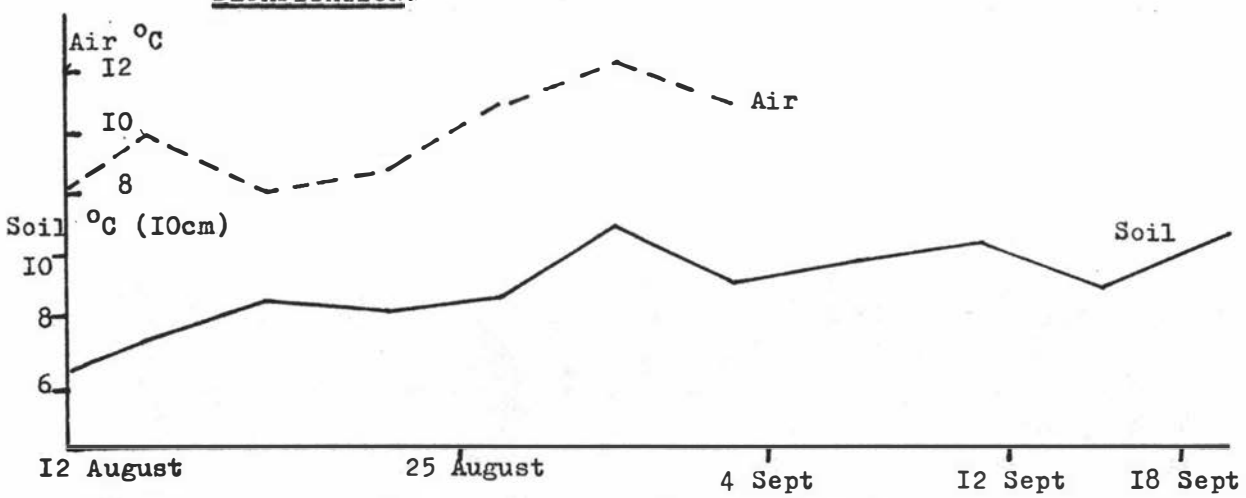


FIG. 2I. SOIL AND AIR TEMPERATURES. (4-day means.)

were about two weeks ahead of development on the lower tier.

In considering any relationship of sap flow to weather conditions one should note the following.

Temperature.

Soil temperature (at 10cm depth) in an adjacent field rose from about 6°C at the time of the first harvest, to 8°C prior to the second harvest peak, and reached 9° - 10°C during the following harvests of declining sap flow. See Fig. 21.

Before the first sap collection air temperatures were variable, but the trend was for temperatures to rise from about the time of the second harvest viz. from a mean of around 8.4°C up to 23 August, and then 11°C in the following week.

Rainfall

A moderate amount of rain fell in mid August which could have ensured adequate moisture supply to support any demands made by the high sap flow at the second harvest. The very heavy rain on 27, 28 August did not appear to increase sap flow on those days, or to prevent a decline in flow rate at the third harvest.

Plant characteristics

Brief reference has been made to the stage of plant development as related to sap flow rates, and in addition a record was kept of the size of each shoot at the point of removal.

Assuming a circular cross-section, there appeared to be a positive correlation between flow rate and size of the cut surface, when expressed as either the surface area or shoot circumference. This was shown for days 1, 2, and 3 following decapitation where the apparent linear relationship applied to all except the 4th harvest. The relationship varied between each harvest date, the slope being lowest for the first harvest, and greatest for the

second one - a reflection of the change in actual flow rate. The relationship for any plant remained the same throughout the period of observation. Flow rate appeared not to be related to the height above ground of the cut surface.

Diurnal flow rates

At the time of the 4th and 5th harvests on 12 and 18 September respectively a single-shoot plant was decapitated, and sap collected usually morning and evening. Unfortunately their flow rates were relatively low, and the infrequent collections did not permit a clear indication of any diurnal fluctuations. On most days flow rate was a little higher from 9am - 5pm than from 5pm - 9am.

When two plants were decapitated on 17/8/76 when only terminal buds were moving, and the sap collected over 3 days, a peak flow rate was recorded around noon each day usually in the period 1230 - 1530 hours.

Decapitation of a plant as done for these sap collections did not prevent it from producing strong new shoot growth. Most plants that had been bled had a new shoot emerging by the end of September, and all stumps had produced several strong new shoots by mid November. This was in spite of the large quantities of sap lost, and the marked blackening and necrosis of the bark where sap had flowed down over it. Similarly on vines where some lateral shoots had been removed eg. for grafting, bleeding produced bark damage but did not prevent new growth occurring. Bleeding from a cut delays the growth of buds on the cut shoot, compared with those on uncut shoots.

B Sap Growth Regulators

It may well be universal for plant sap to contain a number of growth regulators, some of which could have been synthesised in the roots, and these hormones no doubt contribute to the control of growth and development in all parts of the plant. By determining growth regulator activity in the spring sap flow at different stages of development, it was hoped to gain some idea of their possible contribution to growth control, especially the influence of root hormones on the top of the plants.

Using the sap collected at bud burst on 4 September 1972 from established plants at Levin, preliminary tests investigated the possibilities of detecting gibberellin and cytokinin activity in the sap.

With a standard extraction procedure and bioassay (using the barley endosperm and radish cotyledon tests), activity in sap extracts could be detected after paper chromatography. It was also possible to detect gibberellin activity following gradient elution from a silicic acid column.

This work indicated the acidic ethyl acetate phase contained gibberellin-like substances which ran to Rf 0.6 - 0.8 on paper with the solvent isopropanol-ammonia-water (IAW) 8/1/1. When the sap extract was eluted through a silicic acid column with a ethyl acetate/n-hexane mixture, gibberellin-like activity was located in fractions 14-18 (elution volume 650-900cc). These fractions were eluted with a mix containing 42-65% ethyl acetate, and comparison with GA_3 standards indicated a total equivalent activity of 1.1 µg/litre of sap.

A limited number of bioassays with radish cotyledons indicated the presence of cytokinins but the response was variable.

With this background, observations were continued

in following years.

Methods.

Sap was collected and immediately frozen, it was then stored in the deep freeze until it was freeze-dried The extraction method then used is shown in Fig. 22. Re-distilled solvents were used throughout. Extraction and determination of hormone activity in sap extracted from the kiwifruit vine in the spring of 1973 was almost identical with the methods used in the previous year.

(i) Gibberellin-like activity

The acidic ethyl acetate extract was brought down to dryness on a rotary evaporator. The dry residue was taken up in absolute methanol and an aliquot (usually equivalent to about 25-60cc of sap) streaked on pre-run chromatography paper Whatman No. 3MM. The descending chromatogram was usually run in isopropanol-ammonia-water 10/1/1 (IAW), dried and cut into 10 Rfs for bioassay.

Acidic ethyl acetate has frequently been used to remove gibberellin from aqueous solution, but Jones (pers. comm) has obtained greater recovery from apple tree sap with the use of butanol. Inhibitors often partition with the gibberellins, including ABA which interferes with GA_3 in the barley endosperm bioassay (Chrispeels and Varner 1967) - although it's level in kiwifruit sap is low (Davidson and Young 1973).

Bioassay

Gibberellin-like activity was determined by the barley endosperm bioassay (Jones and Varner 1967), usually using the Crop Research Division (Lincoln) Research variety. Dehusked barley seeds were soaked in water at $3^{o}C$ for 24 hours, then cut into uniform halves with white cut surfaces ready for use. Each Rf strip was placed in a 25cc Erlenmeyer-flask with 1.5cc distilled water, 10µg chloramphenicol and three endosperm half-seeds. The flasks were

sealed and incubated in high humidity in the dark at 30°C for about 48 hours. On each occasion a replicated series of GA_3 standards was also incubated with blank paper strips. Four replicates of each standard solution was used, and two or three sample replicates.

After incubation 1.5cc or 2.5cc water was added to each flask, and a 2cc aliquot put in a test tube. After adding 2cc of fresh dinitrosalicylic acid reagent (Miller et al. 1960) the test tube was placed in a boiling water-bath for 8 minutes, and then cooled. As required, a constant volume of water was then added to each, and the absorbance at 640mμ measured on a Hitachi spectrophotometer.

(Reagent mixture: Dissolve 4gm NaOH in 200cc distilled water. Then add and dissolve 4gm 3, 5-dinitrosalicylic acid, 0.8gm phenol. 0.20gm sodium sulphite and 80gm potassium tartrate. Make up to 400cc, and filter).

In some instances the acidic ethyl acetate phase was further purified before bioassay, and an attempt made to make a separation between some of the gibberellin-like substances. This involved the use of a gradient-elution technique, but unlike the preliminary work, the extract was first eluted from a Sephadex column.

Column purification on Sephadex

The acidic ethyl acetate extract was purified on a Sephadex column, following the method of Crozier et al. (1969), except that Sephadex G15 was used (Reynolds 1970). 20gm of Sephadex was swelled in phosphate buffer, and a slurry settled into a 2 x 15cm column by eluting 75cc of 0.1M phosphate buffer. A paper disc was placed on top of the packing 0.3cc blue dextran dye washed into a column, and then the sample was added.

The dry sample was taken up in 0.10M phosphate

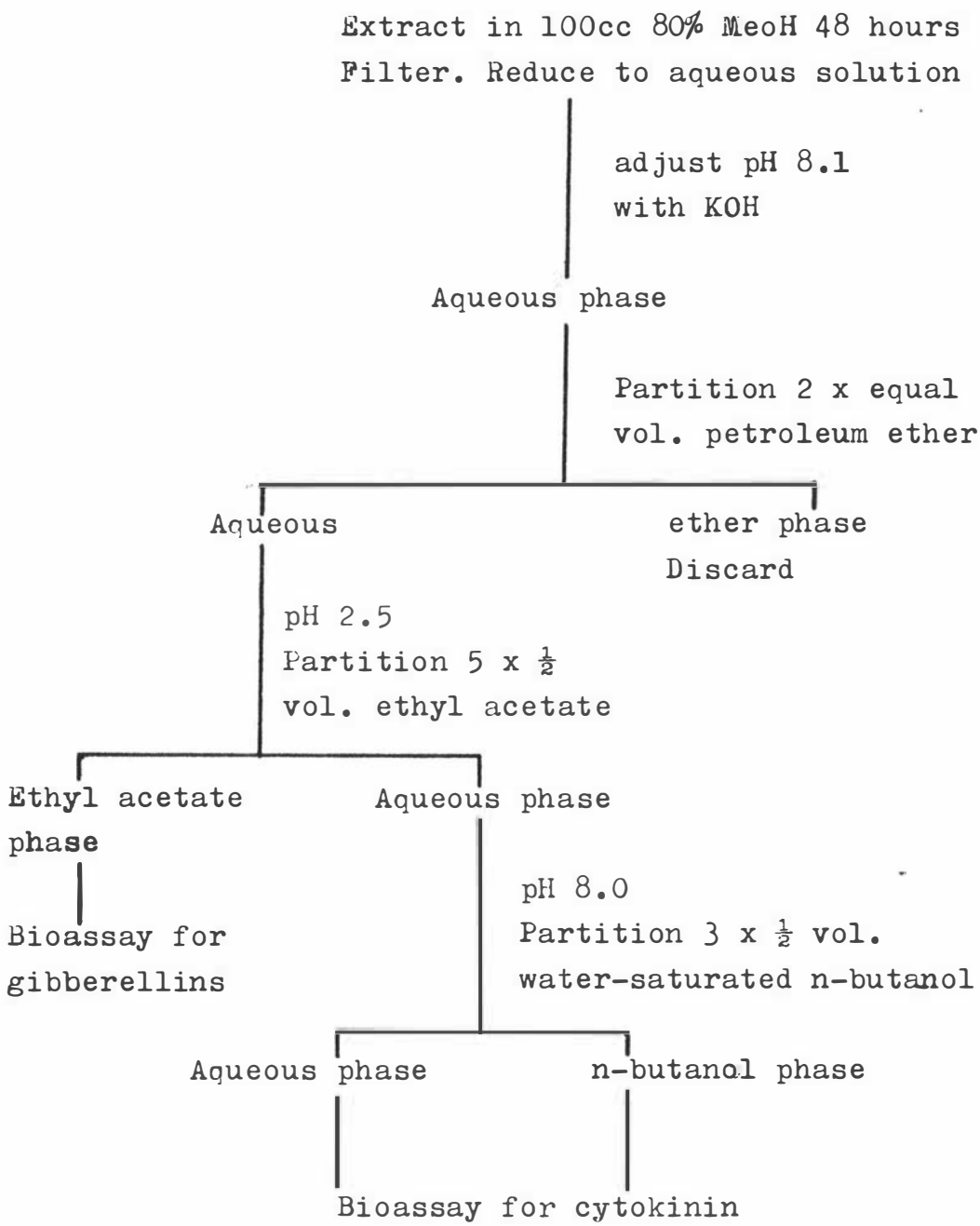


Fig. 22 Extraction of Gibberellins and Cytokinins from Sap

buffer, and added to the column in 1.5cc solution. Elution of the column then proceeded with 0.1M buffer, at 1.5cc/minute from a Mariotte flask. After the dye had been eluted the next 150cc was collected, adjusted to pH 2.5 and partitioned with ethyl acetate. (In a preliminary test there was no activity in the elution volume 150 - 350cc).

This ethyl acetate extract was taken down to dryness, then taken up in a small quantity of methanol and put on an absorbent chromatographic disc for gradient elution.

Separation on a silica gel column

The sap extract was eluted through a silicic acid column according to Powell and Tautvydas (1967), although a similar technique has been used by others (Crozier et al. 1972, Gaskin et al. 1973, Khalifah et al. 1965, Powell et al. 1969).

First, 64gm dry acid-washed silicic acid (M&B) 80-100 mesh was hydrated with 43cc 0.5M formic acid, and mixed over 1-2 hours to give a free-flowing powder. This was slurried with n-hexane saturated with 0.5M formic acid, and packed into a column 28 x 2cms, care being taken to remove air bubbles. Then 100cc of the formic-saturated n-hexane was passed through the column. The disc containing the dry extract was held on top of the column by glass beads, and eluted with an increasing concentration of ethyl acetate in n-hexane in the following manner.

Two glass vessels were joined at the base by a tube and stopcock. The first vessel contained 420cc 0.5M formic acid-saturated ethyl acetate, and fed into the second vessel containing 600cc 0.5M formic-acid saturated n-hexane. A magnetic stirrer gave constant mixing, and under gravity the mixture was passed to the top of the silica gel column. In this way the sample was eluted

with an increasing concentration of ethyl acetate at 2.5cc/min.

The 20 x 50cc fractions were collected, dried in the vacuum oven for at least 36 hours, and the extracts then tested in a bioassay with barley endosperm.

(ii) Cytokinin activity

The aqueous and n-butanol phases (Fig. 22) were each reduced to dryness on a rotary evaporator. Aliquots were then streaked on to a thin-layer plate of silica gel GF_{254} and run 10cms in distilled water pH 5.6. After air-drying the plates, the 10 Rfs were scraped into a 4.5cm petri dish and covered with a filter paper disc. To this was added 1cc distilled water and 8 or 10 radish cotyledons. Dishes of samples and standards on a tray were sealed in a moist plastic bag and incubated at 25°C under fluorescent lights for about 69 hours. The fresh weight of the cotyledons was then determined.

(Long Scarlet radish seeds were germinated on moist paper in the dark, for 24-28 hours; the two smaller cotyledons were then carefully removed, and selected for size uniformity for use in the bioassay. (Letham 1968).).

Experiment No 1 1973

Using the above procedures the change in gibberellin-like activity was assessed in the sap samples collected in the spring of 1973. Purification was by solvent partitioning and paper chromatography, followed by bioassay.

Very limited tests of cytokinin activity were made, using only sap from the first two collections.

Results:

(a) Gibberellin-like activity.

This was assessed for each sap sample on different dates using two or three replicates of sap extract. The results of the bioassay are shown in Fig. 23, which indicated the sap activity of GA-like substances in GA_3 equivalents (µg/l) as:

Harvest No.	1	2	3	4	5
	0.67+	1.11	0.47	0.53	0.39

(+ A test with 5.5cc sap equivalents indicated 1.38 µg/l).

These values agree with the level of activity indicated in the preceding year. These data suggest a relatively constant level of activity in the sap from early bud movement to early shoot extension, with possibly a decline in concentration from the first to the last harvest. Because of the variation in bud development and sap flow over the vine the results are difficult to interpret, and no attempt was made to assess the amount of gibberellins being transported.

(b) Cytokinin activity

Bioassays indicated cytokinin activity was present in the butanol phase extract of sap from each of the first two collection dates with similar levels of activity in each, namely the equivalent of 4.5 and 2.4µg BAP per litre of sap respectively.

Experiment No 2 1973

Further determinations of sap-GA were made, in a comparison of bleeding sap from root systems in the presence or absence of growing shoots.

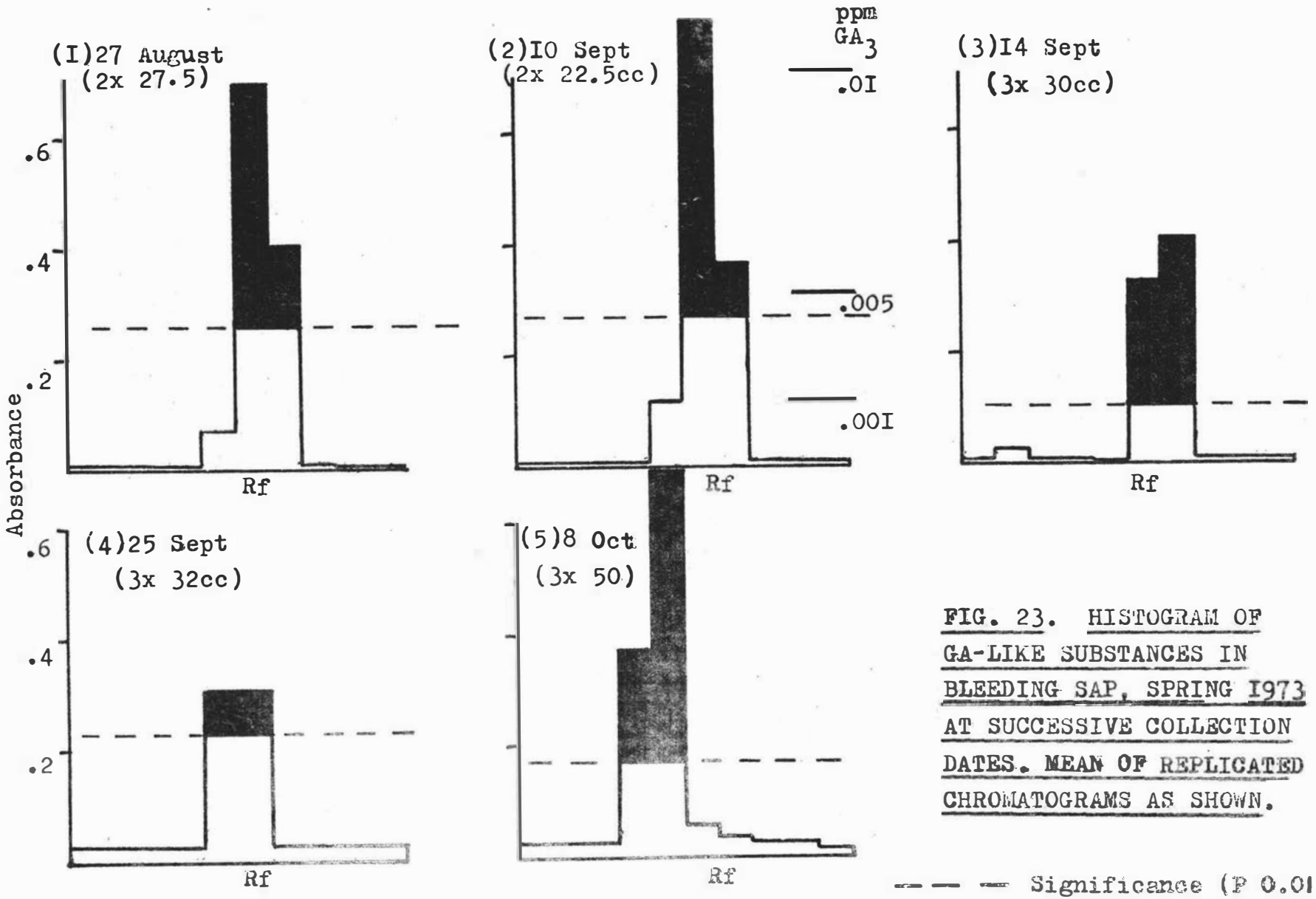


FIG. 23. HISTOGRAM OF GA-LIKE SUBSTANCES IN BLEEDING SAP, SPRING I973 AT SUCCESSIVE COLLECTION DATES. MEAN OF REPLICATED CHROMATOGRAMS AS SHOWN.

Method.

A number of one-year old Abbott plants growing in the field were selected on 25/9/73, when the new seasons shoots were usually less than 5cm long. Each plant had been trained with two leaders.

On the above date, 14 plants were cut off near ground level ("Decapitated") and the xylem sap subsequently collected from the two stumps. Another 18 plants had only one leader removed, from which the bleeding sap was collected. Using sap from each treatment, collected on the first, second and fifth day after cutting, gibberellin-like activity was assessed after purification by solvent partitioning (Fig. 22), and separation on a Sephadex column and a silica-gel column as described earlier.

Results.

The results of the barley endosperm bioassay with the 19 fractions obtained from each sample are shown in Fig. 24, and as the tests were carried out on different days, quantitatively they are only roughly comparable. Again there was rather a large variation between replicates of the active fractions, but the histograms show one clear trend.

In both treatments, in each of the first two days, gibberellin-like activity was found only around fractions 15 - 17 as one broad peak. However on the fifth day of sap bleeding, activity occurred in fraction 4 in both treatments. In Fig. 24 activity occurred in fractions 9 - 15 from the partially-decapitated plant on Day 5, but when a smaller amount of extract of both treatments was tested again, no significant activity was detected in these fractions.

Gibberellin-like activity was relatively similar in all samples tested. The overall low level of activity, and the variability makes proper quantitative comparisons impossible, but bioassay indicated sap activity of GA-

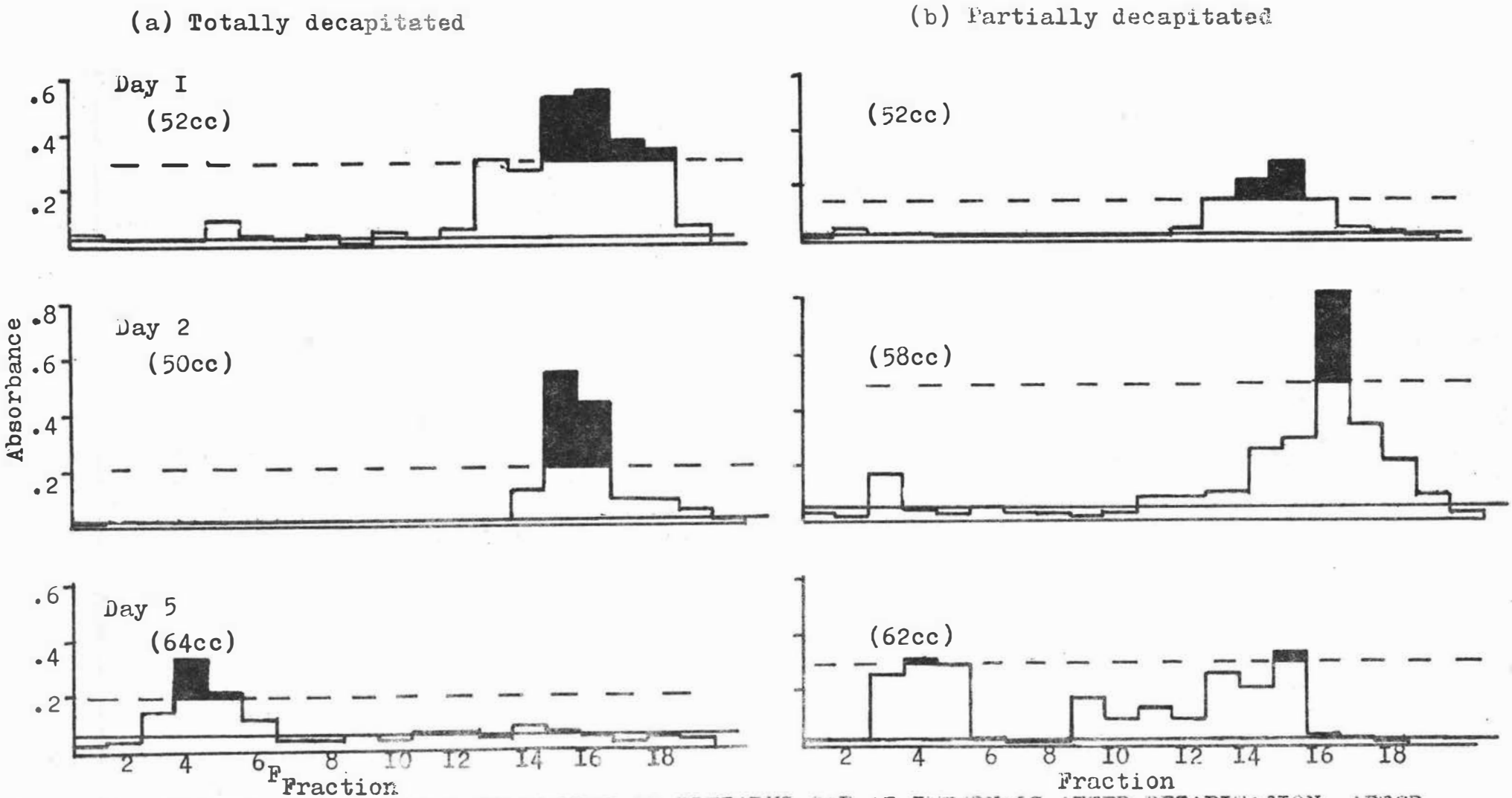


FIG. 24. HISTOGRAM OF GA-LIKE SUBSTANCES IN BLEEDING SAP AT INTERVALS AFTER DECAPITATION, AFTER GRADIENT ELUTION FROM SILICA GEL COLUMN.

like substances, in GA_3 equivalents µg/litre as:

	Day 1	Day 2	Day 5++
Total decapitation	0.23+	0.05	0.05
Partial decapitation	0.05	0.03	0.03

+ Subsequent tests indicated activity equivalent 0.04 µg/litre.

++ Activity occurred in fractions different from the earlier samples.

In further work with endogenous growth regulators, the extraction method and bioassay methods were changed.

The extraction procedure is shown in Fig. 25 where the gibberellins were partitioned into ether, rather than ethyl acetate as in earlier experiments (Goren et al. 1971).

(i) Gibberellins

In a preliminary test, this acidic ether extract contained the same gibberellin-like activity as an acidic n-butanol extract (following a neutral ethyl acetate extraction), which latter method was used by Jones (pers. comm.). So the method based on ether was used for convenience. The dried extract was taken up in methanol, streaked on Whatman 3MM paper, and run (descending) 10cms in 10/1/1 isopropanol-ammonia-water. After drying, the 10 individual Rf's were eluted overnight with absolute methanol, and the eluants made up to 10ml. Suitable aliquots (about 50cc sap equivalent) were dried for 24 hours in a vacuum oven in 25ml Erlenmeyer flasks, ready for bioassay.

The flasks contained 0.75µg sodium metabisulfite, 1.5cc solution, and four barley endosperm half-seeds. They were sealed and incubated at 29.4° for about 46 hours, then the sugar content of each solution determined with an Abbe

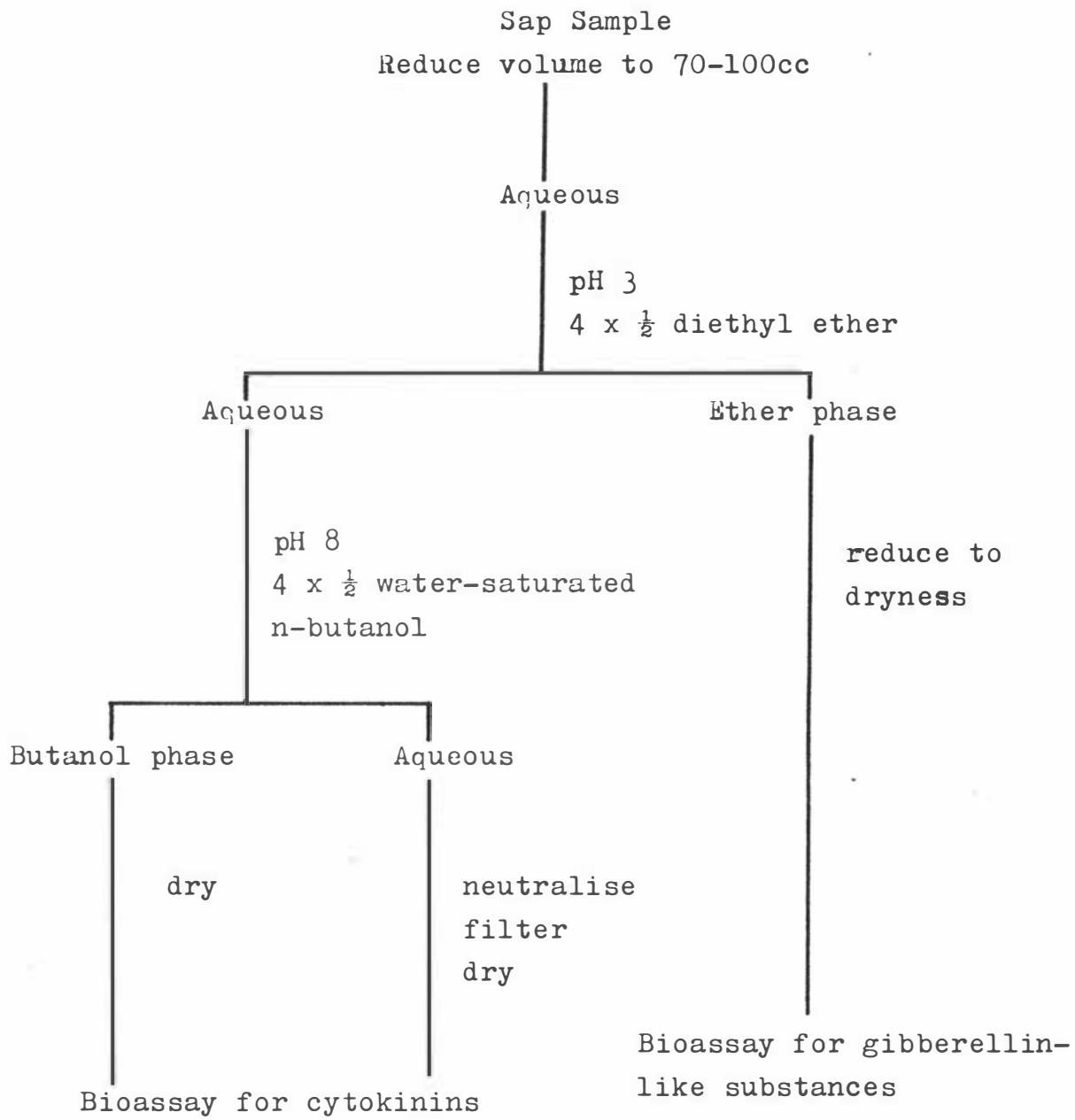


Fig. 25 Extraction of Gibberellins and Cytokinins from Sap.

refractometer, recording the refractive index. This method was used by Faull et al. (1974) although not described by them, and was also found by Jones (pers. comm.) to be satisfactory.

Several tests showed the feasibility of this rapid method of assessing the results, and the results of one comparison are shown in Fig. 26. The barley endosperm bioassay was performed with the varieties 'Research' and 'Black Skinless', and four replicates of each were incubated with a series of gibberellic acid standards. The results of two replicates each were assessed with dinitrosalicylic acid (as described earlier) and with the refractometer.

(ii) Cytokinins

The alkaline butanol extract was taken down to dryness on a Buchi rotary evaporator. The residue was then taken up in methanol, streaked on pre-run chromatography paper Whatman 3MM, and run in a descending manner with distilled water pH 5.6. The aqueous phase was not always purified by paper chromatography.

The chromatograph strips were placed in a 100ml Erlenmeyer flask with 25ml media, and cytokinin activity assessed by soybean callus (three or four pieces to a flask), (Miller 1963). The flasks were held in low intensity fluorescent light at 22° for 3 or 4 weeks, then weighed. Samples were compared with the response to replicate flasks of Kinetin standards.

Experiment No. 3 1975/76

Sap collected in the spring of 1975 was purified as in Fig. 25 and cytokinin and gibberellin activity determined. The five sap collections were made from 12 August (before bud burst began) to 18 September (buds

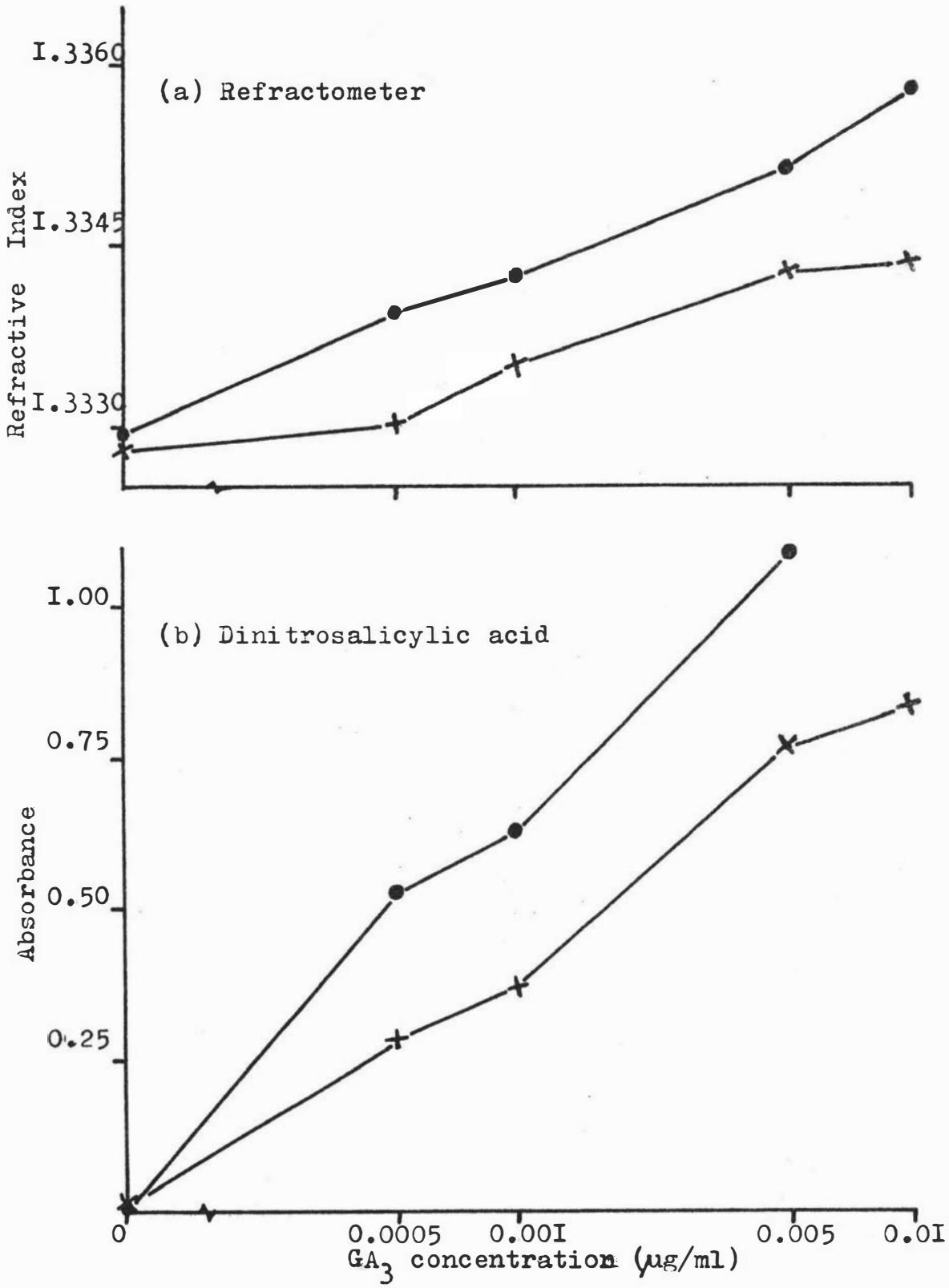


FIG. 26. STANDARD CURVES FOR BARLEY ENDOSPERM RESPONSE TO GA_3 DETERMINED REFRACTOMETRICALLY AND COLOURIMETRICALLY.

●——● Research variety ×——× Black Skinless variety

just beyond bud burst), and for each sample the sap collected in the first 24 hours after decapitation was used. Analysis were completed in November/December.

Results.

(a) Gibberellins

The results of the replicated bioassays with these sap samples are shown in histogram form in Fig. 27 . In Table 5 sample activity is expressed in terms of equivalent GA_3 activity by comparison with a series of standard solutions.

Table 5: Gibberellin-like activity in sap

	Activity equivalents GA_3 µg/litre sap			Equivalents GA_3 µg/day		
Harvest Date	Plant A	Plant B	Plant C	Plant A	Plant B	Plant C
12/8/75	0.01	0.14	-	0.0018	0.0296	-
25/8/75	0.02	0.05	-	0.0146	0.0376	-
4/9/75	0.06/ 1.81+	0	-	0.0205/ 0.60+	0	-
12/9/75	0.11	0.22	18.58	0.0216	0.1026	1.0776
18/9/75	0.73+	2.34+	0.10	0.0669+	0.5415+	0.0080

+Based on extrapolation of the standard curve.

These values indicate a low level of gibberellin-like activity in the sap in the early spring, and a wide variation between plants at a given harvest date and similar stage of development. The concentration of gibberellin-like substances in the sap, and the computed amounts being transported in the sap in one day, show a pattern of being higher in the final two collections - at which time most buds had completed bud burst, and shoot

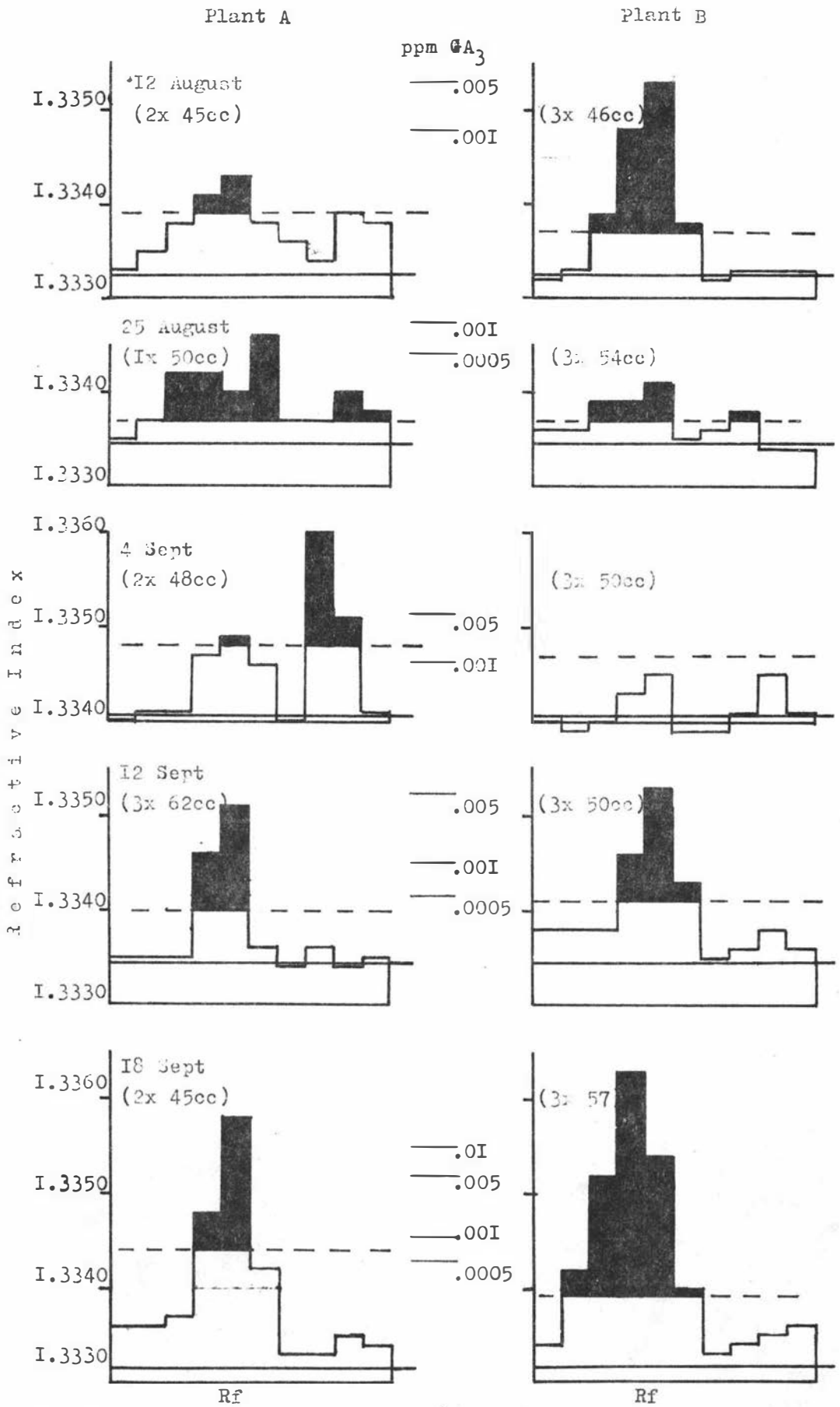


FIG. 27. HISTOGRAM OF GA-LIKE SUBSTANCES IN BLEEDING SAP, SPRING 1975 AT SUCESSIVE DATES FROM DIFFERENT PLANTS. REPLICATED BIOASSAYS AS SHOWN.

——Control level, — — — significance level (P 0.01)

elongation was beginning.

Limited tests indicated similar, or lower activity in sap collected on the second day after decapitation.

The other effect was for there to be two peaks of activity in the sap collected on the first three harvest dates. In a repeat bioassay of extract of the third harvest, Plant B, two peaks were probably present although some uncertainty arises from the inadvertent mixing of some test solutions. In a duplicated test of the second-day's sap flow from one of these plants, there was no indication of a second peak.

(b) Cytokinins

Sap collected at each of the five decapitation dates gave significant activity when extracts were bio-assayed with soybean callus, (Fig. 28). Activity occurred mainly at Rf 0.6 - 0.8 in the butanol-phase, but the level of activity showed considerable variation between the two plants sampled at the same time. There was, in addition, in half of the samples a very low level of activity in the same position on chromatograms of the aqueous phase; except for some unknown reason the aqueous phase of Plant 22 (Harvest 4) contained far more activity than the butanol-phase.

By comparison with a standard curve prepared with kinetin, values for cytokinin activity in the sap are calculated and shown in Table 6. In addition, reference to the daily sap flow rate permits an indication of the amounts of cytokinin being transported in a day. In a few instances, sample activity was beyond the range of the standards. This was especially so for Plant 20, the most active extract, and it seems best to only indicate a minimum level of activity.

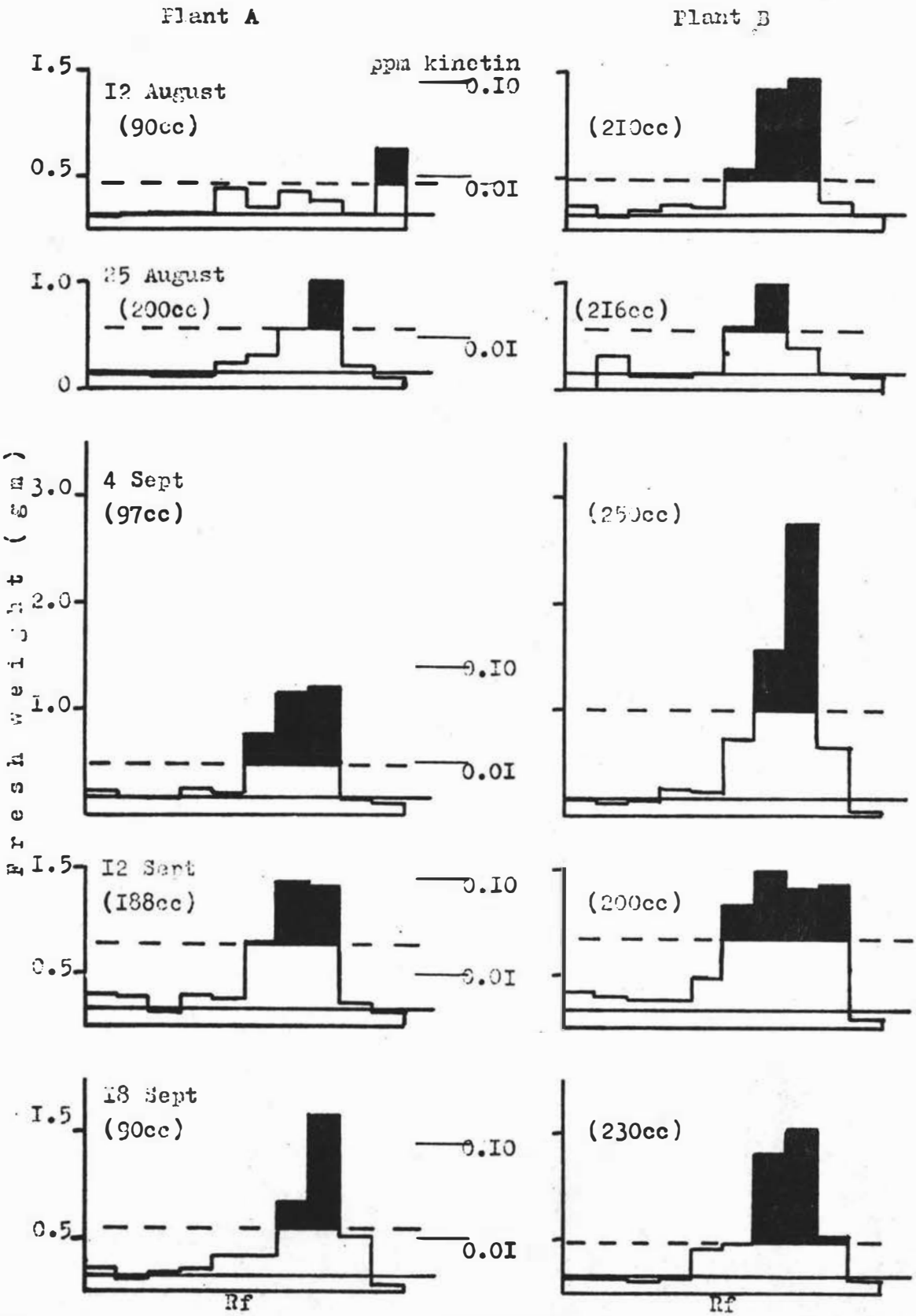


FIG. 28. HISTOGRAM OF SOYBEAN BIOASSAY OF CYTOKININS FROM BLEEDING SAP, SPRING 1975, FROM DIFFERENT PLANTS.

——— Control level, — — — significance level (P 0.01)

Table 6 Cytokinin-like activity in sap, kinetin equivalents
(Total activity, BuOH, Aqueous phases)

Harvest	Plant No.s	Activity µg/litre Plant A	B	Activity µg/day A	B
1	32+(A) 35(B)	3.9	70.2	0.78	14.77
2	45 (A) 37(B)	6.0	5.4	4.51	4.67
3	25 (A) 20(B)	52.6	>500++	17.42	>335++
4	42 (A) 22(B)	64.9	2349.0++	12.39	1110.85++
5	51 (A) 30(B)	261.7	82.5	24.07	19.15

+ Most of this sample was collected on the second day of bleeding

++Based on a linear extrapolation of the standard curve.

There was no obvious difference within the pairs of plants that would explain the difference in activity between plants at the same harvest date, although the variation is reduced a little by relating the computed daily cytokinin transport to a unit stem size. There appears to be a trend for the concentration and levels of cytokinin-like materials in the sap to be highest in the later harvests. These increased levels occur at the time most of the buds on the vine are visibly bursting ie. advanced bud swell - advanced bud burst (Brundell 1975a), with the lowest levels occurring prior to this time. The results may also indicate a lowering of activity in the final harvest, when shoot elongation is beginning ie. buds at the tight cluster - open cluster stage.

Cytokinin activity was also determined on sap extracts of bleeding sap taken from one plant separately over five days following decapitation. For each day's sap the same extraction procedure was followed except that

there were five partitions with n-butanol, and duplicate chromatograms were bioassayed from each extract. None of the Aqueous phases gave significant activity, and the results of the bioassays of the butanol phase are shown in Fig. 29 and Table 7.

Table 7 Cytokinin activity in sap, over 5 days after Decapitation
(Plant number 20, Kinetin equivalents)

Day	Activity µg/litre		Activity µg/day	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	493.8	841.7	330.8	564.0
2	1224.2	633.7	1286.7	666.0
3	424.1	790.5	623.4	1162.0
4	131.5	307.5	189.4	442.8
5	90.0	10.8	132.3	15.9

This shows the initially high levels of activity have decreased with time, especially in the fourth and fifth days after decapitation when cytokinin-concentration in the sap has fallen markedly, although high sap flows are being maintained. Activity was usually at Rf 0.6 - 0.8, although in the final collection (Day 5), there was apparently a second peak, and most of the total activity (as in Table 7) was in a different position ie. 0.2 - 0.5.

Purified sap extracts taken from four different plants on a total of five different occasions at the time of early bud burst were subjected to high pressure liquid chromatography (HPLC). Consistently a peak was obtained which had the same retention time as zeatin riboside, and placed samples in the same order of concentration as the soybean callus bioassay. Fig. 30 shows the bioassay results for each of the peaks collected from an extract of

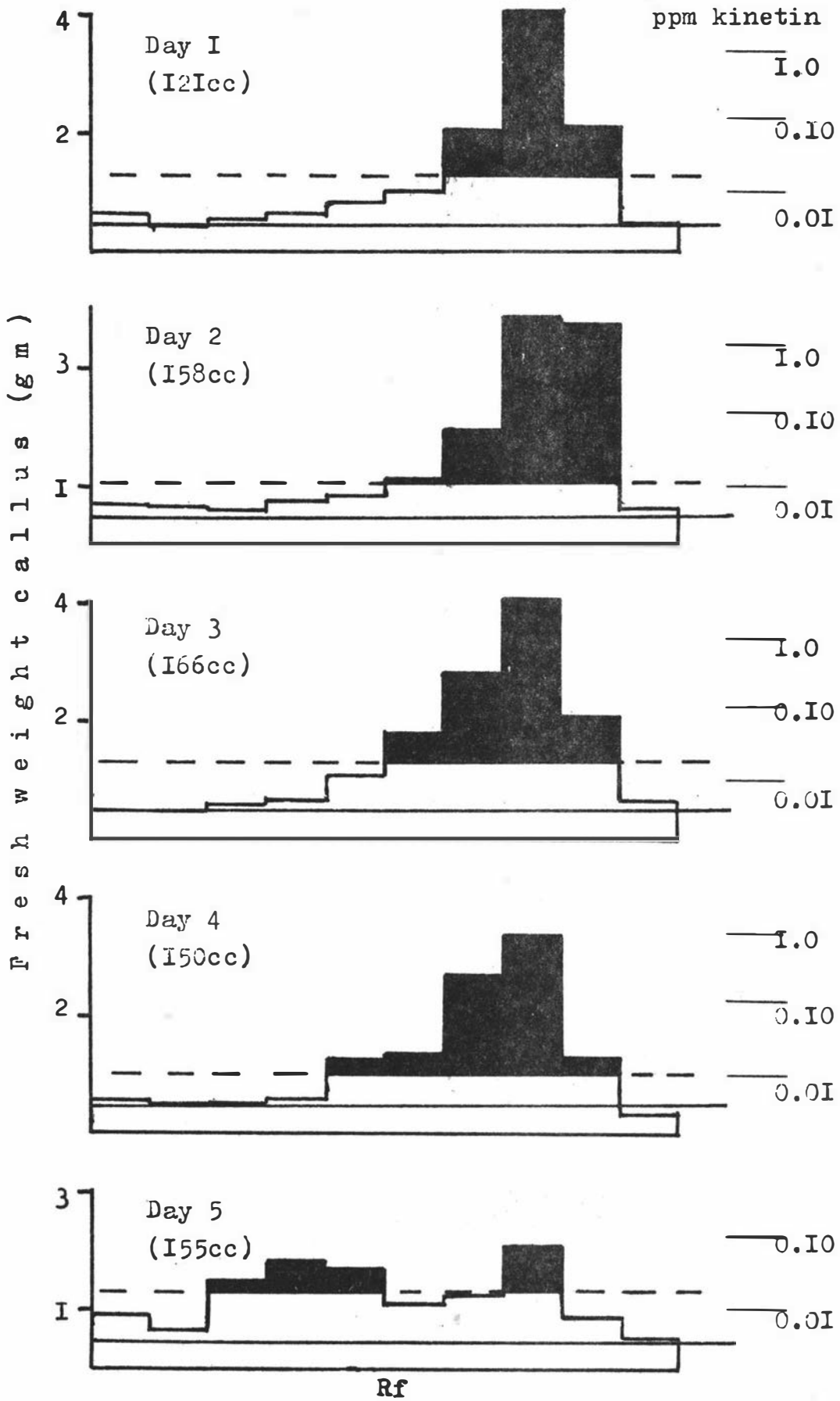
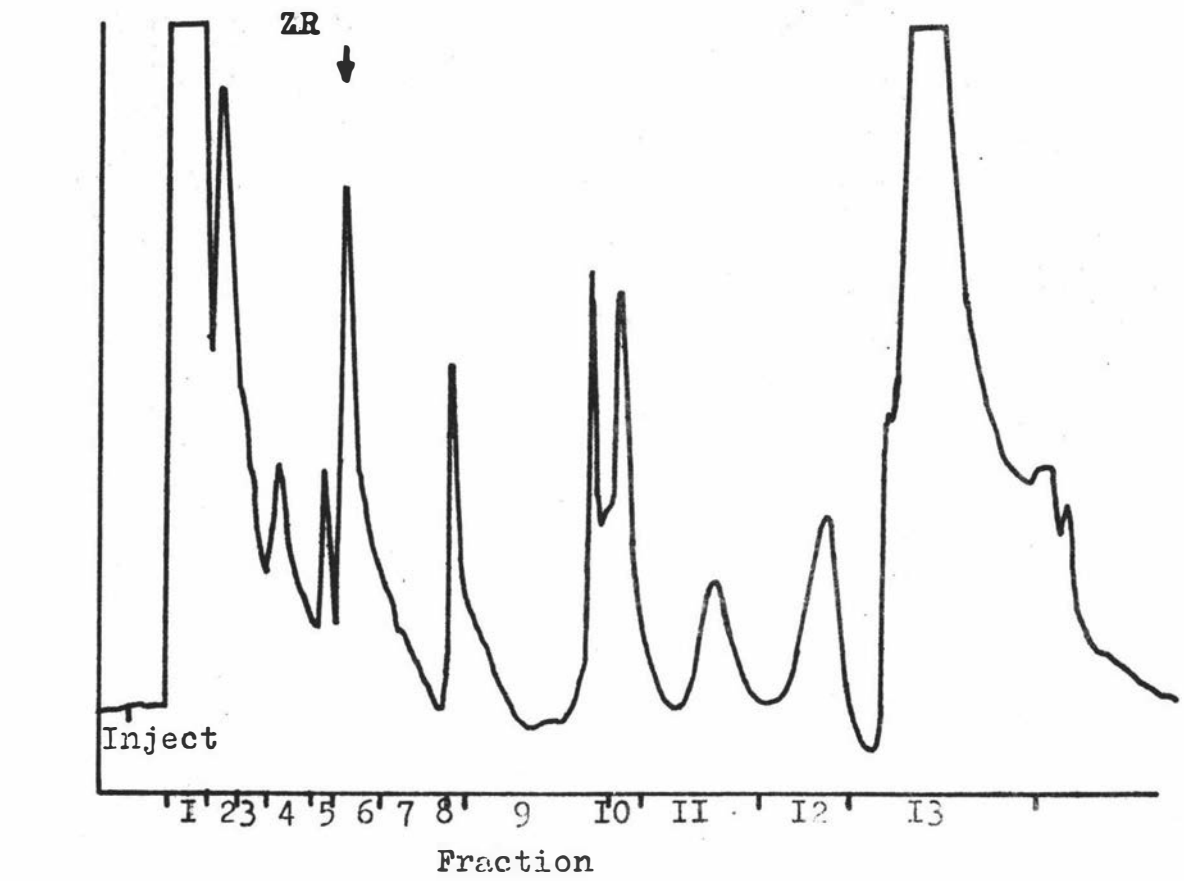


FIG. 29. HISTOGRAM OF SOYBEAN BIOASSAY OF CYTOKININS FROM BLEEDING SAP IN SUCESSIVE DAYS. PLANT 20.

——— Control level, — — — significance level(P 0.0I)

sap collected on 8/9/75 when most of the buds were bursting (See Appendix 2)



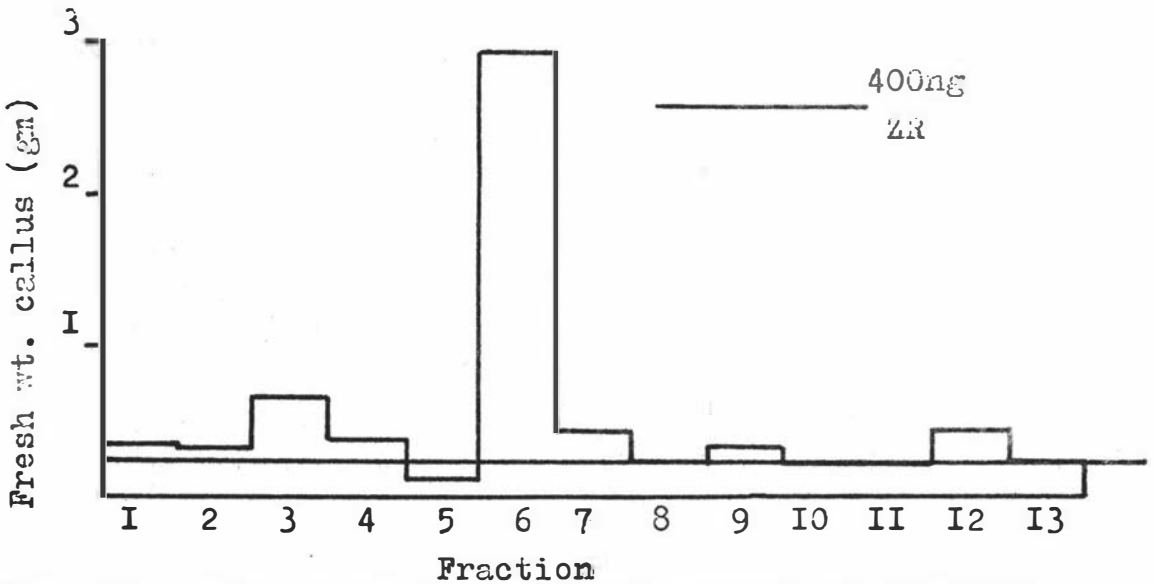


FIG. 30. HPLC TRACE OF CYTOKININ EXTRACT OF KIWIFRUIT SAP, PLANT 20, DAY 4, AND SOYBEAN CALLUS BIOASSAY RESULT FOR EACH FRACTION (BIOASSAY RESULT FOR ONLY 50% OF FRACTION 6).

DISCUSSION

A. Sap Flow

The period during which a vine will produce a quantity of sap exudate from a freshly-cut shoot is limited to about 6 weeks, (Figs. 18, 20), and beyond that time the flow rate and the length of time that bleeding occurs, is very limited.

Records show that the rate of sap flow from the stump of a removed shoot was very variable between shoots on the same plant, or between plants, and was also affected by the time of harvest. These effects could be attributable to environmental conditions, or to characteristics of the plant material sampled. One factor that varied somewhat was the diameter of the stem that was cut for sap collection, and it was found that generally there was a reasonable positive linear correlation between the flow rate of the bleeding sap, and the cross-sectional area of the shoot at the collection surface. The relationship varied with the date of the first collection, but for a given collection period it was similar over the period of collection. During the first and last collection periods when flow rate was lowest, the size of the shoot had little effect on flow rate, but during the period when sap flow was highest, the relative level of flow increased markedly with increasing stem diameter (or circumference). The significance for the plant of such an observation is not clear, nor the relationship of the bleeding sap flow to the sap flow in the intact plant. However, it may suggest that at the time the buds are bursting the larger-sized plant is more effectively able to send large quantities of sap up the plant. Also at this stage flow rates were always highest, and since the transpiring leaf area was essentially nil it might be inferred the sap was important in contributing something essential for bud burst, or for other processes occurring within the buds at that time.

For some unexplained reason, the sap flow data for the fourth harvest period in 1975 were inconsistent with the pattern shown by the other harvests.

Clearly plant size affects sap flow, and indicates the need to select plants for uniformity to reduce the variability in sap flow between them (Morrow 1963). It may also have been more accurate to express the data for sap flow in relation to plant size, although the trends would be the same as those shown by using the actual flow rates.

Limited observations showed the mean flow rate of decapitated plants was greater during daylight hours, especially around noon, and such a pattern was also found by Phillips and Jones (1964). Grossenbacher (1939) studied sap exudation from excised sunflower roots, and showed clearly the existence of such an autonomic cycle of exudation, where maxima and minima of flow rate occurred at 24-hourly intervals of midday and midnight respectively. So root activity seemed related to the light period, whereas when plants were grown in continuous light it was the time of decapitation that determined the time of peak and minimum flow rates in the 24-hourly cycle.

The plants used in 1973 were trained horizontally on several tiers which differed in their development, so that a large portion of the plant was considerably delayed in its spring growth compared with those parts being sampled. This, and the fact that only a very small proportion of the shoots were removed for sap collection, could account for differences in bleeding behaviour from that observed in 1975.

Relationship to vine development

It must be considered whether the pattern of bleeding sap flow is related to growth and development in the plant. In the kiwifruit vine, bleeding is limited to

a short period in the spring, beginning shortly before bud burst when the first buds are noticeably swelling, and ceasing in the early stages of shoot elongation - hence behaving similarly to the grape vine (Skene 1967).

In 1973 the peak flow occurred when in the upper sampled part of the vine, new shoots were at the open cluster stage and beginning to elongate, and bud movement was occurring throughout the vine. When young whole vines were decapitated in 1975 peak flow coincided with bud burst of the very first few buds on the vine, and flow had fallen substantially by about the tight cluster stage - whether expressed as flow rate per cut shoot, or per unit of area of cut surface.

Following removal of the shoot, the sap flowed more slowly, showing in 1973 a marked drop over the first 24 and 48 hours; except for the first harvest when sap flow was just beginning and when flow rate usually increased over the first one or two days. Although similar sized shoots were sampled, flow rates varied considerably - up to 7 times at the same sampling time. Sap production was usually from 100 - 500cc a day, from each cut shoot of a mature vine. Complete decapitation of a young vine yielded 100 - 850cc a day, or 20 - 120cc/cm^2 stem cross-sectional area/day. This indicates the capacity of the stem to transport very large volumes of sap, and it would seem surprising if it didn't have a specific function. One cannot therefore say such a sap flow is essential to transport from the root system substances required for early growth and development in the shoots, as bud burst, shoot elongation and leaf expansion, and flower differentiation can all occur in stem cuttings detached from the parent plant in the winter. Continued growth and development required the presence of a root system, but in the initial stages the stem itself may contribute the required metabolites and hormones, where they may have been previously accumulated from all parts of the plant. In the spring, sap actively flows from a fresh cut only when

it has roots attached, but when stem cuttings taken in summer or winter are placed in water in warm conditions, within 24 hours they produce copious quantities of clear viscous fluid - indicating changes are occurring in the stem.

The rate of sap flow recorded in 1975 similarly showed a wide variation between the shoots on a plant, and between plants (Fig. 19). Usually the mean daily flow rate increased during the first three days, and then declined, with the earliest and latest harvest periods having the lowest flow rates. In the sunflower plant also, the daily sap flow has shown a decline each day following decapitation, although there may initially be an increase in flow rate (Grossenbacher 1939, Phillips and Jones 1964). Fig. 20 shows over the relatively short period of bleeding sap production how the relative level of sap flow increases during each successive day after decapitation, and how flow was greatest from the second and third harvest dates. This means the buds were swelling and bursting at the time of greatest sap flow, and the flow had declined to very low levels when the leaves were unfolding. Page 164 shows how the moisture content of field-chilled buds soon rises when placed in warm temperatures, and one could compare this roughly with sap flow rates in Fig. 20. This suggests that up until the buds are on the point of emerging, when sap flow is increasing rapidly, moisture content of buds is just beginning to increase. Then while the buds are bursting and their moisture content is increasing rapidly, relatively high bleeding sap flow is occurring, and then it declines. So increase in sap flow appears to run ahead of bud growth, and once a transpiring leaf surface appears a steady decline of bleeding sap production occurs.

If flow rate is related to the stage of bud growth this tends to contrast with the results of 1973 when high flow rates were associated with a later stage of development, although the fact that high flows persisted later

could have been explained by the delayed development of the lower half of these vines.

Because of the variation between plants further sampling is required to establish the relationship of sap flow and vine development. It does appear the pattern of flow is related to early-season development of the buds, although other relationships may also exist. Since this plant has a low chilling requirement (Brundell 1976) it could appear that sap flow and its constituents are not involved in the mechanism of breaking bud dormancy, although they could be involved in bud burst and early shoot development. If gibberellin-like substances or cytokinins were detected in the spring sap, this could suggest an association between the supply of these hormones and the commencement of spring growth, as has been found in other plants. In the case of stem cuttings, both growth and development are limited (Brundell 1975c, 1976). Such development as occurs would be based on the background level of substrate in the stem tissue, but unlike the situation in the intact plant, growth would not continue due to the absence of a continuing supply of substrate in the xylem sap, as also considered by Bachelard and Wightman (1974).

The utilisation of reserves (carbohydrate?) appears to be essential for continued sap exudation, and in the presence of such reserves auxin increases the sap flow rate (Skoog et al. 1938). Thus the initial increase in spring flow rates may result from an increasing auxin activity in the tops of the plants, supported by the rapid mobilisation of stored reserves to support bud burst and early growth, which then influences the absorption and translocation of water. The beginning of shoot elongation coincides with a low level of wood-reserves, and as could perhaps be predicted, from this stage sap flow rates decline. One could then consider the possibility that high flow rates are simply a consequence of a particular transient set of circumstances, and not necessarily an essential require-

ment for vine development.

In trying to correlate sap solute level with shoot development there is a problem of sampling, since the level of a sap component may vary along a shoot, or within a plant. This is known to occur in a number of plants with nitrogenous constituents (Cooper et al.1972, Pate 1968) and calcium, and it appears sap composition along a shoot is in a state of flux, changing in response to the activity of the cells surrounding the xylem. Endogenous gibberellin and auxin levels change along the length of a stem (Wareing and Phillips 1970). Within the sap, growth substances may not be the only materials important for bud development, and changing in concentration during the start of spring growth. Nitrogenous substances are important reserve substances in fruiting plants, and show marked changes around bud burst, suggesting they have a role in this process (Cooper et al. 1972, Hill-Cottingham and Bollard 1965, Possingham 1970), and others could also be involved.

The loss of sap from a cut surface does have an effect on the growth of nearby shoots, and this effect is seen early in a shoot's development. Buds near a bleeding surface burst later than those on an uncut shoot - apparently the removal of the sap deprives the buds of an adequate level of some stimulus. There may subsequently be a loss of some potential sites of shoot growth as those areas of bark over which bleeding sap flows become blackened, necrotic, and cracked open. Otherwise a detrimental effect of high sap losses was not noticeable later in the season, so that two months after decapitation stumps had produced several strong new shoots which continued in active growth. Also where bleeding occurred from fresh grafting wounds, the grafts took and made good growth in the early summer.

Environmental Effects

Environmental factors affecting plant growth can be expected to modify plant metabolism, altering meristem activity and "sink strength", and substrate turnover. Increasing the supply of salts in the external medium greatly increases the rate of bleeding sap production (Skoog et al. 1938). When young potted kiwifruit plants in the glasshouse were watered with CCC, sap flow was reduced by 50% over each of the following two days, presumably through some effect on plant development (Skene 1970).

Temperature has a major influence on the rate of development of plants, and could thereby affect demand for substrates by active tissues, and also the level of production in other sites. In the maple, the beginning of sap flow is related to air temperature, and occurs between autumn and spring whenever warm temperatures follow very low temperatures, and below a certain temperature the flow declines. Field temperatures tend to fluctuate widely, and while Fig. 18 indicates that sap flow rate increased once mean air temperature showed a steady increase above 10°C, observation under controlled conditions would be required to confirm this apparent effect. In A. kolomikta growth starts when the mean daily temperature reaches 10°C (Gorokhova 1976). In 1975 mean air temperature had increased substantially during the second and third harvest dates, but an increase preceding the peak second harvest date was less evident (Fig. 21). Soil temperatures prior to the first sap collection had been about 6°C, but the mean rose to 8°C before the second harvest, and the increased sap flow might reflect a temperature-induced rise in root activity. Mean soil temperatures continued to rise during successive harvests, but sap flows declined presumably due to the changing requirements/characteristics of the plant. Temperatures could affect the actual flow rate at any given time, and by affecting root activity indirectly stimulate the beginning

of bleeding. Soil temperature effects could have considerable adaptive significance as it is a more dampened indicator of incoming radiation than air temperature. In citrus the effects of soil and air temperature on plant development can be very different, so that the level of bud burst is greatly increased withhigher soil temperatures, whereas air temperature has little effect (Hall et al. 1977). In a study with a wide range of soil temperatures (Hammond andSeeley 1978) there was no difference in the time of anthesis, indicating this process is not controlled by an upwards spring movement of root elaborated hormones. Hence root temperature did not play a major role in spring bud development, while ambient temperature was important.

Grossenbacher (1939) found that the periodicity of sap exudation was not affected by temperature variations, but flow rates were greater at higher temperatures, and a large sudden increase in temperature immediately resulted in a rapid increase in flow rate. So in the uncontrolled field situation, many factors can account for differences in flow levels.

Working with seedling, Clarkson (1976) found that as temperatures were increased, xylem sap exudation was faster, and furthermore that temperatures preceding decapitation had a marked initial effect on sap exudation rate. The use of reduced pre-treatment temperature conditions apparently induced changes within the vascular system, resulting in greatly increased sap flows in the detached root system, and also increasing the amount of solute moving into the xylem vessels from the root tissue. This shows clearly that environmental conditions can affect the characteristics of the root system, and also the sap coming from it - something also demontrated with respect to temperature and root cytokinins (Skene 1972b).

In his study of temperature effects on the rate of water uptake, Kuiper (1964) distinguished between two phases. At the lower temperature range the rate of water

uptake increased sharply with increasing temperature, and he suggested that at these lower temperatures the root cell membrane acts as a high energy barrier with water transport being limited by the capacity of the metabolic processes involved in establishing this membrane. However, above a critical temperature, water apparently moves freely through the root cell membranes, and is largly affected only by the viscosity of the water. Thus one can envisage the importance of early plant metabolic activity in controlling sap flow rate, and hence a marked increase in sap flow rate with small rises in temperature, to be followed by a period when a continuing rise in temperature does not further stimulate water uptake and sap flow.

Sap might be expected to flow as long as the soil is not dry, and in the absence of any control of moisture supply the observations here do not indicate any relationship between the occurrence of heavy rainfall and the sap flow rate immediately following. Soil moisture levels in the spring were probably relatively high and it did not appear that heavy rain altered the pattern of sap exudation.

B. Sap Growth Regulators.

Observations of kiwifruit and other plants have indicated a requirement for the movement of materials from the root system to the shoots in order that normal growth and development occurs. In the control of growth, hormones are one of the important agents, and Brundell (1976) has suggested a role for a hormonal root factor in the kiwifruit vine. Early tests indicated the presence of substances with cytokinin and gibberellin-like activity in the bleeding sap of kiwifruit plants, and so further observations were made.

Although GA glucosides may play a role in controlling the status of free GAs within a plant, or function in the transport of GA's over long distances in spring sap (Barendse et al. 1969), no record of such substances was made here after some initial unsatisfactory attempts to detect them.

Gibberellin-like Substances.

Bioassays clearly showed the existence of active substances in extracts of sap collected from mature vines in the spring of 1973. The levels of activity in the sap showed only small differences between collection dates over a six-week period (Fig. 23) so no clear pattern related to date or plant development was discernible. If there was any trend it was for activity to be declining in the later collection, when new shoot growth was under way. Since the sap flow rates were higher in the later harvests, it could appear that the greater quantities of gibberellin-like substances were being transported into the shoots from about the time of bud burst, and during the early stages of shoot elongation.

With all samples significant activity occurred in the region of Rf 0.4 - 0.7 with IAA 10:1:1., and different solvent systems were not used in order to detect qualitative differences.

Similar observations were made of sap collected in the spring of 1975, but a number of changes were made:

(a) The solvents used for extraction were changed.

(b) After running two replicate chromatograms, individual Rf's were eluted, and aliquots of each used in the bioassay-previously replicated chromatogram strips were bioassayed directly.

(c) Assessment of the sugar content of the bioassay incubate was based on the use of refractometer, instead of a colorimetric method. Although a more sensitive refractometer was desirable, both methods were found to be satisfactory (Fig. 26), and the refractometric method was used because of its greater convenience.

(d) The sap was collected from younger established vines usually with two leaders of about 3cms. diameter at the base which were both removed close to ground level. This contrasts with 1973 when the bleeding sap was collected from a very few shoots removed from the upper part of older established vines.

While significant activity was shown to be present (Fig. 27), gibberellin-like substances appeared to be present in only low concentration in the spring sap. Activity at a given harvest date varied between plants of apparently similar size and stage of development. No doubt such differences are due in part to the small differences in stage of vine growth, to the differences in relative rate of sap production, and to other plant differences - certainly plant C (Table 5) which was not typical of the other plants used, produced contrasting bioassay results. The results were for the concentration of sap gibberellin-like substances to be higher following bud burst than prior to it, and as in 1973, for the apparent amounts of gibberellins being transported to be highest when shoot growth was beginning. It was of interest that sap collected at a time immediately before most of the buds were full emerged or burst, showed two peaks of activity - something not shown in any of the other samples.

Limited amounts of sample prevented further observation of this apparent qualitative change in gibberellins at that time.

Although gibberellin activity was not followed through the winter and thus the period when dormancy was broken, the results could suggest the rise in GA level was as a result of bud movement and so supporting the new growth, rather than causing bud break. Similarly in other plants the level of GA-like substances is greater at about the time of bud burst (Lavender et al. 1973, Luckwill and Whyte 1968), and they are known to be important in the promotion of shoot extension growth. Working with the flower buds of coffee, Browning (1973a) found that the gibberellins involved in dormancy release originated within the buds themselves, and that gibberellins supplied by the xylem sap were not involved in this process.

The initiation of rapid growth may well require the build up of high GA-levels which would be detected in the early stages of extension growth, but once elongation is proceeding GA-turnover may be of greater importance, and so extractable-GA levels at this time would fall (Robitaille 1970). This would fit the idea of an early increase of sap-gibberellins for whatever role they play in initiating shoot elongation, but which levels could then diminish with a falling sap flow and with their metabolism within a growing shoot, and an increasing role of the young leaves in the GA-metabolism of the shoot. This is compatible with observations on changes in sap-GA levels, GA metabolism in elongating shoots, and the role of leaves in extension growth - and with the observation that in buds GA levels rose as dormancy was released, but stopped rising when rapid expansion began, and then decreased as this proceded (Browning 1973a). Changes in GA activity may also indicate a change in the role of GA, from stimulating mitotic activity in the sub-apical meristem of the incipient shoot (Sachs et al. 1957), to a more secondary function of maintaining cell division and con-

trolling cell elongation in a growing shoot in association with other tissues and hormones (Cleland 1969). In assessing the contribution of gibberellins to shoot growth in the kiwifruit, it would be helpful to determine their effect on the processes of cell division and cell elongation. Furthermore the early spring rise in sap-GA may be related to the stimulation of cambial activity at this time, since division can be initiated in dormant twigs by GA application (Digby and Wareing 1966); gibberellin also enhances the mobilisation of stored carbohydrates (Saniewski and Pieniazek 1972), and can affect the distribution of C-assimilates within the plant (Quinlan and Weaver 1970).

In using a gradient elution technique to make qualitative comparisons of gibberellin-like substances there was the problem of variability associated with the use of a bioassay to measure activity, although the patterns of response were consistent. It is also commonly found that growth regulator levels vary between samples, and between seasons, and activity in these samples was much lower than that in sap samples previously analysed. Whether this was due to the younger age of the plants used, or to greater losses associated with the use of the Sephadex and silica gel columns is not clear.

Gibberellin levels in all sap samples analysed were consistently low, occurring as a relatively broad peak emerging from the final column (Fig. 24). However over the period of observation a qualitative change occurred, which could well have been the result of the pruning treatment. In each of the first two days of bleeding, activity was in the same position of both samples, and presumably reflected the situation in the intact plant. But in the fifth day, in the decapitated plant all activity occurred in a very different position, in fractions emerging from the column earlier, indicating the appearance of less-polar gibberellins. At this time activity was present in the same fractions of the partially-decapitated

plant, but in the initial test activity also occurred at the usual position.

These results indicated that in plants with young elongating shoots, complete decapitation resulted in a marked qualitative change in the gibberellin-like substances present in the bleeding xylem sap. But when sap was collected from a plant still bearing intact shoots both types of gibberellins were obtained. Apparently leafy shoots alter the type of gibberellins in the sap rising from the roots, and even partial removal of the shoots brings about a change in the gibberellins emerging from a cut shoot. It would be interesting to know whether partial decapitation alters the gibberellins in all the shoots, or just in the cut ones - presumably it is the former. Clearly the roots and shoots are not functioning independently, and apparently are interacting with respect to GA metabolism. An interconversion system has been proposed by Crozier and Reid (1971), and the kiwifruit vine would be suitable for further studies of the circulation of root - and shoot gibberellins.

Cytokinins

Low levels of cytokinin-like activity in sap were detected by the radish cotyledon bioassay, in extracts prepared from the two earliest sap collections in 1973.

More detailed study in 1975 showed the large variation in sap activity of plants sampled at the same time that appears characteristic of this sort of investigation, and this makes the recognition of trends and relationships difficult (Fig. 28). Thus at each harvest date, one plant produced sap of low cytokinin content that showed only a moderate increase with time; whereas the second series of plants had a higher cytokinin content which increased considerably over the experimental period. The trend was for the concentration of cytokinin in the bleeding sap, and the estimated daily cytokinin transport,

to increase markedly at the third and fourth harvests, and then to decline at the final harvest. That is, peak activity occurred from the time most of the buds on the vines were partially emerged from their enclosing tissues, and activity was stabilising or starting to decline when most buds were at the advanced bud burst-tight-cluster stage. The lowest cytokinin levels were early in the bleeding period, before any visible bud burst had occurred.

It is no surprise that substances with cytokinin-like activity were detected in the sap of kiwifruit vine, as they are widely found in plant sap, and cytokinins of root origin are considered to be involved in developmental changes taking place in the shoot (Beever and Woolhouse 1974, Davey and van Staden 1976, Kende and Sitton 1967).

High cytokinin content of root exudate of the sunflower and tomato is associated with vegetative growth (Davey and van Staden 1976, Sitton et al. 1967), and in the apple tree activity is highest during active shoot growth although concentration in the sap fluctuates over this period (Luckwill and Whyte 1968). Sap cytokinins in sycamore fluctuated in the spring, and activity was not related to visible bud movement (Purse et al. 1976), although as in apple (Luckwill and Whyte 1968) cytokinin activity appeared at a time prior to bud swelling. The suggestion of a requirement for root cytokinins for bud burst is supported by the data of Alvim et al. (1976) which showed a considerable increase in sap cytokinins prior to bud burst. Cytokinin activity in the roots and stems of the sugar maple increases after a large surge in root activity, and then hormone level falls abruptly several days later as bud break begins (Dumbroff and Brown 1976). This seems to reflect the importance of these hormones in budbreak and growth,and indicates an important role for the roots in supplying these hormones. The early low levels of sap cytokinin in the kiwifruit, coming either directly from the root tips or from storage in the root xylem may play a role in bud movement, but the late appear-

ance of high activity suggests sap cytokinins are not playing a major role in the process of breaking bud dormancy.

Working with sugar maple, Dumbroff and Brown (1976) found cytokinins and gibberellins begin to increase after most, if not all, of the chilling requirement has been met, and suggest the synthesis of cytokinins is not the initial step that leads to emergence from dormancy. The role, if any, of these hormones is not clear, but high root cytokinin levels increase the amount of photosynthates transported to the roots and to the shoot tips (Shindy et al. 1973), and the main function of these substances in the spring may be to control the translocation of photosynthates and other substances. Bachelard and Wightman (1974) in their scheme for bud growth in the spring suggest an early movement of cytokinins from the roots which shortly before bud break activate cell division in the apical meristem, and then subsequently supports shoot development in undefined ways. Various authors have indicated a requirement for cytokinins in the maintenance of normal shoot growth (Aung and Byrne 1976, Jones O.P. 1967, 1973, Luckwill and Whyte 1968, Railton and Reid 1973), although its function may not be given. In order that normal differentiation of xylem tissue can occur it is necessary that a combination of hormones, including a cytokinin, are present (Pieniazek and Saniewski 1968 quoted in Jankiewicz 1972), and like gibberellin, cytokinin acts synergistically with auxin in stimulating cambial activity (Pieniazek et al. 1970).

Further determinations of sap cytokinin activity (Fig. 29) again show the difficulty of making accurate quantitative assessments of hormone level based on bioassay, due to the variation between replicates. However cytokinin activity is shown to be present in the sap for five days following decapitation of a plant, at levels essentially unchanged over the first three days. While qualitative changes may have occurred in this period,

the bioassay indicated all samples were uniform in composition, and presumably indicates the continuing ability of a detached root system to synthesise or release cytokinins. By the fifth day the concentration of cytokinin in the sap had declined markedly to very low levels, although the sap flow rate was still being maintained at a high level (Fig. 19). There also appeared to have been a qualitative change by the fifth day, to give two peaks of activity. It is suggested the loss of the shoots resulted in a change in the cytokinin metabolism of the roots, which is also supported by the presence of a single peak of high activity occurring in sap collected four days later when fresh plants were decapitated. Skene (1972b) also recorded a qualitative change in the cytokinins in bleeding sap of the grape vine, from the second day after decapitation.

Section 3

Dormancy and Bud Burst

A. Winter Dormancy and Bud Burst

In most plants, dormancy is an incompletely understood condition, and in the kiwifruit plant there is little detailed information on its development and loss. It is becoming clear that environmental conditions during dormancy are very important in the development of the vine, and because of its importance to commercial growers these environmental factors must be fully understood.

Temperature is frequently of paramount importance in the development and termination of dormancy, but there is also the possibility of chemical methods of controlling bud dormancy.

The development and loss of dormancy was studied, and observations made of changes in endogenous hormone activity.

Experiment 1 1975, 1976, 1977, 1978.

Preliminary observations in the winter of 1973 with single-node stem cuttings of Bruno had indicated an increase in the rate of bud burst as spring approached. Thus material collected in early July took nearly twice as long to give 100% bud burst, as material collected in late July or early August - and less time still was required with a late August harvest.

(a) Further observations were made in 1975, including the application of growth regulators by standing the explants in a hormone solution. Material was held under continuous fluorescent light at 31°C, and solutions were

changed twice a week.

Results

When Bruno wood was collected on 29/5/75 many leaves were still on the vines, but they could readily be detached. After a period of 39 days there was no sign of bud movement in any of the 18 buds on cuttings in each treatment: water, 50ppm GA_3, 50ppm BAP, or BAP+GA_3 (50ppm each).

When a similar second collection was made on 11th August, after two weeks the controls showed 94% bud burst, and 89% in the presence of GA_3. At that time several of the cuttings receiving cytokinin had dried out, but there was no sign of bud movement. Gibberellic acid markedly increased shoot length, and the increased rate of bud development is shown below:

Effect of Gibberellic Acid on rate of Bud Burst

	No. buds at least fully burst. (max. 18)		
	+7 days	+9 days	+14 days
Control	0^{++}	3^{++}	17 NS
+GA	7^{++}	13^{++}	15 NS

Significance determined at each date by analysis of variance, and indicated: NS Non-significant
$^{++}$ Significant (1%)

This experiment repeated with the Abbott variety collected on 25/8/75 at a stage when the first one or two buds on the vine were showing signs of bud movement. After 26 days a similar proportion (52 - 67%) of the buds had moved in each of the four treatments, most to beyond the bud burst stage, and in those explants receiving GA_3 shoot growth was greater (Plate 2).

(b) On 26 May 1977 Abbott explants were prepared, and both ends dipped in 4000ppm GA_3 in 50% ethanol, and again four days later, then held in water at 23°C. Bud burst began at the same time as the controls, and rose to 26% compared with 38% in the controls. Whereas stem cuttings usually produced a short shoot with large, dark green leaves, gibberellin treatment markedly altered bud growth - there was pronounced stem elongation, and the leaves were very small and light green, with marked hairiness on their upper surface.

(c) In 1976 and 1977 records of bud burst behaviour of one-node Abbott cuttings held under continuous light at 23°C showed the loss of bud dormancy.

Table 8 presents the results of the control (defoliated) treatment of Experiment 2 in Section 3B. Similar results are also shown for material collected at 18 day intervals in 1977 (see also Fig. 32). The time required to achieve 50% bud burst is a useful figure for comparison, and also indicates the mean time to bud burst for a group of cuttings (Antcliff and May 1961).

Table 8: Bud Burst at Different Harvest Dates

(i) Abbott 1976

Harvest Date	% Bud burst at 23 days	% moving at 23 days	Days to 50% bud burst
22/1	73	87	14
23/2	59	62	16
12/4	38	60	27
1/6	3	40	28
5/7	98 (21 days)	100	15
23/7	93 (18 days)	93	13
4/8	94 (15 days)	94	11
7/12+	100 (16 days)	100	8

+Held 25°C

Table 8: Bud Burst at Different Harvest Dates (Cont.)

(ii) Abbott 1977

Harvest date	Percentage bud burst after (days) 11	14	21	28	38	Days to 50% BB	Max. % BB
4/4	4	20	55	73	83	20	83
22/4	0	5	30	56	83	26	83
10/5	0	0	0	6	46	44	51
26/5	0	0	6	28	38	-	38
13/6	0	0	13	41	45	46	49
26/5 +Cooler[θ]	0	2	46	68	72	22	72
1/7	0	4	58	62	64	18	64
18/7	0	15	57	61	63	17	63
28/7	20	80	87	-	-	12	87

[θ]Held at 0^{o} - 1^{o}C for 25 days.

Deepest dormancy in 1976 occurred in the buds at 1 June, the behaviour of which buds contrasted markedly with buds at subsequent collections. Using actual temperature records and the nomograph of Spreen (1956) the degree of chilling was estimated from 1 March, so that for maximum bud burst (ie. >90%):

	Harvest date (1976) 1 June	5 July	23 July
No. Hours < 7.2^{o}C	312	768	970
No. days 22^{o}C	34	21	18

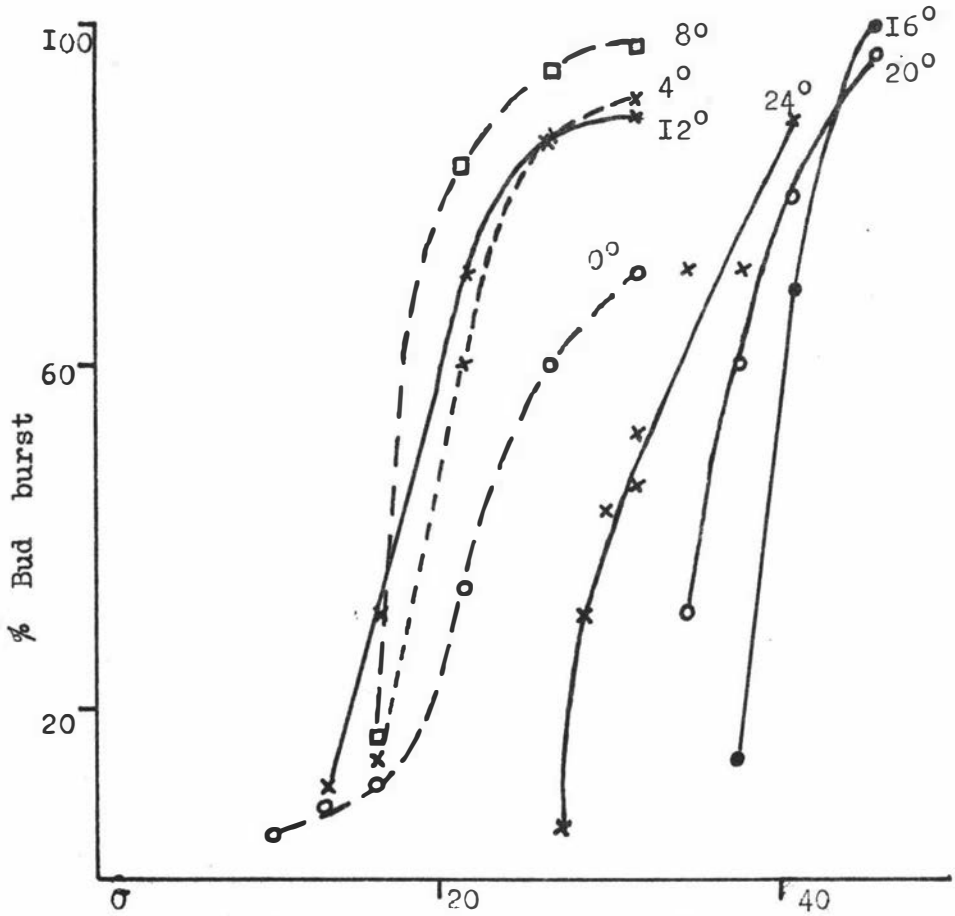
This indicates that after exposure to a relatively low (approaching 768 hours) level of chilling, rapid bud burst occurs. But all samples showed almost total bud burst eventually, and the calculations above suggest warm temperatures are more effective than chilling, in breaking

dormancy eg. buds taken at 1 June (cf 5 July) and given an additional 312 hours (13 days) at 22°C behaved like buds given 456 hours of additional chilling. This response is the same as dormant grape buds (Antcliff and May 1961), in that the hours of chilling given is greater than the resultant reduction in time at warm temperatures needed for bud burst. However, when buds were collected on 26/5/77 and held at 33°C, there was no sign of bud movement after 23 days, at which time 22% were moving where held at 23°C - ie. very high temperatures did not promote bud burst of dormant buds.

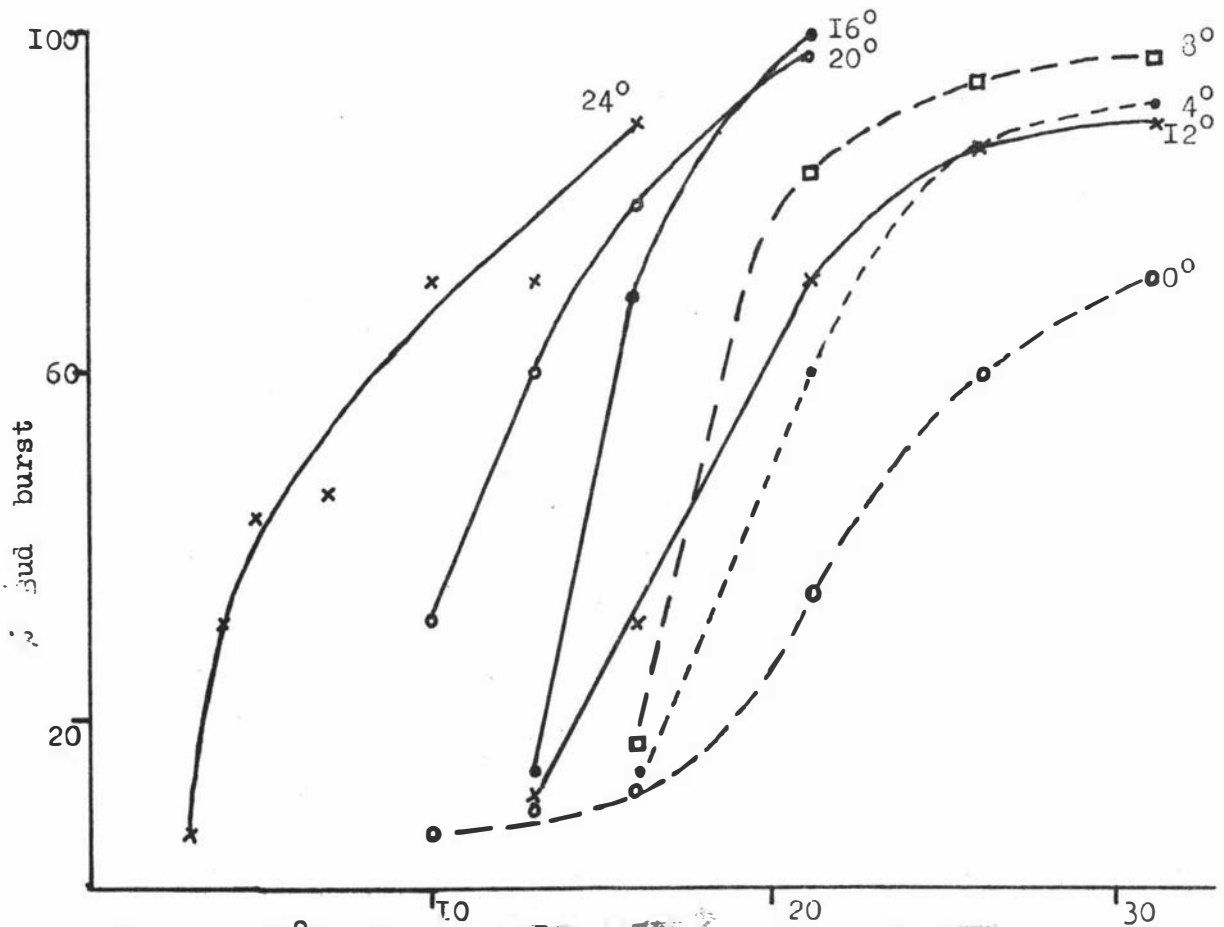
During the winter of 1977 buds collected on the 1st July burst more rapidly than those taken earlier. By calculation it was estimated there had been 520 hours chilling (at less than 7.2°) to 13 June, and 670 hours to 1st July, and the characteristics of buds from the latter harvest appeared different from the typically dormant June buds. Again it appeared as if in late June, only after about 700 hours chilling do dormant buds behave as they do in early autumn and spring, with a high degree of bud burst that begins early and proceeds rapidly.

(d) The ability of warm temperatures to substitute for cold temperatures in the breaking of dormancy was studied in the autumn of 1978. One-node Abbott cuttings were harvested on 23 May (130 hours field chilling) and held in water for 24 days (576 hours) at 0, 4, 8, 12, 16, 20 or 24°C in the dark, before being transferred to 24°C and continuous light.

Bud burst of the 35 buds per treatment is shown in Fig. 31. This shows the rapid bud burst following storage in temperatures ≤12°C, and delayed bud burst at warmer temperatures where the buds had not previously received such a chilling treatment. When the data is rearranged (Fig. 31) to show bud burst from the end of the pre-treatment (storage) temperature, it can be seen that bud burst was high in all treatments and that cold tempera-



Number of days at temperatures ≥16°C for each temperature regime as shown.



Days at 24°C after 24 days pre-treatment at temperatures shown.

FIG. 3I. EFFECT OF TEMPERATURE ON KIWIFRUIT BUD BURST.

ture storage did not increase bud burst. There was a suggestion that bud burst was reduced by storage at the lowest temperature of 0°C.

Experiment 2 1975, 1977.

In one test of growth regulator effect on bud burst, the hormones were applied in lanolin to ensure they were absorbed into the bud tissues. Firstly, Abbott cuttings were taken in early August and maintained at about 31°C under continuous fluorescent light. The lanolin mixtures were applied on the upper cut surface of the explants, and closely around the bud after the swollen stem tissue enveloping the bud had been removed. After two weeks, limited bud burst had occurred in the controls, but no movement had occurred in the lanolin treatments, in which latter all the buds were dead.

With material harvested on 16/8/75 an experiment was laid down to determine whether it was the lanolin or the stem mutilation that delayed bud burst. Again one-node cuttings (13/treatment) were maintained as above, and treated thus:

Treatment 1 Controls
Treatment 2 Remove tissue enclosing bud.
Treatment 3 As in (2), but lanolin applied to top of explant and around bud.

Results

From an early date it was clear buds in treatment one were more advanced, and remained ahead of those in treatment two. Where lanolin was also applied there was an even greater delay, and many buds were killed - as shown in the following table:

Number buds at least fully burst (max. 18)

	+9	+12	+18 days
Treatment 1	12	18	18
Treatment 2	0	8	18
Treatment 3	0	4	8
			(9 dead)

Clearly bud burst was delayed by removing the tissue around a bud, and lanolin was very detrimental to bud growth.

Further observations were made in the winter of 1977 on the effect on bud burst of the tissues surrounding the bud.

One-node Abbott cuttings were held under continuous light at 23°C from harvests made on 10 May, 26 May, and 28 July. On each date one treatment consisted of removing all the tissues surrounding the bud in comparison with untreated controls and at the two earlier dates other cuttings had only enough tissue removed to allow the outer 5 - 6 bud scales to be removed.

Results.

On all occasions bud burst was strongly promoted by removal of the tissue surrounding the bud, especially at the earliest harvests when the buds were at their most dormant stage. It was also clear that this pruning treatment resulted in almost total bud burst, compared with the lower levels achieved in the controls (Table 9, Fig. 32). An almost identical response followed the removal of the bud scales.

Table 9: Bud Burst (%) Following Pruning Treatment

Harvest	Treatment	12 days	22 days	28 days	38 days	Max. % Bud Burst
10 May	Control	0	0	0	46	51
	- Bud scales	0	67	93	-	93
	- Stem tissue	0	77	100	-	100
26 May	Control	0	9a	28x	38	38
	- Bud scales	0	39b	69y	-	69
	- Stem tissue	0	47b	68y	-	69
28 July	Control	33	87	-	-	87
	- Stem tissue	100	100	-	-	100

Analysis of variance, numbers buds burst:

(a) Harvest 10 May. Descale cf. Control - treatment differences significant (p=0.01) at 22, 28, 38 days

(b) Harvest 26 May. Treatment differences significant (p=0.01) at 22, 28 days. Treatment effects shown above by Duncan's Multiple Range test (1%).

(c) Harvest 28 July. Treatment differences significant at 12 (1%) and 22 (5%) days.

There is no obvious explanation for the contrasting behaviour shown in 1977 with that demonstrated above in 1975.

Experiment 3 1978

A further study of bud burst following wounding was made, and the possible invovlement of ethylene investigated.

Stem cuttings of Abbott were prepared in the usual way after harvesting on 24 May, and with eighteen cuttings each the following treatments were applied:

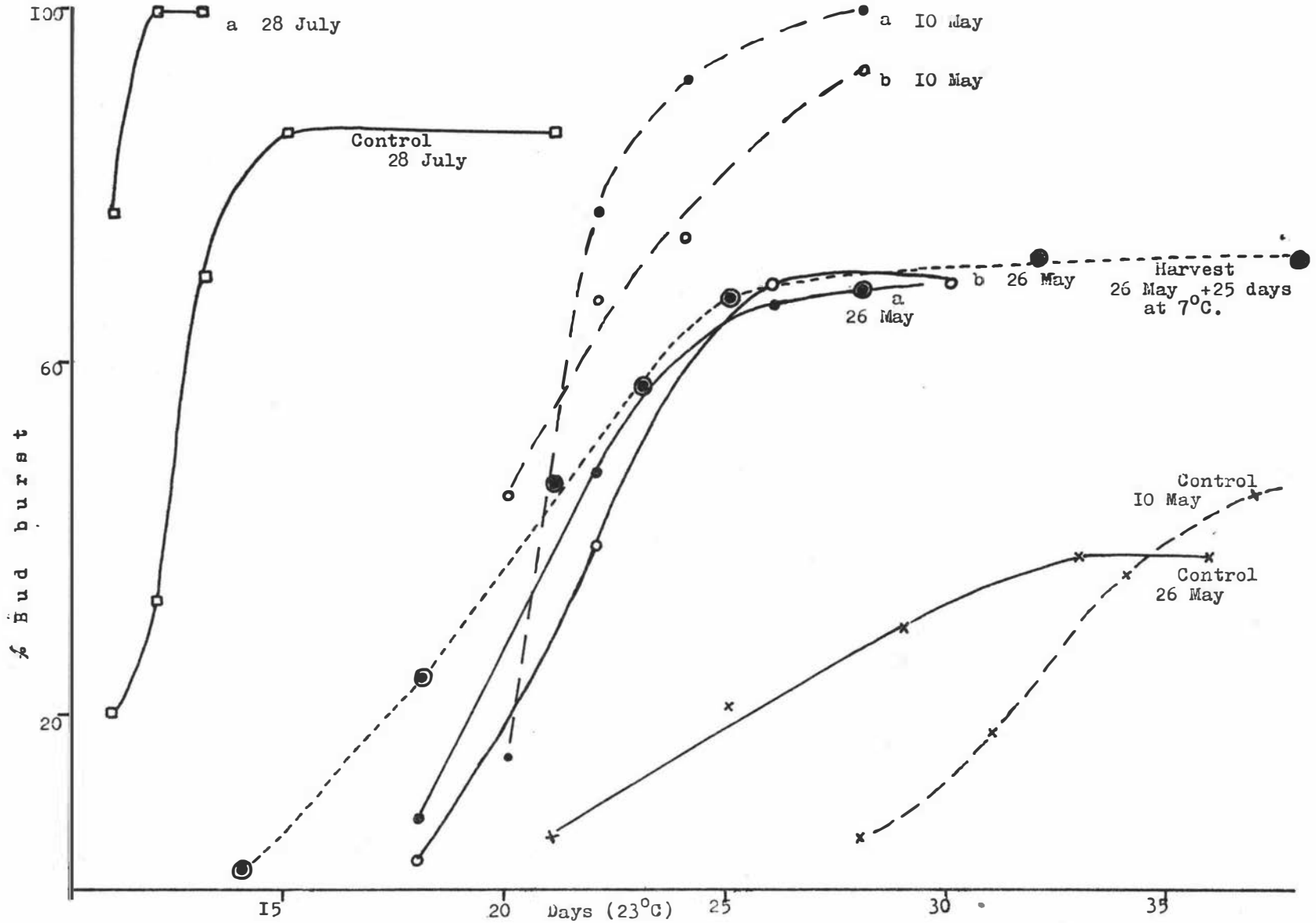


FIG. 32. EFFECT OF PRUNING TREATMENT ON BUD BURST AT THREE HARVEST DATES.
(a) Tissue removed around bud. (b) "Bud scales" removed.

Treatment 1 - Controls
Treatment 2 - Tissue around bud removed
Treatment 3 - Two cuts made on either side of the bud, in the swollen petiole base.
Treatment 4 - Soaked for 30 mins. in Ethrel+ 200ppm
Treatment 5 - " " " " " " 2000ppm
Treatment 6 - " " " " " " 10000ppm
Treatment 7 - " " " " " " 20000ppm
Treatment 8 - As in (2) with Parafilm and lanolin applied around bud.

+48% ethephon

Results.

The rate of bud burst was affected by Ethrel (ethephon) or treatment number two (Fig. 33). Bud burst was enhanced by removing the bud's surrounding tissues, an effect that was lost if lanolin was applied frequently around the exposed bud. Physical wounding by cutting was ineffective, and ethylene treatment delayed bud burst at all concentrations - totally preventing bud burst over a 7-week period in the two highest concentrations.

Experiment 4

It was also of interest to determine whether the presence of roots was favourable to bud burst in the spring. Initially this was observed in the winter of 1973 by taking a number of rather weak and variable Abbott plants. Plants were taken into a heated glasshouse in early July, early August and mid August, and on each occasion the tops were removed from half the plants and placed in water.

In each sampling, buds burst at about the same time in each treatment ie. in the presence or absence of root system. However the rate of bud burst increased with the later samplings, total response taking 45 days in early July, and 18 days in mid August.

In January 1976 cuttings of Abbott were rooted, and

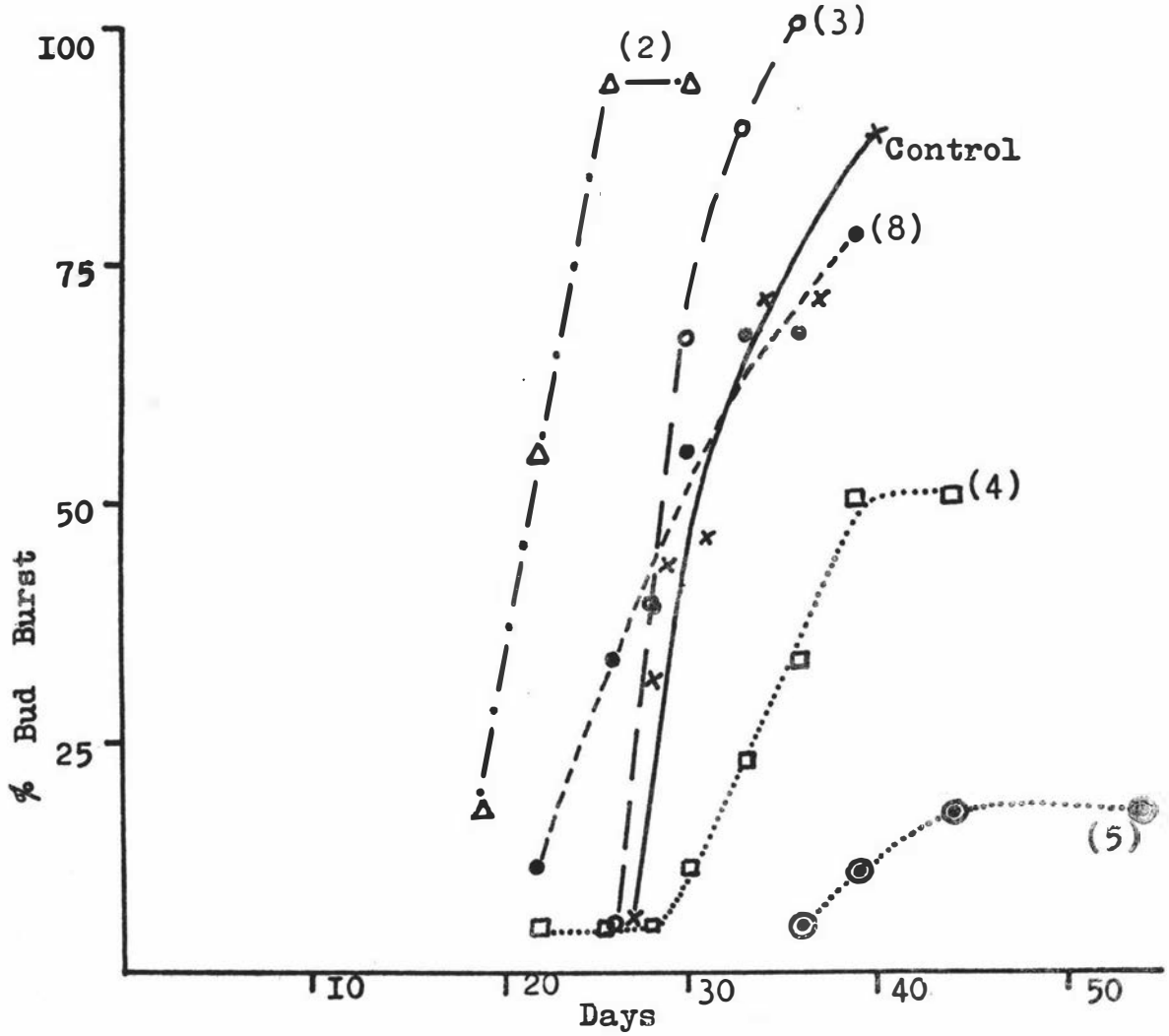


FIG. 33. EFFECT OF ETHEPHON AND PRUNING TREATMENT ON BUD BURST. TREATMENT NUMBERS SHOWN:

(2) Remove tissue. (3) Side cuts.

(4) Ethephon 100ppm. (5) Ethephon 1000ppm.

(8) Remove tissue, seal with lanolin.

Harvest 24/5/78.

grown in a glasshouse in plastic bags of peat and sand to make up to 4' of growth. They were placed outside in the winter, and cut back to about 9". On the 10th July (5 weeks after the leaves were frosted off) and also on 7 August, 48 plants were taken into a room held at 21°C and with continuous fluorescent light. On each date the tops of half the plants were removed, and placed in water, and bud movement recorded for all plants.

Results.

The rate of bud burst was similar, and very rapid in each of the two samplings, being only a few days faster in the second sampling.

Table 10: The effect of Root Removal on Kiwifruit Bud Burst

(a) Buds moving (%)

	Sample 1. 10 July			Sample 2. 7 August		
	9	11	13	6	9	12 days
- Roots	74	83	95	67	91	91
+ Roots	62	100	100	96	100	100

(b) Bud burst (%)

- Roots	56NS	74NS	91NS	46NS	91+	91+
+ Roots	33NS	92NS	100NS	67NS	100+	100+

Significance test by analysis of variance,
NS Non-significant + Significant at 5% level.

Clearly, on each occasion the rate of bud burst was not altered by the absence of the root system, although subsequent growth rate was reduced. In the intact plants sap flow (and bleeding) occurred soon after placing plants in the warmth, but it is suggested it did not contribute to the early stages of bud burst, as bud burst was not affected by the absence of the sap flow.

Without disturbing the root system of these plants it was not possible to observe very much of the root system to ascertain when new root growth occurred. When plants were taken into the glasshouse no active root tips were present within three days, before there were visible signs of bud movement - although moisture content of the buds would have been increasing (see later).

Further observations of root growth were made in 1977 using well established multi-shoot Abbott plants which were transferred into root-observation tins at the end of April. Seven plants were brought inside under continuous low fluorescent light at 23°C ± 1°C on 12/6/77, and another seven on 10/8/77. following which early bud and root growth was recorded.

The plants varied somewhat in the number of new roots recorded, and when root growth began, but certain trends were clear.

(i) Early (June) sample

Newly extended roots were usually present after 15 days, at which time the one or two most advanced buds were at complete bud burst, and lateral roots appeared at 26 days. The number of elongating roots showed an increase at about 19 days, the time at which the number of buds visibly bursting was rapidly rising, and shoot elongation soon followed.

(ii) Late (August) sample

Root growth and bud burst occurred sooner than it did in the June sample, - by about 6 and 12 days respectively. This may suggest a differential effect of winter chilling on bud development and root development.

Root growth occurred by the eighth day, and became most rapid in the 11th day. All plants had some fully burst (emerged) buds on the third day, and marked stem elongation occurred on the 11th day. Over the period of

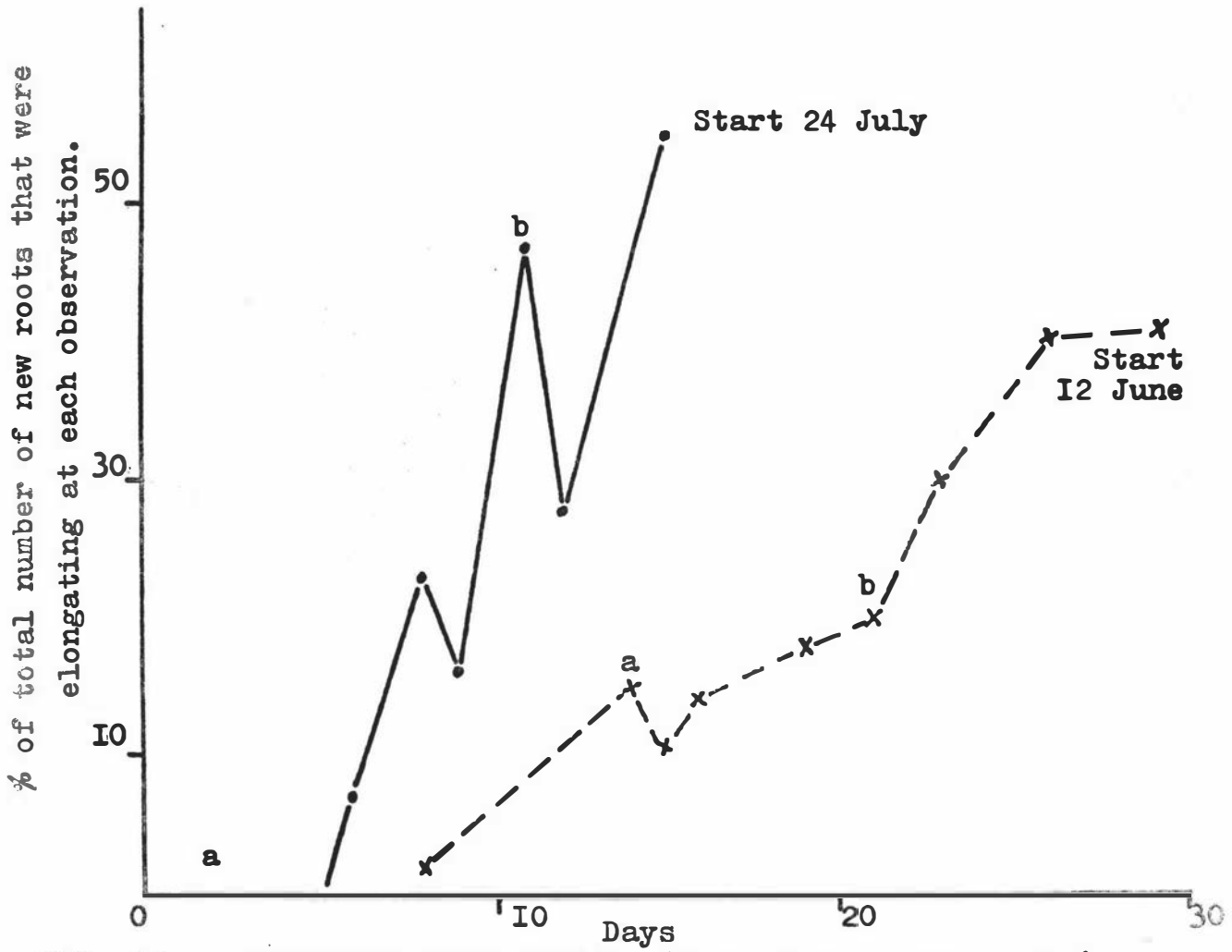


FIG. 34. KIWIFRUIT ROOT GROWTH. (Mean of 7 plants each.)

"a" First bud on plants just visibly moving.

"b" Several shoots on each plant starting to elongate.

observation plants of both samples produced a similar total number of roots, the pattern of growth of each being shown in Fig. 34.

Hence, with both samples root and shoot growth were linked, root extension beginning as the first buds showed signs of emerging (June), or else following bud movement by 5 days (August); and where individual plants had more advanced root development, they also had more advanced bud/shoot development. A noticeable rise in root activity coincided with the presence of a large number of bursting buds (June), or the early rapid extension of shoots (August). Although root elongation is not a measure of root metabolic activity, these results suggest root growth was supporting the simultaneous growth of the buds and shoots, rather than preceding it and thereby initiating bud development.

Experiment 5 1975

Using material of the variety Abbott, changes in the cytokinin level of buds during bud burst were followed. A comparison was made between buds receiving natural chilling in the field while attached to the parent plant, and buds on detached shoots chilled in the cold room.

Methods.

On 29/5/75 canes were cut in the field, and only the middle portions of individual shoots were used. Then 150 buds were collected, and as for all samples analysed they consisted of the bud and its surrounding swollen petiole base. All bud samples were frozen, and later freeze-dried, then held in a deep freeze until all samples were extracted and analysed together in January and February following.

From this harvest, lengths of shoot were also placed in a plastic bag in a cool room at 3^{o} - $4^{o}C$, for artificial chilling. After 6 weeks chilling, on 11/7/75,

these shoots were cut into one-node cuttings and placed in water in a cabinet at 23° - 24°C with a 16 hour daylength to induce bud burst. At intervals 132 buds were harvested and stored, as described earlier.

A second collection of wood was made from the field on 11th August at which time sap bleeding was occurring but there was no sign of bud burst. Explants were held as above, and similar bud collections made to determine cytokinin activity.

Extraction and purification of these samples was based on the method of Hewett and Wareing (1973a), and is shown in Fig. 35.

Notes

PVP slurry - The use of insoluble polyvinylpyrrolidone (PVP) as a highly effective purification of gibberellin-like substances in plant extracts was described by Glenn et al. (1972) and has subsequently found widespread use (Bowen et al. 1973, Williams et al. 1974).

The sample in about 50cc water, 60cc methanol was added to 5gm Plyclar AT (250 - 500 μM) and shaken 30 minutes. After filtration, 75cc 70% methanol was added to the PVP, shaken 30 minutes, filtered, and the solutions mixed.

Biorad column - The acidified methanolic extract was then passed through the column. This was a 17 x 1.7cms packing of Bio-Rad AG 50W - x8 100 - 200 mesh in the H^+ form. The resin had been washed with 100cc 1N HCl and then washed with water until acidified eluant showed no reaction with silver nitrate solution. Then 40cc 70% methanol (pH4) was passed through the column, before adding the sample.

After the sample had been applied it was eluted

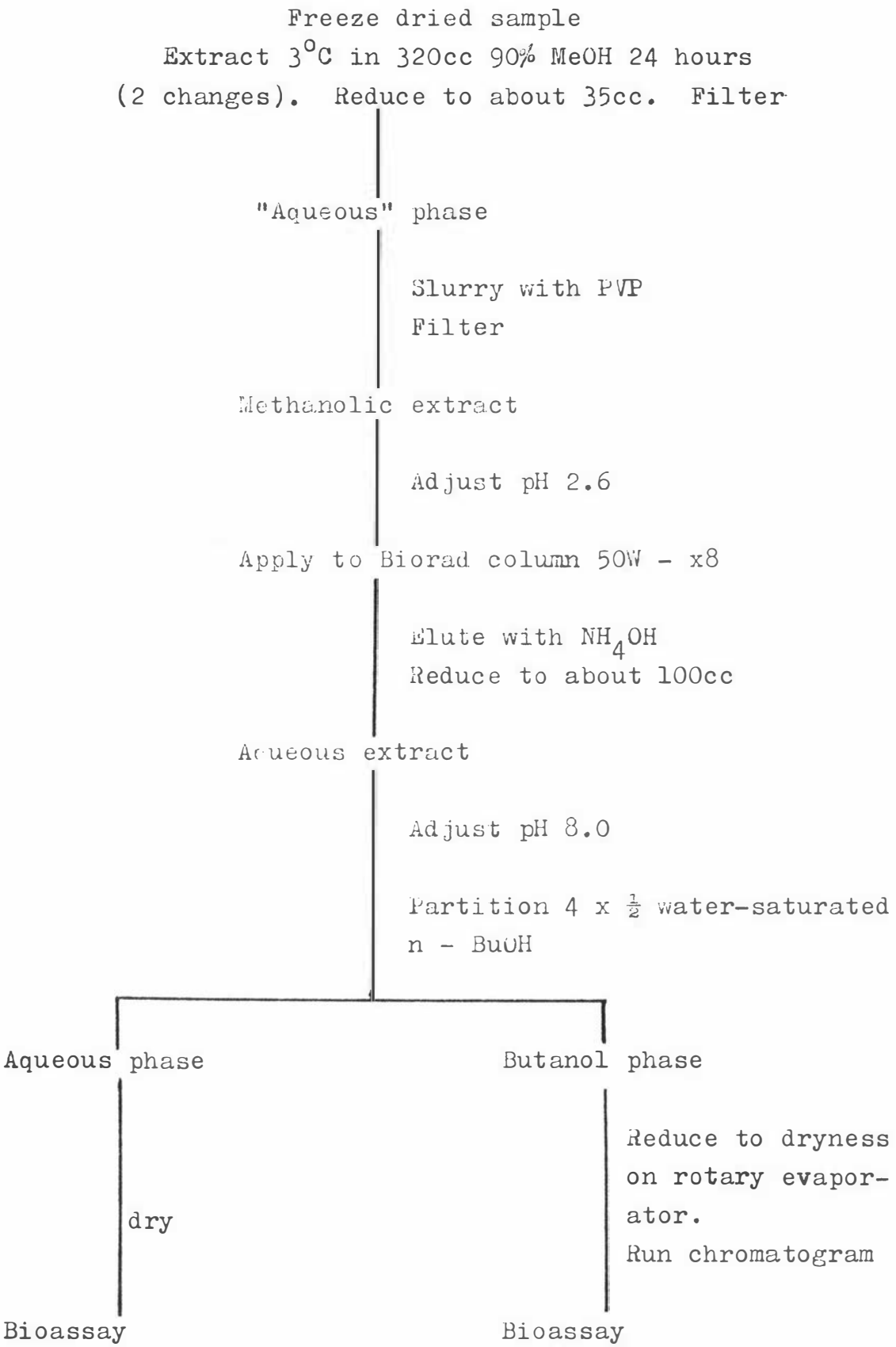


Fig. 35 Extraction of Cytokinins from woody tissue

at 4cc/minute with 75cc 2N NH_4OH followed by 330cc 5N NH_4OH, and the eluate collected.

Bioassay - After partitioning, the alkaline-butanol phase was dried, taken up in methanol, and streaked on paper, Whatman 3MM pre-run in distilled water. One ascending chromatogram of each sample was run in distilled water (pH5.6), and 10 Rfs from each bioassayed with soybean callus (Miller 1963). The aqueous residue of each sample was taken up in methanol, split in two and dried, and also tested with soybean callus.

Results.

The stages of bud development at each sampling are given here:-

P_H harvested 29/5/75. Dormant, minimal winter chilling.

A. Artificial chilling

P_0 harvested 11 July. Dormant, after 6 weeks chilling 3^o - 4^oC.

P_5 harvested 16 July. No sign of bud movement.

P_9 harvested 20 July. 48% just showing visible signs of the start of bud burst.

P_{13} harvested 24 July. 38% buds fully burst, 52% swelling.
(On 31 July 100% of buds at least fully burst)

B. Field chilling

P_0 harvested 11 August. Dormant (15 days before visible bud movement in field).

P_5 harvested 16 August. <5% buds just visibly moving.

P_7 harvested 18 August. 44% buds just showing visible signs of the start of bud burst.

P_{10} harvested 21 August. 30% buds fully burst, 60% swelling.
(On 24 August 93% buds at least fully burst).

Other data about the bud samples used in the analysis is given below:

Harvest	No. buds	Fresh wt(g)	Dry wt(g)	Water content %
29.5 P_H	150	33.8	9.403	72.2
11/7 P_0	188	35.06	10.320	70.6
16/7 P_5	132	23.6	6.638	71.9
20/7 P_9	132	26.1	6.902	73.5
24/7 P_{13}	131	29.45	7.025	76.2
11/8 P_0	130	27.9	8.384	69.9
16/8 p_5	128	26.0	7.618	70.7
18/8 P_7	128	28.9	7.865	72.8
21/8 P_{10}	128	38.2	8.872	76.8

It can be seen that within each series, sampling was at similar stages of development, but the rate of bud development was noticeably faster in the field-chilled material. This is also reflected in the data showing the greater rate of accumulation of water in the field-chilled sample.

The results of the bioassay of the individual chromatograms are given in Fig. 36 and Table 11. Unfortunately there was some activity in the non-chromatogrammed waste aqueous phase, although it was generally very low compared with the alkaline n-butanol phase.

Table 11: Comparison of Cytokinin levels in buds during bud burst (kinetin equivalents, butanol phase).

Treatment	Harvest	µg/Bud	µg/gm F.Wt.	µg/gm D.Wt.
	P_H 29/5	0.05	0.22	0.80
Artificial chilling	P_0 11/7	0.09	0.50	1.70
	P_5 16/7	0.02	0.11	0.41
	P_9 20/7	1.18	6.00	22.67
	P_{13} 24/7	0.28	1.26	5.27
Field chilling	P_0 11/8	0.29	1.35	4.50
	P_5 16/8	1.28	6.33	21.59
	P_7 18/8	2.16	9.58	35.19
	P_{10} 21/8	1.09	3.64	15.69

The results show that bud cytokinin levels were low when collected in the autumn but that following either chilling treatment, activity increased during bud burst, and then declined as shoot growth began. However activity tended to be higher in buds following natural winter chilling in the field, and this was most noticeable in their earlier marked increase in cytokinin activity (ie. P_5)

In addition there appeared to be a change in the type of cytokinins present, from the mid-Rf's in autumn the earlier Rf's after field chilling and during bud burst. The changeover occurred later, when the buds were artificially chilled, although such an apparent qualitative effect would require further investigation.

Experiment 6 1976

During bud burst in the spring of 1976 a comparison was made of cytokinin activity in both the buds and the xylem sap of established Abbott vines.

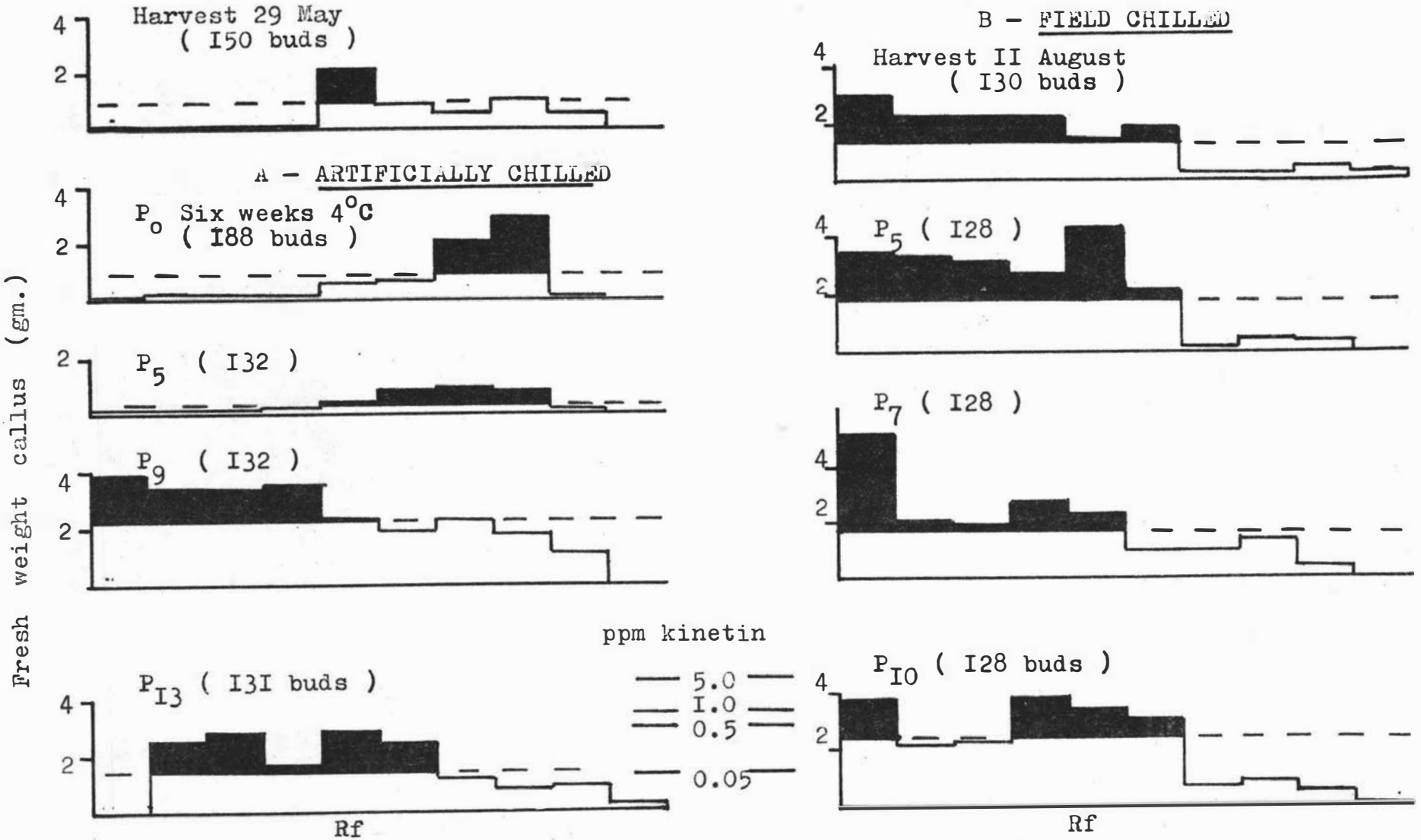


FIG. 36. SOYBEAN BIOASSAY OF BUTANOL– SOLUBLE CYTOKININS IN KIWIFRUIT BUDS DURING BUD BURST. A – Artificially B – Field chilled.
— — — — Significant (P 0.0I)

Methods.

On each of three dates one vine was cut off near ground level and buds and bleeding sap collected. The buds were separated from their surrounding tissue and frozen, and extracts purified essentially as in Fig. 35 before paper chromatography. Sap from the first day's bleeding was frozen before the cytokinins were extracted as in Fig. 25. After chromatography, duplicates of each sample were bioassayed with soybean callus.

Results

Details of the samples collected are:

Harvest Date	Bud Stage	No. buds	Fresh wt.(g)	Dry wt.(g)	Moisture % F.wt.
14/8	About to emerge.	135	12.42	4.35	65.0
18/8	Starting to emerge	145	12.93	3.75	71.0
23/8	Almost emerged	103	11.83	2.91	75.4

	Mls. sap/plant/day	Area cut shoots (cm^2)
14/8	1138	10.37
18/8	502	10.87
23/8	412	7.3

The results of the bioassay of the butanol fractions are shown in Fig. 37, and relative activity compared with kinetin standards is shown in Table 12. The non-chromatographed Aqueous extracts were inactive, except for the second sap sample which just reached significance.

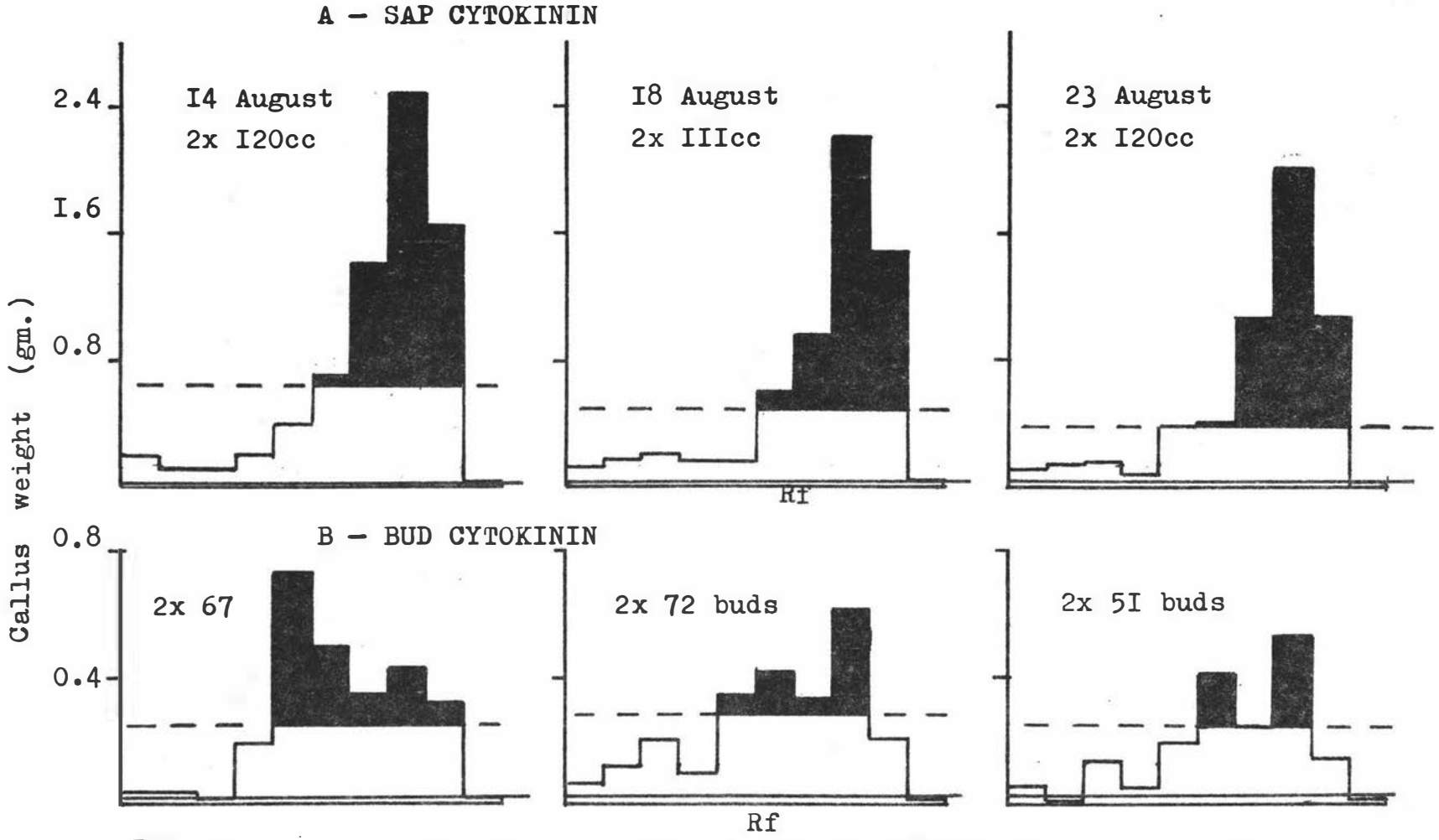


FIG. 37. SOYBEAN BIOASSAY OF BUTANOL SOLUBLE CYTOKININS IN BUDS AND SAP DURING SPRING BUD BURST.

——— Control level, — — — significance (P 0.0I)

Table 12: Comparison of Cytokinin levels in sap and buds during bud burst (kinetin equivalents)

Harvest	14 August	18 August	23 August
Sap µg/litre	80	52	32
µg/day	91	29	13
Buds µg/100 buds	1.02	0.69	0.68
µg/Kg F.Wt	55.6	38.7	29.6

All three sap samples contained a moderate level of activity, and as bud burst proceeded both the concentration in the sap, and the amount transported in one day diminished; - this is still true if one allows for the increased area of bleeding-stem at the first sample. The same downward trend occurred in the buds taken from these separate vines at the same time, the highest levels occurring in buds not yet splitting the enclosing bark, although the first few buds were just emerging on the vine.

Experiment 7 1976

As woody stem tissue is a possible source of growth regulators influencing bud movement, cytokinin activity was determined in stem material harvested at different times.

Method

Using a single Abbott vine, one-year old wood of moderate length likely to produce flowering shoots was collected on four dates. At sampling buds were removed and discarded, and 20gm fresh weight of woody stem was extracted with 90% methanol, purified and bioassayed together in August as in Fig. 35. In addition disbudded surface-sterilised wood collected in the autumn (25 May)

was held in a plastic bag at 2°C for 82 days.

Results

Details of the stem samples are:

Harvest No./Date	Stage Vine Development	Dry Wt.(g)	Moisture % F.Wt.
(1) - 18/2	In active growth, negligible number of fruits	4.763	76.2
(2) - 3/5	Most leaves beginning to yellow.	7.145	64.3
(3) - 25/5	Total defoliation by frost 3 days earlier.	7.535	62.3
(4) - 25/5	Placed in cold storage for 82 days.	7.320	63.4
(5) - 14/8	First few buds on vine emerging.	6.450	67.7

All field samples showed a similar level of activity in the butanol fractions, but it tended to be lowest in the spring sample (Fig. 38). The highest activity was shown by the sample given artificial chilling, and it was unfortunate the replicate chromatograms responded so differently at the position of highest activity(Rf 9). Hence the values for sample number four in the table below may over-estimate cytokinin content. The residual aqueous phases had low but significant activity in the most active samples.

Stem cytokinin (butanol soluble) activity (kinetin equivalents)

Harvest	18/2	3/5	25/5	+Cooler	14/8
Sample No.	1	2	3	4	5
Activity µg/10g F.Wt	5.5	3.2	3.6	13.1	2.6

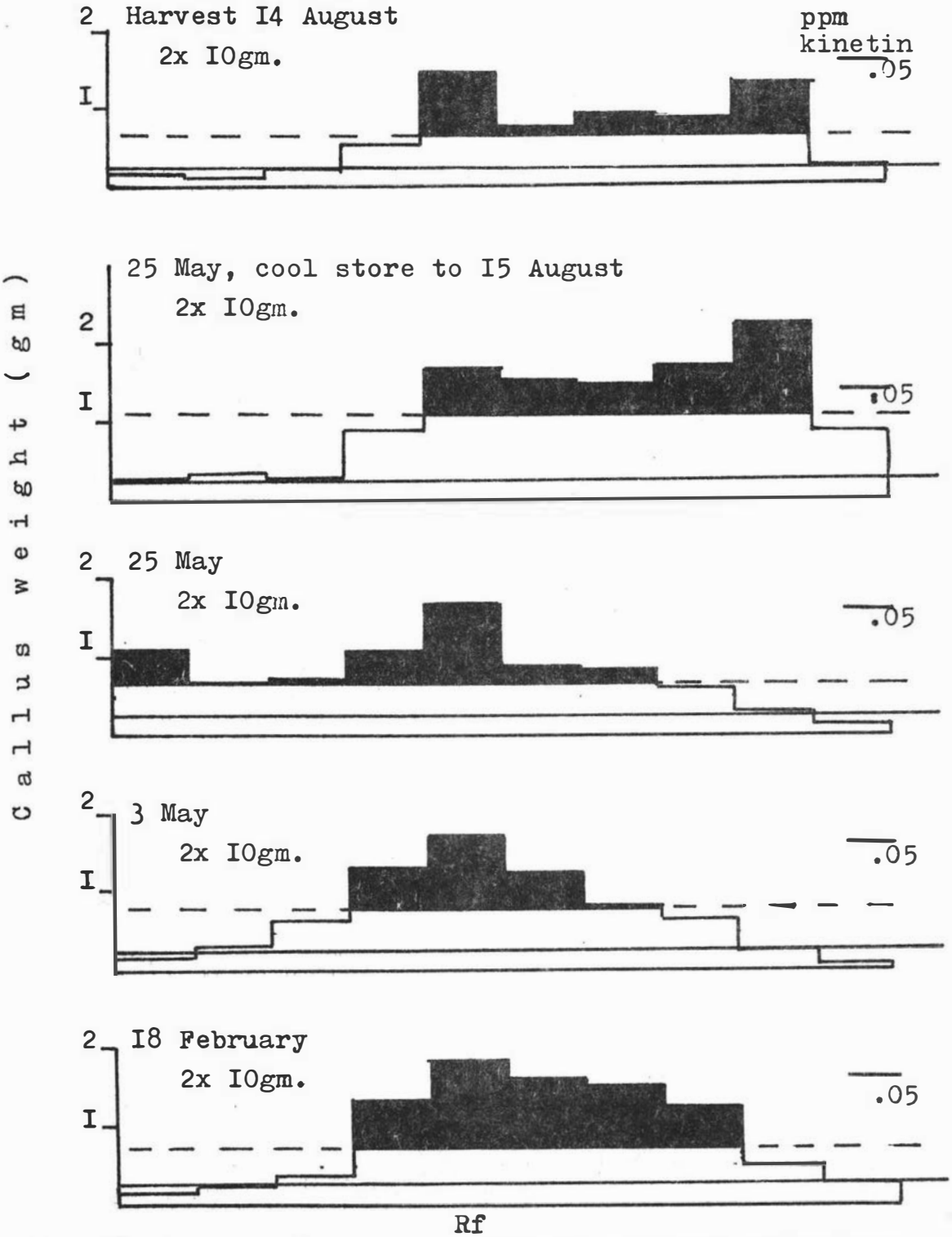


FIG. 38. SOYBEAN BIOASSAY OF BUTANOL-SOLUBLE CYTOKININS IN KIWIFRUIT STEMS.

_____ Control level, _ _ _ significance (P 0.0I)

Experiment 8 1977

A single mature Abbott vine was sampled at intervals during the winter and spring of 1977, and cytokinin activity determined in the buds and one-year old stem tissues, including wood stored at 5° - 6°C for 66 days after being harvested in the autumn.

Extraction and purification was similar to that in Fig. 35 except that:

(a) a slurry with 10gm Polyclar AT was used.
(b) the samples were not eluted from a cation exchange column.
(c) following the PVP slurry, the aqueous extract (pH 3.0) was partitioned with petroleum ether.

Extracts from 30gm stem tissue and 110 buds were bioassayed for cytokinin activity with soybean callus, and compared with BAP standards.

Results

The stages of vine development over the sampling period were:

24 May. Leaves slightly yellowed, light leaf fall, buds at deepest dormancy.
13 June. Most leaves now fallen (frost).
30 June. Bud dormancy broken.
29 July. No visible bud movement, some bleeding occurring.
12 August. First bud visibly bursting.
18 August. 20% buds visibly bursting (bud swell).

The bioassay results are shown in Fig. 39 and they indicate very low activity in all samples, possibly due to omitting the use of a cation exchange column in the purification. Activity was just beyond the range of the standard curve, but by extrapolation the following values were calculated as an indication of the trend shown:

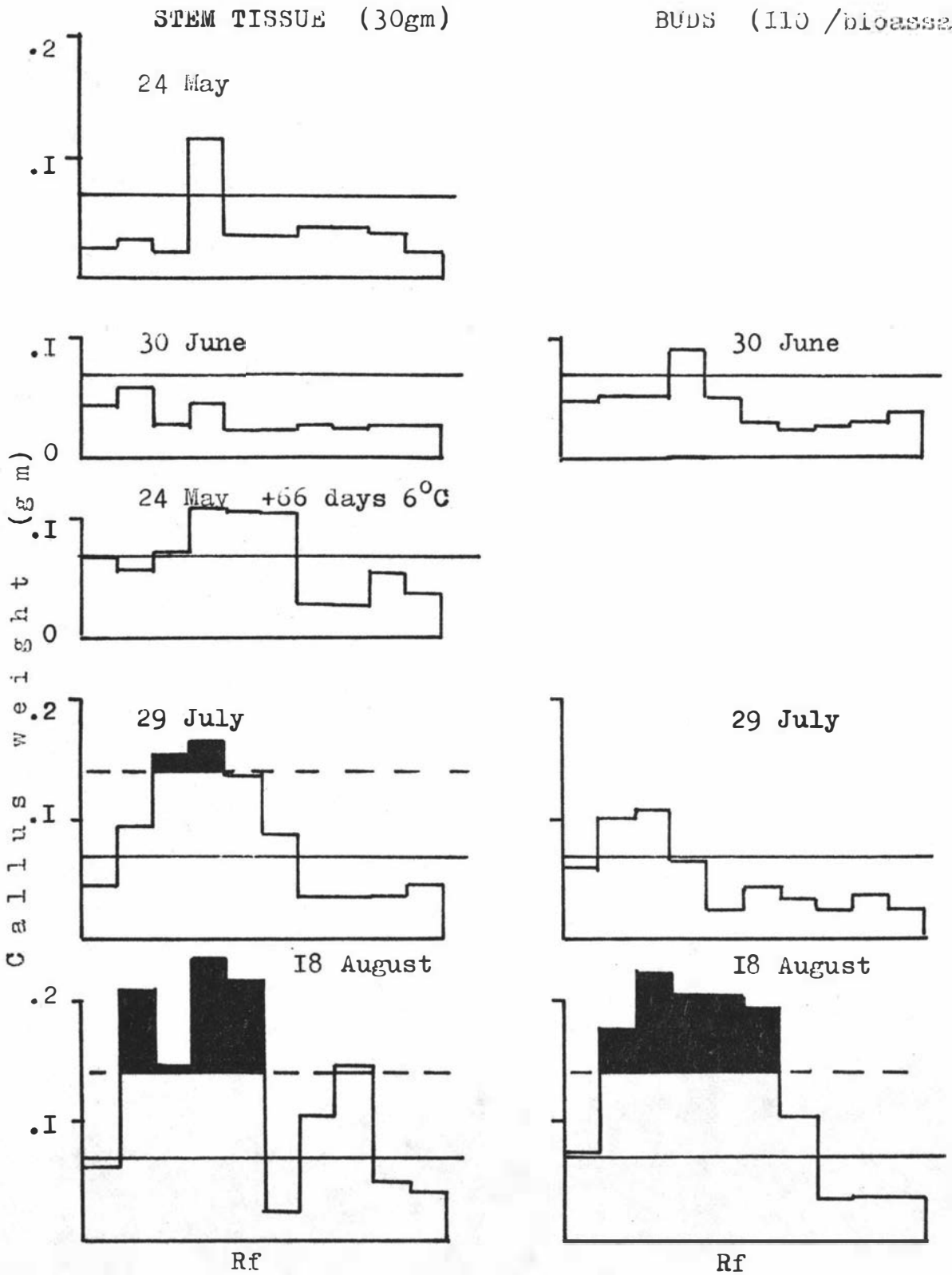


FIG. 39. HISTOGRAMS OF CYTOKININ ACTIVITY IN KIWIFRUIT STEMS AND BUDS DURING DORMANCY.

——— Control, — — — significance P 0.05

Cytokinin activity, BAP equivalents

Harvest date	Wood - µg/kg	Buds - µg/1000 buds
24 May	0.5	-
30 June	NIL	0.14
24 May +cold room	1.6	-
29 July	2.2	0.30
18 August	3.5	0.96

Cytokinin activity in the stem tissue was very low during bud dormancy in May and June, but showed an increase after July or following cold treatment - although the change in activity following artificial chilling was, as in 1976, not great. Similarly in the buds, cytokinin activity was low in the winter, and only showed a noticeable increase when visible bud movement occurred in August.

Experiment 9 1978

Stem tissue of Abbott wood was again used to determine cytokinin activity following different degrees of winter chilling.

Method

One-year old wood was harvested on 18 May (90 hours field chilling) debudded and held in water at 4°C for 168 or 960 hours before extracting as in Fig. 35 and bioassaying the purified extract with soybean callus.

Results.

Bud burst of intact shoots was enhanced only following 1050 hours chilling, and cytokinin activity is shown in Fig. 40. Both chilling treatments gave only a marginal increase in cytokinin activity over the level

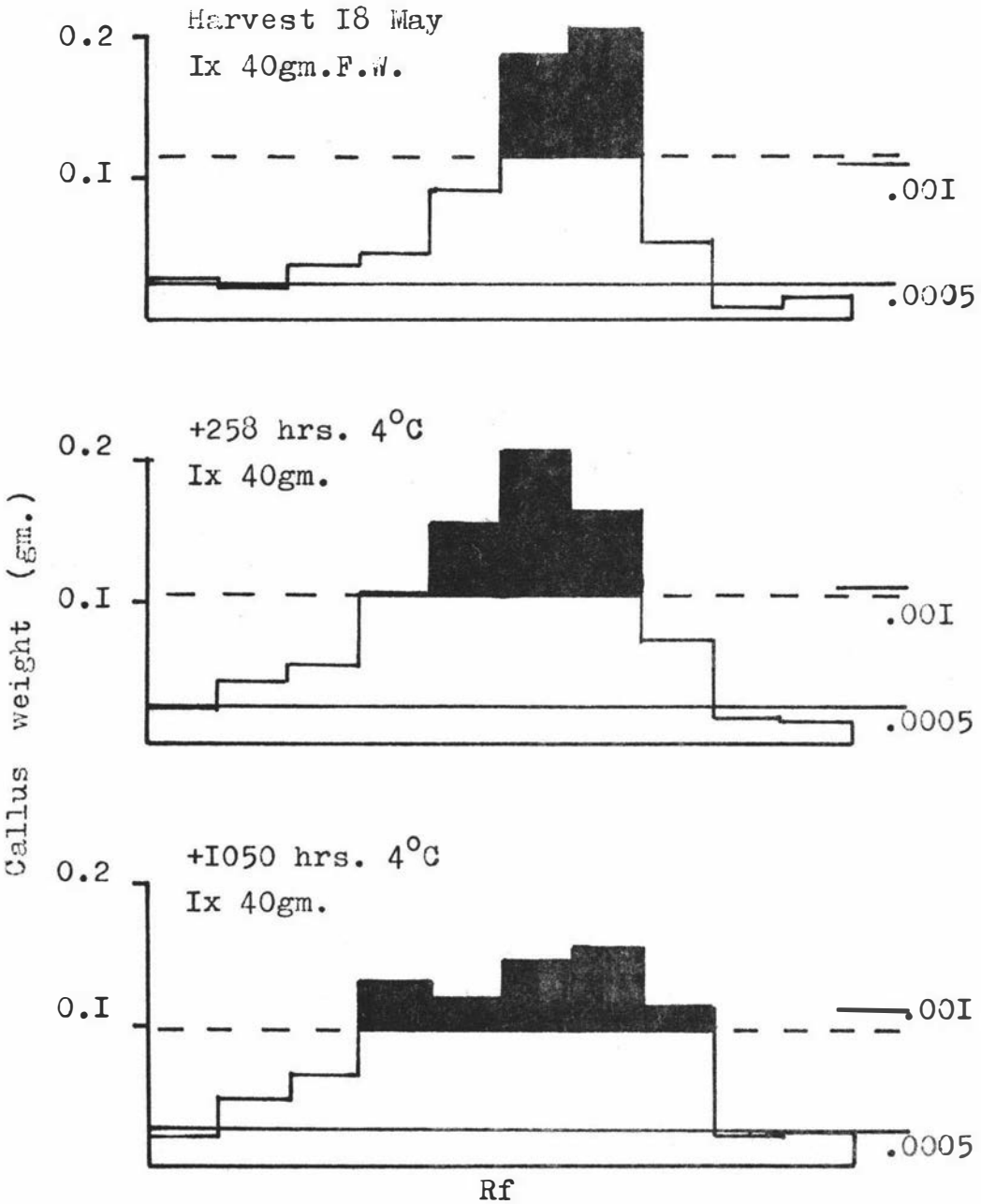


FIG. 40. COMPARISON OF CYTOKININ ACTIVITY IN STEM TISSUE FOLLOWING DIFFERENT LEVELS OF CHILLING.

———— Control, — — — Significant (P 0.0I)

present at harvest in May. The low activity of 6.5 µg BAP equivalents/Kg fresh weight at harvesting, increased to 7.8 and 7.2 µg/Kg for the 950 and 1050 hours cold treatments respectively.

Experiment 10 1978

Buds were extracted and ABA activity determined in the autumn and winter to study the possible relationship of bud-ABA activity to bud dormancy.

Method.

Buds were harvested from mature Abbott vines at the dates shown below and purified as in Fig. 41 before bioassaying with wheat coleoptiles. Similar extractions were performed after storage of the buds.

Treatment 1 - Harvest 2 April
2 - Harvest 23 May (130 hours $<7^{o}C$).
3 - Harvest 10 July (685 hours $<7^{o}C$).
4 - Harvest as (2), then store $4^{o}C$ to 10 July.
5 - Harvest as (2), then store $4^{o}C$ to 6 June.
6 - Harvest as (2), then store $24^{o}C$ to 6 June.

PVP Column -

Polyclar AT (125 - 250 mesh) washed with water and fines decanted off. Pack column 15 x 1.5cms, eluted at 3cc/min.

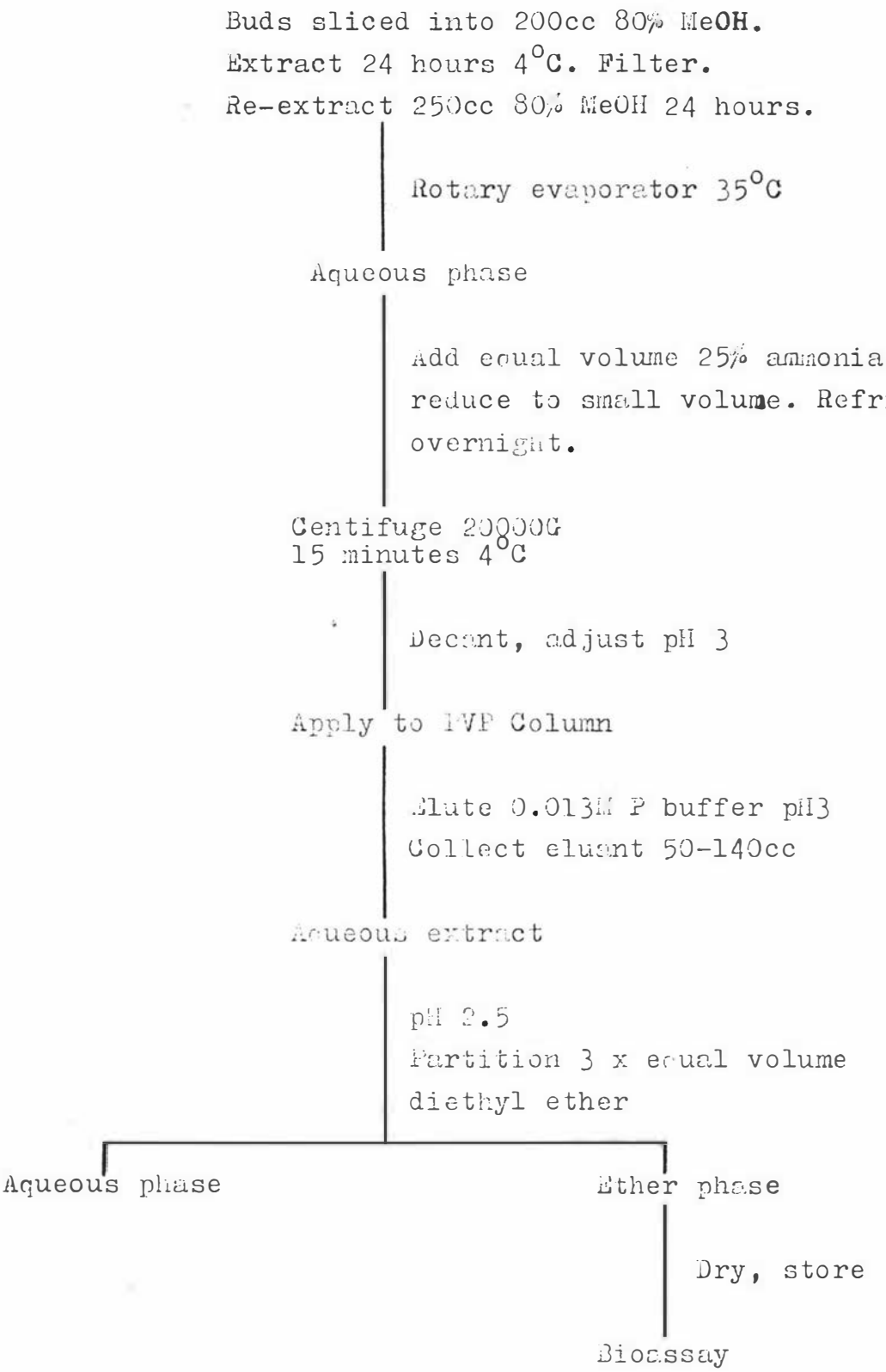


Fig. 41 Extraction of Abscisic acid from buds.

Chromatography -

The dry ether extract was taken up in methanol and streaked (15cm) on pre-run Whatman No.1 paper. Run 20 cms in isopropanol: ammonia : water 10/1/1, dry in vacuum oven 12 hours then cut into strips of 10 Rf's.

Bioassay -

'Aotea' wheat seed soaked 2 hours, then germinated in dark at 25°C for 3 days until coleoptiles 2 - 3cm long. Thn 10mm coleoptile sections cut from 3mm below the tip (done under green safe light) placed in a 40 x 25mm vial with Rf strip. Add 2ml phosphate - citrate buffer pH 5.3, rotate at 1rpm in dark 25°C for 20 hours. Sections then killed with methanol and measured on an enlarger at 3.3 x magnification, and compared with a series of ABA standards.

Results

Bud burst was slow in buds collected in April, May, or cool stored to 6 June (496 hours), but it was rapid when they were harvested on 10 July (685 hours chilling) or cool-stored until that date (1280 hours chilling).

The ABA-like content of the buds is shown in Fig. 42 which indicates an increase in ABA activity in the autumn, followed by a decline with storage or the end of dormancy in the field. The values indicated for ABA content refer to that activity active in the bioassay, and so could be doubled if one assumed a 1:1 mixture of ABA:ABA isomers.

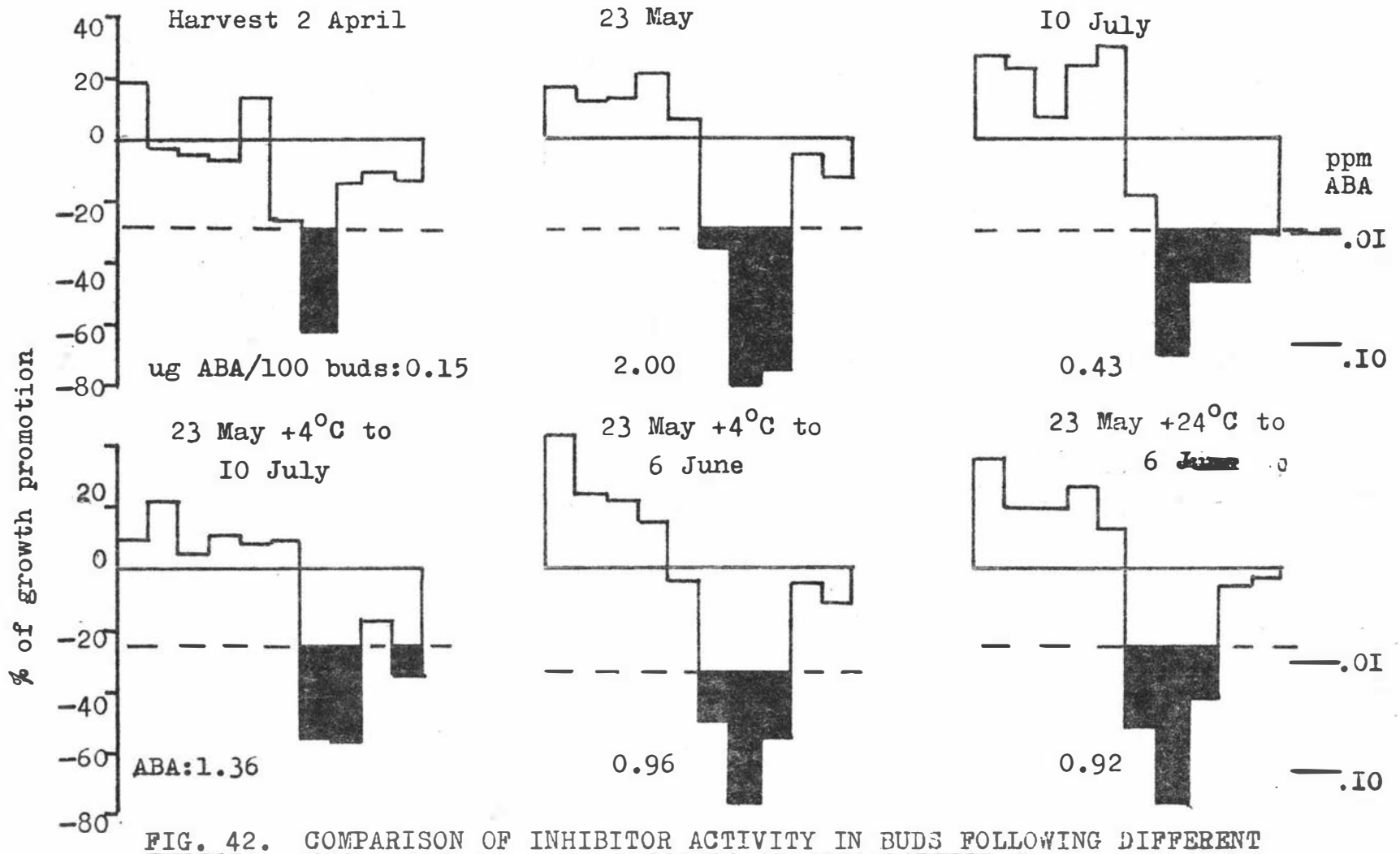


FIG. 42. COMPARISON OF INHIBITOR ACTIVITY IN BUDS FOLLOWING DIFFERENT LEVELS OF WINTER CHILLING. (100 buds each.)

— — — — Significance (P 0.01)

B. Correlative Inhibition (Summer dormancy)

A series of observations were made on the effect of roots and leaves on bud burst in the summer, of single-bud plants or cuttings.

Experiment 1 1974

In mid January a number of single-node cuttings were taken from the mid shoot positions of Abbott vines growing in the field and the leaf on each cutting was cut in half.

The treatments consisted of:

Treat. 1 Single-node cuttings as above.
2 Explants held under mist propagation 12 days to give marked basal callus.
3 As in (2), but callus removed on removal from mist unit.
4 Explants given IBA (0.8%) basal dip, and rooted after 29 days under mist.
5 Explants as in (1) placed in a BAP solution (10ppm).

At the required time, explants in randomised blocks were placed in a glasshouse with tungsten lights extending the daylength to midnight. Explants for treatments 1 - 4 were held in a peat/sand mixture (50/50) and watered frequently.

In addition to these treatments, explants which had only callused after 29 days under mist were used in a comparison (Treatments 6, 7) of bud burst in the presence or absence of a half-leaf - which was removed from half the explants on 17/2/74.

Results.

The results for treatments 1 to 5 were:

Number of buds moving in presence of leaf.

Treatment	(1) Control	(2) +Callus+	(3) Decallus	(4) +Roots	(5) +BAP
No. plants/treat.	24	10(-L) 10(+L)	23	34	29
At 30 days number burst	0	3(-L) 0(+L)	0	19	8

+Callused explants from which the basal callus was not removed were markedly prone to water stress in the glasshouse. This resulted in leaf burn, so that within 7 days half the explants had lost their leaf, and it was only in these ones that bud movement occurred.

Thus no bud break occurred over 30 days on rootless cuttings, or when callused on the base - so long as they bore a half-leaf. It had been noted that on leafless non-rooted cuttings under mist, and explants that became defoliated in the glasshouse, the buds often burst, and so after a period the leaf was removed from all explants and bud burst again determined. The effect of defoliation on the material above is shown:

Number buds burst after removing mature half-leaf

Treatment	(1) Control	(2) +Callus	(3) Decallus	(4) +Roots	(5) +BAP
At defoliation	0 (45)	8 (31)	0 (31)	23 (36)	13(45)
20 days after defoliation	6	20	21	30	27

The figure in brackets indicates the period in the glasshouse before defoliation was performed.

Clearly when the leaf was removed rapid bud movement occurred to give almost complete bud burst in all except treatment one.

This inhibitory effect of a subtending leaf on bud burst was reduced by the presence of roots on the cuttings where 64% of such buds were moving after 30 days. It was interesting that the response was greater in those explants that initially had the larger amount of root ie. 83% cf 44% bud burst.

Supplying BAP to the rootless cuttings induced some response, as 41% and 58% of the buds were moving after 30 and 45 days treatment.

The results for treatments 6, 7 made it clear that callus did not affect the behaviour of the bud - the presence or absence of a leaf determined the bud's response:

Effect of callus and leaf tissue on bud movement

	+ Callus		De-callused	
	-L	+L	-L	+L
No. explants	9	9	8	9
No. moving after 36 days	7	0	7	0

Experiment 2 1976

At various times from mid-summer - onwards, bud burst was observed in single-node cuttings of Abbott collected from the field. Twenty four cuttings were used for each treatment, arranged in randomised blocks. Cuttings were placed in small jars of aqueous solution, and held under continuous light (fluorescent and tungsten) at 22° - 23°C.

Using the more mature part of each shoot, the first cuttings (24/treatment) were prepared on 22/1/76 as follows:

Treatment 1 Defoliated + water
2 Defoliated + 20ppm BAP
3 Defoliated + 20ppm BAP + 10ppm IAA
4 Defoliated + 20ppm BAP + 1ppm ABA
5 + Half-leaf + water
6 + Half-leaf + 20ppm BAP

In the case of treatments 3 and 4, either IAA or ABA alone, was also applied to a pad of cotton wool held around the cut stem immediately above the bud with polythene tape. All solutions were changed each five days.

Results

Initially only treatment 1 (defoliated) showed active bud movement, and after 23 days it showed the highest level of bud burst, although a number of cuttings had since died. (Table 13). In comparison, the presence of mature leaf tissue was partially inhibitory to bud movement (Treatment 5); and surprisingly cytokinin inhibited bud movement (Treatments 2, 6). Treatments 3, 4, 6 were totally inhibitory to bud burst, hence both IAA and ABA and also leaf tissue are strongly inhibitory to bud movement.

Table 13: Bud movement in response to hormone treatment in different seasons

(a) Harvest 22/1/76

	No. buds burst +11 days. (max 24)	At +23 days Bud burst	No. moving	Max.
Treatment 1	5	11	13	15
2	0	0	7	18
3	0	0	1	13
4	0	1	1	15
5	0	6	8	24
6	0	0	0	8

Table 13: Bud movement in response to hormone treatment in different seasons (Cont.)

(b) Harvest 23/2/76

	At +13 days No. buds moving	No. burst (max 28)	At +23 days No. burst	Max.
Treatment 1	13	5	16	27
2	6	1	6	25
3	0	0	0	23
4	1	0	0	26
5	3	1	3	28

(c) Harvest 1/6/76 (No. of buds burst)

Days 22°C	24	27	30	34
Control	1	15^{NS}	26^{+}	32^{++}
+ BAP	2	9^{NS}	18^{+}	20^{++}

(d) Harvest 23/7/76 (No. of buds burst)

Days 22°C	10	12	14	18
Control	3	12^{+}	39^{++}	42^{++}
+ BAP	0	3^{+}	16^{++}	32^{++}

Significance of difference between treatments at each date determined by analysis of variance.
NS - Non significant $^{+}$Significant 5% $^{++}$Significant 1%

(e) Harvest 7/12/76 (% bud burst)

	+10	+23 days
Treatment 1	70 a	98
5	15 b	51
6	0 c	6$^{\Theta}$

Analysis of variance of number of buds burst at 10 days significant (1%) treatment differences determined by Duncan's Multiple Range test (1%) shown
$^{\Theta}$ Six days later, after removing the leaf and BAP, this had increased to 72%.

When repeated with material collected on 23 February, after one week most of treatment 6 had been lost. Treatments 1 - 5 were continued except that BAP was reduced to 5ppm in (2), and excluded from treatments (3) and (4). The results again showed the inhibition of bud movement in the presence of leaf tissue, and also in the presence of BAP, IAA or ABA (Table 13).

When shoots were collected on 12 April, about three weeks before leaves on the vines were becoming yellow, the leaves showed total inhibition of bud movement of cuttings over four weeks, and the level of bud burst in the water controls was less than in the earlier harvests.

Similar samples were taken at intervals through the winter, and bud burst recorded. On two occasions a BAP (20ppm) treatment was compared with controls, and again this hormone delayed bud burst (1/6/76 and 23/7/76).

Within these winter samplings, the rate of bud burst varied. The pattern over all the collection dates for the control treatment No. 1 showed a slowing of bud burst through the summer until June, with a sharp rise in bud growth rate from early July.

A final collection of the current season's shoots, was made on 7/12/76 and 144 cuttings prepared from the mid-part of each, and held at 25°C as in treatments 1, 5, 6 using 5ppm BAP. Bud movement occurred in the presence of a leaf although it was somewhat inhibited, and at this time of the year the cutting back of shoots in the field results in a high level of axillary bud break. When a cytokinin was supplied some mortality (33% at 23 days) of cuttings occurred, and there was a strong inhibition of bud movement.

Experiment 3 1976

On the 5 April 1976 leafy Abbott shoots were harvested, and one-node cuttings were held at 22°C under continuous light, after removing the leaves. Half the cuttings were prepared in the usual way with the bud at the top, and for the other cuttings of similar total length the bud was left up from the base. This was repeated with material collected on 7 December and held at 25°C.

Results

Visible signs of bud movement occurred earlier in buds when at the top of the cutting, and they also gave the highest level of bud burst.

Table 14: Effect of bud position on cutting on bud burst

(a) Harvest 5/4/76 (Number of buds at least fully burst)

	+15	+18	+21	+24days	% (at +24)
Bud at base (42 explants)	2	2	5	13	31
Bud at top (37 explants)	8	17	19	24	65

(b) Harvest 7/12/76

	+7	+10	+16	% (at +16 days)
Bud at base (48)	5^{++}	12^{++}	29^{++}	60
Bud at top (48)	17^{++}	40^{++}	47^{++}	98

$^{++}$Treatment differences at each date significant (1%).

Experiment 4 1977

On 5 December single-node cuttings were prepared from the mature part of shoots taken from established Abbott vines. The leaves were removed, and the cuttings held in aqueous solution under continuous light at about

27°C. One half of the cuttings had cotton-wool moistened with IAA held on the distal cut surface, and they also stood in IAA solution (10ppm) which was replaced daily.

Results

Auxin was very effective in inhibiting bud burst of leafless cuttings in the summer (Table 13), and the samples for bioassay were collected several days before any visible sign of bud burst in untreated cuttings.

After 42 and 72 hours the upper stem tissue and its bud was collected from 12 cuttings of each treatment to give 25gm fresh weight, and these samples were purified as in Fig. 35 and cytokinin activity determined by bioassay with soybean callus.

Cytokinin activity was very low in all samples (Fig. 43), estimated as being (µg/Kg Wt. in BAP equivalents):

At harvest		6.9			
After 42 hours	-IAA	8.8	and	+IAA	4.7
After 72 hours	-IAA	6.7	and	+IAA	3.4

Hence, while the cytokinin activity of stem tissue showed a temporary rise shortly after collection and defoliation, in the presence of IAA the stem-cytokinin level stedily declined.

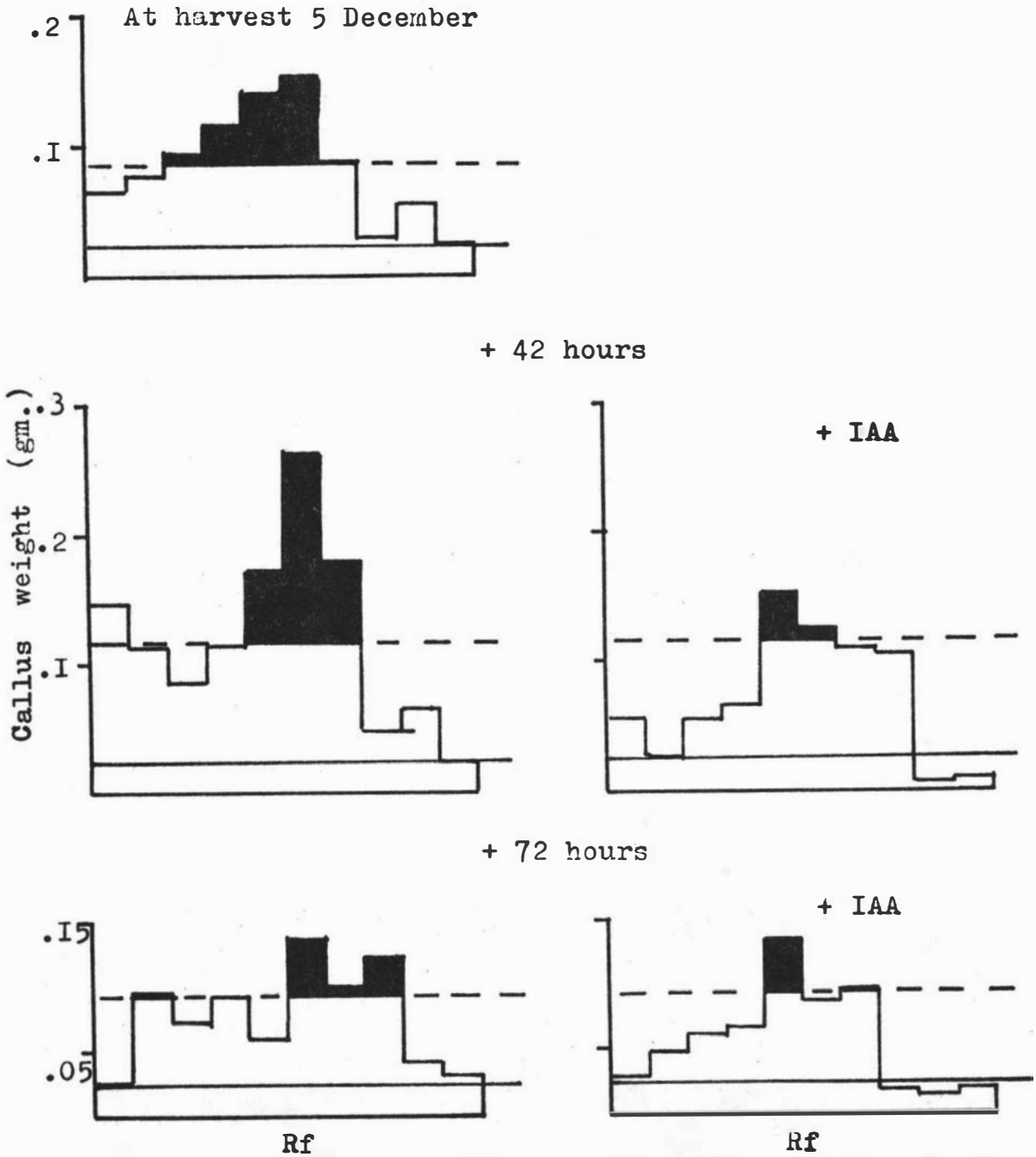


FIG. 43. SOYBEAN BIOASSAY OF CYTOKININ ACTIVITY IN KIWIFRUIT SHOOT (25gm FW), AS AFFECTED BY AUXIN APPLICATION.

——— Control, — — — Significant level (P 0.0I)

DISCUSSION

A. Bud Dormancy and Bud Burst.

Buds of Bruno collected at the end of May (Experiment 1 1975), before leaf fall, were dormant, and neither GA_3 or BAP alone or in combination was effective in inducing bud burst, and BAP applications in the winter were also ineffective in breaking dormancy (Section B). No attempt was made to determine whether this was due to inadequate uptake of hormone into the bud's tissue. Where similar applications were made later in the winter (August), dormancy had already been broken as control buds burst rapidly. Adding gibberellic acid to such quiescent buds promoted bud growth resulting in earlier bud burst and increased shoot elongation, indicating that at this time uptake of exogenous hormone did occur readily. The indication is that while GA did not break dormancy, it stimulated the growth of buds that had previously had their rest period broken, as in pears (Brown et al. 1960), although in stonefruit GA breaks bud dormancy (Donoho and Walker 1957), and cytokinins and gibberellins are effective on apple buds (Williams and Billingsley 1970). Exogenous GA releases coffee buds from dormancy, in which plant gibberellins appeared to be the mechanism through which environmental factors overcame the dormancy effect; but exogenous cytokinin was effective only if applied after a water stress, indicating a need for other promotive influences also to be present. Hence, while neither GA nor cytokinin, unlike chilling temperatures, were found to overcome the mid-winter dormancy of kiwifruit buds, it is still possible the presence or absence of each of them could control the breaking of dormancy. Gibberellin activity in kiwifruit buds was not determined, but in various species endogenous levels of bud-gibberellins rise towards the end of the dormant period, but before the end of rest (Ramsay and Martin 1970), and many authors support the hypothesis that the end of rest is regulated by the

balance between growth promoters and inhibitors.

In 1976 Abbott buds were most dormant on 1 June, and with closer sampling in 1977 deepest dormancy occurred on 10 May with only a small change over the next month. Using a smaller number of cuttings Brundell (1976) similarly found the time for 50% bud burst was greatest for three other varieties in Auckland when they were harvested in May. Hence maximum bud dormancy occurs when most green leaves are still on the vine, one month before total leaf fall; and within 3 weeks of leaf fall the buds are capable of rapid bud burst. Cold temperature treatment of detached shoots overcomes the dormancy of May buds, as also was found to occur after about 700 hours chilling of vines in the field. In the latter half of June in the field, the vines apparently undergo major changes. Only then do they develop the capacity for rapid bud burst, and also for the production of flower buds.

Examination of bud burst data for three varieties in Auckland also indicates a requirement for a level of winter chilling of this magnitude. Table 15 presents data of Brundell (1973), and emphasises the distinction between buds receiving more than, or less then, 770 hours chilling treatment. If 21 days (500 hours) or less at 25°C, for 50% bud burst, is taken as a reasonable guide to loss of dormancy, this usually only occurred following more than 770 hours below 45°F (7.2°C) - bud burst taking up to 23, 20, 20 days respectively for Hayward, Alpha, and Monty.

Table I5: Bud Burst response to varying levels of winter chilling.

Hayward.

Harvest	Hours <7.2°C (field + artificial) Treatment a	b	c	d	e	Hours at 25°C for 50% bud burst. Treatment a	b	c	d	e
2/5	50	290	530	770	I0I0	I0I0	740	550	480	4I0
23/5	I40	380	620	860	–	II00	9I0	580	460	–
I3/6	290	530	770	I0I0	–	860	670	650	550	–
4/7	480	720	960	I200	I440	840	670	530	530	360
25/7	625	865	II05	I345	I585	700	500	430	260	220

Alpha.

2/5	50	290	530	770	I0I0	600	620	380	360	360
23/5	I40	380	620	860	–	840	620	500	460	–
I3/6	290	530	770	I0I0	–	790	620	480	460	–
4/7	480	720	960	I200	I440	770	530	480	3I0	240
25/7	625	865	II05	I345	I585	530	340	290	260	220

Monty.

9/5	80	320	560	800	890	890	580	460
30/5	I70	4I0	650	890	890	670	550	4I0
20/6	360	600	840	I080	700	500	430	380
II/7	530	770	I0I0	I250	650	480	380	3I0
I/8	670	9I0	II50	I390	460	340	240	240

I. Experimental data from Brundell (I973).
2. Hours chilling computed(Spreen I956) in part, using meteorological data for Oratia I972.
3. Data in shaded areas received greater than 770 hours below 7.2°C.

It has already been indicated that warm temperatures appeared to be as effective as low temperatures in inducing bud burst in 1976. Reference to Table 15 and dormant buds harvested on 23 May shows that as the period of cold treatment was reduced, the total period of warm and cold treatment for the same level of bud burst declined slightly. Hence a temperature of 25°C was as effective as a temperature of<7.2°C in inducing bud burst, because if warm temperatures were ineffective substantially increased total time periods would have been required. A similar statement could be made about the buds harvested on the other dates during the autumn and winter.

If the time period is excluded from a definition of dormancy (p. 27), the fact that 50% bud burst could occur in 50 days with as little as 140 hours chilling would mean buds of the kiwifruit vine do not show true dormancy. Unfortunately Brundell does not indicate whether substantially more than 50% bud burst could occur, and my results for 1977 suggest total bud burst may not have been very high. Thus the buds exhibit a behaviour like those showing true dormancy, although weakened in degree, but a full study of the ability of warm temperatures to substitute for winter chilling would be best made with intact plants in a controlled environment.

The 1978 study with dormant buds stored at different temperatures (Fig. 31) provides support for the greater effectiveness of warm temperatures in breaking dormancy. Then pre-treatment with limited field chilling (harvested 23 May) plus 580 hours low temperature storage gave rapid and high bud burst, but a pre-treatment of 580 hours storage at 16°, 20° or 24°Cinduced even more rapid bud burst.

The swollen tissues that enclose a bud were shown to exert a strong effect in inhibiting bud burst of the dormant bud, an effect which was diminished but still present in the late winter. The bud scales themselves did

not appear to confer any additional inhibitory effect, whereas in many plants they are the source of bud inhibition. Apparently freeing the bud tissues from the totally enveloping "stem" tissue, exposing them to the air, is a potent stimulus to rapid and complete bud burst. The manner in which the buds are enclosed no doubt provides good protection from the cold winter temperatures the otherwise naked buds would be subjected to, but also appears to form part of the control mechanism for bud burst.

Fig. 32 also shows the promotion of bud burst by tissue removal was almost identical to that resulting from cold treatment (25 days 7°C) of the stems. This supports the idea of the enclosing tissues controlling bud burst in the field, with dormancy-breaking low temperatures somehow modifying their effect on the buds.

It is difficult to suggest the manner in which the enclosing tissues limit bud break. While in some plants there is light requirement (Erez et al. 1966) this does not seem very likely in the kiwifruit, and in August exposed buds burst as rapidly when light was totally excluded. The likely change in bud temperature as a result of removing the surrounding tissue may not be adequate to account for the relatively large change in bud behaviour, and compared with spring buds, it can take a considerable period for the bud burst response to develop (Fig. 32). The development of winter dormancy appeared as an extension of leaf-inhibited bud break in the summer, and so as with dormancy induced by a short-day leaf stimulus it does not seem likely dormancy resulted from a limited oxygen diffusion into the bud tissues (Wareing 1969). Hence, it is difficult to imagine that the bud's surrounding tissues behave as a seed coat may in interfering with oxygen uptake. Although such an effect of the bud scales of Acer has been recorded (Pollock 1953), in the blackcurrant inhibition of oxygen uptake did not appear to account for the inhibitory effect of the bud scales on bud burst (Tinklin and Schwabe 1970). In fact kiwifruit buds in August, when exposed and

sealed with Parafilm and lanolin tended to make the most rapid growth.

However the results of Experiment 3 in Section 3A suggest that in the early winter lateral buds are prevented from bursting by a lack of air. Exposing the buds by pruning results in rapid bud burst, but this does not occur if lanolin is used to exclude air from the bud. Wounding by cutting was not effective, although in detached apple shoots dormant buds develop as a result of cutting (Paiva and Robitaille 1978). Similarly such injury as resulted from immersing cuttings in running water for two or five days largely prevented bud burst, unlike Westwood and Bjornstad (1978) who found this treatment reduced winter dormancy.

The non-involvement of ethylene in the promotion of bud burst by stem tissue removal was indicated by the inhibitory effect of even the lowest ethephon application. This is similar to the results of Paiva and Robitaille (1978) who found no evidence for a role of ethylene in wound-stimulated bud break of dormant buds.

Observations in two years showed that in the absence of a root system, buds burst as rapidly, and to the same extent as those on an intact plant. This indicates that using shoot cuttings to study bud burst is an acceptable way of studying bud burst in this plant, and also shows the roots are not contributing to early bud growth. Any root contribution must be residual, having been previously stored in the stem tissue and capable of being drawn upon; but the retarded growth after bud burst in the absence of roots indicates they are important in supporting subsequent shoot extension. Observations in 1977 of bud and root growth indicated active root growth coincided with, rather than preceded shoot development and elongation, expecially after long winter chilling, which suggests root activity does not promote early bud growth - although it could well support shoot extension growth. The results of

Brundell's (1976) study of flower bud development suggest the roots may contribute to developmental changes within the bud. It rather appears any environmental effect promoting bud burst (ie. in overcoming dormancy, or accelerating the rate of bud burst) does so by directly affecting the buds or stems, and not indirectly via the roots. In fact winter chilling advanced bud burst more than it advanced the commencement of root growth in 1977, which could indicate a degree of independence of these two processes. So even though sap flow and root growth began soon after placing the plants in warm temperatures, there was no indication that it facilitated bud burst. The possibility that root-produced cytokinins present in the stem enable the bud on explants to grow was demonstrated by Woolley and Wareing (1972a), although Peterson and Fletcher (1975) considered that in addition to this possibility bud growth required some other stem factor.

Mature shoots collected in the autumn gave rapid and complete bud burst following artificial chilling (Experiment 5), behaving similarly to material given a longer period of chilling in the field while still attached to the parent plant. In this respect there was no suggestion that removing the buds from the influence of the roots at the end of May, put them at a disadvantage - an effect also indicated above. Hence when material was harvested at the time of deepest dormancy, bud burst only occurred after chilling, and so bud burst was stimulated by a cold-induced change in the stem/bud, and not by a root-factor.

Changes in cytokinin activity

In the autumn, bud-cytokinin activity was very low, but showed an increase after field chilling, and also a possible qualitative change (Fig. 36) which would need to be confirmed. Browning (1973b) found a change from a single peak of activity in dormant buds, to two broad zones of activity when dormancy was broken. During bud burst, cytokinin activity in the buds increased, reaching

a maximum when almost half the buds were partly burst (bud swell), and then beginning to decline (Table 11). In the previous spring, sap cytokinin levels similarly were highest at the time that the buds were between bud swell and advanced bud burst. It appeared unlikely activity was any different in the two types of bud sample (artificially or field chilled), except that in this single test activity was unusually low immediately before visible bud movement (P_5) in the artificially-chilled material, and also had not yet undergone the apparent qualitative change already seen in the field material (Fig. 36).

However this latter effect was possibly an artifact as no such type of activity was recorded in buds in the following year (Fig. 37). The buds used in Experiment 6 contained lower levels of cytokinin than those above, a major difference between the samples being that the earlier sample consisted of both the bud tissues and the enclosing stem tissue. As with the field material used the previous year, there was little change in activity over the nine days of early bud movement. Again the trend was for cytokinin concentration (and perhaps content) to decline during the later stages of bud burst. Over this period the plants had a declining level of activity in the sap - a level possibly lower than might have been expected from earlier observations (Table 6). Hence the quantities of hormones being transported daily in the sap declined markedly, while there was only a small change in bud cytokinin activity - but since different plants were used each time, and they can vary markedly in cytokinin activity at any given time (Table 6) it is difficult to draw conclusions. The effect was for high sap flow rate and sap cytokinin level to coincide with high bud-cytokinin activity and the stage when the first few buds on the vine were beginning to emerge. It could not be shown that the build-up in sap-cytokinin level preceded slightly the bud-cytokinin maximum, as found by Hewett and Wareing (1973a), but rather activity in both tended to be higher during the very early stage of bud growth, and decreased once bud burst

was under way. It is unlikely an earlier peak in activity was missed since sap bleeding began not more than about 10 days before the first sampling, when activity was previously found to be particularly low. If cytokinins do contribute to early bud growth, normal bud burst in the absence of a sap flow means that in its absence the buds must have access to adequate quantities of this hormone from other sources. However bud burst was not induced in dormant buds by treatment with BAP, (Experiment 1 Section 3A), and even delayed bud burst of winter buds (Experiment 2, Section 3B).

Bud cytokinin was a little higher following field chilling, and particularly in the early stages of bud growth activity was greater in field-chilled material than artificially-chilled buds (Table 11), an effect also recorded by Hewett and Wareing (1973a). The difference between buds field-chilled on an intact plant and those artificially-chilled on detached shoots was also shown by the more rapid bud burst of the former (although the level of chilling differed), and Brundell (1976) also found artificially chilled buds were slower to resume growth at elevated temperatures.

Because it appeared possible the cytokinins increasing in the buds during bud burst came from sources other than the active root tips, it was desirable to determine their activity in stem tissue, which is considered by other authors (Browning 1973b, Hewett and Wareing 1973a, Skene 1972) to be an important source of cytokinins in the spring. In considering plant behaviour it is also well to remember the field situation, and the seasonal variations in microclimate of a deciduous vine. In the absence of a leaf canopy, bark, cambium, and xylem temperatures are higher during the day on a bright sunny day in the cool season than in the summer (Gerber et al. 1974). With the warmest and coldest zones usually being near to the ground, very large temperature gradients can occur then across a twig or trunk and produce physiological stress. Such stress

conditions, and the stimulation of cellular activity in the cambium layer by the warm temperatures could contribute to seasonal changes in the tissues which changes are of importance in early plant growth.

It has been postulated that lateral buds may be sites of cytokinin synthesis, although the increased bud-cytokinin activity could also be accounted for by their accumulation from other tissues. In the willow it was shown that lateral buds have the ability to hydrolyse glucosylated zeatin derivatives, and then to use them for bud development (van Staden and Brown 1978). These authors suggested the lateral buds do not synthesise cytokinins de novo, but have the capacity to convert cytokinins that are transported to them.

A similar level of activity was found in the shoots of all field samples (Fig. 33), and there was no evidence of increased activity over the late summer and autumn. In the poplar, leaf cytokinins are converted to glucosides (Hewett and Wareing 1973b), and as they have also been detected in phloem sap (van Staden 1976) it is possible for shoot cytokinins to be transported to the stems or roots in the autumn, for use at a subsequent date - as in the support of bud burst. There was no suggestion in the results here of either an autumn accumulation of stem cytokinins, or the presence of a cytokinin glucoside. But cold storage of wood resulted in increased activity, which was not the result of bud or root activity since they had been removed 12 weeks earlier - and a similar but more dramatic change in the sap activity of grape canes following cold storage was found by Skene (1972a).

Determinations of cytokinin activity in 1977 showed that bud activity was very low at the time that their dormancy had just been broken (30 June), then tended to rise over the following month when sap bleeding began and the buds developed the capacity for very rapid bud burst. But as in 1975 (Fig. 36) a marked increase in activity

occurred only at bud swell. In the stem tissue, cytokinin activity was very low when dormancy had been recently broken (June), although the buds were now capable of rapid bud burst - suggesting stem-cytokinin did not contribute to the breaking of dormancy. When stem tissue was chilled (Experiment No. 9) there was only a slight change in cytokinin activity, and it could not be related to the cold treatment that promoted bud burst.

It was suggested earlier that important changes occur in the vines from the end of June when rapid bud burst can again occur, and it could also be that only from that time do buds and stems develop the capacity to produce cytokinins that appear from that date. Stem-cytokinins increased during July, and this activity could favour the very rapid bud burst the buds were then capable of; and over the next 3 weeks leading up to bud swell the stem tissue continued to offer increased quantities of cytokinin. Any effect of stem-cytokinins on bud burst would appear to be to facilitate or support bud growth, rather than to cause it, since there was no dramatic increase in stem-cytokinin activity preceding bud burst.

Stem-cytokinin activity showed an increase from the start of sap bleeding in the same manner as sap-cytokinin content rises - an effect one could expect where cytokinins were being released into the sap from woody tissue, rather than reflecting production in the root tips.

Hence there is some evidence the shoots themselves could, following winter chilling, make cytokinins available for developing buds from cytokinins originating in the woody tissue. Such stem-cytokinin could be transported in the sap flow, and contribute to bud growth along with other controlling factors. However in both field-chilled and artificially-chilled stem tissue, only small increases in cytokinin activity were detected. In the buds of coffee

Browning (1973b) sees the dormancy-breaking stress resulting in cytokinins being released into the xylem sap from the vascular cambium, and taken to the buds where the increased bud-cytokinin in conjunction with increasing bud-gibberellin activity results in bud growth.

Bud-ABA and Dormancy

Further studies of changes in bud-ABA activity would be of value to assess the effects of temperature on dormancy and bud-ABA. The results in Fig. 42 show a marked rise in inhibitor activity in the autumn to reach a high value at about the time of deepest dormancy. This high bud-ABA content had fallen by the time dormancy was broken in the field in early July, and also following cool storage.

ABA activity was not related to the capacity for rapid bud burst, being somewhat lower in Treatments 5, 6, 3, 1 than in Treatment 4, although the former all had delayed bud burst. A decrease in ABA-like activity does not necessarily result in a loss of dormancy (Treatment 5).

The loss of ABA activity with chilling and with the breaking of dormancy are not therefore shown to be related.

It is also of interest that bud-ABA activity was reduced by storage at either warm or cold temperatures, and indicates winter chilling is not an essential pre-requisite for a reduction in the ABA content of kiwifruit buds. This is similar to the result of Mielke and Dennis (1975b) who showed that temperature had no effect on the disappearance of ABA from dormant cherry buds.

Hence in kiwifruit buds ABA-like activity increased in the autumn with the development of bud dormancy, and activity declined during the winter to reach a lower level when the buds were capable of bursting. This is typical of many other plants. Mielke and Dennis (1975a) found the

autumn increase was not coincident with the onset of dormancy, but with the start of leaf abscission. The same may be true of kiwifruit where maximum-inhibitor content was found when most of the leaves were yellowing, and abscised relatively freely.

Bud-ABA activity was not reduced to a greater extent by increasing the degree of chilling, and rapid bud burst could occur where bud-ABA levels were still relatively high. The similarity of the effect also recorded by Mielke and Dennis (1975b) of a temperature of 24°C and 4°C equally reducing bud-ABA content, could suggest winter chilling does not promote bud burst by lowering ABA activity. But the complex nature of the effect of temperature in overcoming dormancy (Fig.31) makes more detailed study necessary to confirm the idea that a loss of ABA is not essential for the loss of dormancy. The detailed study of Wright (1975) indicates it would also be helpful in such an investigation to study the distribution of ABA within the bud, and the form in which it is present.

B. "Summer Dormancy"

The kiwifruit vine shows strong apical dominance, with the growing tip of a shoot preventing the growth of the lateral buds, which remain inhibited until the following spring when they burst to produce a flowering shoot. During the early summer severe defoliation of the actively growing shoot, or loss of the growing tip by decapitation or dieback - after a maleic hydrazide spray, results in the rapid growth of a number of axillary buds. As the summer advances, this tendency, as in other plants become less marked and finally no bud break follows decapitation. This can be observed in the field, but a similar effect was shown by defoliated single-node cuttings held at warm temperatures where buds collected in early December had a complete and rapid bud burst, and through the summer until mid April bud burst became slower. When material was collected in the autumn and winter almost complete bud burst could sometimes be induced, indicating low temperatures were not necessary for bud burst although they could increase it, and always hasten it. Hence the summer correlative inhibition of buds continued into the winter, without an obvious dramatic change indicating the so-called winter dormancy is simply an extension of summer correlative inhibition, and Tinklin and Schwabe (1970) suggested that summer and winter dormancy did not differ qualitatively. Since this pattern of increasing bud inhibition was found in detached material, it appears the inhibitory condition is residual in the plant shoot and it takes time for it to disappear - either by actual loss or being counteracted by some promotive influence.

From many studies of correlative inhibition, two main points are that in the promotion of bud burst, other factors being equal nearness to the roots confers some advantage, which appears to be hormonal (Smith and Wareing 1964a, 1964b), and that some other factor modifies the distribution or activity of this root factor throughout the plant shoots. Auxin has a primary role in correlative inhibition (Phillips 1975), and of all the other

growth regulators the cytokinins are the most active in antagonising or relieving the inhibitory effect of IAA or correlative inhibition (eg. Wickson and Thimann 1958), and cytokinins arise to a large extent in the root system. In the presence of auxin there appears to be effectively a deficiency of cytokinin at the lateral bud, and this is apparently brought about by the apex (or apically-applied IAA) having a controlling influence on cytokinin transport and metabolism (hence distribution and utilisation) in the stem (Woolley and Wareing 1973a). Sachs and Thimann (1967) however suggest that following a decline in auxin from the apex, the lateral buds initiate auxin production which then stimulates the buds to synthesise cytokinins.

The presence of leaf tissue - either on the intact plant, or on a single-node cutting - was found to inhibit bud burst during the summer. Hence defoliation of a stem cutting immediately after collection, or its subsequent loss through dessication or pruning promptly resulted in bud growth. During the summer the inhibitory factor continued to accumulate in the stem and/or bud, so that it took progressively longer to disappear at warm temperatures.

Using data in the tables presented in Section 3B one can indicate the relative effect of a leaf on bud burst (after 23 days) -

	December	January	February	April
+leaf	51%	25	11	0
-leaf	98	73	59	38

Hence a bud itself (in the absence of a leaf) becomes increasingly inhibited through the summer, on top of which a leaf always imposes a strong degree of inhibition of bud growth. In the early summer the shoot apex may be the dominant cause of bud inhibition (apical dominance), as was claimed by Tinklin and Schwabe (1970).

However the results of Cozens and Wilkinson (1966) show that in mid summer when the apex has its maximum inhibitory effect in the blackcurrant shoot, the effect of the apex is considerably less than that of the leaves. Apparently the leaves are acting either as a source of inhibitor production, or else cause a promotive influence not to exert its effect.

Tinklin and Schwabe (1970) suggested that an inhibitor was produced in the leaves, which then passed into the buds and bud scales until finally at the end of the summer the buds were dormant. Their inhibitor could have been ABA, and the inhibitory factor was also readily transported basipetally. Wright's (1975) work with blackcurrant suggested ABA has an important role in the induction of dormancy. In kiwifruit ABA applied to defoliated cuttings inhibited bud burst, and the levels of ABA-like activity in buds increased in the autumn (Fig. 42), hence it would be possible for ABA produced within a mature leaf to inhibit bud burst in the intact plant. There is limited evidence that ABA acts as a correlative inhibitor of bud growth in the pea (White and Mansfield 1977) and Xanthium (Tucker and Mansfield 1973), and it is possible the presence of auxin could also stimulate the local synthesis of ABA (Tucker 1977). Further work is required to determine the extent to which ABA produced by maturing leaves results in correlative inhibition, and what other growth regulators are also involved.

There is considerable evidence for a prime role of IAA in the correlative inhibition of lateral bud growth by the leading shoot, and possible mechanisms of this effect were considered above. It has also been suggested that in mid-summer it is auxin produced by the leaves that maintains the developing bud as a resting bud, since auxin prevents bud burst yet permits normal bud development in several crop plants (Fulford 1970). IAA applied to defoliated cuttings was strongly inhibitory of bud burst (Experiment 2

Section 3B). As is typical of auxin, in Experiment 3 the inhibitory factor was found to move in the stem tissue in a basipetal direction, so that its presumed accumulation inhibited bud burst, and its disappearance allowed bud burst. The same effect was recorded by Peterson and Fletcher (1975) who claimed their results with decapitated stem cuttings indicated a possible auxin involvement in bud inhibition. Over the period that leaves are producing auxin, leaf correlative bud inhibition could occur in a similar manner to that proposed for the maintenance of apical dominance.

The inhibition of bud burst in the presence of a leaf could be due to its continuing production of auxin, which inhibits bud growth directly, or indirectly by preventing the normal activity of a substance promoting bud burst. The existence of such promotive material was suggested in Experiment 1 (Section 3B) where in the presence of a leaf, a large proportion of buds burst when the cutting also had roots - particularly when the root system was relatively large and extensive. The promotive effect of a newly-established root system was not present in basal callus and any root primordia it might contain, but exogenous BAP was partially effective. The relative effectiveness of the roots may have been exaggerated since they would have been present from some time prior to the start of observation. The limited bud burst in the presence of a leaf, and with no exogenous cytokinin (Experiment 2, Section 3B) could reflect the presence of a residual promotive root factor(Woolley and Wareing 1972a).

Woolley and Wareing (1972c) found that when apical dominance was removed, the action of cytokinins was antagonised by IAA, and that BAP metabolism by stem tissue was different in the presence of IAA (Woolley and Wareing 1972a). When Kinetin -8^{14}C was applied to decapitated pea seedlings (Morris and Winfield 1972), ^{14}C accumulated in the internodes but not in the axillary buds if the plants were treated with IAA. In Experiment 4 although changes in

cytokinin activity were not great, it appeared that endogenous stem-cytokinin metabolism and activity was altered in the presence of elevated levels of auxin. It may then be that the changed higher tissue cytokinin following decapitation and defoliation is related to the subsequent bud burst, and that IAA level is important in initiating such a change.

In the presence of IAA, cytokinin activity in the combined stem/bud sample was reduced over the 72 hours following decapitation. Woolley and Wareing (1972a) found reduced radioactivity form ^{14}C-BAP 12 hours after rootless cuttings were treated with IAA - but not after 24 hours. In the absence of IAA the surge of cytokinin-like activity at only 42 hours in kiwifruit, is similar to the occurrence of relatively higher level of cytokinin metabolism 12 hours after treatment but not after 24 hours in the potato (Woolley and Wareing 1972a), and could suggest any causal changes in endogenous hormones are of relatively short duration, and hence a need for close-interval sampling in this work. It has similarly been noted that partial root pruning of seedlings increased xylem-sap cytokinin activity after 24 hours but not after 48 hours (Carlson and Larson 1977).

It was because of the effectiveness of exogenous cytokinin that it was initially used in Experiment 2 (Section 3B), but it was then found that in both summer and winter (December, January, February, June, July) BAP inhibited bud burst in both the presence and absence of leaf tissue.

When cuttings were collected on 25/8/75 immediately before bud burst in the field (Experiment 4, Section 1B) a high concentration of BAP (50ppm) either alone, or in combination with GA_3 did not delay bud movement, or reduce the level of bud burst. The reason for this conflict in results is not known. Initially cytokinins have delayed

bud growth, although subsequently such buds have made strong shoot growth (Woolley and Wareing 1972a). They also found that under some conditions of handling their stem cuttings, BAP reduced the percentage bud burst and slowed their growth, hence the variable results obtained here may reflect subtle differences in the conditions of the cuttings used at different times.

Part 2.

INTRODUCTION.

JUVENILITY.

There is now some evidence that shoot juvenility is intimately linked to root activity and hormones produced in the roots, hence a limited study of juvenility appeared a useful extension of work on the kiwifruit, although the species chosen for this investigation was the blackcurrant (*Ribes nigrum*).

Flowering in many higher plants is preceded by a period of juvenility, which can be defined as the period from seed germination during which no flower initiation can take place under conditions that are favourable during a later stage. This topic has been reviewed many times (see Zimmerman, 1972), and been the subject of Symposia (e.g. ISHS 1976), but it is still a phenomenon we neither fully understand nor can control. A range of characteristics are associated with the juvenile state, and they have been studied in a number of different plants, together with the changes occurring at the time of 'phase-change' or conversion to the adult form, at which stage the plant achieves the capacity to become sexually reproductive. While it may often appear clear what juvenility is, and when the sudden switch to the adult stage has occurred, Borchert (1976) considered juvenility a vague concept, and doubted the existence of one uniform juvenile state.

While in nature the juvenile period of some species lasts for many years, Higazy (1962) working with herbaceous species found no fixed juvenile phase exists i.e. it can be modified, and similarly in woody plants, it was found that growing them continuously under long days

reduced the length of the juvenile phase so that flowering occurred sooner (Robinson and Wareing, 1969).

It has often been noted that the transition from juvenile to adult takes place when the seedling has reached a certain minimum size, so that size would seem important to predispose the plant to respond to inductive conditions. Blackcurrant seedlings will initiate flower buds after short-day treatment, but only if they have previously attained a certain minimum stem height (Robinson and Wareing, 1969). A similar situation is shown on individual shoots of established plants growing in the field. Blackcurrant shoots with fewer than 20 nodes fail to initiate flowers in short-days (Tinklin et al. 1970), and a similar apparent size requirement is shown by the shoots of the raspberry (Williams, 1960) and the bines of the hop (Thomas and Schwabe 1969).

In the raspberry and blackcurrant, in response to inductive conditions, flower initiation first occurs in buds at some position below the terminal shoot bud, and initiation then proceeds in both a downwards and upwards direction to all except the lowest few buds (Keep 1961, Tinklin et al. 1970). In the kiwifruit if the most basal buds of a one year old shoot grow out they are usually not floral, but strongly vegetative. In these plants it is these lower buds growing away in the following season that produce a shoot with a juvenile-like condition, and this pattern seems characteristic of phase change - the transition occurs in the distal parts of the branches, and the basal parts remain juvenile. This phenomenon suggested to Robinson and Wareing (1969) that phase change is something that occurs in the extending meristematic tip of the shoot, and not the whole of the stem, and led them to study the role of the apex. They concluded the attainment of a certain size is correlated with other changes which are more directly responsible for the phase change, and that in this, the number of cell divisions undergone by the shoot apical meristem is important. Hence the meristem

appeared as an autonomous unit in which the phase change initial event occurred, seperate from conditions within the differentiated parts of the plant. There appears to be intrinsic differences between the meristematic cells of the apices of juvenile and adult shoots (Wareing and Frydman 1976), and also indications that the size and shape of the apex may be involved in the transition to the mature condition (Stein and Fosket 1969, Thomas and Schwabe 1970). Generally, grafting a juvenile scion on to an adult tree does not hasten the maturation of the scion - which is to be expected if it is the meristem that is important (Hackett, 1976). How phase change comes about, or how to specify it in terms of gene activity is very unclear, and has been discussed by Sussex (1976) who reminded us that two plant hormones (GA and ABA) are candidates as the activators of the genetic mechanism.

Another aspect of juvenility is that its disappearance occurs following a period of growth which has led to a plant system of increased size and complexity, with progressively larger distances occurring between the growing shoot meristems, and the root system - and this is considered by Borchert (1976) to be important in explaining the eventual loss of juvenile characters, and caused him to doubt that there is one uniform juvenile state.

Working mainly with ivy, Wareing and Frydman (1976) believed the juvenile condition is promoted and maintained by the occurrence of relatively high levels of endogenous gibberellins in the shoot apices, possibly arising from the proximity of the shoot apices to the roots. Early workers suggested an involvement of hormones in sexual maturity in plants (see Higazy 1962), and in ivy the juvenile condition has been related to GA level (Frydman and Wareing 1973a, Hess 1964). The work of Frydman and Wareing (1973b) indicated the roots were important in providing the high apical GA levels associated with the juvenile condition, and Schwabe (1976) also believes shoot juvenility is linked to root proximity and GA-like factors produced there.

The stability of rooted cuttings of the adult phase of ivy, in which the shoot meristem is very close to the roots, is presumably due to the maturation changes having permanently affected cell metabolism and need not conflict with the hypothesis of Wareing and Frydman (1976).

The first appearance of flowers cannot be taken to indicate when the juvenile phase ends as there may be an intervening period when some limitation to initiation still remains. This period has been called a transition period by Zimmerman (1973), and during this period only a small interference with, or suppression of, plant growth may be adequate to induce flowering.

It is often found that young plants vegetatively-propagated from mature trees do not flower, and I have already alluded to the blackcurrant in which aerial shoots on mature plants fail, for a time, to initiate flower buds. These observations present a situation very similar to juvenility. However, Zimmerman (1973) has suggested such plants/shoots are temporarily in the invigorated transition stage, and before they can respond to inductive conditions, they must have gone through the process of ageing. This is the loss of vigour usually associated with plant development, and which progressively facilitates flower induction - it does not cause phase change changes, but occurs in parallel with them (Wareing and Frydman 1976). Robinson and Wareing (1969) showed how flowering in an adult scion may be prevented in a vigorously-growing system, and ageing, not phase change, is required. A reduction in shoot growth rate is commonly associated with the beginning of flower initiation in woody plants, including the blackcurrant (Tinklin et al. 1970). Sachs (1977) proposes that a critical part of the shoot apical meristem is relatively deprived of nutrients during vegetative development, and it is only when an increased availability of assimilates to the meristem central zone occurs, that flower initiation can occur. This effect may be seen in blackcurrant, where shoot growth rate drops, and then rapid axillary bud growth

and flower initiation occur (Nasr and Wareing 1961a). In addition to such a nutrient diversion theory of flowering in adult plants, Hackett (1976) concluded it is also likely to be important in accounting for phase change.

The blackcurrant plant was seen to offer an opportunity to study a juvenile-like condition, and likely to provide some information on the way in which size could affect the physiological process of attaining the ability to flower (Schwabe and Al-doori 1973). When apical soft wood cuttings of blackcurrant are rooted and grown on to various sizes before receiving short-day treatment, only the longer rooted shoots initiate flowers (Tinklin et al. 1970). Also when a shoot originates near ground level on established blackcurrant plants or from plants mown at ground level, that shoot will not initiate flower buds if it is below a minimum size - although a short shoot arising from a point high in a bush will flower the following year (Wilkinson pers. comm.). Hence in this plant, shoots arising from established bushes, or plants grown from rooted cuttings from older stock plants fail to flower following inductive conditions until after they have made a certain minimum amount of growth. This size effect is very similar to that of juvenility. Since juvenility is a term used to describe the stage during which it is not possible to induce flowering in seedling plants, the condition described above in the blackcurrant is better referred to as a juvenile-like condition.

In a similar way to work on juvenility already discussed, it was found that in clonally-propagated blackcurrant material the proximity of the shoot tip to the roots controlled whether flowering could be induced, and that the root effect could be traced to gibberellin-like activity coming from the roots (Schwabe and Al-doori 1973).

Such an integration of the shoots and the roots of a plant is not unreasonable, and it had been demonstrated

in several plants that the roots are important in maintaining the level of GA-like substances in the shoot - substances which are strongly inhibitory to flowering in many plants. The gibberellins are considered to be the main inhibitors of flower induction in fruit trees (Luckwill 1970), exogenous GA_3 prevents flowering in mature blackcurrant shoots (Schwabe and Al-doori 1973), and the gibberellins are important in phase change in ivy (Frydman and Wareing 1973a). In contrast, in a number of conifer species the gibberellins will induce precocious flowering (Pharis and Ross 1976).

Root-GA levels do not seem to be the only factor affecting initiation as the juvenile-like condition of short shoots is not removed by treatment with a growth retardant or loss of their roots (Schwabe and Al-doori 1973), and similarly Wareing and Frydman (1976) concluded that for phase change low GA-levels are necessary but not sufficient. Fulford (1970) suggested that in the autonomous axillary meristem the onset of the maturation phase depends on the balance of endogenous gibberellins and auxins.

Clearly there are similarities between juvenility, and the juvenile-like condition studied by Schwabe and Al-doori (1973), although it has not been shown that they are identical, and one could enquire whether in the latter instance the problem is one of invigorated transition-phase growth and not one of juvenility. If that is the case, then it is ageing and not maturation that is required. Fulford (1970) found flowering was prevented in the lower parts of the main limbs of off-year apple trees by correlative inhibition between vegetative growth and bud development; Nasr and Wareing (1961) put forward the idea that in clonally-produced blackcurrant, correlative inhibition prevented flower initiation in the early part of the growth cycle. Generally grafting a juvenile scion on to an adult tree does not affect maturation of the scion, yet Schwabe and Al-doori (1973) found that such a graft combination

with vegetatively-propagated material strongly promoted flowering. This is similar to observations made with larch (Robinson and Wareing 1969), and one could similarly conclude the graft-induced change in the shoot system was one of ageing and not maturation - i.e. the scion never was truly juvenile. It should also be noted that the work showing a decreasing gradient in GA-activity down a blackcurrant stem (Schwabe and Al-doori 1973), and used to support a role for root-GA's in maintaining the juvenile state, was not done with plants showing the typical juvenile/adult phase change. Such a gradient may be significant for phase-change if it is also found to occur in woody plants grown from seed and showing the typical juvenile/adult transition.

Thus a number of correlations have been found with phase change, and they have at times been considered to reflect causal relationships. Hence the correlated increase in physiological age, and ageing are not now considered essential, and increasing plant size itself is not now seen as important.

The properties of the shoot apex could be determined by influences from more remote parts of the plant, and there is evidence of hormonal influences from the roots maintaining the juvenile state. For instance in blackcurrant, failure to respond to short-days is due to the proximity of the roots to the growing points, and the operative activity of the roots depends almost entirely on their gibberellin production (Schwabe 1976); and in ivy apical buds of juvenile shoots contain higher gibberellin levels than adult shoot tips (Frydman and Wareing 1973a). Hence high GA levels in the shoot apex appear to be required to maintain the juvenile condition, and when the GA level falls the change to the adult can occur.

But distant hormonal influences may not be the only factor involved as phase change appears to result in a more stable adult state than changes in hormonal level would

seem to allow. Wareing and Frydman (1976) believe, on good evidence, that phase change is accompanied by changes in the meristematic cells, so that the adult and juvenile meristematic cells have intrinsic differences, and study of the apex itself becomes important. Once the stable adult condition has arisen it is not directly controlled by prevailing GA levels, and hence meaningful changes in GA level may be relatively short-lived.

Sussex (1976) indicates that at phase change there must be the selective activation of specific genes, which need not occur simultaneously - for it would be possible for a developmentally-linked series of genes to be co-ordinately activated. Wareing (1976) also indicates phase change may involve differences in the rate of transcription of specific genes, and that some DNA sequences transcribed in the adult phase are not active in the juvenile phase. Plant hormones could be the activators of this genetic mechanism and so factors such as the environment, plant size, and distant plant parts could affect maturation. ABA can block gene activation, and the gibberellins which have appeared so important in relation to juvenility can lead to the initiation of gene activity (Sussex 1976).

EXPERIMENTAL

This work was carried out at Wye College, Kent, in the United Kingdom in 1974/75, mainly using the black-currant variety Wellington XXX which has been intensively studied in relation to flower initiation. Established plants in the field were available, and they had been cut back close to ground level in the previous winter to provide strong shoot growth that typically displays the juvenile-like condition. As required, shoot tips were harvested from outdoor plants, or those maintained under continuous light in the glasshouse, and rooted under mist to provide plant material for growing in pots in the glasshouse.

Short-day treatments were provided by covering the plants with a double layer of thick black plastic, and flowering response subsequently determined after defoliation stimulated the growth of the lateral buds. Plant samples were analyzed for activity of gibberellin-like substances, by bioassay.

When shoots of the current season's wood were harvested for analysis they divided into four parts: an apical sample of 3cm stem and its leaves, and the balance of the stem tissue which was divided into three equal lengths. Field samples were first collected in mid June 1974, when each shoot had 16 - 21 visible internodes.

Extraction Methods

Initially plant samples were harvested in the morning, and sealed in a plastic bag in a deep freeze at $-15^{\circ}C$ until weighed and samples ground in an Atomiser with cold 80% methanol, in which they were extracted for 48 hours. After removing the methanol and partitioning with petroleum ether (at pH 8), the acidified (pH 2.6) aqueous phase

was extracted with ethyl acetate. All solvents were re-distilled before use.

Determinations of GA-like activity were made using the basal stem section, as this sample was expected to have the highest activity (Schwabe and Al-doori 1973). Purification by solvent partitioning and paper chromatography (Whatman No.42) with isopropanol-ammonia-water (10/1/4) did not result in any detectable activity in the GA_3 position, and at most Rf positions there was severe inhibition of seedling root growth in the lettuce hypocotyl bioassay. Similarly when this sample was purified and chromatographed using the method of Schwabe and Al-doori (1973) there was marked inhibition of hypocotyl growth. and the low level of GA-like activity that occurred was at an early Rf.

As these ethyl acetate extracts of woody tissues were very impure, further purification with PVP (Polyclar AT) was investigated. PVP was packed into a column, and then the extract eluted through it with phosphate buffer. However, the recovery of GA_3 from the column was very low unless the eluate was chromatographed before bioassay - a difference that was absent if acid-washed PVP was used. Hence, for all further work the Polyclar AT was boiled for 8 minutes in 10% HCl, and washed well with glass distilled water (Loomis et al. 1966) to remove the fines and all chloride, and stored in the refrigerator in phosphate buffer. When the above acetate extract was passed through the PVP column before chromatography and bioassay, the stem-base sample was active at the GA_3 position. Hence purification with PVP was used for all future samples as it was essential to the demonstration of GA-like activity, and reduced the severity of the damage to the seedling roots.

When required, a slurry at room temperature was settled into a column 15 - 1.9cms, and the sample applied to the top in 2ml of buffer, and eluted with 0.1 M phos-

phate buffer pH 8.0. The column eluate was adjusted to pH 2.5 and partitioned into ethyl acetate which was dried down for chromatography - for the later samples diethyl ether replaced ethyl acetate. Chromatography was usually on a thin-layer plate of Merck silica gel H (0.25mm) that had been pre-run in the solvent system used.

It was also found that when the column eluate was collected in two fractions, more inhibitory substances were present in the first fraction (F1) 0-60cc, than in F2 60-260cc. (Figs 48, 49). All plant samples were separated into these two fractions and bioassayed separately and indicated this effect - possibly reflecting the early elution of ABA from this type of column, and with only one exception any significant GA-like activity detected occurred in F2. The presence of inhibitory substances and pigments in this second fraction still remained something of a problem, and as chromatography on a thin-layer plate with 8/1/1 EBM (diethyl ether-benzene-methanol) offered a partial solution, it was used in all the main GA-determinations. A completely satisfactory separation of GA-activity and inhibitors was not achieved by any of the following methods:

(a) two other chromatography solvent systems, (di-isopropyl ether- acetic acid 95/5; isopropanol - ammonia - water 10/1/1).
(b) eluting the TLC with water before bioassay (Eeuwens 1973).
(c) including 5ppm kinetin in each bioassay solution.

Changing the initial sample purification method gave some reduction in the quantity of inhibitory substances, and using the modified extraction method with different blackcurrant tissues, followed by TLC with 8/1/1 EBM, the inhibitory substances ran very close to the solvent front - at which position they interacted strongly with exogenous GA_3. The blackcurrant gibberellin activity occurred in the region of Rf 0.5 - 0.8 where any inhibitory substances present had slight or nil interaction with

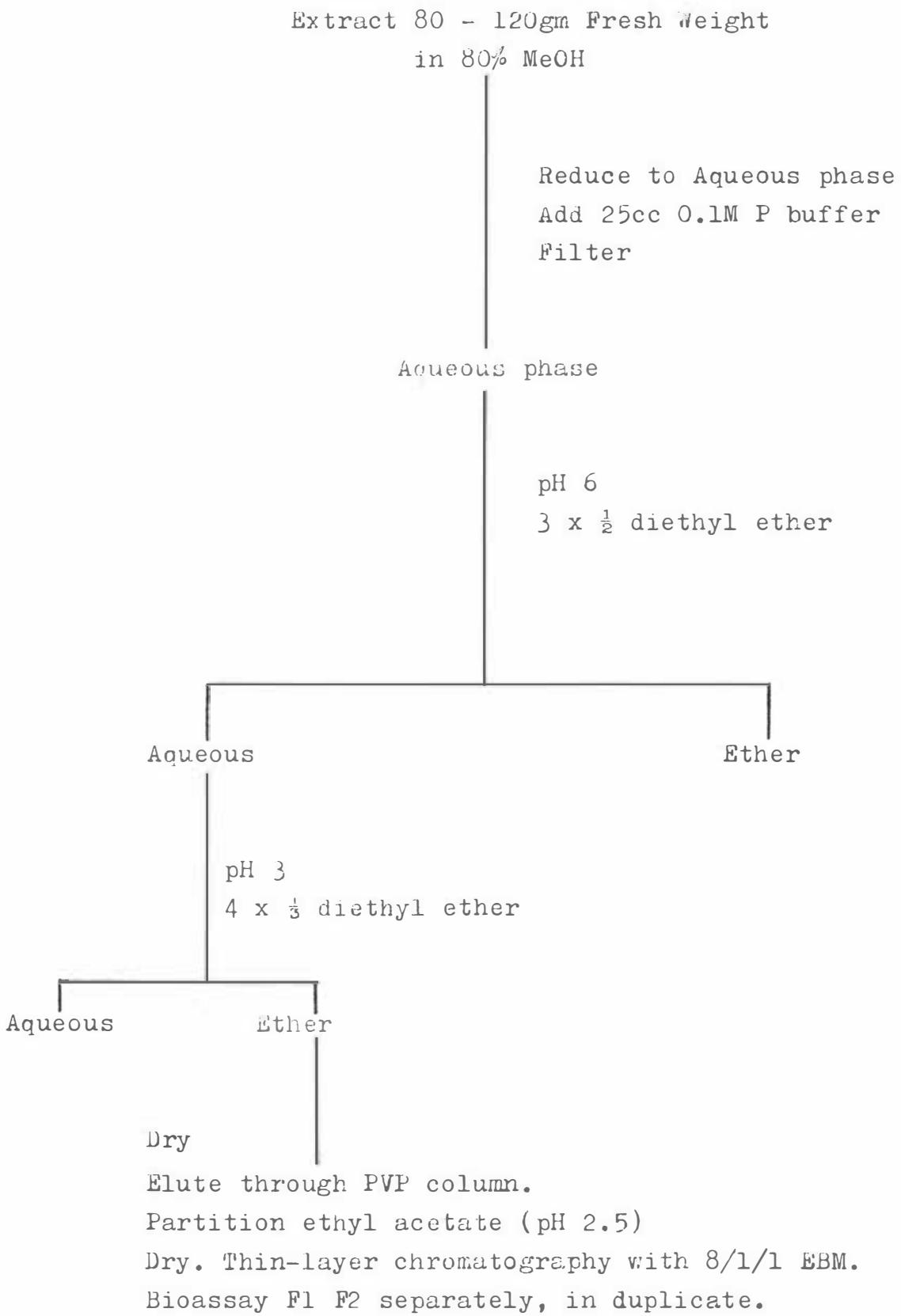


Fig. 44 Purification of blackcurrant tissue for gibberellin determination.

exogenous GA_3. The purification methods used for the plant tissues are shown in Fig. 44, and are based on the method of Goldschmidt and Monselise (1968).

When 5µg GA_3 was eluted through a PVP column, and various eluate fractions bioassayed after partitioning into ethyl acetate, activity was detected only in the first 60cc after adding the sample - no activity was present in the next 150cc. Subsequently stem tissue with exogenous gibberellins was extracted and bioassayed following the usual procedure. It was found that the greater proportion of the GA_3 and inhibitory substances recovered was in F1 i.e.the first 60cc of eluate, and $GA_{4/7}$ was mainly in the next 200cc (F2) Fig. 45).

In all cases unless stated, the results refer to Fraction 2 (F2) from the PVP column, it having been found in each case that F1 was inactive in the bioassay for gibberellin-like substances.

Bioassay

After drying, the plate was divided into ten strips which were scraped into plastic Repli-dishes for bioassaying individually using the lettuce hypocotyl bioassay (Frankland and Wareing 1960). To each was added 0.6cc distilled water or GA_3 standard, and eight germinated "Arctic King" lettuce seeds.

They were incubated with high humidity under fluorescent lights at 22°C for about four days when the length of the hypocotyl was measured. Significance levels were again calculated by the method of Link and Wallace (1952), and are shown on the histograms.

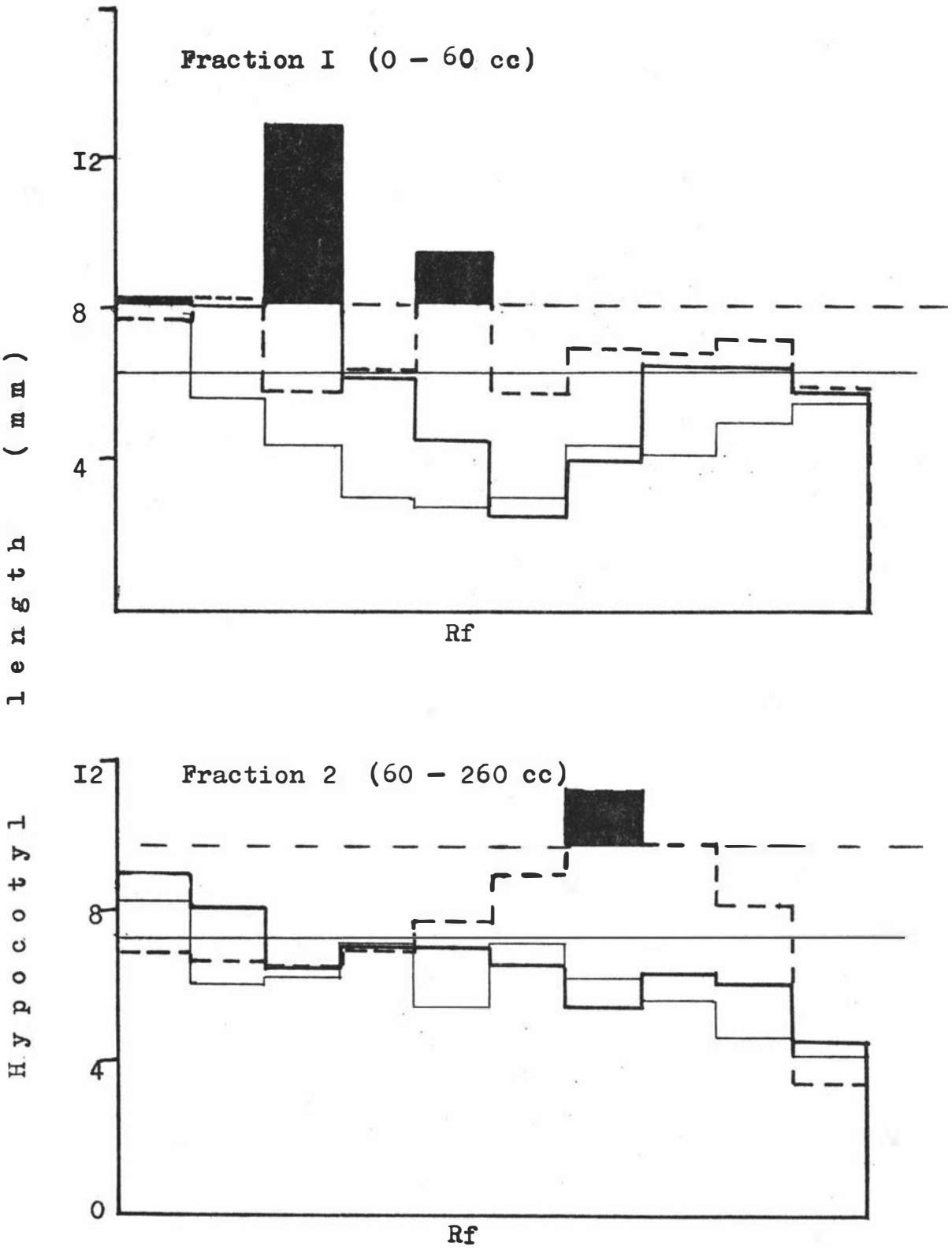


FIG. 45. BIOASSAY OF 25gm. STEM TISSUE ±GA AFTER EXTRACTION AND CHROMATOGRAPHY WITH 8/I/I EBM.

——— Control ——— $+GA_3$ — — — $+GA_{4+7}$
——— Blank — — — significance (P 0.0I)

Results:

(a) 'Adult' Plants

(i) Outdoor Plants

The current season's shoots of severely-pruned Wellington XXX were harvested on 11/6/74 and 10/7/74. At this time harvested shoots had 16 - 21 and 22 - 25 nodes respectively, and when bud burst was induced in the glass-house, it was shown no flowers had been initiated at the latter harvest date.

Bioassay results with two different solvent systems showed the presence of GA-like activity in all parts of the stem, with a gradient from the highest concentration in the basal 33% length to the lowest concentration in the upper one-third length (Fig. 46.).

Usually no activity was found in the upper 3cm stem and its apical leaves when harvested in June or July, nor in mature, fully-expanded mid-shoot leaves in July. In one sample there was a similar very low level of activity in each type of leaf.

On the 10 July current season's shoot tissue was collected from established fruiting bushes on a nearby fruit farm. Strong shoots with about 20 nodes, and originating near the base of the plants were selected, and the upper and lower one-third's used for analysis. It was found the GA-like activity was almost the same in both parts of these shoots (Fig. 47), the gradient having almost disappeared due to the relatively high activity in the upper part of the shoot. Although it was not determined, it was expected these shoots were of a size that would permit them to become floral in spite of their basal origin (Wilkinson per. comm.).

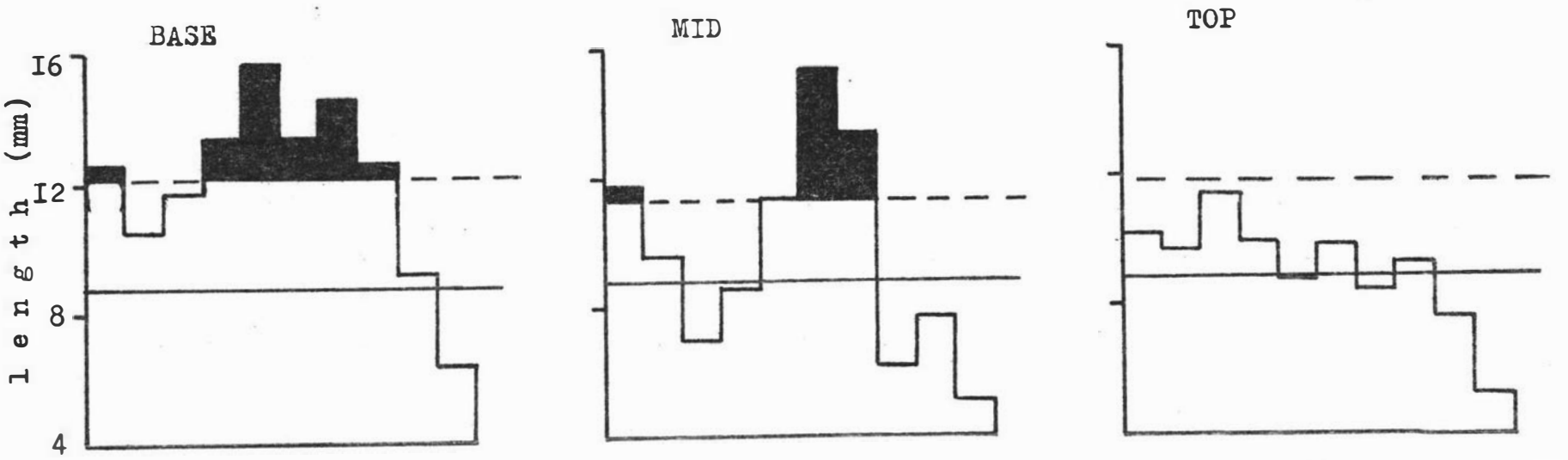


FIG. 46. HISTOGRAMS OF BIOASSAYS OF GA- LIKE SUBSTANCES IN ADULT BLACKCURRANT STEM. (30gm.)

——— Control level, — — — — significance level (P 0.05)

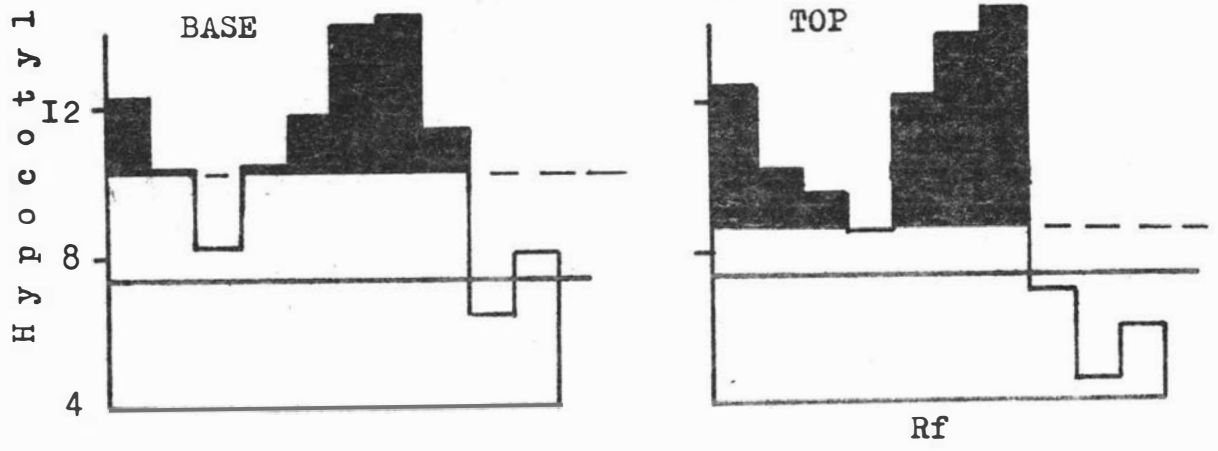


FIG. 47. HISTOGRAMS OF BIOASSAYS OF GA- LIKE SUBSTANCES IN ADULT STEMS FROM FRUITING BUSHES.
(2x 45gm.)

——— Control level,
— — — significance (P 0.0I)

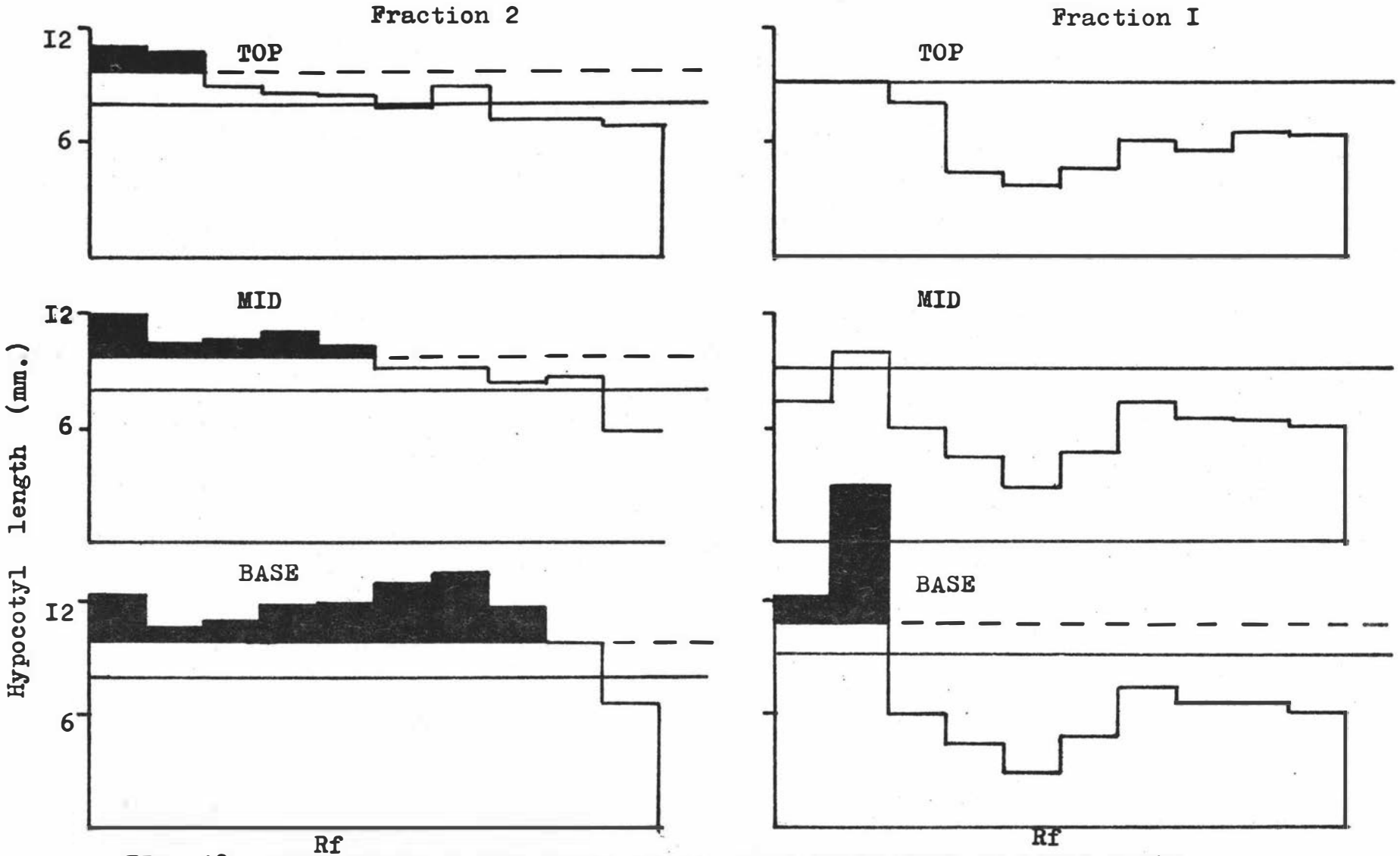


FIG. 48. HISTOGRAMS OF BIOASSAYS OF GA- LIKE SUBSTANCES IN ADULT STEMS.

Two replicates FI F2 50gm.

——— Control level, — — → significance (P 0.05)

(ii) Glasshouse Plants

Stem samples were harvested in the usual manner from Wellington XXX plants grown from tip cuttings, and grown in pots as single-stem plants in the glasshouse. Natural daylight was supplemented by 60w tungsten lights overnight, and material was harvested on 3 November when it had 23 - 30 nodes.

The histograms in Fig. 48 show the decreasing concentration of GA-like activity in the stem as it is sampled further from the root system, and the high level of activity compared with field-grown plants. Further more, this was the only sample in which F1 (ex PVP) contained significant activity - which appeared qualitatively different to the usual blackcurrant gibberellin. This latter effect may result from the inadequate separation of inhibitor and gibberellin, although in an earlier test an interaction did not occur over such a large part of the chromatogram.

(b) 'Juvenile' Plants

Plants similarly grown in the glasshouse were harvested when they had 8 - 12 nodes, to give a single stem sample - these plants were found not to initiate flowers when given a 6 week short-day treatment. One batch of these plants was grown in 'Durite' in order that root samples could easily be obtained.

Analysis showed that substances inhibitory in the bioassay were present, and significant GA-like activity was detected in some samples of stems and roots, but activity was absent from the apical leaves and older leaves (Fig. 49). Significant GA-like activity was detected in samples harvested on 18 June and 18 July but not 31 July, but the level of activity was low being of the same order as that found in the upper one-third of adult stems of the same variety.

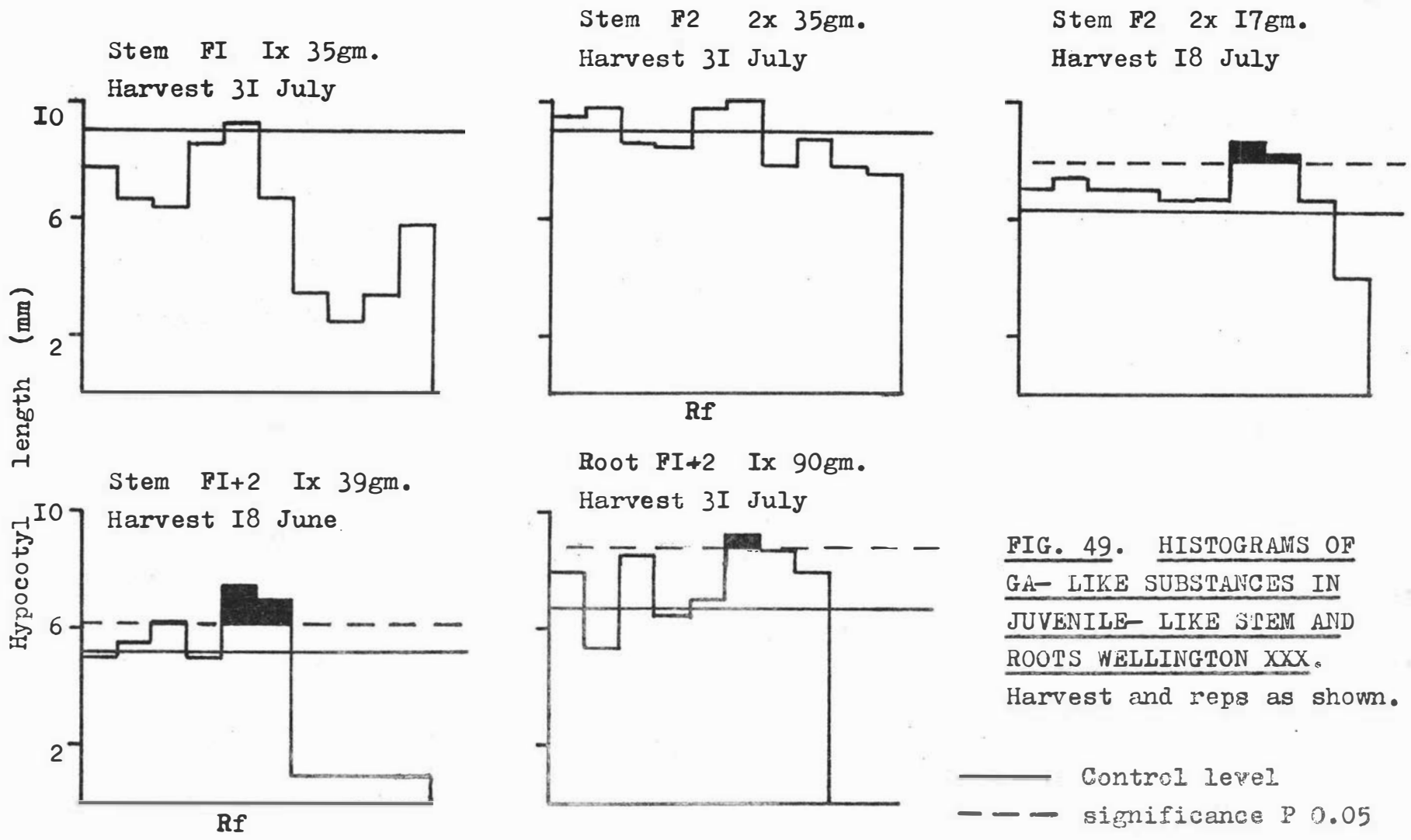


FIG. 49. HISTOGRAMS OF GA- LIKE SUBSTANCES IN JUVENILE- LIKE STEM AND ROOTS WELLINGTON XXX.
Harvest and reps as shown.

(c) Baldwin Seedlings

In contrast to the vegetatively-propagated material used in the above work, similar analyses were also carried out on seedlings of the variety Baldwin. Seed was germinated and the seedlings grown in containers of "Eff" potting compost in the glasshouse, under continuous light. They were separated into three similar groups, and each was harvested over 3 - 4 dates when the plants reached the desired size. Plants typical of each group were also given a 5 week short-day treatment, followed by defoliation, to assess its physiological state after almost every bud was induced to burst:

Plant Type	No.	Mean Ht (cms)	Mean No. Internodes	Flowering Response
Juvenile	36	40.5	16.6	Nil
Intermediate	31	79.8	26.6	Nil
Adult	32	115.0	38.0	5 plants

Duplicate tests of all parts of the stems of these plants at the three stages of development failed to show more than a trace of GA-like activity (Fig. 50), except for a trace of activity in mature leaves from the 'adult' plants. Significant activity was present in root extracts prepared from the 'adult' seedlings - other Baldwin samples were not analyzed. Root extract activity again occurred as two distinct peaks, and at a slightly higher concentration than in the roots of "juvenile" glasshouse-grown Wellington XXX plants.

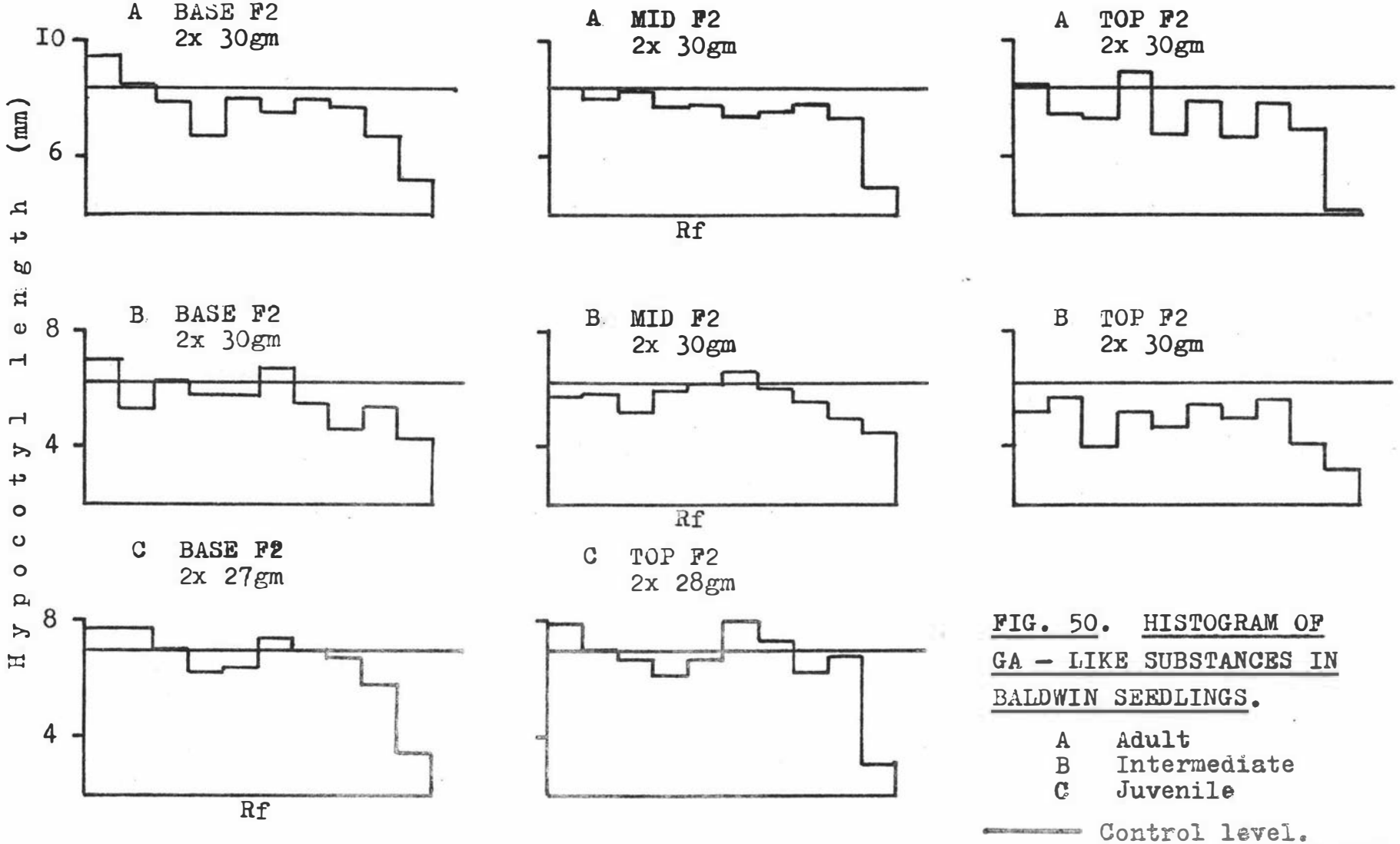


FIG. 50. HISTOGRAM OF GA – LIKE SUBSTANCES IN BALDWIN SEEDLINGS.

A Adult
B Intermediate
C Juvenile

Control level.
Samples NS (P 0.05)

DISCUSSION

Gibberellin-like activity was found in tissue extracts, particularly of stem tissue, but this was only possible with a greater degree of purification than that used by Schwabe and Al-doori (1973). The presence of inhibitory substances, phytotoxic in the bioassay, appeared to be a far greater problem, although it is believed their effect on the results was minimized by the techniques used. However, it must be agreed they could have had some effect on the bioassay results.

With the exception of the one sample that contained unusually high GA-like activity, the blackcurrant gibberellin came off the PVP column only in the second fraction. By comparison with gibberellin standards the endogenous-GA behaved more like a GA_{4+7} mixture than GA_3 which was eluted mainly in the first fraction. In a number of extractions of stem and root, both juvenile and adult, the bioassay clearly distinguished between two zones of activity, the faster-running one being the greater. The slow-running peak shows similar chromatographic behaviour to GA_1, GA_3, and the other is as for GA's 4, 7, 9 (Eeuwens 1973). This suggests the gibberellins present in the stem could have been transported there from the roots, as suggested by Schwabe and Al-doori (1973), and may indicate there has been no major change in the types of gibberellins in the change from 'juvenile' to adult - as in the ivy, where Frydman and Wareing (1973a) suggested the same or similar GA-like substances occurred in adult and juvenile tissue. No accurate quantitative estimation of endogenous GA was made as the levels detected showed some variation from extract to extract, and levels were too low for the preparation of a dose-response curve.

Total GA-like activity in basal 'adult' stem material was equivalent to 2.6 - 10 μg GA_3/Kg fresh weight, and

lower in the upper part by a factor of about eight - with an intermediate value in the middle one-third of the stem. By contrast, in a single test of similar stem tissue taken from fruiting bushes, a similar level of activity was found in the top and the base of the shoots. While it was expected both types of tissue responded in the same way to inductive conditions, they differed in the distribution of GA-like activity. Using glasshouse-grown 'adult' material, activity of 22 µg/Kg was found in the shoot base, and in 'juvenile' plants, levels of up to about 0.5 µg/Kg in both the stem and roots.

In long shoots from heavily-pruned bushes harvested in June/July i.e. immediately preceding flower induction, GA-like activity was consistently higher nearer the stem base - as already recorded by Schwabe and Al-doori (1973) Activity was greater in material grown under greenhouse conditions. In such long shoots, short-day treatment caused flowers to develop in the upper five lateral buds in both Wellington XXX and Baldwin - in contrast to the evidence that in the former, initiation begins near the 20th bud below the tip (Tinklin et al. 1970). Hence the results appear to support the proposition that a bud's nearness to the roots and the higher gibberellin concentrations there discourages flower initiation, and that this explains the minimum-size requirement for the end of juvenility. Similarly, it is not surprising that while in a single-shoot plant at least 12 nodes must be present in order for it to be induced to flower, in a fruiting bush shoots of 8 - 11 nodes that originate high in the bush well away from the root system, produce flowers at almost every node. However, flowering in the latter shoot may simply reflect its origins from a mature adult meristem.

The high GA-like activity in the upper parts of long shoots from normal fruit-bearing bushes may suggest a greatly diminished upward movement of root-GA's is not critical for the maturation type of change to the 'adult' form in this juvenility-like behaviour of blackcurrant.

Alternatively shoots on fruiting bushes do not exhibit the same minimum node number requirement of heavily-pruned bushes - this point has not been resolved.

However, short juvenile-like shoots and their roots were found to have low GA-like activity - similar to that in the top of 'adult' shoots. This is in marked contrast to the results of Schwabe and Al-doori (1973), and does not indicate a likely role of high root and stem GA levels causing the juvenile-like behaviour of short shoots. The inhibitory substances present in all the plant extracts were found to antagonise the effect of gibberellin in the bioassay, and they could then reduce the apparent GA activity in the sample. While this effect could mean the GA activity of the juvenile plant samples was grossly underestimated, it is believed the inhibitor effect was not so marked. This is because most of the inhibitors were removed in Fraction 1 off the PVP column (and black-currant-gibberellin rarely occurred in F1), the chromato-gram solvent gave reasonable separation of inhibitor and gibberellin-like activity, and in the region of the black-currant gibberellin and GA_3 on the chromatogram there was only a slight interaction with inhibitors in the plant extract. So it is considered the bioassay results give a reasonable indication of the level of GA-like activity present in the various plant tissues. The absence of leaf and apical GA activity, and the similarity of the chromatographic behaviour of the stem and root GA's suggests the stem activity could have originated in the root system.

In the blackcurrant, the 'juvenility' of short shoots is not removed by CCC or ABA treatment, or loss of their roots (Al-doori 1972), which could also suggest that root-GA level is not a major factor affecting initiation in these shoots. It is, therefore, very possible that in this plant system, low GA levels are necessary but not sufficient for the ability to flower, which is the general view of phase change of Wareing and Frydman (1976) i.e. there is far more

to attaining the ability to initiate flowers, than simply a reduction in GA concentration in the stem tip. The growth hormone studies made here involved the determination of pool size, but other hormone studies may have been more meaningful since pool size is often poorly correlated with a specific physiological process. A clearer explanation of the effect of leaf and root-GAs on flower initiation would undoubtedly result from studies on GA metabolism, and tissue sensitivity, since they are so important in understanding hormone effects (Dennis 1977).

The results obtained with Baldwin seedlings of different ages, with negligible GA levels at all stages also suggests the inaccuracy of claiming low GA-like activity as the cause of gaining the ability to flower. Because these plants exhibited true juvenility, their contrasting results is strong evidence that the work by Schwabe and Al-doori (1973) with vegetatively-propagated plants cannot be taken as a reliable guide to the mechanism of phase change. This makes the flowering response of vegetatively-propagated plants a problem different from that of juvenility, as claimed by Zimmerman (1973). Further support for this comes from the conclusion of Schwabe and Al-doori (1973) that the 'juvenile-like' condition is not an inherent property of the bud or shoot; whereas in true juvenility it apparently is, phase change occurring in the apex and hence flowering in the later lateral buds (Robinson and Wareing 1969).

Fulford (1970) has considered the importance of correlative inhibition in preventing flower initiation, and stressed the likely importance of high auxin activity in delaying bud maturation. Wareing (1959) discussed the importance of a loss of vigour before a plant responds to inductive conditions. In an invigorated growth state drastic changes in growth must precede flower initiation, and in the blackcurrant it is only after shoot growth rate drops that flower initiation begins and a period of rapid growth of axillary buds occurs (Nasr and Wareing 1961a).

Hence release from correlative inhibition appears important to permit buds to initiate flowers, and could well be a major factor in the eventual response to inductive conditions of vegetatively-propagated plants. Wardell (1976) suggested that changes in auxin level in shoot apices might produce quantitative shifts in DNA which then changed the developmental capabilities of the shoot apices. Hence increased auxin levels would reduce the tendency to flower, and decreasing auxin would increase the tendency to flower and reduce vegetative growth. In studying this non-juvenile condition, perhaps the role of the apex and correlative inhibition should receive no less attention than the nearness of the root system, and the gibberellins produced there.

FINAL DISCUSSION

In many plants control over stem growth or plant development is desirable, and this is also true of the vigorous kiwifruit vine. But in order to consider alternative methods of growth control it is necessary to understand the mechanism of growth control within the plant, and the way in which external factors modify growth and development.

A study of the literature indicates that the leaves, growth regulators, carbohydrates, and the roots are all involved in the control of growth, although there is some uncertainty about the actual role of each, and the various inter relationships that might exist.

This study considered neither the involvement of growth regulators in the production of determinate as distinct from indeterminate shoots - the proportions of which vary with the clone, nor their involvement in apical bud abortion - a characteristic of kiwifruit shoot growth. There is evidence the gibberellins may be involved in these phenomena in plants, and in this study the gibberellins were found to be active in the control of stem elongation.

The function of growth regulators, especially the gibberellins appears to be intimately associated with the distribution and utilisation of carbohydrates. For instance it was found (a) stem growth was directly related to stem cutting size, and that this growth resulted in about a 50% reduction in the starch reserves.

(b) growth stimulation by GA was associated with a re-distribution of carbohydrate, and that after a certain level of growth the response ceased.

(c) a leaf had its greatest effect on

internode elongation when the leaf was very young. At that time one would expect its growth regulator activity to be greatest, and this is also the time when a leaf's import of carbohydrate is very high (Thrower 1962).

Further support is found in the results of other studies: (a) GA_3 reduces starch in proportion to the amount of extension growth induced (Nanda and Purohit 1965).

(b) A GA_3 spray to part of a plant causes a marked transfer of assimilate from the unsprayed part (Quinlan and Weaver 1970).

(c) Hoad and Monselise (1976) proposed that reduced shoot growth from SADH is a result of a reduction in GA-induced translocation to that area.

(d) Grochowska (1973) indicated that high starch content in a plant is an indicator of low auxin and GA in the tissues.

(e) Luis and Guardiola (1974) believed the increased transport of reserves caused by GA resulted from GA increasing the capacity of the shoot to act as a sink for nutrients.

and (f) When exogenous GA increases shoot elongation, either a plant's shoot weight is not increased (Powell et al. 1959), or the increased top weight is associated with a reduction in root dry weight (Brian 1959).

Thus stem growth appears as being totally dependent on photosynthate supply - and this is mediated by the growth regulators. While GA had a strong effect in promoting stem growth in seedlings, and detached shoots, the requirement indicated in the literature for the presence of auxin for GA action, and for the release of starch reserves, was supported by the results here of a reduced GA-response if auxin was reduced. IAA did not increase growth of stem cuttings, but in the presence of PCIB there was less response to exogenous GA. No doubt carbohydrate mobilisation was affected, since an important role of auxin appears to be in initiating the degradation of stored carbohydrates - a process which is then enhanced by GA's (Saniewski and

Pieniazek 1972). Hence, while auxin did not stimulate growth of excised shoots, like cytokinin it is involved in the GA-induced growth response. Hence in growth control one is concerned with an interplay of different hormones, and an interplay of different parts of the plant. These two characteristics will be noticeable throughout this discussion.

Cytokinins are probably involved in growth control since the three hormone groups together most favour nutrient mobilisation. There is evidence root-cytokinins play a role in shoot growth by affecting GA turnover (Railton 1974), and the two together could form an effective growth control system. A low level of cytokinin is associated with poor plant growth (Reid and Railton 1974), and normal growth and development requires its presence also (Moraszczyk et al. 1974). In kiwifruit cuttings the GA-response was over within six weeks, and it resulted from a re-distribution of leaf dry weight to new stem tissue. In the absence of roots (and hence cytokinins?) no new leaf primordia were produced, and to this extent the GA-induced growth was not normal. Exogenous cytokinin was found to modify the GA-response, and this could reflect the situation in the intact plant.

It appears that GA interacts with auxin to mobilise carbohydrate, and that the roots supply factors (possibly including gibberellins) that maintain adequate GA levels for continued growth, although young leaves are intimately involved in the control system for internode growth, probably via GA metabolism, and hence photosynthate transport. In detached stems the amount of carbohydrate available for translocation limits the level of response to GA. Cytokinins too are involved and the roots could also be a source of supply of this growth factor.

In the kiwifruit plant it was found that removing an apical leaf had a large effect in reducing internode

length - acting particularly in an acropetal direction. This promotive leaf effect was apparently reduced by the presence of the shoot apex, since decapitation finally (but not initially) increased the growth of the upper internodes - however this may merely have been an expression of compensatory growth. The stem-growth promoting influence of a leaf was therefore localised, and possibly reflects a localised deficiency of assimilates due to a local deficiency of growth regulators. Shein and Jackson (1972) have similarly suggested the importance of local hormone balance in determining stem growth.

Stem growth was affected by the apex and by the leaves (especially young apical leaves), although severe defoliation was shown to have a more far-reaching effect. The youngest internodes were the ones most affected by removal of their subtending leaf, and it was also the youngest internodes that responded most to exogenous GA. The evidence of the literature is that growth regulator and photosynthate availability is affected by the presence/absence of leaves, and it is likely that in kiwifruit these factors also account for the leaf contribution to stem elongation. The reduction in internode growth from defoliation may be due to the removal of a local source of specific GA's, since GA frequently replaces the leaf-effect, and SADH reduces internode growth in the shoot tip by reducing apical - GA levels (Hoad and Monselise 1976). In practical terms, it means that to maximise the reduction in stem growth by any treatment, it must be applied at an early stage when the leaves are still very small.

Field applications of SADH and maleic hydrazide were effective in reducing shoot growth, and limited fruit residue tests indicated SADH residues in fruit were likely to be within tolerable limits. More recent trials have also shown the effectiveness of early-season SADH sprays on kiwifruit vines, without any significant reduction in fruit size at harvest (Anon. 1978). The evidence presented here

of a SADH or maleic spray increasing post-harvest fruit respiration indicates that this aspect must be more fully studied before commercial use of these chemical sprays could be recommended.

The compensatory growth noted (increased internode number and leaf size) is important in indicating there is a mechanism for controlling the relative growth of the shoot and root - which implies a mobile hormone is involved, and that the roots are a source of specific shoot-growth factors. Similarly to other authors, Richards and Rowe (1977) postulated the limit on top growth set by the roots involves an internal regulation by the roots, especially the production and supply of growth substances.

In this study the roots were seen to contribute to stem elongation, as shoot growth ceased three days after removing the roots, although growth resumed within three days when GA was applied to the shoot base. But while this may indicate root-GAs are involved in stem growth, they could normally be synthesised in the tops and only undergo qualitative changes in the roots. Identification of individual gibberellins in different parts of the plant would be necessary to resolve this question. The type of growth produced in this shoot system, and hence its control system, appears as incomplete though when compared with a normal intact plant. Hence some other growth factor than GA and carbohydrate seems required for plant growth - as already indicated.

The data presented here showed that large volumes of sap can be transported up the stem of the kiwifruit vine. Active sap bleeding from the cut stem occurred for a limited period in spring, and the flow rate depended on the stage of vine development, plant size, temperature, the degree of decapitation and the time of day. Bleeding began just before bud movement, and the flow rate was highest during the period that the buds were first visibly moving (advanced swell), and thereafter declined. High

sap flows tended to run ahead of bud growth, and the timing of increasing sap flows could suggest sap provided essentials for bud burst and growth. However it has been noted these processes can occur in the absence of a root system and the associated high sap flow. It is also of interest that sap flows decreased as the leaf area developed - and when transpiration losses were probably increasing.

While the pattern of sap flow appears to be related to the early-season development of the buds, there must be some doubt about its relationship to the start of spring growth.

The increased shoot auxin associated with increased cambial and bud activity, and the ready availability of carbohydrates released from storage would favour sap exudation (Skoog et al. 1938), and so high sap flow rates appear more as a byproduct if plant metabolism than as a stimulus of it, or an essential requirement for it. Further, at the low temperatures prevailing in early spring, with water absorption largely being determined by the level of metabolic activity, there is an explanation of the substantial increases in flow rate with relatively small rises in temperature (Kuiper 1964). Subsequently, with the start of shoot growth and reduced carbohydrates, and a reduced temperature effect on water uptake and sap flow, sap flow rates would decline - as in fact they do.

In the uncontrolled field situation many things can account for differences in flow rates, and it is desirable to work with controlled environments and to use more direct methods of assessing sap movement.

The spring bleeding sap was shown to contain cytokinins and GA-like substances. Gibberellin-like activity rose at the time most of the buds were at advanced bud burst, with the amounts being transported towards the shoots being highest when shoot elongation was just beginning.

Trunk sap-GAs rose in the apple from the start of growth, and levels parallelled (but ran 2 weeks ahead of) the extension growth of the annual shoots (Grochowska and Karaszewska 1978). Within the shoots themselves high GA occurred at the start of shoot growth, but levels were at their lowest during the most rapid extension growth. This may indicate that during growth the shoot-GAs were consumed, or were recirculated and found their way back into the conducting tissues of the trunk as gibberellins more responsive in the bioassay.

The kiwifruit "root-GA" was possibly supporting the growth of quiescent buds and the new season's shoot growth In the absence of any earlier assessments of plant or bud GA it is not possible indicate whether GA's may have been involved in the breaking of dormancy - which would have occurred much earlier. Browning (1973a) indicated the GA's involved in dormancy release were not supplied by the xylem sap, but originated within the buds themselves.

How rapid shoot elongation continued through the summer - with the apparent reduction in sap flow, and the decreased concentration of sap-GA is not clear. If the initial high levels of sap-GA had a function it could have also been to stimulate the start of cambial activity (Digby and Wareing 1966). Once stem growth is established lower levels of sap-GA in association with other tissues and hormones may be adequate to maintain cell division and control cell elongation (Cleland 1969). Qualitative changes in sap GA's and cytokinins following decapitation indicated the roots and shoots were not functioning independently, but were interacting with respect to GA and cytokinin metabolism.

As with gibberellins, sap-cytokinin activity varied between plants at a similar stage of development. Cytokinin concentration and the amounts being transported peaked slightly earlier than did gibberellins, being at a maximum at advanced bud burst. Cytokinin levels then decreased as

shoot elongation began. The sap-cytokinin was found to be possibly identical with zeatin riboside, and may have played a role in both bud burst and shoot growth. In the apple a high level of trunk sap cytokinin seemed to act as a triggering factor for the initiation of spring growth, which it preceded (Grochowska and Karaszewska 1978). In this, cytokinins could act similarly to GA in controlling the translocation of assimilates to support shoot growth (Shindy et al. 1973), and subsequently generally support normal shoot development (Bachelard and Wightman 1974, Jones, O.P., 1967, 1973).

During the summer the inhibition of lateral bud growth became progressively stronger from January, to a maximum about the end of May which lasted through June. Hence this summer correlative inhibition appeared to merge into the condition known as winter dormancy.

ABA, IAA, and a mobile auxin-like substance that moved down a stem were potent inhibitors of bud growth, whereas the presence of roots favoured bud outgrowth. Applications of cytokinin generally inhibited bud growth, although on one occasion they acted similarly to roots in promoting bud burst during the summer. The leaves were inhibitory of bud burst, and this probably reflects their production of auxin, which is widely accepted as being the primary correlative inhibitor.

What was originally described as summer dormancy then is probably a correlative inhibition due to auxin produced in the shoot tip and also in the leaves. In the presence of elevated auxin levels it is likely factors such as cytokinin which are promotive of bud burst cannot act. In the blackcurrant, bud growth was rapid in a period in late summer when correlative inhibition was less strong (Nasr and Wareing 1961). In this study stem-cytokinin levels in December were depressed in the presence of IAA, and Woolley and Wareing (1972a) have shown

that auxin can affect a bud's ability to receive and utilise cytokinins for growth.

The problem of the correlative summer inhibition of bud growth appears similar to that of winter dormancy - both possibly being the result of an inability to utilise cytokinins: in effect a cytokinin deficiency.

The breaking of winter dormancy is apparently related to a rise in the level of growth promoters which may be cytokinins and/or gibberellins. Once dormancy has been broken GA strongly promotes shoot elongation.

Temperatures of 4^{o} - $8^{o}C$ were best for producing efficient and rapid bud burst of dormant winter buds, and $0^{o}C$ was relatively inaffective, - and in this respect the buds were typical of true dormancy. However it seemed weaker in degree as storage temperature of 16^{o} - $24^{o}C$ for 24 days induced more rapid budburst than storage at 4^{o} - $12^{o}C$. It would appear that in some manner chilling renews the supply of growth promoters - and the stress that results from incubation at warm temperatures, or from removing the bud's surrounding tissues acts in the same manner.

Gibberellins could be an important part of these growth promoters as it is known that both chilling and also GA treatment restore growth in physiologic dwarfs (Powell 1978). Also other authors have attributed the emergence of the buds from rest to the build-up of endogenous GA's and Ramsay and Martin (1970) found an increase in apricot bud GA-like activity shortly prior to the end of rest. It appeared that about 700 hours chilling was adequate to result in rapid bud burst, typical of the end of dormancy, and this has normally occurred by 1 July. Thus it is necessary for any studies on the involvement of growth regulators in the breaking of dormancy to be done in May and June.

The role of inhibitors in bud dormancy is not clear, but some theories regard dormancy as being controlled by a growth regulator balance including inhibitor concentrations falling below a threshold concentration. In kiwifruit, as in other plants, rest intensity increases through the summer, and autumn applications of growth regulators do not promote bud outgrowth - suggesting there is the accumulation of an inhibitor. In this study, autumn bud-ABA had fallen by the time dormancy was broken in early July, but ABA activity was not related to the capacity for rapid bud burst, or the breaking of dormancy The fact that the decline in bud ABA was not temperature - related, suggests winter chilling does not promote bud burst by reducing ABA levels.

The kiwifruit roots were not shown to contribute directly to bud burst in the spring, although they could have had a residual effect. In a study of root growth, it was found to occur along with, or following bud movement, and so while the roots did not appear likely to initiate top growth they did support the subsequent shoot growth. Studies of stem and sap cytokinin and GA activity did not indicate they were related to the breaking of dormancy, but they increased with the start of shoot growth - in which process they are likely to have played a controlling role. Unlike Hewett and Wareing (1973a) the build-up in sap-cytokinin level did not precede the rise in bud-cytokinin - both being highest at an early stage of bud growth and decreasing once bud burst was under way.

Stem tissue was found to be able to make cytokinins available to the buds, and would form an alternative source of this hormone, as was found to occur following a dormancy-breaking stress in coffee (Browning 1973b). It would seem that any factor promoting bud burst, or overcoming dormancy, does so by directly affecting the buds or stems, and not indirectly via the roots.

Juvenility and a Juvenile-like condition in blackcurrant.

Juvenility has been much studied, but the concept has led to difficulties in definition. Traditionally it is used to describe the non-flowering condition characteristic of young seedlings, as distinct from other plant systems, It appears to be largely for this reason that Schwabe and Al-Doori (1973) describe a condition with similar characteristics, but in blackcurrant cuttings, as a juvenile-like condition. However Schwabe (1976) suggests one should regard a plant as in a juvenile condition when it fails to become reproductive even when exposed to environmental conditions which would normally induce flowering. It is of interest that Schwabe (1976) makes no reference to Zimmerman's (1973) "transition period", which was proposed to account for the flowering response of the young vegetatively-propogated plant system that Schwabe has usually worked with.

There is a further reason to believe that the juvenile-like condition of blackcurrant being considered is distinct from true juvenility. Robinson and Wareing (1969) have indicated that phase change occurs in the shoot's meristematic tip, distinct from the differentiated part of the shoot, and hence affects subsequent tissues. Schwabe and Al-Doori (1973) found their juvenile-like condition was not an inherent property of the bud or shoot, but was related to the bud's position relative to the roots. Further, once this blackcurrant shoot is large enough to respond to inductive conditions, flower initiation then occurs in a basipetal position. Hence, while juvenile characters may appear in a vegetatively - propagated plant, it is inaccurate to describe it as being juvenile. Describing it as being in an invigorated transition phase type of condition of the adult, or juvenile-like, seems to be more correct. Such a distinction is also seen by Robinson and Wareing (1969) in their reference to a 'ripeness-to-flower' state that occurs with phase change, and a condition of the shoot that requires ageing in order for it to be able to respond

to inductive conditions.

In this study using vegetively-propagated plants, in 'adult' plants there was a marked gradient in stem-GA level, from high basal levels to very low levels in the upper part of the shoot. As the first buds to initiate flowers were those in the upper part of the stem, it appeared as if high-GA was discouraging initiation. However, in contrast to the results of Schwabe and Al-Doori (1973) the roots and stems of short juvenile-like shoots in which flower initiation was not possible contained very low levels of GA. It was therefore not possible to support the idea that the juvenile-like condition of the blackcurrant is due to the proximity of the shoot tip to the roots, and that this is due to gibberellins coming from the roots (Schwabe and Al-Doori 1973).

Wareing and Frydman (1976) believe that juvenility in ivy is maintained by relatively high GA in the shoot apices. But the results with blackcurrant seedlings which detected no stem-gibberellins in the shoots at any stage suggests it is inaccurate to claim low GA-like activity is the cause of gaining the ability to flower. Hence a prime function for the roots in the control of this aspect of plant development was not indicated. It has been suggested elsewhere also, that low GA alone is probably inadequate for phase change (Wareing and Frydman 1976). In discussing clonally-produced blackcurrant Nasr and Wareing (1961) proposed that correlative inhibition prevented flower initiation in the early part of the growth cycle. Perhaps then the GA/auxin balance is important both in phase change and in the ageing required by the juvenile-like blackcurrant, and this aspect should receive further consideration.

APPENDIX 1.

Determination of Alar residue in kiwifruit.

METHOD: Place in a 2 litre flask 50 gm. of homogenized sample of frozen, pulped whole fruit. Add 100 ml. 50% NaOH, 50 gm. NaOH pellets, 8.4 ml. 12% $TiCl_3$, 1 ml. Antifoam A, and 1 gm. gran. zinc. This mixture was distilled and 40 ml. distillate was collected in 5 ml. 2% citric acid containing 1 drop phenolphthalein solution.

If pink colour appeared, sufficient citric acid solution was added dropwise, to discharge it. The distillate solution was then acidified to 3.5 with dropwise addition of 2% citric acid.

When ready to develop the colour, dilute NaOH was added dropwise to give a pH of 5.0. Then 5.0 ml. of 1% Trisodium Penta Cyanoamine ferroate was added, the whole made up to 50 ml. and shaken well. After one hour the solution was filtered through Whatman No. 1 paper and the absorbance measured on a Hilger Biochem Absorptiometer at 490 and 460 mu in 1 cm cells using a reagent blank.

The Net Absorbance was calculated as (Ab_{490} - Ab_{460} and this was plotted on a Standard Graph using 200, 400, 800, and 1000 µgm dimas.

Untreated samples of kiwifruit were analysed after the addition of aliquots of dimas and the % recovery determined.

The treated samples were then analysed, without fortification, and the results corrected for % recovery.

APPENDIX 2.

HPLC of cytokinin extract of kiwifruit sap.

High pressure liquid chromatography was performed with ISCO equipment model 1440C3N, incorporating high pressure (2000 psi) Dialagrad pumps. The column, 25 cms. x 2 mm i.d. was packed with microparticulate silica (Varian Aerograph Si - 5). The system was linked to a UA5 monitor with built-in recorder, and micro flow cells, with 10 mm path length and 19 µl hold-up volume.

The dried sample extract was taken up in chloroform-acetic acid (9:1 90% saturated), and 250 µl injected through a sample loop. The sample was eluted with a final solvent 80% from pump A and 20% from pump B, at $20^{o} \pm 2^{o}C$ and 1450psi, and 20 ml/hr.

Solvent preparation.

The solvents used were:

Pump A - Chloroform/Acetic acid 9:1 (90% saturated).

Pump B - Chloroform/Acetic acid 8.5 : 1.5 (90% saturated) + 20% methanol.

The 90% water saturated solvent was prepared by saturating dry solvent in a separating funnel, and then adding 10% of dry solvent.

LITERATURE CITED

Abbott, D.L., 1962. J. hort. Sci. 37: 272 - 284.

_____, 1970. In "Physiology of Tree Crops". Eds. Luckwill, L.C., and Cutting, C.V., p. 65 - 82.

Al-doori, A.H., 1972. M.Phil. thesis, Univ. London.

Ali, A., and Fletcher, R.A., 1970. Can. J. Bot. 48: 1989 - 1994.

_____, 1971. Can. J. Bot. 49: 1727 - 1731.

Alvim, R., Hewett, E.W., and Saunders, P.F., 1976. Pl. Physiol., Lancaster. 57: 474 - 476.

Anonymous, 1978. Trial Results 1977-8, Ispray Ltd.; 116 - 118.

Antcliff, A.J., and May, P., 1961. Vitis 3: 1 - 14.

Arney, S.E., and Mancinelli, P., 1966. New Phytol. 65: 161 - 175.

Atkin, R.K., Barton, G.E., and Robinson, D.K., 1973. J. exp. Bot. 24: 479 - 487.

Aung, L.H., and Byrne, J.M., 1976. J. Amer. Soc. hort. Sci. 101: 189 - 192.

Avery, D.J., 1969. New Phytol. 68: 323 - 336.

Bachelard, E.P., and Wightman, F., 1974. Can. J. Bot. 52: 1483 - 1489.

Barendse, G.W.M., Kende, H., and Lang, A., 1968. Pl. Physiol. Lancaster 43: 815 - 822.

Barlow, H.W.B., and Hancock, C.R., 1955. Rep. E. Malling Res. Stn. for 1955 (1956). 89 - 100.

_____, 1956. In "The Growth of Leaves", ed. F.L. Milthorpe, p. 107 - 118.

Beever, J.E., and Woolhouse, H.W. 1974. In "Mechanisms of regulation of plant growth". Eds. Bieleski, R.L., Ferguson, A.R., and Cresswell, M.M., Bull. 12 Royal Soc. N.Z. 681 - 686.

Bollard, E.G., 1953. J. exp. Bot. 4: 363 - 368.

Boothby, D., and Wright, S.T.C., 1962. Nature. 196: 389 - 390.

Borchert, R., 1976. Acta Hort. No. 56. p. 21 - 33.

Bowen, M.R., and Wareing, P.F. 1969. Planta 89: 108 - 125.

Brian, P.W., 1959. Biol. Rev. 34: 37 - 84.

_____, and Hemming, H.G., 1957. Ann. appl. Biol. 45: 489 - 497.

_____, 1958. Ann. Bot. N.S. 22: 1.

Brown, D.S., Griggs, W.H., and Iwakiri, B.T., 1960. Proc. Am. Soc. hort. Sci. 76: 52 - 58.

Browning, G., 1973a. J. hort. Sci. 48: 29 - 41.

_____, 1973b. J. hort. Sci. 48: 297 - 310.

Brundell, D.J., 1973. M. Hort. Sc. thesis, Massey University.

_____, 1975a. N.Z. J. Bot. 13: 473 - 483.

_____, 1975b. N.Z. J. Bot. 13: 485 - 496.

_____, 1975c. N.Z. J. agric. Res. 18: 365 - 370.

_____, 1975d. N.Z. J. agric. Res. 18: 371 - 374.

_____, 1976. Scientia Hort. 4: 175 - 182.

Buttrose, M.S., 1966. Aust. J. biol. Sci. 19: 247 - 256.

_____, and Mullins, M.G., 1968. Aust. J. biol. Sc. 21: 1095 - 1101.

Carlson, W.G., and Larson, M.M., 1977. Physiologia Pl. 41: 162 - 166.

Carr, D.J., and Burrows, W.J., 1966. Life Sci. 5: 2061 - 2077.

_____, and Pate, J.S., 1967. Sym. Soc. Biol. No. 21. p. 559 - 600.

_____, and Reid, D.M., 1968. In "Biochemistry and Physiology Plant Growth Substances" Ed. F. Wightman, G. Setterfield, p. 1169 - 1187.

_____, and Skene, K.G.M., 1964. Planta 63: 382 - 394.

Cheffins, .J., Howard, B.H., and Priestley, C.A., 1974. Rep. E. Malling Res. Stn. for 1974 (1975), 41 - 42.

Chrispeels, M.J., and Varner, J.E., 1967. Pl. Physiol. Lancaster 42: 1008 - 1016.

Clarkson, D.T., 1976. Planta. 132: 297 - 304.

Cleland, R., 1964. Physiologia Pl. 17: 126 - 135.

Cleland R.E., 1969. In "Physiology of plant growth and development". Ed. M.B. Wilkins, McGraw-Hill,London. 49 - 81.

Cooper, D.R., Hill-Cottingham, D.G., and Shorthill, M.J. 1972. J. exp. Bot. 23: 247 - 254.

Cooper, W.C., Young, R.H., and Henry, W.H., 1969. Proc. First Internat. Citus Symposium. 1: 301 - 314.

Cozens, I.G., and Wilkinson, E.H., 1966. Nature 211: 867 - 868.

Crabbe, J., 1972. Hort. Abs. 42: 2946.

Critchfield, W.B.,1970. Bot. Gaz. 131: 150 - 162.

Crozier, A., Aoki, H., and Pharis, R.P., 1969. J. exp. Bot. 20: 786 - 795.

_____, Kuo, C.C., Durley, R.C., and Pharis, R.P., 1970. Can. J. Bot. 48: 867 - 877.

_____, and Reid, D.M., 1971. Can. J. Bot. 49: 967 - 975.

_____, Bowen, D.H., MacMillan, J., Reid, D.M., and Most, B.H., 1971. Planta. 97: 142 - 154.

_____, Reid, D.M., and Reeve, D.R., 1973. J. exp. Bot. 24: 923 - 934.

Davey, J.E., and van Staden, J., 1976. Planta. 130: 69 - 72.

Davison, R.M., 1971. Orchardist of N.Z. 44: 443 - 444.

_____, and Young, H., 1973. Planta. 109: 95 - 98.

Dennis, F.G., Jr. 1977. HortScience, 12: 217 - 220.

_____., and Nitsch J.P., 1966. Nature 211: 781 - 782.

Digby, J., and Wareing, P.F., 1966. Ann. Bot. 30: 539 - 548.

Domanski, P., and Kozlowski, T.T., 1968. Can. J. Bot. 46: 397 - 403.

Donoho, C.W. Jr., and Walker, D.R., 1957. Science 126: 1178 - 1179.

Doorenbos, J., 1953. Meded. Landb.hogesch. Wageningen 53: 1 - 24.

Dorffling, K., 1976. Physiologia Pl. 38: 319 - 322.

Dumbroff, E.B., and Brown, D.C.W., 1976. Can. J. Bot. 54: 191 - 197.

Dunberg, A., 1976. Physiologia Pl. 38: 186 - 190.

Durley, R.C., Crozier, A., Pharis, R.P., and McLaughlin, G.E., 1972. Phytochemistry 11: 3029 - 3033.

Eagles, C.F., and Wareing, P.F., 1964. Physiologia Pl. 17: 697 - 708.

Leuwens,C.J., 1973. Ph.D. thesis. Univ. London.

Eliasson, L., 1969. Physiologia Pl. 22: 1288 - 1301.

Emmerson, J.G., and Powell, L.E., 1978. J. Amer. Soc. Hort. Sci. 103: 677 - 680.

Erez, A.,Lavee, S., and Samish, R.M., 1971. J. Amer. Soc. Hort. Sci. 96: 519 - 522.

_____, 1974. Proc. 19 Internat. Hort. Congress Warsaw. 3: 69 - 78.

_____, Samish, R.M., and Lavee, R., 1966. Physiologia Pl. 19: 650 - 659.

Faull, K.F., Coombe, B.G., and Paleg, L.G., 1974. Aust. J. Plant Physio. 1: 183 - 198.

Faust, M., Shear, C.B., and Williams, M.W., 1969. Bot. Rev. 35: 103 - 134.

Fletcher, R.A., and Malik, S., 1964. Pl. Physiol. Lancaster 39: 328 - 331.

Flood, A. E., and Priestley, C.A., 1973. J. Sci. Fd Agric. 24: 945 - 955.

Frankland, B., and Wareing, P.F., 1960. Nature 185: 255 - 256.

Freeman, B.M., and Kliewer, W.M., 1978. Am. J. Enol. Vitic. 27: 36 - 39.

Frydman, V.M., and Wareing, P.F., 1973a. J. exp. Bot. 24: 1131 - 1138.

_____, 1973b. J. exp. Bot. 24: 1139 - 1148.

Fulford, R.M., 1970. Proc. 18 Internat. Hort. Congress 4: 143 - 150.

Fulford, R.M., Quinlan , J.D., Lacey, H.J., and Barlow, H.W.B. 1968. In "Biochemistry and Physiology of Plant Growth Substances". Eds. Wightman F., and Setterfield G., p. 1187 - 1203.

Gaskin, P., MacMillan, J., and Zeevaart, J.A.D. 1973. Planta 111: 347 - 352.

Gerber, J.F., Martsolf, J.D., and Bartholic, J.F., 1974. HortScience 9: 569 - 572.

Glenn, J.L., Kuo, C.C., Durley, R.C., and Pharis, R.P., 1972. Phytochemistry 11: 345 - 351.

Goldschmidt, E.E., 1976. HortScience 11: 95 - 99.

_____, and Monselise S.P., 1968. Pl. Physiol. 43: 113 - 116.

Goren, R., Goldschmidt, E.E., and Monselise, S.P., 1971. J. hort. Sci. 46: 443 - 451.

Gorokhova, G.I., 1976. Hort. Abs. 46: No. 188.

Grauslund, J., 1972. Physiologia Pl. 27: 65 - 70.

Greenhalgh, W.J., and Edgerton, L.J., 1967. Proc. Am. Soc. hort. Sci. 91: 9 - 17.

Griggs, W.H., and Iwakiri, B.T., 1961. Proc. Am. Soc. hort. Sci. 77: 73 - 89.

Grochowska, M.J., 1973. J. hort. Sci. 48: 347 - 356.

_____, and Karaszewska, A., 1978. ActaHort. (80): 457 - 464.

Grossenbacher, K.A., 1939. Am. J. Bot. 26: 107 - 109.

Grunwald, C., and Lockard, R.G., 1973. Physiologia Pl. 29: 64 - 67.

Gunckel, J.E., and Thimann, K.V., 1949. A . J. Bot. 36: 145 - 151.

_____, and Wetmore, R.H., 1946. Amer. Jour. Bot. 33: 285 - 295.

Hackett, W.P., 1976. Acta Hort. No. 56. 143 - 154.

Hall, R.H., 1973. A. Rev. Pl. Physiol. 24: 415 - 444.

Hall, A.E., Khairi, M.M.A., and Asbell, C.W., 1977. J. Amer. Soc. Hort. Sci. 102: 261 - 263.

Hammond, M.W., and Seeley, S.D., 1978. J. Amer. Soc. Hort. Sci. 103: 655 - 657.

Hansen, P., 1967. Physiologia Pl. 20: 720 - 725.

_____, 1971. Physiologia Pl. 25: 469 - 473.

Hess, C.E., 1964. "Reg. Nat. de la Croiss. Vege." 15th Internat. Conf. Plant Growth Substances p. 5 - 17.

Hewett, E.W., and Wareing, P.F., 1973a. Physiologia Pl. 28: 393 - 399.

_____, 1973b. Physiologia Pl. 29: 386 - 389.

Higazy, M.K.M.T., 1962. Meded. Landbouw. 62: (8).

Hill-Cottingham, D.G., and Bollard, E.G., 1965. N.Z. Jl. agric. Res. 8: 778 - 787.

Hoad, G.V., and Monselise, S.P., 1976. Scientia Hort. 4: 41 - 47.

Horgan, R., Hewett, E.W., Purse, J.G., Horgan, J.M., and Wareing, P.F., 1973. Plant. Sci. Letters, 1: 321 - 324.

Humphries, E.C., and French, S.A.W., 1969. Planta 88: 87 - 90.

Ibrahim, I.M. and Dana, M.N., 1971. HortScience 6: 541 - 542.

ISHS 1976. Acta Hort. No. 56 Symposium on Juvenility in Woody Perennials.

Jackson, D.I., and Field, R.J., Ann. Bot. 36: 525 - 532.

Jankiewicz, L.S., 1972. Biologia Plantarum (Praha). 14: 52 - 61.

_____, Plich, H., Barkoswka, B., and Moraszczyk, A., 1973. Acta. Hort. 34: 107 - 116.

Jones, O.P., 1967. Nature 215: 1514 - 1515.

_____, 1971. Ann. Bot. 35: 825 - 836.

_____, 1973. J. hort. Sci. 48: 181 - 188.

Jones, R.A., and Kaufman, P.B., 1971. Physiologia Pl. 24: 491 - 497.

Jones, R.L., 1973. A. Rev. Pl. Physiol. 24: 571 - 598.

_____, and Phillips, I.D.J., 1966. Pl. Physiol. 1966. 41: 1381 - 1386.

_____, and Varner, J.E., 1967. Planta. 72: 155 - 161.

Junttila, O., 1976. Physiologia Pl. 38: 278 - 286.

Kato, T., and Ito, H., 1962. Tohoku J. Agri. Res. 13: 1 - 21.

Kaufman, P.B., 1965. Physiologia Pl. 18: 424 - 443.

_____, 1967. Ann. N.Y. Acad. Sci. 144: 191 - 203.

_____, Ghosheh, N.S., Nakosteen, L., Pharis, R.P., Durley, R.C., and Morf, W., 1976. Pl. Physiol. 58: 131 - 134.

Keep, E., 1961. J. hort. Sci. 36: 174 - 185.

Kefford, N.P., 1962. Pl. Physiol. 37: 380 - 386.

Kende, H., 1965. Proc. natn. Acad. Sci. U.S.A. 53: 1302 - 1307.

_____, and Sitton, D., 1967. Ann. N.Y. Acad. Sci. 144: 235 - 243.

Kender, W.J., and Carpenter, S., 1972. J. Amer. Soc. Hort. Sci. 97: 377 - 380.

Khalifah, R.A., Lewis, L.N., and Coggins, C.W., 1965. Analyt. Biochem. 12: 113.

Kliewer, W.M., 1967. Am. J. Enol. Vitic. 18: 126 - 137.

Kozlowski, T.T., 1964. Bot. Rev. 30: 335 - 392.

Kuiper, P.J.C., 1964. Meded. Landbouwhogeschool Wageningen, 64 - 4.

Lang, A., 1970. A. Rev. Physiol. 21: 537 - 570.

Lavee, S., 1973. Acta Hort. 34: 225 - 234.

Lavender, D.P., Sweet, G.B., Zaerr, J.B., and Hermann, R.K., 1973. Science. 182: 838 - 839.

Lee, C.I., and Hackett, W.P., 1976. J. Amer. Soc. Hort. Sci. 101: 236 - 240.

Lenton, J.R., Perry, V.M., and Saunder, P.F., 1972. Planta 106: 13 - 22.

Letham, D.S., 1968. In "Biochemistry and Physiology of Plant Growth Substances". pp. 19 - 31. Runge Press, Ottawa.

Link, R.F., and Wallace, D.L., 1952. Report Princetown University, March 2.

Lockard, R.G., 1956. PhD. Thesis Univ. London.

Lockhart, J.A., 1957. Pl. Physiol. 32: 204 - 207.

_____, 1964. Planta. 62: 97 - 115.

Lockwood, D.W., and Sparks, D., 1978. J. Amer. Soc. Hort. Sci. 103: 38 - 45.

Loomis, W.D., Battaile, J., 1966. Phytochemistry 5: 423 - 438.

Looney, N.E., McGlasson, W.B., and Coombe, B.G., 1974. Aust. J. Plant Physiol. 1: 77 - 86.

Luckwill, L.C., 1968. J. hort. Sci. 43: 91 - 102.

_____, 1970. In "Physiology of Tree Crops", pp. 237 - 253. Eds. Luckwill, L.C., and Cutting, C.V. Academic Press.

_____, and Whyte, P., 1968. In "Plant Growth Regulators". Soc. Chem. Ind. (London). Monogr. No.31, 87 - 101.

Luis, A.G., and Guardiola, J.L., 1974. Proc. 19th Internat. Hort. Cong. 1A.87.

Mer, C.L., 1969. New Phytol. 68: 275 - 294.

Mielke, E.A. and Dennis, F.G., 1975. J. Amer. Soc. Hort. Sci. 100: 287 - 290.

_____, 1978. J. Amer. Soc. Hort. Sci. 103: 446 - 449.

Miginiac, E., 1971. Physiologia Pl. 25: 234 - 239.

Milborrow, B.V., 1974.A.Rev.Pl. Physiol. 25: 259 - 307.

Miller, C.O., 1963. Modern Methods of Plant Analysis, 6: 194 - 202.

Miller, G.L., Blum, R., Glennon, W.E., and Burton, A.L., 1960. Analyt. Biochem. 2: 127 - 132.

Modlibowska, I., 1966. Rep. E. Malling Res. Stn. for 1965, (1966). 88 - 93.

Monselise, S.P., and Halevy, A.H., 1964. Proc. Am. Soc. hort. Sci. 84: 141 - 146.

Moraszczyk, A., Jankiewicz, L.S. and Pilch, H., 1974. Proc. 20th Internat. Hort. Congress. Warsaw. Vol. 1A 416.

Morris, D.A., and Winfield, P.J., 1972. J. exp. Bot. 23: 346 - 355.

Morrow, R.R., 1955. J. Forestry. 53: 24 - 25.

Morrow, R.R., 1963. Bull. 982 Cornell Univ. Agr. Exp. Stn., Ithaca, N.Y.

_____, 1972. New York Food and Life Sciences Bulletin No. 14. Cornell University, Ithaca, N.Y.

Mullins, M.G., 1967. J. exp. Bot. 18: 206 - 214.

_____, 1970. Ann. Bot. N.S. 34: 897 - 909.

Nagao, M.A., and Rubinstein, B., 1975. Bot. Gaz. 136: 366 - 371.

Nanda, K.K., and Purohit, A.N., 1965. Planta 66: 121 - 125.

Nasr, T.A.A.,and Wareing, P.F., 1961. J. hort. Sci. 36: 1 - 10.

Nitsch, J.P., 1970. "The Biochemistry of Fruits and their Products". ed. A.C. Hulme. Chap. 15. 428 - 474.

Nooden, L.D., 1969. Physiologia Pl. 22: 260 - 270.

Obbink, J.G., Alexander, D.McE., and Possingham, J.V., 1973. Vitis. 12: 207 - 213.

Ockerse, R., and Galston, A.W., 1967. Pl. Physiol. 42: 47 - 54.

Oland, K., 1959. Physiologia Pl. 12: 594 - 648.

Paiva, E., and Robitaille, H.A., 1978. J. Amer. Soc. Hort. Sci. 103: 101 - 104.

Paleg, L.G., 1965. A. Rev. Pl. Physiol. 16: 291 - 322.

Pate, J.S., 1968. In "Recent aspects of nitrogen metabolism in plants". Ed. Hewitt, E.J., and Cutting, C.V. Academic Press. 219 - 240.

Perry, T.O., 1971. Science 171: 29 - 36.

Peterson, C.A., and Fletcher, R.Z., 1975. Can. J. Bot. 53: 243 - 248.

Pharis, R.P., and Ross, S.D., 1976. Outl. Agric. 9: 82 - 87.

Phillips, I.D.J., 1968. J. exp. Bot. 19: 617 - 627.

_____, 1971a.J. exp. Bot. 22: 465 - 471.

_____, 1971b.Planta 96: 27 - 34.

_____, 1972. Planta 105: 234 - 244.

_____, 1975. A. Rev. Pl. Physiol. 26: 341 - 367.

Phillips, I.D.J., and Jones, R.J., 1964. Planta 63: 269 - 278.

_____, and Wareing, P.F., 1958. J. exp. Bot. 9: 350 - 364.

_____, 1959. J. exp. Bot. 10: 504 - 514.

Phinney, B.O., and West, C.A., 1960. A. Rev. Pl. Physiol. 11: 411 - 436.

Pieniazek, J., and Saniewski, M., 1968. Bull. de L'Academie Polonaise des Sciences. 26: 39 - 46.

_____, and Jankiewicz, L.S., 1970. Physiologsche Probleme im Obstbau.

Poll, L., 1973. Hort. Res. 13: 83 - 88.

Possingham, J.V., 1970. In "Physiology of Tree Crops". Ed. Luckwill, L.C., and Cutting, C.V. Academic Press. p. 335 - 349.

Powell, L.E., 1960. Pl. Physiol. 35: 256 - 261.

_____, 1976. HortScience. 11: 498 - 499.

_____, 1978. Acta Hort. (80): 27 - 37.

_____, Cain, J.C., and Lamb, R.C., 1959. Proc. Am. hort. Sci. 74: 82 - 86.

_____, and Seeley, S.D., 1970. HortScience. 5: 327.

_____, and Tautvydas, K.J., 1967. Nature 213: 292 - 293.

Pollock, B.M., 1953. Physiologia Pl. 6: 47 - 64.

Priestley, C.A., 1960. Rep. E. Malling Res. Stn. for 1959 (1960). 70 - 77.

_____, 1965. J. Sci. Fd Agric. 16: 717 - 721.

_____, 1970. In "Physiology of Tree Crops". Eds. Luckwill, L.C., and Cutting, C.V. p. 113.

Purse, J.G., Horgan, R., Horgan, J.M., and Wareing, P.F., 1976. Planta. 132: 1 - 8.

Quinlan, J.D., and Preston, A.P., 1968. J. hort. Sci. 43: 373 - 381.

_____, and Weaver, R.J., 1970. Pl. Physiol. 46: 527 - 530.

Radin, J.W., and Loomis, R.S., 1971. Physiologia Pl. 25: 240 - 244.

Railton, I.D., 1974. Planta. 120: 197 - 200.

_____, and Reid, D.M., 1973. Planta 111: 261 -266.

_____, and Wareing, P.F., 1973. Planta 112: 65 - 69.

Ramsay, J., and Martin, G.C., 1970. J. Amer. Soc. Hort. Sci. 95: 569 - 574.

Reid, D.M., and Carr, D.J., 1967. Planta 73: 1 - 11.

_____, and Crozier, A., 1970. In "Plant Growth Substances". Ed. Carr, D.J. 420 - 427.

_____, 1971. J. exp. Bot. 22: 39 - 48.

_____, and Harvey, B.M.R., 1969. Planta. 89: 376 - 379.

_____, and Railton, I.D., 1974. In "Mechanisms of regulation of plant growth". Ed. Bieleski, R.L. Ferguson, A.R., and Cresswell, M.M. Bull. 12. The Royal Soc. of N.Z. p. 789 - 792.

Reynolds, T., 1970. J. exp. Bot. 21: 702 - 711.

Richards, D., and Rowe, R.N., 1977. Ann. Bot. 41: 729 - 740.

Robinson, J.C., 1975. PhD. thesis. Wye College, University of London.

Robinson, L.W., and Wareing, P.F., 1969. New Phytol. 68: 67 - 78.

Robitaille, H.A., 1970. PhD. thesis. Michigan State University.

Rogers, W.S., and Booth, G.A., 1964. J. hort. Sci. 39: 61 - 65.

Ryugo, K., and Sansavini, S., 1972. J. hort. Sci. 47: 173 - 178.

Sachs, R.M., 1965. A. Rev. Pl. Physiol. 16: 73 - 96.

_____, 1977. HortScience. 12: 220 -222.

_____, Bretz, C.F., and Lang, A., 1959. Am. J. Bot. 46: 376 - 384.

_____, Hackett, W.P., Maire, R.G., Kretchun, T.M., and deBie, J., 1970. Bull. 844 Calif. Agr. Exp. Stn. Univ. Calif.

Sachs, R.M., and Kofranek, A.M., 1963. Am. J. Bot. 50: 772 - 779.

_____, and Lang, A., 1957. Science. 125: 1144 - 1145.

_____, and Thimann, K.V., 1964. Nature. 201: 939 - 940.

Sachs, T., and Thimann, K.V., 1967. Am. J. Bot. 54: 136 - 144.

Saniewski, M., and Pieniazek, J., 1972. Bull. de L'Academie Polonaise des Sciences, 20: 501 - 507.

Schwabe, W.W., 1976. Acta Hort. No. 56 45 - 56.

Schwabe, W.W., and Al-Doori, A.H., 1973. J. exp. Bot. 24: 969 - 981.

Sembdner, G., Weiland, J., Aurich, O. and Schreiber, K., 1968. In "Plant Growth Regulators", Mongr. 31 Soc. Chem. Ind. p. 70 - 86.

Seth, A.K., and Wareing, P.F., 1967. J. exp. Bot. 18: 65 - 77.

Shein, T., and Jackson, D.I., 1971. Ann. Bot. 35: 555 - 564.

_____, 1972. Ann. Bot. 36: 791 - 800.

Shindy, W.W., Kliewer, W.M., and Weaver, R.J., 1973. Pl. Physiol. 51: 345 - 349.

_____, and Weaver, R.J., 1970. Nature 227: 301 - 302.

Singha,S., and Powell, L.E., 1978. J. Amer. Soc. Hort. Sci. 103: 620 - 622.

Sitton, D., Itai, C., and Kende, H., 1967. Planta. 73: 296 - 300.

_____, Richmond, A., and Vaadia, Y., 1967. Phytochemistry 6: 1101 - 1105.

Skene, K.G.M., 1967. Planta 74: 250 - 262.

_____, 1968. Science. 159: 1477 - 1478.

_____, 1970. J. exp. Bot. 21: 418 - 431.

_____, 1971. Ann. Bot. 35: 277 - 286.

_____, 1972a. Planta 104: 89 - 92.

_____, 1972b. In "Plant Growth Substances". Ed. Carr, J.D. 476 - 483.

Skene, K.G.M., 1975. In "The Development and function of roots". Eds. Torrey, J.G., and Clarkson, D.T., Chap. 17. p. 365 - 396.

_____, and Antcliff, A.J., 1972. J. exp. Bot. 23: 283 - 293.

_____, and Kerridge, G.H., 1967. Pl. Physiol. 42: 1131 - 1139.

Skoog, F., Broyer, T.C., and Grossenbacher, K.A., 1938. Am. J. Bot. 25: 749 - 759.

Smith, H., and Wareing, P.F., 1964a. Ann. Bot. N.S. 28: 283 - 295.

_____, 1964b. Ann. Bot. N.S. 28: 297 - 309.

Spreen, W.C., 1956. J. Met. 13: 351.

Stein, O.L., and Fosket, E.B., 1969. Am. J. Bot. 56: 546 - 551.

Sussex, I., 1976. Acta Hort. No. 56. p. 275 - 280.

Thomas, G.G., and Schwabe, W.W., 1969. Ann. Bot. 33: 781 - 793.

_____, 1970. Ann. Bot. 34: 849 - 859.

Thomas, T.H., 1972. J. exp. Bot. 23: 294 - 301.

Thrower S.L., 1962. Aust. J. biol. Sci. 15: 629 - 649.

Tinklin, I.G., Wilkinson, E.H., and Schwabe, W.W., 1970. J. hort. Sci. 45: 275 - 282.

Tinklin, I.J., and Schwabe, W.W., 1970. Ann. Bot. 34: 691 - 706.

Tromp, J., 1970. In "Physiology of Tree Crops". Ed. Luckwill, L.C., and Cutting, C.V., p. 143 - 159.

_____, 1975. Physiologia Pl. 33: 87 - 93.

Tucker, D.J., 1977. Ann. Bot. 41: 181 - 190.

_____, and Mansfield, T.A., 1973. J. exp. Bot. 24: 731 - 740.

Uriu, K., Crane, J.C., and Henderson, D.W., HortScience. 8: 49 - 50.

Van Staden, J., 1976. Physiol. Plant 36: 225 - 228.

Van Staden, J., Brown, N.A.C., 1978. Physiologia Pl. 43: 148 - 153.

_____, and Smith, A.B., 1978. Ann. Bot. 42:

_____, Webb, D.P., and Wareing, P.F., 1972. Planta 104: 110 - 114.

Walker, D.R., and Seeley, S.D., 1973. Acta Hort. 34: 235 - 239.

Wardlaw, I.F., 1968. Biol. Rev. 34: 79 - 105.

Wardell, W.L., 1976. Acta Hort. No. 56. p. 281 - 285.

Wareing, P.F., 1954. Physiologia Pl. 7: 261 - 277.

_____, 1959. J. Linn. Soc. Land. (Bot.) 56: 282 - 289.

_____, 1969. Symposia. Soc. Exper. Biol. No. 23 241 - 262.

_____, 1970. In "Physiology of Tree Crops". Eds. Luckwill, L.C., and Cutting, C.V. p. 1 - 21.

_____, and Frydman, V.M., 1976. Acta Hort. No. 56 p. 57 - 69.

_____, and Nasr, T.A.A., 1961. Ann. Bot. 25: 321 - 340.

_____, and Phillips, I.D.J., 1970. In "The Control of growth and differentiation in plants". Pergamon Press.

_____, and Ryback, G., 1970. Endeavour 29: 84 - 88.

Weaver, R.J., and McCune, S.B., 1959. Am. J. Enol. Vitic. 10: 189 - 190.

_____, and Coombe, B.G., 1961. Am. J. Enol. Vitic. 12: 131 - 142.

Webb, D.P., Van Staden, J., and Wareing, P.F., 1973. J. exp. Bot. 24: 105 - 116.

Weinberger, J.H., 1969. HortScience. 4: 125 - 126.

Went, F.W., 1938. Pl. Physiol. 13: 55 - 80.

_____, 1943. Pl. Physiol. 18: 51 - 65.

Westwood, M.N., and Bjornstad, H.O., 1978. J. Amer. Soc. Hort. Sci. 103: 142 - 144.

White, J.C., and Mansfield, T.A., 1977. Ann. Bot. 41: 1163 - 1170.

Wholey, D.W., and Cock, J.H., 1975. Trop. Agr. 52: 187 - 189.

Wickson, M., and Thimann, K.V., 1958. Physiologia Pl. 11: 62 - 74.

Williams, I.H., 1960. J. hort. Sci. 35: 214 - 220.

Williams, M.W., and Billingsley, H.D., 1970. J. Amer. Soc. Hort. Sci. 95: 649 - 651.

_____, and Stahly, E.A., 1968. HortScience. 3: 68 - 69.

Williams, P.M., Bradbeer, J.W., Gaskin, P., and MacMillan, J., 1974. Planta 117: 101 - 108.

Wittwer, S.H., and Dedolph, R.R., 1963. Am. J. Bot. 50: 330 - 337.

Woolley, D.J., and Wareing, P.F., 1972a. New Phytol. 71: 781 - 793.

_____, 1972b. New Phytol. 71: 1015 - 1025.

_____, 1972c. Planta 105: 33 - 42.

Wright, S.T.C., 1968. In "Biochemistry and Physiology of Plant Growth Substances". Eds. Wightman, F., and Setterfield, G., 521 - 542.

_____, 1975. J. exp. Bot. 26: 161 - 174.

Wu, L.L., and Overcash, J.P., 1968. Proc. Am. Soc. hort. Sci. 93: 235 - 241.

Zeevaart, J.A.D., 1971. Pl. Physiol. 47: 821 -827.

Zimmerman, R.H., 1972. HortScience. 7: 447 - 455.

_____, 1973. Acta Hort. No. 34. 139 - 142.