PHYSIOLOGY OF PRUNING EFFECTS ON BUD DORMANCY IN APPLE

by

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I dedicate this Thesis to my parents

JEAN and ALBERT WILLIAMS

whose unassuming support and encouragement

over many years enabled me to pursue my studies.

TABLE OF CONTENTS

III EXPERIMENTATION (continued)

SUMMARY

An analysis of the effects of pruning on bud development in apple has revealed that pruning treatments involve at least three components; the removal of growing points (lateral buds), the removal of the terminal bud and the introduction of a pruning cut. These components of pruning influence different aspects of the response to pruning. The removal of buds stimulates other buds to burst, removal of the terminal growing point modifies the distribution of growth along the shoot and bud-burst is hastened in the proximity of a pruning cut.

Wounding experiments, with and without the isolation or removal of the distal portion of a shoot, indicated that the stimulation of bud-burst may be attributed to the removal of correlative inhibition mediated by an agent, presumably auxin, produced by all buds and transported basipetally through the peripheral stem tissues. The promotion of more rapid bud-burst is due to the breaking of bud dormancy in response to a stimulus associated with the disruption (wounding) of wood tissue at the pruning cut.

Observation of bud-burst on single-bud segments collected regularly through the year, when compared with bud-burst on whole shoots, confirmed that the winter dormancy of lateral buds has three distinct phases similar to those formerly established for terminal buds namely, correlative inhibition, rest and environmentally imposed dormancy.

Isolation of buds on single-bud segments overcame correlative inhibition and rest and reduced the effect of environmentally imposed dormancy. This effect could also be attributed to proximity of the bud to a pruning cut.

It was hypothesised that wound-induced ethylene breaks bud dormancy and thereby promotes the burst of buds proximal to a pruning cut. A single application of Ethrel mimicked the effect of pruning but repeated application delayed bud-burst suggesting that an initial burst of ethylene produced following cutting overcomes bud dormancy but prolonged exposure to the gas inhibits subsequent growth. Quantitative measurements of ethylene production from segments and intact or pruned shoots supported the above hypothesis.

The promotion of bud-burst and the production of wound-induced ethylene are most pronounced during the period when pruning breaks rest. It is therefore proposed that the hastening of bud-burst following dormant pruning results from the breaking of rest by wound-induced ethylene.

The implications of these findings, with respect to our understanding of the physiology of pruning effects on shoot growth, are discussed.

STATEMENT

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

(RICHARD RANDOLPH WILLIAMS)

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LIST OF ABBREVIATIONS

The following abbreviations have been used in this thesis:

ABA	Abscisic acid
B.D.H.	British Drug Houses
^{o}C	Degrees Celsius
C.I.G.	Commonwealth Industrial Gases Ltd
cm	Centimetre (s)
c.v.	Cultivar
fc	Foot Candles
GA	Gibberellic acid
G.L.C.	Gas-Liquid chromotography
gm	Gram (s)
hr	Hour (s)
M	Molar
max	Maximum
min	Minute (s)
ml	Millilitre (s)
mm	Millimetre (s)
N	Normal
nl	Nanolitre (s) (10^{-9} litres)
ppm	Parts per million (μg per gm)
'Tween 20'	Polyoxyethylene (2) sorbitan monolaurate
μl	Microlitre (s) (10^{-6} litres)
v/v	Proportions by volume

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The cyclic nature of the growth of deciduous, woody trees in temperate climates is readily apparent. A period of active growth and accumulation of photosynthate reserves alternates with a period when activity is limited to slow development of the next season's shoots within the buds, drawing on the reserves of carbohydrates previously accumulated within the plant. Even during the active growth period the distribution of growth and utilization of reserves changes from early leaf production and stem elongation, to fruit growth, then root growth, stem thickening and accumulation of reserves. Each of these phases of growth involves a change in the pattern of plant development. It is of fundamental importance to the study of woody plant growth to understand both the control of development during each growth phase and the regulation of changes from one phase to the next.

One of the most dramatic of these phases is shoot growth. This involves the change in development from the dormant phase to the non-dormant phase leading to active growth of the new shoot axis within the bud. This change is associated with the warmer temperatures and increasing daylength of spring and is usually dependent on the prior occurrence of suitable winter (chilling) conditions for completion of the dormancy phase. In terms of shoot morphogenesis this change involves the resumption of activity in the shoot's cambium, mobilization of stored reserves in the stems and a change from a low level of activity, (mainly cell division and differentiation), within the bud to rapid growth, predominantly by cell expansion.

Since the whole shoot system of a tree is derived from the expansion of shoots from individual buds of successive growth cycles, it is useful initially to study the growth from a single bud to form a shoot. Attention is focussed on the lateral bud.

A lateral bud may be defined as a primordial or unexpanded form of a lateral shoot enclosed by bud scales. It represents an intermediate stage in the development of a lateral shoot between the initiation and differentiation of a lateral shoot axis and the expansion of this axis into a lateral shoot. The word "bud" will be used to describe the morphological entity while "bud phase" will be used ontogenetically to describe the period of shoot growth and development between initiation of bud scales and the emergence of the growing shoot from within the bud (bud-burst). This includes the formation of the bud, its transition into the dormant state, the dormant state and the subsequent release from dormancy and resumption of active shoot growth.

The boundaries of the bud phase are not distinct. There is a transition from the stage of leaf formation to that of scale formation and this transition is associated with a general slowing down of shoot growth. Similarly there is a transition between the end of bud dormancy and the emergence of the shoot. Likewise, distinction between the stages of bud development within the bud phase are largely arbitrary (see Section II B 1).

The final contribution of an individual bud to the total shoot system depends not only on factors regulating its growth directly but also on the influence of concurrent development in other parts of the shoot system, including other buds. The balance between shoot elongation and increase in girth and between shoot and root growth also have an influence. The horticultural practice of pruning modifies shoot growth by removal of part of the shoot or root system. Growth and development of the remaining buds may be modified both in time and form.

The object of this study was to examine the influence of manipulation of a shoot and its environment on the development of individual lateral buds located on the shoot, particularly the regulation of changes between dormant and non-dormant states in the bud, with special reference to the effects of pruning. Apple trees and rootstock cultivars (*Malus* species) were used in this work because of their horticultural significance and because they produce one-year-old, long-shoots on which few lateral buds elongate during the first growth season thereby providing a good source of lateral buds.

II. A REVIEW OF BUD DORMANCY

A. THE SCOPE

This work was a study of lateral bud development on individual apple shoots in response to pruning treatments. This involved the release of lateral buds from their otherwise dormant state. The aim of the review is to provide background information on the phenomenon of bud dormancy.

Although the main emphasis is on the release of bud dormancy and the resumption of lateral shoot growth, the earlier phases of bud formation and the induction of dormancy are also reviewed because a knowledge of these is essential to an understanding of the regulation of bud dormancy. Where possible published reviews provide the basis for discussion and specific references are included only where a particular viewpoint has not been adequately covered in these reviews.

The topics are discussed in general terms with specific information on apples included where available. The role of plant growth regulators is not discussed comprehensively. (They are dealt with where they are thought to play a mediatory role in the responses to the factors controlling bud dormancy). This aspect of the subject has been covered in detail by numerous reviews (Khan 1975; Kozlowski 1971; Lavee 1973; Phillips 1975; Rubinstein and Nagao 1976; Smith and Kefford 1964; Walker and Seeley 1973; Wareing 1969; Wareing and Saunders 1971). Discussion of the literature pertaining directly to the experimental work has been included in the relevent section of the experimentation chapter.

B. THE DORMANT STATE

B1. The Concepts of Bud Dormancy

The concepts of dormancy and its regulation have been reviewed by a number of authors (Kozlowski 1971; Romberger 1963; Samish 1954; Smith and Kefford 1964; Vegis 1964; Wareing and Saunders 1971). The following presents a consensus of these reviews and defines the terminology adopted in this thesis.

The term dormancy is used in its broadest sense to mean a lack of visible activity. A dormant bud is one which has not burst i.e. the foliage leaves have not protruded through the bud scales.

There are many factors which may prevent bud-burst. Some arise as a direct response to unfavourable environmental conditions such as extremes of temperature, an inadequate light regime or a lack of water or nutrients available to the roots. Others result from physiological conditions within the plant such as the production of inhibitors or the inadequate supply of essential growth factors, whether by overall reduced levels or by differences in distribution or availability. There may be interactions between environmental conditions and the physiological processes within the plant.

Although there may be interaction between the factors causing bud dormancy they still provide a basis for the classification of the types of formancy which may occur (see Table 1). In the past some confusion has been caused by the use of different nomenclature for the types of dormancy.

TABLE 1 CLASSIFICATION OF THE TYPES OF BUD DORMANCY

BUD DORMANCY

When a bud does not undergo visible growth leading to bud-burst.

ENVIRONMENTAL DORMANCY	PHYSIOLOGICAL DORMANCY
When a bud does not grow because of unfavourable prevailing environmental conditions.	When a bud does not grow under favourable environmental conditions i.e. the cause lies within the plant.

CORRELATIVE INHIBITION	REST
When the source of physiological inhibition arises from outside of the bud	When the bud is dormant because of the conditions within the bud itself.

WINTER DORMANCY is the long period of bud dormancy from the end of the summer growth period through to bud-burst in spring.

SUMMER DORMANCY includes periods of reduced growth during the growth season, often under environmental conditions favourable for growth and not involving rest.

Romberger (1963) reviewed this nomenclature and presented a table of the synonyms used by various authors. In this thesis the nomenclature used corresponds to that adopted by Romberger (1963) with one exception; the term quiescence is replaced by environmental dormancy. This term was considered more appropriate because this type of dormancy is imposed by the environment and does not originate within the plant. In contrast to environmental dormancy there is physiological dormancy in which the inhibition of bud growth arises within the plant. Care must be taken to distinguish between dormancy due to prevailing unfavourable environmental conditions and that which has been induced by previous exposure to inductive conditions. Dormancy is said to be physiological when it is caused by physiological conditions which persist under environmental conditions suitable for growth, even though these physiological conditions were, in some cases, induced by environmental factors.

Physiological dormancy may be further divided into two categories on the basis of where the inhibition of bud growth arises within the plant (Table 1). Where the inhibition arises from tissues outside of the bud it is called correlative inhibition; this includes the particular phenomenon of apical dominance. When bud-burst is prevented by physiological conditions within the bud itself the bud is said to be in the state of rest.

If these categories of bud dormancy are to have physiological significance they must be experimentally demonstrable.

Various methods have been used to determine the state of dormancy on experimental material the most common being the observation of bud-burst on detached shoots placed in an environment known to permit bud growth. This method was first reported by Krasan (1873) and Askenasky (1877) and later used for apple (Eggert 1951; Howard 1910; Konstantanov 1972) and peach (Corgan and Peyton 1970; El-Mansy and Walker 1969; Hendershott and Walker 1959) and various other species (e.g. Bachelard and Wightman 1973; Hewitt and Wareing 1973; Kefeli and Turetskaya 1965). Other methods include the response to applied gibberellic acid (Hatch and Walker 1969), measurement of electric potentials (Filinger and Cardwell 1941), the location and density of calcium oxlate crystals (Chirilei and Molea 1970), the observation of the state of plasmolysis of the tissues (Konstantinov 1972) and the determination of ^{32}P uptake into the buds (Semin and Madis 1964). None of these methods are very satisfactory.

The use of observations made on whole shoots is considered unsatisfactory for the determination of lateral bud dormancy status because it does not take into account the involvement of correlative inhibition (see Section III D 1). The response to gibberellic acid cannot be used as a general test since no response was obtained with apple (Hatch and Walker 1969). In other methods cited above no distinction may be made between rest <u>per se</u> and the accompanying development of cold hardiness. Changes in ^{32}P uptake by the buds may be associated with a period of dormancy but may not

necessarily involve rest. The elucidation of a suitable method of assessing the state of dormancy of lateral buds is reported in Section III D.

The above categories of dormancy are not mutually exclusive. A particular bud may be subject to more than one type of dormancy at the same time. Environmental dormancy and correlative inhibition may act synergistically in the development of dormancy, particulary in the establishment of rest. Furthermore there is usually a progression from the predominance of one type of dormancy to the next.

During the annual cycle of shoot growth of temperate species there is a long period of bud dormancy from the end of the summer growth period through to bud-burst in spring. This is referred to as winter dormancy and it is the part of the cycle to which this thesis mainly relates. However, even during the spring and summer months shoot growth is not usually continuous. There are periods when shoot growth ceases, and commonly a terminal bud is formed, but this is only of short duration and is followed by another flush of growth. Such periods of dormancy are called summer dormancy. This differs from winter dormancy in that rest does not occur and the prevailing environmental conditions are generally favourable for shoot growth (although inhibitory high temperatures may be involved as discussed in Section II C 2). Winter dormancy includes a sequence of phases in which correlative inhibition, environmental dormancy and rest differ in their contribution to the overall state. Summer dormancy is predominantly due to correlative inhibition.

B2. The Pattern of Dormancy Development

The generally accepted concept of winter bud dormancy proposes that dormancy is initiated by correlative inhibition of buds by the expanding leaves or by correlative inhibition arising from competition for a limited supply of some growth factor and in the case of lateral buds, by the additional effect of apical dominance. This correlative inhibition results in the formation of the bud structure within which primordia production and differentiation at the shoot apex may continue without the rapid expansion involved in shoot growth. Environmental conditions may contribute to the commencement of winter dormancy both by inductive processes and by direct inhibition of shoot growth leading to bud formation.

After formation of the first bud-scale commences the development of subsequent primordia is modified so that a series of bud-scales and scale-like appendages is produced before the apex resumes the production of leaf initials. This developmental sequence is not irreversible and is dependent on the maintenance of the inducing conditions. Manipulation of the plant (Dostal 1952; Fulford 1966, 1970; Goebels 1905) or the environmental conditions (Abbott 1970) may cause a cessation of scale formation and a resumption of shoot growth.

Following bud formation and in response to physiological and environmental factors, the activity of the apical meristem is suppressed and the bud enters the state of rest.

At this stage removal of the sources of correlative inhibition and environmental dormancy does not release the bud from dormancy. Only after exposure to particular environmental conditions, usually chilling temperatures, is this physiological block to bud development removed and then the bud may resume active growth and development if correlative inhibition and unfavourable environmental conditions do not prevail.

C. THE IMPOSITION OF DORMANCY

C1. Correlative Inhibition

Correlative inhibition plays an important role in the regulation of dormancy in both terminal and lateral buds. This inhibition may arise from the leaves and from other buds or active shoot apices. It may involve the production of an inhibitor of shoot growth, reduced production of a growth promoting factor or an inadequate supply of some growth factor to the bud because of preferential transport to other growth centres.

The term correlative inhibition refers specifically to an inhibition arising in one part of the plant and affecting another but there are some physiological factors, such as a low root : shoot ratio, nutrient deficiencies or a high water deficit, which result in a more general inhibition of shoot growth. These are not considered separately because they are important mainly as modifying influences on the general regulatory mechanisms in the plant and they will be referred to in relation to the mechanisms they affect. For example, a low root: shoot ratio may result in an inadequate supply of root produced factors required for shoot growth. This will enhance the effect of correlative inhibition between buds because they must compete for this limited resource.

The leaves play a major role in the regulation of bud development and the imposition of dormancy in both terminal

and lateral buds. Fulford (1965, 1966, 1970) has studied the relationship between leaves and the growth and development of the apex on apple shoots. He proposes a scheme in which successive leaves and foliar organs exert an inhibitory influence on the younger primordia developing at the apex and thereby regulate the sequence of appendages produced. This sequence of correlative inhibition regulates the rate of primordia production and differentiation.

The eventual cessation of visible growth at the shoot apex and the establishment of the dormant state, may be due to correlative inhibition of the bud by mature leaves. When the leaves are exposed to inductive environmental conditions, usually decreasing daylength (Wareing 1954), they produce an inhibitor which has been found to accumulate in the bud (Eagles and Wareing 1964; Kawase 1961a, b; Phillips and Wareing 1959; Varga 1957). This inhibitor was defined as "inhibitor B" by Bennet-Clark and Kefford (1953) on the basis of paper chromotography and the main component has been identified as ABA (Cornforth, Milborrow, Ryback and Wareing 1965; Milborrow 1967). However the more recent use of G.L.C. for ABA determinations has raised doubts about the validity of the earlier work based on less specific bioassays (Alvim and Saunders 1974; Harrison and Saunders 1975; Hillman, Hocking and Mc Wha 1974; Lenton, Perry and Saunders 1972). None of these workers found a correlation between ABA levels and bud dormancy.

Lenton et al. (1972) suggest that the activity of the inhibitor may depend on other factors, the production of which is reduced when the leaves are exposed to short days. A gibberellin is a likely candidate (Bowen 1969; Brian 1957) as it has been shown to antagonise the ABA inhibition of buds (Wareing 1969). Harrison and Saunders (1975) have demonstrated a correlation between the ratio of free ABA : esterified ABA and the degree of dormancy in Birch buds (as indicated by the time taken for bud-burst under long days at 20°C) and they suggest that this ratio may be important rather than the absolute levels of ABA. Possibly GA mediates the inhibitory effect of the ABA present in the buds by influencing the formation of the ABA ester? There is also some evidence to suggest that ABA may reduce the levels of GA_3 present, possibly by blocking its synthesis (Wareing 1969), which emphasises the complexity of the interaction between these hormones. Khan (1975) has elaborated on the concept of the interaction between inhibitors and various promotors present such that absolute levels of these substances are less important than their overall balance.

Correlative inhibition arising from the leaves also plays an important role in lateral bud dormancy. The differentiation of a lateral meristem appears to be dependent on the presence of a subtending leaf primordium (Garrison 1955) but after it has been established the subsequent growth of this axillary meristem is suppressed by the subtending leaf. This inhibition of the newly established (lateral) shoot meristem presumably regulates bud formation. It is not known when the leaf first

exerts its inhibitory influence on the axillary shoot but in apple Barlow and Hancock (1962) have shown that the first scale-like appendages have been completely determined in the axils of very young leaves suggesting that bud formation has been initiated before this stage.

In the presence of the subtending leaf the axillary meristem forms an axillary bud. This bud usually remains dormant unless the leaf is removed either artificially or by natural senescence. If the shoot is defoliated before rest is established the lateral bud may burst but on most shoots it is prevented from doing so by apical dominance. By the time of natural leaf fall the bud is dormant because of rest.

Leaves also contribute to the regulation of shoot growth and development by the supply of essential growth factors, nutrients, substrates and hormones. Young leaves are thought to be the main source of gibberellins required for internode elongation and leaf expansion (Barlow and Hancock 1956b; Lockwill 1968). They may also regulate the distribution of root factors particularly cytokinins (Skene 1975). The net contribution of leaves to the developing apex will depend on the balance between these growth promoting substances and the inhibitors mentioned earlier. This balance may be determined by the age of the leaf and the environmental conditions to which they are exposed. This is an example of the interaction between correlative inhibition, environmental factors and the developmental sequence of the shoot.

Correlative inhibition between shoot growing points is the other main form of correlative inhibition. This may be

considered at two levels; the competition between growing points on different shoots, i.e. between shoots and the balance of growth between buds on the same shoot. Both these forms of correlative inhibition have been referred to as apical dominance but this term will be used here to refer specifically to the suppression of lateral buds by the terminal (distal) bud.

Competition between shoots arises from the limited supply of essential growth factors within the plant particularly from the roots. When the plant has an adequate supply of nutrients and water this competition is reduced and more buds develop into shoots. A particular shoot may gain greater access to the supply of growth factors by virtue of its position or orientation within the tree (Mullins 1967; Mullins and Rogers 1971) or its distance from the roots (Maggs 1964a; Wareing and Nasr 1961) or the apex (Mullins and Rogers 1971). In addition there may be competition between shoot elongation and fruit growth or leaf expansion. A balance between the supply of growth factors from the roots (and hence root activity) and shoot growth also exists.

Phillips (1975) has recently reviewed the subject of apical dominance including a discussion of the nature of the correlative signal and its mode of action plus a section on the arrest of bud development. Rubinstein and Nagao (1976) have reviewed the literature with an emphasis on the control mechanism involved in the release of lateral buds of Vicia faba from correlative inhibition. The relevent aspects of these reviews are outlined here.

Apical dominance is readily overcome by removal of the apical bud although usually the distal remaining bud eventually assumes the dominant role. Interruption of the continuum of cells along the stem (by girdling or heat girdling) releases the buds below from apical dominance suggesting that the inhibitive signal passes along the shoot via the outer tissues. There is usually some reduction in the growth of buds distal to a ring but this is not sufficient to support the idea that lower buds are stimulated by greater availability of growth factors which are otherwise utilized by the distal growing point. This, plus other evidence cited by Phillips (1975), suggests that nutrition plays a secondary role in the correlative inhibition associated with apical dominance.

The primary signal in apical dominance appears to be the basipetal passage of auxin produced by the shoot apex. Application of auxin to decapitated stems usually replaces the apex in suppression of lateral bud growth and treatments which are considered to specifically inhibit basipetal transport of auxin reduce apical dominance. After a consideration of the evidence for and against the direct involvement of other endogenous growth regulators (GA, cytokinins, ABA) Phillips (1975) concludes that these only play secondary roles in apical dominance. Auxin acts as the primary signal and may subsequently stimulate the supply of other factors to the buds, particularly cytokinins, or it may interact with other hormones present in the bud (Khan 1975).

The extent to which apical dominance suppresses lateral bud growth varies between species (see Phillips 1975) but in apple most lateral buds are inhibited by the combination of apical dominance and leaf inhibition (discussed earlier) at least until their second growth season. In some instances buds near the base of a shoot burst in the first growth season, particularly following defoliation, suggesting that apical dominance is reduced. This is considered further in Section II D. As with other forms of correlative inhibition, environmental factors may have a modifying influence on apical dominance. This may be brought about by modification of the balance of hormones within the plant or the overall supply of growth factors. Also the imposition of dormancy on the apical bud may reduce its dominance.

C2. Environmental Factors

Studies of the influence of environmental factors on bud growth and development have been concerned almost entirely with the terminal bud. References to lateral buds are only indirect and usually involve modification of correlative inhibition and apical dominance (reviewed by Phillips 1969; Rubinstein and Nagao 1976). Usually short days promote lateral bud development and temperature may interact with photoperiod (see Phillips 1969). There is also an interaction between the supply of nutrients and apical dominance (Phillips 1975; Rubinstein and Nagao 1976).

The lack of information on the effects of environmental factors on lateral buds may be attributed to the fact that such buds are prevented from responding to changes in the environment because of correlative inhibition. However terminal buds are not subject to this restriction and since the development of lateral buds is analogous, apart from the origin of the apical meristem, studies made on terminal buds may be relevent to lateral buds and these are reviewed here.

Photoperiod has been widely acclaimed as an environmental factor controlling the formation of resting buds (Eagles and Wareing 1963; Nitsch 1957; Wareing 1956). In many species the formation of terminal buds is promoted by exposure of the leaves to short days (Wareing 1954, 1956). As little as 2-3 short days has been found to reduce shoot growth of Norway Spruce whilst growth stopped completely after 4 short days (Heide 1974) and Birch and Alder required only 12-13 short

days to induce terminal bud formation (Hillman *et al.* 1974). In *Betula pubescens* (Wareing 1954) and *Cornus florida* (Waxman 1957) this effect may be attributed to an inhibitor arising from the leaves exposed to short days rather than a reduced supply of some promotor.

The nature of the response in apple varies with the quality of light used. Garner and Allard (1923), using natural light, reported that shoot growth in general appears to be greater under intermediate daylength (10 hrs). Stahly and Piringer (1952) found that main shoot length was reduced under short days but that total growth was increased because of greater lateral shoot growth. Fluorescent supplementary light was ineffective but supplementary incandescent light to give 16 hours daylength, increased the main shoot length (Piringer and Downs 1959; Visser 1956). Thus short daylengths favour reduced shoot extension growth which is probably associated with earlier terminal bud formation (Waxman 1957).

Although terminal bud formation may be influenced by controlled light periods, under field conditions terminal bud formation may commence well before daylength declines (*Acer pseudoplantanus*, Phillips and Wareing 1958) or elongation growth may continue until the short days of autumn (*Salix* and *Populus*, Wareing and Saunders 1971). Similarly, in apple terminal bud formation commences during summer while the days are still long. Thus it would appear that photoperiod is not a major factor in regulation of terminal bud formation although, as discussed later, it may have an important role in the induction of rest subsequent to bud formation.

Another environmental factor which may regulate shoot growth and hence terminal bud formation is temperature. Most metabolic and physiological processes within plants may be influenced by temperature therefore the responses of plant growth to temperature are numerous. Apart from the injurious effects of extremes of temperature on plant growth (reviewed by Kramer and Kozlowski 1960; Levitt 1969; and Kozlowski 1971) normal fluctuations in temperature may regulate tree growth. Bud-burst in spring may be dependent on both the prior occurrence of chilling temperatures during winter dormancy and the prevailing temperatures at the time of bud-burst (see Section II D 2). The rate and duration of shoot growth is influenced not only by the prevailing and cumulative effects of temperature but also by its range and periodicity.

Studies of the influence of temperature on the shoot growth of trees (Pinus, Quercus, Pseudotsuga) have shown that although high daytime temperatures increase growth rate and high night temperatures reduce growth rate, maximum growth rate is more a function of the day-night temperature differential (Kramer 1957 and Hellmers 1962). Increased temperature, either day or night, was also found to increase the number of growth flushes i.e. there was a greater tendency to form terminal buds although the buds did not remain dormant (Hellmers 1962). Pollock (1953) and Vegis (1956) suggested that exposure to high summer temperatures, especially warm nights, may lead to terminal bud formation but development of rest requires prolonged exposure.

More recent work has also suggested a strong correlation between cessation of shoot growth and temperature. In Eucalyptus regnans grown in a cool-temperate climate, shoot extension growth was correlated with mean maximum weekly temperatures and was found to be essentially continous except for a period of quiescence imposed by low temperatures during winter (Cremer 1975). Conversely E. obliqua seedlings grown in a warm environment (constant temperatures) entered temporary quiescence when subject to high temperatures during summer (Blake 1976).

There appears to be little information available on the effects of temperature on terminal bud formation in horticultural species. Williams (1959) found that shoot extension growth of Raspberry (Rubus idaeus L.) was more dependent on temperature than photoperiod. At warm temperatures (70°C) extension growth was similar under short days (9 hrs) or long days (14 hrs) and at 50°C growth ceased regardless of photoperiod but at 60°C, short days resulted in earlier cessation of shoot growth. Hancock and Barlow (1958) investigated the relationship between daylength, accumulated mean temperature and the growth of apple shoots. Daylength and temperature had a synergistic effect on shoot growth rate but they found no correlation between these factors and final shoot length.

Thus the precise contribution of temperature and photoperiod to terminal bud formation is not clear. In situations where trees are subject to long, hot summers terminal bud formation may be induced by the high (night) temperatures. If these

temperatures continue the bud may persist and eventually enter rest. A break in the temperature may often result in a flush of growth as is often observed. Where the temperature falls prior to the onset of rest low temperatures may contribute to the subsequent induction of rest (Piringer and Downs 1959). The formation of winter terminal bud is usually under way before daylength declines but it is possible that where moderate temperatures occur late into autumn the reduction in daylength may contribute to the cessation of shoot growth.

The supply of nutrients and water available to the plant roots has a marked effect on shoot growth and bud formation. High levels of nutrition reduced the effects of apical dominance (Loeb 1924) particularly an ample supply of nitrogen (Gregory and Veale 1957). Low soil moisture may reduce shoot growth (Goode 1956) and it has been claimed that internal water stress is a prime cause of growth cessation in apple (Kovalev, Gluščenko and Tupicyn 1956). Irrigation to relieve water stress increases shoot number rather than length (Goode 1960) which suggest that bud dormancy has been released. However, as discussed by Browning (1973), the response to water stress is dependent on other conditions prevailing within the bud so this effect may be indirect.

C3. Rest

The factors which regulate the induction of rest are not distinct from those already discussed above. Entry into rest is preceeded by a period of dormancy due to correlative inhibition or environmental dormancy and the bud becomes progressively less able to respond to manipulative treatments which remove these external sources of growth inhibition. Changes occur within the bud, physiological and morphological, which eventually prevent the bud from actively growing even when the external constraints are removed; the bud is then in the state of rest.

There are three main aspects of the rest mechanism (Wareing 1969) although these are not mutually exclusive:

(1) The restriction of gaseous exchange caused by the bud scales (Vegis 1964).

(2) Hormonal control based on the interaction between growth promoters and inhibitors.

(3) Control of gene activity at the molecular level (Bonner 1965).

Hypotheses based on restricted gaseous exchange presuppose the existence of the bud structure. The regulation of bud formation by correlative inhibition and imposed dormancy has already been discussed. It may well be that gaseous exchange, particularly the restricted supply of oxygen, may limit or modify metabolic activity at the enclosed meristems and thereby contribute to rest induction. At higher temperatures anaerobiosis may inhibit bud activity or may lead to

inhibitor production (Pollock 1953). Wareing (1969) doubted the importance of the bud scales in controlling rest because reports of scale removal stimulating bud growth often do not clearly establish that the response involved rest rather than other types of dormancy but recent work on Tung (Spiers 1972) and Douglas-fir (Roberts, Tomasovic and Fuchigami 1974) indicate that scale removal does break rest. However scales may inhibit bud growth because of their inhibitor content, whether imported from the leaves under short days (Eagles and Wareing 1964) or produced de novo (Pollock 1953). Alternatively they may act as competitive sinks for growth factors (cytokinins from the roots- Abbott 1970).

Current theories on the hormonal control of rest (Lavee 1973; Wareing and Saunders 1971) are based on the interaction between growth promotors and inhibitors (Section II C1). The responses to exogenous hormones and the correlations between the balance of endogenous hormone levels and the development of dormancy leave little doubt about their regulatory involvement. However the hormonal balance within the plant appears to be in a continuous flux and the primary control of rest cannot be ascribed to a particular hormonal relationship. Also the high levels of inhibitors which have been associated with rest occur mainly within the bud scales rather than in the tissues which are inhibited suggesting that their role may be indirect.

Rest involves the suppression of growth of the shoot axis within the bud. This must involve suppression of one or more

of the following; cell division, cell elongation or metabolic processes pre-requisite to growth. Clearly we cannot expect to explain the mechanism of the control of rest until we know how these basic processes are regulated. Perhaps the nearest we have come to this is the involvement of hormones in the regulation of DNA and RNA and hence gene expression. Khan (1975) and Lavee (1973) have reviewed some of the evidence for an interaction between hormones and nucleic acid activity in relation to dormancy development and Lavee (1973) presents a scheme of the general pattern but considerably more information is needed to complete this picture.

Since neither restriction of gaseous exchange nor a specific change in the hormonal balance have been shown to control rest, it would seem that these processes enable the development of rest by regulating bud dormancy in relation to correlative inhibition and induced dormancy, rather than by a direct participation in rest. The concept of reversible and irreversible processes in dormancy development, proposed by Smith and Kefford (1964) is consistent with such a situation. They suggest that correlative inhibition and environmental dormancy involve reversible processes since bud activity may resume following the removal of their influence but whilst a bud is suppressed by these factors some irreversible processes occur which, if allowed to proceed for a sufficient time, eventually lead to rest. If this is so the induction of rest is a consequence of the dormant state caused by correlative inhibition or environmental dormancy rather than a direct response to

specific regulatory factors. Furthermore, the mechanism of rest control must be sought at the level of the regulation of cell growth and development. There are several possibilities; the control of gene expression (mentioned above), changes in the nature of cell constituents e.g. proteins (Perry 1963; Van den Born 1963) or membranes (Wurzburger and Farkash 1976), changes in the endogenous production of hormones by the tissues entering rest or most likely, by a combination of these. Whichever of these mechanisms are invoked they must be consistent with the nature of the treatments which have been shown to break rest (Section II D1).

D. THE RESUMPTION OF GROWTH

When considering the resumption of shoot growth after a period of dormancy one must first distinguish which types of dormancy are involved. It has already been pointed out that a bud may be subjected to one or all of the types of dormancy at a particular time. Obviously when environmental conditions are favourable and rest has not been imposed, the resumption of growth will depend on the removal of correlative inhibition. At other times environmental dormancy may be the limiting factor although this is largely confined to the end of winter dormancy because at other times rest and correlative inhibition are usually involved too. Whatever the case, bud-burst only occurs after all forms of dormancy have been overcome, thus the termination of one particular type of dormancy may not be followed by bud-burst.

The factors which regulate the resumption of growth are discussed in relation to the types of dormancy they influence. A number of authors have reviewed this phase of the shoot growth cycle (Khan 1975; Phillips 1975; Rubinstein and Nagao 1976; Smith and Kefford 1964; Wareing and Saunders 1971) but there is a paucity of information on the processes involved in the resumption of bud growth, particularly the sequence of events following the release of dormancy.

D1. Termination of Rest

Once the state of rest has been established bud growth can only be resumed when rest has been broken, usually by exposure

to particular conditions which in themselves are not conducive to bud growth but which modify the physiological conditions within the bud to overcome the inhibition of growth. Under natural conditions rest is usually overcome by exposure of the bud to chilling temperatures (less than 10°C) for a minimum period of time which varies widely between species and varieties (Howard 1910; Wareing 1969). For apple the chilling requirement is between 500 - 3000 hours depending on the temperature with 2°C being the optimum (Thompson, Jones and Nichols 1975). The effect of chilling may be partially nullified by intervening periods of non-chilling conditions (Chandler, Kimball, Phillip, Tufts and Weldon 1937; Vegis 1964). In some species termination of rest may be promoted by long daylengths e.g. Fagus sylvatica, Betula pubescens and Larix decidua (see Wareing 1969) whilst exposure to water stress has been effective in others e.g. Coffea arabica L (Browning 1973).

The mechanism of breaking rest cannot be enunciated until we understand the mechanism of rest in buds but several general changes in bud activity have been associated with the end of rest. The effect of chilling has been attributed to the reduction in the level of endogenous inhibitors (Hemberg 1949; Phillips and Wareing 1958) although there is not always a correlation between the depth of dormancy and inhibitor levels (Dennis and Edgerton 1961; Pieniazek 1964) and observed correlations may not indicate a causal relationship (Perry and Hellmers 1973). Endogenous levels of gibberellins may increase during chilling (Eagles and Wareing

1963; Smith and Kefford 1964; Vegis 1964). Laties (1957) and Pollock (1960) reported increases in respiratory activity following the end of rest and various other metabolic changes have been reported (Bachelard and Wightman 1973; Smith 1954; Usciati, Codaccioni and Guern 1972). Abbott (1970) suggests that enhanced senescence of bud-scales at low temperatures may be the mode of breaking rest whilst Coville (1920) suggested that changes in membrane permeability could be important.

It is not clear which of the above effects are directly involved in the end of rest and which occur subsequent to it largely because of the problem of determining the precise time at which rest ends. Obviously any treatment which stimulates bud-burst during the rest period must also terminate rest but care must be taken to establish that the buds were actually at rest. This problem is discussed further in Section III D.

D2. Environmental Factors

The involvement of environmental factors in the induction of dormancy and the release of rest has been discussed (Sections II C2 and II D1 respectively). This section refers specifically to environmental dormancy where the prevailing environment has a direct effect on the bud.

Temperature appears to be the major factor in the imposition of environmental dormancy in buds. Bud-burst time at the end of winter dormancy has been closely correlated with the prevailing air temperatures (Kozlowski 1971). This appears to be a direct effect of temperature on shoot growth within the bud. All growth processes are influenced by temperature and during the winter months the temperature is often low enough to prevent bud growth, even when all other factors are favourable. Thus, although rest may be broken in early winter, buds do not grow until spring because of environmental dormancy. This fact has been established at least for terminal buds (discussed in Section III D) by the observed stimulation of bud-burst on dormant twigs or intact plants placed in a warm environment during the latter part of the period of winter dormancy.

After the end of rest, bud growth and development may proceed when the temperature is high enough. The actual time of bud-burst will depend on the stage of development of the bud at any particular time as well as the prevailing temperature. Thus the use of the parameter time-to-burst in relation to different temperatures as an indicator of the degree of

rest (Vegis 1964) involves two factors; the effect of the prevailing temperature on the rate of growth of the shoot axis within the bud and the extent to which prior conditions have enabled bud development to proceed. This should be borne in mind when considering correlations between temperature and the time of bud-burst.

Vegis (1964) discusses at length, the relationship between temperature and the end of dormancy in seeds and buds. He indicates that the end of rest involves a period of increasing response to a wider range of temperatures. This is attributed to the need for a period of after-rest during which the factors inhibiting growth during rest are progressively removed. High temperatures during this time may induce secondary dormancy; the inhibition of shoot growth by high temperatures was discussed earlier in relation to dormancy induction (Section II C2). Low temperatures may simply slow down the rate of development. An alternative explanation of this effect may be that the sensitivity of the bud to temperature changes as the development of the new shoot axis progresses within the bud. This would be consistent with the need for a minimum degree of development within each phase of shoot development as suggested by Smith and Kefford (1964).

Bud-burst is not generally influenced by the prevailing light conditions since buds may burst in darkness (Pauley and Perry 1954; Wareing 1953, 1956), however long days do promote bud-burst in some species (Kramer 1936).

As mentioned above, soil conditions may influence bud-burst indirectly by their influence on root growth. However the supply of water or nutrients from the soil via the roots may have a more direct effect on bud growth. Water deficits within the bud, whether caused by reduced supply from the roots or increased losses by transpiration, may reduce cell methabolism and growth (Kozlowski 1971) and thereby reduce shoot expansion which is involved in bud-burst. On the other hand, if senescense of the bud scales is important in the release of rest (Abbott 1970), a period of water deficit may promote bud-scale senescence and thereby enhance bud-burst when the water balance is restored (i.e. an inductive process). This may provide an alternative explanation to the effect of water stress on bud break as found in Coffea arabica L. (Browning 1973).

The supply of nutrients from the soil or the supply of organic substrates within the plant may limit bud growth (Kozlowski 1971). This may occur under conditions of absolute deficiency but for carbohydrate at least, it would appear to involve mobilization and movement of these substances to the buds since the levels present in the wood are usually high prior to the resumption of shoot growth (Kozlowski 1962; Priestley 1962a). The availability of these factors may be influenced by the effect of temperature on their uptake, mobilization or transport.

D3. Correlative Inhibition

Correlative inhibition is present in some form during all stages of bud development although it may not be the primary cause of dormancy during rest. At the time of the resumption of growth following winter dormancy of deciduous species no leaves are present (apart from unexpanded leaves within the buds) therefore this type of correlative inhibition is not involved. Likewise competition between active growth centres is minimal until growth has resumed in some parts of the tree. Thus apical dominance and possibly correlative inhibition between buds are the main contributors to the correlative inhibition at the end of winter dormancy.

Under natural conditions apical dominance is rarely prevented entirely except where apical abortion occurs and even here the distal remaining bud assumes a dominant role. However the degree of apical dominance may be reduced under some circumstances e.g. high nutritional status, horizontal orientation of the shoot or when the apical bud is dormant (see Section II C1).

Correlative inhibition is particularly important in relation to the response of buds to manipulative treatments applied to plants, especially pruning. Such treatments remove the source of correlative inhibition. Other treatments such as bending or ringing may modify apical dominance, probably by changing the movement of growth hormones within the plant. This aspect will be considered further in the experimentation section (Section III C).

E. CONCLUDING REMARKS

The occurrence of different types of bud dormancy has been recognised and these have been defined but many workers investigating the physiology and control of dormancy have not clearly distinguished which type of dormancy they have been studying, particularly in lateral buds. This is largely due to the lack of a suitable experimental procedure for determining the types of dormancy prevailing in the buds at a particular time. In this work such a procedure was developed and it was used to determine the pattern of occurrence of different types of dormancy during the period of winter dormancy of lateral buds (Section III D).

In horticultural practice the main method of regulating the growth of a bud, and hence its release from dormancy, is by pruning treatments. There is a lack of information on the relationship between pruning treatments and the types of dormancy they influence. The following experimental sections reveal specific relationships between particular aspects of pruning treatments and the release of lateral buds from different types of dormancy.

III EXPERIMENTATION

A GENERAL MATERIALS AND METHODS

B THE DORMANT STATE

C THE IMPOSITION OF DORMANCY

D THE RESUMPTION OF GROWTH

E CONCLUDING REMARKS

II EXPERIMENTATION

A GENERAL MATERIALS AND METHODS

A1 INTRODUCTION

This section describes the three basic types of plant material used in the experimental work, small hedge-pruned, apple rootstocks, mature orchard trees and pot grown seedlings, the method adopted for the culture of detached shoots and segments and the three general types of environmental conditions employed (Sections A2, A3, A4). The types of pruning treatments used repeatedly and the parameters measured are defined (Sections A5, A6).

A detailed description of the techniques developed for the collection, accumulation and quantitative measurement of ethylene evolved from shoots is presented in Section A7. These represent the end-product of a series of developments and therefore do not pertain directly to all the results reported.

Finally, the general approach to statistical analyses is discussed in Section A8.

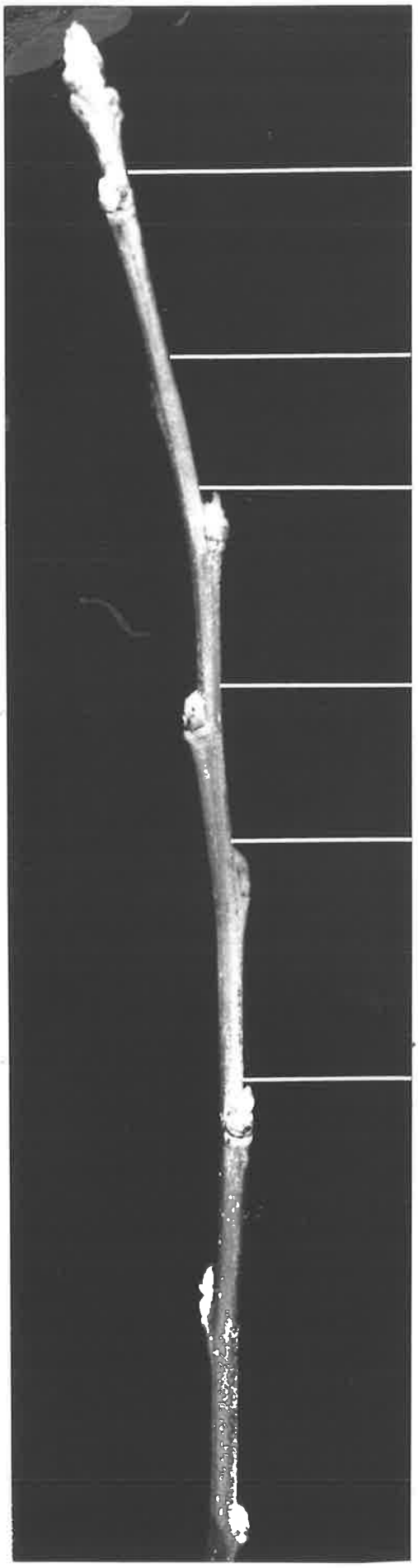


Fig. A 1

The position of buds sampled on one-year-old apple shoots.

The numbers indicate the designation used to refer to the bud position or the excised segment.

A2 PLANT MATERIAL

Apple rootstocks

These were growing in the Claremont Experimental Orchard at the Waite Institute. The planting consisted of a range of apple rootstock cultivars spaced 1.0 x 1.2 meters and planted in 1965. Since establishment they were maintained as small, hedge-pruned trees by heavy annual pruning. This results in the growth of many vigorous, new shoots each year, all with vertical orientation and arising at a similar distance (approximately 1 metre) from the base of the tree.

Mature Trees

The mature apple trees (c.v.Jonathan on various rootstocks) were part of an established planting in the Alverstoke Experimental Orchard at the Waite Institute. These trees were planted in 1963 and were not routinely pruned. Uniform shoots were selected from particular groups of trees.

Pot-grown Seedlings

Apple seed (c.v.Granny Smith) was removed from fruit obtained from local cold-stores (Lenswood Co-operative). About ¼ of each seed was cut from the end opposite the embryo and the seeds were imbibed in aerated tap water overnight before being germinated in a mixture of washed river sand + Perlite (50:50 v/v) at 20°C with 16 hrs light (fluorescent) per day.

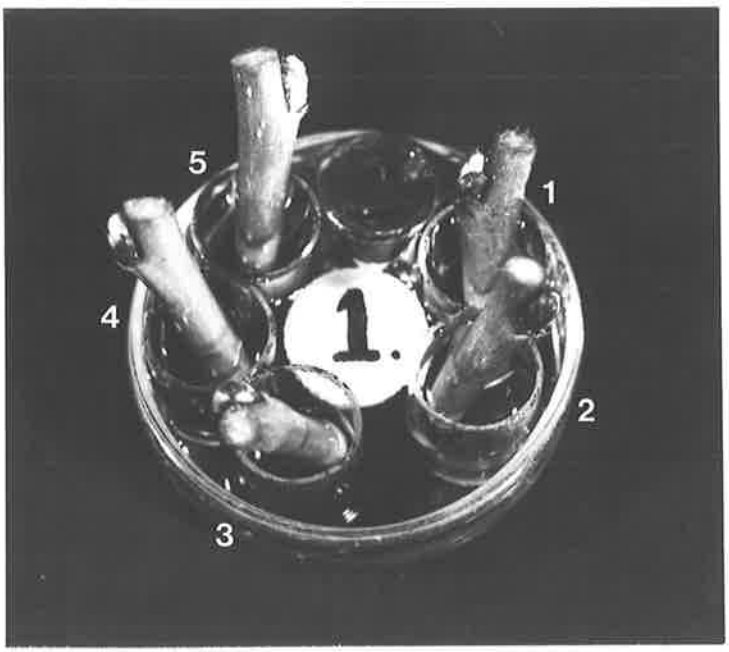
After the emergence of two true leaves (about 2 weeks after germination) the plants were transplanted into 15 cm plastic pots filled with John Innes potting soil, using a 2 cm layer

of course gravel to aid drainage. They were then kept in the glass-house except for those used in the Dormancy Studies (Section D III 5) where the plants were placed outside after 7 weeks in the glass-house.

Fig. A2 Culture of Segments.

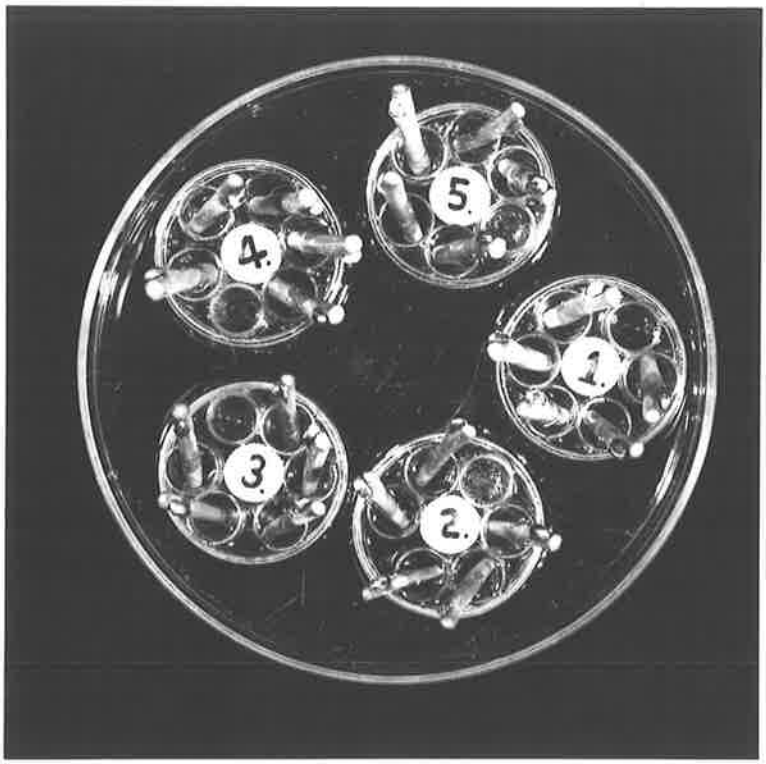
a One experimental unit:

Segments [1] – [5] from shoot (rep) 1.



b Unit receiving each treatment:

Segments from each of 5 shoots.



A3 SHOOT AND SEGMENT CULTURE

Shoots

Detached shoots were immediately defoliated and stood with their basal ends immersed in water. After the treatments were applied the shoots were cut to a uniform length then stood in de-ionised water in 250 ml beakers and covered with a clear polythene sleeve. The water was maintained above the cut shoot ends and was replaced after one week.

Segments

Shoots were detached and stood in water until used. Each shoot was cut into an apical segment plus a series of single-bud, internode segments each with the bud at the distal end (Figure A1). The apical segment was cut above the first lateral bud subtended by an internode longer than 1 cm. Each segment was stood in a 1 x 1 cm vial filled with de-ionised water.

For routine dormancy testing the distal 5 segments were used from each shoot and they were placed in the order of their position (clockwise) around a small petri dish (Figure A2 (a)). Each treatment unit included segments from 5 shoots (replicates from particular trees) which were enclosed in a covered 15 cm petri dish with water added to maintain a high humidity (Figure A2 (b)).

In other experiments the 1 x 1 cm vials were placed in 4 oz screw-top jars, with up to 8 segments per jar, or individually in glass vials, usually 5 x 2.5 cm, which were kept in a tray covered with polyvinyl chloride film (Glad Wrap).

A4 ENVIRONMENTAL CONDITIONS

Outside

Potted seedlings were stood on gravel in an open area sheltered from the wind. Detached shoots (in beakers with polythene covers) and segments (in petri dishes) were placed in an open shade-house.

Glass-house

The glass-house was heated by steam pipes and cooled by evaporative coolers to maintain a minimum temperature of 10°C with daytime temperatures in the range 20-25°C except on very hot days. Natural light was used for all experiments with partial shading during the summer months.

Growth Cabinets

Close control of temperature and lighting conditions was possible within growth cabinets (4 x 3 x 3.5 ft) using fluorescent light (approximately 2000 ft.c.) unless otherwise specified. Conditions used were combinations of 20-25°C continuous temperature with continuous/16 hrs per day light.

A5 PRUNING TREATMENTS

Decapitation[++]

Cutting off the terminal portion of the shoot, removing the terminal (apical) bud and small lateral buds which are not separated from the terminal bud by a node at least 5 mm long.

Pruning[++]

Cutting off the distal portion of a shoot including the terminal bud, some lateral buds, woody stem and leaves when present.

Disbudding

The removal of individual lateral buds by a single blade cut through the base of the bud.

Girdling[++]

The removal of a complete cylinder, either narrow or wide, of all tissues external to the secondary xylem (Noel 1970).

Partial Girdling[++]

The removal of an incomplete cylinder leaving the external tissues either directly above or above but on the opposite side to the bud.

++ All these treatments were carried out on the distal side of a node bearing a lateral bud and adjacent to the bud unless otherwise stated.

Fig. A3 *Single-bud Segments at Sampling and at Time of Bud-burst.*

a *Segments when cut.*

b *Segments at bud-burst.*

A6 ASSESSMENT OF BUD-BURST

Bud-burst

A bud was considered to have burst when the green tips of the new leaves were first visible through the bud-scales (Figure A3(b)). Observations were usually made daily and recorded for each bud.

Bud-burst Time

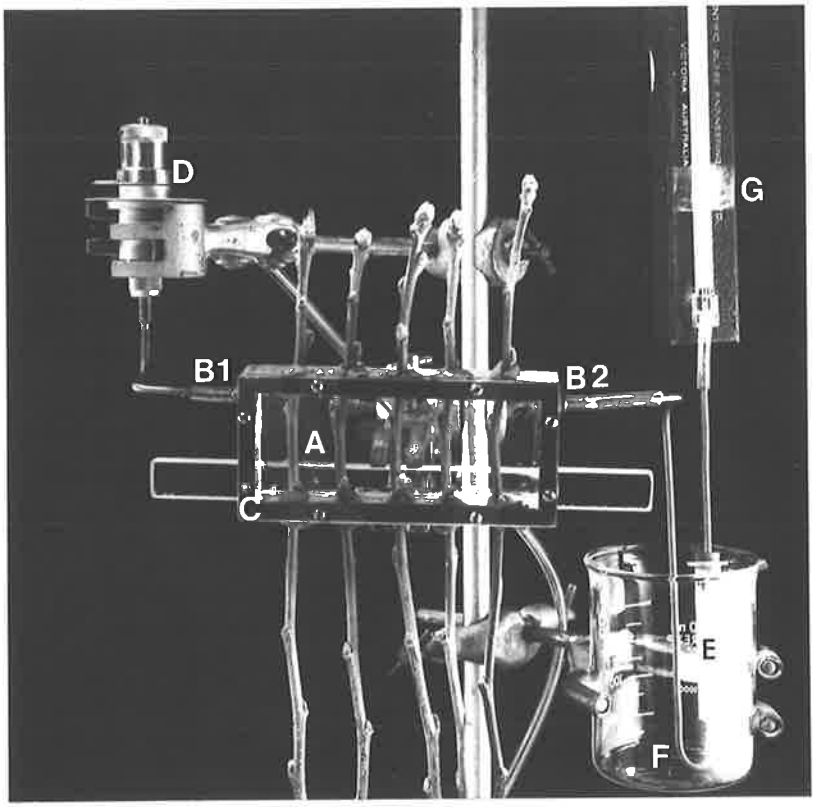
Bud-burst time was taken as the number of days between the application of a treatment and the occurrence of bud-burst. For small samples (usually 5 buds) the median burst-time was used because of the skewed distribution of individual bud-burst times with some buds not bursting within the duration of the observations, usually a maximum of 50 days.

Bud-burst Number

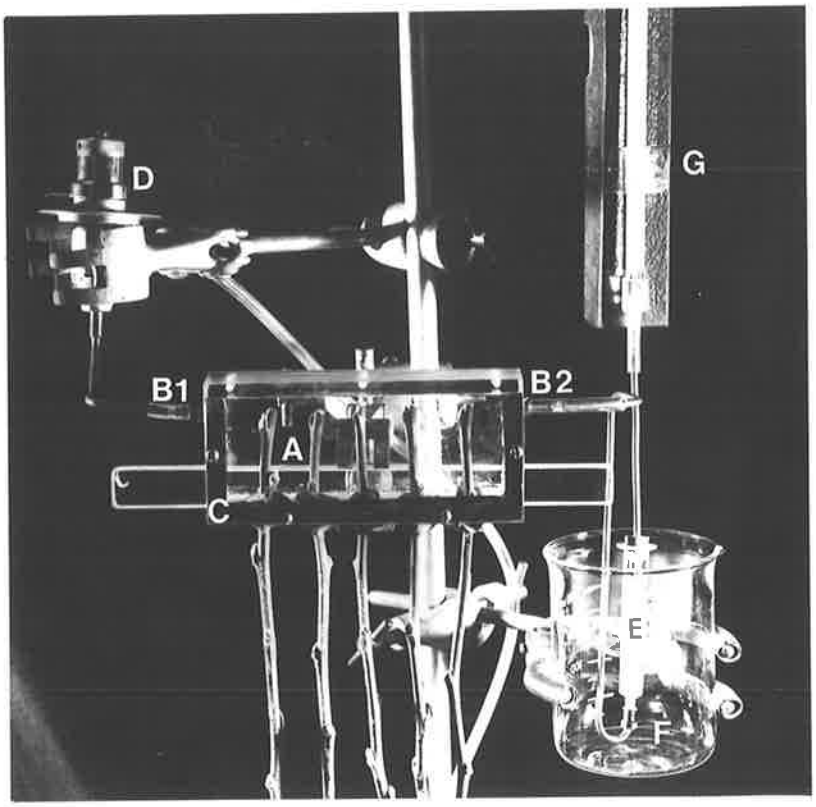
Bud-burst number was the number of buds which had burst at the end of the observation period.

Fig. A4 Apparatus for Collecting Ethylene from Shoots (Refer to text opposite).

a Intact shoots.



b Pruned shoots.



A7 ETHYLENE DETERMINATIONS

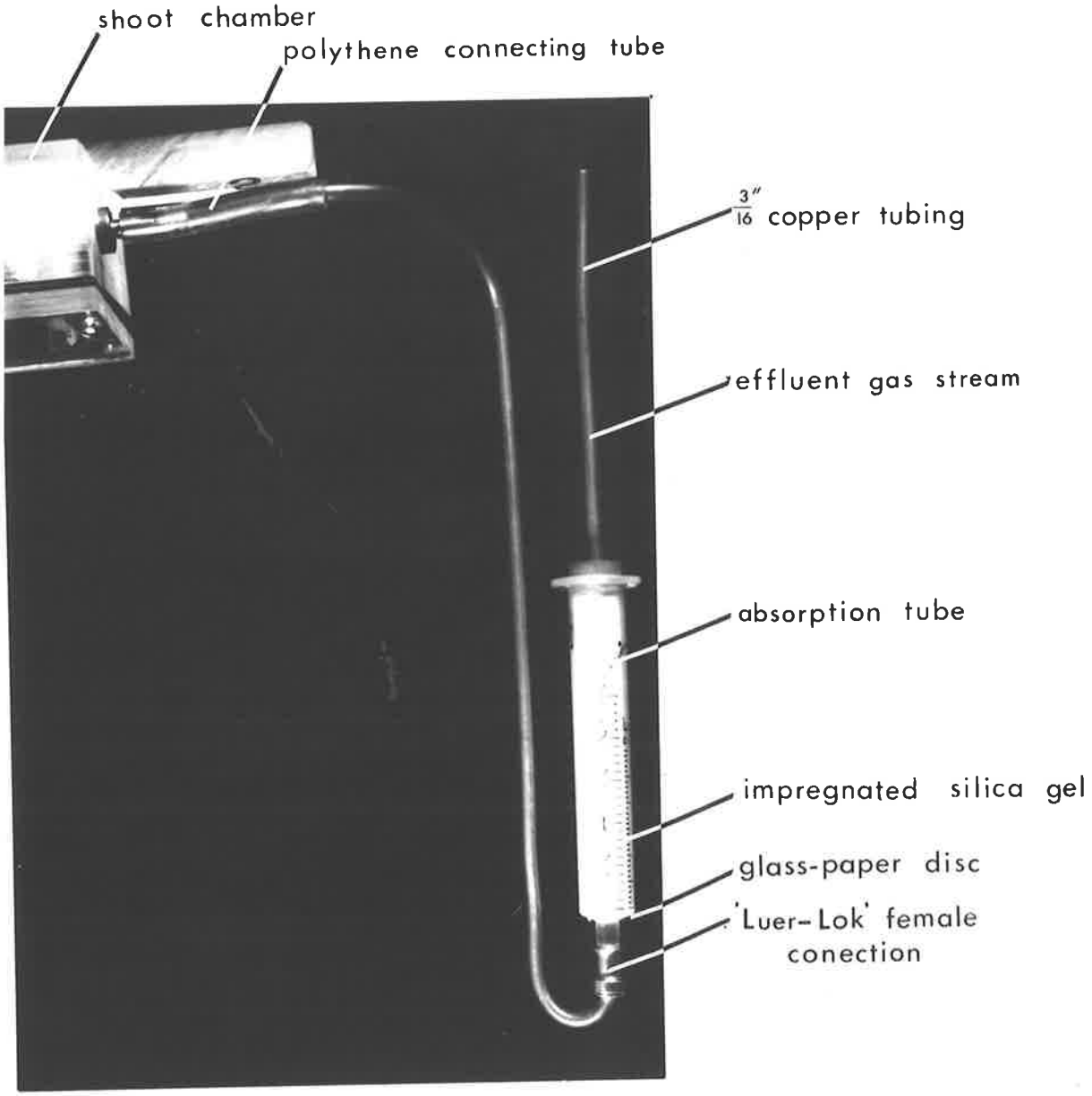
Experimental Chambers

The aim was to collect the ethylene evolved from stem internode sections on whole and pruned shoots. A continuous, controlled air stream was required with 5 shoots per unit to provide measurable quantities of ethylene in the air stream. The apparatus is shown in Figure A4.

Perspex chambers (A), of internal dimensions 11 x 4.5 x 2 cm (volume 99 ml), were constructed with air inlets (B_1) and outlets (B_2) and a removable front panel (C). Slots were cut in the base to accommodate pruned shoots (Figure A4 (b)) and in the base and top for intact shoots (Figure A4 (a)). Plasticene was used to form an air-tight seal around the stem in the slot. Compressed air (C.I.G. Medical Air) was delivered to the chamber via a pressure regulating valve (Fisher Governor Co., 0-35 p.s.i.) and a needle valve (D) (Edwards, high vacuum) in series to provide a steady flow rate, usually 1 ml min^{-1} measured at the outlet of the absorption tube (E).

The shoots were placed in position in the chambers with the slots packed with plasticene, then left over night with the front panel removed. At the commencement of an experiment the front panel was rapidly secured in position (immediately following pruning where applicable) and an absorption tube was attached to the chamber outlet and immersed in ice-water (F). The effluent air flow rate was measured (G) and adjusted using the needle valve.

Fig. A5 An Ethylene Absorption Tube.



Ethylene Accumulation

In order to measure ethylene at low rates of evolution a method of accumulation was developed. This was based on the method of Phan (1965) in which ethylene is collected by reaction with 0.25 M mercuric perchlorate in 2.5 M perchloric acid dispersed on silica gel over which the gas passed, then released as the gas by the addition of 1 N HCl. The technique used was as follows :

A. Preparation of the solution (Vogel 1962):

(1) 35.4g of (71%) perchloric acid were diluted with water to give 100 mls final volume

(2) 5.145g of mercuric oxide red (Ajax Chemical Co) was added to (1) and the mixture shaken vigorously, frequently for one hour.

(3) This solution was stored in darkness under refrigeration.

B. Preparation of absorption tubes:

(1) A single, 7mm diameter, glass-paper (Whatman GF/A) disc was placed in the bottom of each 2 ml disposable, plastic syringe (Figure A5).

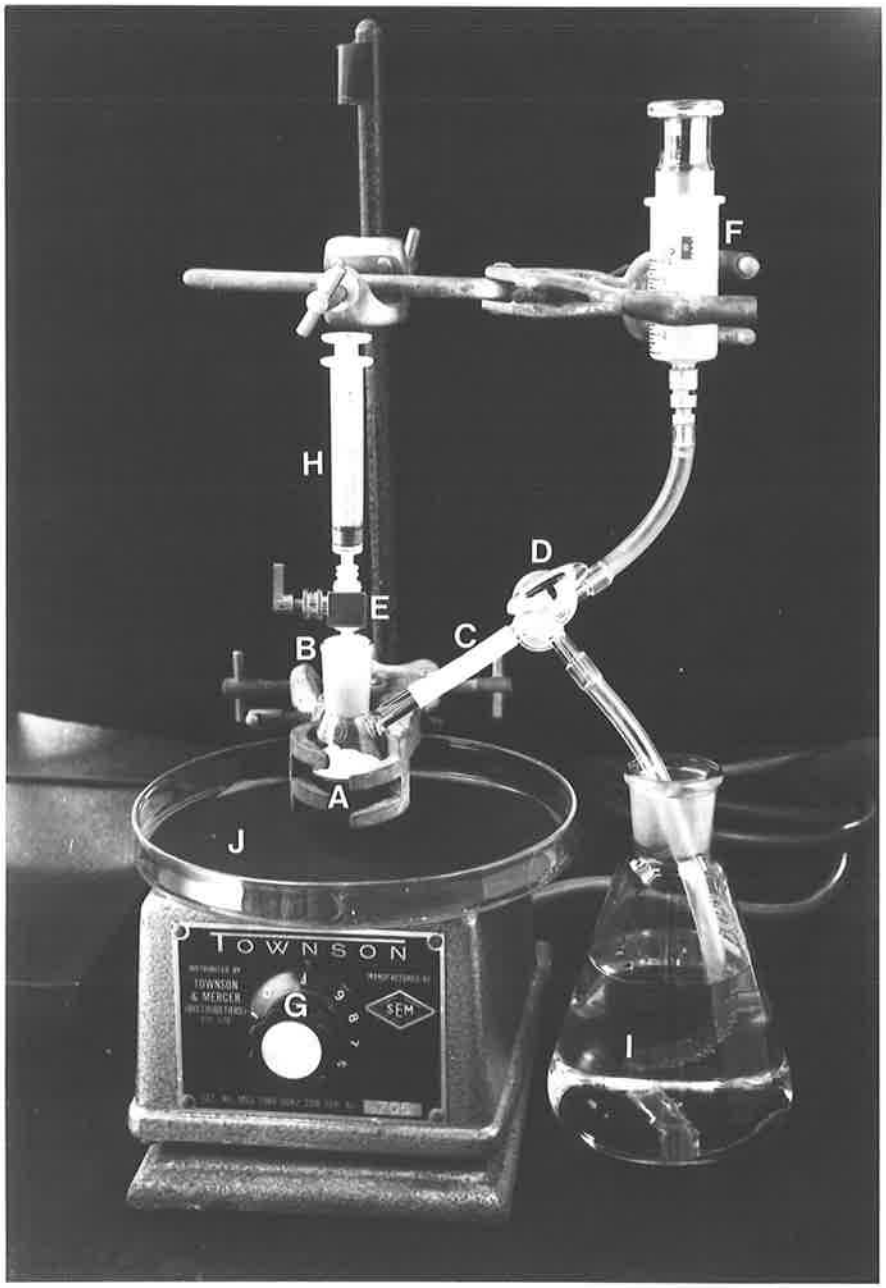
(2) 2.5g of oven-dried, silica gel (60-120 mesh, B.D.H. Ltd., England) was added to each syringe and compacted by gently tapping the syringe.

(3) 1 ml mercuric perchlorate solution (from A) was pipetted onto the gel and the syringe was capped using its rubber tipped plunger.

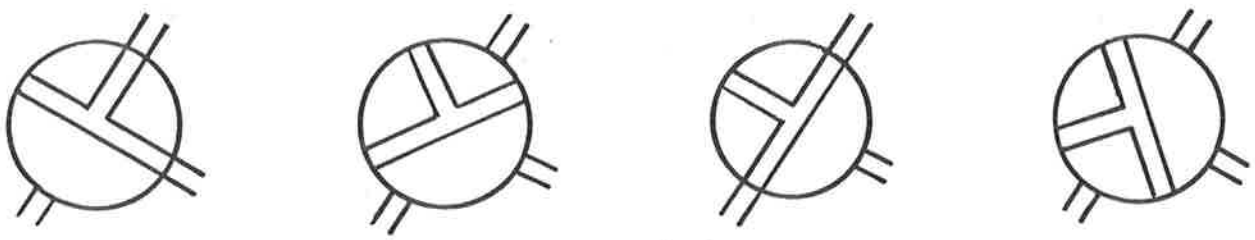
(4) Prepared syringes (absorption tubes) were stored over-night in a desiccator containing granular

Fig. A6 *Apparatus for the Release and Collection of Ethylene after Absorption on Impregnated Silica Gel.*

(Refer to text opposite.)



Positions of the Stopcock "D".



silica gel and placed in a freezer.

C. Absorption of ethylene:

(1) Prepared absorption tubes were removed from storage just prior to use and a needle point was inserted through the base of the syringe to pierce the filter paper which tended to become sealed by frozen solution.

(2) The absorption tubes were fitted into the connecting tube (Figure A5) and connected to the chamber. They were immersed in iced water during the collection period (Figure A4).

(3) At the end of the collection period the tubes were removed from the apparatus, capped and replaced in the freezer.

D. Release of ethylene from the gel (refer to Figure A6):

(1) The impregnated silica gel was removed from the tube by tapping the sides and placed in the reaction flask (A). A small magnetic stirring rod was added to the flask and the teflon plug (B) placed into position.

(2) The reaction flask was connected, via a teflon tube (C) to a 3-way stopcock (D). The 1-way, teflon, Luer-Lok valve (E) was inserted into the teflon plug and closed.

(3) With the stopcock in position 1, acid was drawn into a 50 ml syringe (F) by raising the plunger, then the stopcock was moved to position 2.

(4) With the stopcock in position 3 , the valve was opened briefly to permit acid to flow into the flask up to the base of the neck, then the valve was closed.

(5) A timer was started and the magnetic stirer (G) activated. The sample syringe (H) was placed in position with the plunger in.

(6) At the end of the reaction time the stirer was stopped and the valve opened. The entire gas sample was drawn into the syringe as acid filled the reaction flask. The valve was closed and the stopcock placed in position 4.

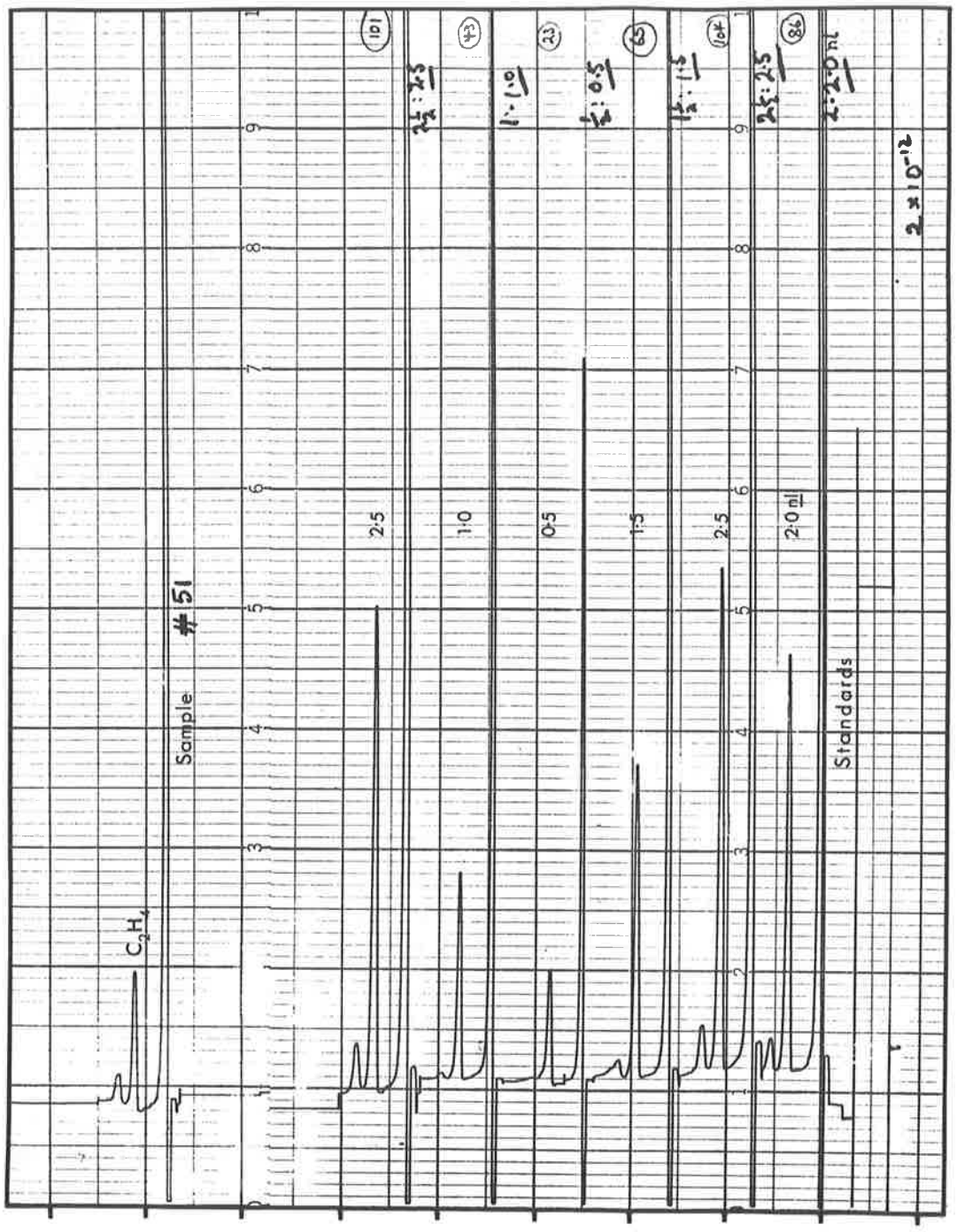
(7) The sample syringe was removed with the valve attached. A needle was attached at the valve and the sample injected into the G.L.C.

(8) The reaction flask was removed and cleaned for the next run.

Note: (a) 1 N HCl was stored in the reservoir (I)

(b) The drip-tray (J) contained $NaHCO_3$ to neutralize spilt acid.

FIGURE A7. G.L.C. DETECTION OF ETHYLENE



Ethylene Measurement

The level of ethylene present in the gas samples was determined by quantitative, gas-liquid chromotography (G.L.C) using a Varian Aerograph 2700 instrument fitted with a Flame Ionization Detector. The glass column (150 x 0.3 cm) was packed with 100-120 mesh Porapak Q (Waters Assoc. U.S.A.) and the carrier gas was nitrogen.

The operating conditions were:

Flow rates :	N_2	-	30 ml min^{-1}
	H_2	-	30 ml min^{-1}
	AIR	-	300 ml min^{-1}
Temperatures :	Column-		40^{o} C
	Detector		100^{o} C
	Injector		60^{o} C
Chart Speed :	2 cm min^{-1}		

Standard gas samples were obtained by dilution of compressed ethylene (C.I.G.) with nitrogen in 20 litre honey tins fitted with 'Suba Seal' septa. These were calibrated by comparison of a fixed volume of each with different volumes of the same concentration and by reference to an authenticated sample (Attech Assoc. U.S.A). The detector response (peak height) was calibrated using different sample volumes of a known standard concentration of ethylene.

The retention time for ethylene was 1.5 minutes and with maximum attenuation, 0.02 ml of ethylene produced a 2 mm peak. Figure A7 presents the chart tracing for a series of standards plus an unknown sample

A8 STATISTICAL ANALYSES

The data for the experiments in Section III B were analysed using the Waite Institute's computing facilities with the assistance of a staff biometrician (Mr. T. Hancock). Most of the data were analysed using parametric statistical methods but a non-parametric technique (Kruskal Wallis) was used for discreet data (e.g. numbers of buds or shoots) and for tests of correlation (Kendall's τ).

In Section III C bud-burst number data were not subject to statistical analysis because the sample size was usually only 5. Analysis of variance was carried out on mean burst-time data for all positions at which bud-burst occurred on more than 2 out of 5 shoots.

The data presented under Dormancy Studies (Section III D) were not subjected to statistical analyses. The inferences drawn from these experiments were based mainly on consistent trends in the data and the absolute occurrence or absence of bud-burst.

In Section III E the least significant differences (L.S.D.) were calculated following one-way analysis of variance. Incomplete data precluded comprehensive analysis of the measurements of ethylene levels.

B PRUNING EXPERIMENTS

B1 GENERAL INTRODUCTION

Pruning treatments are commonly used to modify shoot form in woody plants. Shoot form is determined by two groups of factors:

(a) the number and position of the growing points and

(b) the relative growth made from these points. The amount of growth from any one point is a function of both the rate and duration of growth.

The response to any pruning treatment will depend on effects on the above factors and the basic aim of this section is to examine how pruning influences these factors particularly in apple root-stock cultivars.

The term "growing point" as used above is essentially synonymous with "shoot apex". Most shoot apices follow a cyclic growth pattern including a period of dormancy as outlined in the introduction to this thesis, and in the case of deciduous, temperate species (including apple) the growth cycle includes a bud phase. Shoot pruning influences shoot form largely by determining which shoot apices resume active growth after the bud phase. This involves effects on factors in group (a) above.

The factors in group (b) may also be influenced by pruning in a less direct manner. The duration of shoot growth will depend partly on the time of resumption of growth i.e. the time of bud-burst. Pruning may promote earlier bud-burst (Maggs 1959a;

Oskamp 1931). The rate of growth will be influenced by the number of active growing points relative to the plant's capacity to support shoot growth. Pruning may regulate the number of growing points (Chandler 1957; Maggs 1959a, 1963. 1964a, 1965b; Moorby and Wareing 1963). Also, where a particular shoot apex resumes growth before others (including in response to pruning), it may gain some initial advantage by virtue of there being less competition for resources (Jankiewicz 1972). Thus the effects of pruning on shoot form may be largely analysed in terms of the effects on the regulation of the resumption of active growth from dormant buds.

The effect of pruning on shoot growth is not confined to the growth made by shoot apices. A portion of the shoot growth increment, in terms of increase in mass, is also distributed between the expansion of leaves and an increase in girth of the existing shoot system. Furthermore, pruning may influence the distribution of the total tree growth increment between the shoot system and the roots.

The general pattern of the distribution of the growth increment has been studied in some detail, particularly for apple (Maggs 1965b *et seq.*). This distribution varies in such a way as to maintain an overall balance between the centres of assimilation (leaves and roots) and the sites of growth increment (mainly stems and fruit). There appears to be a fixed relationship between the portion of the growth increment occurring in the leaves plus roots and that contributing to stem growth (Maggs 1959a, 1962). In young apple rootstocks

(cultivar MXXV) 42% of the total dry weight increment for one season was found in the leaves plus roots (Maggs 1959a). This relationship can be greatly modified in older trees (see Kozlowski 1971) particularly when fruiting is involved (Avery 1969; Kozlowski 1971; Maggs 1963; Rogers and Booth 1964).

The growth increment occuring in the stem is distributed between the production of new shoots (i.e. growth from shoot apices) and the expansion in girth of the existing shoots (resulting from cambial activity). This aspect of growth distribution is markedly influenced by pruning treatments. Maggs (1959a) found that the weight of new stem growth relative to old stem increment was proportional to the severity of pruning (i.e. to the proportion of the old stem removed).

In the final analysis then, the effect of pruning on shoot growth will be related to its effects on new shoot production. This in turn will be determined by the number of buds which burst and subsequently produce shoots and the amount of growth made by these shoots (Maggs 1959a). Therefore a study of the effects of pruning on bud development should have relevance to the overall understanding of tree growth responses.

The responses to pruning treatments are partly determined by the stage of the shoot growth cycle at which they are applied. This relates mainly to the state of activity or dormancy of the buds. There is little difference in the

effects of pruning at any time during the winter dormancy period (dormant pruning) but there is a marked difference between pruning in the shoot growth period (summer pruning) compared to dormant pruning (Chandler 1957; Gardener, Bradford and Hooker 1953).

Dormant pruning involves a reduction in the number of buds available and moves the zone of response to a different group of buds. Maggs (1963) found a greater propensity to grow in buds in the top half of shoots. Therefore the removal of more distal buds may contribute to a reduction in the number of buds which elongate, even though a similar number of buds burst within the response zone regardless of the severity of the pruning treatment. This difference appears to be predominantly a position effect, possibly related to cambial area along the shoot (Maggs 1964a), since the buds have similar growth potential when placed in a comparable position on another shoot (Maggs 1959b).

An important difference between summer pruning and dormant pruning is that during the shoot growth season lateral buds are dormant mainly because of apical dominance and decapitation (light pruning) may stimulate the burst of many buds along the shoot (Barlow and Hancock 1960, 1963, Gardener *et al.* 1953; Pieniazek, Saniewski and Jankiewicz 1970) whereas in late summer or autumn buds cannot respond because of rest. After light dormant pruning apical dominance may be re-established during the period of slow development which precedes bud-burst so that only distal buds respond as found by Pieniazek *et al.* (1970).

Summer pruning has the additional effect of removing leaves. If carried out early in the growing season the leaf area may be re-established, usually by a larger number of smaller leaves from buds along the shoot (Maggs 1965b), and overall growth is not affected but late summer pruning results in reduced shoot increment (Maggs 1964b, 1965b).

Although this thesis is concerned mainly with the study of new shoot growth, it is important to bear in mind the relationship that exists between new shoot growth and growth activities in other parts of the plant. Cambial activity may influence new shoot growth both directly, as a site of meristematic activity and hence a potential site of hormone production and indirectly, as an alternative growth centre and by its role in the establishment of translocation pathways. Roots not only provide water and nutrients from the soil, they may also produce growth regulatory substances. Cambial tissues in both shoots and roots (Kozlowski 1971) and root elongation (see Kozlowski 1971; Romberger 1963) are subject to seasonal fluctuations in activity which may influence their interaction with new shoot growth.

Another aspect of shoot growth regulation which should be mentioned is the storage and mobilization of nutrients and assimilates. The supply of stored assimilates from the roots or stem is particularly important at the commencement of growth in spring until leaf productivity is established. The leaves themselves may play a transient role in the storage and distribution of assimilates, nutrients and

growth regulators as well as providing sites of photosynthesis and transpiration. Pruning may influence these processes by directly removing the tissues or by affecting the build-up of reserves during the active period.

This section has presented a general outline of the responses to pruning and considerable information is available on this aspect of the subject. The literature also contains many references to the principles and practices of pruning and the empirical responses obtained. But what is known about the underlying physiological processes involved? Again, this discussion is confined to the context of effects on bud development.

The current understanding of the physiology of pruning responses is based mainly on inferences drawn from general studies of shoot growth regulation (reviewed in Section II). Much less is known about the effects of removing the distal parts of the shoot and the cutting involved, therefore this aspect has been emphasised in this work.

B2 THE COMPONENTS OF PRUNING

B2(a) Introduction

Shoot pruning involves the removal of part of the shoot including various proportions of leaves, buds and wood. The removal of different parts of the shoot may influence the growth pattern of the remaining shoot in different ways. This experiment was conducted to analyse the contribution to the pruning response of the various parts of a shoot.

The effects of pruning may be attributable to one of more of the following five factors:

i) removal of the shoot terminal apex,

ii) removal of lateral apices or buds,

iii) removal of leaves, if present,

iv) removal of wood (necessarily distal to the pruning cut),

v) the pruning cut itself.

These are regarded as the components of pruning. Their relative importance may depend on the state of activity of various parts of the shoot or of the whole plant. This in turn will depend on the time of the year (or the stage of the growth cycle) at which treatments are carried out (Maggs 1964b, 1965b and other references cited in Section B1).

In this experiment (and in subsequent experiments in this section) plants were treated either in the leafless, winter dormant state or early in the growth cycle to minimize the involvement of the leaf component and removal of new growth.

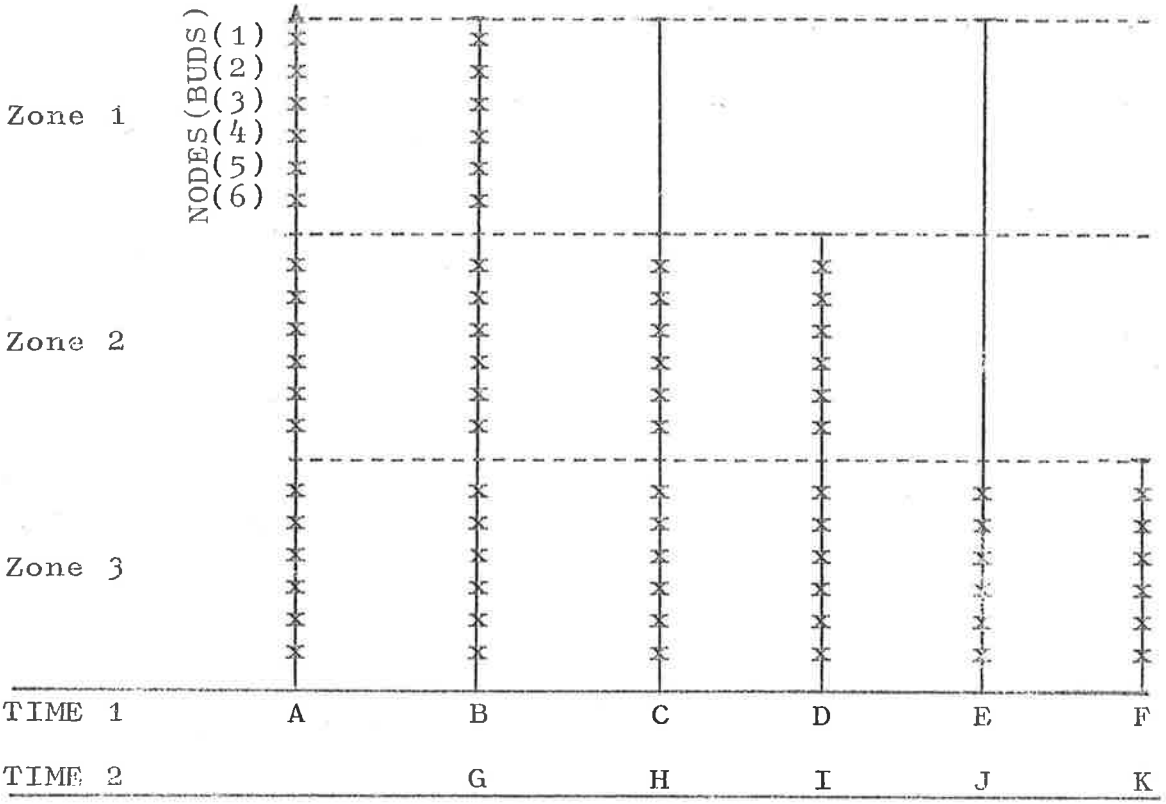
Considerable work has been reported on the effects of leaf removal on bud development (Barlow and Hancock 1963 *et seq*; Fulford 1965, Haas and Hein 1973; Maggs 1964b, 1965a, b) and this aspect is not dealt with.

Although one may categorise the components of pruning, it is not possible(in practice) to manipulate them independently. The removal of any portion of the shoot involves the injury of tissues. Wood cannot be removed without involving all the other components.

The approach taken was to commence with the simplest treatment, decapitation, which involves (i) and (ii), and then to progressively include others. In this way the contribution of individual components can be assessed (by the effect of their inclusion) though the possibility of their interaction should not be overlooked. In order to determine the responses in both active (growing) and inactive (non-growing) shoots, two treatment times were used. The first was during winter dormancy and the second shortly after natural bud-burst had commenced.

As stated earlier (Section III B 1) the effect of pruning on shoot growth will depend on the number and position of buds which burst and subsequently elongate and the duration and rate of growth from the buds. Observations were made on all these factors.

Figure B1 Diagramatic presentation of the treatments



A Untreated (control).

B Decapitation.

C Decapitation & top zone disbudded (LIGHT DISBUDDING).

D Distal zone pruned off (LIGHT PRUNING)

E Decapitation & two zones disbudded (HEAVY DISBUDDING)

F Two zones pruned off (HEAVY PRUNING)

x Lateral bud

▲ Apex present

\- Pruning cut

vertical lines represent shoots

B2(b) Methods

An experiment was carried out in 1972 in the Claremont Orchard on the small, hedge-pruned, apple rootstocks described under General Materials and Methods (Section III A). The rootstock cultivars MM102, MM104, MM106, MM109, MXII and Northern Spy were used. During the winter period 10 one-year-old shoots were selected on each tree to provide, as near as possible, uniform length and node number for all shoots within a block. The other previous year's shoots were removed. Each shoot was divided into top (distal), middle and bottom (proximal) zones, each containing one third of the lateral buds present. Since the lowest number of buds per shoot was 18, the top 6 buds in each zone were used for all observations.

Five treatments were applied on each of the two dates and untreated shoots were used as controls. The treatments were:

i) decapitation

ii) decapitation and the top zone disbudded

iii) the top zone pruned off

v) the top and middle zones pruned off. (see Figure B1).

Time 1 was the first week of September (before natural bud-burst), and Time 2 the last week of October (after natural bud-burst).

Each treatment was applied to all 10 selected shoots on one tree in each block of rootstock cultivar used, i.e. 11 treatments (one per tree) in 6 blocks. All the shoots on a

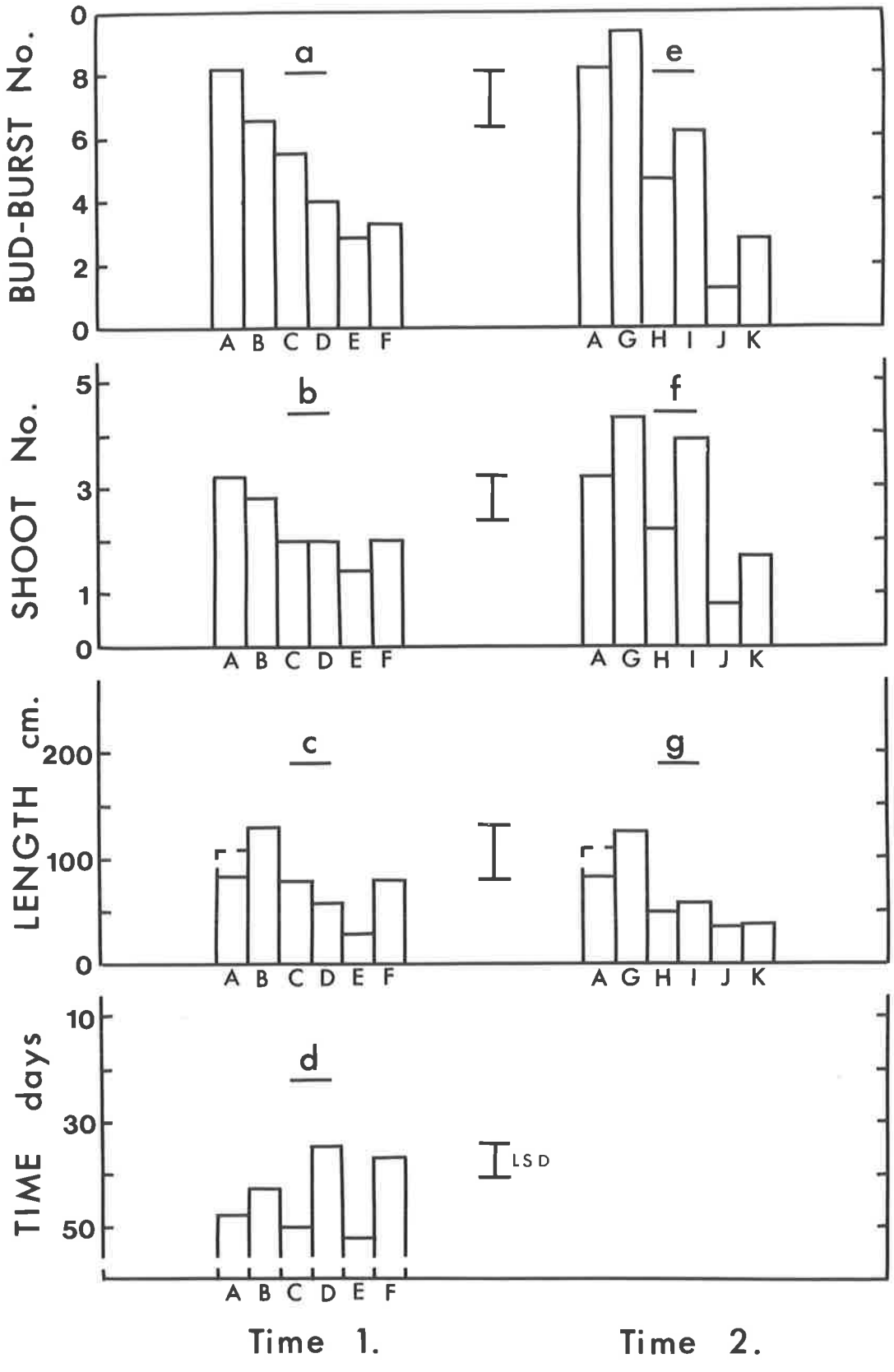

BUD-BURST No.
0
8
6
4
2
0
a
e
A B C D E F
A G H I J K
SHOOT No.
5
3
1
0
b
f
A B C D E F
A G H I J K
LENGTH cm.
200
100
0
c
g
A B C D E F
A G H I J K
TIME days
10
30
50
d
LSD
A B C D E F
Time 1.
Time 2.

FIGURE B2 OVERALL SHOOT GROWTH

A	Untreated
B, G	Decapitated
C, H	⅓ Disbudded
D, I	⅓ Pruned
E, J	2/3 Disbudded
F, K	2/3 Pruned

Time 1 - treatment before natural bud-burst

Time 2 - treatment after natural bud-burst

a, e number of buds which burst along a shoot

b, f number of lateral shoots at least 2 cm long

c, g total length of new shoot growth, including terminal growth on untreated shoots (----)

d mean bud-burst time for all buds which burst; - all buds bursting at Time 2 did so within 14 days.

L.S.D. P=0.01

tree were given the same treatment to avoid the interaction between different treatments. Chandler (1957) found that the growth of a single pruned branch may be suppressed by the growth of other unpruned branches.

Bud-burst was recorded for each bud by observation at weekly intervals until late November. At the end of the growing season the lengths of all new shoots were measured to the nearest centimetre.

B2(c) Results

The Pattern of Bud-burst and Elongation

i) The Overall Pattern

On untreated shoots 8.1 buds burst with a mean burst time of 48 days from Time 1. Of these buds 3.1 subsequently elongated to produce a lateral shoot 2 cms or longer.

Pruning or disbudding at Time 1 reduced the number of buds bursting and also the number which elongated (Figure B2(a), (b)). Pruning hastened bud-burst but disbudding did not (Figure B2(d)). When treatments were carried out at Time 2 (i.e. after natural bud-burst), disbudding or heavy pruning had the same effect as at Time 1 (Figure B2(e), (f), (g)) but decapitation or light pruning resulted in higher numbers of buds bursting and elongating (Figure B2(e), (f)). Also the number of lateral shoots was higher following pruning than for disbudding at the same level (Figure B2(f)).
There was a general correlation between the number of buds remaining after treatment and the number which burst or

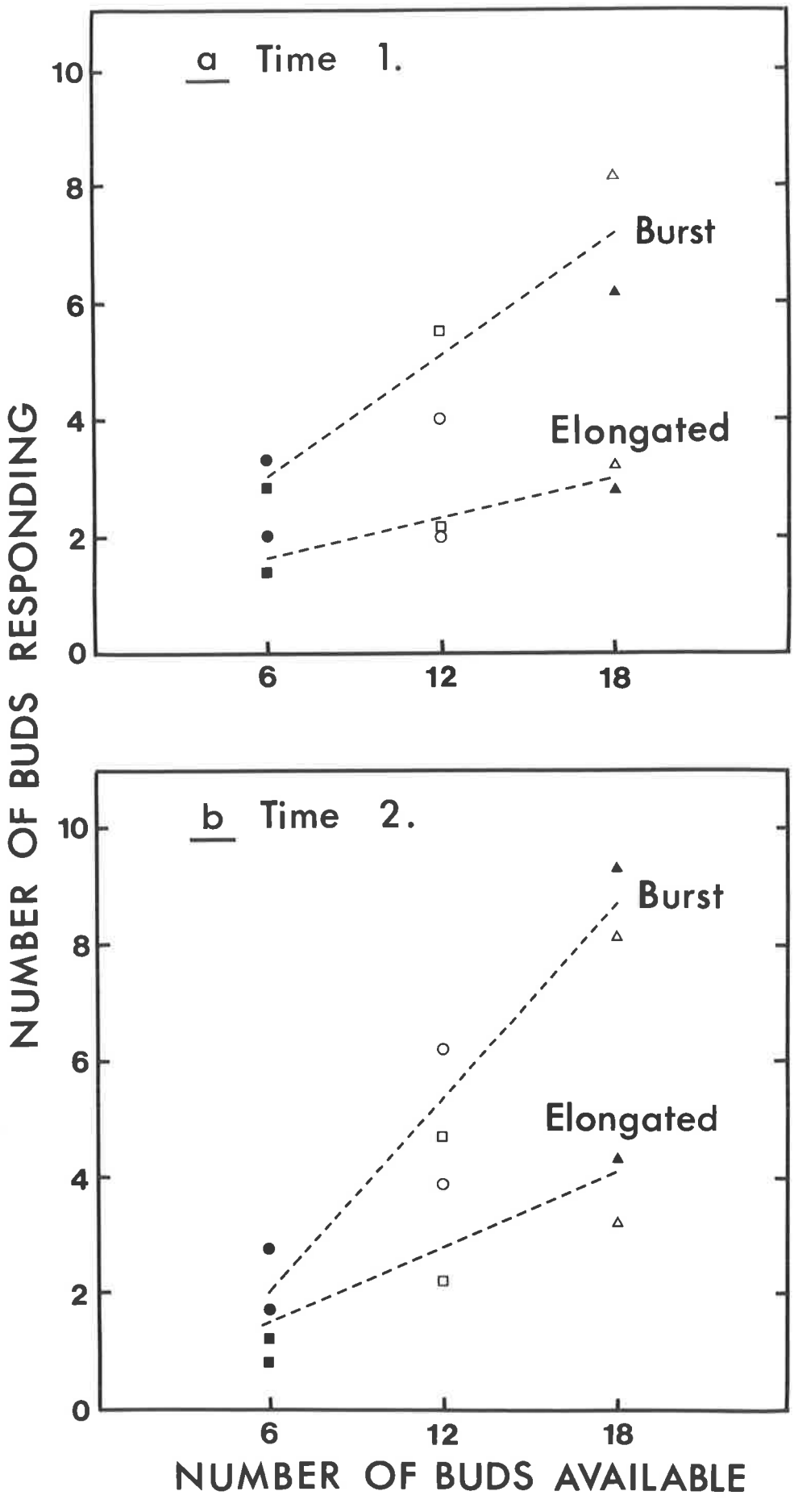

a Time 1.
Burst
Elongated
b Time 2.
Burst
Elongated
NUMBER OF BUDS RESPONDING
NUMBER OF BUDS AVAILABLE
0
2
4
6
8
10
6
12
18

FIGURE B3 CORRELATION BETWEEN THE NUMBER OF BUDS WHICH BURST OR ELONGATED AND THE NUMBER AVAILABLE

△ Untreated

▲ Decapitated

□ 1/3 Disbudded

○ 1/3 Pruned

■ 2/3 Disbudded

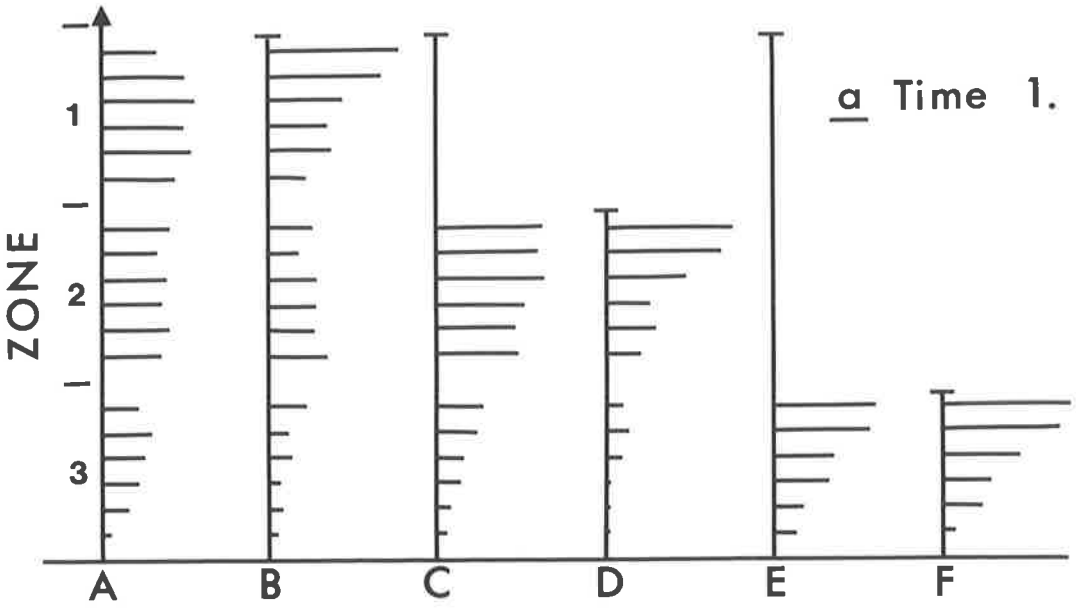
● 2/3 Pruned

Time 1 - Treatment before natural bud-burst

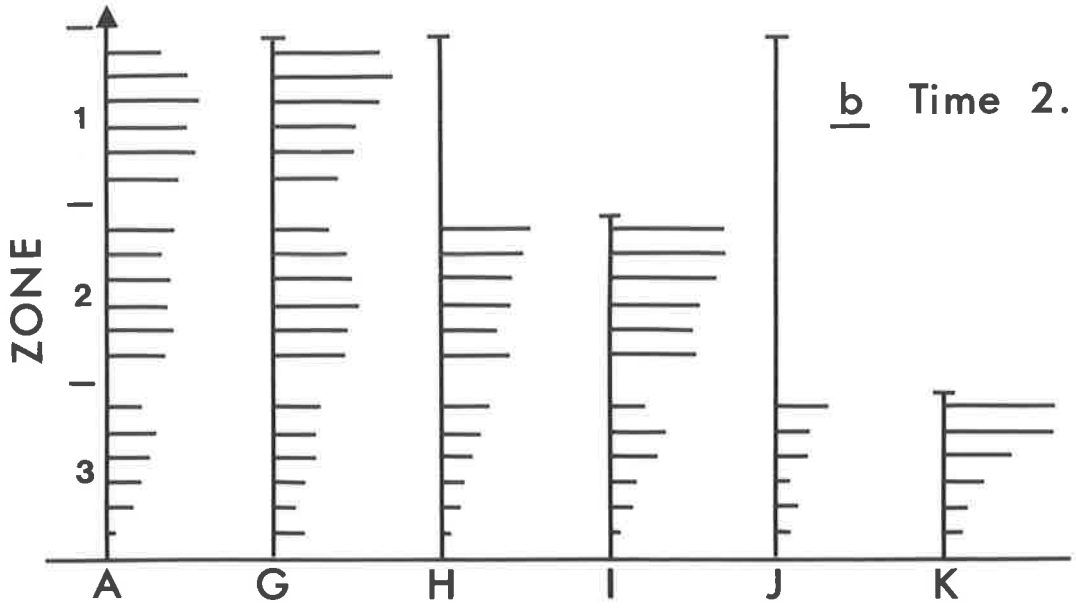
Time 2 - Treatment after natural bud-burst

BUD-BURST FREQUENCY

Scale: 100 %



a Time 1.



b Time 2.

FIGURE B4 FREQUENCY OF BUD-BURST AT NODES ALONG A SHOOT

The length of horizontal line represents the frequency at which bud-burst occurred at that node. There were 6 nodes per zone and 1, 2 or 3 zones of buds remaining.

A	Untreated
B, G	Decapitated
C, H	1/3 Disbudded
D, I	1/3 Pruned
E, J	2/3 Disbudded
F, K	2/3 Pruned

a - Time 1 - Treatment before natural bud-burst

b - Time 2 - Treatment after natural bud-burst

Each value is the mean of 6 blocks with 10 shoots per block.

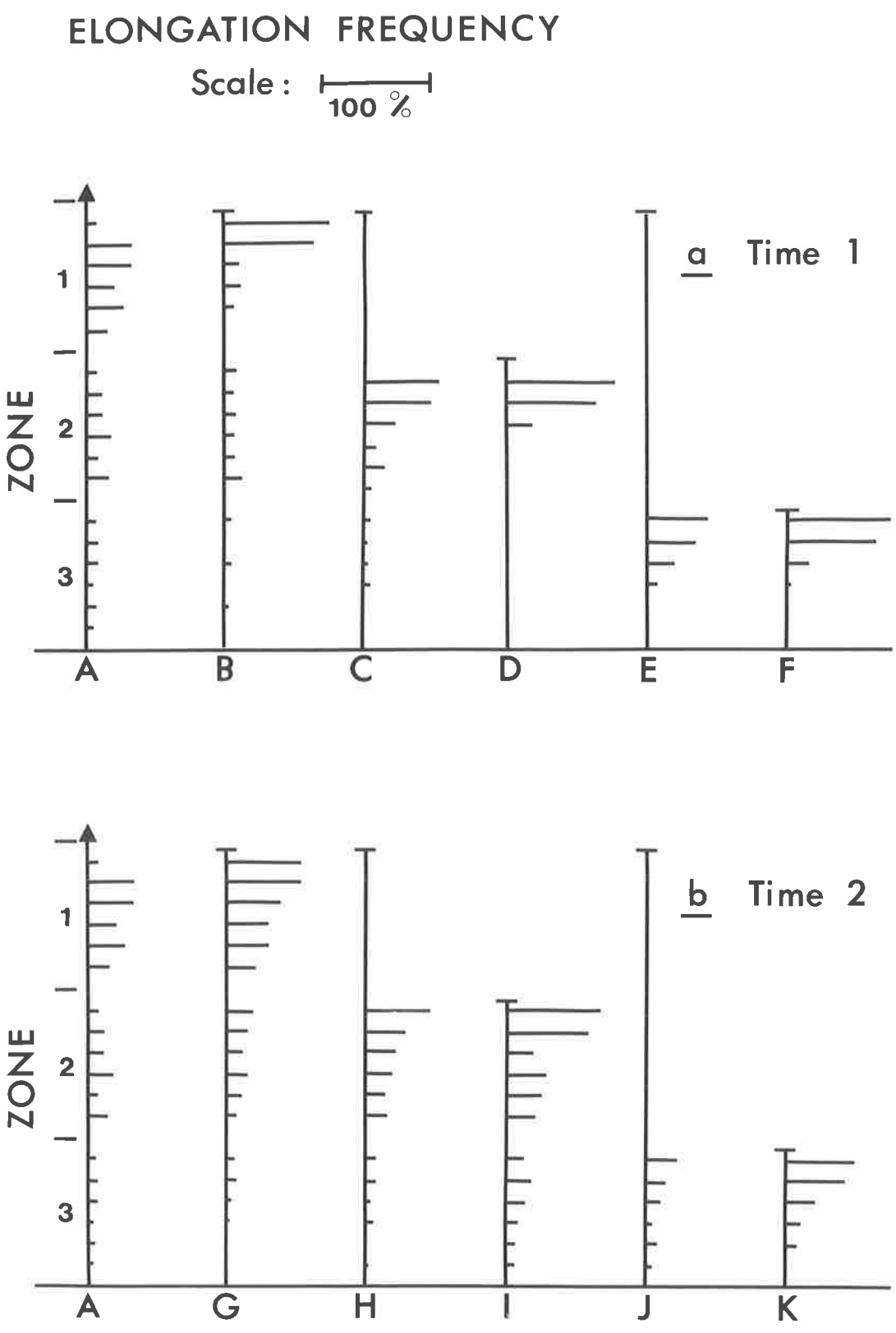

ELONGATION FREQUENCY
Scale : 100 %
ZONE
1
2
3
A
B
C
D
E
F
a Time 1
ZONE
1
2
3
A
G
H
I
J
K
b Time 2

FIGURE B5 FREQUENCY OF BUD ELONGATION AT NODES ALONG A SHOOT

The length of horizontal line represents the frequency at which the bud at that node elongated to produce a lateral shoot at least 2 cm long. There were 6 nodes per zone and 1, 2 or 3 zones of buds remaining.

A	Untreated
B, G	Decapitated
C, H	1/3 Disbudded
D, I	1/3 Pruned
E, J	2/3 Disbudded
F, K	2/3 Pruned

a - Time 1 - Treatment before natural bud-burst

b - Time 2 - Treatment after natural bud-burst

Each value is the mean of 6 blocks with 10 shoots per block.

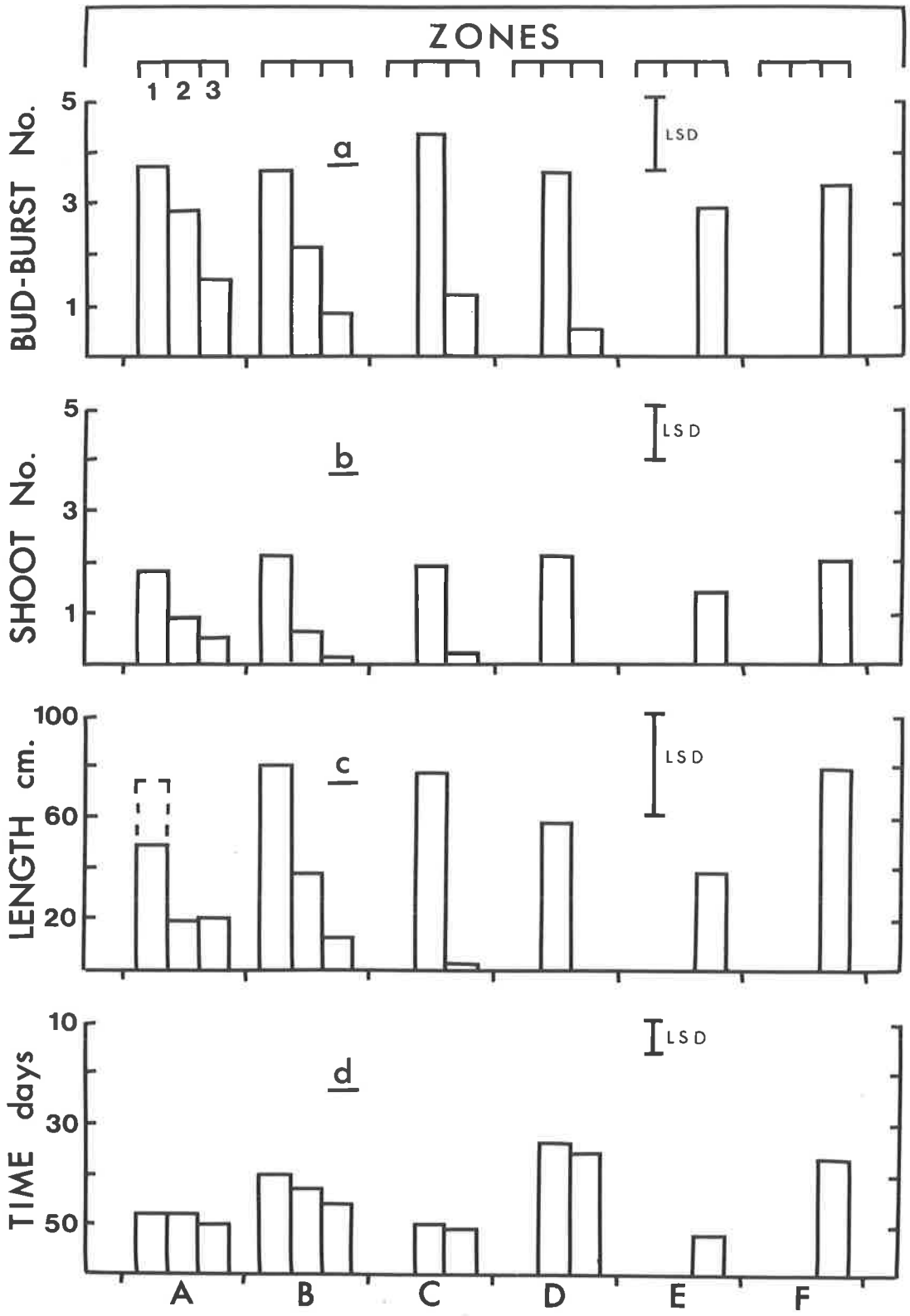

ZONES
1 2 3
BUD-BURST No.
5
3
1
a
LSD
SHOOT No.
5
3
1
b
LSD
LENGTH cm.
100
60
20
c
LSD
TIME days
10
30
50
d
LSD
A
B
C
D
E
F

FIGURE B6 COMPARISON OF RESPONSES BETEEEN ZONES - Time 1.

Shoots treated before natural bud-burst

A	Untreated
B	Decapitated
C	1/3 Disbudded
D	1/3 Pruned
E	2/3 Disbudded
F	2/3 Pruned

a Number of buds which burst within a zone

b Number of lateral shoots at least 2 cm long within a zone

c Total length of new shoot growth within a zone;
- including terminal growth on untreated shoots (--)

d mean bud-burst time for buds which burst within a zone

L.S.D. P=0.01

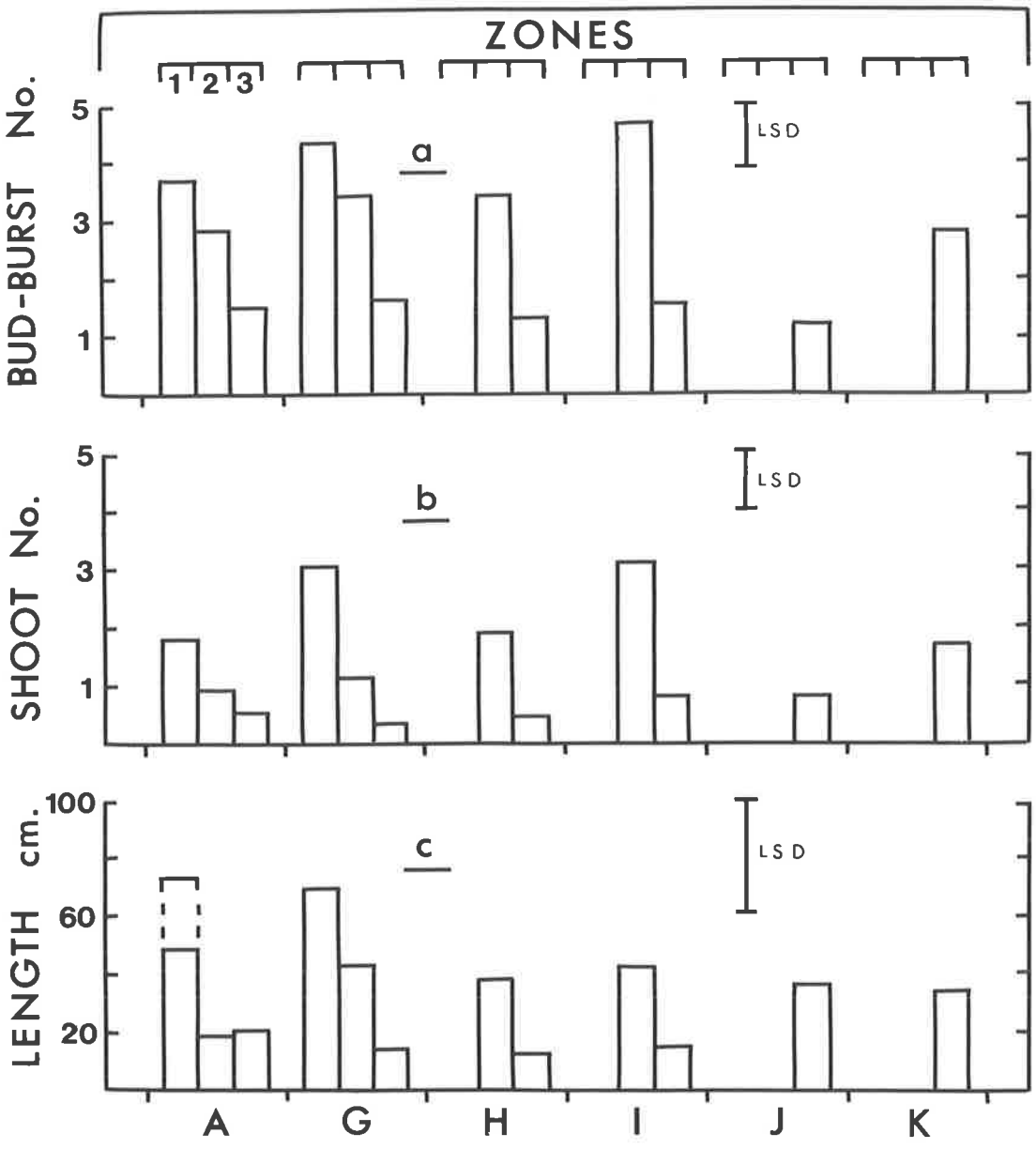

ZONES
1 2 3
BUD-BURST No.
5
3
1
a
LSD
SHOOT No.
5
3
1
b
LSD
LENGTH cm.
100
60
20
c
LSD
A
G
H
I
J
K

FIGURE B7 COMPARISON OF RESPONSES BETWEEN ZONES - Time 2

Shoots treated after natural bud-burst

A	Untreated
G	Decapitated
H	1/3 Disbudded
I	1/3 Pruned
J	2/3 Disbudded
K	2/3 Pruned

a Number of buds which burst within a zone

b Number of lateral shoots at least 2 cm long within a zone

c Total length of new shoot growth within a zone;
- including terminal growth on untreated shoots (--)

L.S.D. P=0.01

elongated (Figure B3). As the number of buds present was reduced, fewer buds burst and elongated but a greater proportion of those which burst subsequently elongated.

The general pattern of bud-burst is illustrated by Figure B4. On untreated shoots there is a gradual decrease in frequency of bud-burst at nodes basipetally along the shoot except that burst occurred less frequently at the top (distal) node. Treatments modified this pattern in two main ways; the distal zone (where burst frequency is high) occurred at a lower position on the shoot and the gradation in bud-burst frequency basipetally along the shoot was steeper.

The frequency of bud elongation on untreated shoots shows a similar pattern to burst frequency but with an overall reduction in magnitude (Figure B5). There is a greater distal localisation of the response, especially at Time 1 where elongation is almost confined to the distal 2-3 nodes. At Time 2 elongation frequency was enhanced at the distal nodes but with less reduction at the lower nodes. Bud-burst time did not display any consistent pattern along the shoot.

ii) Comparison between zones

The number of buds which burst or elongated per zone tended to decrease basipetally (Figure B6, B7). When the buds in more distal zones were removed by pruning or disbudding at Time 1, bud-burst and elongation within the distal remaining zone was increased to the same level as found in the distal zone of untreated shoots (Figure B6 a, b). At

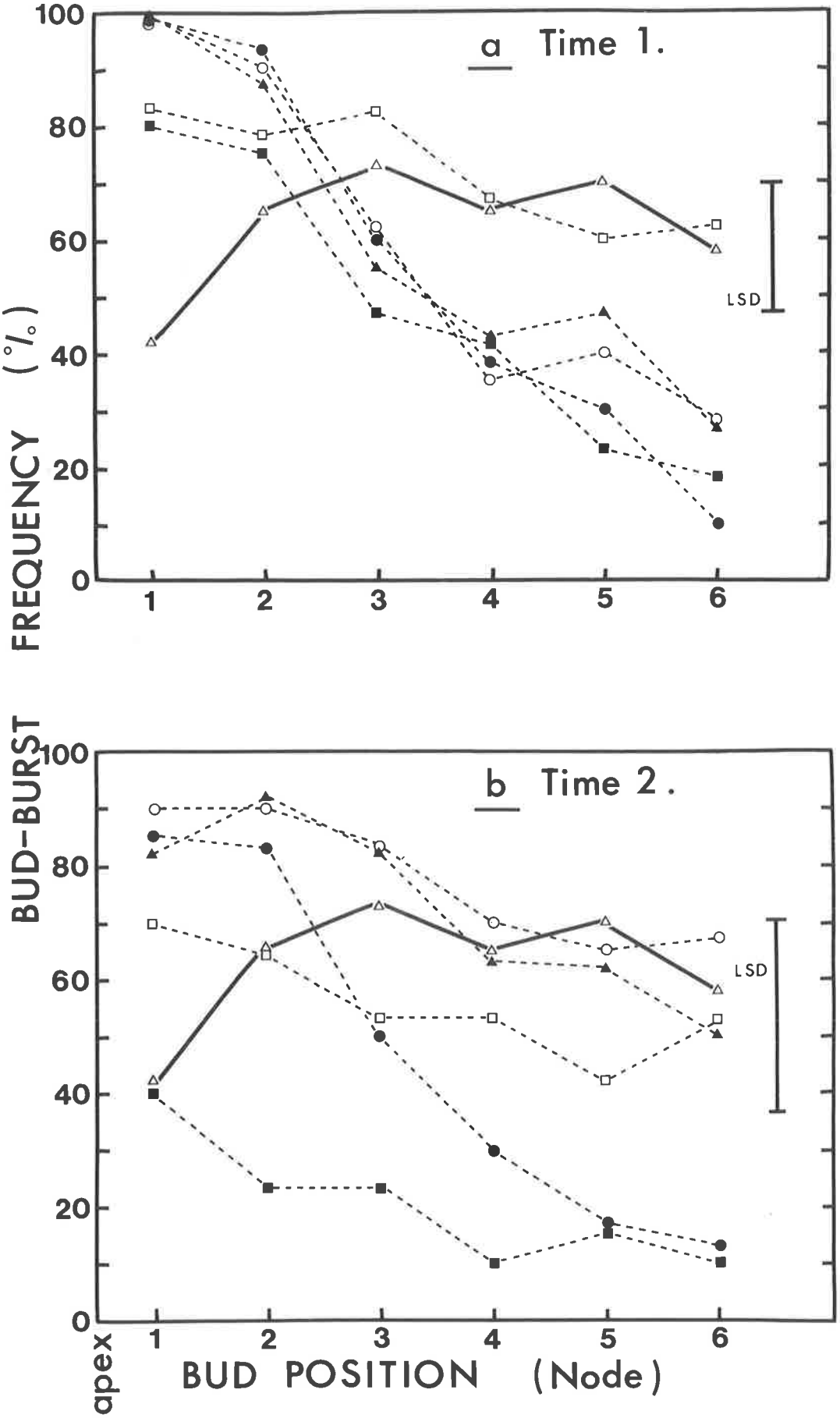

a Time 1.
100
80
60
40
20
0
FREQUENCY (%)
LSD
1
2
3
4
5
6
b Time 2.
BUD-BURST
LSD
apex
BUD POSITION (Node)

FIGURE B8 BUD-BURST FREQUENCY AT NODES WITHIN THE DISTAL ZONE

△ Untreated

▲ Decapitated

□ 1/3 Disbudded

○ 1/3 Pruned

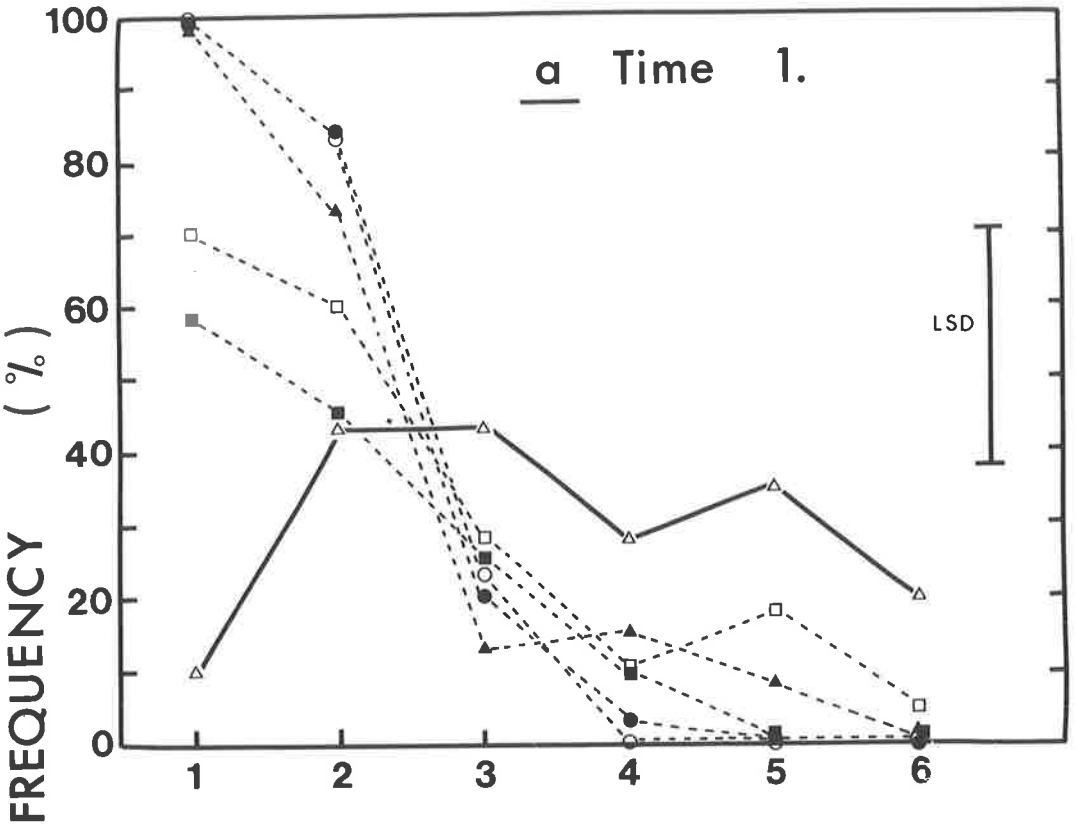
◪ 2/3 Disbudded

● 2/3 Pruned

a - Time 1 - Treatment before natural bud-burst

b - Time 2 - Treatment after natural bud-burst

L.S.D. P=0.01


a Time 1.
100
80
60
40
20
0
FREQUENCY (%)
LSD
1
2
3
4
5
6

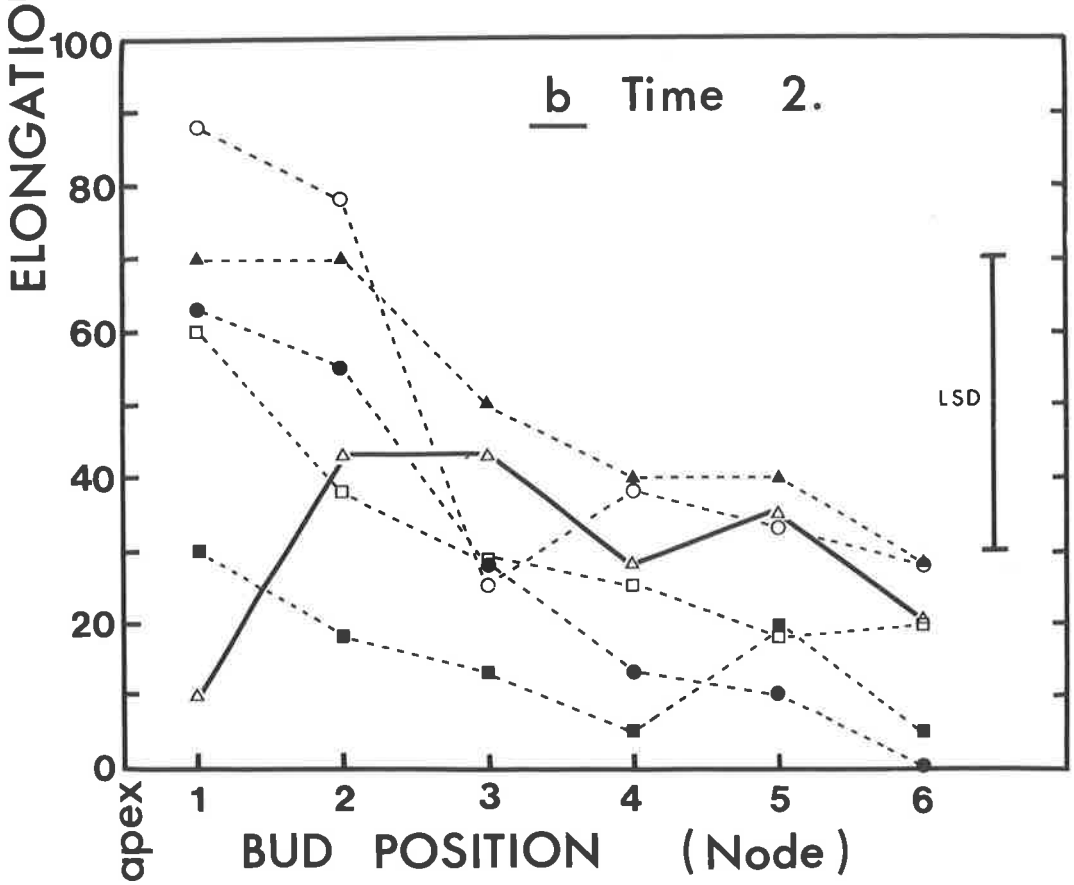

b Time 2.
ELONGATION
100
80
60
40
20
0
LSD
apex
1
2
3
4
5
6
BUD POSITION (Node)

FIGURE B9 FREQUENCY OF BUD ELONGATION AT NODES WITHIN THE DISTAL ZONE

△ Untreated

▲ Decapitated

□ 1/3 Disbudded

○ 1/3 Pruned

■ 2/3 Disbudded

● 2/3 Pruned

a - Time 1 - Treatment before natural bud-burst

b - Time 2 - Treatment after natural bud-burst

L.S.D. P=0.01

Time 2 heavy disbudding reduced the number of buds which burst whilst decapitation or light pruning increased the number which elongated (Figure B7 a,b). There was insignificant growth of buds in the lower zones except on decapitated shoots.

The pattern of bud-burst time responded to treatment in a manner distinctly different from that for the other parameters in two ways (Figure B6 d). Firstly, there were no significant differences between zones on a shoot within any treatment. Secondly, decapitation or pruning at Time 1 promoted earlier bud-burst in all zones but disbudding had no net effect (even though the shoots were also decapitated). All buds which burst following treatment at Time 2 did so within 14 days and no observations were made during this time.

Since no treatments caused a significant change in these parameters below the distal zone, the pattern of bud-burst and elongation was mainly determined by the effects of the treatments within the distal zone.

iii) Within the distal zone

Within the distal zone the general pattern of response from node to node was similar for bud-burst frequency (Figure B8) and elongation frequency (Figure B9). On untreated shoots the distal lateral bud was less active than those below. All treatments at Time 1 promoted this distal bud to a level of activity above those below; the effect was always less for disbudding treatments. The second bud was usually promoted slightly but the lower buds tended to be less active than on

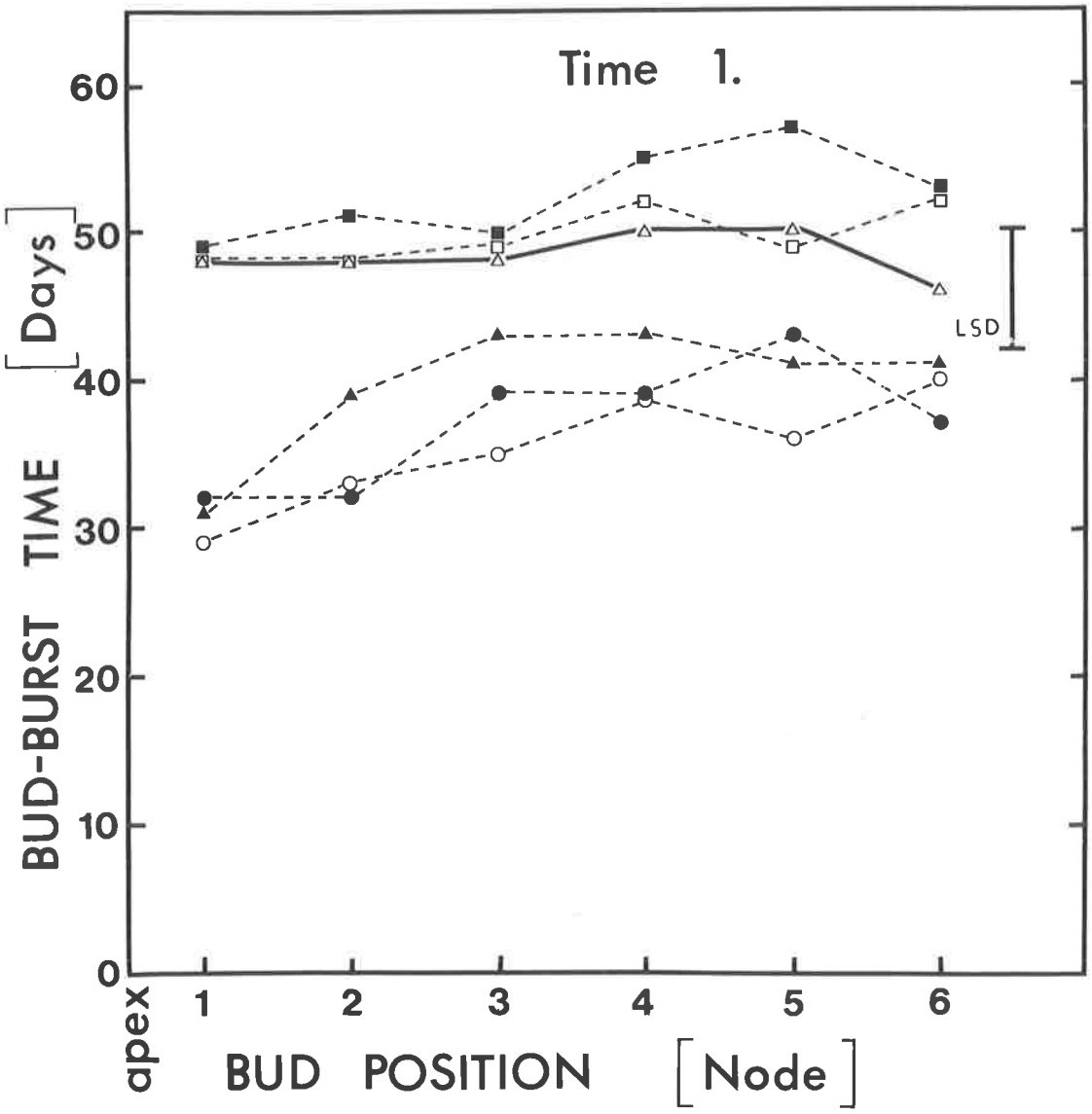

Time 1.
60
50
40
30
20
10
0
[Days]
BUD-BURST TIME
LSD
apex
1
2
3
4
5
6
BUD POSITION [Node]

FIGURE B10 BUD-BURST TIME AT NODES WITHIN THE DISTAL ZONE

The mean bud-burst time for those buds which burst after treatment before natural bud-burst (Time 1). All buds which burst following treatment of shoots after natural bud-burst did so within 14 days.

△ Untreated

▲ Decapitated

□ 1/3 Disbudded

○ 1/3 Pruned

■ 2/3 Disbudded

● 2/3 Pruned

L.S.D. P=0.01

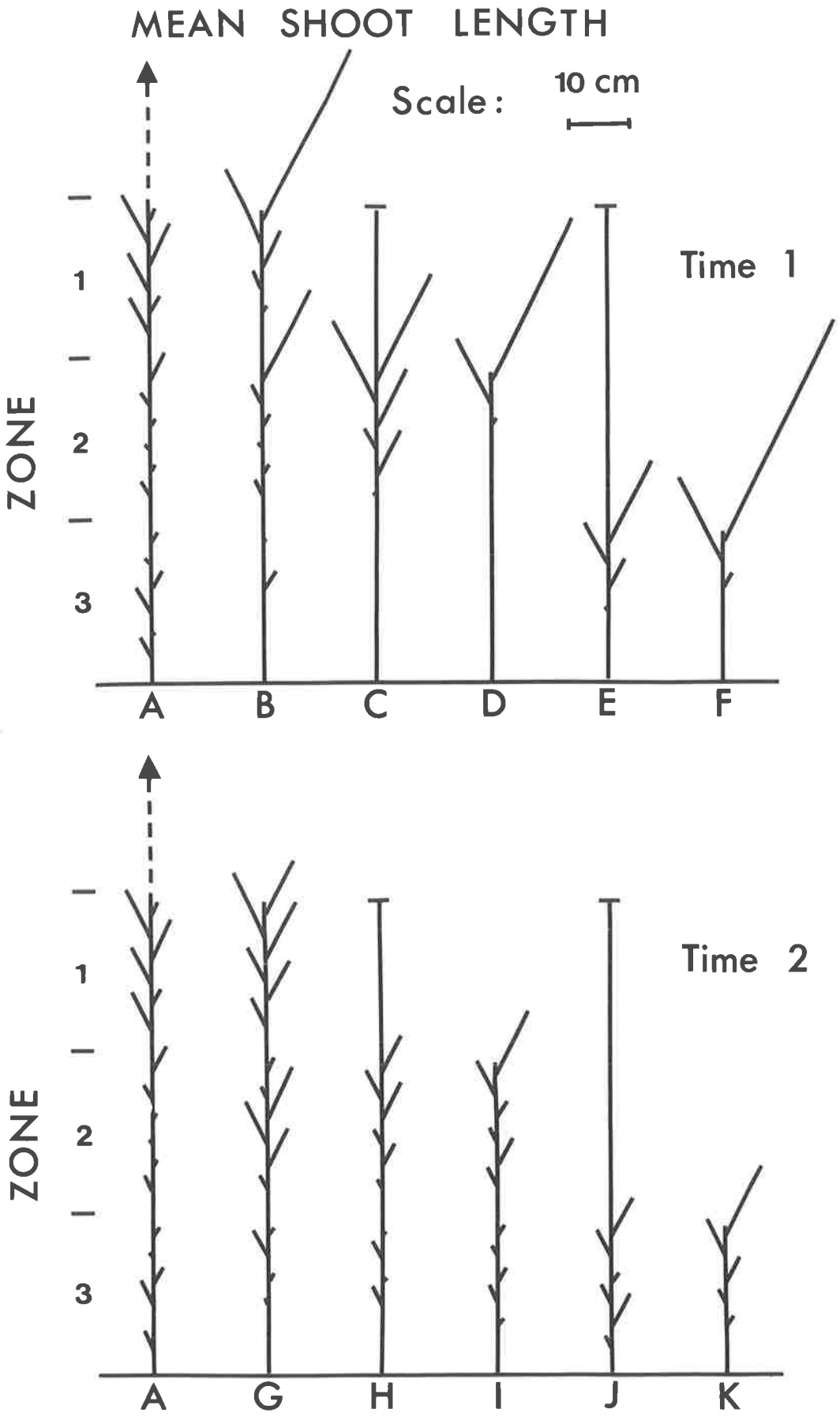

MEAN SHOOT LENGTH
Scale :
10 cm
Time 1
ZONE
1
2
3
A
B
C
D
E
F
Time 2
ZONE
1
2
3
A
G
H
I
J
K

FIGURE B11 MEAN SHOOT LENGTH AT NODES ALONG A SHOOT

Length of lateral line represents the mean length of shoot at that node, including terminal shoot growth on untreated shoots (--). There were 6 nodes per zone and 1, 2 or 3 zones of buds remaining.

A	Untreated
B, G	Decapitated
C, H	1/3 Disbudded
D, I	1/3 Pruned
E, J	2/3 Disbudded
F, K	2/3 Pruned

Time 1 - Treatment before natural bud-burst

Time 2 - Treatment after natural bud-burst

Each value is the mean of 6 blocks with 10 shoots per block

untreated shoots. The net effect of treatments was the transfer of the overall activity within the distal zone to the distal remaining buds since the total zone effects were the same as on untreated shoots (Figures B6, B7).

Following treatment at Time 2 the same general responses were obtained but their magnitude was less, particularly below the distal bud. There was an overall reduction in bud-burst frequency following disbudding at Time 2 compared with Time 1 (Figure B8).

As found in the comparison between zones, the pattern of bud-burst time at the nodes within the distal zone responded differently to pruning compared with disbudding. Decapitation or pruning promoted earlier burst at all nodes while disbudding tended to delay it, at least at lower nodes (Figure B10). On decapitated or pruned shoots bud-burst occurred earlier at the distal 1-2 buds than those below.

The Distribution of Growth

Pruning reduced the total increase in shoot length during the following growth season but the length of lateral shoots was unchanged (Figure B2). Decapitation increased the growth of lateral shoots but disbudding prevented this increase.

There was a marked change in the pattern of shoot growth after pruning in winter. On untreated shoots lateral shoot growth occurred from any bud along the main shoot although it tended to be greater from the more distal buds (Figure B11). After winter pruning the growth was almost entirely

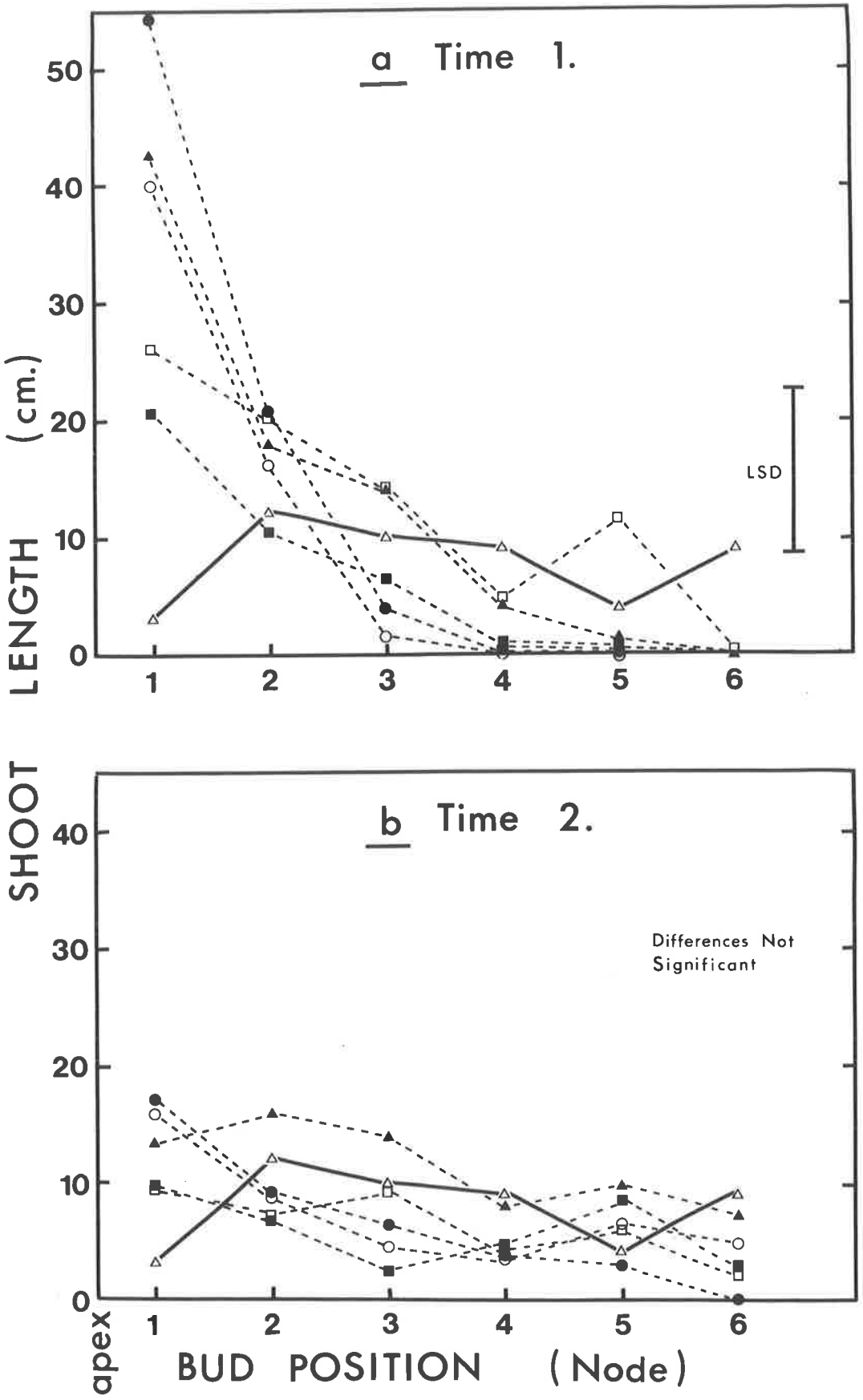

a Time 1.
SHOOT LENGTH (cm.)
50
40
30
20
10
0
LSD
1
2
3
4
5
6
b Time 2.
Differences Not Significant
apex
BUD POSITION (Node)

FIGURE B12 MEAN SHOOT LENGTH AT NODES WITHIN THE DISTAL ZONE

△ Untreated

▲ Decapitated

□ 1/3 Disbudded

○ 1/3 Pruned

▣ 2/3 Disbudded

● 2/3 Pruned

a - Time 1 - Treatment before natural bud-burst

b - Time 2 - Treatment after natural bud-burst

L.S.D. P=0.01

from the distal 2-3 buds. A similar change occurred with decapitation, with and without disbudding. The length of the main new shoot was increased by all treatments but was significantly less with disbudding (Figure B12).

Spring pruning had much less effect on the pattern of shoot growth (Figure B11). The length of the distal remaining bud was always increased but did not exceed growth from the terminal bud on untreated shoots (Figure B12). Growth from lower buds did not differ from that on untreated shoots.

B2(d) Discussion

Winter Pruning

Winter pruning of one-year-old woody shoots on apple rootstock cultivars hastened bud-burst but reduced the number which burst and elongated. Similar responses were obtained by earlier workers who pruned one-year-old rootstocks in winter i.e. hastened burst (Maggs 1959a; Oskamp 1931) and reduced shoot number (Bedford and Pickering 1919; Maggs 1959a, 1963, 1965b; Magness, Edmeister and Gardener 1917; Oskamp 1931). Bud-burst and elongation was enhanced at the distal end of the remaining shoot with a corresponding reduction at lower nodes. Decapitation promoted earlier bud-burst along the shoot and increased bud-burst and elongation within the distal zone. These effects were similar to the effects of pruning. Therefore, removal of the distal portion of the shoot, with the concomitant pruning cut, is sufficient to induce this part of the pruning response. The magnitude of the promotion of early burst was greater with pruning

than decapitation but was not proportional to the amount of shoot removed as reported by Maggs (1959a).

The total number of buds which burst or elongated was not significantly altered by decapitation. However when the shoots were also partially disbudded these total numbers were reduced and the numbers were correlated with the number of buds present (or removed) as was also the case with pruning. Only a few of the buds which burst subsequently elongated although the proportion of buds elongating tended to increase as the total burst was reduced. These results are similar to those reported by Edwards (1969) who also found that an increasing proportion of buds which burst subsequently elongated as the total burst was reduced. He found no difference between bud removal by pruning or disbudding. Maggs (1959a) and Barlow and Hancock (1960) reported a correlation between the amount of shoot removed and the number of buds bursting whilst Magness et al. (1917) reported a correlation with the number of shoots produced. This suggests that the removal of buds may contribute to the overall pruning response by reducing the number of buds available to respond.

The proportion of buds elongating after burst appears to be dependent on the plants growth capacity. The data presented by Maggs (1963) indicate a proportionate increase in shoot number with increasing burst number on established stool-bed trees (see Maggs' Figure 4, Experiment 2).

The reduction in the total number of buds which burst was less than the reduction in the number of buds available because not all buds burst. The frequency at which bud-burst occurred at any node decreased basipetally along the shoot but following pruning there was a compensatory increase in the frequency of bud-burst at nodes within the distal remaining zone. Thus the net result was a reduction in the number of buds present at nodes where burst frequency is low.

There was a slight reduction in the total elongation growth following winter pruning but this growth occurred almost entirely at the top one or two buds so that the length of the main shoot was increased. The pattern of lateral shoot growth is determined by the number of buds which elongate, the position of these buds and the distribution of the total growth between these buds. Maggs (1960) found that the total length of elongation growth was dependant on the number of growing points. The results of this experiment are consistent with this since total shoot growth varied with treatment in a similar manner to the number of buds elongating. If this relationship is valid then reduction in the total elongation growth following winter pruning may be a consequence of the removal of buds or growing points. The actual total growth will of course be dependant on the overall vigour of the main shoot.

On untreated shoots bud-burst and elongation tend to occur uniformly along the length of the shoot although it is more frequent at the higher nodes (except the top node)and

greatest at the apex. Winter pruning results in a general increase in bud activity at the distal remaining nodes with a corresponding reduction at the lower nodes; bud elongation is almost confined to the distal two nodes. The promotion of growth at the distal nodes would appear to be an example of the release of buds from the dominance by the more distal buds. Conversely the reduction in growth at lower nodes may be due to increased apical dominance caused by enhanced growth at the distal nodes.

On untreated shoots most of the new extension growth occurred from the apical bud or the top lateral bud but buds lower down the shoot commonly formed lateral shoots too. When a shoot was winter pruned the top remaining bud usually grew vigorously with little growth from the buds below. Decapitation, with and without disbudding, had a similar effect on this response as it had on the overall increase in bud-burst and elongation within the distal zone. Therefore the same mode of response is likely to be involved.

Spring Pruning

In spring, after natural bud-burst, decapitation or light pruning no longer reduced the number of buds which burst but increased the number which elongated. These increases in total bud-burst and elongation, compared to winter treatment, were the result of less promotion of the distal bud so that the lower buds were no longer suppressed. The distribution of new growth was similarly affected and more lateral shoot growth occurred along the length of the shoot though the total shoot length was unchanged.

The difference in response to pruning between spring and winter was associated with reduced dominance of the distal buds over those below. Following winter pruning bud-burst was earlier at the top one or two nodes than at those below even though there was a general promotion of early burst. Cannon (1941) and Maggs (1959a, 1963) found that part of the difference in shoot growth following pruning could be attributed to the time of bud-burst. Also differences in shoot growth between rootstock cultivars may be due to the time of natural bud break (Maggs 1958). Jankiewicz (1972) has proposed a scheme whereby a small initial advantage of earlier commencement of growth may result in a large long term advantage. Thus, although the difference in bud-burst time was less than a week, such early differences may persist throughout the growth cycle.

There was no indication of the promotion of early bud-burst following spring pruning since all burst occurred within 14 days. Also, many buds had burst before the treatments were applied. Thus the distal bud did not gain an early advantage over the others and this may explain why bud activity was not confined to the distal nodes.

Another possible explanation cannot be overlooked. Bud activity at the commencement of the growth season is dependent on the plant's reserves of substrates (Harley, Regeimbal and Moon 1958; Kozlowski 1962a; Priestley 1962a, b). The supply or mobilization of these reserves may be a limiting factor (Kozlowski 1971; Murneek 1930; Taylor 1966). At the time of

spring treatments the shoots had leaves present and the supply of substrates may have been greater although the demand for substrates for growth would also have been high. A greater supply of these substrates could reduce the competition between growing points and so widen the distribution of growth. The reduction of apical dominance under conditions of high nutritional status has been reported (Gregory and Veale 1957; Wareing and Nasr 1961). However the fact that total shoot growth was relatively unaffected by the different pruning treatments (excluding inhibition associated with disbudding) suggests that some factor essential for growth was limiting in all cases.

The Disbudding Effect

An unexpected complication in this experiment was an effect associated with disbudding. The presence of disbudded wood distal to the buds caused a general suppression of their activity. When a shoot was partially disbudded following decapitation the promotion of early bud-burst along the shoot no longer occurred and the stimulation of bud-burst and growth of the distal remaining bud was reduced. This suppression of bud activity may be attributed directly to the presence of the disbudded wood itself or to the separation of the remaining buds from the distal end of the shoot and the pruning cut.

The influence of disbudding also varied with the time of treatment. There was a difference between disbudding and pruning on the total bud-burst and elongation following

winter treatment but pruned shoots had greater bud-burst and elongation after spring treatment. This could be due to a difference in the responsiveness of the buds to the disbudded wood effect or a change in the effect of disbudding on the distal part of the shoot (see Section III B 5).

There was a consistent difference between the levels of disbudding such that a greater inhibitory effect occurred with a large length of disbudded wood present. This again may relate to the length of disbudded wood itself or to the distance between the cut and the buds.

The next section of this work describes an attempt to distinguish between an effect due to the presence of the disbudded wood itself and one caused by separation of the buds from the (distal) cut end of the shoot.

Summary

The components of pruning do contribute differently to the overall response to pruning and their contribution varies with the time of pruning, as follows:

a) The removal of the apical portion of the shoot (decapitation) stimulates greater growth from the distal remaining buds; it was not possible to determine whether this was due to removal of the terminal apex itself or to the concomitant pruning cut.

b) The removal of lateral buds contributes to the pruning effect in two ways:

   i) the reduction in the number of buds present

quantitatively reduces the number of buds which burst and hence the number of lateral shoots formed.

ii) the removal of the more distal buds stimulates growth from buds at lower positions along the main shoot. This was a qualitative response.

c) The presence of disbudded wood distal to the buds causes a reduction in the growth of the buds immediately below.

It was not possible from this experiment to determine whether distal wood itself influences bud growth below it because of the abnormal effect associated with the disbudding treatments.

B3 THE EFFECT OF DISBUDDING

B3(a) Introduction

In the previous experiment (Section III B 2) it was found that disbudding the distal portion of a shoot resulted in less activity of the buds below than did removal of the same portion by pruning. This was particularly evident in the time of bud-burst (Figure B2). Cannon (1941) reported that maiden shoot growth was less where 4 inches of stock was left distal to the bud union until summer rather than cutting back to the bud in spring. The implication is that the presence of disbudded wood distal to the buds may have a deleterious effect on their growth. This effect has been examined in more detail.

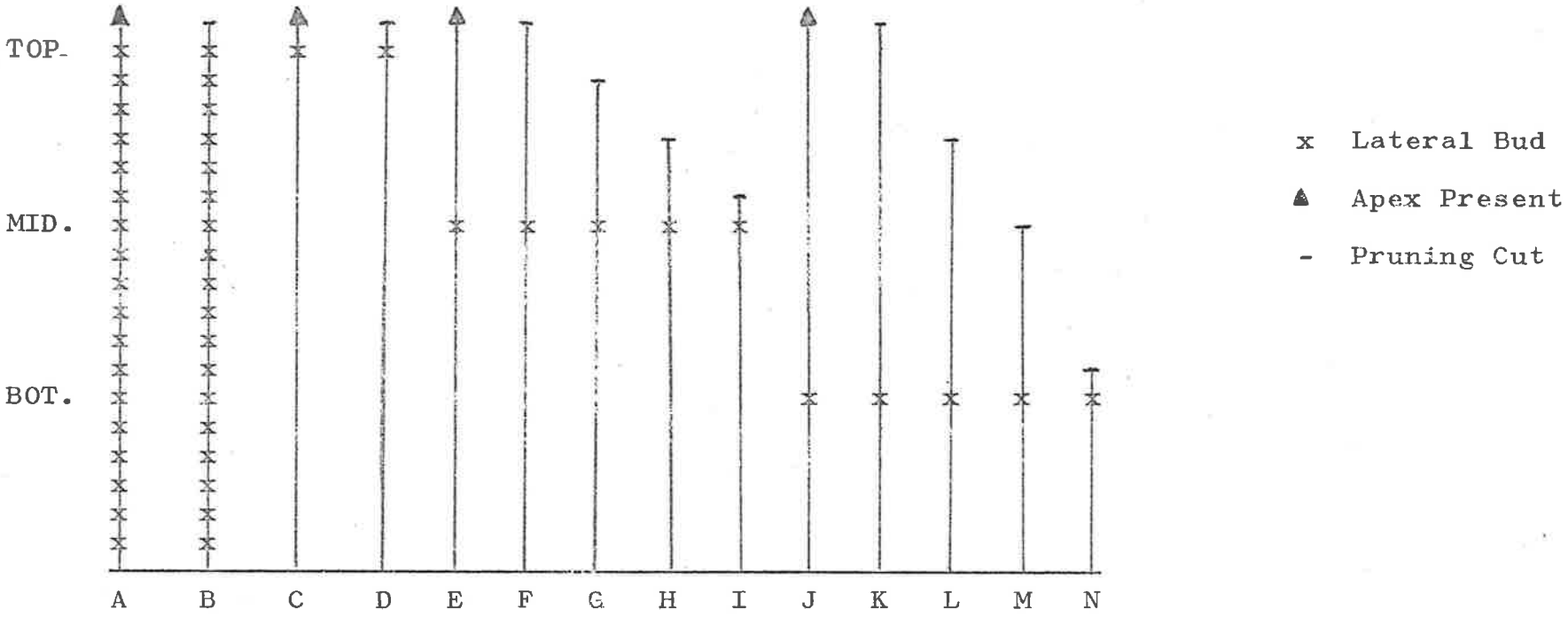
The effect of disbudding was previously observed only in conjunction with decapitation of the shoot. In this experiment shoots were disbudded with and without decapitation to determine whether disbudding has an independent effect on bud growth as opposed to the reduction or prevention of the response to decapitation.

The earlier results (Figure B4, B5) suggested that there may be a quantitative relationship between the length of disbudded wood distal to the buds and the suppression of bud activity. This relationship, and the importance of the position of the disbudded wood relative to the bud, were investigated by varying both the length of disbudded wood present and the position of the bud.

FIGURE B13

THE TREATMENTS

Vertical lines represent length of shoot.



This experimental approach was limited in two ways; (a) it is impossible to have the apex present on pruned shoots and (b) the pruning cut occurred at, and was necessarily associated with, a varying distance from the bud. Only one bud was left on each shoot (except for controls) to avoid the occurrence of different numbers of buds.

B3(b) Methods

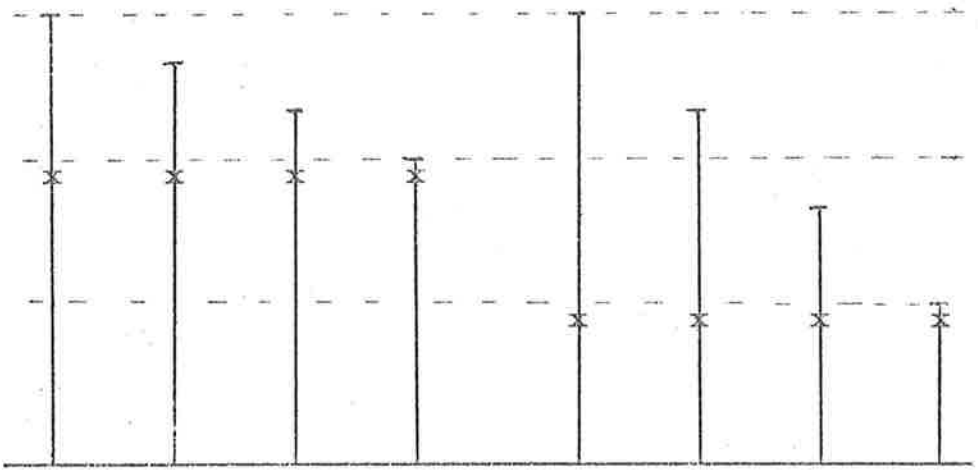
One-year-old, single stemmed shoots with a few basal roots were cut from stool-bed Northern Spy rootstocks and planted in 10 inch plastic pots using John Innes potting soil with ¼ inch gravel added to aid drainage. The plants were potted on the 13th September 1973 (early spring, before bud-burst) and treated two weeks later. They were ranked for shoot length and grouped to give six replicates.

The shoots were disbudded to leave only one lateral bud except for the untreated and decapitated control plants. The single bud was left at the top, middle or bottom position along the shoot (see Figure B13) with and without decapitation. Different lengths (all, 2/3, 1/3 or none) of the distal disbudded wood were removed by pruning to provide different proportions of disbudded wood distal and proximal to the bud. The fourteen treatments are illustrated by Figure B13.

Bud-burst was recorded daily until day fifty and then periodically until seven months after treatment time. Analysis of variance was carried out over all treatments but because the experimental design was unbalanced, separate analyses were carried out for comparisons between groups of treatments.

FIGURE B14

DIFFERENT LENGTH OF DISTAL DISBUDDED WOOD - POSITION CONSTANT



LENGTH OF DISTAL DISBUDDED WOOD - POSITION AVERAGED

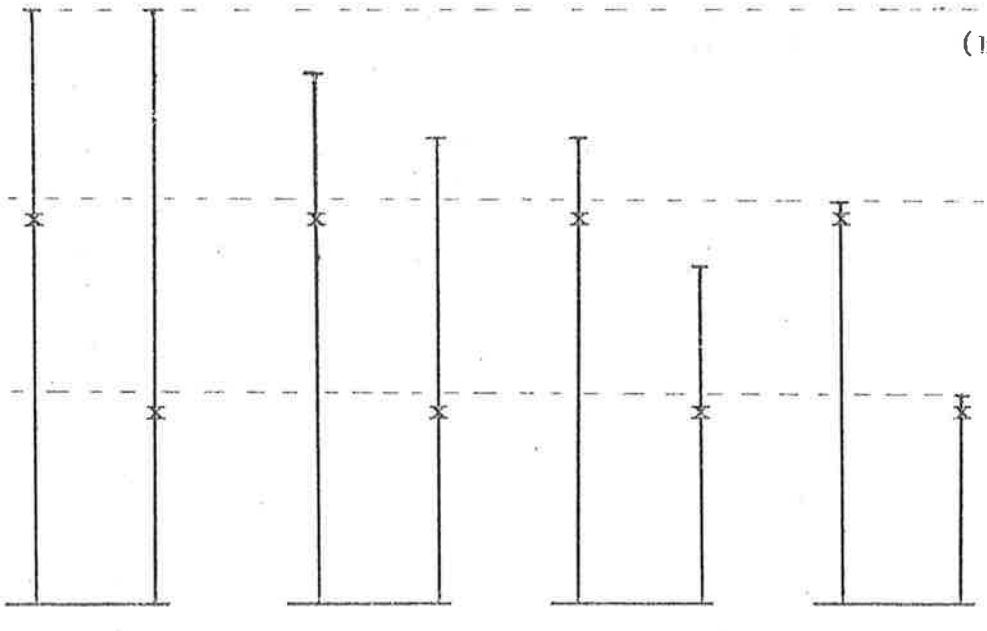


FIGURE B14 THE QUANTITATIVE EFFECT OF DISBUDDED WOOD

Bud-burst time (days) is compared for buds with different lengths of distal disbudded wood present. The diagrams illustrate the treatments between which comparisons are made with the mean bud-burst time indicated below. Similar letters beneath the figures indicate values not significantly different (P=0.05)

There was no significant difference in bud-burst time between buds with different lengths of distal disbudded wood present for buds at either position (a) or with the effect of position averaged (b)

x lateral bud

\- pruning cut

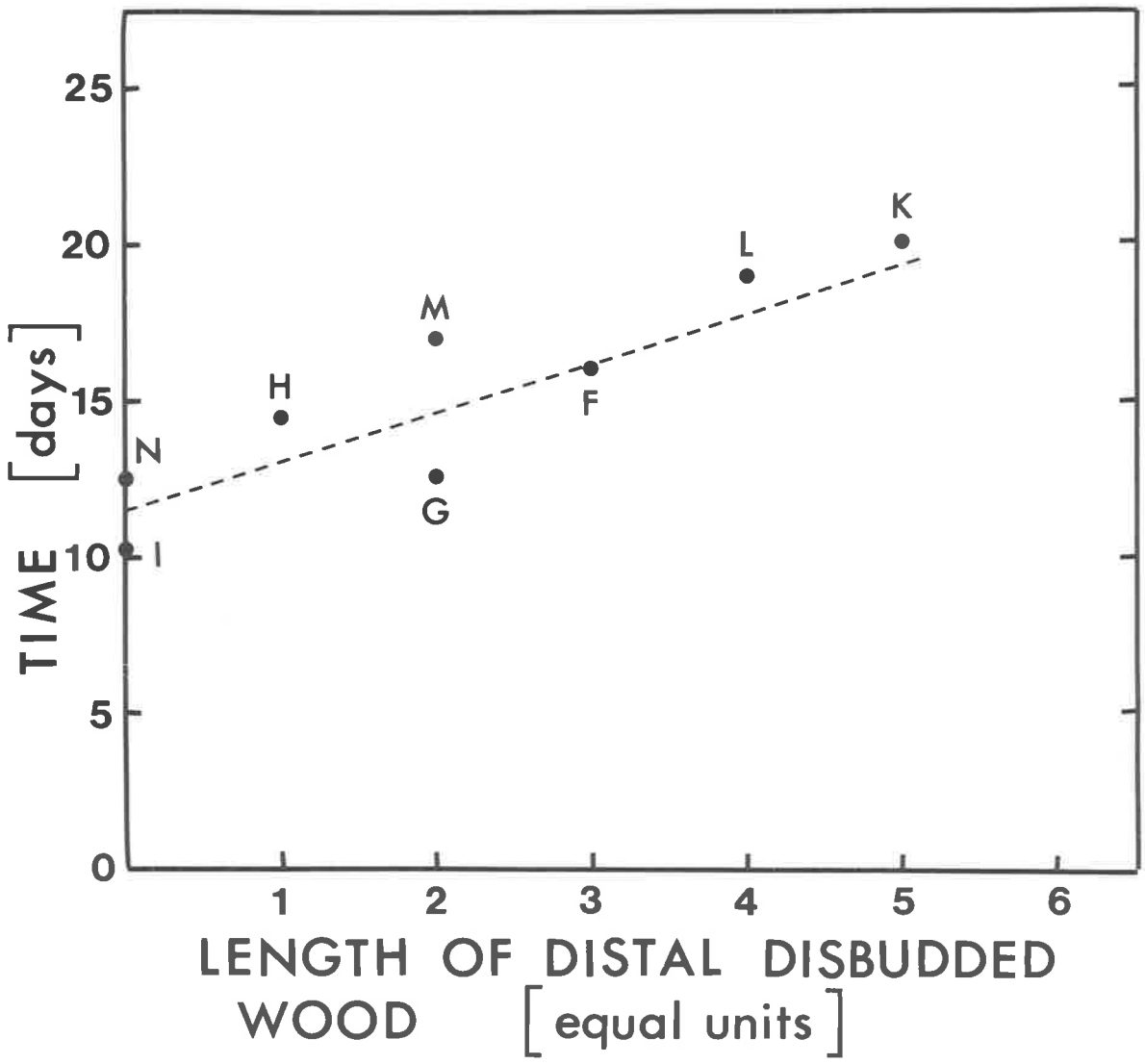

25
20
15
10
5
0
TIME [days]
K
L
M
H
F
N
G
I
1
2
3
4
5
6
LENGTH OF DISTAL DISBUDDED WOOD [equal units]

FIGURE B15 CORRELATION BETWEEN BUD-BURST TIME AND LENGTH OF DISTAL DISBUDDED WOOD

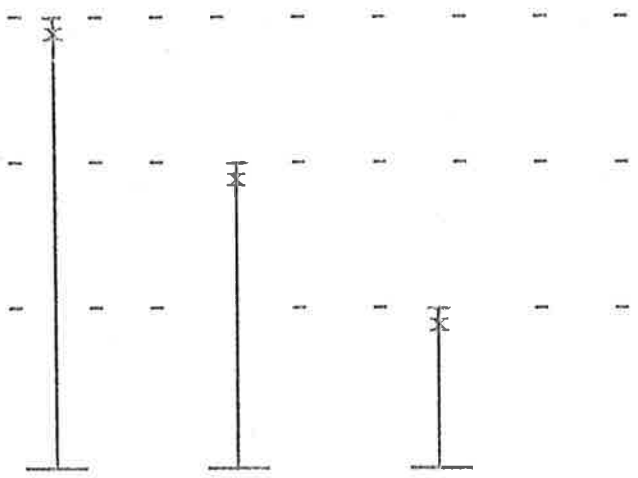
The letters indicate the treatment from which the point was obtained - these correspond to Figure B13.

The unit of length was 1/9 the mean shoot length within a block such that each zone of a shoot was approximately 3 units long.

FIGURE B16

THE DIFFERENCE BETWEEN

BUD POSITIONS



DISBUDDING DELAYS BURST WITH OR WITHOUT

DECAPITATION

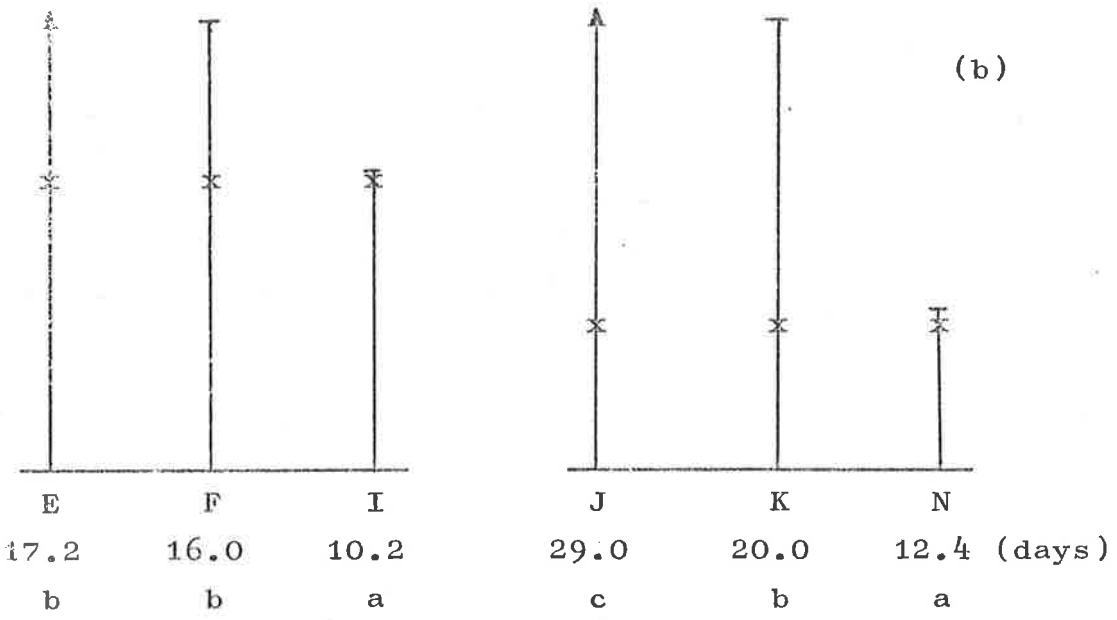


FIGURE B16

(a) THE POSITION EFFECT

Bud-burst time (days) is longer at lower nodes in the absence of distal disbudded wood

(b) THE INFLUENCE OF THE APEX ON THE EFFECT OF DISTAL DISBUDDED WOOD

Distal disbudded wood delays bud-burst irrespective of the presence or absence of the apical bud.

Different letters below the figures indicate differences significant at P=0.05

x	Lateral bud
▲	Apex present
-	pruning cut

TABLE B1

THE TIME OF BUD-BURST (days)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Top	7.4	9.0	12.6	5.4										
Mid.	10.2	11.0	-	-	17.2	16.0	12.6	14.4	10.2					
Bot.	**	**	-	-	-	-	-	-	-	‡‡29.0	20.0	18.8	16.8	12.4

L.S.D. (p=0.05) = 4.3 days

** Only two buds of B had burst after 7 months.

‡‡ This mean may be unreliable because some apices abscissed but the difference between this value and the others is regarded as significant because loss of the apex would be expected to bring the value closer to treatment K.

Only differences significant at the 95% level were accepted.

B3(c) Results

The mean burst time for each bud is presented in Table B1. There was no difference between the top and middle position on the untreated shoots but bud-burst did not occur at the bottom position (A). Decapitation alone had no significant effect (B). Disbudding to leave only one lateral bud tended to delay bud-burst at all positions (A vs C, E, J and B vs F, G, H or K, L, M), except where the bud was adjacent the pruning cut (B vs D, I, N). However disbudding stimulated bud-burst at the bottom position where it did not usually occur on the untreated shoots.

If a portion of disbudded wood remained distal to the bud, burst was delayed compared to other buds at the same position where all the distal wood was removed by pruning (Figure B14 a). There was a general correlation between the length of disbudded wood distal to the bud and the time of burst (Figure B15) but when the effect of bud position (i.e. the distance from the base of the shoot) is removed by taking the average value for buds at different positions there is no significant difference between different lengths of distal disbudded wood (Figure B14 b; F, K vs G.L. vs H.M).

A comparison between buds at different positions but with no distal wood present (Figure B16 a) shows that buds at lower positions burst later. Factorial analysis indicated that there was no interaction between the position of the

bud and the length of distal disbudded wood or the presence or absence of decapitation.

The delay of bud-burst associated with the presence of disbudded wood distal to the bud occurred with or without decapitation of the shoot (Figure B16 b, i.e. both E + F are $>$ I and J + K are $>$ N). Decapitation of the disbudded shoots promoted earlier bud-burst at the top and bottom positions (Table 1. C vs D, J vs K) but not at the middle position (E vs F).

B3(d) Discussion

The delay of bud-burst as a result of disbudding the distal portion of a shoot was reported earlier (Section III B2) and has been confirmed here. This delay occurred on both decapitated and non-decapitated shoots (Figure B16 b) indicating that the effect of disbudding involves more than the prevention of the response to decapitation.

The results in the preceding section (Section III B2) showed that there tended to be a greater delay of bud-burst when a larger portion of distal disbudded wood was present. Similarly, in this experiment there was a general correlation between the length of distal disbudded wood and the time of bud-burst (Figure B15). However, with the bud at the same position, there was no significant difference in burst time in the presence of different lengths of distal disbudded wood (Figure B14). The disbudding effect would therefore appear to be qualitative rather than quantitative.

Where all shoots have the same total length, variation of the length of disbudded wood distal to a bud can only be achieved by using buds at different positions. The comparison of buds at different positions on disbudded shoots, other factors being equal (Figure B16 a), showed that buds at the lower positions tend to burst later and this position effect was independent of the other factors. This explains why there was an apparent correlation between the length of distal disbudded wood and the time of bud-burst.

There was no indication of a significant difference in bud-burst time for buds in different zones on shoots in the earlier experiment (Figure B7 d) or at different positions on the non-disbudded shoots of this experiment (Table B1). This suggests that the position effect is associated with the disbudding of the shoots. It has already been shown (above) that the length of distal disbudded wood has no effect therefore the position effect must be due to the length of disbudded wood proximal to the bud.

Thus the overall delay of bud-burst associated with the disbudding of shoots appears to be the result of two factors:

i) the occurrence of disbudded wood distal to the bud, which tends to be an all or none effect,

ii) the disbudding of the shoot proximal to the bud, which causes a greater delay of bud-burst for buds at lower positions.

In the context of analysing the effects of pruning only the first factor above is relevant since only the portion of the shoot distal to the bud can be manipulated by pruning. The role of disbudding itself, as opposed to the presence of wood irrespective of disbudding, cannot be established because isolation of the wood necessarily involves removal of the lateral buds by disbudding. In any case, distal disbudded wood cannot be involved in the pruning response therefore only an effect due to removal of the distal wood would be of importance.

The removal of distal wood, regardless of disbudding, may involve removal of a source of inhibition or conversely, removal of a more distal (and hence competitive) sink for some factor(s) which promotes bud growth. In the process of removing the wood a pruning cut is formed adjacent to the distal bud. The presence of distal disbudded wood may prevent the response to pruning because the wood is still present or because the cut is no longer adjacent to the bud. The latter possibility has been investigated further in sections III B4 and III C.

B4 THE PRUNING CUT

B4(a) Introduction

Although Section B3 established that the presence of disbudded wood distal to a bud delays burst, no distinction was made between the effect of the disbudded wood *per se* and the concommitant separation of the bud from the pruning cut. Several observations have been recorded which suggest that a pruning cut promotes earlier burst of the bud proximal to the cut :

i) The overall delay of bud-burst associated with disbudding did not occur when the bud was adjacent to the pruning cut (Section III B 3).

ii) Removal of the distal portion of the shoot by pruning stimulated earlier burst of the remaining buds, particularly the one adjacent to the cut (Section II B 2).

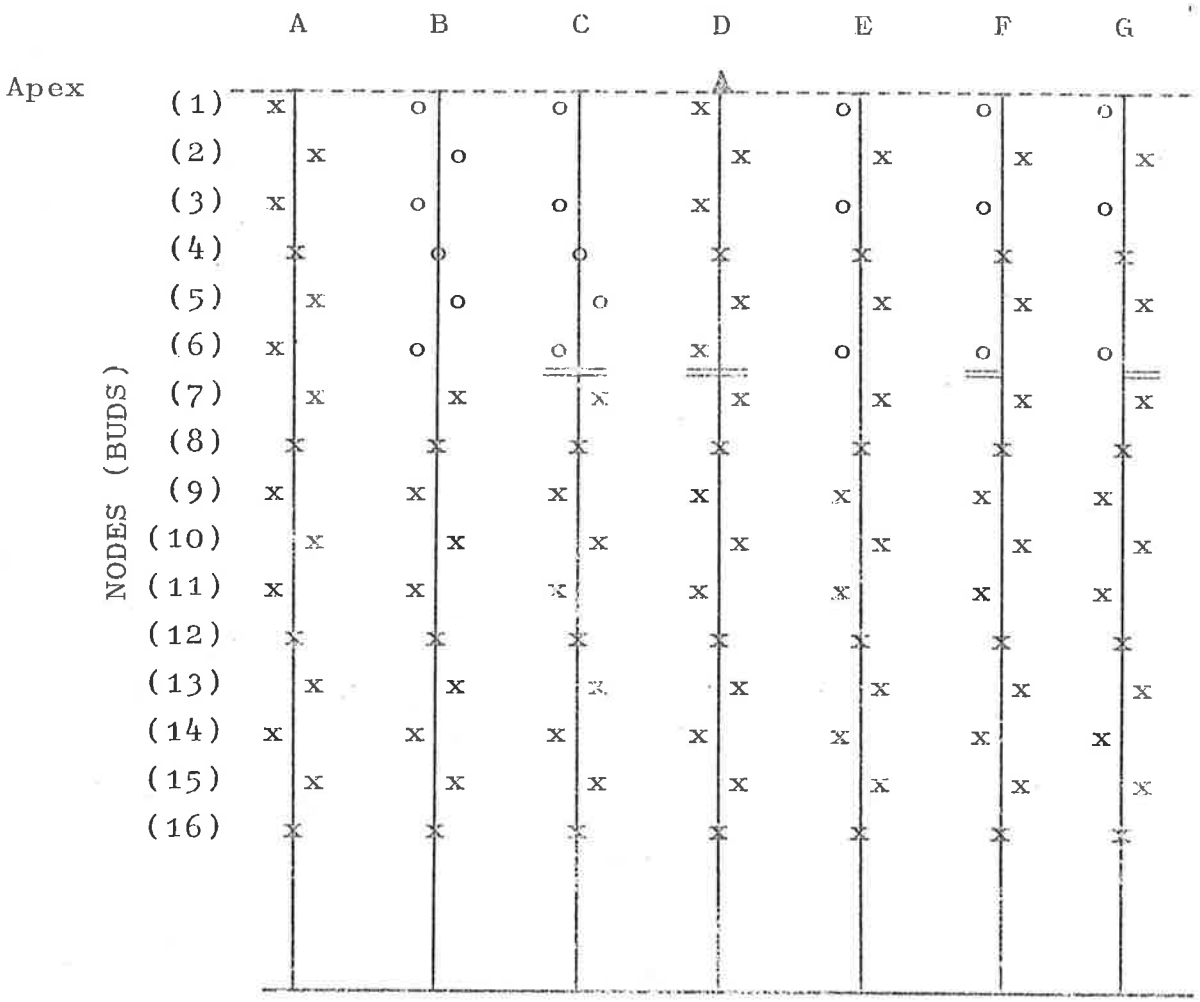
iii) Cutting a shoot into single-bud segments, so that each bud is adjacent to a pruning cut, stimulates bud-burst even when the buds on intact shoots are dormant (Section III D 3).

If proximity to a pruning cut promotes bud-burst, the difference between pruning and disbudding above a bud may be due to the separation of the pruning cut from the buds.

An experiment was designed to test the above proposal but the results were inconclusive. There was no indication of the delay of bud-burst as was usually associated with the

FIGURE B17

THE TREATMENTS



A	DECAPITATED CONTROL	▲	Apex present
B	DISBUDDED	x	Lateral bud
C	DISBUDDED + GIRDLE	o	Bud removed
D	GIRDLE ONLY	═	Girdling position
E	½ DISBUD		
F	½ DISBUD + ½ GIRDLE BELOW		
G	½ DISBUD + ½ GIRDLE OPPOSITE		

Bud positions on opposite sides of the line are on opposite sides of the shoot.

presence of disbudded wood. The experiment has been reported here because it is still considered to be a valid approach to the problem but there was no opportunity to repeat it. Some possible explanations for the lack of response to disbudding are discussed later (Section III B 4(d)).

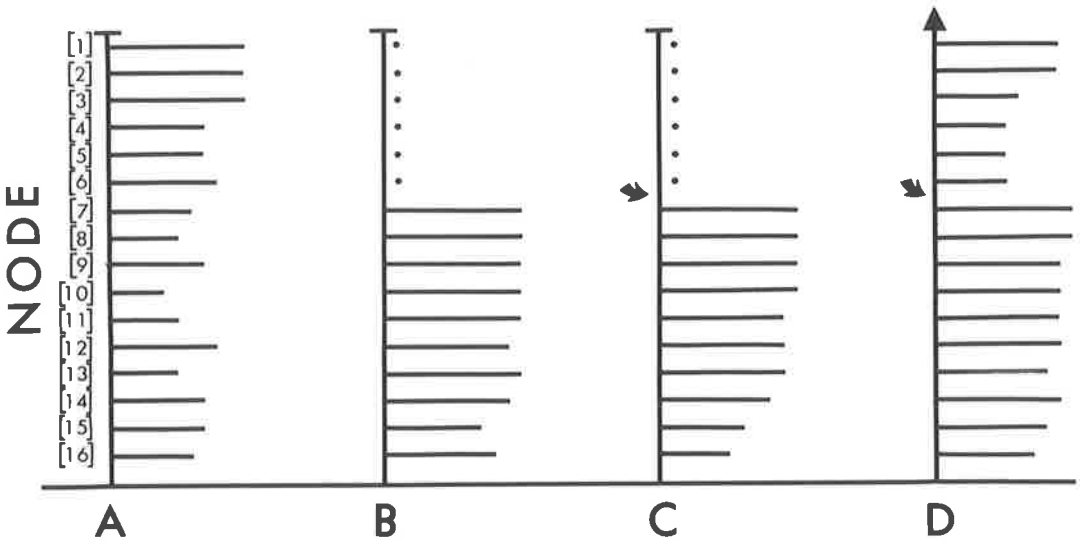
The hypothesis for this experiment was that the effect of distal disbudded wood could be overcome by a cut adjacent to the bud. Since a pruning cut could not be used without removing the distal wood, girdling was used to simulate a cutting wound above the bud. The effect of girdling itself was allowed for by including girdled, non-disbudded shoots. Also, combinations of disbudding one side with half-girdling below, on the same or opposite side of the shoot, were included to determine whether isolation of disbudded wood by a girdle had any effect on the response to disbudding.

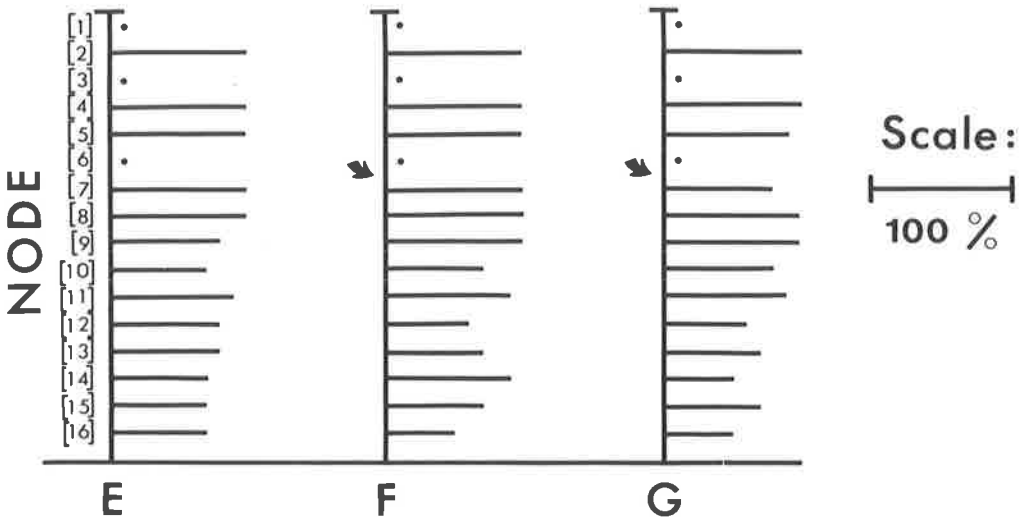
B4(b) Methods

Stool-bed cuttings of Northern Spy rootstocks were collected and potted in the previous year (as in Section III B 3(b)). They were cut back to 3-5cm. above the soil and only one new shoot was allowed to grow. These plants were left outside over winter and were given one application of John Innes supplementary nutrient solution during late winter. Prior to the experiment they were ranked for shoot length and grouped into 10 blocks of 7 plants.

Treatments involved combinations of girdling and half-girdling above the seventh lateral bud from the apex and

BUD-BURST FREQUENCY





Scale:

100 %

FIGURE B18 FREQUENCY OF BUD-BURST AT EACH NODE

Length of horizontal line represents the frequency at which bud-burst occurred at the node.

A	Untreated
B	Disbudding
C	Disbudding + Girdle
D	Girdle only
E	One side disbudded
F	One side disbudded + ½-girdle same side
G	One side disbudded + ½-girdle opposite side

Arrows indicate the position of girdling treatments.

Dots indicate nodes where buds removed.

Each value determined from 10 shoots.

complete or partial disbudding of the shoot above this point. They are summarised in Figure B17.

The treatments were applied on 3rd October 1974 and bud-burst was recorded at daily intervals over the following 4 weeks. Analyses of variance were carried out on bud-burst time data for buds at particular positions and on means for groups of buds.

B4(c) Results

All buds at the distal 2-3 nodes burst except where the apex was present (Figure B18 D). Disbudding and/or girdling, resulted in 100% bud-burst at the more distal nodes below the treatment zone (Figure B18: nodes (1), (3) & (6)) but there was no significant difference between treatments. Partial disbudding increased the frequency of bud-burst at nodes within the treatment zone.

TABLE B2 BUD-BURST TIME FOR TWO BUDS BELOW TREATMENT ZONE

(days)

	A CONT.	B DISBUD	C DISBUD + GIRDLE	D GIRDLE ONLY	E ½DISBUD	F ½DISBUD + ½GIRDLE BELOW	G ½DISBUD + ½GIRDLE OPP.
Node (7)	9.3	9.4	9.8	9.8	8.5	8.3	9.5
Node	12.0	9.3	8.7	9.2	9.1	9.3	10.0

No difference significant (p=0.05)

The mean burst-time for the two buds below the treatment zone did not differ significantly between treatments (Table B2). The presence of distal disbudded wood did not cause a delay of bud-burst (Table B2: A vs G).

Table B3 presents the mean burst times for the buds anatomically (directly) below the treated positions. There was no significant difference between the buds on opposite sides of the shoots within any treatment, or between treatments for either side.

TABLE B3 BUD-BURST TIME FOR BUDS ANATOMICALLY BELOW TREATED POSITIONS (MEAN) (days)

	A CONT.	B DISBUD	C DISBUD + GIRDLE	D GIRDLE ONLY	E ½DISBUD	F ½DISBUD + ½GIRDLE BELOW	G ½DISBUD + ½GIRDLE OPP.
Below 1,3,6	11.5	9.0	10.3	11.2	11.2	9.5	9.8
Below 2,5	9.6	10.5	11.6	12.0	11.1	9.3	9.7

No difference significant (p=0.05)

B4(d) Discussion

The shoots used in this experiment did not display a delay of bud-burst in the presence of distal disbudded wood. Bud-burst occurred readily on all shoots and mostly within 1-2 weeks from treatment time. This behaviour was quite different to that observed in the previous experiments on one-year-old

shoots. The reason for this difference is not known but some possibilities are discussed below. Whatever the cause, it indicates that the responses normally observed are dependent on the balance of other physiological or environmental factors.

The shoots used for Section III B2 were located on small trees where competition between shoots is possible. Those used for Section III B3 were newly planted stool-bed shoots which had limited root development at the time of treatment. In both these cases the supply of nutrients or assimilates to the treated shoots was limited. On the other hand, the shoots used in this section were single-stemmed plants with established root systems. Also they were given an application of supplementary nutrient solution during the winter dormancy period, therefore they were well supplied with nutrients and there was no competition between shoots.

It has already been suggested that pruning regulates shoot growth by modifying the supply or distribution of nutrients (Moorby and Wareing 1963) or assimilates (Maggs 1965a) between growing points. Where the supply is sufficient to enable growth from all the growing points the relative distribution of nutrients or assimilates is not likely to be important. The high frequency of bud-burst and the even burst-times in this experiment are consistent with the idea that there were no differences between treatments because of the high nutritional status in the plants.

Another possible source of difference between the experiments is the balance between the roots and the number of shoots (growing points). In Section III B2 there were numerous one-year-old shoots which were pruned off the trees prior to the experiment. The hard pruning of these shoots stimulated early growth of buds not involved in the experiment and these could have competed with the later pruned, experimental shoots. Conversely, in Section III B3 the scarcity of roots on the plants could have favoured root growth in preference to shoot growth to maintain the balance between roots and shoots (Maggs (1959a 1961). However the plants used in this section had established root systems therefore any reduction in the shoot system would enhance shoot growth if the balance is to be restored.

A variation in climatic conditions from year to year could influence the response of the plants but an examination of mean daily maximum and minimum temperatures over the experimental periods did not reveal any marked differences. The conditions prevailing during the preceding winter period could influence the physiological state of the shoots at treatment time but there was no significant difference in the time of burst of buds on the control shoots of these experiments. Thus it would appear that seasonal variation was not an important contributor to the differences between experiments.

The only difference between the treatments was in the pattern of bud-burst frequency (Figure B18). In general total burst

was the same and removal of buds resulted in a slight increase in burst frequency at other positions. The occurrence of a girdle on whole shoots (Figure B18 D) stimulated higher burst frequency at the nodes below, presumably by releasing them from apical dominance. The involvement of apical dominance is examined more closely later in Section III C3.

Thus, as stated in the introduction, although the treatments could be used to test the hypothesis the material used did not display the required response to disbudding.

TABLE B4 THE RELATIONSHIP BETWEEN THE COMPONENTS OF PRUNING AND THE TYPES OF RESPONSES

	TYPES OF RESPONSES	COMPONENTS INVOLVED
(1)	Changes in bud-burst number and shoot number	- removal of lateral buds
(2)	Time of bud-burst	- the pruning cut or - the removal of wood
(3)	The distribution of bud-burst and growth	- removal of the terminal bud - removal of all distal buds
(4)	Overall shoot elongation growth	- the removal of lateral buds

TABLE B5 THE RESPONSES TO PRUNING DORMANT AND ACTIVE SHOOTS

	PARAMETER	DORMANT SHOOTS	ACTIVE SHOOTS
(1)	Bud-burst Number	reduced	unaffected
	Shoot Number	reduced	increased
(2)	Bud-burst Time	may be hastened	unaffected
(3)	Distribution of Burst and growth	enhanced at distal nodes, reduced below	even distribution

B5 DISCUSSION OF PRUNING EXPERIMENTS

The aim in this section of the experimental work was to analyse the relationships between the components of pruning and the responses of buds to pruning treatments. As anticipated in the introduction, the components of pruning were found to influence different aspects of the pruning response and their influence varied with the time of pruning. The responses to pruning may be divided into 3 general groups and these are summarised in Table B4 along with the components of pruning which effect them. The responses obtained following pruning dormant or active shoots are summarised in Table B5.

When the final shoot form is considered the most marked effect of dormant pruning is the stimulation of vigorous growth from the distal remaining 1-3 buds. This general pattern is evident regardless of the degree of pruning (see Figures B5, B12). On untreated shoots extension growth occurs randomly from buds along the shoot although there is a tendency for more growth to occur at distal buds and the apex. Thus the response to pruning is essentially the promotion of growth from distal (remaining) buds and the suppression of buds at lower (proximal) nodes.

The promotion of the distal remaining buds would appear to involve the removal of correlative inhibition from more distal buds, including the apex, since disbudding was sufficient to obtain this response. Conversely the suppression of the lower buds suggests that correlative inhibition of

these buds has been increased. This may be attributed to the enhanced growth of the distal buds on pruned shoots. Why then, do the distal buds on pruned shoots grow more vigorously?

There are a number of factors which could contribute to the enhanced growth of the distal buds following dormant pruning:

1) Buds proximal to a pruning cut burst earlier. This earlier commencement of growth may provide a competitive advantage which is perpetuated (Jankiewicz 1972; Maggs 1959a).
2) Pruning reduces the number of buds which burst and elongate thereby increasing the availability of growth factors to those which grow.
3) On untreated shoots the apical bud remains at rest longer than the lateral buds (Figures D2, D4, D6). This might enable the lateral buds to compete with the apical bud and thereby reduce apical dominance. The random distribution of bud-burst along the shoot is consistant with this suggestion.

When shoots were pruned in spring, after natural bud-burst, the pattern of bud growth was not markedly altered (Figure B12) except for the loss of a portion of the buds. Correlative inhibition between buds does not appear to be significant at this stage of bud development. In contrast to the earlier situation (dormant pruning) where the distal bud was promoted, spring pruning:

1) Did not hasten bud-burst near the pruning cut.
2) Increased the number of buds elongating.

Furthermore it will be shown in Section III D6 that rest is completed in all buds by this time.

The relative importance of the above factors in the regulation of the pruning response cannot be ascertained from these results. However, having elucidated particular treatment component-response relationships (Table B4) different aspects of the overall effects may now be studied separately. The relationship between the pruning cut or wood removal and the time of bud-burst are investigated further.

The other aspect of this analysis of pruning effects is the lack of hastening of bud-burst when the distal part of the shoot is disbudded rather than removed by pruning. Three modes of action are suggested:

1) the distal wood (when disbudded) is the source of an inhibitor of bud-burst.
2) the distal wood provides a competitive sink for growth factors transported acropetally in the stem.
3) isolation of the distal buds from the pruning cut prevents the hastening of burst that occurs when the buds are proximal to a pruning cut.

One must also consider the different responses obtained following dormant versus spring treatments. In spring there was no difference in bud-burst time following pruning or disbudding but pruning increased bud-burst and elongation within the distal zone whilst disbudding did not. Either the nature of the influence of distal (disbudded) wood is different or the responsiveness of the buds changes.

The third possible mode of action was favoured in the light of the finding (in Section III D) that cutting a shoot into segments may break rest and reduce environmental dormancy. Furthermore the difference between dormant and spring treatments with respect to burst-time could be explained by the absence of dormancy at the later time. There can be no promotion of buds due to localised release of dormancy near a pruning cut unless dormancy is present. This is also consistent with the first proposal to explain the promotion of distal bud growth following dormant pruning but not after spring pruning i.e. the distal buds gain an initial advantage over those below because dormancy has been overcome by the adjacent pruning cut.

There is some evidence available to support the second mode of action of distal wood, particularly with respect to the effects of pruning and disbudding on the numbers of buds which grow. The resumption of cambial activity following winter dormancy has been shown to depend on the presence of active buds along the shoot (Maggs 1959a; Wareing 1968). If the distal part of the shoot is disbudded during winter, cambial activity may not be initiated in this part of the stem. This could reduce the sink effect of this distal wood (i.e. there is no growth) so that its removal does not constitute the removal of a competitive sink for growth factors required by the buds. Conversely in spring cambial activity has already commenced and the distal wood may continue growth following disbudding and thereby reduce the number of buds growing below by competition for the growth factors. Alternatively active cambia may produce a substance

which inhibits the buds below i.e. the first mode of action.

The occurrence of later bud-burst at lower positions on shoots disbudded proximally to the observed bud (Figure B16 (a))cannot be explained by the above suggestions. Since no such position effect was found when the bud was adjacent to a pruning cut one might suspect that bud dormancy is involved. Possibly dormancy remains later or is deeper in lower buds but this is not evident in non-disbudded shoots (Figure B6 (d); Table B1).

Alternatively bud-burst may be dependent on the supply of some factor arising from the proximal part of the shoot and associated with the presence of proximal buds. Removal of the proximal buds may restrict the rate of supply of this factor so that the amount available becomes dependent on the length of proximal wood present. Thus on whole shoots, this factor is not limiting and bud position relative to the proximal wood is not important. After disbudding, reduction in the length of proximal wood reduces the supply of this factor and thereby delays bud-burst. The production or supply of this factor may be associated with cambial activity which is in turn influenced by the presence of buds (Maggs 1959a; Wareing 1968).

Peterson and Fletcher (1975) found a similar effect of proximal stem length on the growth of lateral buds on pea segments. The factor was not replaced by a cytokinin (Benzyladenine).

C ANALYSIS OF THE PRUNING CUT

C1 GENERAL INTRODUCTION

An analytical approach to the study of shoot pruning, by separation of the various components involved in a pruning treatment, has indicated that some physiological advantage is conferred on the bud adjacent to the pruning cut (Section III B). Pruning results in three main changes in the physiological habitat of such a bud:

i) the bud assumes a terminal position on the shoot

ii) all parts of the shoot distal to the bud are removed (a converse of i)).

iii) the bud is in close proximity to the pruning cut.

Disbudding experiments (Section III B2) suggest that the two parameters of bud-burst, the occurrence of burst and the time of burst, may be influenced by different aspects of the pruning effect. In particular the occurrence of bud-burst appears to be associated with i) or ii) above whereas the time of burst is possibly influenced by iii). This distinction between the effects of pruning on the stimulation (occurrence) of bud-burst and the promotion of bud-burst time is examined in more detailed in this section.

C2 COMPARISON OF GIRDLING AND PRUNING

C2(a) Introduction

It has been found that pruning not only stimulates to grow, a bud which would otherwise remain dormant, it also promotes earlier burst, particularly in the distal remaining bud, compared with buds induced to grow without the introduction of a pruning cut nearby (Section III B). To what extent is the promotion of bud-burst due to removal of the possible influence of the distal part of the shoot as opposed to an effect of the pruning cut per se?

Since there is no way of removing the distal part of the shoot without introducing a pruning cut, girdling (the removal of a complete band of tissue from around the stem external to the wood) and partial girdling treatments were used to isolate a bud from the distal parts of the shoot. The assumption was that a stimulus moving basipetally from the distal part of a shoot passes through the phloem and is therefore interrupted by a girdle; however it is possible that basipetal transmission could occur via the xylem. Acropetal transport through the xylem should not be prevented by a girdle. Kurtzmann (1956) has confirmed that girdling does not interrupt the passage of xylem sap. By comparing girdling treatments with pruning it should be possible to gauge the involvement of isolation of a bud from the distal part of the shoot.

One possible effect of pruning is the direct effect of severing or disruption of the stem tissues (i.e. wounding).

Girdling treatments also involve wounding. If the girdling response is due to isolation of the bud rather than wounding, then partial girdling without isolation of the bud but still involving wounding, may distinguish between the two possibilities. The assumption here is that lateral movement of a stimulus around a partial girdle does not occur.

C2(b) Methods

The experiment was carried out on detached, one-year-old shoots, collected during mid winter (late July) from mature apple trees (c.v.Jonathan on Northern Spy rootstocks) growing in the Alverstoke Orchard at the Waite Institute. Each shoot was disbudded leaving only the top (distal) 5 lateral buds plus the apex. All the shoots within each replicate were cut to the same length then placed in covered beakers as described under General Materials and Methods (Section III A). They were all held in a growth cabinet maintained at 25°C/16 hrs daylight (fluorescent light).

Each of the following treatments++ was applied to 5 shoots (replicates):

A Untreated control

B Decapitated

C Girdled above bud (3)

D ½-Girdled above bud (3), on the same side

E ½-Girdled above bud (3), on the opposite side.

Bud-burst was recorded for each bud. Analysis of variance was carried out on the burst-time data for positions where burst occurred on more than 2/5 shoots.

++ Details of treatment procedures are presented under General Materials and Methods (Section III A).

TABLE C1 EFFECT OF GIRDLING ON BUD-BURST

(a) THE NUMBER OF BUDS WHICH BURST OUT OF 5

BUD POSITION	A UNTREATED	B DECAPITATED	C GIRDLED	D ½-GIRDLE SAME SIDE	E ½-GIRDLE OPPOSITE SIDE
Apex	2		0	1	2
(1)	0	5	3	2	2
(2)	2	2	0	3	2
(3)	2	2	5	5	1
(4)	1	0	0	1	5
(5)	1	2	2	1	1

(b) MEAN DAYS TO BURST -OF THOSE BUDS WHICH BURST

BUD POSITION	A UNTREATED	B DECAPITATED	C GIRDLED	D ½-GIRDLE SAME SIDE	E ½-GIRDLE OPPOSITE SIDE
Apex	(12.5)++		-	(19.0)	(9.5)
(1)	-	9.2	12.0	(14.5)	(10.5)
(2)	(13.0)	(11.5)	-	13.3	(10.5)
(3)	(12.5)	(12.0)	12.3	11.2	(15.0)
(4)	(12.0)	-	-	(10.0)	10.8
(5)	(11.0)	(12.0)	(10.0)	(9.0)	(11.0)

++ Values in parenthesis derived from less than three buds; these were not included in the statistical analysis

LSD (P=0.05)=2.0 days

===== Treatment Position

C2(c) Results

On untreated shoots bud-burst was infrequent (Table C1 (a)) but those which burst did so within a similar time of about 12 days (Table C1 (b)). Decapitation stimulated the distal lateral bud and promoted this bud compared to untreated shoots, with no effect on lower buds. A complete girdle above node (3) stimulated burst of all the lateral buds at this node but did not influence the time of burst. Lower buds were unaffected. Similarly a ½-girdle stimulated burst of the bud directly below on the same side but did not influence the bud on the opposite side.

C2(d) Discussion

On the detached shoots in this experiment, the occurrence of a pruning cut near (and by necessity distal to) a bud, following decapitation, stimulated that bud to burst and promoted earlier bud-burst in a similar manner to that found on attached shoots in Section III B. Therefore, detached shoots should be suitable experimental units for investigation of the phenomenon. The effect of the pruning cut was confined to the buds adjacent to the cut.

When a shoot was girdled distal to a particular node the bud at that node always burst (Table C1 (a)) but the time of burst did not differ from that of buds which burst naturally (Table C1 (b)). Thus girdling mimicked the effect of a pruning cut on the occurrence of burst but did not promote more rapid burst. The ½-girdle treatments indicate that this girdling effect only occurs at the bud directly

below the treated side of the shoot. The bud proximal to a ½-girdle but not directly below it usually did not burst. Therefore the stimulation of a bud to burst would appear to involve the interruption of the longitudinal passage of some agent through the peripheral stem tissues. Alternatively a stimulus may arise from the treatment wound and move basipetally to the bud. Proximity of the wound to the bud would not appear to be important in this effect because in some cases the responding bud was some distance from the wound.

Promotion of more rapid burst only occurred near a pruning cut. Removal of a complete girdle of tissue did not influence bud-burst time (Table C1 (a)). Either cutting through the mature xylem tissue or complete removal of the more distal parts of the shoot is necessary to induce this response. The disbudding treatments reported in Section III B2 indicate that the removal of all the more distal buds, including the apex, was not sufficient to promote earlier bud-burst therefore the presence of more distal stem tissue appears to prevent the response.

In summary, this experiment has confirmed the finding in Section III B of a distinction between the effect of pruning on the stimulation of a bud to burst and the promotion of more rapid burst viz;

a) Bud-burst occurred whenever the treatment removed a portion of the shoot tissue directly distal to the bud (i.e. ½-girdle, girdling or pruning).

b) Bud-burst was hastened only when the distal portion of the shoot was completely severed.

This suggests that the stimulation of bud-burst involves interruption of the continuity of the peripheral tissues of the shoot directly distal to the bud, although a wound stimulus may be involved, whereas the promotion of earlier burst involves the additional severing of the mature xylem (wood) and possibly the removal of the distal part of the shoot.

C3 INVOLVEMENT OF APICAL DOMINANCE

C3(a) Introduction

It has been established that the stimulation of lateral bud-burst near a pruning cut involves a discontinuity in the peripheral tissues distal to the bud (Section III C2). Girdling the shoot or removal of all the more distal buds, including the apex, has a similar effect to pruning (Section III B3). Does this effect involve isolation of the bud from the apex or all the more distal buds, or is it an independent response due to the wounding associated with these treatments? In this section the response to girdling is examined in the presence or absence of the apex to determine the involvement of the apex.

It was found in Section III B2 that the response to pruning was dependent on the time of application of treatment. A comparison between girdling treatments carried out in this experiment with similar treatments in the previous section, provides some indication of the influence of the time of year (and hence stage of development of the buds) on the response to girdling.

C3(b) Methods

The materials and methods were similar to those used in the previous experiments (Section III B2 (b)) except that the shoots were collected in early spring (early September). The following treatments were applied :

A Untreated control

B Girdled above bud (4).

TABLE C2 EFFECT OF THE APEX ON BUD-BURST OCCURRENCE

(Number of buds which burst out of 5)

BUD POSITION	CONTROL	GIRDLING	½-GIRDLE SAME SIDE	½-GIRDLE OPPOSITE SIDE
	(a) APEX PRESENT ON ALL SHOOTS			
	A	B	C	D
Apex	0	3	1	0
(1)	4	3	0	2
(2)	5	2	2	3
(3)	5	1	2	3
		=====	=====	=====
(4)	5	5	5	3
(5)	2	2	5	4
	(b) ALL SHOOTS DECAPITATED			
	E	F	G	H
(1)	5	5	5	5
(2)	3	1	1	4
(3)	2	0	2	1
		=====	=====	=====
(4)	0	5	5	0
(5)	1	4	1	3

===== Treatment Position

TABLE C3 EFFECT OF THE APEX ON BUD-BURST TIME

(Mean days to burst - of those buds which burst)

BUD POSITION	CONTROL	GIRDLING	½-GIRDLE SAME SIDE	½-GIRDLE OPPOSITE SIDE
		(a) APEX PRESENT ON ALL SHOOTS		
	A	B	C	D
Apex	-	8.3	(14.0)	-
(1)	9.0	8.7	-	(9.0)
(2)	7.4	(10.5)	(8.5)	11.0
(3)	8.8	(11.0)	(8.5)	8.3
		=====	=====	=====
(4)	8.4	8.0	9.6	10.0
(5)	(10.0)++	(8.5)	9.0	9.0
		(b) ALL SHOOTS DECAPITATED		
	E	F	G	H
(1)	7.8	8.4	7.4	8.0
(2)	8.3	(9.0)	(9.0)	8.5
(3)	(10.5)	-	(9.0)	(9.0)
		=====	=====	=====
(4)	-	8.2	8.3	-
(5)	(9.0)	9.8	(9.0)	8.0

++Values in parenthesis derived from less than three buds; these were not included in the statistical analysis.

LSD (P=0.05) = 2.1 days

===== Treatment Position

C ½-girdled above bud (4); on the same side

D ½-girdled above bud (4); on the opposite side

E Decapitated

F Decapitated + girdled above bud (4)

G Decapitated + ½-girdled above bud (4); on the same side

H Decapitated + ½-girdled above bud (4); on the opposite side

C3(c) Results

When untreated shoots were collected and placed in the growth cabinet, bud-burst occurred readily at nodes (1) - (4) but no apical buds burst (Table C2 (a), C3 (a)). The precocious burst of these lateral buds left little scope for detecting any stimulatory effects due to girdling treatments on non-decapitated shoots. On the other hand, girdling treatments reduced the number of buds which burst distal to the treatment with no indication of a dependence on the position of the ½-girdle (Table C2 (a)).

Decapitation resulted in a reduction in bud-burst at nodes below position (1) (Table C2 A vs E) but girdling of decapitated shoots stimulated burst of the buds directly below the treatment positions with little effect on the buds above (Table C2 (b), E vs F,G,H).

There was little difference in bud-burst times between the treatments (Table C3).

C3(d) Discussion

Lateral bud-burst occurred readily on untreated shoots in this experiment whereas in the earlier work (Section III C2) bud-burst on similar shoots was infrequent and generally slower. This difference may be attributed to the time of the year and hence the phase of development of the shoots or buds. In Section III C2 the shoots were collected in the middle of winter dormancy when rest was probably completed but environmental dormancy had prevented the resumption of activity (see Section III D, particularly Figure D1). This experiment commenced in early spring when winter dormancy was almost over.

It is axiomatic that where bud-burst is occurring readily on untreated shoots no enhancement of the burst process by manipulative treatments can be expected, hence the lack of response to girdling on shoots without decapitation (Table C2). Conversely, the response to girdling must involve the release of the bud from some influence which is limiting bud-burst. The earlier observation of enhanced bud-burst proximal to a girdle (Section III C2) was made at a time when bud-burst on untreated shoots was limited, presumably by winter dormancy. In this section the response to girdling was observed only when the burst of lower lateral buds was suppressed following decapitation (Table C2). This suggests that girdling may overcome lateral bud dormancy arising under different circumstances.

The response to girdling on decapitated shoots (Table C3) indicates that the response does not necessarily involve isolation of the bud from the apex. In Section III B2 the occurrence of bud-burst (as distinct from the promotion of earlier burst) was promoted by removal of all the more distal buds including the apex. Thus isolation of a bud from all more distal buds would appear to be involved in this response. This is consistent with the notion that a lateral bud may be subject to correlative inhibition mediated by some agent passing along the shoot between the buds via the peripheral tissues of the stem. Apical dominance is a specific case of this phenomenon.

The alternative hypothesis that bud-burst is stimulated by a direct effect of wounding cannot be dismissed. The effectiveness of disbudding in promoting bud-burst may also be due to the wounds inflicted when buds were removed.

C4 THE SOURCE OF LATERAL BUD INHIBITION

C4(a) Introduction

Girdling distal to a bud releases it from some form of correlative inhibition (Section III C3). This effect appears to be dependent on the isolation of the bud from the distal parts of the shoot by the occurrence of the girdling treatment directly distal to the bud (Section III C2). If this is so then some regulatory agent of bud-burst must pass basipetally or acropetally along the stem via the peripheral tissues immediately distal to the bud.

There is a strong case for a primary role for auxin passing basipetally through the stem in the correlative inhibition of lateral buds (Khan 1975; Phillips 1975; Rubinstein and Nagao 1976). Polar transport of auxin appears to be confined to the tissues either side of the cambium in woody shoots (Sheldrake 1973; Soding 1937, 1940), although non-polar transport may occur in either the phloem or xylem (Goldsmith 1968), therefore disruption of the cambium and adjacent tissues could be expected to interrupt the polar, basipetal transport of auxin.

Huber (1948) found that auxin levels rose in the stem distal to a girdle and declined proximal to it. Also the effects of girdling on lateral shoot growth have been overcome by the application of auxin below the girdle (Jankiewicz 1956) and the application of an inhibitor of auxin transport (T.I.B.A.) to a shoot stimulates lateral bud-burst (Baldini, Sansavini and Zocca 1973; Luckwill 1968). Therefore the

stimulation of a bud to burst proximal to a pruning cut, which was mimicked by girdling treatments distal to the bud (Section III C5), could be due to release of the bud from correlative inhibition mediated by basipetal transport of auxin in the tissues associated with the cambium.

If the source of inhibition of lateral bud-burst on defoliated shoots is the most distal bud (be it the terminal bud or a lateral bud) isolation of that bud by a girdle proximal to it should release all the lower buds from apical dominance. Furthermore, if the effect of girdling is to release a bud from this apical dominance, there should be no response to concurrent girdling at lower positions. These inferences were tested by multiple girdling treatments.

Another explanation for the stimulation of bud-burst proximal to a girdle is that some agent which promotes bud-burst is transported acropetally in the stem and accumulates below the girdle. The supply of nutrients or growth factors from the roots may influence apical dominance (Phillips 1975) and Went (1936) suggested that distal buds were dominant because they were more effective sinks for such factors. The removal of the more distal buds by disbudding, as in Section III B2, could promote the burst of lateral buds by removing these competitive sinks. However the continuing supply of these substances from the roots cannot be vital because bud-burst occurs readily on rootless cuttings.

It is possible that a stimulatory agent is present in the xylem sap or is released from the stem tissues of detached

shoots. The release of cytokinins into the xylem sap of rootless vine cuttings, in response to chilling, has been reported (Skene 1972) and cytokinins have been shown to promote bud-burst on woody shoots including apple (Chovjka, Travnicek and Zakourilova 1962; Kender and Carpenter 1972; Pieniazek 1964; Williams and Billingsley 1970) therefore cytokinins may be involved but these substances appear to play a secondary role in the control of lateral bud dormancy (Khan 1975; Phillips 1975). However, the occurrence of bud-burst on single internode segments (Section III D) where the volume of shoot present is small suggests that the accumulation of a stimulatory substance at the distal cut end is unlikely to be the major controlling influence.

The possibility still remains that the wounding associated with girdling treatments results in the production of a stimulus of bud-burst which is transmitted basipetally. It is plausible that this wound effect may occur via an interaction with the correlative inhibitor suggested earlier i.e. auxin from the distal buds.

In the following experiment multiple girdling treatments were used to investigate the source of the stimulus of bud-burst. Additional ½-girdle treatments were included for comparison with earlier experiments because it was found that the response to girdling varies with the physiological state of the material.

TABLE C4 EFFECT OF MULTIPLE GIRDLING ON BUD-BURST OCCURRENCE

(The number of buds which burst out of 5)

BUD POSITION	A CONTROL	B 1-GIRDLE	C 2-GIRDLE	D 3-GIRDLE
Apex	5	5	4	5
(1)	0	0	4	3
(2)	0	0	2	2
(3)	1	0	0	1
(4)	0	0	0	0
(5)	0	0	0	1
(6)	0	0	0	1
(7)	1	5	5	5
(8)	0	5	5	3
(9)	0	4	3	1
(10)	0	1	0	0
(11)	0	0	0	1
(12)	1	0	0	0
(13)	0	0	1	5
(14)	0	0	0	5
(15)	1	0	0	2
(16)	0	0	0	1
(17)	0	0	0	1
(18)	0	0	0	0

===== Treatment Position

TABLE C5 EFFECT OF MULTIPLE GIRDLING ON BUD-BURST TIME

(Mean days to burst - of those buds which burst)

BUD POSITION	A CONTROL	B 1-GIRDLE	C 2-GIRDLE	D 3-GIRDLE
Apex	6.4	5.4	11.0	8.2
(1)	-	-	8.8	7.3
(2)	-	-	8.5	9.0
(3)	(12.0)++	-	-	(10.0)
(4)	-	-	-	-
(5)	-	-	-	(10.0)
(6)	-	-	-	(11.0)
(7)	(7.0)	6.2	7.4	7.6
(8)	-	6.8	7.8	8.7
(9)	-	7.3	10.3	(7.0)
(10)	-	(6.0)	-	-
(11)	-	-	-	(5.0)
(12)	(8.0)	-	-	-
(13)	-	-	(11.0)	8.0
(14)	-	-	-	9.8
(15)	(8.0)	-	-	11.0
(16)	-	-	-	(11.0)
(17)	-	-	-	(11.0)
(18)	-	-	-	-

++ Values in parenthesis derived from less than three buds; these were not included in the statistical analysis

LSD (P=0.05)=2.4 days

===== Treatment Positions

C4(b) Methods

Single-stemmed apple seedlings (c.v. Granny Smith), were grown as described in Section III A. Plants with a minimum of 18 lateral buds were selected to give 5 groups (replicates) each having uniform size and total bud number. The few remaining leaves were removed. All the plants remained in the glasshouse in which they were grown (i.e. under non-chilling conditions).

The following treatments were applied in mid-September:

A Untreated control

B 1 girdle, above bud (7)

C 2 girdles, above buds (7) and (1)

D 3 girdles, above buds (13), (7) and (1)

E ½-girdle above bud (7), on the same side

F ½-girdle above bud (7), on the opposite side

Bud-burst was recorded for all buds.

C4(c) Results

The data from this experiment have been presented in two groups to simplify the discussion. Tables C4 and C5 contain the results for comparison between multiple girdling treatments. The data in Tables C6 and C7 provide a comparison with the results obtained earlier (Tables C1, C2, C3) with similar treatments carried out on different material.

Consider first the multiple girdling treatments (Table C4 and C5). Bud-burst was stimulated at the 2-3 nodes below a girdle regardless of the number of girdles present (Table C4). The response was limited to about 3 buds below a

TABLE C6 EFFECT OF GIRDLING ON BUD-BURST OCCURRENCE

(Number of buds which burst out of 5)

BUD POSITION	A CONTROL	B GIRDLE	E ½-GIRDLE SAME SIDE	F ½-GIRDLE OPPOSITE SIDE
Apex	5	5	4	5
(1)	0	0	0	0
(2)	1	0	0	0
(3)	0	0	0	0
(4)	0	0	0	0
(5)	0	0	1	0
(6)	1	0	0	0
		=====	=====	=====
(7)	0	5	5	0
(8)	0	5	0	5
(9)	0	4	0	0

===== Treatment Position

TABLE C7 EFFECT OF GIRDLING ON BUD-BURST TIME

(Mean days to burst - of those buds which burst)

BUD POSITION	A CONTROL	B GIRDLE	E ½-GIRDLE SAME SIDE	F ½-GIRDLE OPPOSITE SIDE
Apex	6.4	5.4	5.5	6.4
(1)	-	-	-	-
(2)	-	-	-	-
(3)	(12.0)++	-	-	-
(4)	-	-	-	-
(5)	-	-	(5.0)	-
(6)	-	-	-	-
		=====	=====	=====
(7)	(7.0)	6.2	6.6	-
(8)	-	6.8	-	6.8
(9)	-	7.3	-	-

++ Values in parenthesis derived from less than three buds; these were not included in the statistical analysis

LSD (P=0.05)=2.4 days

===== Treatment Position

girdle. There was less response below a girdle at the top position which may be associated with the position of the buds or the existence of other girdles below.

The time of bud-burst adjacent to a girdle did not vary significantly (Table C5; except between the extremes i.e. treatment B, bud (7) and treatment C bud (1)) but burst tended to occur later at nodes further from the girdle and on shoots where more than one girdle was present.

The pattern of bud-burst in response to girdling treatments evident in Table C6 is similar to that found in Section III C2 (Table C1) and on decapitated shoots in Section III C3 (Table C2 (b)). The bud below a girdle always burst and bud-burst below a ½-girdle only occurred at the bud directly below the treatment position i.e. on the same side of the shoot. On the other hand the pattern of bud-burst on untreated shoots was distinctly different between experiments.

On untreated shoots in this experiment apical buds burst readily (within 7 days) following removal of the few remaining leaves but there was little lateral bud activity; yet when stimulated by a girdle lateral buds also burst rapidly (Table C7). In Section III C2 overall bud activity was low and bud-burst did not occur until about 12 days after treatment of a shoot (Table C1) whereas in Section III C3 lateral bud activity predominated (Tables C2 and C3).

C4(d) Discussion

It is clear from this experiment that the promotion of bud-burst may occur independently below more than one girdle on the same shoot (Table C4). This rules out the possibility that this response is directly dependent on interruption of the movement of an inhibitor of bud-burst through the peripheral stem tissues from the terminal bud or of a stimulus from the basal end of the shoot (including the roots). If the interception of such an agent is involved this agent must arise from along the length of the shoot.

The proposed agent of correlative inhibition, auxin (see Section III C (a)), may arise from all the buds along a shoot. The stimulation of bud-burst at a particular node by the removal of all more distal buds (Section III B) supports this suggestion.

Another feature of the response to girdling is exemplified by the results in this section. Stimulation rarely occurs at more than three nodes below a girdle (Table C4). This localisation is similar to that of bud-burst below a pruning cut (Section III B2). In fact buds below the one adjacent to a pruning cut are often suppressed (Figure B8 and Table C2 (b)).

It was argued in Section III B2(d) that the promotion of earlier bud-burst of the bud adjacent to a pruning cut enables it to gain an advantage over the buds below. This does not appear to be true for the bud below a girdle because it was not promoted significantly compared with

buds on untreated shoots (Tables C5, C7). However it does tend to burst earlier than those below.

The localisation of the stimulation of buds below a girdle (to within 2-3 nodes of the girdle) and the later burst of buds further (proximal) from the girdle may have a common explanation. Lateral buds appear to be a source of correlative inhibition of other buds proximal to them (Section III C3). Presumably those buds with a greater number of more distal buds will be subject to greater inhibition. It would appear that inhibition from about 3 more-distal buds is usually sufficient to prevent lateral bud-burst. If a distal bud is promoted by proximity to a pruning cut it must have a greater inhibitory effect on more proximal buds and thereby increase the localisation of bud-burst.

If the alternative hypothesis of a response to wounding causing bud-burst is true, then the decline in response with distance from the girdle could be attributed to the limited passage of the wound stimulus. In fact the two proposals are not incompatible because the production of auxin may be enhanced in buds adjacent to the wound so that those buds exert a greater correlative influence over the more distant buds. Also the localisation of the girdling effect at any of several positions along the same shoot is consistent with the wound being the centre of stimulus production.

The results of the second part of this experiment confirm the existence of a spatial relationship between the position of the girdling treatment and the stimulation of bud-burst i.e. the response to girdling treatments is associated with a discontinuity in the peripheral tissues of the stem directly above (distal to) the bud.

The comparison between experiments revealed the occurrence of markedly different distributions of bud activity on untreated shoots in different experiments. In spite of this difference between samples of shoots the basic response to girdling treatments was reproduced. Any proposal to explain the mode of action by which pruning or girdling modifies bud-burst must take this into account. A more detailed discussion is deferred until the general discussion (Section III C6) to avoid detracting from the main sequence of argument.

C5 THE INVOLVEMENT OF TISSUE WOUNDING

C5(a) Introduction

It has been established that the stimulation of lateral bud-burst in response to girdling involves the disruption of the peripheral stem tissues distal to a bud (Section III C2). The effect appears to be localised since it may occur independently at several sites along the same shoot (Section III C4), and in the absence of a portion of a girdle directly above (distal to) the bud there is no response (Section III C2). The minimum amount of disruption (wounding) of the tissue required to invoke this response has not been determined.

It was found earlier (Section III C2) that the promotion of bud-burst required severing of the wood as well as the peripheral tissues distal to the bud. Does exposure of the sub-epidermal tissues as a result of pruning or girdling contribute to the stimulation of bud-burst or the promotion of earlier bud-burst?

The extent of the wounding required to elicit these responses and the importance of exposure of the sub-epidermal tissues has been investigated in this section by inflicting a variety of wounds above a bud and by attempting to reduce the exposure of the tissues by covering the wounds.

C5(b) Methods

Two separate experiments are included in this section.

Experiment 1

This experiment was conducted on shoots *in situ* on trees in the orchard (the small, hedge-pruned apple rootstocks described in Section III A). Each treatment was applied to a separate shoot on each of 5 trees (replicates). All treatments were applied just above (distal to) the 6th lateral bud from the apex.

Two types of wounds were used apart from pruning:

(i) Notching - a small wedge of tissue removed by two blade cuts penetrating to the wood.

(ii) Nicking - one inclined blade cut similar to notching but without the removal of tissue.

The wounds were covered with grafting wax (Shell) or wrapped with polyvinyl chloride film ('Glad Wrap').

The following treatments were applied in early spring (September) before natural bud-burst:

A Untreated control

B Pruned

C Pruned + wax applied

D Pruned + wound wrapped

E Notch above the bud

F Notch above the bud + wax applied

G Notch above the bud + wound wrapped

H Nick above the bud

I Nick above the bud + wax applied

Bud-burst was recorded only for bud (6).

Experiment 2

Single-stemmed apple seedlings (c.v. Granny Smith), grown as described in Section III A, were used in this experiment. 10 groups (replicates) of similar plants were selected and each treatment was applied above a bud of comparable size and position on one plant per group.

Some treatment wounds were covered with a liberal application of silicone vacumn grease applied immediately after the wound was made. All treatments were applied on the same day in early spring (September) and the plants remained in the glasshouse. Bud-burst was recorded for all buds but only the data for the bud proximal to the treatment is presented.

The treatments were :

A Pruned

B Pruned + grease

C Girdled

D Girdled + grease

E 2/3-girdle, on the opposite side to the bud

F Intermittent girdle above the bud

G Shallow girdle

An intermittent girdle involved the removal of several narrow, longitudinal strips of tissue directly above the bud and to the depth of the wood to provide a wound area equivalent to a 1/3 girdle but leaving strips of tissue intact. A shallow girdle removed only epidermal and cortex tissues with minimal disruption of phloem.

TABLE C8 EFFECT OF DIFFERENT DEGREES OF WOUNDING ON BUD BURST

TREATMENT	No. OF BUDS WHICH BURST (OUT OF 5)	MEAN TIME TO BURST (DAYS)		
A UNTREATED	0	-		
B PRUNED	5	9.6	x	a
C PRUNED + WAX	5	11.6	y	
D PRUNED + WRAP	5	12.4	z	
E NOTCH	4	17.8		b
F NOTCHED + WAX	5	18.8		
G NOTCH + WRAP	5	17.6		
H NICK	4	22.0		c
I NICK + WAX	5	21.0		

Different letters indicate a significant difference (P=0.05):

x,y,z comparison between pruning treatments only

a,b,c comparison between all treatments

TABLE C9 EFFECT OF COVERING THE WOUND WITH GREASE

	PRUNING		GIRDLING	
	A	B	C	D
	NO GREASE	WITH GREASE	NO GREASE	WITH GREASE
No. OF BUDS WHICH BURST (OUT OF 10)	10.0	10.0	10.0	10.0
MEAN TIME TO BURST (DAYS)	9.4	7.9	10.7	8.3

LSD (P=0.05) = 1.3 days

TABLE C10 EFFECT OF THE EXTENT OF THE WOUND

	No. OF BUDS WHICH BURST (OUT OF 10)	MEAN TIME TO BURST (DAYS)
A PRUNED	10	9.4
C GIRDLE	10	10.7
E 2/3-GIRDLE (OPPOSITE SIDE)	1	-
F INTERMITTENT GIRDLE	0	-
G SHALLOW GIRDLE	1	-

C5(c) Results

Experiment 1

On untreated shoots the observed lateral bud (the 6th from the apex) did not burst (Table C8). All wounding treatments (pruning, a notch or a nick) stimulated the bud below to burst with a nick tending to be less effective. The time taken for the bud to burst was dependent on the type of wound (Table C8). Following pruning the distal bud burst within an average of 10 days. The time to burst increased to 18 and 22 days respectively with a notch or a nick.

Covering the wounds with grafting wax or wrapping with polythene film had no effect on the response to a notch or a nick but covering the pruning cut delayed burst (Table C8).

Experiment 2

In this experiment complete girdling or pruning treatments stimulated bud-burst and pruning promoted earlier bud-burst than girdling as observed previously (Table C9). With either treatment the immediate application of grease to cover the wound promoted earlier bud-burst (Table C9).

Where the girdle was not continuous directly above the bud (treatment E and treatment F) or where the girdle did not remove all the tissue external to the primary xylem (treatment G) the bud did not usually burst (Table C10).

C5(d) Discussion

The results of experiment 1 indicate that the stimulation of a bud to burst requires only minor disruption of the

peripheral tissues directly above the bud. A nick i.e. an incision without the removal of any tissue, was sufficient to induce bud-burst (Table C8). However it is clear from the second experiment (Table C10) that the disruption of the peripheral stem tissues must be continuous above the bud and that it must penetrate through to the wood.

These minimum requirements for the stimulation of bud-burst are consistent with the proposal that the stimulation of bud-burst involves the interruption of the basipetal passage of an inhibitor of bud growth, presumably auxin, through the peripheral stem tissues (see Section III C3(a)). Any treatment which disrupts the continuity of the peripheral tissues directly distal to a bud would interrupt the passage of auxin to the bud and so release it from this correlative inhibition. The effectiveness of partial girdles indicates that there can be little lateral dispersion of the auxin, at least over the short distance between the girdle and the bud.

There does not appear to be any direct relationship between the amount of tissue injury and the occurrence of bud-burst. Covering the wounds to reduce exposure of the tissues had no effect (Tables C8, C9) and there was no difference between treatments with different amounts of tissue disruption i.e. pruning versus girdling (Table C9) or notching (Table C8). Treatments involving more extensive wounding than a notch or nick did not necessarily stimulate bud-burst (Table C10, E & F).

In contrast, the promotion of earlier bud-burst in response to wounding treatments is dependent on the extent of the

disruption of the tissues. A notch, where a small but complete portion of tissue is removed distal to the bud, results in slower burst than pruning (Table C8). It is possible that callus may bridge the wound early enough to restore the continuity of the tissue. Likewise recovery from a nick would be rapid.

If the rate of re-establishment of this tissue continuity is critical it would be expected that covering the wound to reduce dehydration, which promotes callus growth (Swarbrick 1927), would further delay bud-burst. This is not evident in the results (Table C8). On the contrary, covering the wound was only effective in the case of pruning where establishment of connections to distal parts of the (original) shoot cannot be involved.

The delay of bud-burst caused by covering the pruning cut (with wax or Glad Wrap) suggests that exposure of the wounded tissues to the atmosphere may be involved in the promotion of earlier burst. It was suggested earlier (Section III C2 (d)) that disruption of the xylem tissues may be important in determining the rate of bud-burst. A notch or a nick would have little effect on the exposure or disruption of the xylem tissues and this could account for the later burst and lack of response to covering such wounds. Similarly girdling usually results in later burst than a pruning cut where the xylem is cut too.

Where the pruning or girdling wound was covered immediately with grease, bud-burst occurred earlier (Table C9). This

difference may be explained by the nature of the covering materials. The P.V.C. film and the grafting wax both form barriers against dehydration of the tissues and possibly gas exchange but neither would be expected to permeate the tissues. Grease, on the other hand, is more fluid and may penetrate between or even into the cells. This could have physical or chemical effects on the tissue. The fact that grease influenced the response to girdling whilst the other covers did not effect the response to a notch (a similar type of wound), is at least consistent with the argument that the covering materials have different effective properties.

Thus, in summary, this section has lead to two main conclusions about the influence of the extent of wounding on bud-burst:

i) the occurrence of bud-burst is dependent on the position of the wound. The wound must disrupt the peripheral tissues continuously across the stem distal to the bud and through to the wood.

ii) the promotion of earlier bud-burst is necessarily dependent on the occurrence of burst and therefore on i) above. The magnitude of the hastening of burst is influenced by the extent of the disruption and exposure of tissues particularly the wood.

C6 DISCUSSION OF EFFECTS ASSOCIATED WITH THE PRUNING CUT

In the introduction to this group of experiments (Section III C1) pruning was said to change the physiological habitat of a bud in three ways:

i) the bud assumes a terminal position on the shoot

ii) all parts of the shoot distal to the bud are removed

iii) the bud is in close proximity to the pruning cut

The results of the experiments support the proposal that the occurrence of bud-burst is associated mainly with ii) above. Bud-burst occurs when the bud is released from correlative inhibition arising from the distal part of the shoot and transmitted basipetally (Section III C4). The attainment of a terminal position does not contribute to the occurrence of bud-burst per se ; a terminal bud cannot be subject to basipetally transmitted inhibition however the occurrence of burst is not influenced by bud position relative to the proximal part of the shoot (see Section III C4(a)).

Disruption of the tissues (wounding) does not necessarily stimulate bud-burst (Section III C5) however it does influence the time of burst. Promotion of early bud-burst only occurs where wounding is sufficient to affect the wood tissues (Sections III C2, III C5). This response appears to involve exposure of the tissues since covering a wound reduced its effectiveness (Section III C5). The apparently anomalous

effect of applying grease to the wound may be due to a direct effect of this substance on the exposed tissues. This is discussed further in Section IV.

In light of the preceeding information, the distinction which has been made (Section III B) between the two parameters bud-burst occurrence and bud-burst time, in relation to responses to pruning, appears to have some physiological basis. The stimulus of bud-burst occurrence is the removal of correlative inhibition. The hastening of burst appears to involve a positive effect resulting from tissue disruption and exposure. The nature of the stimulus in the latter case and its mode of influencing bud-burst are the subject of Section III E.

Some insight into the aspects of the physiology of bud development involved in the responses to pruning may be gained from the comparison between responses obtained on shoots in different physiological states. In Section III B bud-burst time was promoted when shoots were pruned while dormant but not when they were pruned after natural bud-burst. Similarly in Section III C2 the lateral buds on shoots, collected during mid winter dormancy and placed under conditions conducive to bud-burst (in a growth cabinet), remained inactive. Terminal buds on these shoots also rarely burst suggesting that the bud dormancy was not only due to correlative inhibition. Decapitation of these shoots resulted in hastened bud-burst (Table C1 (b)). However when similar shoots were collected in early spring the lateral buds burst readily in the growth cabinet indicating that their dormancy

was only environmental at this stage (Tables C2, C3). No hastening of bud-burst was observed when these shoots were decapitated.

These observations on the variation in the response to pruning indicate that the hastening of bud-burst on pruned shoots only occurs when the buds are subjected to dormancy which cannot be entirely attributed to environmental dormancy or correlative inhibition i.e. when they are subject to rest (see Section II B1). The corollary to this is that the promotion of bud-burst in the proximity of a pruning cut (or wound) is due to the breaking of rest in these buds. The results of the concurrent investigation into the pattern of bud dormancy (Section III D) confirm these conclusions.

The stimulation of bud-burst can only be observed when the buds are initially dormant (Section III C3(d)). When shoots are placed under favourable environmental conditions the bud proximal to a pruning cut always burst. Presumably rest, when present, is broken by the wound effect therefore rest and environmental dormancy are not prerequisite to this response. Correlative inhibition must be the type of dormancy overcome when bud-burst is stimulated. This is supported by the fact that proximal buds on active shoots do not respond to girdling (i.e. the buds grow naturally), except when apical dominance is induced by decapitation of the shoot (Table C3).

D DORMANCY STUDIES

D1 GENERAL INTRODUCTION

The experimental work reported in this thesis involved the study of the resumption of active growth from dormant lateral buds. Bud dormancy may occur with the bud under different physiological conditions (see the review, Section II) and the responses to treatments may depend on the type or extent of the dormancy prevailing in the buds at the time of treatment. Thus in order to relate the observed responses to the underlying physiology of bud development it is necessary to have some measure of the dormancy status of the material used.

In the past the most commonly used method of assessing bud dormancy on field material has involved removal of a shoot or twig and placing it under warm conditions in a glasshouse or growth room with its basal end immersed in water(references in section II B1). Bud-burst is then observed and some parameter of the time taken for bud-burst is used to compute an index. This may be the number of buds which burst (or the number of shoots on which bud-burst occurs) within a specified period of time (e.g. Eggert 1951; Little and Bonga 1974) or it may be the average time taken for a sample of buds to burst; usually the time taken for 50% of the buds to burst since not all buds burst within the test period (e.g. Corgan and Peyton 1970; Hendershott and Walker 1959). This method provides a measure of the general state of bud dormancy on the shoots but it does not distinguish between all the types of dormancy involved particularly in the case of lateral buds.

Bearing in mind the types of dormancy defined in the review, environmental dormancy, correlative inhibition and rest (see Table 1), which types of dormancy may be assessed by the above method? The influence of environmental dormancy may be assessed by comparing the responses obtained under natural environmental conditions (mainly light and temperature) with those obtained under controlled conditions which favour bud-burst. Any differences may be attributed to environmental dormancy.

Correlative inhibition may arise from the leaves or the other buds on the shoot. Usually the shoots are leafless when collected, otherwise they may be defoliated thereby eliminating correlative inhibition due to the leaves. Any remaining bud dormancy may be attributed to either correlative inhibition between the buds or rest. Thus it is these two types of dormancy which are involved in the determination of indices of dormancy status when shoots or twigs are tested by this method.

If we consider a whole shoot, all buds except the terminal bud (or distal lateral bud in its absence) may be subject to apical dominance therefore any dormancy detected in the lateral buds may be due to apical dominance or rest. Decapitation of the shoot eliminates apical dominance by the terminal bud. However, correlative inhibition may still exist between the lateral buds. Therefore, for lateral buds in particular, the commonly used method of assessing dormancy by placing whole or decapitated shoots in a favourable environment does not completely distinguish between correlative inhibition and rest.

Since this work mainly involved the responses of lateral buds a more precise method of assessing lateral bud dormancy was required. It follows from the preceding paragraphs that if lateral buds are isolated from each other any remaining dormancy could be attributed to rest. This was the basic premise for the use of single-bud, internode segments for assessing dormancy in conjunction with the field experiments reported earlier (Section III B). Unexpected results were obtained using single-bud segments therefore the system was investigated more closely and the experiments are reported in this section.

See the appendix (Section V).

D2 DORMANCY OF ISOLATED BUDS

D2(a) Introduction

Some indication of the state of dormancy of the lateral buds on apple shoots was needed in conjunction with the field experiment reported in Section III B2. The first treatment time was during the winter dormancy period, i.e. the buds in the field were inactive, but it was not known whether the buds were subject to rest, dormancy imposed by correlative inhibition or environmental dormancy. In order to determine the presence of rest the other types of dormancy need to be overcome. Correlative inhibition was overcome by isolating buds on internode segments (for reasons discussed in the previous section) and environmental dormancy was avoided by placing the segments in a glasshouse. Any remaining dormancy could be attributed to rest.

D2(b) Methods

Representative samples of shoots were collected from the apple rootstock cultivars used in the field experiment (Section III B2). The shoots were cut into single-bud segments (Section III A2(b)) and kept in the glasshouse (Section III A3(b)). Bud-burst was recorded daily.

Samples were collected on four dates from early winter (June) until spring (September) when the experimental treatments were applied. Buds in the field remained dormant during this period.

D2(c) Results

The buds burst on all segments within 11-12 days regardless of the collection date (Table D1).

TABLE D1 BUD BURST OF ISOLATED BUDS COLLECTED DURING WINTER DORMANCY

SAMPLING DATE		DAYS TO BURST (RANGE)		
June	29	12	-	21
July	20	11	-	20
August	29	13	-	19
September	6	11	-	21

D2(d) Discussion

The consistent, rapid burst of all buds on segments regardless of the collection date indicated that no dormancy was present in these isolated buds. This suggests that the dormancy of buds in the field was due to environmental dormancy or correlative inhibition both of which were overcome by the treatment of the single-bud segments. There was no indication of rest during the sampled period. However, the possibility remained that rest was present in the buds *in situ*. but the sampling and testing procedure broke this rest. This possibility is investigated in the next section.

D3 DETECTION OF PHYSIOLOGICAL DORMANCY

D3(a) Introduction

The results of the preceding experiment suggested that winter dormancy from early winter (late June) until early spring (September) is due to either correlative inhibition or environmental dormancy. However the possibility remained that rest may have occurred *in situ* but was not present in the test system. This experiment examines the dormancy testing system to see whether rest has in fact been overcome by the experimental procedures.

Several hypotheses were formulated to explain the apparent lack of rest in the samples tested in Section III D2.

(1) that the dormancy of buds *in situ* was due to correlative inhibition

(2) that bud dormancy was due to the environment during winter

(3) that rest was broken before the first sample date

(4) that rest was operating during winter but was broken by cutting shoots into segments.

D3(b) Methods

In this and subsequent experiments in Section III D field material was obtained from the current years shoots on mature Jonathan apple trees (Section III A1(b)). Samples of shoots were collected from each of five trees (used as 5 replicates) on a succession of dates from early autumn (March) until mid spring (October). All shoots were defoliated when leaves

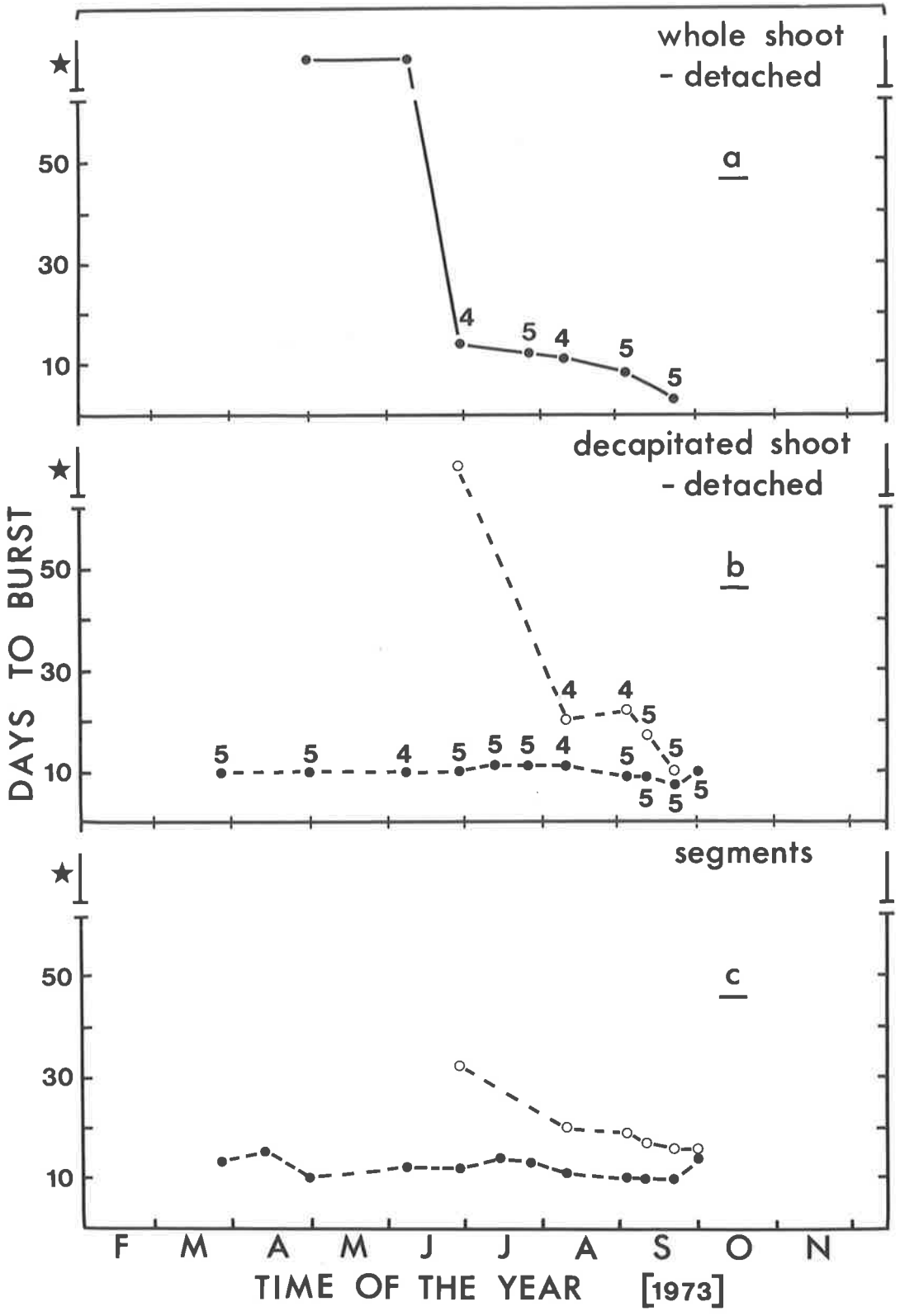

whole shoot
- detached
a
decapitated shoot
- detached
b
segments
c
DAYS TO BURST
50
30
10
F
M
A
M
J
J
A
S
O
N
TIME OF THE YEAR [1973]

FIGURE D 1 THE PATTERN OF LATERAL BUD DORMANCY (1973)

Median bud-burst times for the buds on samples of 5 shoots on each date. Numerals indicate the number of shoots on which bud-burst occurred. Most buds on segments burst.

★ If no buds burst by day 50, the buds were considered to be dormant.

o outside

• cabinet, 25°C

a Dormancy evident on whole shoots outside.

b Dormancy evident on decapitated shoots outside but absent on decapitated shoots in the cabinet.

c Slight dormancy evident on segments outside but absent on segments in the cabinet.

were present and one shoot from each tree was assigned to each of the following treatments:

(1) Shoots cut into segments and kept in a growth cabinet

(2) Shoots cut into segments and kept outside

(3) Shoots decapitated and kept in a growth cabinet

(4) Shoots decapitated and kept outside

(5) Whole shoots kept in a growth cabinet

The cultural details for detached shoots and segments are presented in Section III A2. Bud-burst was recorded daily and number of days-to-burst was determined by the procedure described in Section III A5.

D3(c) Results

Lateral buds on segments and decapitated shoots kept under growth cabinet conditions burst readily, within 10 days, for all collection dates (Figure D1 b, c). Lateral buds on whole shoots grown in the cabinet did not burst when collected during May-June, then burst with decreasing days-to-burst until late September (Figure D1 a).

The buds on decapitated shoots kept outside did not burst in the June sample but those on segments did. All bud-burst took longer outside than did the corresponding treatment inside (Figure D1 b, c).

D3(d) Discussion

The dormancy present in buds on whole shoots placed in the cabinet during May-June must have been physiological

dormancy i.e. the buds did not grow under favourable environmental conditions (Figure D1 a). This physiological dormancy was not evident on segments or decapitated shoots (Figure D1 b, c). No distinction could be made between correlative inhibition and rest because decapitation to remove the apex and prevent apical dominance may also have broken rest or other forms of correlative inhibition.

The delay of burst on both decapitated shoots and segments outside compared with in the cabinet, indicated an environmental component of winter dormancy but the occurrence of bud dormancy on whole shoots inside was evidence for a physiological component as well. The lack of distinction between rest and correlative inhibition prevented conclusions being drawn about hypotheses (3) and (4); but if they were broadened to include physiological dormancy rather than rest *per se*, then they were supported. Hypotheses (1) and (2) are disproved since neither correlative inhibition nor environmental dormancy alone accounted for the whole period of winter dormancy.

Thus it can be concluded that winter dormancy of lateral buds on apple shoots involves both environmental dormancy and physiological dormancy and that this physiological dormancy may be overcome by decapitation of the shoot or cutting the shoot into segments.

D4 DISTINCTION BETWEEN CORRELATIVE INHIBITION AND REST

D4(a) Introduction

The previous section established that there was a period of physiological dormancy during early winter dormancy of lateral buds but it failed to distinguish between rest and correlative inhibition. It was considered that demonstration of a period before winter dormancy, when lateral buds burst on defoliated shoots without decapitation, would indicate that apical dominance was not then present. Also, if there was no indication of new activity in the apex until after winter dormancy, resumption of apical dominance during winter would not be expected. Occurrence of physiological dormancy during winter might then be attributed to rest.

Two hypotheses were proposed :

(i) that apical dominance ceases before winter dormancy is established

(ii) that the apex remains inactive until after the winter dormancy period.

Since it had been shown that environmental dormancy does not cause winter dormancy during July (Section III D3), it was considered necessary to establish the duration of environmental dormancy so that the relative contribution of physiological dormancy and environmental dormancy to winter dormancy, at any one time, could be assessed.

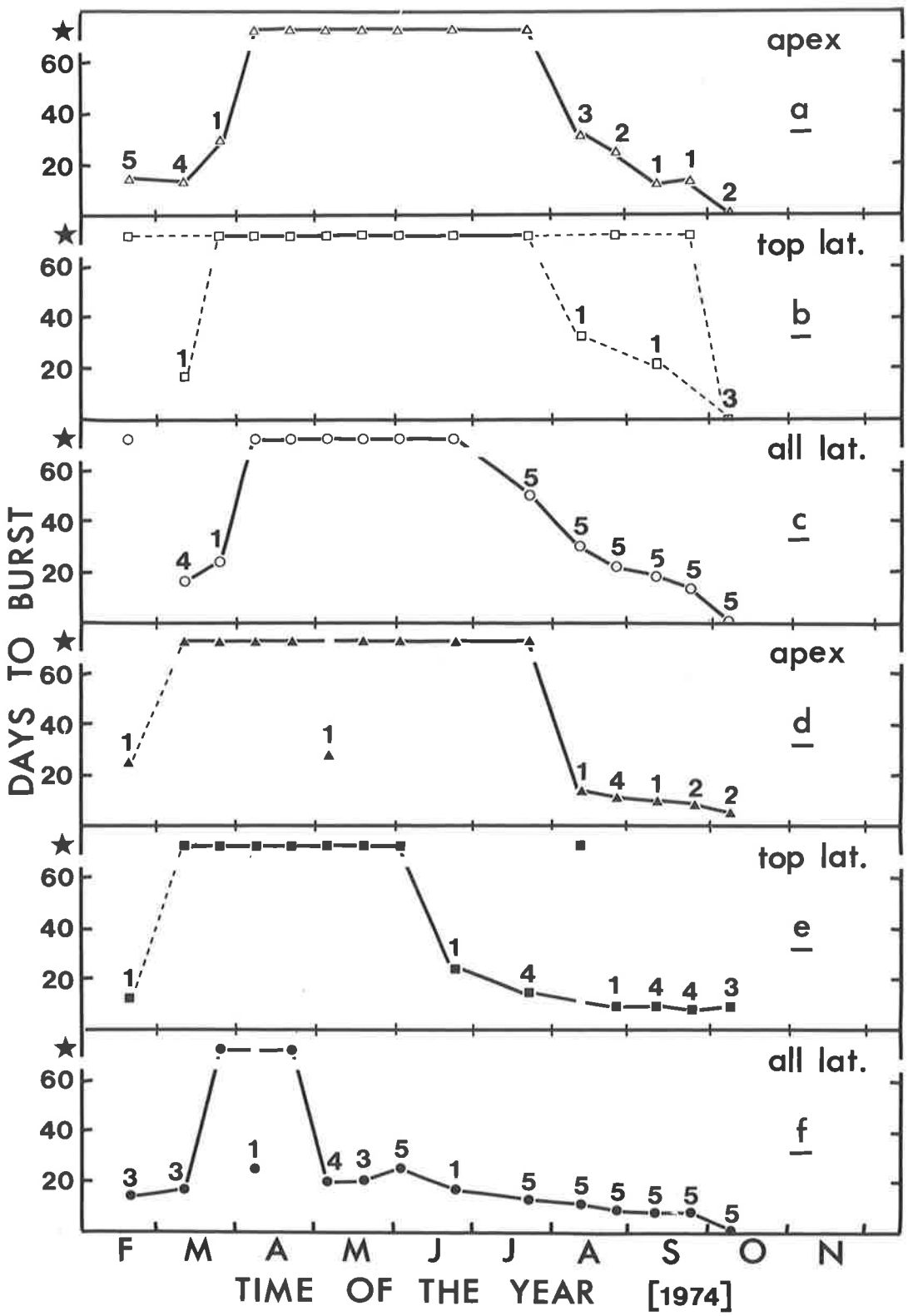

apex
a
top lat.
b
all lat.
c
apex
d
top lat.
e
all lat.
f
DAYS TO BURST
60
40
20
F
M
A
M
J
J
A
S
O
N
TIME OF THE YEAR [1974]

FIGURE D2 THE PATTERN OF BUD DORMANCY ON WHOLE, DETACHED SHOOTS (1974)

Median bud-burst times for the buds on samples of 5 shoots on each date. Numerals indicate the number of shoots on which bud-burst occurred.

★ If no buds burst by day 50, the buds were considered to be dormant.

o	outside
•	cabinet, 20°C
a, d	Apex (terminal bud) only.
b, e	Top lateral bud.
c, f	All lateral buds

Note: Broken lines are used where the position of the curve is not distinct because bud-burst only occurred on 1 shoot. However, the consistency of response between experiments (see Figures D4, D6) indicates that the values obtained from single shoots fit the normal pattern.

D4(b) Methods

The sampling procedure and testing methods were similar to those used in the preceding section. The sampling times were extended to include the end of the summer period (February). In addition, whole shoots were also tested outside and bud-burst data were collected for the terminal (apical) bud.

D4(c) Results

The data for bud-burst on whole shoots (Figure D2) showed that there was a period between the end of active shoot elongation and winter dormancy when lateral buds burst following defoliation without decapitation.

A comparison of the time to burst of the apex and the top lateral bud with that of all lateral buds, tested in the cabinet (Figure D2 d, e, f) suggested that physiological dormancy occurred at the top buds at least two weeks before the lower buds. Also the end of this dormancy period occurred first in the lower lateral buds then in the top lateral bud and last in the apex. The pattern of bud-burst time on shoots outside was similar for the buds in different positions (Figure D2 a, b, c). In contrast to the shoots in the cabinet, there was no clear difference in the time at which dormancy ended in different buds on shoots outside (Figure D2 a, b, c vs d, e, f). When the number of shoots on which burst occurred was considered it was clear that burst occurred more frequently at lower lateral buds than the others (Figure D2 c, f).

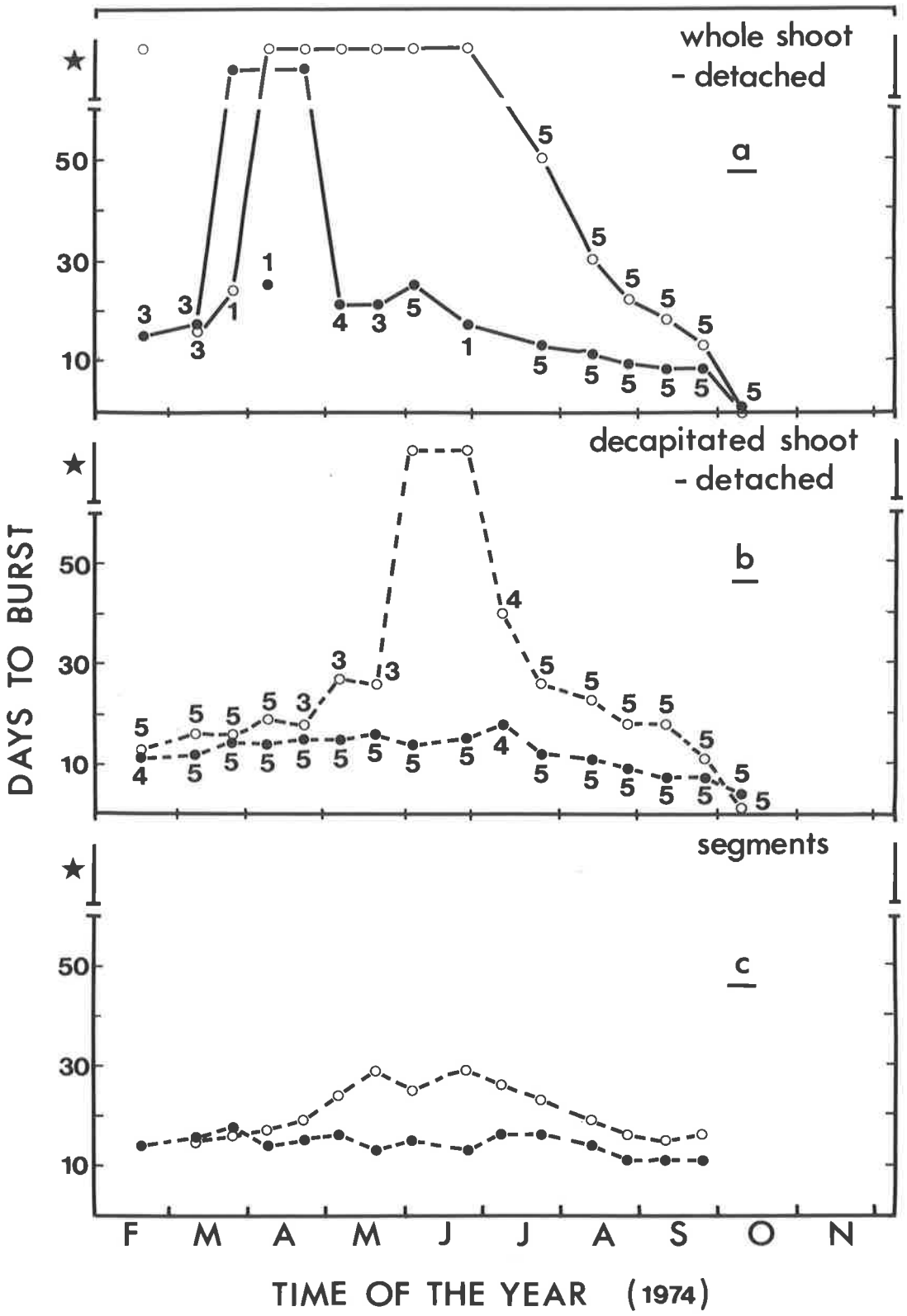

whole shoot
- detached
a
decapitated shoot
- detached
b
segments
c
DAYS TO BURST
50
30
10
F
M
A
M
J
J
A
S
O
N
TIME OF THE YEAR (1974)

FIGURE D3 DISTINCTION BETWEEN PHYSIOLOGICAL AND ENVIRONMENTAL DORMANCY OF LATERAL BUDS ON DETACHED SHOOTS (1974)

Mean bud-burst times for the buds on samples of 5 shoots on each date. Numerals indicate the number of shoots on which bud-burst occurred. Most buds on segments burst.

★ If no buds burst by day 50, the buds were considered to be dormant.

o outside

● cabinet, 20°C

a Whole shoots outside indicate winter dormancy.
Whole shoots in cabinet indicate physiological dormancy.
The difference is environmental dormancy.

b Physiological dormancy is absent on decapitated shoots.
Environmental dormancy (the difference) is reduced.

c Physiological dormancy is absent on segments.
Environmental dormancy (the difference) is only slight.

The comparison of median burst times for all lateral buds in the cabinet or outside with different treatments (Figure D3), showed that the physiological and environmental components of winter dormancy predominate at different times. The whole shoots inside (Figure D3 a) were dormant during March-April whilst similar shoots outside remained dormant until July. Thus physiological dormancy contributed to winter dormancy of the lateral buds on whole shoots only during the early weeks. When shoots were decapitated physiological dormancy was overcome (as shown in Section III D3), and no period of dormancy was found inside (Figure D3 b). Furthermore, decapitated shoots placed outside did not display dormancy at the time when physiological dormancy was the main component (i.e. March - April) but were dormant during June when environmental dormancy was prevalent.

Segments did not display complete dormancy in the cabinet or outside although the rate of bud-burst was reduced for segments outside during winter (Figure D3 c). The period of longest burst times coincided with the environmental dormancy period for decapitated shoots.

D4(d) Discussion

Hypothesis (i) was supported; i.e. lateral buds burst without decapitation before winter dormancy commenced therefore apical dominance could not have been prevailing. Hypothesis (ii) was also supported; assuming that bud-burst is an indication of bud activity associated with apical dominance, once winter dormancy was established the apex did not resume activity until after the lateral buds. Since (i) and (ii)

were verified, it is probable that the physiological dormancy observed during early winter dormancy is due to rest.

The absence of apical dominance before and after winter dormancy, as indicated by the growth of lateral buds, may be associated with the occurrence of rest at the apex. Rest was evident at the apex at the time when lateral buds responded to defoliation of the shoot in late summer. It is also broken in lateral buds some weeks before bud-break at the apex (Figure D2 d, e, f) and this may enable the lateral buds to develop before the apex asserts its dominance.

When environmental dormancy and physiological dormancy were distinguished by using decapitated shoots outside (environmental dormancy only) and whole shoots inside (physiological dormancy), it became apparent that these two types of dormancy contributed to winter dormancy at different times (Figure D3 a, b). At the beginning of winter dormancy lateral buds <u>in situ</u> are inhibited by correlative inhibition arising in the leaves since defoliation in late summer (February) stimulates lateral bud-burst. Inearly autumn (March) lateral buds no longer respond to defoliation because of some form of physiological dormancy i.e. the buds do not grow on shoots in the cabinet (Figure D3 a). This would appear to involve rest since apical dominance would not be expected (as discussed above). By the end of autumn (May) the buds will grow in the cabinet although they remain dormant outside (Figure D3 a) therefore, at this stage, they must be inhibited by environmental dormancy only. This dependence of late winter dormancy on

environmental conditions may explain why bud-burst outside occurs at the same time for all buds (Figure D2 a, b, c) and is not related to the time of bud-break, i.e. the end of rest (Figure D2 d, e, f).

The absence of dormancy on decapitated shoots inside (Figure D3 b) during the period when physiological dormancy was evident on whole shoots (March - April) indicates that decapitation breaks the dormancy of lateral buds which has been attributed to rest. A similar response was obtained for all buds on segments (Figure D3 c). Decapitation also appears to reduce the effect of environmental dormancy apart from during the middle period (June) and cutting the shoot into segments markedly reduced environmental dormancy over the whole period.

Thus the evidence strongly supports the proposal that early winter dormancy of lateral buds involves rest and that cutting a shoot breaks rest in adjacent buds. This evidence has been obtained with detached shoots and its validity for buds on shoots <u>in situ</u> is examined in the next section.

D5 COMPARISON OF DETACHED SHOOTS AND SEEDLINGS

D5(a) Introduction

The pattern of winter bud dormancy suggested in the previous section is based on evidence obtained from detached shoots. The possibility remains that isolation of a shoot from the remainder of the plant may influence bud dormancy although Eggert (1951) found no difference in overall bud activity on apple shoot cuttings compared with similar shoots *in situ*. This possibility was tested by carrying out a similar set of treatments on pot grown seedlings with their roots intact.

D5(b) Methods

Single-stemmed apple seedlings (c.v. Granny Smith) were grown as described under General Materials and Methods (Section III A1(c)). They were 4 months old at the first sampling date and had been growing outside since early summer. Mineral nutrients were applied in June to those plants remaining in the experiment.

Random samples of 20 plants were selected from a large supply of potted seedlings at intervals of 2 weeks from mid-summer (January) until natural bud-burst in mid-spring (September). After defoliation, where necessary, 10 plants were left outside whilst 10 were placed in a growth cabinet (20°C, 16 hrs light). 5 plants in each environment were decapitated. Bud-burst was recorded for all buds.

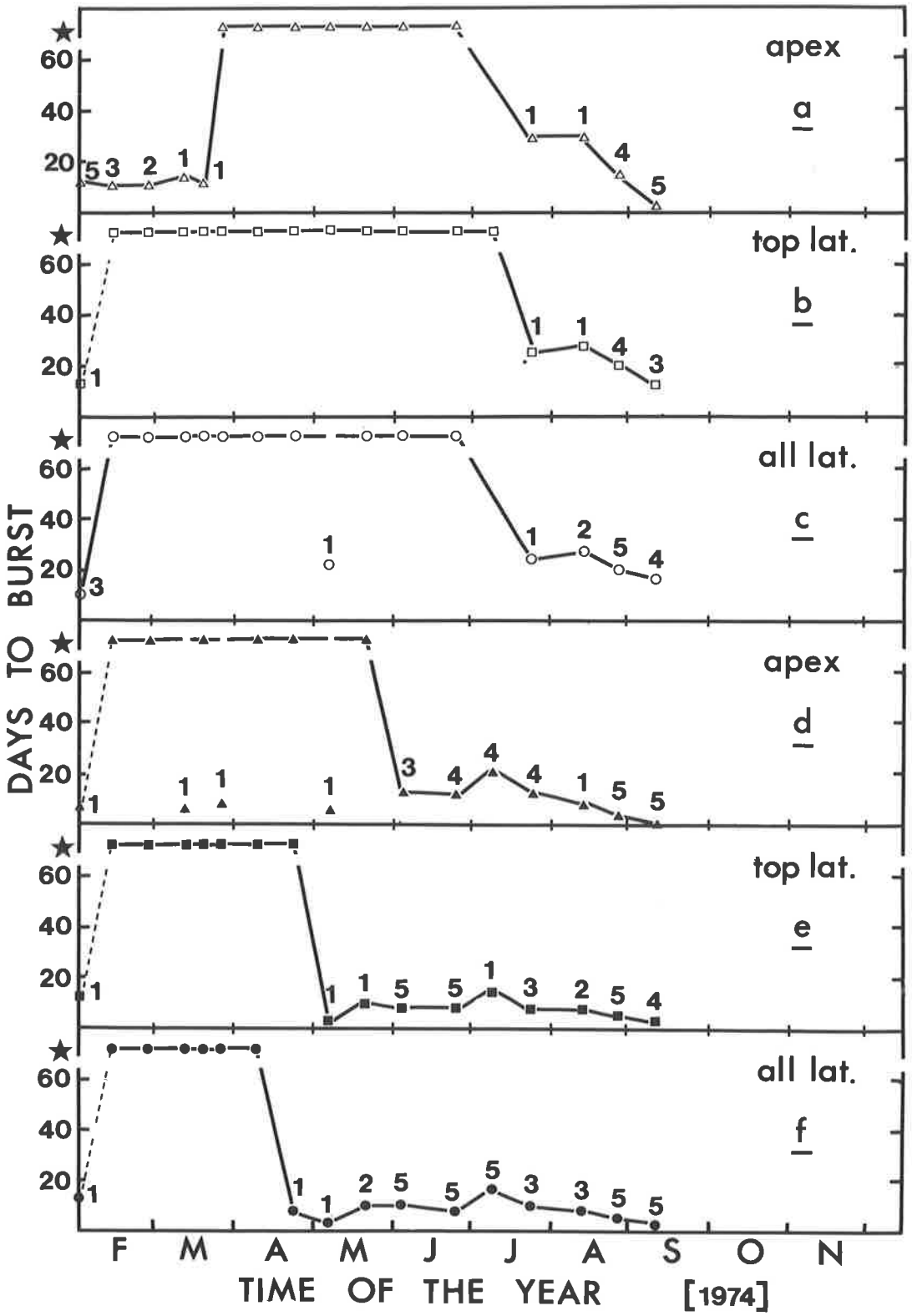

apex
a
top lat.
b
all lat.
c
apex
d
top lat.
e
all lat.
f
DAYS TO BURST
60
40
20
F M A M J J A S O N
TIME OF THE YEAR [1974]

FIGURE D4 THE PATTERN OF BUD DORMANCY ON INTACT SEEDLINGS

Median bud-burst times for the buds on samples of 5 seedlings on each date. Numerals indicate the number of seedlings on which bud-burst occurred

* If no buds burst by day 50, the buds were considered to be dormant.

o outside

• cabinet, 20°C

a, d Apex (terminal bud) only.

b, e Top lateral bud.

c, f All lateral buds

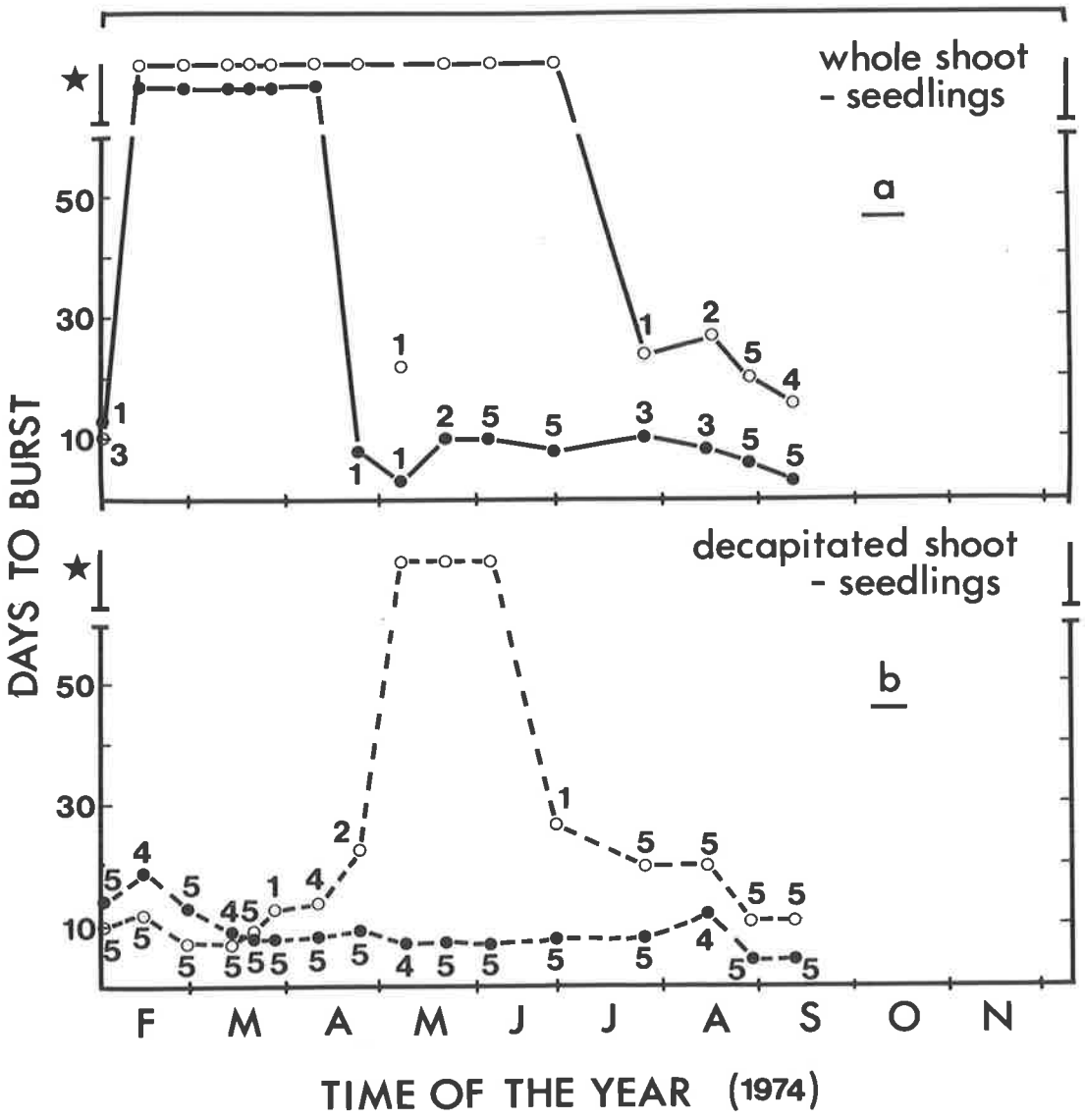

whole shoot
- seedlings
a
decapitated shoot
- seedlings
b
DAYS TO BURST
50
30
10
F
M
A
M
J
J
A
S
O
N
TIME OF THE YEAR (1974)

FIGURE D5 DISTINCTION BETWEEN PHYSIOLOGICAL AND ENVIRONMENTAL DORMANCY OF LATERAL BUDS ON SEEDLINGS

Median bud-burst times for the buds on samples of 5 seedlings on each date. Numerals indicate the number of seedlings on which bud-burst occurred.

★ If no buds burst by day 50, the buds were considered to be dormant.

o outside

● cabinet, 20°C

a Whole seedlings outside indicate winter dormancy.
Whole seedlings in cabinet indicate physiological dormancy.
The difference is environmental dormancy.

b Physiological dormancy is absent on decapitated seedlings.
Environmental dormancy (the difference) is reduced.

D5(c) Results

In seedlings the general pattern of bud-burst (Figure D4) and the distinction between environmental and physiological dormancy, as shown by the comparison of whole and decapitated shoots (Figure D5), was similar to that of detached shoots (c.f. Figures D2, D3). The main differences were the displacement of the bud-burst curves to the left (i.e. dormancy occurred earlier in the year), and a change in the relationship between the commencement of dormancy in lateral buds and apices.

On detached shoots inside, lower lateral buds entered dormancy later than the apex (Figure D2 d, e, f) but in seedlings there was no difference (Figure D4 d, e, f). Apical buds and lateral buds on detached shoots outside entered dormancy the same time (Figure D2 a, b, c) but on seedlings apical buds remained active about 40 days later than the lateral buds (Figure D4 a, b, c).

D5(d) Discussion

The overall similarity between bud-burst patterns (Figure D4 vs D2) and the distinction between environmental and physiological dormancy (Figure D3 vs D5) in seedlings and detached shoots indicates that detached shoots provide a useful indication of bud-dormancy in situ.

The bud-burst data for lateral buds on these seedlings indicate that the buds were physiologically dormant before the first sampling date. The results for the apices are

contradictory; apices on shoots in the cabinet did not burst suggesting that they were at rest but those on shoots outside did burst until early autumn (March). The general pattern of early physiological dormancy may be due to the restriction of root growth and the supply of water or nutrients to pot grown seedlings. This difference between detached shoots and seedlings was not evident in spring presumably because the seedlings were given nutrients during winter. The higher day temperatures, cooler night temperatures or greater light intensities outside may have enabled apices to resume growth following defoliation.

The earlier occurrence of physiological dormancy of lateral buds on seedlings, compared with detached shoots, may have been due to correlative inhibition induced by the restriction of growth imposed by the pot. The time of cessation of activity at the apices, which corresponds with the commencement of rest on detached shoots (c.f. Figures D4 a, D2 d), may indicate the onset of rest in the seedlings. The pattern of release from rest, after the seedlings had been given nutrients, was similar to that for detached shoots, supporting the suggestion that the initial differences may have been due to growth restrictions within the pots.

Thus it is concluded that the responses of buds on detached shoots are representative of those on shoots *in situ* when overall plant growth has not been restricted, particularly by root or soil volume limitations.

D6 THE CHILLING RESPONSE

D6(a) Introduction

It has been established (Section III D3) that winter dormancy of lateral buds involves distinct periods of physiological and environmental dormancy. The evidence also suggests that the physiological dormancy during early winter dormancy is due to rest rather than apical dominance (Section III D4). However this evidence is only inductive and a more direct test is needed to verify the proposal that rest is involved.

Horticulturalists have long recognised the need for a period of exposure to chilling temperatures to overcome winter dormancy in buds of deciduous trees (Chandler *et al.* 1937; Howard 1910). Chilling overcomes a physiological block to enable growth to resume i.e. it breaks rest. The amount of chilling needed to permit bud-burst has been taken as an indication of the depth of rest in the buds (Howard 1910). Conversely then, a response to chilling in the release of bud dormancy may be considered as an indication of the occurrence of rest.

If the hastening of bud-burst in response to chilling is accepted as a specific indication of the occurrence of rest, and if the period of physiological dormancy detected during early winter dormancy is true rest, then the following hypothesis should be true:

> that buds burst more rapidly in response to chilling only during the period of winter dormancy attributed to physiological dormancy.

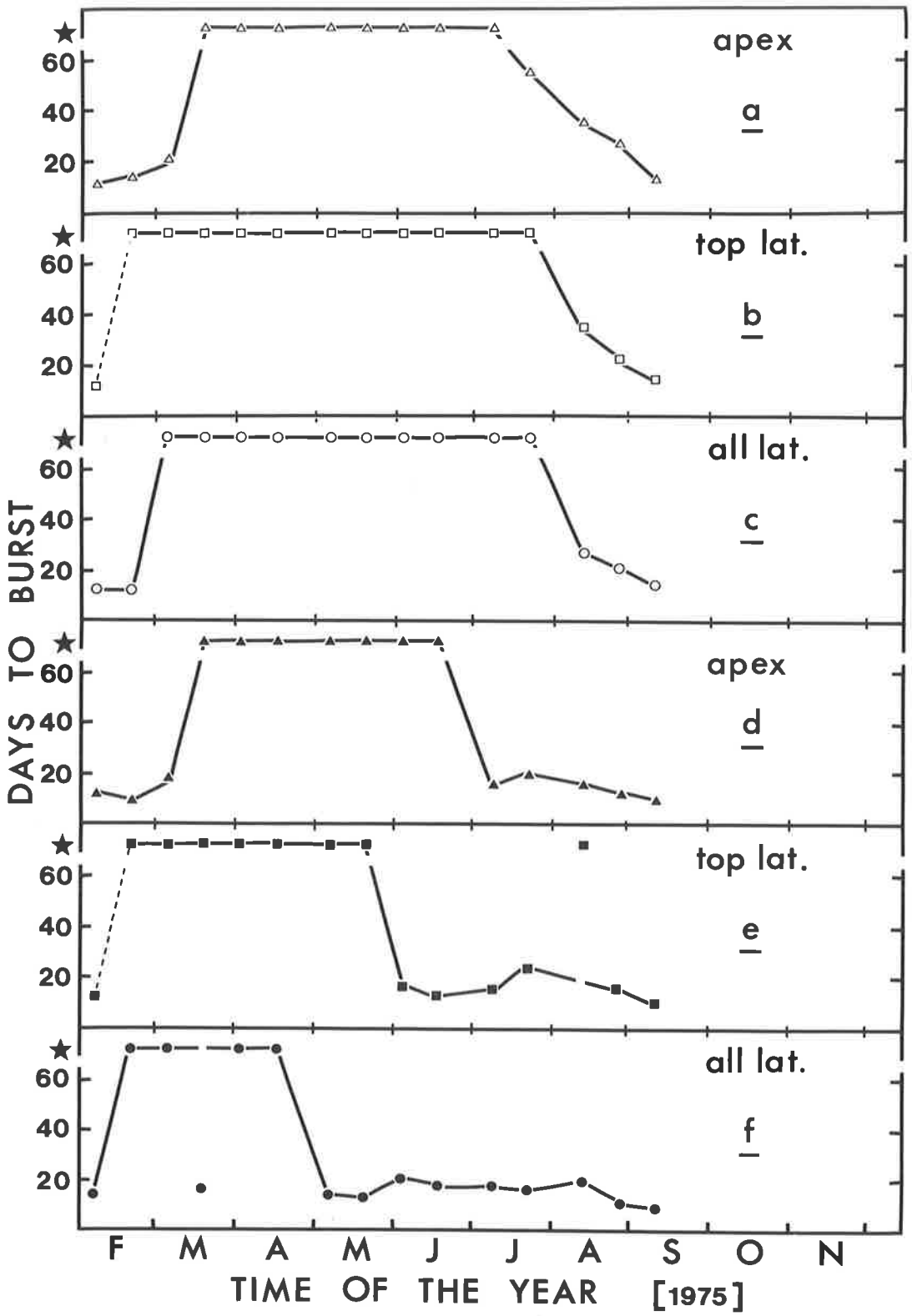

apex
a
top lat.
b
all lat.
c
apex
d
top lat.
e
all lat.
f
60
40
20
DAYS TO BURST
F M A M J J A S O N
TIME OF THE YEAR [1975]

FIGURE D6 THE PATTERN OF BUD DORMANCY ON WHOLE, DETACHED SHOOTS (1975)

Median bud-burst times for the buds on samples of 5 shoots on each date.

★ If no buds burst by day 50, the buds were considered to be dormant.

o outside

• cabinet, 20°C

Note the similarity in the patterns of dormancy between years; compare with Figure D2 (1974).

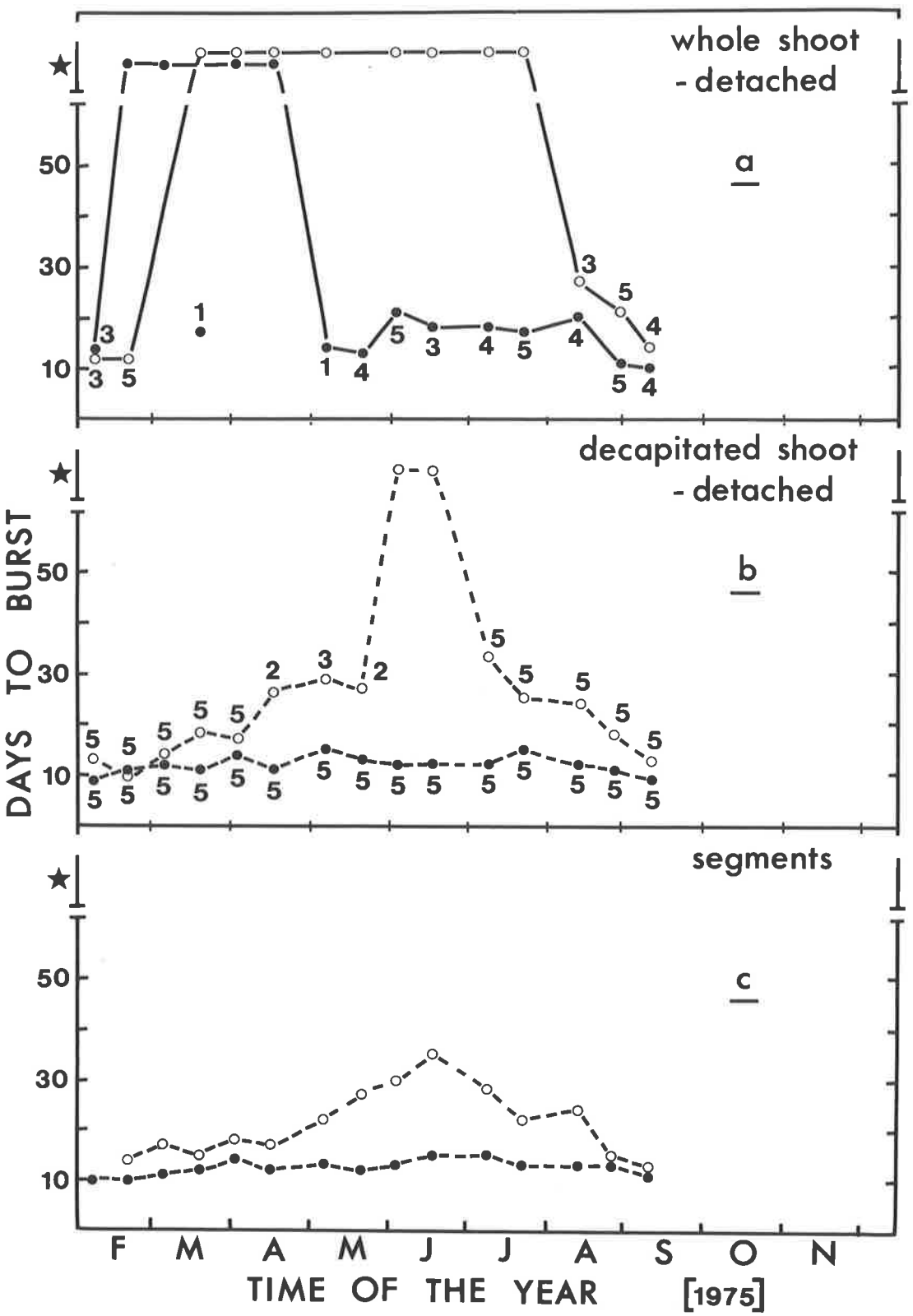

whole shoot
- detached
a
decapitated shoot
- detached
b
segments
c
DAYS TO BURST
50
30
10
F M A M J J A S O N
TIME OF THE YEAR [1975]

FIGURE D7 DISTINCTION BETWEEN PHYSIOLOGICAL AND ENVIRONMENTAL DORMANCY OF LATERAL BUDS ON DETACHED SHOOTS (1975)

Median bud-burst times for the buds on samples of 5 shoots on each date. Numerals indicate the number of shoots on which bud-burst occurred. Most buds on segments burst.

★ If no buds burst by day 50, the buds were considered to be dormant.

o outside

● cabinet, 20°C

Note the similarity in the patterns of dormancy between years; compare with Figure D3 (1974)

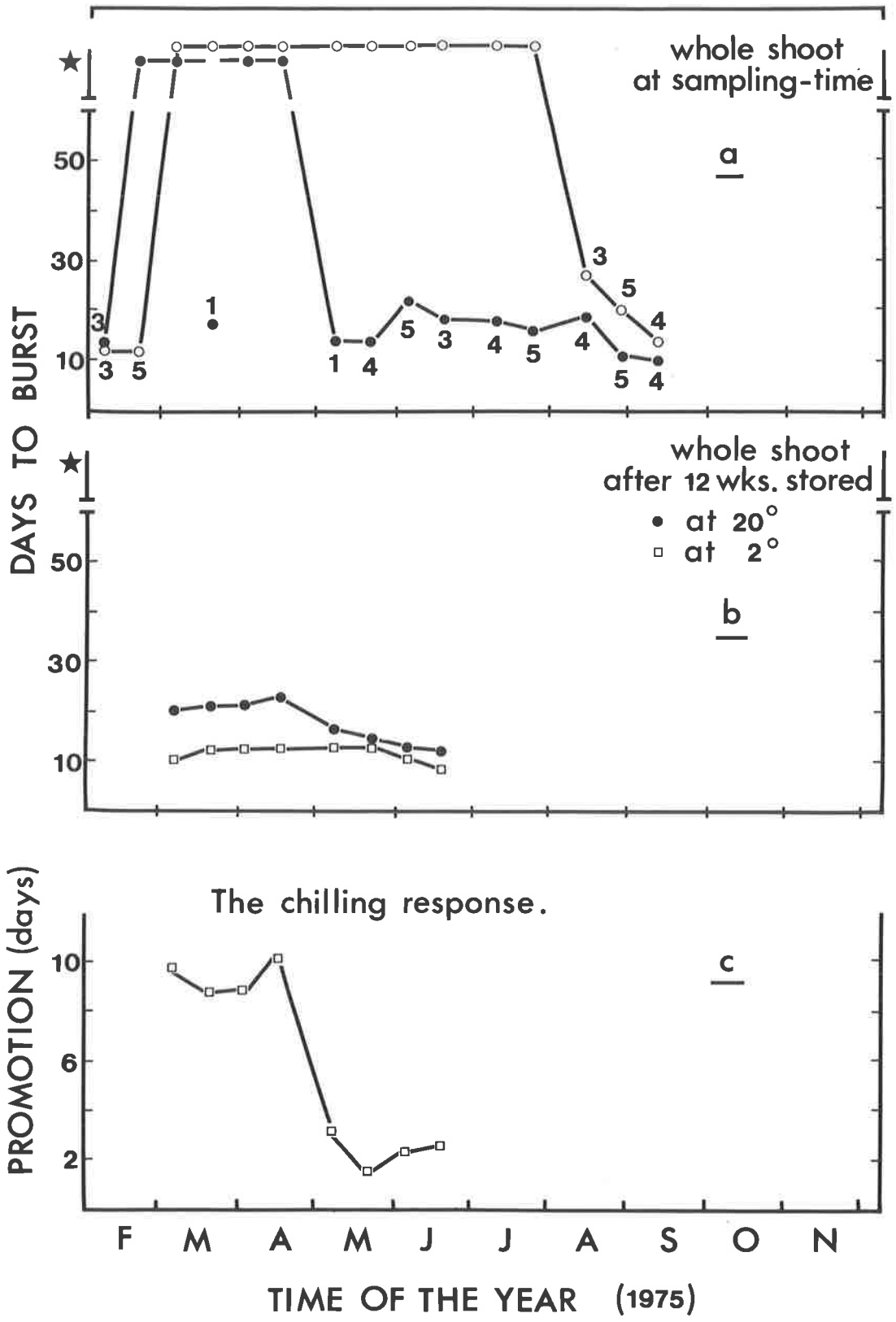

whole shoot
at sampling-time
a
50
30
10
DAYS TO BURST
whole shoot
after 12 wks. stored
• at 20°
▫ at 2°
b
50
30
10
The chilling response.
c
10
6
2
PROMOTION (days)
F
M
A
M
J
J
A
S
O
N
TIME OF THE YEAR (1975)

FIGURE D8 A DEMONSTRATION OF REST - THE CHILLING RESPONSE

a The pattern of Dormancy on whole detached shoots tested at the time of collection. Physiological dormancy is indicated by the dormancy present on shoots in the cabinet (•).

b Bud-burst on whole shoots after storage for 12 weeks in darkness at 20°C (•) or 2°C (▫).

c Buds on shoots stored at 2°C burst more rapidly than on those stored at 20°C; the difference is due to chilling and is called the Chilling Response.

Note the correlation between the occurrence of a chilling response (c) and the occurrence of physiological dormancy (a). This indicates that the physiological dormancy involves rest.

The following experiment was carried out to test this hypothesis.

D6(b) Methods

Apple shoots were collected regularly from summer to spring and tested for dormancy as described earlier (Section III D4). During the period from autumn to mid-winter (March - July) additional, similar samples of shoots were collected and were stored in covered beakers for 12 weeks at either 2°C or 20°C, in darkness, before they were tested for dormancy under the standard growth cabinet conditions (20°C/LD). The difference in time-to-burst for buds stored at 2°C or 20°C was taken as an index of the response to chilling.

D6(c) Results

The pattern of bud dormancy obtained this year (Figures D6, D7) was almost identical to that obtained for similar material the preceding year (Figures D2, D3). The only difference was that physiological dormancy of the lateral buds commenced about a month earlier (c.f. Figures D2 f and D6 f).

Lateral buds on the shoots used in this experiment were physiologically dormant from late February - early May (Figure D8 a). Shoots collected between early March - late June, but stored for 12 weeks under either warm or chilling conditions, no longer displayed this dormancy (Figure D8 b). However, shoots stored at 2°C consistently burst more rapidly than did those stored at 20°C.

The chilling response, i.e. the difference in burst time for chilled and unchilled buds, is presented in Figure D8 c. When this is compared with Figure D8 a, a close correlation may be seen between the magnitude of the chilling response and the occurrence of physiological dormancy.

D6(d) Discussion

If the assumption that chilling during winter dormancy specifically breaks rest is accepted, then the close correlation between the occurrence of a chilling response and the period of physiological dormancy (Figure D8) proves that the early period of winter dormancy in lateral buds is due to rest. Furthermore, it can be said that a pruning cut (involved in decapitation or cutting segments) breaks rest in buds nearby.

After this work had been undertaken Thompson et al. (1975) published their work on the chilling requirements of Jonathan apple buds which clearly establishes that such buds have a quantitative chilling requirement. They found that the most effective time for chilling treatments was during mid or late winter whereas the results reported here (Figure D8 a) indicate that, in the field, rest has broken before mid-winter. This difference may be attributed to the fact that Thompson et al. (1975) had stored their plants (under warm conditions) for some weeks before they were exposed to chilling. It has been shown (Figure D8 b) that

physiological dormancy is reduced during storage at non-chilling temperatures and therefore plants would require less chilling when treated on the later dates. Thus their experiments do not accurately demonstrate the occurrence of rest.

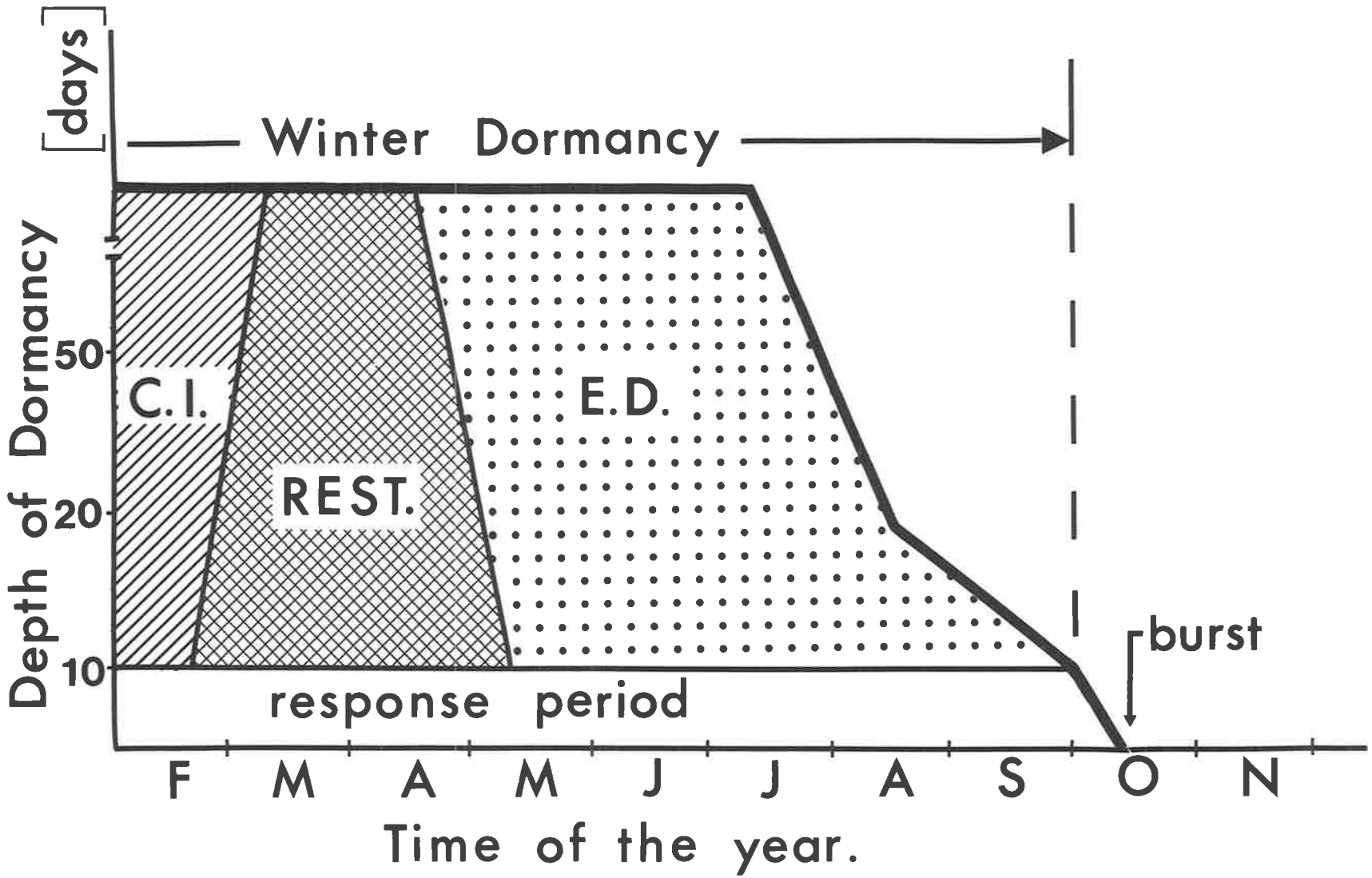

[days]
Winter Dormancy
Depth of Dormancy
50
20
10
C.I.
REST.
E.D.
burst
response period
F
M
A
M
J
J
A
S
O
N
Time of the year.

FIGURE D9 THE CONTRIBUTION OF THE TYPES OF DORMANCY TO WINTER DORMANCY OF LATERAL BUDS

The schematic model indicates which type of dormancy predominates during the different phases of winter dormancy of lateral buds *in situ*. The depth of dormancy is the bud-burst time on detached shoots.

The response period is the time taken for a bud to burst once it has been released from dormancy i.e. the time required for the new leaves to expand and emerge from the bud.

C.I. - Correlative Inhibition

E.D. - Environmental Dormancy

D7 DISCUSSION OF DORMANCY STUDIES

This study of bud dormancy arose from the need to assess the nature of the dormancy present in lateral buds on woody shoots used in physiological studies. The initial supposition, that isolating buds on single-bud segments would eliminate correlative inhibition, was correct but a concommitant, secondary factor, the pruning cut, prevented the detection of rest by this method (Section III D2). However, the finding that cutting a shoot removed physiological dormancy was utilized to distinguish between physiolocical and environmental components of winter dormancy in lateral buds (Section III D4). The introduction of an additional factor, the effect of chilling on the breaking of rest, provided a means of distinguishing between correlative inhibition and rest (Section III D6). Thus by a combination of treatments, the involvement of the different types of dormancy during the period of winter dormancy of lateral buds has been elucidated (Figure D9).

The scheme presented here for lateral bud dormancy (Figure D9) is essentially the same as that proposed earlier for buds in general (e.g. Samish 1954; Vegis 1964) but this work has validated such schemes for the specific case of lateral buds. The demonstration of a period of chilling response corresponding to the period of physiological dormancy on defoliated shoots (Figure D8) substantiates the claim that rest is involved. Furthermore, all dormancy was removed from the chilled buds since they burst within 10 days (Figure D8),

the rate obtained with non-dormant buds (on segments in the cabinet Figure D3 or D7) therefore correlative inhibition was not contributing to bud dormancy at this stage.

From the results of this study it would appear that observation of bud-burst on shoots under two conditions is the minimum required for the assessment of physiological dormancy: a comparison of whole shoots and decapitated shoots in a favourable environment (20°C, long daylength). The existence of winter dormancy is evident from the lack of activity of buds *in situ*. The test indicates the duration and extent of physiological dormancy and the remainder of winter dormancy may be attributed to environmental dormancy.

The detection of rest by the occurrence of a chilling response is not suitable for routine assessments because of the time and material required. However, the consistency in the pattern of dormancy from year to year makes it reasonable to assume that the physiological dormancy detected by the above method is in fact rest. This must be verified for other combinations of environment and plant material. Further refinement of the testing procedure is dependent on the availability of a method of eliminating correlative inhibition without cutting. From the data of Semin and Madis (1964) it would appear that ^{32}P incorporation into the buds on twigs is not influenced by correlative inhibition. If this can be verified, for lateral buds in particular, then the distinction between rest and correlative inhibition may be achieved by this technique which only requires 2-3 days instead of the 3 months required for chilling treatments.

The experimental verification of the breaking of rest in the proximity of a pruning cut is consistent with the earlier conclusion that bud-burst is hastened in the proximity of a pruning cut (Section III B2) and the proposal that this involves the breaking of rest (Section III C5).

It should also be noted here that bud-burst on decapitated shoots was confined to the distal bud on shoots collected before the end of the rest period (i.e. before the end of June). Although after this time burst occurred frequently at other buds along the shoot. When all the buds were near a pruning cut, i.e. on segments, they all burst even during the rest period. These observations indicate that the pruning effect on rest is localised near the cut (wound).

The results obtained in Section III D4 (Figure D2 d, e, f) suggested that rest occurred in the apex and top lateral buds before the lower buds but this was not substantiated in subsequent experiments (Figures D4, D6). On the contrary, terminal buds on shoots tested outside burst on later dates than did the lateral buds. This was also found by Chandler (1960). Therefore the earlier suggestion (Section III D4(d)) that apical dominance is reduced in early winter dormancy because the apex enters rest first, is not tenable.

The end of rest, as indicated by bud-burst on shoots in the cabinet, followed a consistent pattern in each experiment. It occurred first in the lower buds (compare d, e, f in Figures D2, D4, D6). However bud-burst on shoots outside occurred similtaneously for all buds (a, b, c in Figures D2,

D4, D6) indicating that bud-burst *in situ* is more closely related to prevailing environmental conditions than to the physiological state of the buds. This may not be the case where buds have not received adequate chilling to overcome rest (Chandler *et al*. 1937; Howard 1910). Eggert (1951) reported that lateral buds burst later than terminal buds but he was dealing with older shoots.

E THE ROLE OF ETHYLENE

E1 GENERAL INTRODUCTION

E1(a) The Case for Ethylene Involvement

The hastening of bud-burst in the proximity of a pruning cut (Section III B) is a response to the wounding (disruption) of stem tissues, particularly the wood (section III C). The wounding of tissues has often been accompanied by enhanced levels of plant hormones in the tissues including auxins, gibberellins, ethylene and probably cytokinins (reviewed by Sheldrake 1973). A localised change in the hormone balance of the stem may contribute to the promotion of earlier burst of the adjacent bud.

Although each of the hormones mentioned above has been reported to stimulate bud-burst on apple shoots, the available evidence suggests that ethylene is the one most likely to cause the promotion of earlier bud-burst following pruning which involves the breaking of rest (Section III D). Auxin applied to a decapitated shoot (i.e. to the wound) usually inhibits lateral bud development (Phillips 1975; Rubinstein and Nagao 1976) including apple buds (Pieniazek et al. 1970) and gibberellin application has often failed to stimulate apple buds (Brown, Griggs and Iwakiri 1960; Hull and Lewis 1959; Leibster and Kettner 1959; Williams and Billingsley 1970). Cytokinin will only stimulate the burst of dormant buds after their chilling requirement has been partly met, e.g. apple (Kender and Carpenter 1972) and peach (Weinberger 1969).

suggesting that this hormone does not break rest but is involved in subsequent events. Thus these hormones may influence correlative inhibition rather than rest.

Ethylene does not appear to have a regulatory role in the correlative inhibition of lateral buds (see reviews by Phillips 1975; Rubinstein and Nagao 1976). High levels or prolonged exposure to ethylene inhibits lateral bud development in pea, and pulse treatments may be stimulatory (Burg 1973), but there is no correlation between endogenous ethylene levels and the release of apical dominance (Burg and Burg 1968). However increased lateral bud development following treatment with ethylene, or ethylene releasing chemicals, has been observed for many species (references in Abeles 1973) including apple (Chandler 1957; Dozier and Barden 1973).

Ethylene may overcome rest in dormant potato tubers (Vacha and Harvey 1927; Rylski, Rappaport and Pratt 1974) and gladiolus cormels (Ginzburg 1974) but it delayed the germination of non-dormant gladiolus cormels (Ginzburg 1974). It also prolonged the dormancy of Vitis vinifera buds (Weaver 1973). These findings suggest that ethylene may promote the end of rest but that it inhibits later stages of bud development.

Ethylene production is the most widely reported hormonal response to tissue injury whether by pathogenic infection or pest attack (Williamson 1950; Archer and Hislop 1975) or by excision of various tissues including fruit flesh

(Norris, Craft and Liberman 1960), cotton petioles and leaves (Mc Afee and Morgan 1971) mung bean hypocotyle (Mullins 1972) and woody citrus shoots (Cooper 1972). Blanpied (1971) measured the evolution of ethylene from apple shoots cut into segments and claimed that it was not associated with wounding because there was no difference in evolution when a 60 inch length of shoot was cut into 30 (2 inch) pieces compared with 240 (0.5 inch)pieces but he did not have a comparison with uncut shoots. On the other hand Robitaille (1975) detected enhanced ethylene production from detached apple shoots. Thus it appears likely that ethylene production (or evolution) may be enhanced in the vicinity of a pruning cut.

Cooper (1972) obtained a 12 fold increase in the level of ethylene evolved from citrus stem following cutting. Furthermore the wood produced more than twice the amount of ethylene compared with peeled bark. This is consistent with the earlier finding (Section III C) that the wound effect on bud-burst involved cutting through the wood (xylem) not just the outer tissues and may explain why girdling does not promote early bud-burst.

On the basis of this evidence it was proposed that the hastening of bud-burst in proximity of a pruning cut is mediated by wound-induced ethylene. This proposal gives rise to the following hypothesis :

1) that ethylene promotes hastened bud-burst
2) that ethylene production increases following pruning
3) that inhibition of ethylene production or action

reduces the response to pruning.

An alternate hypothesis could be:

4) that ethylene is produced (or evolved) as a result of or coincidental to pruning induced bud-burst.

Apart from the work of Blanpied (1971), Cooper (1972) and Robitaille (1975) little is known about ethylene production following the cutting of woody shoots. The responses to exogenous ethylene are discussed below (Section III E1(b)). The remainder of the experimental work in this thesis extends this knowledge and tests some of the above hypotheses.

E1(b) Responses to Exogenous Ethylene

Little is known about the influence of ethylene treatments on the development of lateral buds on woody shoots. Spraying shoots with ethylene releasing chemicals (Ethrel or Ethephon)causes a general reduction in shoot growth, largely due to reduced internode elongation, accompanied by widespread leaf abscission (Dozier and Barden 1973; Edgerton and Blanpied 1968). Ethephon (4000 ppm) applied in early summer or autumn but not during mid-summer, stimulated lateral bud growth, mainly at the base of the shoot unless the apex abscissed (Dozier and Barden 1973). Cummins and Fiorino (1969) observed delayed lateral shoot growth in spring following an autumn application of Ethrel (2000 ppm). These results indicate that ethylene inhibits shoot growth but do not provide any information on the effects on bud dormancy.

The effect of exogenous ethylene in other plant systems has been found to depend on the level applied and the duration of the exposure (reviewed by Pratt and Goeschl 1969). Ethylene treatment shortens the dormancy period of potato tubers (Rylski, Rappaport and Pratt 1974) and rhubarb (Bjornseth 1946) and promotes the growth of dormant gladiolus cormels (Ginzburg 1974) but in all cases it inhibits the subsequent growth of the shoots. Similarly hypocotyl growth in bean and cocklebur is initially stimulated by ethylene but inhibited by prolonged application (Goto and Esashi 1974). In pea 500-1000 ppm Ethrel stimulated lateral buds but

1000 ppm was inhibitory (Skytt-Anderson 1970) and 2-24 hours exposure to ethylene (100 ppm) releases petunia buds from apical dominance whilst 7 days exposure was completely inhibitory (Burg 1973). Thus short term exposure to ethylene appears to overcome dormancy in various organs although growth itself is inhibited by prolonged exposure.

The following experiments (Sections III E 2,E3,E4) investigate the effects of short term and prolonged applications of Ethrel on apple bud-burst.

E2 APPLICATION OF ETHREL TO SEGMENTS

E2(a) Introduction

The first hypothesis proposed earlier (Section III E1(a)) was that ethylene promotes hastened bud-burst. This hypothesis was tested in the following 3 experiments (including Sections III E3 and III E4) by the application of Ethrel which has been shown to release ethylene at the pH levels present in plant tissues (Edgerton and Blanpied 1968).

Single-bud segments provide convenient experimental units for the study of lateral bud responses under controlled conditions but they have the disadvantage of involving wounding of the stem and may therefore have high endogenous ethylene levels. None-the-less responses were obtained when Ethrel was applied to segments and these are presented here.

E2(b) Materials and Methods

Dormant, current-year's apple shoots (c.v. Jonathan) were collected from the orchard during mid-winter and were cut into segments in the standard manner (Section III A).
Two groups of treatments were applied as follows :

a) segments immersed for 24 hours in :

A H_2O

B 5 ppm Ethrel

C 50 ppm Ethrel

D 500 ppm Ethrel

E 5000 ppm Ethrel

b) application of 10 µl drops of Ethrel (5000 ppm) or H_2O to the distal end of the segment or at the bud axil i.e. between the bud and the stem

F Ethrel to end, H_2O to bud

G H_2O to end, Ethrel to bud

H H_2O to both positions

I Untreated

After treatment each segment was stood in a 1 x 1 cm vial of water placed inside a 5 x 2.5 cm vial and these were kept in a tray covered with 'Glad Wrap' to prevent dehydration. The trays were placed in a growth cabinet at 25°C with 16 hours fluorescent light per day (approximately 2000 ft. c.). Bud-burst was recorded daily for three weeks.

TABLE E1 EFFECT OF ETHREL ON BUD-BURST ON SEGMENTS

(a) APPLIED BY 24 HRS IMMERSION

	Ethrel ppm	BUD-BURST No.(Max. 10)	Time (days)
A	0	9	13.6
B	5	8	14.3
C	50	10	13.1
D	500	10	14.2
E	5000	0	-

(b) APPLIED AS 10 µl DROPS

	TO DISTAL END	TO THE BUD	BUD-BURST No.(Max 10)	Time (days)
F	++ Ethrel	H_2O	10	10.0
G	H_2O	Ethrel	9	10.3
H	H_2O	H_2O	10	11.7
I	-	-	10	11.7

++ Ethrel 5000 ppm

For comparison between time means

LSD (P=0.05)=1.5 days

E2(c) Results

Ethrel (5-500 ppm) had no significant effect on the time of bud-burst on segments immersed for 24 hrs (Table E1 (a)). At the highest concentration (5000 ppm)Ethrel completely inhibited bud-burst and caused marked swelling of the cortex tissues near each end of the segments. This swelling was also found at the basal end of segments treated with 500 ppm Ethrel. By comparison with untreated segments (Table E1 (b), treatment I) bud-burst was delayed on all segments as a result of immersion.

When Ethrel was applied as a drop, either to the bud or the distal end of the segment, bud-burst was hastened (Table E1 (b)).

E2(d) Discussion

These results indicate that localised increases in ethylene levels may promote earlier bud-burst (Table E1 (b)).

Since immersion itself delayed bud-burst (Table E1 (a)) the effect of widespread application of Ethrel cannot be ascertained but at high concentrations (500-5000 ppm) there was a toxic reaction. Hypertrophy of cortex tissues following exposure to an ethylene-rich atmosphere was reported by Janick (1975) who noted that the response occurred mainly at the acropetal end of shoots and only in association with a wound.

E3 EFFECTS OF CONCENTRATION AND DURATION OF ETHREL TREATMENT

E3(a) Introduction

A single localised application of Ethrel to the distal end of segments enhances bud-burst (Section III E2). In other systems (Section III E1(a)) Ethrel has been found to have an initial stimulatory effect on shoot growth but to be inhibitory with high concentrations or prolonged application. The response pattern of apple segments is examined here.

E3(b) Materials and Methods

The materials and methods were similar to those used in Section III E2 except that the shoots were collected in early spring, about one month before natural bud-burst. The treatments involved application of Ethrel solutions by drops onto the distal end of segments. There were two groups of treatments :

a) a single 25 μl application immediately after cutting with the following concentrations (ppm):

   A 0 (H_2O)

   B 5

   C 50

   D 500

   E 5000

b) 25 μl of 500 ppm Ethrel applied at the time of cutting (day 0) or distributed over 6 days as shown in Table E2.

TABLE E3 EFFECT OF CONCENTRATION AND DURATION OF ETHREL TREATMENTS

(a) DIFFERENT CONCENTRATIONS

	Ethrel ppm	BUD-BURST No.(Max. 10)	Time (days)
A	0	10	7.7
B	5	9	7.6
C	50	10	7.0
D	500	10	7.3
E	5000	10	7.9

(b) DIFFERENT DURATION (25 µl, 500 ppm)

	TREATMENT	BUD-BURST No.(Max. 10)	Time (days)
F	Untreated	10	7.8
G	1 application	10	7.4
H	2 applications	10	8.2
I	3 applications	9	8.6
J	4 applications	9	8.0

Comparison between mean times

LSD (P=0.05) = 0.3 days

TABLE E2 ETHREL APPLICATION SCHEDULE

TREATMENT	μl ALIQUOTS ETHREL DAY 0	DAY 2	DAY 4	DAY 6
F CONTROL	-	-	-	-
G 1 APPLICATION	25	-	-	-
H 2 APPLICATIONS	20	5	-	-
I 3 APPLICATIONS	15	5	5	-
J 4 APPLICATIONS	10	5	5	5

E3(c) Results

Bud-burst occurred rapidly on all segments but in spite of this small but statisically significant differences (P=0.05) were detected. Ethrel promoted earlier bud-burst at moderate concentrations (50, 500 ppm) but not at the highest concentration (Table E3 (a)). When the application was spread over 2 or more days bud-burst was delayed (Table E3 (b)).

E3(d) Discussion

Although the differences in bud-burst time are only marginal they were significant because of the uniformity of the responses. These results suggest that ethylene may have an initial stimulatory effect but inhibit later stages as discussed earlier (Section III E1(b)). This needs to be

verified using segments collected during winter when the response to cutting segments is slower or by similar experiments on intact shoots.

The dual response to Ethrel application may be caused by a change in the sensitivity of the buds to ethylene. Cutting the shoot will break rest (Section III D) and the promotion of earlier burst when Ethrel is applied may be due to a more rapid release from rest. This is supported by the greater effect observed at the earlier date (Table E2 (b)) when rest would be deeper, compared with the lack of effect following later applications when rest is completely absent and bud-burst occurs in less than 7 days (unpublished data). Once bud-burst has been initiated by cutting or exogenous ethylene the subsequent expansion of the young leaves leading to bud-burst may be inhibited.

This association between the response of segments to Ethrel and the time of year is consistent with the earlier suggestion that the wound response involves the breaking of rest (Section III C6).

E4 RESPONSES OF BUDS IN SITU

E4(a) Introduction

In the preceeding experiments Ethrel was applied to segments where cutting is involved and buds burst readily on the controls. A better test for the effect of ethylene on buds is the application of Ethrel to buds in situ. without involving a pruning cut. One difficulty is that apical dominance may prevent the buds from responding. An experiment was conducted in the field and included decapitation of shoots to remove apical dominance.

E4(b) Materials and Methods

One-year-old shoots on the hedge-pruned apple rootstocks (c.v. MM106) were used in this experiment. Shoots were selected for uniformity of vigour with a minimum of 15 lateral buds on each. Treatments were applied to 2 groups of 5 buds, at nodes (1) - (5) and (11) - (15), except for treatment E where buds at nodes (1), (3), (6), (10), (13), (15) were treated. All observed buds were treated with either water or Ethrel (500 ppm) by injection of 10 μl into the base of bud scales using a micro-syringe.

The treatments were :

A) Water only

B) Decapitated plus water

C) Ethrel

D) Decapitated plus ethrel

E) Ethrel to buds distributed along the shoot

TABLE E4 APPLICATION OF ETHREL TO BUDS IN SITU.

POSITION	CONTROL	DECAPITATED	ETHREL	DECAPITATED + ETHREL
	A	B	C	D
		MEAN BURST-TIME (DAYS)		
TOP	15.8	13.4	12.9	12.0
BOTTOM	20	-	14.0	12.2
		MEAN BUD-BURST NUMBER (MAX. 5)		
TOP	2.0	2.2	4.0	4.4
BOTTOM	0.6	0	4.3	3.6

LSD (P=0.05)

BURST TIME - 2.1 days

NUMBER - 1.6

Treatments were applied in October when natural bud-burst was commencing. Each treatment was applied to a single shoot on each of 5 trees (replicates). Bud-burst was recorded daily for one month.

E4(c) Results

This experiment was carried out at the time of natural bud-burst and some lateral buds burst on control shoots. These were confined mainly to the top group of buds although a few buds in the bottom group burst later (A - Table E4).

The main effect of decapitation was to promote earlier bud-burst in the top group and suppress those below (B - Table E4). Ethrel applied to the buds stimulated most buds to burst regardless of their position and promoted earlier bud-burst compared to the control (C - Table E4). Decapitation and Ethrel application tended to act synergistically with Ethrel overcoming the suppression of lower buds usually observed following decapitation (D - Table E4).

When Ethrel was applied to individual buds distributed along the shoot 50% of the treated buds burst with a mean burst-time of 14.5 days whilst only 6% of the untreated buds burst and with a mean burst-time of 24 days. This illustrates the localised nature of the response to Ethrel applied to the buds.

E4(d) Discussion

These results confirm the earlier finding (Section III E3)

that Ethrel application in the vicinity of a bud hastens bud-burst and indicate that the effect is localised (treatment E). In addition, since Ethrel application stimulated lateral bud-burst on intact shoots, the treatment must overcome correlative inhibition, as well as rest when present. This is particularly evident where Ethrel prevents the suppression of lower buds following decapitation.

Similar experiments should be conducted on buds *in situ*. during the rest period to confirm that rest is in fact broken by exogenous ethylene.

C5 ETHYLENE EVOLUTION FROM SEGMENTS

E5(a) Introduction

The second hypothesis proposed earlier (Section III E1(a)) was that ethylene production increases following pruning. If this is true then freshly cut segments would be expected to produce ethylene. This was tested by measuring the evolution of ethylene following the cutting of segments. The assumption here is that the evolution of ethylene is a reflection of endogenous production and not only the release of the gas present in intact tissues.

E5(b) Materials and Methods

Two experiments are reported here. In both instances segments were stood in 1 x 1 cm vials and placed into 4 oz glass jars (approximately 130 mls air space) and the lids were sealed immediately. Samples of gas were removed at intervals using 2 ml disposible syringes and the quantities of ethylene were determined by G.L.C. (see Section III A).

Experiment 1

The jar contained 8 segments of Jonathan apple shoot collected from the Alvestoke orchard in January. This system was closed so that the cummulative levels of ethylene were obtained. Sampling commenced 6 minutes after cutting and continued for 70 minutes.

Experiment 2

Each jar contained 7 segments obtained from one-year-old

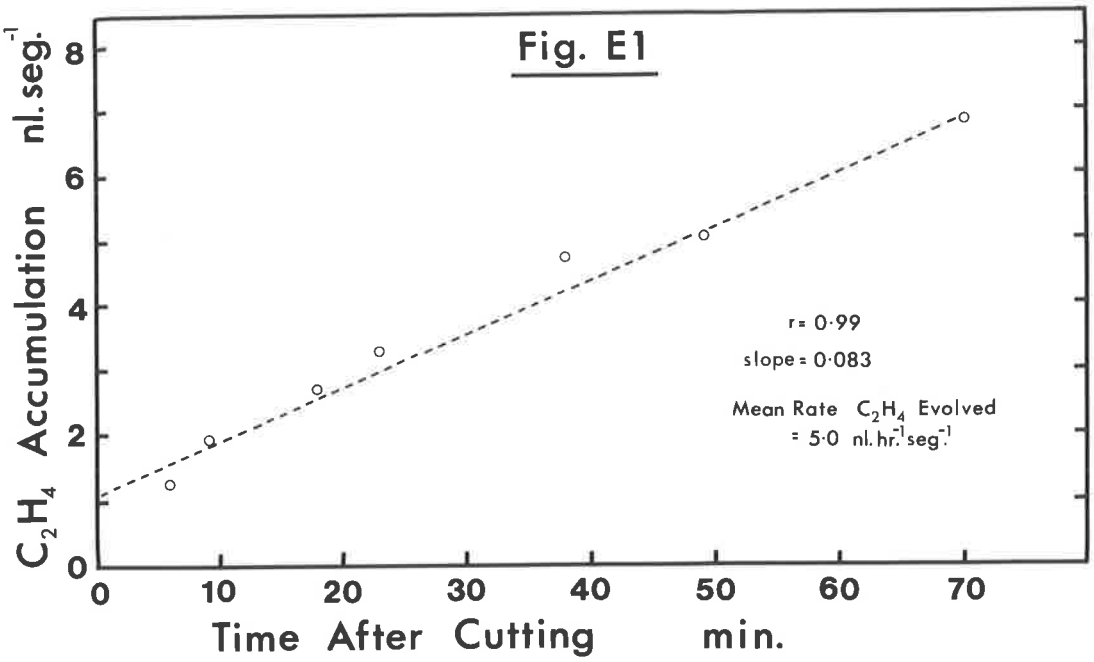


Fig. E1
C_2H_4 Accumulation nl. seg.$^{-1}$
0
2
4
6
8
0
10
20
30
40
50
60
70
Time After Cutting min.
r = 0·99
slope = 0·083
Mean Rate C_2H_4 Evolved
= 5·0 nl. hr.$^{-1}$ seg.$^{-1}$

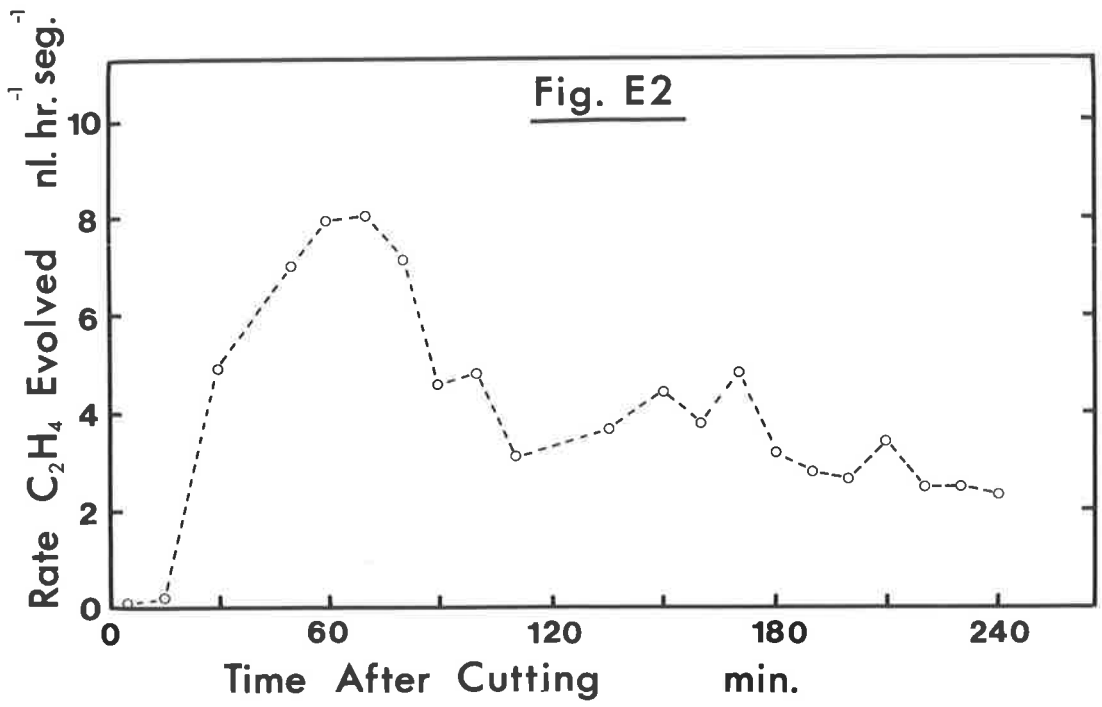


Fig. E2
Rate C_2H_4 Evolved nl. hr.$^{-1}$ seg.$^{-1}$
0
2
4
6
8
10
0
60
120
180
240
Time After Cutting min.

FIGURE E1 CUMULATIVE ETHYLENE EVOLUTION BY SEGMENTS

Ethylene levels produced by segments within a closed space were measured by sampling at intervals.

FIGURE E2 RATE OF ETHYLENE EVOLUTION BY SEGMENTS

The level of ethylene released by segments in a flowing air stream was determined by sampling at intervals

L.S.D. Between samples (P=0.05) is 2.6 nl $hr^{-1}seg^{-1}$

apple seedlings (c.v. Granny Smith) grown in the glasshouse. The jar lids were fitted with inlets and outlets and a continuous stream of air was passed through (1 ml/min; C.I.G. Medical Air). Samples were collected at approximately 10 minute intervals for 4 hours after cutting.

E5(c) Results

The quantities of ethylene measured have been expressed on a per segment basis, i.e. relative to the number of pruning cuts present rather than on a per tissue weight basis which would not be relevent for decapitated shoots. (Individual segments weigh approximately 0.6-0.8 gm).

The levels of ethylene accumulated in experiment 1 are plotted in Figure E1. The line of best fit was obtained by linear regression (correlation coefficient r=0.99) and from the slope of this line the mean rate of ethylene production was calculated to be 5.0 nl/segment/hour.

The data for experiment 2 are plotted as the rate of ethylene evolution at intervals over 4 hours (Figure E2). There was a distinct peak in ethylene evolution at 60-70 minutes after cutting after which the level declined to about 30% of the maximum at 240 minutes.

Although the two sets of data were obtained under different conditions there is close agreement between them. The rate of evolution of ethylene after 35 minutes in the second experiment (Figure E2), which approximates the mean rate for the first 70 minutes, is between 5-6 nl/segment/hour

compared with 5.0 obtained from Figure E1.

E5(d) Discussion

These results demonstrate that ethylene is evolved from apple shoot segments. The peak in production rate after about 1 hour (Figure E2) could possibly be due to an initial release of ethylene present in the stem tissues but even so the steady rate of evolution 2-4 hours after cutting is still at physiologically active levels. Direct comparison of these rates of ethylene evolution with those made by other workers is difficult because of differences in technique and units. Blanpied (1971) extracted ethylene from apple segments under vacuum and found approximately 0.3 nl/gm with a ½-hr extraction period. This is approximately 0.42 nl/segment/hour, somewhat less than detected above. However Robitaille and Leopold (1974) reported ethylene levels between 0.1 - 1.0 ppm in the atmosphere extracted from apple shoots, which compares with the 0.4 ppm detected in the closed jars of experiment 1 after 70 minutes. Also Cooper (1971) detected about 4 nl/gm/hour of ethylene after cutting citrus stem and this is equivalent to approximately 2.8 nl/segment/hour which is similar to the rate 3 hours after cutting apple segments (Figure E2). Thus the ethylene levels determined here for apple segments are of the same order as found in comparable tissues by other workers.

In a recent paper Jackson and Campbell (1976) reported a delay of 20-30 minutes before ethylene levels rose rapidly in response to cutting petiolar segments of tomato. Their peak

rate of production, (approximately 1.8 nl/gm/hr) occurred at about 80 minutes after cutting. Jackson and Osborne (1970) observed a similar pattern of ethylene production following excision of bean petioles. The data of Figure E2 indicate a similar lag phase (between 16-30 minutes) and time of maximum rate (60-70 minutes) suggesting that the pattern of wound-induced ethylene production is similar in different tissues. This lag phase could account for the lower levels detected by Blanpied (1971) who only collected for the first 30 minutes.

These results establish that cutting apple shoots into segments causes a flush of ethylene production 1-2 hours later and that levels produced are in the range of physiological activity. However methods which use segments of tissue in containers do not permit a comparison with uncut shoots. The next section deals with a system which permits this.

E6 COMPARISON OF WHOLE AND PRUNED SHOOTS

E6(a) Introduction

Measurements of ethylene evolution from segments indicated that a peak of ethylene was produced 1-2 hours after cutting the shoot (Section III E5). To justify the claim that pruned shoots produce more ethylene than unpruned shoots it is essential to have a direct comparison between the two. Furthermore, to test the 4th hypothesis proposed earlier (Section E1(a)), that ethylene is produced as a result of or coincidental to pruning induced bud-burst, it is necessary to have a system where ethylene production in the vicinity of a bursting bud may be monitored without the introduction of a pruning cut. For these reasons a new technique was developed. Several requirements placed constraints on the technique being developed.

1) A portion of the shoot must be sealed within the collection chamber without including the apical portion because this may produce high levels of ethylene on uncut shoots whilst it is absent from pruned shoots.
2) The air within the chamber must be changed often enough to remove the ethylene produced and maintain the levels of CO_2 and O_2.
3) The levels of ethylene sampled must be within the quantitative range of the GLC.

The first constraint was met by the design of the chamber (see Figure A4). The high flow rate required because of 2)

resulted in dilution of the ethylene produced. Inclusion of 5 shoots did not solve this problem therefore a method of trapping and accumulating ethylene was required. This was achieved by a modification of the technique of Phan (1965) which involved the absorption of ethylene on silica gel impregnated with mercuric perchlorate then its release from the gel by HCl. The system eventually developed is described in Section III A.

The following results were obtained from a series of experiments carried out during the development of the experimental apparatus. They provide an indication of the pattern of ethylene evolution by intact and pruned shoots.

E6(b) Materials and Methods

Although the details of the apparatus and techniques used were progressively modified in the course of these experiments the basic system was similar. In each case 5 single-stemmed apple seedlings (c.v. Granny Smith) were used per chamber. The air flow rate was 1 ml/min(C.I.G. Medical Air) and ethylene was accumulated for 1 hour periods. The final experimental system is described in detail in Section III A.

Each experiment involved the comparison between pruned and intact shoots, usually with 2 replicates. Ethylene production was expressed as nl/hr/shoot which represents the production from a 4 cm section of a shoot, usually including 2 nodes, with or without a pruning cut at the distal end.

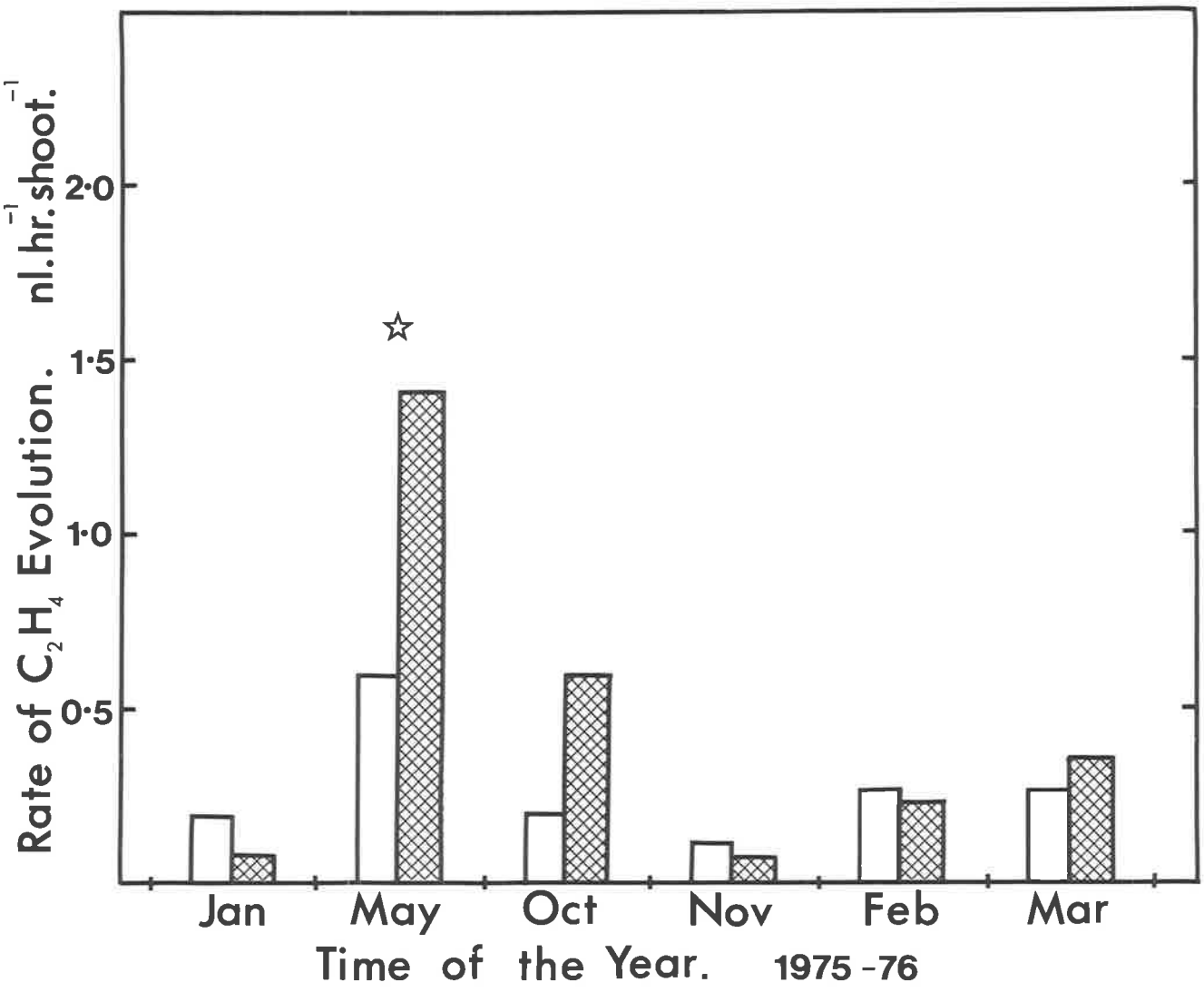

Rate of C_2H_4 Evolution. $nl.hr.shoot.^{-1}$
2·0
1·5
1·0
0·5
Jan
May
Oct
Nov
Feb
Mar
Time of the Year. 1975-76

FIGURE E3 RATE OF ETHYLENE EVOLUTION BY INTACT AND PRUNED SHOOTS

Ethylene evolution from *in situ* segments of a shoot with and without the distal portion of the shoot removed by pruning i.e. with and without a pruning cut.

Values for different dates obtained from separate experiments, usually the means of 2 replicates

without pruning

with pruning

☆ difference significant (P=0.05)

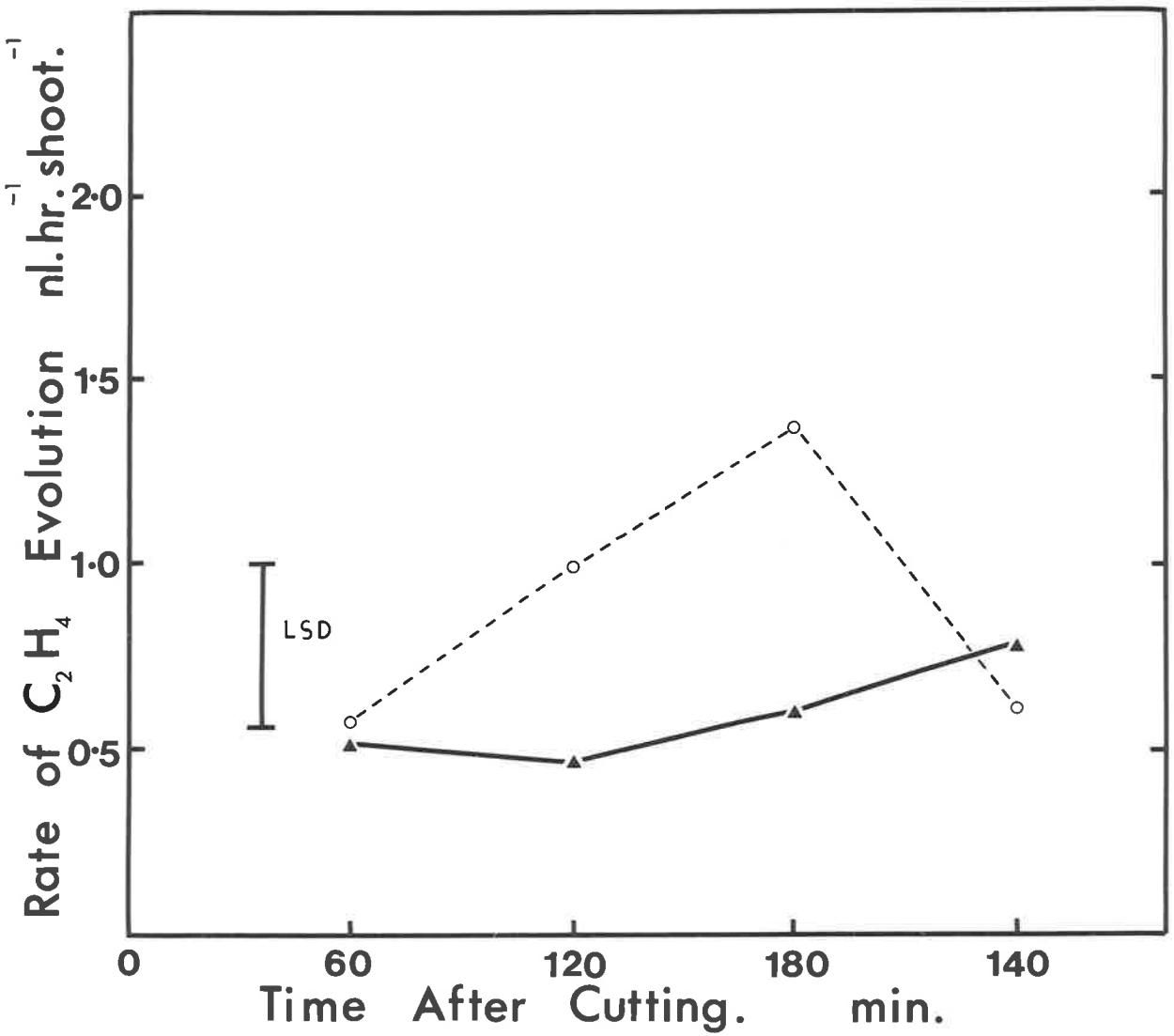

Rate of C_2H_4 Evolution nl.hr.$^{-1}$.shoot.$^{-1}$
2·0
1·5
1·0
0·5
LSD
0
60
120
180
140
Time After Cutting. min.

FIGURE E4 TIME-COURSE OF ETHYLENE EVOLUTION FOLLOWING PRUNING

Data obtained from an experiment during winter dormancy (May). Ethylene was accumulated for 60 minute periods and mean rates of evolution determined.

▲——▲ unpruned control

o- - - -o with pruning

L.S.D. P=0.05

E6(c) Results

The data presented in Figure E3 are the mean values for the rates of ethylene production estimated for intact versus pruned shoots in a series of experiments over 16 months. The results from individual experiments appeared inconsistent but when they are compared overall (as in Figure E3) several points become apparent:

i) The rates obtained in January of the first year are similar to those which may be extrapolated for the second year. This supports the validity of the measurements.

ii) The rate of ethylene evolved by intact shoots displays a distinct seasonal pattern, low during the growing season (January - February) and increasing to a maximum in the winter dormancy period (May).

iii) The occurrence of greater ethylene production by pruned shoots is evident during the winter dormancy period and conversely those experiments in which this response was not apparent all occurred during the spring - summer period.

The time-course data for the May experiment, when the enhancement of ethylene following pruning was greatest, are presented in Figure E4. The rate of ethylene evolution from the pruned shoots is more than double that of the intact shoots during the third hour. The lack of data for the second hour does not permit a more precise interpretation

but it would appear that a peak of ethylene production by pruned shoots may occur within the 1-3 hours after cutting.

The actual rates of ethylene measured in these experiments with shoots is several fold lower than found for segments but this may be due to the inefficiency of the technique. This was not investigated in detail but one estimate, based on the recovery of a known volume of ethylene, indicated only a 20% efficiency.

E6(d) Discussion

Although the data combined to produce Figure E3 are not strictly comparable because of differences in the technique used they do provide an estimate of the rates of ethylene production at different times of the year. The data are at least consistent with the proposal that both the occurrence of wound-induced ethylene and the levels produced from uncut shoots display a seasonal pattern. Pruning a shoot does induce ethylene production from that part of the shoot in the vicinity of the pruning wound but only during the winter dormancy period.

A seasonal pattern of ethylene production by xylem tissues, with a maximum during winter, has also been described for Pinus radiata (Shain and Hillis 1973). These workers obtained higher levels of ethylene from the outer sap wood (plus the cambium) than from the inner xylem but provide no data for the peripheral tissues (bark). All their

determinations were made using tissue slices and therefore include wound induced ethylene.

The time-course of wound-induced ethylene production from shoots (Figure E4) is in agreement with the pattern obtained for segments (Figure E2) therefore segments should provide satisfactory experimental units for further studies of the wound response. In the next section they are used to investigate the relationship between wound-induced ethylene and the rate of bud-burst.

E7 EFFECT OF ETHYLENE INHIBITION

E7(a) Introduction

The third hypothesis proposed earlier (Section III E1(a)), based on the proposal that the hastening of bud-burst in proximity of a pruning cut is mediated by wound-induced ethylene, was that inhibition of ethylene production or action reduces the response to pruning. This hypothesis has been tested from two approaches :

a) reduction of ethylene production at the wound

b) inhibition of ethylene activity.

Burg and Thimann (1960) found that washing the surface of tissue slices after cutting reduces the level of ethylene produced. It follows from this that washing a pruning cut should reduce the level of wound-ethylene produced and therefore reduce the wound stimulation of earlier bud-burst. This has been investigated using apple shoot segments.

For the second approach to the problem a synthetic growth regulator, Du 17623 (a Philips - Duphar product), was used. This chemical has been found to inhibit the action of ethylene rather than its production (Simons and Dilley 1971). Application of Du 17623 to the pruning wound should also reduce the wound response and delay bud-burst on segments.

E7(b) Materials and Methods

Experiment 1 - the effect of washing the wound.

Apple shoots (c.v.Northern Spy rootstock) were collected

from the field in mid-spring and cut into segments. The distal end of each segment was flushed with a jet of tap water for 2 minutes immediately after cutting. These segments were then stood in 1 x 1 cm vials of water with 8 of these units per 4 oz jar and kept at 25°C and 16 hours light. Unwashed segments were used as controls with 5 jars (replicates) per treatment and bud-burst was recorded for each segment.

Experiment 2

Segments similar to those in experiment 1 were placed in 1 x 1 cm vials of water and each unit was placed in an open 5 x 2.5 cm glass vial. 2 drops (approximately 11 µl) of test solution was applied to the distal end of the segment then the vials were placed in two unsealed, glass-covered trays under 25°C/16 hours light conditions. The treatments included the following solutions :

A Control (5% Ethanol/H_2O + 0.02% Tween 20)

B Ethrel 10 ppm

C Ethrel 100 ppm

D Ethrel 1000 ppm

E Du 17623 10^{-9}M

F 10^{-6}

G 10^{-3} (288 ppm).

Each tray (block) contained three groups (replicates) of 5 segments per treatment arranged to allow for interference between adjacent treatments.

Bud-burst was recorded for each segment.

TABLE E6 EFFECT OF EXOGENOUS ETHYLENE AND INHIBITION OF ETHYLENE ACTION

		BUD-BURST	
		No.(Max 30)	Time (Days)
A	CONTROL	22	8.4
B	ETHREL 10 ppm	27	8.6
C	100	27	7.8
D	1000	28	7.3*
E	Du 17623 10^{-9} M	29	8.0
F	10^{-6} M	26	8.6
G	10^{-3} M	27	8.9

*only value significantly different from control

E7(c) Results

Washing the pruning wound (experiment 1) caused a significant delay of bud-burst (Table E5).

TABLE E5 EFFECT OF WASHING THE WOUND

	BUD-BURST		
	NO. (max 40)	TIME (DAYS)	
A UNWASHED	31	9.1	++
B WASHED	30	12.0	

++ Difference significant at P=0.05

The results of experiment 2 are summarised in Table E6. The differences in burst time between the control and treated shoots are only small but Ethrel (1000 ppm) caused a significant (P=0.05) hastening of bud-burst whilst Du 17623, at the highest concentration (10^{-3}M), tended to delay it.

E7(d) Discussion

Although the results of these experiments are not conclusive they are consistent with the notion that treatments which reduce wound-ethylene production or action reduce the wound response. The validity of this conclusion needs to be verified possibly by using higher concentrations of Du 17623. Application of chemical inhibitors of ethylene production e.g. ethylene oxide (Lieberman and Mapson 1962), benzyl

isothiocyanate (Patil and Tang 1974) or Rhizobitoxine (Owens, Lieberman and Kunishi 1971) with monitoring of ethylene production and observation of bud-burst would be particularly useful.

E8 DISCUSSION OF ETHYLENE STUDIES

In the introduction to this section it was proposed a priori, that the hastening of bud-burst in proximity of a pruning cut is mediated by wound-induced ethylene (Section III E1(a)). Several hypothesis derived from this proposal have been tested and the results support the proposal, namely:

i) a transient increase in ethylene levels in the vicinity of a bud hastens bud-burst (Sections III E2,E3,E4).

ii) pruning may stimulate enhanced ethylene production near the wound (Sections III E5,E6).

iii) bud-burst proximal to a pruning cut is delayed if ethylene production or action is inhibited (Section III E7).

The alternate hypothesis that ethylene is produced as a result of or coincidental to pruning-induced bud-burst has not been tested. Lack of time and suitable material prevented the completion of this part of the work but the technique has been developed. The proposal is that ethylene production from unpruned shoots be measured (using the technique of Section III E6) with or without bud-burst induced by cytokinin application. Cytokinin has been shown to stimulate bud-burst (unpublished data supported by references in Section III E1).

The stimulation of bud-burst on whole shoots by either girdling (Section III C) or disbudding the distal part of the shoot (Section III B) does not cause the hastening of bud-burst associated with a pruning cut. It follows then, that ethylene levels should not be enhanced in the vicinity of the bud under these circumstances. This could also be examined by the above technique.

The apparent seasonal occurrence of the responses involving ethylene has important implications. Firstly it emphasises the need to consider and specify the state of the material used in physiological studies of bud development. In addition, the seasonal occurrence of the wound-ethylene response and the promotion of bud-burst in response to wounding treatments coincide (Section III C). This supports the contention that the two are causally linked. Furthermore as discussed in relation to the responses to wounding treatments (Section III C6), the seasonal pattern of the phenomena suggests that the hastening of bud-burst results from the breaking of dormancy due to rest.

IV GENERAL DISCUSSION AND CONCLUSIONS

The objective in this research project was to further our understanding of the way in which pruning modifies the growth pattern of woody shoots. The existing understanding was based largely on inference from general studies of the regulation of shoot growth and the responses to the removal of different amounts of the distal part of a shoot, without consideration of the different parts of the shoot. It had been inferred from studies of apical dominance, mainly on non-woody plants, that the buds remaining after pruning were stimulated to grow because they were released from apical dominance. The more vigorous growth made by the new shoots was attributed to the reduction in the number of growing points. Thus the essential effects of pruning were attributed to the removal of the distal growing point plus the reduction in bud numbers i.e. the removal of buds. In this project the effects of pruning were examined directly by distinguishing between the different components of pruning (Section III B2(a)) and this has lead to a re-evaluation of the existing concept of responses mediated through bud removal only.

An analysis of the relationships between the components of pruning and the responses obtained, as indicated by different parameters of shoot growth (Section III B), revealed that bud removal was not the only factor contributing to the response (Table B4). In particular, the hastening of bud-burst following pruning was not induced by bud removal only

i.e. disbudding (Figure B2(d)). It was due to either the removal of more distal wood (stem) or the introduction of a pruning cut distal to the responding buds.

The analysis of pruning supported the view that pruning induced bud-burst on the remaining part of the shoot by releasing buds from apical dominance (Section III C3). However, multiple girdling experiments (Section III C4) show that this view must be broadened to include the release of buds from correlative inhibition from all more distal buds, not just the distal growing point.

The promotion of more vigorous growth from the distal remaining buds may be due to either a direct stimulation of these buds or a reduction in the competition from other growing points. A reduction in the number of growing points should enhance the growth of all the remaining points rather then only the distal 1-3 buds. In addition, the growth of the lower lateral buds is suppressed suggesting that the distal buds have some advantage conferred upon them. Thus the vigorous growth of the new shoots may largely be explained by the stimulation of the distal remaining buds.

There is evidence (discussed in Section III B2(d)) to suggest that an initial small advantage in the rate of development of a shoot may result in a persistent difference in the growth rate. It is proposed here that the distal remaining buds gain an initial advantage because of the hastening of bud-burst in the proximity of a pruning cut. This is consistent with the differences in the distribution

of new shoot growth following dormant pruning compared to spring pruning and with pruning compared to disbudding (Figure B11). The enhanced vigour of the growth from the distal buds and the suppression of the lower buds is most marked where the treatment also promoted earlier bud-burst at the distal nodes i.e. following dormant pruning.

The final shoot form is dependent on the number and distribution of the new shoots as well as the total extension growth. On unpruned shoots more buds burst and elongated (Figure B2) and these were distributed along the shoot (Figure B5) compared with pruned shoots. Decapitation alone markedly altered the distribution of new growth and additional pruning or disbudding had little effect (Figure B5) suggesting that removal of the terminal bud was the main cause of the change in growth distribution. Proximity to a pruning cut was not involved. Thus, the distribution of the new shoot growth appears to be regulated by true apical dominance (i.e. correlative inhibition specifically from the distal bud).

The conclusion from this analysis of the pruning effect is therefore, that the responses to pruning cannot be explained simply in terms of the amount of shoot or the number of buds removed. At least three separate factors are involved:

1) the removal of growing points (buds)
2) the removal of the terminal bud
3) the introduction of a pruning cut.

These factors influence different aspects of the shoot

growth response. The recognition of these distinct treatment-response relationships is pre-requisite to any attempt to explain the underlying physiology of the responses to pruning.

Further investigation of the influence of the pruning cut, using cutting treatments with and without the removal or isolation of the distal portion of the shoot (Section III C) supported the contention that the hastening of bud-burst in the proximity of a pruning cut was due to a stimulus arising from the wounded tissues. On the basis of the available literature (Section III E1) it was proposed that the wound-induced ethylene might regulate this response. Subsequent experiments indicated that :

a) exogenous ethylene promotes earlier bud-burst (Sections III E2, E3)

b) endogenous ethylene production increases in the vicinity of a pruning cut (Sections III E5, E6)

c) treatments which would be expected to reduce the ethylene effect also reduce the response to pruning (Section III E7).

This is regarded as strong evidence in favour of the above proposal.

In the course of developing the technique for assessing lateral bud dormancy (Section III D), it was discovered that cutting a shoot into segments overcomes physiological bud dormancy (Section III D3) and it was subsequently shown that this included rest (Section III D6). This discovery

provided a possible explanation for the promotion of earlier bud-burst in the vicinity of a pruning cut, i.e. wound-induced ethylene may overcome bud dormancy and thereby enable more rapid development of the buds.

One further line of evidence which supports the proposed role of wound-induced ethylene production in the hastening of bud-burst following pruning, is the correlation between the seasonal occurrence of different aspects of this phenomenon in separate experiments. The difference in bud-burst time following pruning versus disbudding was observed following dormant pruning but not spring pruning (Section III B2). Similarly decapitation of detached shoots only resulted in hastened bud-burst when the buds were initially subject to some degree of dormancy i.e. when shoot were collected during mid winter (Section III C6). These observations indicate that the response is associated with the breaking of winter dormancy.

On the other hand, wound-induced ethylene production (Section III E6) and the response to exogenous ethylene (Sections III E2, E3, E4) were evident mainly during the winter dormancy period. Thus the response (the hastening of bud-burst), the mode of action of the treatment (wound-induced ethylene production) and the sensitivity of the system (response to exogenous ethylene), all have seasonal patterns of occurrence which coincide with the occurrence of winter dormancy.

Thefact that cutting shoots into segments stimulated lateral bud-burst at all times and under both cabinet and outside conditions (Section III D), indicates that both rest and environmental dormancy were overcome. Buds in situ. burst following the application of Ethrel (Section III E4) therefore correlative inhibition must also be overcome. Thus the response to pruning and, or enhanced ethylene levels, involves the release of lateral buds from all types of dormancy.

The tentative conclusion from this section of the work is that the hastening of bud-burst in the vicinity of a pruning cut is due to the release of the buds from dormancy as a result of enhanced ethylene levels in response to wounding. However, several alternative explanations of the results must be considered :

i) enhanced ethylene production may be concomitant with the occurrence of bud-burst

ii) the apparent enhanced ethylene production may be due to more rapid release of the gas already present in the tissue

iii) following ii), the release of buds from dormancy may be due to a reduction in endogenous ethylene levels near a pruning cut.

The first alternative i), cannot be eliminated by available evidence but experiments to test this possibility have been suggested (Section III E8) based on the technique developed for the measurement of ethylene from intact shoots. The second alternative ii) is possible but the occurrence of

a 20-30 minute lag phase in ethylene production following cutting (Section III E5) suggests that it is not simply release of the gas from the severed tissues. The application of inhibitors of ethylene production (see Section III E7(d)) immediately after cutting may establish whether or not ethylene production is involved.

Ethylene production, commonly associated with auxin application, has been reported to inhibit lateral bud development in pea (Rubinstein and Nagao 1976) and prolonged application of Ethrel delayed bud-burst of apple (Section III E3) therefore the removal of ethylene from the tissues near a bud may promote bud development. This would support alternative iii) above. However single applications of Ethrel (Sections III E2, E3) or pulse treatments (Phillips 1975), promote bud-burst and the rise in ethylene levels following cutting is transitory, therefore the above conclusion is not disproved. The existence of two modes of action of ethylene, initially stimulatory and later inhibitory as discussed earlier (Section III E1), may explain this difference in response.

The mechanism by which ethylene production is enhanced following cutting is not known. Exposure of the tissue appears to be important (Section III C5; Phan 1960) and this may involve modified gas exchange or dehydration of the tissue. Either of these factors could enhance ethylene production. Ethylene production within intact shoots may be limited by anaerobic conditions which have been shown

to inhibit ethylene synthesis (Leopold 1972; Pratt and Goeschl 1969) and cutting may overcome this effect. Water stress has also been found to enhance ethylene production (Ben-Yehoshua and Aloni 1974; Guinn 1976; Wright 1974).

Leopold (1972) cites evidence indicating that peptides of methionine, rather than methionine itself, may be the precursor of induced ethylene production and he suggests that wounding may increase the supply of these peptides by releasing hydrolytic enzymes. This could result from the rupture of cells during cutting. Washing the wound may reduce ethylene production (Section III E7) by removing these enzymes or substrates.

The biosynthesis of stress-induced ethylene appears to follow a similar pathway to other types of ethylene production (Abeles and Abeles 1972) which includes two steps (Imaseki and Sakai 1972):

a) induction of the synthetic system involving protein (enzyme) synthesis (Liberman and Kunishi 1975; Steen and Chadwick 1973).

b) stimulation ofthe synthesis from precursors, methionine (Abeles and Abeles 1972) or peptides (Leopold 1972).

Specific inhibitors of the first step have been reported e.g. ethylene oxide (Liberman and Mapson 1962), benzyl isothiocyanide (Patil and Tang 1974). Application of these should indicate whether the pruning effect involves induc-

tion or synthesis. Alternatively application of precursors should only enhance ethylene production if the system is already in existence.

The facility with which cutting overcomes all types of bud dormancy indicates either a common link in the processes regulating the different types of bud dormancy or that more than one mode of action is involved. The effect on environmental dormancy may be indirect. Vegis (1964) proposes that entry to and exit from rest involves a progressive change in the range of environmental conditions, particularly temperature, under which the bud will grow. Rest may not be completely broken by cutting during the period of maximum intensity so that, although the buds grow, they do so over a narrow range of environmental conditions. The persistence of some degree of physiological dormancy during late winter is evident from the longer bud-burst times on whole shoots in the cabinet compared with bud-burst times after winter (Figures D3 (a), D7 (a)). The difference between bud-burst times on segments in the cabinet compared with outside (Figures D3 (c), D7 (c)) could be a direct effect of temperature on the rate of growth leading to bud-burst after rest has ended.

It has already been established that cutting overcomes correlative inhibition by the removal of more distal buds. Exogenous ethylene may overcome correlative inhibition on intact shoots by interfering with auxin transport (Morgan and Gausman 1966; Burg and Burg 1966) and possibly auxin metabolism (Abeles 1972). Numerous modes of action of

ethylene have been suggested (reviewed comprehensively by Abeles 1972; Pratt and Goeschl 1969) but since the physiological basis of rest has not been elucidated it would be premature to postulate how ethylene might overcome this type of dormancy.

In summary, several treatment-response relationships have been distinguished within the overall effect of pruning on bud development on apple shoots. Physiological studies on the particular relationship between the pruning cut and the promotion of bud-burst time indicated that wound-induced ethylene mediates this aspect of the pruning response by breaking rest. This hitherto unrecognised component of pruning may be significant in that it provides a mechanism by which the bud proximal to a pruning cut may establish its dominance over the remaining buds and thereby determine the subsequent pattern of shoot growth. To the horticulturalist this suggests that pruning during rest will result in strong growth of the distal remaining bud whilst a similar treatment applied at the end of winter dormancy will lead to more even distribution of growth between the remaining buds.

V APPENDIX

The following is a discussion of a particular paper (Sochet 1974)a translation of which was not available until after the body of this thesis was completed. It has been included here because the work reported parallels that in the Dormancy Studies (Section III D) in this thesis.

Sochet (1974) investigated the existence of a whole-shoot factor in bud dormancy of apple by observing bud-burst on single-bud segments collected during different phases of the growth cycle. She found that lateral buds on segments under warm conditions burst on all sample dates as was found in this study. The results in this thesis are in agreement with Sochet's interpretation of her results in as much as correlative inhibition could be considered as the whole-shoot factor and this is overcome by isolation of the buds. However, Sochet does not allude to the fact that buds *in situ*. are also subject to an additional type of dormancy (rest) which is overcome as a result of proximity to a pruning cut, although a comparison between her apical segments (with no distal cut), which exibit dormancy during autumn, and the other segments on which bud-burst always occurred, would support this notion.

The other aspect of Sochet's work was the application of water to buds via a wick at the junction of the buds with the stem. This, she found, could stimulate the burst of buds *in situ*. at any time of the year. From this she concludes that the buds are never subject to deep dormancy (rest)

and that the dormancy which does occur does not lie within the bud meristem. It is not evident from the published information whether or not wounding may have also contributed to this breaking of bud-dormancy. If in fact water does release buds from rest, then this may be a contributing factor to the ready burst of buds on segments cultured in water as in this study. However this does not negate the proposal that wounding breaks rest because this effect was also observed on decapitated shoots where the water supply to the buds was not enhanced.

Thus, although Sochet used a similar experimental system, her attention was focussed on the role of correlative inhibition and she did not concieve of the role of cutting in overcoming rest.

SOCHET, M-J (1974). Etude de la ramification du rameau d'un an de pommier cultivar "Golden Délicious"
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