FRUIT QUALITY AND PRODUCTIVITY ON APPLE REPLACEMENT BRANCHES

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ABSTRACT

Three different bud types were identified on vigorous horizontal to upright (replacement) branches growing on the outer tree canopy of several apple (*Malus domestica* Borkh.) cultivars ('Granny Smith', 'Royal Gala' and 'Braeburn'). These bud types were termed two-year spur, one-year lateral and one-year terminal buds. Fruit quality and productivity characteristics of these bud types, and those of old spur buds (> three years) located inside the canopy, were investigated and compared.

Final fruit set on the replacement branch was consistently greater for buds on two-year old wood than for those on one-year wood. However, there was little difference in budbreak or flowering characteristics between wood ages. When three different bud types were compared, fruit set was greatest on two-year spur buds, intermediate on one-year terminal buds and lowest on one-year lateral buds. A similar pattern in the timing of flower bud opening during bloom was also measured for the different bud types. In contrast, flower number per bud, primary leaf area at bloom and bourse leaf area after bloom were greatest on one-year terminal, lowest on one-year lateral and intermediate on two-year spur buds.

Fruit from two-year spur buds were larger at harvest than those borne on one-year lateral buds. Differences in average size ranged from 12 to 36%, depending upon cultivar and year. Fruit on one-year terminal buds were intermediate in size ('Granny Smith' only). There was no difference in seed

number per fruit between fruit of various bud types. Fruit on old spurs were also consistently smaller than fruit on two-year spur buds.

Cumulative fruit growth followed a sigmoidal curve for fruit from two-year spur buds and one-year lateral buds (fruit from one-year terminals were not considered). Absolute growth rate was greater for fruit from two-year spurs compared with fruit from one-year laterals, although relative growth rates were similar. Flower receptacle size at bloom was consistently larger on two-year spurs than on one-year lateral buds. These differences in receptacle size probably accounted for differences in fruit size at harvest.

Fruit from two-year spur buds had higher internal ethylene concentrations and starch index score at commercial harvest and were softer and had yellower flesh ('Royal Gala' and 'Braeburn') or skin colour ('Granny Smith') than fruit from one-year lateral buds. There was little influence of bud type on fruit soluble solids concentration, amount of red blush coverage on the fruit or intensity of red blush ('Royal Gala' and 'Braeburn').

Fruit on old spurs inside the canopy had lower internal ethylene concentrations than fruit from two-year spurs or one-year lateral buds for all cultivars at commercial harvest. Fruit from old spurs also had lower soluble solids concentration, poorer red skin colour development and intensity ('Royal Gala' and 'Braeburn'), greener flesh colour ('Royal Gala' and 'Braeburn') and greener skin colour ('Granny Smith') than fruit on the replacement branch.

Fruit mineral concentrations from different bud types of 'Braeburn' and 'Granny Smith' were also compared at commercial harvest. One-year terminal

buds on 'Granny Smith' produced fruit which had higher calcium, potassium and magnesium concentrations than fruit on two-year spurs, one-year lateral and old spur buds. When fruit of the same size was compared, fruit calcium concentrations, Ca:K and Ca:Mg ratios were generally highest for one-year terminal buds, lowest for one-year lateral buds and intermediate for the other bud types. For 'Braeburn', fruit on the replacement branch had similar mineral concentrations, but had lower calcium concentrations than fruit from old spurs inside the canopy.

One-year lateral buds had the lowest fruit calcium, magnesium and potassium contents for 'Granny Smith' and 'Braeburn'. One-year terminal buds produced fruit with the highest fruit mineral content for 'Granny Smith' whilst for 'Braeburn' two-year spurs had the highest mineral content. Differences in 'Granny Smith' fruit calcium content between bud types on the replacement branch were associated with similar differences in bourse leaf area.

Manual reduction in leaf area at bloom on two-year spurs reduced fruit calcium content on 'Gala' and 'Royal Gala' throughout the season. Partial removal of primary leaves reduced calcium accumulation earlier than total bourse shoot removal. On a per leaf basis, removal of primary leaves was more effective in reducing calcium uptake than removal of the bourse shoots. However, neither fruit growth, magnesium nor potassium accumulation during the season were generally affected by such treatments.

These results are discussed in terms of (1) physiological limitations to productivity and fruit quality on apple replacement branches and trees; (2)

refining current management techniques so that yield and fruit quality are maximised on such branches and trees.

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CHAPTER ONE

GENERAL INTRODUCTION

The New Zealand apple industry has been successful by selling top-quality fruit in overseas markets. Currently, markets require fruit to be large in size, of even colour and shape, and blemish-free. Colour, flavour and texture must be typical of the cultivar. Fruit also must store adequately at low temperatures, maintaining high quality while in transit to international markets. Physiological disorders, which may develop in apple fruits during cool storage, can have a particularly harmful influence on the marketing of New Zealand's apple crop. The major aim of modern apple growers, therefore, is to produce the optimum sustainable quantity of high quality fruits as efficiently as possible from their orchards.

Light affects overall yield and is of major importance to the production of high quality fruit. Within-canopy shading can reduce apple flower bud production and fruit set, as well as inhibiting fruit growth, decreasing red colour development and influencing acid and sugar levels within fruit (Jackson et al., 1977; Jackson, 1980). Therefore it is very important that tree canopies be designed so that buds, fruit and leaves surrounding the fruit, receive optimum light quantities.

In New Zealand, good light interception in apple orchards is achieved by growing hedgerows of trees trained as centre-leader pyramids. Permanent or

semi-permanent branches are arranged up the main single leader, with temporary side laterals produced on these branches. Orientation of the side laterals may have a marked effect on fruit productivity and quality. Pendant laterals are exposed to low light levels and generally produce small fruit of poor quality (Tustin et al., 1988). To overcome this problem, a renewal pruning system is usually practised under New Zealand conditions. Renewal pruning in winter removes pendant branches from the tree and encourages laterals, termed "replacement" branches, to grow vertically or horizontally in a high light environment. When large numbers of replacement branches are produced on a tree the number of fruit harvested from these branches can be a substantial proportion of total production. This is especially so for precocious commercial cultivars, such as 'Royal Gala', 'Gala', 'Braeburn' and 'Fuji'.

Nevertheless, considerable variation in fruit size and set can occur within the replacement branch. Fruit on one-year old wood set less and were smaller than fruit on two and three-year wood for 'Golden Delicious' (Lespinasse, 1977) and 'Laxton's Superb' (Jackson, 1970a), although in both cases the replacement branch was not considered *per se*. In a more detailed study, Calleson (1988) examined fruit productivity on horizontal two or three-year old branches for nine cultivars over three years. For most cultivars two and three-year spurs produced more fruit which were heavier than fruit on one-year old shoots. However little quantitative information is available on the variation in fruit quality within the replacement branch. An understanding of the extent and causes of this variation in productivity and fruit quality may enable apple

tree management to be further refined to maximise numbers of high quality fruit on the tree.

Therefore the objectives of the following study were:

(i) To examine fruit quality and productivity within the replacement branch system. This was done by comparing the characteristics of several different types of buds including subsequent fruit produced from these buds.

(ii) To understand how bud types might account for variation in fruit productivity and quality on the replacement branch.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Plant Material

2.1.1 Hawkes Bay

'Braeburn' (13 yrs) and 'Granny Smith' trees (10 yrs), on two different commercial orchards were chosen for the study. Trees in a block of 'Royal Gala' (5 yrs) were also used, which were growing on the same property as the 'Braeburn' trees. In addition, 'Golden Delicious' trees (27 yrs) were selected for uniform cropping and vigour characteristics in a block located at the DSIR Orchard, Havelock North. All orchards were located on deep fertile silt loams (Twyford or Hastings silt loam).

All trees were growing on MM 106 rootstock, except for the 'Golden Delicious' trees which were on M 16. Tree spacings were 6.5 x 5.4m ('Braeburn'), 5.4 x 3.9m ('Granny Smith'), 5.6 x 5.6m ('Golden Delicious') and 5.0 x 3.75m ('Royal Gala').

2.1.2 Nelson

All trees used were planted at the DSIR Research Orchard, Appleby, and were grown on a heavy Mapua clay loam. 'Gala' (18 yrs) on MM 106 rootstock, 'Golden Delicious' (14 yrs) on M 12 rootstock and 'Royal Gala' (9

yrs) on M 793 rootstock were used in the experiments. Tree spacings were 5.5 x 5.5m ('Gala'), 5.5 x 2.75m ('Golden Delicious') and 5.5 x 4.0m ('Royal Gala').

2.1.3 Tree Management

Trees on the commercial orchards and at DSIR Orchard, Appleby, were trained as three-tier centre-leader pyramids. The 'Golden Delicious' trees at DSIR Orchard, Havelock North, were trained as vase-shaped multi-leader trees.

Renewal pruning was practised on all trees and large numbers of replacement branches were present on each tree. There was no fruit thinning in some experiments. However, in other experiments fruit number at each fruiting cluster was standardised by hand-thinning trees/clusters to one flower/fruit per cluster at or after flowering. Pesticide, herbicide and calcium spray programmes were carried out according to standard commercial practice. Irrigation was carried out on all trees from December to harvest using drippers.

2.2 Replacement Branch Sampling

Measurements and counts of leaves, flowers and fruit were made on replacement branches. Branches were randomly sampled from those on the outside of the tree canopy, 1.5m above ground and growing outwards from the tree centre located in a "high" light environment (300-700μmolm^{-2}s^{-1} estimated from Tustin et al., 1988). Branch lengths ranged from 0.5-1.5m.

2.3 Description of Bud Types

Several types of buds are located on a replacement branch which are capable of bearing flowers, fruits, leaves and shoots. Three flowering bud types were distinguished on replacement branches (Plate 1). Botanical descriptions of each bud type are adapted from Gur (1985).

1) Two-year spur bud - terminally borne flower bud at the tip of a short shoot of less than 5cm (spur). This shoot grew the previous season from the two-year old section of the replacement branch.

2) One-year terminal bud - terminally borne flower bud at the tip of a long (> 25cm) extension shoot. This shoot also grew the previous season from the two-year old section of the replacement branch.

3) One-year lateral bud - laterally borne flower bud on the side of a long extension shoot (as above).

In addition, fruit from inside the tree canopy, growing in a "poor" light environment (150-225μmolm^{-2}s^{-1} calculated from Tustin et al., 1988), were also sampled for fruit quality comparisons. These were from buds defined as:

4) >Three-year spur bud - terminally borne flower bud at the tip of a spur. This spur was borne on the tip of a spur which grew in the previous season.

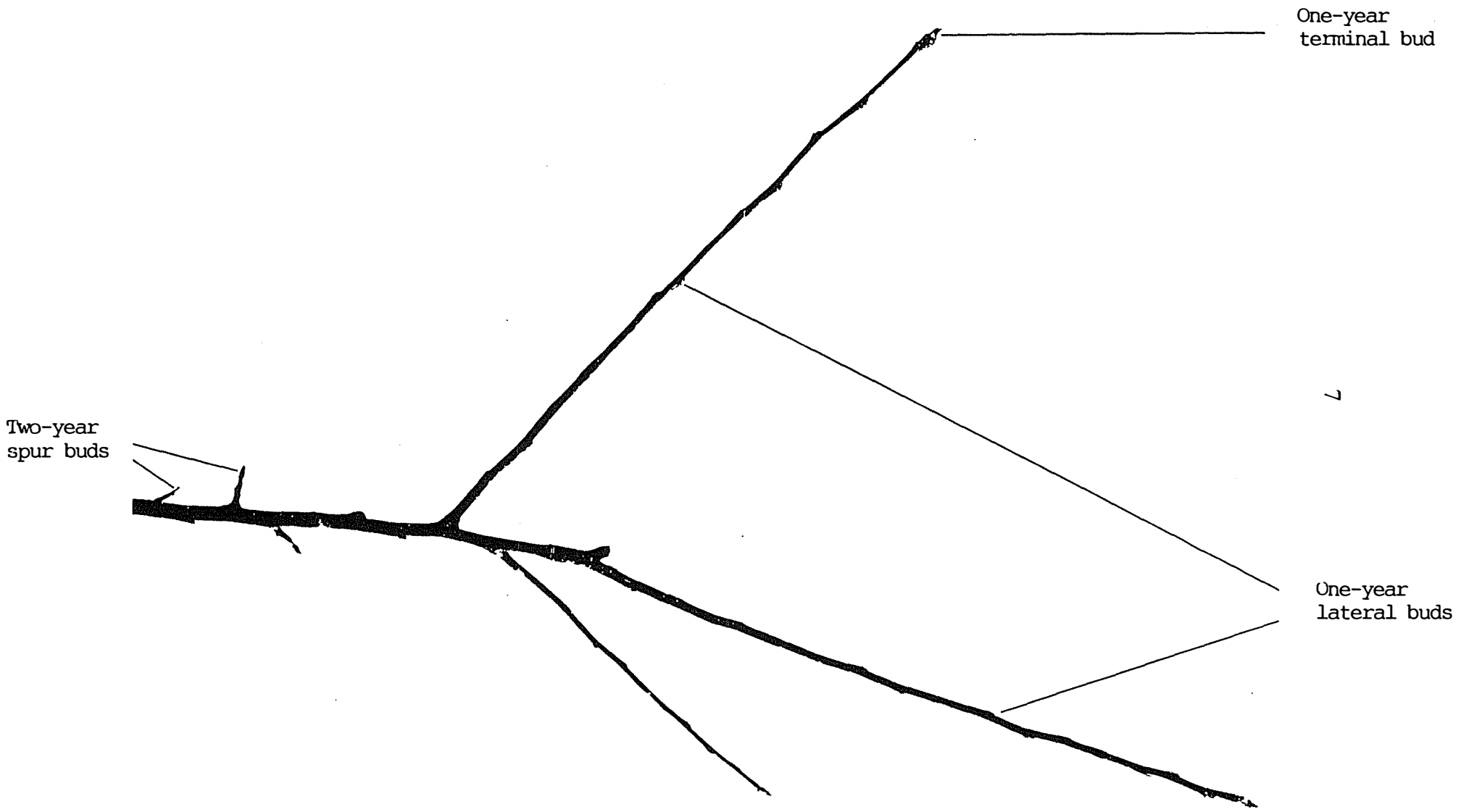


Plate 1: Example of a replacement apple branch showing position of different bud types for apple cv. 'Royal Gala'.

2.4 Statistical Analyses

The SAS statistical package was used for analysis of variance, calculation of Fisher-protected least significant differences (LSD), and linear and step-wise regressions.

CHAPTER THREE

FLOWERING AND FRUIT ABSCISSION

3.1 Introduction

The number of fruit harvested from different bud types on replacement branches are determined by three factors:

(1) Number of buds of each type on branches.

(2) The propensity of buds to produce flowers

(3) The capacity of flowers to set fruit

3.1.1 Shoot growth dynamics

The greater the number and length of extension shoots produced on a branch, the greater the number of lateral and terminal buds will be present. Spur numbers will also be reduced if long shoots are generated from potential spur sites on two year wood.

Shoot dynamics of the replacement branch are influenced by the overall vigour of the tree, which in turn, is limited by the availability of carbohydrate, nutrients and water. However the capacity of the shoot from the terminal bud to regulate development of lower lateral and spur buds (ie apical dominance) is also important. Axillary buds usually fail to elongate during the growing season in which they are initiated. Some exceptions do occur naturally where

axillary buds on vigorous extension shoots "escape" apical dominance and outgrow as lammas shoots (Crabbe, 1984). In the following year, the capacity of previously inhibited shoots borne on spur and lateral buds to compete with (dominate) the shoot from the terminal bud determines the shoot growth characteristics of the branch (Brown et al, 1967).

Examples of different shoot growth characteristics on branches can be observed between cultivars. Lespinasse (1977) described in some detail growth characteristics of different cultivars based on natural positioning of shoots on branches and trees (ie basal or apical) and intensity of branching. Thus spur-type strains of 'Red Delicious' bear only a few shoots at the base of branches but many spurs. For 'Granny Smith', vigorous shoots are produced in the apical part of the branch and few spurs are formed. Thus 'Granny Smith' bears a large number of flowers on one-year wood whereas most flowers for 'Red Delicious' types are on spurs.

Branch bending can also influence the position and vigour of new shoots. Thus branches placed in a vertical position produce more longer shoots at the apex of the branch compared with horizontally placed branches (Mullins, 1967). Otherwise little is known of environmental or cultural factors which influence shoot growth characteristics of branches and thus the numbers of buds from different bud types borne on these branches.

3.1.2 Flowering

Variation in the propensity of different bud types to produce flowers can also occur. One-year old lateral and terminal buds are often vegetative when heavy flowering occurs on nearby older spurs (Auchter, 1919). However on trees in warm climates heavy flowering can occur on young trees (Buban and Faust, 1982).

Factors influencing flower production have been well studied on apple, although most work has occurred on spurs with scant attention paid to buds on one-year wood. Fulford (1966) showed that the inception of flower buds occurs soon after initial leaf growth of the spur. Development and growth within the outer bud scales continues throughout the season culminating in flowering approximately ten months after inception.

Effect of Leaves and Shoots on Flower Initiation

Spur leaves subtending buds are critical for flower bud initiation. Their removal early in the growing season inhibits flowering (Huet, 1973). However, the nature of this effect is not clear. Pools of flower-promotive plant hormones such as cytokinin (Hoad and Abbott, 1986) may reside in mature spur leaves and be transported to the developing bud. Alternatively, spur leaves might direct movement of hormones synthesised in the roots and transported to the spur bud *via* the transpiration stream (Luckwill, 1970). Carbohydrate supply to the developing bud might also be affected by leaf removal.

Growing shoots may inhibit flowering. Where shoot growth is inhibited by synthetic growth retardants (Luckwill, 1970; Jackson and Sweet, 1972), horizontal branch bending (Mullins, 1967) and high air/root temperatures (Tromp, 1976, 1984), flower bud initiation on either spurs or one-year wood is promoted. Vigorous trees producing strong shoot growth bear fewer flower buds than less vigorous trees (Williams, 1981). Cessation of growth midway through the growing season followed by some late regrowth can encourage flower bud production on one-year wood (Crabbe, 1984).

Shoot growth may regulate flower formation as a consequence of export of hormones from the developing leaves/shoot tips to the bud. Young leaves and growing tips of apple produce high levels of hormones, especially gibberellins which are inhibitory to flower bud formation (Kato and Ito, 1962). Applications of gibberellin can promote extension growth while decreasing flower bud initiation (Luckwill and Silva, 1979).

Nutrient supply may also limit flower bud initiation and bud development. Soil and foliar applications of nitrogen to the tree during the growing season enhanced flowering (Delap, 1967). Whether this effect is one of direct action of a resource (nitrogen) limiting initiation is not clear. Buban et al. (1978) found that ammonium and nitrate applications on trees to increase levels of zeatin in the xylem sap, although flowering levels were not measured.

Effects of Flowers and Fruit on Initiation

Flowers and fruits have an inhibitory effect on flower bud initiation (Chan and Cain, 1967; Hoad, 1978; Marino and Green, 1981). The growth hormone gibberellin, emanating from developing seeds, may play a key regulatory role. A rise in gibberellin concentration in the seed or from pedicel diffusates occurs several weeks after bloom, the period when fruits are known to inhibit initiation (Luckwill, 1970; Ebert and Bangerth, 1981). However, more recent studies have found that different gibberellins may elicit quite opposite effects on flowering (Tromp, 1982; Looney and Pharis, 1986). Specifically, GA_4 may promote whereas GA_3 may inhibit initiation (Looney and Pharis, 1986).

3.1.3 Flower/Fruit Abscission

Considerable variation in fruit set occurs between different zones of an apple tree and between different bud types. Fruit set has been found to be lower on branches located within the tree canopy compared with branches on the outside (Barritt et al., 1987; Tustin et al., 1988). This may be caused by low levels of light received by spurs inside the tree canopy, as artificial shading after bloom has been shown to reduce fruit set (Jackson and Palmer, 1977). Additionally, the amount of light intercepted by spurs in the previous season may also influence fruit retention (Barden, 1978). Horizontal orientated branches have greater fruit set than vertical branches (Mullins, 1967; Tustin et al., 1988). On the replacement branch, fruit abscission has been reported to

be greater on one year lateral than one year terminal flower buds (Howlett, 1926; Goldwin, 1981). Upper parts of the shoot can have a greater set compared with lower parts (Heinicke 1917 in Dennis, 1986). However little is known of the physiological factors causing such differences in between bud types, or indeed between branches within a canopy. Factors known to influence fruit abscission will now be explored.

Pollination and Fertilisation

Pollination and fertilisation of the apple flower is usually a requirement for seed development and fruit growth, otherwise 'early' flower/fruit abortion occurs 0-14 days after bloom. Both pollination and fertilisation processes may promote a hormonal stimulus to zygotic cells which triggers cell division and initial fruit growth. Natural parthenocarpy in apple is usually not evident. However, depending upon cultivar and external conditions, parthenocarpy has been demonstrated (Wertheim, 1986; Hansen, 1989). Pollinizing cultivars must bloom simultaneously with the cultivar pollinated and produce compatible pollen for successful pollination to occur. Environmental factors may limit fruit set directly by interfering with pollen transfer (*via* insect) between two compatible cultivars or indirectly, by affecting the longevity of the pollen tube/ovules (Dennis, 1986). Thus buds blooming at different times on a tree may have different fruit set capacities because environmental conditions at anthesis are different or because the timing of anthesis is not coincident with that of the bloom time of the pollinizing cultivar/s.

Flower Quality

Flower quality is a term which has often been used to describe innate characteristics of flowers which determine their setting potential (Dennis, 1986). On the other hand, flower bud quality would seem to be a term to describe a bud's capacity to produce flowers of high "quality". For instance, flowers from longer, larger buds often set better than those from smaller buds (Dennis, 1986). Many horticultural factors are thought to be important in affecting fruit set through their influence on flower (bud) quality; summer pruning, crop load and time of harvest in the previous growing season are a few (Dennis, 1986). However a clear physiological definition of flower (bud) quality is not apparent.

Flower quality has been defined in terms of the Effective Pollination Period (EPP) (Williams, 1970). The longer the EPP, the greater chance a flower has of being successfully pollinated, fertilised and of setting. There are several basic components of EPP:

1. pollen germination and pollen tube growth, 2. longevity of the embryo sac. The physical condition of the surface of the stigma can change with time after anthesis. Thus stigma receptivity decreases with time from petal opening so decreasing EPP. Higher temperatures before (Jackson et al., 1983) or during flowering (Williams, 1970; Vasilakakis and Porlings, 1985 on pear) can decreases ovule longevity and fruit set. On the other hand, warm temperatures at bloom may enhance rate of pollen tube growth down the flower style after germination thus increasing EPP (Williams, 1970).

Cultural factors influencing flower bud quality are poorly understood. In a classic study, Williams (1965) applied nitrogen to potted apple trees at different times during the growing season. In the following season those treatments which had highest fruit set also had flowers with larger ovules and longer stigma receptivity for pollen than other treatments. A differential supply of nutrients or carbohydrates to different bud types during development might therefore influence flower quality and therefore potential fruit set.

Resource Limitations, Competition for Resources and Fruit Abscission

Unsuccessful competition for limited resources may also result in flower/fruit abscission. Thus limiting the resource or competitive ability of a fruit for that resource will influence its fruit setting potential.

Photosynthetic resources are supplied by leaves on the same cluster as flowers and developing fruit (Hansen, 1971; Quinlan and Preston, 1971). Factors which therefore reduce area and/or photosynthetic efficiency of spur leaves may therefore increase fruit drop. On an individual spur, removal of spur leaves at flowering (Ferree and Palmer, 1982) or later (Llewelyn, 1968; Proctor and Palmer,1991) increases fruit abscission.

The growing (bourse) shoot emanating from the flowering cluster can compete with developing fruitlets for carbohydrate (Quinlan and Preston, 1971; Tustin and Lai, 1990). Shoot tip removal on the flowering spur can increase initial fruit set (Abbott, 1960; Quinlan and Preston, 1971; Ferree and Palmer,

1982). Applications of growth retardants to growing shoots can increase fruit set (Williams, 1981).

There is considerable variation in photosynthetic efficiency of leaves on different flowering clusters within a tree (Barden, 1978), which is associated with the amount of light intercepted by leaves during their development. Spur leaf area is usually greater for clusters in the upper or outer parts of the tree canopy than inner parts (Barritt et al., 1987; Ferree and Forshey, 1988). However the relationship between the bourse shoot and developing fruitlets for clusters borne in different parts of the canopy has received scant attention. Variation in area or photosynthetic efficiency of spur leaves, specifically from different bud types on replacement branches has not been published.

As fruits develop, assimilate is attracted to them initially from leaves on neighbouring spurs and shoots (Hansen, 1971) and later from more distant leaves (Hansen and Christenson, 1974). It can be argued therefore that the degree of abscission is also influenced by the resources of the tree as a whole. Fruit abscission is heavier when there is a high number of flowers on the tree. Removal of flowers/fruits increases the percentage of flowers setting fruits (Heineke, 1917; Howlett, 1926 in Dennis, 1986). Partial or total shading of whole trees following bloom increases fruit drop (Dennis, 1986). Two or three days of 92% artificial shading applied 14-28 days after bloom caused over 80% fruit drop to whole apple trees (Byers et al., 1991).

The role of carbohydrate reserves in the trunk and branches in supplying carbohydrate after bloom has received scant attention. Starch levels decrease

rapidly within fruit-bearing spurs 5-6 weeks after bloom, coinciding with the period of maximum fruit growth rate (and fruit drop) (Grochowska, 1973). On the other hand, Priestly (1976) found no differences in extractable carbohydrates in discs of bark tissue from deblossomed and cropping trees. More recently, Goldwin (1985) could not induce radioactively-labelled sugars to move from any part of the spur to hormone-treated flowers. This was despite the fact that these flowers set and rapidly increased in dry weight whereas untreated (non-pollinated flowers) abscised. Whole tree shading reduced fruit set considerably but had little effect on soluble carbohydrate levels in the tree (Avery et al., 1979). These authors and others (Luckwill, 1970; Beruter, 1985) proposed that carbohydrate levels in the tree do not limit fruit set. Rather, some (hormonal) stimulus from "successful" fruit competitors induced abscission in other fruitlets.

Generally, successful pollination/fertilisation leads to rapid acceleration of the growth of the receptacle (Goldwin, 1989). If this rapid growth does not occur, then the flower will not set. Similarly, fruitlets which drop several weeks after anthesis slow in relative growth rates some 14 days prior to drop (Goldwin, 1989). Thus factors maintaining "adequate" fruit growth after fertilisation would seem to be vital in ensuring that fruitlets do not abscise.

Maintenance of fruit growth may be controlled by hormonal levels emanating from developing seeds. Hormone sprays at bloom, including GA and auxin components, have successfully stimulated early fruit growth and initial set of non-pollinated apple fruitlets (Goldwin, 1981, 1985, 1989).

Within a cluster successful competition for carbohydrate is probably determined by flower size. The apical king fruit is larger and sets more readily than side lateral flowers (Dennis, 1986). Quinlan and Preston (1971) found that accumulation of radioactively-labelled assimilates exported by a primary leaf occurred only to the subtending flower or fruitlets and to 1-2 other flowers/fruitlets in the same cluster. This was not based upon vascular phyllotaxy in the cluster, but probably the relative sink capacities of fruits for the label. Pre-anthesis factors determining apple flower quality have been previously discussed. However these same factors may also be important in influencing a fruit's capacity to grow and set after fertilisation. Within-tree variation in these factors has not been established.

In summary, the fruit productivity of a bud can be influenced by a number of local factors on the tree. The spatial and temporal relationships of a bud with nearby shoots, leaves and fruits seem to be important in determining whether a bud will flower and the amount of fruit abscission which might occur after bloom. This can lead to large variations in bud productivity within the tree. Factors influencing variation in bud productivity, specifically within the replacement branch, will now be examined.

3.2 Experimental Objectives

Variation in the number of fruit produced on branches of different wood ages may be caused by differences in number of buds burst, number of flower

buds and flowers initiated and/or number of developing flowers/fruitlets which set.

The objectives of the following experiments were:

(i) to quantify differences in bud break, flower bud initiation, fruit set and timing of fruit drop between buds borne on two-year spurs and those on long extension shoots

(ii) to ascertain possible causes for differences in fruit set between various bud types. This was pursued by investigating flower bud quality and leaf growth characteristics of buds on the replacement branch.

3.3 Influence of Wood Age on Bud Productivity

3.3.1 Materials and Methods

Experimental Procedure

Two replacement branches were selected from each of fifteen 'Granny Smith' and 'Golden Delicious' trees and one branch from each of fifteen 'Braeburn' trees at full bloom, in 1986. Flower and fruiting characteristics on one-year lateral and two-year spur buds were compared in a randomised block design, the treatment being wood age and each replacement branch a block. Before analysis of variance data were logarithmically transformed to stabilise the variance, (Steel and Torrie, 1986).

Measurements

Number of nodes, number of burst buds and buds with one or more flowers were counted on each age of wood. Terminal buds on the tips of one-year shoots were not considered. At harvest the number of fruit for each wood age were also counted.

From this data, % bud burst, % flowering buds (flower bud number/number of buds burst x 100) and % fruit set (fruit number/number of flowering buds x 100) were calculated for each wood age.

3.3.2 Results

The proportion of buds bursting was significantly higher for one-year wood compared with two-year wood on 'Granny Smith' only (Table 3.1). Bud burst was considerably less on 'Granny Smith' compared with the other two cultivars (28-38% compared with 72-84% respectively). Flowering occurred on 85% of buds on one-year wood of 'Braeburn' compared with 67% on two-year wood. There was no effect of bud age on % flower buds for 'Royal Gala' and 'Granny Smith'. Large differences existed between cultivars in proportion of flowering buds with a range from 61% to 97%.

Fruit number set as a function of total flower bud number was always substantially greater for two-year wood than for one-year wood. For 'Golden Delicious', this difference in fruit set was considerable (280%) while for the other cultivars it was 160-180%.

Table 3.1 Influence of wood age on bud break, flowering buds and fruit set for apple. All data were transformed (arcsine [sqrt {Ax.001}]) and LSD calculated on the transformed data. Transformed data in brackets.

Cultivar	Wood age	% bud break	% flowering buds	% fruit set
Braeburn	2 yr	75.3 (0.277)	66.7 (0.258)	111.2 (0.336)
	1 yr	83.5 (0.293)	85.2 (0.296)	60.6 (0.246)
	LSD (P=0.05)	(0.017)	(0.029)	(0.035)
Golden Delicious	2 yr	71.8 (0.269)	61.1 (0.245)	176.9 (0.425)
	1 yr	71.5 (0.269)	62.7 (0.251)	109.0 (0.333)
	LSD (P=0.05)	(0.018)	(0.021)	(0.038)
Granny Smith	2 yr	28.1 (0.165)	94.1 (0.311)	121.9 (0.328)
	1 yr	38.2 (0.194)	96.7 (0.316)	42.4 (0.198)
	LSD (P=0.05)	(0.016)	(0.013)	(0.070)

3.4 Influence of Bud Type on Fruit Abscission

3.4.1 Materials and Methods

Experimental Procedure and Measurements

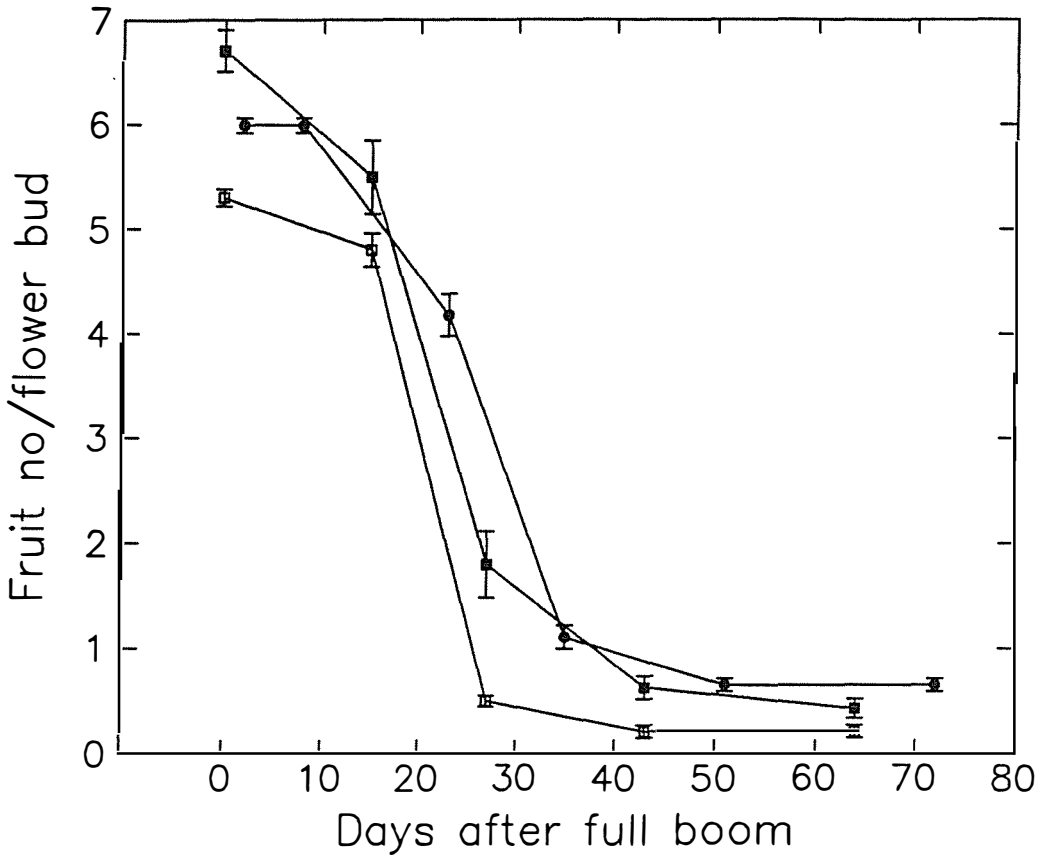
One replacement branch was selected from each of ten 'Granny Smith' trees at full bloom in 1987. Fruitlets retained on each two-year spur, one-year lateral and one-year terminal buds were counted at 7-14 day intervals for eight weeks from full bloom.

3.4.2 Results

Fruit abscission during the 60 DAFB (days after full bloom) for 'Granny Smith' followed a negative sigmoid curve with the greatest fruit drop occurring 20-40 days after full bloom for all three bud types (Figure 3.1).

One-year terminal buds had the greatest potential for fruit set as they had the highest flower number per bud (6.8 flowers/bud) (Figure 3.1). Flower number was lowest for one-year lateral buds (5.2 flowers /bud) while flower number for two-year spur buds was intermediate (6.0 flowers/bud). Fruit drop up to 20 DAFB was similar for all three bud types while the greatest differences between bud types occurred from 20-40 DAFB. One-year lateral buds had the greatest drop, two-year spur buds the lowest and one-year old terminal buds were intermediate. Final fruit set paralleled these differences

Figure 3.1 Fruit drop on apple cv. 'Granny Smith' from different bud types. Bars indicate ± SE. [two-year spur (●), one-year lateral (□), one-year terminal (■)].



(Figure 3.1). Two-year spur buds had the highest fruit set, one-year lateral buds the lowest and one-year terminal buds were intermediate.

3.5 Influence of Wood Age and Bud Type on Flower Bud Quality

3.5.1 Materials and Methods

Experimental Procedure

Three replacement branches were selected from each of ten 'Granny Smith' and 'Braeburn' trees one week before first flowering in 1987. Flowering buds occurred evenly over each age section of the replacement branches for both cultivars except for a non-flowering segment (10-30cms) at the base of one-year wood. Therefore, the flowering buds on each one- and two-year section of a branch were divided into basal, middle and distal classes (the terminal bud on the long extension shoot was not considered). An average of 3 and 4 flower buds in each section were recorded for one and two-year wood respectively for both cultivars.

The experiment was analysed as a split plot design, each branch being a block, the main factor being wood age and the sub-factor being bud position.

The above experiment was also analysed by comparing one-year terminal buds with two-year spur buds and one-year lateral buds as three treatments on the replacement branch. The experiment was analysed as a

randomised block design - each branch being a block and the main factor being bud type.

Before analysis data were logarithmically transformed to stabilise the variance (Steel and Torrie, 1986).

Measurements

The number of flowers per bud was counted and the stage of flower bud opening (tight cluster, pink bud, king full bloom, king petal fall, and petal fall) (see Table 3.2 for descriptions) was assessed once during bloom for each flowering bud ('Braeburn' - 3/10/87 'Granny Smith' - 6/10/87). Each bud opening stage was assigned a numerical value from 1-5 (Table 3.2).

3.5.2 Results

Flower buds on two-year spurs were significantly more advanced at blossom time than both one-year bud types (Table 3.3). On 3/10 for 'Braeburn' and on 6/10 for 'Granny Smith', two-year spur buds were between king full bloom and king petal fall, but one-year lateral buds were only slightly after pink bud. One-year old terminal flower buds on 'Granny Smith' were significantly more advanced than those on one-year lateral buds but this difference did not occur with 'Braeburn'.

A substantial range of flowering stages existed within any one wood age for both cultivars (Table 3.4). The middle and distal flowering buds were more advanced than basal flower buds for both wood ages. This was

Table 3.2 Flower cluster opening stages for apple.

Flower bud opening stage	Numerical 'value'
Tight cluster (TC)	1
Pink bud (PB)	2
King full bloom (KFB)	3
King petal fall (KPF)	4
Petal fall (PF)	5

Table 3.3 Influence of bud type on the stage of flower bud opening and flower number per bud for apple. Flowering is expressed on a scale from 1 (tight cluster) to 5 (petal fall). Assessments were made on 3/10/87 ('Braeburn') and 6/10/87 ('Granny Smith'). All data were transformed (log + 0.5) and LSD values calculated on the transformed data. Transformed data in brackets.

Cultivar	Bud type	Stage of flower bud opening	Flower number per flowering bud
Braeburn	2 yr spur	3.9 (1.47)	5.8 (1.84)
	1 yr lateral	2.3 (0.98)	5.3 (1.72)
	1 yr terminal	2.4 (1.00)	5.6 (1.79)
	LSD (P=0.05)	(0.12)	(0.12)
Granny Smith	2 yr spur	3.4 (1.34)	6.0 (1.86)
	1 yr lateral	2.0 (0.90)	5.3 (1.74)
	1 yr terminal	2.3 (1.06)	6.4 (1.91)
	LSD (P=0.05)	(0.11)	(0.09)

Table 3.4 Influence of wood age and bud position on flower bud opening for apple cv. 'Braeburn' (A) and 'Granny Smith' (B). Flowering is expressed on a scale of 1 (tight cluster) to 5 (petal fall). Assessments were made on 3/10/87. All data were transformed (log + 0.5) and LSD values calculated on the transformed data. Transformed data in brackets.

A.

	Bud position		
Wood age	Basal	Middle	Distal
2 yr	3.70 (1.41)	4.05 (1.51)	4.06 (1.51)
1 yr	2.01 (0.87)	2.47 (1.05)	2.47 (1.05)

The LSD (P=0.05) values used to compare values within a row (bud position) or a column (wood age) were 0.07 and 0.11 respectively.

B.

	Bud position		
Wood age	Basal	Middle	Distal
2 yr	3.2 (1.27)	3.5 (1.37)	3.6 (1.40)
1 yr	1.9 (0.86)	2.0 (0.89)	2.2 (0.94)

The LSD (P=0.05) values used to compare values within a row (bud position) or a column (wood age) were 0.07 and 0.1 respectively.

significant for 'Braeburn' and two-year wood on 'Granny Smith'. Further a flower bud opening gradient was present from the basal to the distal end of each age section of the replacement branch for both cultivars.

One-year old terminal buds and two-year spur buds had more flowers per bud than one-year lateral buds for both cultivars (Table 3.3). These differences were significant only for 'Granny Smith'. There was no consistent pattern for differences between one-year terminal and two-year spur buds.

Basal buds on each age section of the replacement branch tended to have lower flower numbers compared with the middle or distal buds (Table 3.5). This was significant on one-year wood for both cultivars. There was no difference between middle and distal buds for either cultivar.

3.6 Influence of Bud Type on Leaf Growth

3.6.1 Materials and Methods

Experimental Procedure

In 1986 all leaves from one two-year spur bud, one-year lateral and one-year terminal bud which had flowered on each of fifteen 'Granny Smith' trees, were harvested randomly on the 14/11/86 from replacement branches. Six to eight buds of each type did not bear fruit. The experiment was analysed as a complete randomised design, each bud type being a treatment, and analyses of variance computed.

Table 3.5 Influence of wood age and bud position on flower number per bud for apple cv. 'Braeburn' (A) and 'Granny Smith' (B). Assessments were made on 3/10/87. All data were transformed (log + 0.5) and LSD values calculated on the transformed data. Transformed data in brackets.

A.

	Bud position		
Wood age	Basal	Middle	Distal
2 yr	5.7 (1.83)	5.9 (1.85)	5.9 (1.85)
1 yr	4.9 (1.64)	5.5 (1.75)	5.5 (1.75)

The LSD (P=0.05) value used to compare values within a row (bud position or a column (wood age) was 0.08.

B.

	Bud position		
Wood age	Basal	Middle	Distal
2 yr	5.7 (1.82)	6.0 (1.87)	6.2 (1.90)
1 yr	5.0 (1.67)	5.6 (1.80)	5.4 (1.77)

The LSD (P=0.05) values used to compare values within a row (bud position) or a column (wood age) were 0.06 and 0.08 respectively.

In 1987 all leaves from fifteen buds of each type (as above) from ten 'Granny Smith' trees were harvested every 7-14 days from full bloom until 27/11/87. The three bud types had different dates of full bloom in 1987 (two-year, 6/10/87; one-year lateral and one-year terminal, 13/10/87). Changes in leaf characteristics with time were thus standardised by plotting them as a function of days from full bloom for each bud type.

Measurements

All unfolded leaves from each bud type were divided into primary and bourse leaves. The leaf number for each leaf and bud type was determined and total leaf area measured, using a Licor LI 3100 metre. Area per leaf was calculated from this data.

3.6.2 Results

In mid November, one-year terminal buds of 'Granny Smith' in 1986 had a significantly higher number of primary leaves than other bud types (Table 3.6). However there was no difference between two-year spur and one-year lateral buds. Differences in area per leaf and total primary leaf area between the three bud types followed a similar trend.

Bourse leaf numbers and total leaf area were significantly lower for one-year lateral buds compared with both other bud types in 1986. Bourse leaf number and total leaf area were higher on one-year terminal buds than two-year spur buds, although this was not significant. Area per leaf was lowest for

Table 3.6 Influence of bud type on primary and bourse leaf characteristics for apple buds cv. 'Granny Smith' measured 14/11/86.

	Primary leaf			Bourse leaf		
Bud type	Leaf no.	Average leaf area (cm^2)	Total leaf area (cm^2)	Leaf no.	Average leaf area (cm^2)	Total leaf area (cm^2)
2 yr spur	5.4	4.8	26.0	6.7	22.8	151.9
1 yr lateral	5.2	4.6	24.1	2.3	10.0	30.6
1 yr terminal	8.2	9.9	81.9	8.9	21.4	198.4
LSD (P=0.05)	0.9	1.1	10.5	2.8	4.8	64.4

one-year lateral buds but was similar for one-year terminal and two-year spur buds.

Area per leaf and total leaf areas were greater on the bourse than for the corresponding primary leaves for each bud type. However, there was no consistent effect of leaf type on the number of leaves produced across the three bud types.

In 1987 there were differences between the three bud types in the characteristics of primary leaves measured at bloom. One-year terminal buds had the greatest number (Figure 3.2A), area per leaf (Figure 3.2B) and total area (Figure 3.2C) of primary leaves compared with both other bud types. These differences were maintained throughout the following seven weeks. One-year lateral buds had a slightly lower primary leaf number and total area at bloom compared with those from two-year spur buds. However average leaf size was similar.

Primary leaf number and total leaf area on one-year terminal buds tended to increase over the seven week period after bloom. However primary leaf number on two-year spur buds decreased slightly from 23 DAFB so that there was little difference between one-year lateral and two-year spur buds after this time.

The three bud types also had slight differences in bourse leaf characteristics at full bloom in 1987. One-year terminal buds had the greatest leaf number (Figure 3.3A), area per leaf (3.3B) and total leaf area (Figure 3.3C), one-year lateral buds had the smallest, whilst the leaf characteristics of

Figure 3.2 Changes in primary leaf characteristics after full bloom, as influenced by bud type for apple cv. 'Granny Smith'. Bars indicate ± SE. [two-year spur (●), one-year lateral (□), one-year terminal (■)].

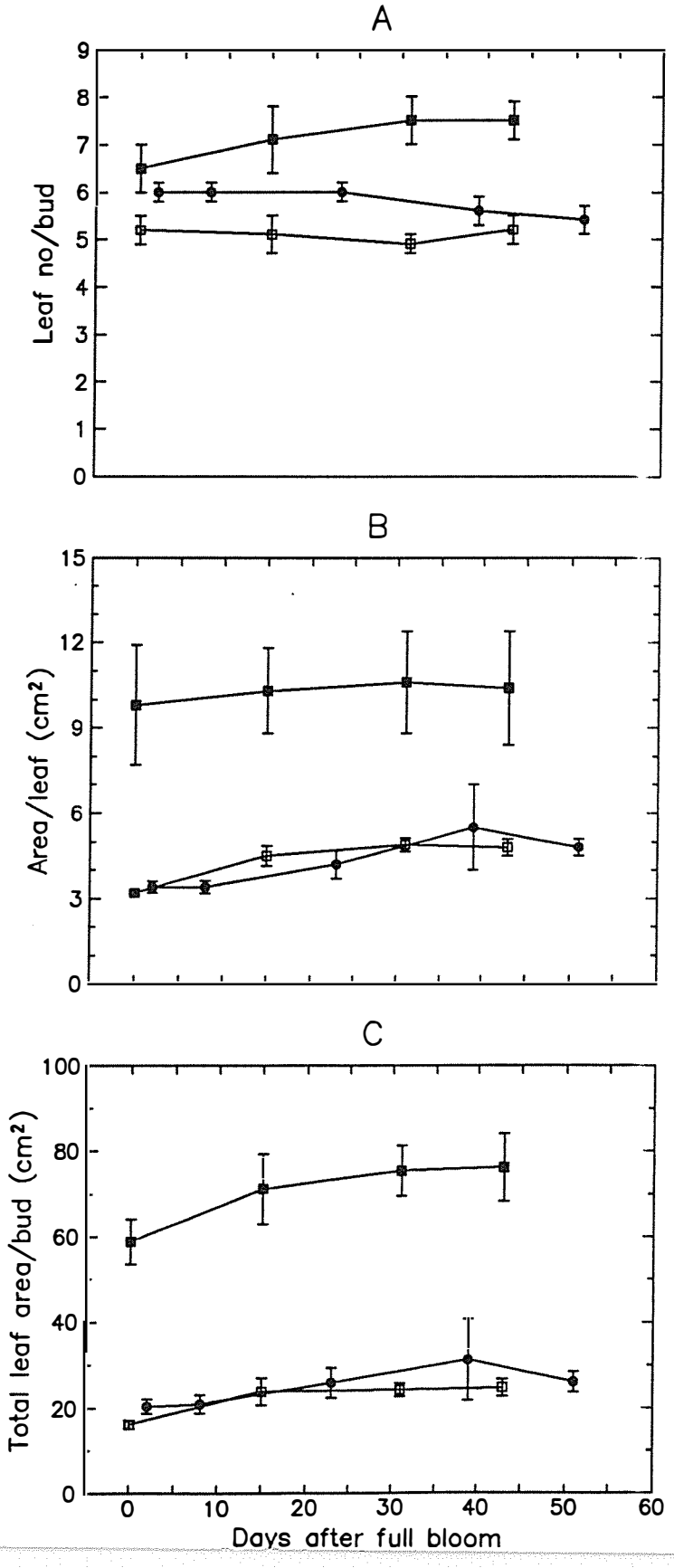
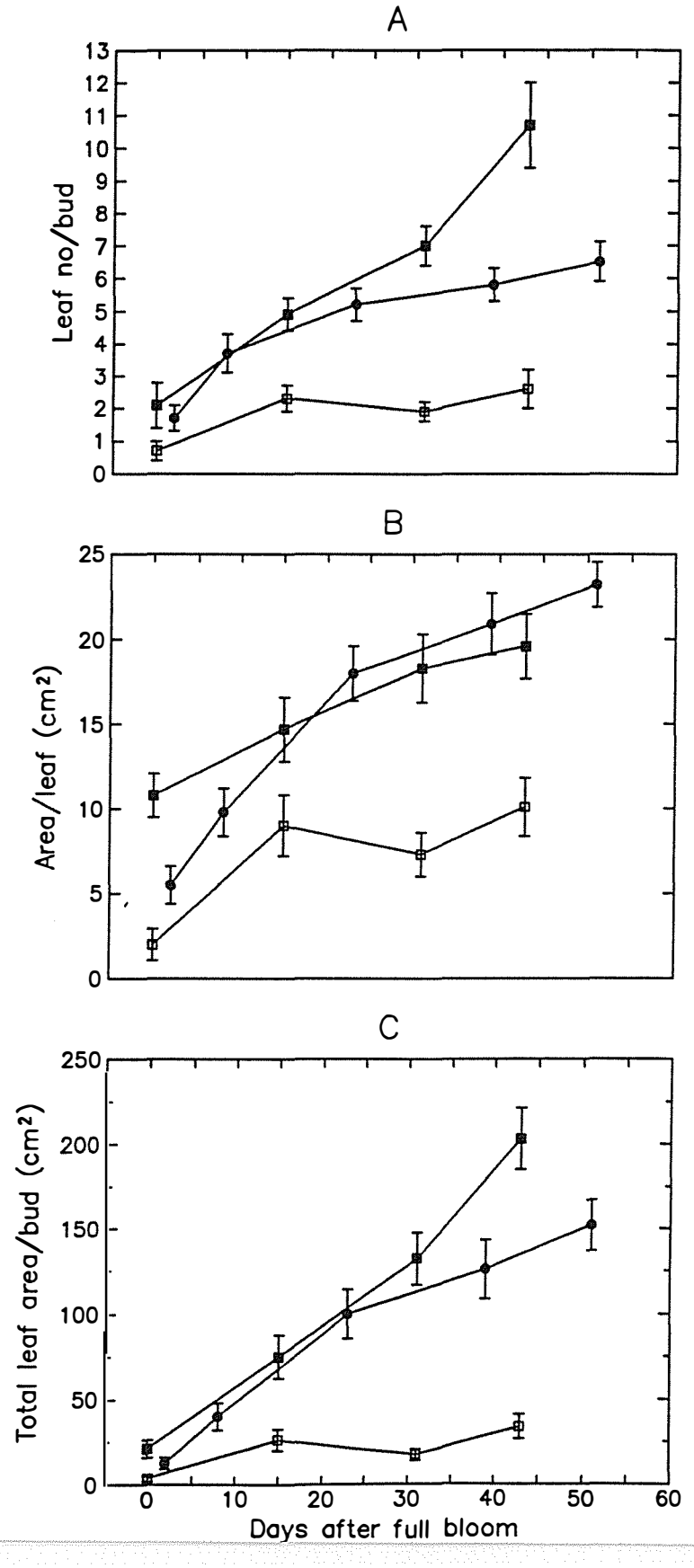


Figure 3.3 Changes in bourse leaf characteristics after full bloom, as influenced by bud type for apple cv. 'Granny Smith'. Bars indicate ± SE. [two-year spur (●), one-year lateral (□), one-year terminal (■)].



two-year spur buds were intermediary. Bourse leaf number increased substantially with time from full bloom for two-year spur buds from 1.5 to 6.5 leaves per bud 42 DAFB. For this bud type rate of bourse leaf production tended to decrease with time. In contrast, bourse leaf number on one-year old terminal buds showed a constant increase reaching 10.6 leaves per bud 42 days after bloom. After an initial increase immediately after bloom, bourse leaf number on one-year lateral buds was maintained at a constant level of 2.5 leaves per bud.

Area per bourse leaf also increased rapidly after bloom for two-year and one-year terminal buds, reaching 20cm^2 at about 42 DAFB. After an initial increase immediately after bloom, bourse leaf area on one-year lateral buds did not increase more than 10cm^2.

Total bourse leaf area paralleled the changes in bourse leaf number and leaf size for the three bud types. A substantial increase in bourse leaf area occurred over time for both one-year terminal and two-year spur bud types although the younger bud type tended to have a more rapid increase from about 25 DAFB. Total bourse leaf area for one-year lateral buds was maintained at a very low and constant level during the 42 DAFB.

3.7 Discussion

3.7.1 Budbreak and Flowering

Budbreak and flower bud production were not consistently affected by wood age on the three cultivars tested in 1986 (Table 3.1). Differences in productivity between bud types do not, therefore, seem to be caused by limitations in budbreak or flowering, at least for trees used in this study.

It has been observed that substantial variation in the propensity of lateral and terminal buds on extension shoots to flower can occur on different orchard blocks; this variation is not necessarily linked to the amount of flowering on older spurs, but also to the amount of flowering on younger wood. There can be substantial cultivar variation as to the position where flowers (and fruits) are borne (Lespinasse, 1977). Buban and Faust (1982) noted that flowering on one-year wood tends mainly to occur on younger trees. Clearly significant flowering can also occur on one-year lateral buds on older trees in Hawkes Bay as the orchard blocks used in this study ranged in age from 10 to 27 years.

3.7.2 Flower Bud Quality

In the present study, differences in flower number per bud (Figure 3.1 and Table 3.3) across the three bud types generally paralleled similar differences in total primary leaf area at bloom (Figure 3.2C) for 'Granny Smith'. One-year old terminal buds had the highest flower number and primary leaf area per bud, one-year lateral buds had the lowest, whilst two-year

spur buds were intermediary. Bud position on the branch also influenced flower number and leaf area. A flower number per bud gradient from the basal to middle end of a branch was apparent for both one and two-year wood. Differences in flower number per bud and primary leaf area measured in the present study should be good indicators of the relative floral strengths of the different bud types and their potential to set. Primary leaves are formed from leaf primordia which develop in the previous autumn (Fulford, 1966), as are the flower initials (Bergh, 1985a). Rom and Ferree (1984a) related differences in yield and productivity across eight cultivars to differences in total spur leaf area at pink bud while a reduction in spur leaf area at pink bud quantitatively decreases fruit set (Ferree and Palmer, 1982). Therefore a larger total spur leaf area should reflect an increased initial source of carbohydrate to the flower and fruit.

A low flower number per spur at bloom clearly reduces the potential number of flowers which can set. However it probably also indicates reduced "quality" of the individual flowers which remain. Increases in flower number per cluster resulting from applications of nitrogen to potted apple trees at various times during the previous season were paralleled by similar increases in ovule longevity and final fruit set (Hill-Cottingham and Williams, 1967). An increase in final fruit set was associated with an increase in flower number per spur cluster following an application of benzyladenine onto fruiting and non-fruiting limbs in the previous season (McLauglin and Greene, 1984). Thus, results from the present study indicate that one-year terminal buds are of

the highest flower bud quality, one-year lateral buds are of the lowest flower bud quality and two-year spurs are intermediate.

The question remains, of course, as to whether factors not necessarily linked to flower number per bud or primary leaf area might be causing more critical differences in flower bud quality between bud types. For instance, flowers from older shorter spurs with low fruit setting capacity have been shown to have irregular ovaries more frequently than flowers from younger longer spurs which have a higher level of set (Milutinovic, 1974 in Buban and Faust, 1982). It would have been interesting to determine possible differences in flower ovule longevity between the three bud types and within any one wood age in the present study.

The mechanism by which the origin of a bud influences flower quality of that bud is not known. 'Braeburn' buds having greater flower bud quality in this study flowered earlier than those having poorer quality (Table 3.3). However this was not the case for 'Granny Smith'. Two-year spur buds were more advanced than one-year terminal buds although one-year lateral buds opened after both bud types. Denne (1963) also found that early opening flower buds had a higher flower number and a greater expanded leaf area than later opening flower buds for cv. 'Dougherty'. The effects of bud type, wood age and position (within the tree canopy) were not distinguished. Nevertheless, these results indicate that factors regulating the timing of flower bud opening (budbreak) and flower bud quality may be similar.

Presumably "stronger" flower buds with greater numbers of flowers and leaves at bloom would have been initiated earlier or they had a greater rate of development in the preceding growing season and/or before budbreak. Some work has been carried out comparing the timing of initiation of different bud types. Buban and Faust (1982) concluded that flowers in one-year lateral buds were initiated several weeks after those in two-year spur buds. However in a detailed study, Luckwill and Silva (1979) found that flowers in 'Golden Delicious' buds on two-year spurs were initiated at the same time as one-year lateral buds (mid-summer). Flowers in one-year terminal buds were initiated one month later. Initiation of flower buds only occurs once the bud is fully dormant in summer (Fulford, 1966). Thus the timing of cessation of shoot and spur growth, which can vary between orchards and seasons, might be expected to determine time of initiation of the three bud types.

Abbott (1984) compared flower bud quality of one-year old lateral buds where buds had developed for various lengths of time from initiation. He found that older flower buds produced a higher number of flowers but a lower number and smaller sized primary leaves with a short bourse length at bloom, than did younger buds. Within the developing bud, an axillary meristem (flower) is regulated by the subtending leaf primordium (Fulford, 1966). Bud (foliage) factors causing an increase in leaf primordial development would inhibit the formation of the associated axillary flower. The inverse relationship between number of primary leaves and flower number predicted from these two studies (Fulford, 1966; Abbott, 1984) suggests that differences in flower bud

quality between the three bud types in the present study are not, in fact, solely based upon different timings of flower bud initiation.

Differences in flower bud quality might be explained by differences in the rate of development of the developing buds - a greater rate of development resulting in an earlier opening flower bud and "stronger" individual parts of a bud. The supply of resources or the capacity of a bud to successfully compete for resources may be critical to its rate of development. In this regard, the amount of shoot growth associated with the developing bud may be important. Several workers have linked growth of the spur in the previous season [measured as spur diameter (Denne, 1963), length (Lespinasse, 1977) and weight (Heinicke 1917 in Dennis, 1986) before flowering] to flower bud quality or fruit set characteristics in the following season. Walsh (1977b) measured a basipetal gradient on 'Delicious' branches in terms of fruit set per spur. In the present study, one-year terminal buds which had the highest flower bud quality are borne on shoots 30-100cm in length whereas two-year spurs are on shoots less than 5cm, by definition (ie spurs). Thus for terminally situated flower buds shoot growth made in the previous season may indeed be a good indicator of bud quality. At any one bud site vegetative activity prior to floral transition, may improve vascular connections with the bearing branch so increasing the potential for further nutrients to be supplied to the developing flower bud.

Whilst this hypothesis may hold true for terminally positioned buds, it would not seem to be the case for one-year laterals. One-year lateral buds

which had the lowest flower bud quality are also situated on long extension shoots. Some other factor would seem to be crucial in limiting flower bud quality for this bud type.

Rate of bud development for one year lateral buds may be determined by the extent of correlative inhibition at the node during the growing season (Walsh, 1977b). In the season before flowering development of lateral buds would be inhibited more by extension shoots than buds on two-year spurs as they would be closer to the growing tip. Similarly lateral buds would be influenced by the growing tip for a longer time period than one-year terminal buds. On one-year wood at least basal nodes would be influenced for a longer period by the growing meristem compared with more distal nodes.

In this regard, it is interesting to note results from a recent study comparing spurs on two, three and four year old wood of two, three and four strains of spur-type 'Red Delicious' (Rom and Barritt, 1990). Little difference in flower number per spur could be found between wood ages nor were there any positional gradients in flower number per spur within any one wood age. It is likely that differences in flower number per bud between wood ages and gradients within wood ages may be confined to one and two-year wood and be specific to non-spur cultivars. Trees of high vigour, as used in the present study, might be expected to exhibit greater degrees of shoot apical dominance on replacement branches and therefore show a greater variation in the characteristics of flowering buds than spur-type 'Red Delicious' trees. A

detailed comparison of bud types from a range of cultivars showing "extreme" differences in growth habit would be useful in exploring this aspect further.

3.7.3 Leaf Growth After Bloom

Total primary leaf area from all three bud types on 'Granny Smith' varied only slightly after bloom (Figure 3.2C). Other studies have also shown that primary leaf area reaches a threshold shortly after bloom (Rom and Ferree, 1986a). The slight reduction in primary leaf number and total leaf area on two-year spur buds 21 days after bloom in 1987 may be indicative of a drop of small leaf 'bracts'. The effect of such leaf abscission on fruit set and fruit growth is not known.

Large differences in bourse leaf area on the three bud types were apparent during the fruit drop period (Figure 3.1 cf Figure 3.3C). The trend was similar to differences in primary leaf area measured at bloom. This variation in total bourse leaf area resulted from both differences in bourse leaf number and individual leaf size. Variable numbers of bourse leaves probably reflect differences in bourse shoot length as bourse leaf primordia are produced by the bourse growing tip. Indeed two bourse shoots were harvested from some one-year terminal buds, from 27 days after bloom.

This variation in bourse shoot growth on the replacement branch may result from the interaction of nearby growing shoots through apical dominance. For instance very active bourse shoots from one-year terminal buds may inhibit growth of bourse shoots on nearby lateral buds and to a lesser extent, the

bourse shoots on two-year spurs. On the other hand, leaf number at the bud (spur) in the previous season may also be important in regulating bourse shoot activity. Partial defoliation on individual spurs in midsummer resulted in a significant reduction in bourse leaf area in the following season (Rom and Barritt, 1990). One-year old lateral buds are subtended by a single leaf in the previous season whereas two-year spur buds are subtended by several. This pattern is consistent with their relative differences in bourse leaf area. Only one leaf is borne from the tip bud of an extension shoot (which later develops into a one-year terminal bud). Internode lengths are often very short at the tip end of an extension shoot with leaves being close together. This would allow considerable resources within the terminal bud to be harnessed for vigorous bourse shoot development in the following growing season.

Size of bourse leaves on one-year lateral buds was substantially smaller than those from the other two bud types in 1986 and 1987, paralleling similar differences in bourse leaf number. In contrast, bourse leaf production and individual leaf growth from one-year terminal and two-spur buds appear to be affected differentially. Bourse leaf size from two-year spur buds was similar to that of one-year terminal buds, however bourse leaf number was lower.

3.7.4 Fruit Abscission

Fruit set was substantially greater for spurs on two-year wood compared with lateral buds on one-year wood (Table 3.1). Further, for 'Granny Smith' in 1987, fruit set was greater for one-year terminal compared with one-year

lateral buds (Figure 3.1). Goldwin (1981) found similar differences in final fruit set between one-year lateral and terminal buds on 'Cox's Orange Pippin' after exogenous hormone sprays at bloom. It may therefore be appropriate to divide fruit set data for apple into that from one-year old lateral buds and that from older spurs when assessing cultural or growth regulator effects on tree productivity (Volz and Knight, 1986).

A closer examination of the timing of fruit drop on 'Granny Smith' (Figure 3.1) showed that only a relatively small amount of flower/fruitlet drop occurred immediately following anthesis from all three bud types (after correction for differences in the time of full bloom for each bud type had been made). Other workers have shown that flower and early fruit abortion is not the major factor limiting fruit set on 'Granny Smith' grown in a warm climate (Pisani et al., 1979). The low flower/fruitlet abortion during this period is indicative of successful pollination and fertilisation of flowers on all three bud types (Williams, 1970) despite differences in the time of flower opening. Indeed the 'Granny Smith' block used in this study was surrounded by 'Gala' and 'Red Delicious' pollinizers, whose flowering times overlapped with those of 'Granny Smith'. Also, warm conditions over the flowering period ensured vigorous bee activity. It is unlikely that inadequate pollination and fertilisation was a factor accounting for the differences in fruit set between the three bud types.

The greatest difference in fruit drop between the bud types which occurred 20-40 DAFB coincided with the period when the greatest overall level

of fruit drop was occurring. This late phase of fruit drop occurred as the growing fruit approached maximum increases in fresh and dry weight (Chapter 4). This result suggests that there may be a differential supply of carbohydrate to growing fruits on the three bud types (Stephenson, 1981; Dennis, 1986). This could be caused by resource limitations and/or differential competitive capacities of the fruit for these nutrients.

In the cultivar 'Granny Smith', differences between the three bud types in final fruit set did not always parallel differences in flower bud quality. One-year lateral buds had the lowest final fruit set, flower number and total primary leaf area. In contrast, one-year terminal buds had the highest number of flowers and the greatest leaf area, but had intermediate fruit set.

Several workers have shown low bourse leaf areas at bloom can reduce final fruit set (Quinlan and Preston, 1971; Ferree and Palmer, 1982). However in the present study, final fruit set did not always reflect the large differences in total leaf area measured on the three bud types during the post-blossom period. For 'Granny Smith', one-year lateral buds had the lowest set and leaf area, but one-year terminal buds had the greatest leaf area, but an intermediary set. It is possible that rapidly growing shoot tips on one-year terminal shoots competed with fruit for resources (Quinlan and Preston, 1971). However, these workers found that drop induced by such competition occurred during the first 20 days after bloom, not during the later drop period as occurred on the one-year terminal buds in the present study. Also, it is probable that rate of bourse shoot growth was similar for the two-year spur buds and the one-year

terminal buds during the first 20 days after bloom, based upon their similar rates of leaf number production.

For a number of horticultural crops the first pollinated flowers are more likely to set than later pollinated flowers (Stephenson, 1981). Clearly for 'Granny Smith' the order of pollination, indicated by the stage of flower bud opening at one date during bloom, does parallel final fruit set (two-year spur > one-year terminal > one-year lateral). Fruit from two-year old spur buds may be able to compete more successfully for nutrients than fruits from the other two bud types because of their developmental advantage. Denne (1963) found that early flowering clusters set better than late flowering clusters, although early clusters also had a greater flower bud quality and leaf area at bloom. For crops, such as tomato, where fruit abscission does not usually take place, early flowering fruit compete more successfully for assimilate than late flowering fruit (Ho, 1988).

On the other hand other workers have suggested that "dominant" fruit sinks may directly influence other fruit sinks *via* direct plant hormone inhibition, rather than competition for nutrients *per se* (Bangerth, 1989). For instance in apple, lateral fruit have a greater tendency to abscise than king fruit on the same cluster. Lateral fruit set is greater when the king fruit is removed at bloom. Gruber and Bangerth (1991) suggested that a greater production and transport of indole ascetic acid (IAA) from dominant sinks may suppress subsequent IAA transport from weaker sinks. Fruit from two-year spur fruit might dominate fruit from those on one-year lateral buds by such a mechanism.

This mechanism of within-tree regulation of fruit abscission and growth is further discussed in Chapters 4 and 8.

In summary, fruit from one-year lateral buds on replacement branches dropped more heavily than fruit from buds on two-year spurs. Fruit drop from one-year terminal buds was intermediate. Bud type effects on bud break and flowering were variable. Large differences in flower bud quality and leaf growth between the three bud types were also apparent. However these differences did not always relate to differences in fruit set between the three bud types. It seems most likely that differences in fruit set between bud types were brought about by their different blossom dates. Early developing fruit on two-year spur buds would be able to compete more successfully for nutrients than later developing fruit on one-year lateral buds and therefore be less "prone" to drop. Whatever the mechanism by which two-year spur fruit exert their effects on one-year lateral fruit (direct competition or hormonal), this study indicates that factors which influence the development of the flower bud and affect the timing of anthesis, have a major bearing on fruit set and the final distribution of fruits on the tree.

CHAPTER FOUR

FRUIT GROWTH AND DEVELOPMENT

4.1 Introduction

Fruit size at harvest is a major determinant of apple fruit quality and has a significant effect on orchard profitability. Generally, small fruit below 110g are not exported, while those fruit between 160-250g gain a premium over other fruit sizes. In order to improve tree management methods, it is essential to understand what factors affect fruit sizing.

Considerable variation in final fruit size is known to occur within the apple tree canopy. Fruit close to the tree trunk are often smaller than those on the outside of the tree canopy (Jackson et al., 1971). This probably relates to light penetration into the canopy. A reduction of light within the tree canopy has been correlated with a reduction in fruit size Heinicke 1966; Morgan et al., 1984; Barritt et al., 1987; Tustin et al., 1988). Artificial shading of whole trees has also reduced final fruit size (Jackson et al., 1977). However fruit close to the trunk are also nearer to the roots and further away from shoot tips compared with fruit on the outer canopy. It has been suggested that concentration gradients of endogenous factors away from the sites of synthesis in roots or shoot tips may cause gradients in plant development (Chalmers, 1985; Dann and Jerie, 1988).

Variation in fruit size can also occur on the outside of the tree canopy although relatively few studies on apple have quantified this. Rom and Barritt (1990) found fruit size of 'Delicious' to be larger on two and three-year old compared with four-year old wood. However, Denne (1963) found no such relationship between fruit size and wood age. Fruit size was also shown to be significantly smaller on one-year shoots (>20cm length) than for those borne on brindales or spurs for six out of nine Northern European cultivars trained as slender pyramids (Calleson, 1988). This result agreed with earlier data collected from 'Golden Delicious' (Lespinasse, 1977).

However, the linkage between positional factors influencing fruit sizing on the tree and physiological factors known to regulate fruit growth have not been explored, other than those connected with shade. To understand those factors which regulate growth of apple fruit, the origin and time sequence of fruit development must be detailed. Fruit development begins with the evocation and initiation of the flower bud in early summer followed by differentiation of individual flowers within the bud in late summer (Bergh, 1985a). Following winter dormancy floral components within individual flowers expand rapidly before bloom (Bergh, 1985a).

After pollination and fertilisation, the receptacle and ovary wall of the flower enlarges to form the apple pome or fruit (MacDaniels, 1940; MacArthur and Wetmore, 1941). The fresh weight curve of apple fruit is described as sigmoidal (Bain and Robertson, 1951; Denne, 1963). An initial exponential growth phase occurs up to 3-5 weeks after bloom (Bain and Robertson, 1951;

Denne, 1963) and coincides with the period of cell division. Cell enlargement begins soon after fertilisation and continues until harvest although at a progressively diminishing rate (Denne, 1963). However a recent study on apple fruit tagged on the tree and measured periodically throughout the season suggests that the growth curve may be double sigmoidal. A one week reduction in growth rate 6-7 weeks after bloom was recorded for two cultivars over five years (Magein, 1989).

Fruit which abscise slow in growth rate two weeks before abscission (Goldwin, 1989; Magein, 1989). Many physiological factors know to regulate abscission are also thought to be important in controlling growth of the remaining fruit on the tree (Chapter 3). Some of these factors will now be explored.

Although water is the main constituent of apple fruit (80-90%), it is dry matter accumulation in the fruit during the growing season which determines final fruit size. Factors controlling inflow of dry matter (mainly carbon) into the fruit are of vital importance when discussing causes of fruit size variation within the tree.

Indeed, an understanding of such regulation has come through the use of terms such as "sources" (net carbon exporters eg. leaves) and "sinks" (net carbon importers eg. fruit) (Warren-Wilson, 1972). Extending this concept further, "sink strength" was defined as the rate of dry matter increase by the sink and a product of sink size and sink activity. "Sink activity" measured the metabolic activity of the sink and its ability to import assimilate.

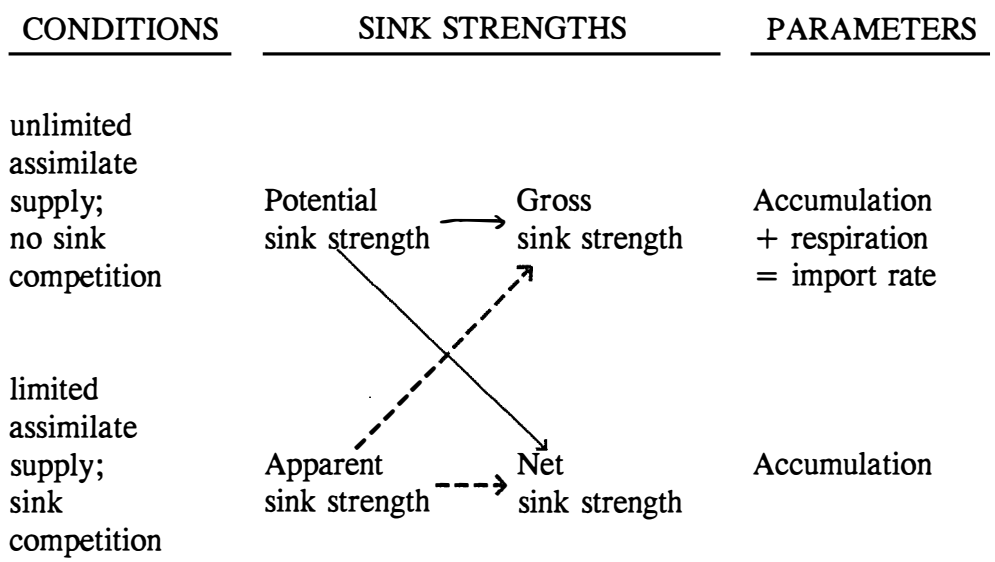
That is,

Sink strength = sink size x sink activity (4.1)

(g/day) = (g) x (g/g/day)

More recently the concept of "competitive" sink ability or the capacity of sinks to compete with other sinks for assimilate has been introduced (Wareing and Patrick, 1975). Estimates of apparent sink strength based upon an organ's growth rate will equal its potential sink strength only when assimilate is supplied under non-limiting conditions (Ho, 1988). Figure 4.1 shows the relationships between different types of sink strength and their estimation.

Figure 4.1 Definitions of sink strength according to various conditions (from Ho et al., 1989).



4.1.1 Regulation of Apple Fruit Sink Size

Cell number is an important determinant of sink size and final fruit size. Indeed, Ho (1988) suggested that cell number provides a physical constraint on importation of assimilate into fruit. In apple, it has been reported that large fruit have higher cell numbers but a similar cell size than smaller fruit on the same tree (Bain and Robertson, 1951; Denne, 1963).

Most of the cells in apple fruit are derived from cell division occurring after fertilisation (Bollard, 1970). However cell number achieved before anthesis may also be important. In tomato, differences in size of fruit borne on the same plant are partially explained by different numbers of cells in the ovary (Ho, 1988). The ratio of cell numbers between fruit of two tomato mutants showing substantially different fruit sizes at harvest was greater at anthesis (2.6) than at final harvest (1.7) (Bohner and Bangerth, 1988). Growth of the apple flower, ovary and embryo sacs before bloom may be limited by nitrogen supply (Williams, 1965; Hill-Cottingham and Williams, 1967). Hormonal factors may also be important in regulating cell division before anthesis (Bunger-Kibler and Bangerth, 1983; Heddon and Hoad, 1985). It may be that significant differences in cell number exist for apple flower receptacles positioned in different parts of the tree.

Cell number in apple fruit increases significantly after anthesis and occurs at the same time as growth and development of seed/s within the enlarging fruit. Seed number has been positively correlated with fruit cell number at

commercial harvest (Denne, 1963) and final fruit weight for apple (Williams, 1977) and many other fruits (Bollard, 1970).

Successful pollination results in pollen tube growth down the style whereupon union with an ovule releases two male gametes from the pollen tube and fertilisation occurs. The first gamete fuses with the egg cell becoming the embryo while the other fuses with the polar nuclei creating the endosperm (Abbott, 1984). This results in immediate and rapid cell division of the endosperm followed by that of the embryo and of maternal tissues surrounding the seed (Martin et al., 1980; Abbott, 1984; Heddon and Hoad, 1985).

Regulation of cell division of the seed and the fruit after fertilisation and during growth seems to occur through the production of growth hormones produced by the developing seed. High levels of auxins, gibberellins and cytokinins are produced by young apple seeds during the cell division phase of fruitlet growth (Luckwill, 1970). Hormones may direct cell division as has been shown to occur *in vitro* (Nitsch, 1970) and/or influence rate of fruit growth by affecting the import rate of assimilate into fruit (Patrick, 1990).

Experimental evidence for this is provided from the action of exogenous applications of growth hormones to apple flowers. A gibberellins/auxin/ cytokinin mixtures at bloom has induced parthenocarpic fruit growth in apple (Goldwin, 1981, 1989). Gibberellin sprays at or immediately after bloom can stimulate growth and development of many seedless and seeded fruit (Looney and Pharis, 1986).

On the other hand, very few studies have successfully correlated levels of endogenous hormones with fruit growth rate for apple or other crops (Martin et al., 1982; Browning, 1989). It has recently been suggested that primary hormonal control of tissue development is exerted *via* tissue sensitivity to hormones (Firn, 1986), rather than hormone concentration *per se*. On the other hand, hormone production within the separate tissues of endosperm, embryo sac and surrounding tissues are likely to be compartmentalised in space as well as time (Browning, 1989). Simple assessment of gross hormone levels diffusing from or within fruitlets/seeds may be misleading and gloss over the real complexities of hormone signals in apple fruit tissue. Nevertheless, variation in the potential of fruit to produce mature seeds may be important in influencing fruit size variation within the tree canopy.

4.1.2 Sink Regulation of Assimilate Import

The degree of sink activity realises the growth potential of the developing fruit. In apple the determinants of sink activity have been little explored. Current theories of phloem transport indicate that a sugar gradient from leaf to fruit may be crucial in determining the rate of assimilate import into the fruit (Salisbury and Ross, 1985). Thus maintenance of high sugar concentrations at the leaf end and/or low concentrations at the fruit end of the pathway in the plant seem important for determining rates of assimilate transport.

Thus sugar import may be regulated at one of several points at the sink end of the pathway. In many storage tissues the transported sugar (often sucrose)

moves from the phloem sieve elements to adjacent sink storage cells *via* the sink inter-cellular free space (apoplast) (Ho, 1988). Apoplastic unloading of the sugar may occur by simple sucrose diffusion and/or by active sucrose transport across the phloem sieve element plasmalemma. Active transport across the membrane may occur *via* a protein/sugar cotransport or antiport system (Ho and Baker, 1982). Specific protein translocation complexes may also be important (Ho, 1988).

Enzymatic hydrolysis of transported sugar in the free space may be crucial in regulating the concentration of sugar in the free space and thus rate of sugar imported *via* diffusion. Extracellular enzymes such as sucrose synthetase and acid invertase have been implicated as regulators of sucrose unloading and import for several fruits (Thomas, 1985).

In apple sorbitol is the major photosynthetically derived carbohydrate which is translocated throughout the tree (Bieleski, 1969) before being converted to fructose, glucose and sucrose in the developing fruit (Hulme, 1958; Chan et al., 1972). Beruter (1985) suggested that acid invertase is important in regulating sucrose concentration in apple fruit, and thus early fruit growth.

Sugars may be preferentially taken up from the free space by adjacent fruit sink cells before being stored in the vacuole. This may occur either in response to a concentration gradient or by active transport across cell membranes. In sugar cane stalks selective diffusion of sucrose occurs across the sink cell plasmalemma and tonoplast membranes according to concentration

gradients in the various compartments of the cell (Ho, 1988). In apple, sorbitol dehydrogenase (SDH) is present in the cytosol of the fruit cortical cell (Yamaki, 1980). This enzyme may regulate fruit growth by controlling sorbitol transport and storage (as fructose) into the cytosol (Beruter, 1985).

Polymerisation of sugars in fruit cells may also be important in creating a demand for assimilate. In tomato fruit, rate of starch accumulation regulates rate of sugar importation and growth rate (Ho, 1988). Starch accumulation in apple occurs in mid-summer while its hydrolysis occurs during fruit maturation. Its rate of increase may be important in determining fruit sizing later in the season. Regulation of assimilate import may occur through hormonal control of enzymes which affect sugar concentration gradients (eg. IAA/GA_3 and acid invertase) (Thomas, 1985). Hormones might also be important in regulating active transport systems across cell membranes (eg. ABA and sucrose translocation through sugar beet cell membranes). An increase in ABA content in apple fruit flesh has been associated with an increase in rate of sorbitol uptake by tissue during development. Beruter and Kalberer (1983) suggested this hormone may be important in affecting assimilate import into the fruit. However, hormones may also influence long distance phloem transport as well as other enzymatic processes (eg. polymerisation of starch) in the fruit (Thomas, 1985). Clearly much work has yet to be done to clarify our understanding of assimilate import into apple fruits.

4.1.3 Regulation by Assimilate and Nutrient Supply

Assimilate for fruit (sink) growth is supplied by an assimilate source. For apple, leaves are the most important assimilate source. Two leaf types subtend the flowering and fruiting apple bud. Primary spur leaves are initiated in autumn. They grow out from the bud and develop between bud burst and full bloom. Bourse leaves are produced on an extension shoot which bursts from the flowering bud between bloom and petal fall. This shoot may stop growth within several weeks of bloom thus producing a spur. Growth may also continue throughout the growing season, so that a brindle or long extension shoot is produced (Pratt, 1990).

Both leaf types associated with developing apple fruit supply the bulk of photosynthate to these fruits during the initial growth phase. Reserve carbohydrate and that derived from non-adjacent leaves are thought to play little part in fruit growth during this period (Hansen, 1971).

The extent to which primary and bourse leaves contribute differentially to fruit growth is not known. However recent work using 14-C labelling techniques indicates that primary spur leaves are probably most important in supplying assimilates to fruits from flowering to several weeks after petal fall. Bourse leaves seem to contribute significant assimilate well after petal fall (Tustin and Lai, 1990). Complete removal of the bourse shoot can increase initial fruit growth (Abbott, 1960).

Source strength is defined as the product of leaf net assimilation rate and leaf area, and is thought to limit growth of meristematic sinks (Patrick, 1988).

Positional factors which affect total spur leaf area and photosynthetic efficiencies may be important in determining carbohydrate supply and growth rates of apple fruit, particularly during the early growth phase.

Starch build-up and/or sucrose formation in leaves regulate photosynthetic rates in source leaves of many plants (Daie, 1985). Loading of sugars from mesophyll cells into the phloem may also be another point for regulation of assimilate rate. Enzymatic control of these processes would seem to be important in determining sugars available for export (Patrick, 1988). Starch/sorbitol metabolism in apple leaves seems crucial in determining sink and source strength in developing apple leaves (Loescher et al., 1982).

It is probable that fruit load also influences the photosynthetic efficiency of apple leaves. Several studies have also shown that the presence of apple fruits can increase the photosynthetic rate of nearby spur leaves (Kennedy and Fujii, 1986). Net photosynthetic efficiency was also shown to be higher on leaves from fruiting trees compared to non-fruiting trees (Avery, 1975). Stomatal resistance has been shown to be lower for fruiting than for non-fruiting trees (Palmer, 1986). Recently Kennedy and Fujii (1986) reported that carboxylation efficiency was increased while mesophyll resistance was reduced on flowering or fruiting spur leaves compared with vegetative spur leaves.

Flowers/fruit might regulate photosynthetic activity by increasing the rate of substrate movement out of leaves. A reduction in apple fruit load has also been shown to result in higher concentrations of leaf sugars and starch (Avery

et al., 1979). Alternatively flowers/fruit may provide hormones which are transported to the leaf where they could stimulate CO_2 assimilation/assimilate loading into the phloem (Treharne, 1986). For instance, ABA has been shown to inhibit sucrose loading in the phloem while IAA can promote the process (Thomas, 1985).

Movement of assimilate can occur over large distances within the apple tree. Areas of the tree having low leaf:fruit ratios are able to attract assimilates from non-fruiting spurs and shoots (Hansen and Christenson, 1974). Nevertheless, inter-fruit competition for resources can reduce fruit growth. Practical evidence for this phenomenon is the size response of apples to fruit thinning. This result may simply reflect a division of resources among less fruit. However in several thinning experiments the effect of high crop loads has been to shown to reduce growth of smaller fruit much more than that of larger fruit (Hansen, 1969; Webb et al., 1980; Volz, 1988). This indicates that large fruit have a competitive advantage over smaller fruit when assimilate is limiting (Ho, 1988). Such interactive sink effects have been demonstrated for apple (Gruber and Bangerth, 1990), wheat (Cook and Evans, 1978; 1983) and kiwifruit (Lai et al., 1990). The mechanism of such competition is not known, however recently it has been suggested that stronger sinks may produce a correlative signal (eg. indole-acetic acid) which inhibits growth of weaker sinks (Bangerth, 1989).

Mineral supply to the fruit can also affect fruit growth. Letham (1961) found that low phosphorous levels in the tree could reduce cell division and fruit size.

4.1.4 Growth Analysis

An understanding of an organ's growth and its apparent net sink strength can be determined by measuring its absolute growth rate over time. At any instant the absolute growth rate can be written as dw/dt where w is the total weight of the plant or organ at time t (Hunt, 1978). However absolute growth rate (sink strength) is often a function of initial (sink) size (see equation 4.1). That is,

absolute growth rate = dry weight x relative growth rate

(g/day) (g) (g/g/day)

Growth rate may not necessarily give much information on the physiological performance (sink activity) of the organ. A better rate parameter is relative growth (R), defined at any instant as

$$R = \frac{1}{w} \cdot \frac{dw}{dt} \qquad (4.2)$$

that is, absolute growth rate divided by the existing weight. Thus relative growth rate can be used as a measure of sink activity and to compare the

efficiencies of different sized organs as accumulators of dry matter. The following experimental work uses absolute and relative growth rate curves to compare growth of fruits on different bud types.

4.2 Experimental Objectives

The first objective of the following experiments was to quantify the influence of bud type, borne on the replacement branch, on final fruit size.

The second objective was to ascertain possible causes for any differences found. Final fruit size will depend on initial size and relative growth rate during its development. The second objective was therefore pursued by measuring initial fruit (receptacle) size at anthesis and calculating relative growth rate curves for fruit from each bud type.

When measuring fruit growth it is important that the sampling system represents true fruit growth. Several studies have shown that growth rates can be calculated either from regular destructive samples of fruit or from on-tree measurements of the same fruit (Griggs and Iwakiri, 1956; Lai, 1987). However it must be noted that heavier fruitlet abscission in one population may decrease the proportion of smaller fruit in that population (Stephenson, 1981). Thus fruit growth measurements based upon harvested fruit samples might reflect a decreased proportion of small fruit within the population as well as an increase in weight of individual fruit remaining on the tree. Fruits on one-year lateral buds drop more heavily than those from two-year spur buds (Chapter 3).

Rate of growth of fruit borne on one-year lateral and two-year spur buds was therefore determined by calculating weights from non-destructive measurements of tagged fruits and including only those which did not abscise in the growth curve.

4.3 Influence of Bud Type on Final Fruit Size and Seed Number

4.3.1 Materials and Methods

In 1986/87, two replacement branches on each of fifteen 'Golden Delicious' and 'Granny Smith' trees and one replacement branch on each of fifteen 'Braeburn' trees were chosen at random in mid-November. One fruiting bud from each of the three bud types (two-year spur, one-year terminal and one-year lateral) was chosen at random from each sample branch at the beginning of commercial harvest. All selected fruit were harvested and fruit number, total fruit weight, and seed number per fruit were measured for each bud type. Mean individual fruit weight was subsequently calculated from this data.

This experiment was analysed as a randomised block design with each bud type being the treatment and each branch being a block.

In 1987/88, three replacement branches were selected at random at the beginning of flowering on each of ten 'Braeburn', 'Royal Gala' and 'Granny Smith' trees. All inflorescences on these trees were hand-thinned to one (king) fruit per bud in December ('Royal Gala' = 1/12; 'Braeburn' = 2/12; 'Granny

Smith' = 3/12). The largest lateral fruit on two-year spur and one-year lateral buds borne on the replacement branches was selected to remain if the king fruit had previously abscised. All king and lateral fruit were harvested at the middle of the commercial harvest ('Royal Gala' = 4/3; 'Braeburn' = 28/3; 'Granny Smith' = 22/4). Fresh weight and seed number per fruit were determined after harvest.

This experiment was analysed as a split plot design, each bud type being the main plot, fruit position (lateral or king) being the sub-plot and each tree being the block.

4.3.2 Results

Fruit from two-year spurs were consistently larger than fruit from both other types in 1987 (Table 4.1) and 1988 (Table 4.2). In contrast one-year lateral buds had the lowest average weight. In 1987 when the buds were left unthinned, one-year lateral buds also had the lowest fruit number per fruiting site and total fruit weight (productivity) per bud compared to both other buds types. The difference in average weight between one-year lateral and two-year buds ranged from 36% ('Golden Delicious', 1987), to only 12% ('Granny Smith', 1987). Average weight of fruit from one-year terminal buds in 1987 was similar to those of fruit from two-year buds for 'Granny Smith' but intermediate between two-year spur buds and one-year lateral buds for 'Golden Delicious'. Fruit number per fruiting site and total bud productivity were

Table 4.1 Bud productivity and seed number per fruit for two apple bud types (1987).

Cultivar	Bud type	Fruit no. /fruiting site	Ave fruit weight (g)	Total fruit weight (g)	Seed no.
Braeburn	2yr spur	1.37	151.3	206.6	6.7
	1yr lateral	1.11	117.6	134.3	5.7
	LSD (P=0.05)	0.30	8.3	37.6	1.2
Golden Delicious	2yr spur	1.91	119.1	227.3	6.7
	1yr lateral	1.20	75.9	91.2	4.6
	1yr terminal	2.68	100.0	268.7	6.6
	LSD (P=0.05)	0.65	14.0	54.8	1.2
Granny Smith	2yr spur	1.50	149.3	223.4	8.0
	1yr lateral	1.00	131.6	131.6	8.1
	1yr terminal	1.68	139.2	234.3	7.8
	LSD (P=0.05)	0.42	17.3	61.2	1.2

Table 4.2 Average fruit weight (g) for different bud types and fruit positions on a bud for apple cv. 'Braeburn' (A) and 'Royal Gala' (B) 1988.

A.

Bud type	Fruit position King	Lateral	Mean
2 yr spur	167.9	179.4	173.7
1 yr lateral	152.3	155.5	153.9
Mean	160.1	167.4	

LSD (P=0.05) for comparisons between bud types = 8.2
LSD (P=0.05) for comparisons between fruit positions = 10.2
LSD (P=0.05) for interactions between bud type/fruit positions = 13.5

B.

Bud type	Fruit position King	Lateral	Mean
2 yr spur	138.9	129.3	133.7
1 yr lateral	109.2	110.6	109.9
Mean	123.2	119.9	

LSD (P=0.05) for comparisons between bud types = 9.7
LSD (P=0.05) for comparisons between fruit positions = 9.1
LSD (P=0.05) for interactions between bud types/fruit positions = 12.1

significantly higher for one-year terminal buds than for two-year spur buds on 'Golden Delicious' but similar to two-year spur buds of 'Granny Smith'.

In 1988, king fruits tended to be larger than lateral fruits on two-year spur buds of 'Royal Gala'. This trend was reversed for 'Braeburn' although the differences were not significant.

There was no influence of bud type on seed number per fruit in either year except on 'Golden Delicious' in 1987 (Tables 4.1 and 4.3). For this cultivar one-year lateral buds had fruit with a significantly lower seed number than fruit from both two-year spur and one-year terminal buds.

4.4 Influence of Bud Type on Flower Receptacle Size

4.4.1 Materials and Methods

In 1987, ten heavy flowering trees of 'Royal Gala', 'Braeburn' and 'Granny Smith' were chosen for this experiment. Three "king" flowers at full bloom from two-year spur buds and three "king" flowers from one-year lateral buds were harvested randomly from each replacement branch on each tree. Flowering on two-year spur buds occurred approximately one week before that on one-year lateral buds (Chapter 3). Therefore flowers from one-year lateral buds were harvested one week after that from two-year spur buds. All flower parts and the pedicel were subsequently removed from the receptacle. The fresh weights of the flower receptacles were measured in the laboratory after harvest.

Table 4.3 Seed number per fruit for different bud types and fruit positions on a bud for apple cv 'Braeburn' (A) and 'Royal Gala' (B) (1988).

A.

Bud type	Fruit position King	Lateral	Mean
2 yr spur	5.9	5.7	5.8
1 yr lateral	6.4	6.3	6.3
Mean	6.1	6.0	

LSD (P=0.05) for comparisons between bud type means = 1.0
LSD (P=0.05) for comparisons between fruit positions = 0.8
LSD (P=0.05) for interactions between bud type/fruit positions = 1.1

B.

Bud type	Fruit position King	Lateral	Mean
2 yr spur	4.7	5.2	5.0
1 yr lateral	5.2	5.5	5.4
Mean	5.0	5.4	

LSD (P=0.05) for comparisons between bud types = 1.3
LSD (P=0.05) for comparisons between fruit positions = 0.7
LSD (P=0.05) for interactions between bud type/fruit positions = 1.0

The experiment was analysed as a complete randomised design, each spur type being a treatment, before analyses of variance.

4.4.2 Results

The fresh weight of king flower receptacles harvested at king full bloom was significantly greater from two-year spurs than from one-year lateral buds for all three cultivars (Table 4.4). Differences between the bud types range from 100% for 'Royal Gala' to 35% for 'Braeburn'.

4.5 Influence of Bud Type on Fruit Growth

4.5.1 Materials and Methods

Growth of Fruit In Situ

In 1987, all king flowers borne on two-year spurs and one-year lateral buds were tagged once during bloom on the same replacement branches on 'Royal Gala', 'Braeburn' and 'Granny Smith' trees as used in Section 4.4. The stage of flower bud opening for each bud was also noted (Table 4.5).

Table 4.4 Fresh weight of king flower receptacles at king full bloom for different apple bud types.

Cultivar	Bud type	Receptacle fresh weight (mg)
Royal Gala	2 yr spur	44
	1 yr lateral	22
	LSD (P=0.05)	7
Braeburn	2 yr spur	31
	1 yr lateral	23
	LSD (P=0.05)	2
Granny Smith	2 yr spur	36
	1 yr lateral	20
	LSD (P=0.05)	9

Table 4.5 Definitions of flower bud opening stages for apple.

Stage	Description
Tight Cluster (TC)	Inflorescence had burst from shoot. Individual flowers not separated - petals not visible
Pink Bud (PB)	Individual flowers separated - petals visible, but flowers not open
King Full Bloom (KFB)	King flower open on an inflorescence - lateral flowers closed
Full Bloom (FB)	All flowers on an inflorescence open
King Petal Fall (KPF)	All flowers on an inflorescence open - king flower without petals
Petal Fall (PF)	All flowers on an inflorescence open and without petals

The diameters and lengths of the receptacles of these king flowers were measured from full bloom. Subsequently the diameters and lengths of fruits which developed from these king flowers were measured at 1-4 week intervals.

Calculations of Fruit Fresh Weight

Weight of tagged king flower receptacles and fruit were calculated according to the procedure of Zilkah and Klein (1987). Thirty flower and fruit samples were collected from each bud type periodically throughout the growing

season (1-4 week intervals) from full bloom until commercial harvest. The average value of diameter, length and fresh weight for each destructive sample was calculated at each harvest date for each cultivar and bud type. A standard curve was calculated for fruit from each cultivar and bud type which related average fruit weight to average diameter and average length (Mitchell, 1986). Fruit fresh weight was then calculated from the following equation:

$$\text{Fruit fresh weight} = (0.33\ [(\text{diameter}/2) \times \text{length}] \times A) \qquad (4.3)$$

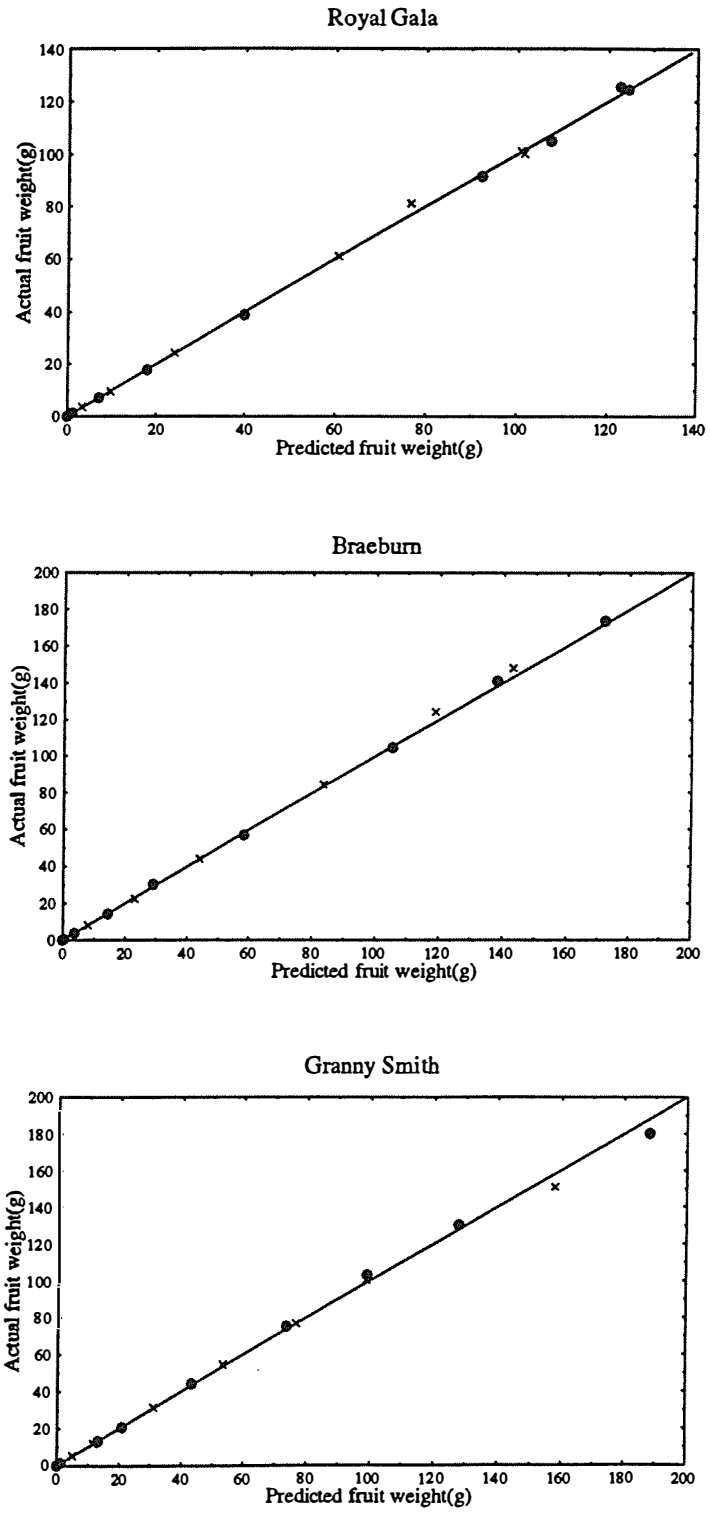
where A is the derived coefficient

The relationship over the growing season between predicted and actual fruit weight at each harvest date is shown in Figure 4.2 for each cultivar. The greatest deviation from the 1:1 line occurred at 14-21 days after full bloom. At this time predicted weights underestimated actual weights by between 4-9% depending upon cultivar and bud type.

Weight of tagged king fruit at each measuring date was calculated using relationships derived from Equation 4.3 and absolute growth curves for fruit from each bud type and cultivar formulated.

At commercial harvest the tagged fruit were picked and seed number per fruit counted.

Figure 4.2 Relationship between predicted and actual fresh fruit weights from samples harvested periodically throughout the growing season for apple. [Solid line is 1:1 line, two-year spur (●), one-year lateral (×)].



Calculation of Average Relative Growth Rates

In the 'classical' approach of growth analysis (Hunt, 1978), relative growth rate is represented mathematically as:

$$R\ (t_2\text{-}t_1) = \frac{\log_e W_2 - \log_e W_1}{t_2\text{-}t_1}$$

where t_1 = Time (1)

t_2 = Time (2)

$R\ (t_2\text{-}t_1)$ = Average relative growth rate between t_2 and t_1 at time $(t_2+t_1)/2$

W_1 = Weight at t_1

W_2 = Weight at t_2

Using this formula, average relative growth rates were determined between each pair of sampling dates for fruit from each bud type and cultivar. Relative growth rate curves were subsequently formulated.

4.5.2 Results

Flower Receptacle Size

The calculated fresh weight of king flower receptacles and their final fruit weight at commercial harvest were higher for those from two-year spur buds compared with those from one-year lateral buds (Table 4.6). This was the case

Table 4.6 Calculated receptacle weight at bloom, calculated fruit weight at the final harvest and seed number per fruit, for two different apple bud types as influenced by flower bud stage (1987). Standard errors in parentheses. (TC = Tight Cluster, PB = Pink Bud, KFB = King Full Bloom, KPF = King Petal Fall, PF = Petal Fall)

Cultivar	Bud type	Flower bud stage	Sample size	Receptacle wt. (mg)	Final fruit wt. (g)	Seed no.
Royal Gala	2 yr spur	KFB	14	32 (2)	128.3 (8.5)	5.0 (0.7)
		KPF	33	42 (2)	151.2 (4.4)	5.4 (0.4)
		Mean	47	40 (2)	144.7 (4.1)	5.3 (0.5)
	1 yr lateral	KFB	9	25 (2)	91.7 (7.8)	5.3 (0.9)
		KPF	22	33 (4)	120.1 (5.2)	5.3 (0.5)
		Mean	31	31 (3)	111.4 (4.7)	5.3 (0.4)
Braeburn	2 yr spur	PB	25	32 (2)	172.5 (5.8)	6.8 (0.4)
		KFB	40	40 (6)	163.7 (5.1)	6.8 (0.5)
		Mean	65	37 (1)	166.2 (3.8)	6.8 (0.4)
	1 yr lateral	TC	9	48 (7)	150.4 (7.3)	5.1 (0.9)
		PB	41	28 (1)	137.8 (4.7)	6.0 (0.4)
		KFB	25	31 (2)	150.3 (5.6)	7.0 (0.4)
		KPF	4	27 (2)	141.3 (5.1)	6.5 (0.3)
		Mean	79	29 (1)	143.3 (3.2)	6.2 (0.3)
Granny Smith	2 yr	PF	12	51 (5)	158.1(13.6)	8.5 (0.2)
	1 yr lateral	PB	2	19 (0)	122.8 (9.7)	10.0 (1.0)
		KFB	8	29 (2)	111.3 (9.8)	6.8 (0.4)
		KPF	3	27 (5)	127.2 (8.3)	7.7 (0.9)
		Mean	13	27 (2)	111.7 (7.0)	7.5 (0.4)

if averaged across all flowering stages for all three cultivars or, for 'Royal Gala' and 'Braeburn', for any one common flowering stage. The differences between the two bud types ranged from 28% ('Braeburn') to 89% ('Granny Smith'). There was no influence of bud type on seed number per fruit.

Causes of variation in final weight for individual fruit were explored in terms of receptacle size and seed number per fruit. Final fruit weight was positively correlated with receptacle weight across both bud types for 'Royal Gala' and 'Granny Smith', but not within a bud type (Table 4.7). For 'Braeburn', final fruit weight of individual one-year lateral fruit was also positively correlated with receptacle weight.

Seed number per fruit was correlated with final fruit weight across and within both bud types for 'Braeburn', from 'Royal Gala' two-year spur buds, but not for 'Granny Smith'.

A mathematical model was created from the individual fruit data across both bud types for each cultivar using stepwise multilinear regression analysis (Table 4.8). The use of higher order terms (parabolic, cubic) did not improve r^2 or F values over those found for linear relationships. Variation in seed number and initial receptacle weight both contributed significantly ($P < 0.05$) to variation in individual fruit weight for 'Royal Gala' and 'Braeburn' (Table 4.9). For 'Granny Smith', fruit size was influenced by receptacle size and not seed number. The predicted relationships between receptacle weight and final weight of fruit 'Royal Gala' and 'Braeburn' (seed number per fruit = 5) is shown in Figure 4.3A and for 'Granny Smith' is shown in Figure 4.3B. The

Table 4.7 Relationships between final fruit weight and seed number per fruit, and between final fruit weight and receptacle weight (expressed as correlation coefficients [r]).

Cultivar	Bud type	n	Seed no.	Receptacle wt.
Royal Gala	2 yr spur	48	0.31*	0.06
	1 yr lateral	33	0.13	0.30
	Total	81	0.22*	0.29**
Braeburn	2 yr spur	65	0.42***	0.17
	1 yr lateral	69	0.44***	0.28*
	Total	134	0.43***	0.15
Granny Smith	2 yr spur	13	0.42	0.34
	1 yr lateral	12	0.11	0.18
	Total	25	0.38	0.55**

*, **, *** Significant at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 \geq P$ respectively.

Table 4.8 Equations developed to describe relationships between final weight, seed number per fruit and receptacle weight for individual fruit from two-year spur and one-year lateral buds.

Cultivar	Equation	r^2	P value
Royal Gala	y=89.2+3.3(x1)+681(x2)	0.14	0.0032
Braeburn	y=94.1+6.4(x1)+558(x2)	0.26	0.0001
Granny Smith	y=79.9+141.4(x2)[1]	0.30	0.0045

y = final fruit weight (g)
x1 = seed number per fruit
x2 = receptacle weight (g)

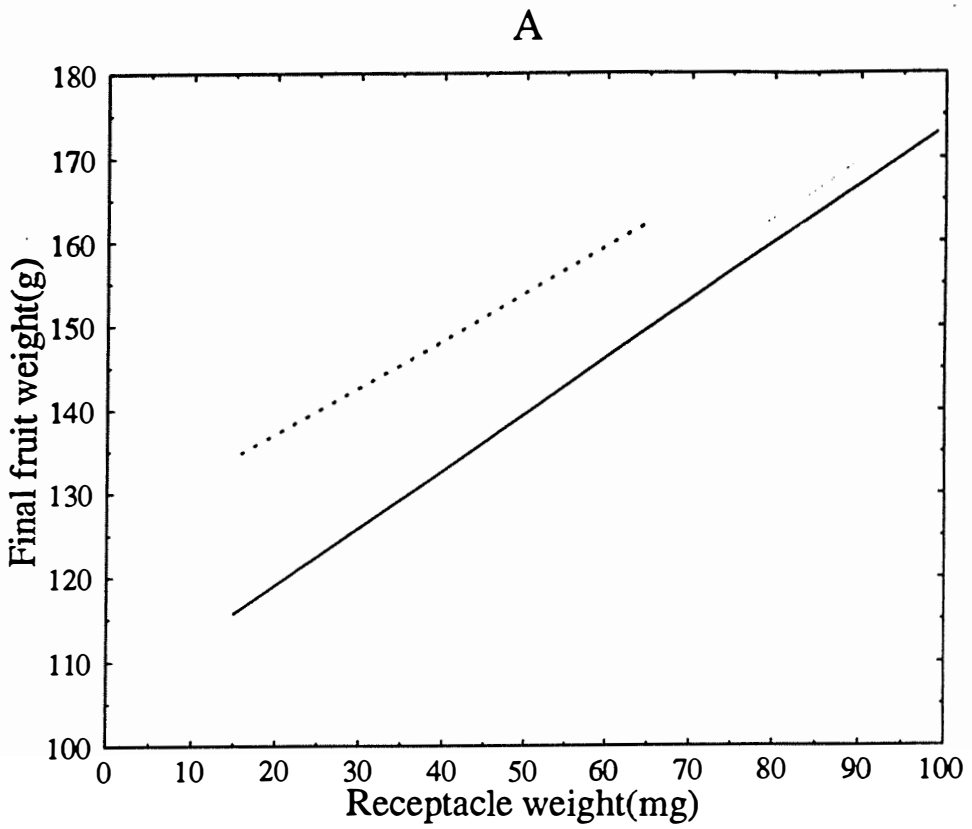
[1] Relationship between final fruit weight and seed number per fruit not significant.

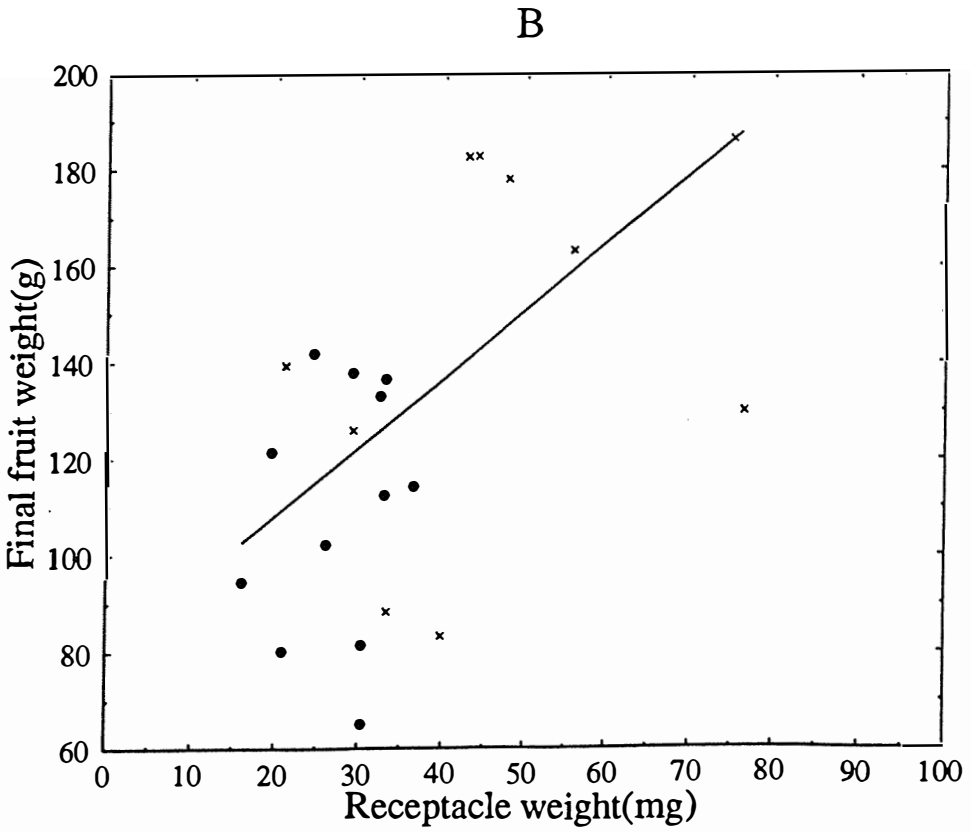
Table 4.9 Summary of stepwise regression procedure relating individual final fruit weight to seed number per fruit and receptacle weight for apple.

Cultivar	Variable	Partial r^2	Model r^2	P value
Royal Gala	Seed number	0.05	0.05	0.03
	Receptacle weight	0.08	0.14	0.006
Braeburn	Seed number	0.24	0.24	0.0001
	Receptacle weight	0.02	0.26	0.04
Granny Smith	Seed number	0.05	-	NS[1]
	Receptacle weight	0.30	0.30	0.02

NS[1] = Not significant.

Figure 4.3 Influence of receptacle fresh weight on final fruit weight for 'Royal Gala' (A- - -) and 'Braeburn' (A——) fruit with 5 seeds, and 'Granny Smith' fruit (B). Linear relationships derived from appropriate equations in Table 4.8. [Individual 'Granny Smith' two-year spur fruit (×), one-year lateral fruit (●)].





range in individual receptacle weights across both bud types was considerable, ranging from 15-99mg. However the predicted increase in final fruit size for such a large increase in receptacle weight was less than double.

Fruit Growth

The cumulative increase in estimated fresh weight of tagged fruit retained on the tree until harvest showed a sigmoidal curve with time for both bud types and three cultivars. This was the case when each growth curve was plotted against date (Figures 4.4A, 4.5A, 4.6A) or time from king full bloom for each bud type (Figures 4.4B, 4.5B, 4.6B). Changes in fruit growth rate are shown in Figures 4.4C, 4.5C and 4.6C. Each growth rate curve can be divided into three general stages:

1) an early phase where there is a rapid increase in growth rate

2) a high more constant mid-season growth rate phase

3) a slow pre-harvest growth rate phase

The early growth phase lasted for 60-70 DAFB (days after full bloom) for 'Royal Gala' and 'Braeburn'. For 'Granny Smith' this phase only lasted up to 40-45 DAFB and was followed by a reduction in growth rate over the following two weeks. For the two red-skinned cultivars, the second mid-season phase of fruit growth was shorter than that of 'Granny Smith', ending 140 DAFB and 160 DAFB respectively. Maximum absolute fruit growth rate was achieved during the constant fruit growth rate phase (1.3-1.6g/day, depending on cultivar).

Figure 4.4 Cumulative growth curves of apple fruit (cv. 'Royal Gala'), expressed as a function of date (A) or days after full bloom (B), and fruit growth rates (C) for two-year spur buds (●) and one-year lateral buds (□). Bars indicate ± SE. For early harvest ± are contained within written symbols.

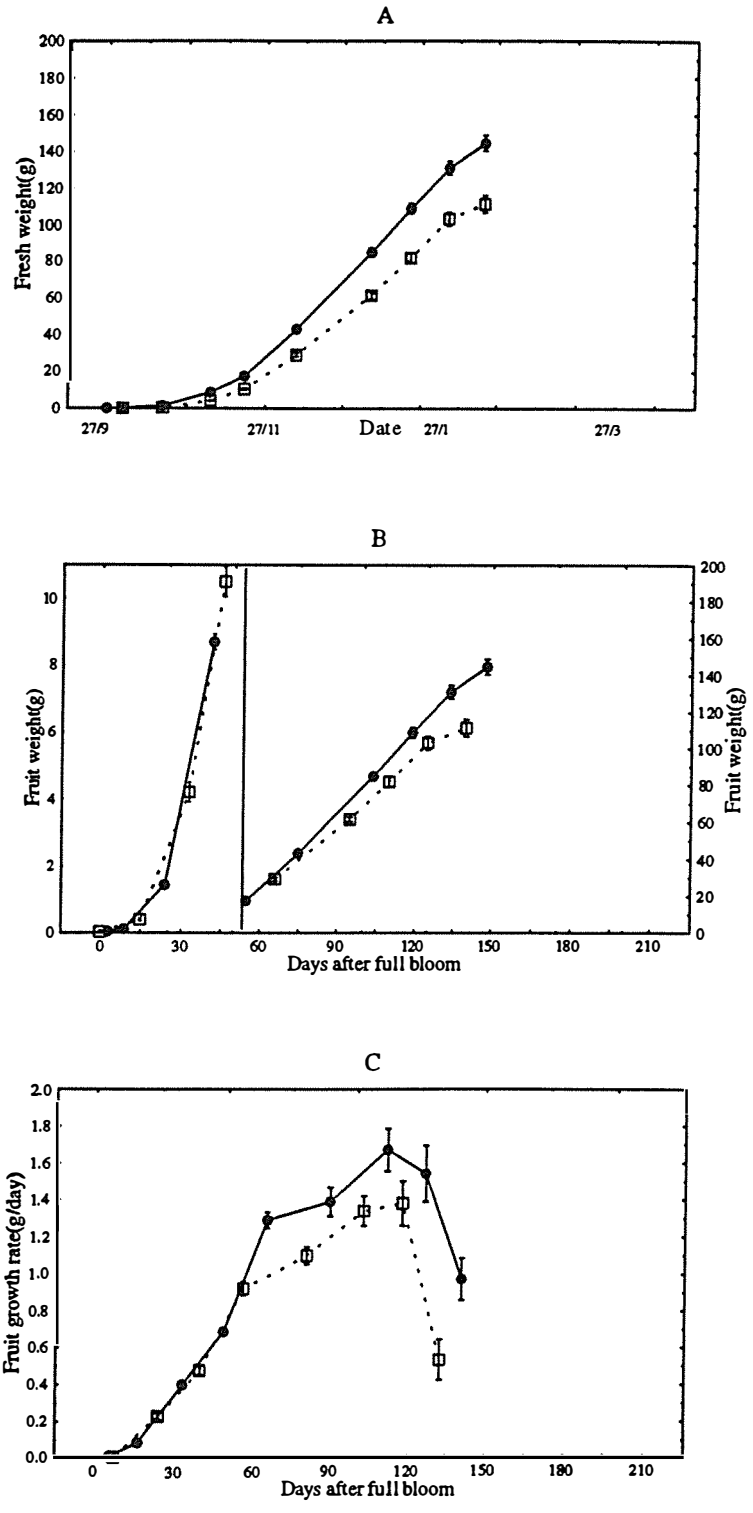


Figure 4.5 Cumulative growth curves of apple fruit (cv. 'Braeburn'), expressed as a function of date (A) or days after full bloom (B), and fruit growth rates (C) for two-year spur buds (●) and one-year lateral buds (□). Bars indicate ± SE. For early harvest ± are contained within written symbols.

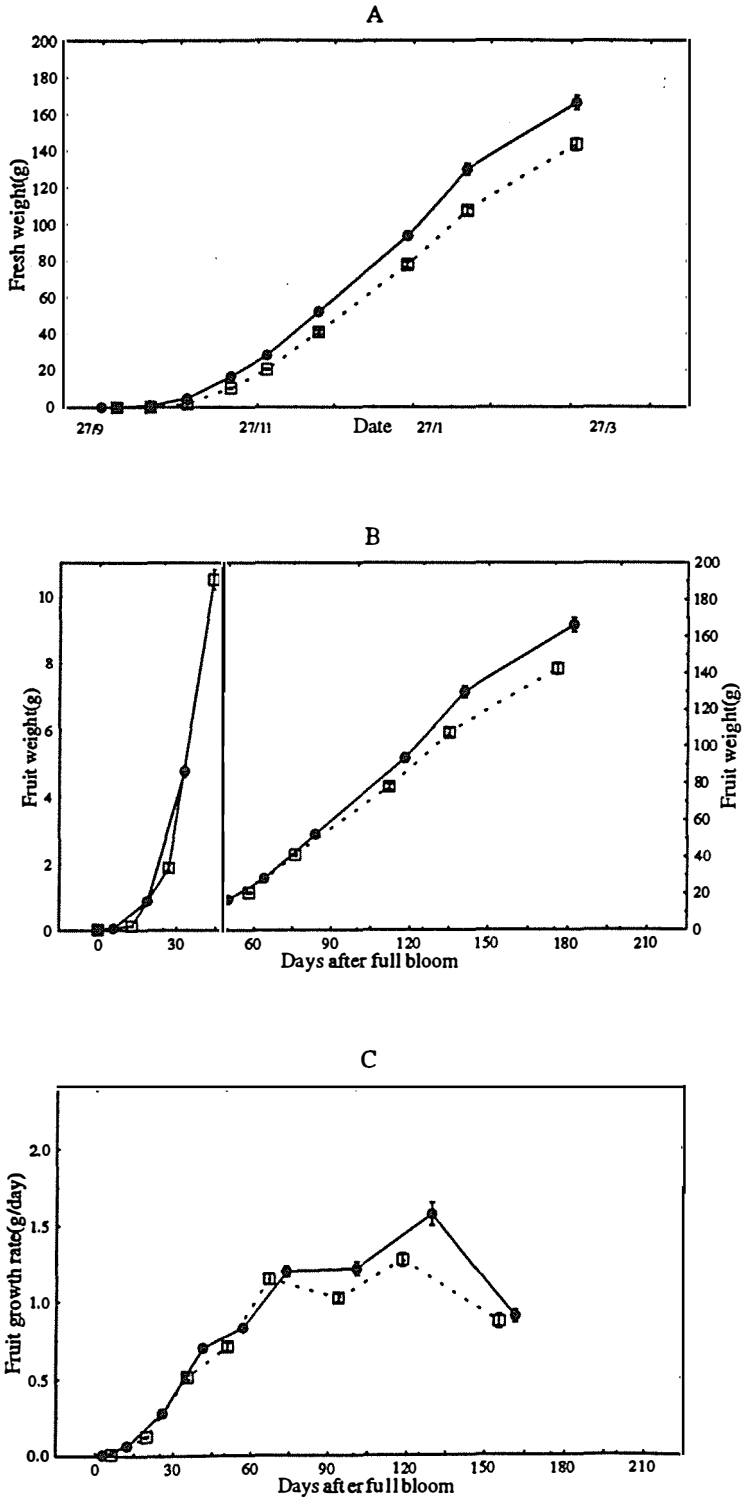
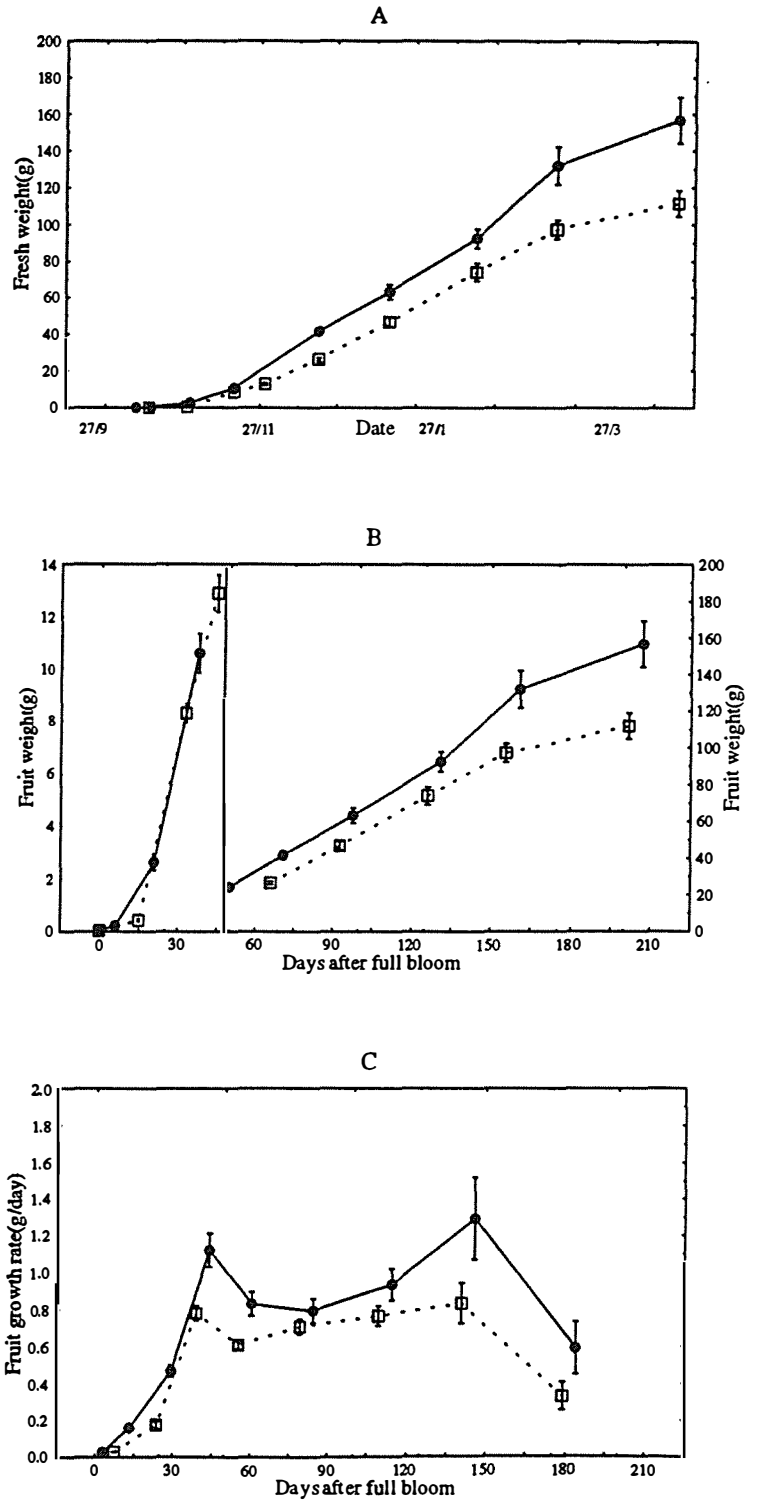


Figure 4.6 Cumulative growth curves of apple fruit (cv. 'Granny Smith'), expressed as a function of date (A) or days after full bloom (B), and fruit growth rates (C) for two-year spur buds (●) and one-year lateral buds (□). Bars indicate ± SE. For early harvest ± are contained within written symbols.



Fruit growth rates were similar for both bud types up to 70 DAFB for 'Royal Gala' and 'Braeburn' or 40 DAFB for 'Granny Smith'. After this time growth rate was lower for fruit from one-year lateral buds than from two-year spurs by 0.2-0.5g/day. This resulted in a subsequent divergence in fruit size such that those from two-year buds were substantially larger at the final measuring date than fruit from one-year buds for all three cultivars. The time when fruit growth rates from the two bud types diverged coincided with the end of the rapid phase of early fruit growth.

The average relative growth rate for fruit from both bud types and three cultivars generally decreased with time in a curvilinear fashion (Figures 4.7, 4.8, 4.9). However for 'Braeburn' fruit and fruit on two-year spur buds of 'Royal Gala', there was an initial rapid increase occurring from full bloom to 10-15 DAFB, before the steady decrease in rate occurred. The maximum average relative growth rate was highest for fruit on two-year spur buds of 'Granny Smith', with a rate of 0.242g/g/day occurring 3 DAFB. This compared with maximum rates of 0.171g/g/day for 'Royal Gala' and 0.203g/g/day for 'Braeburn' fruit on the same bud type.

Generally, average relative growth rate curves during the growing season of fruit from the two bud types were similar. Differences which did occur were small and not consistent between cultivars.

Figure 4.7 Average relative growth rates of apple fruit (cv. 'Royal Gala') from two-year spur buds (●) and one-year lateral buds (□), expressed as a function of days after full bloom, for each bud type. Bars indicate ± SE.

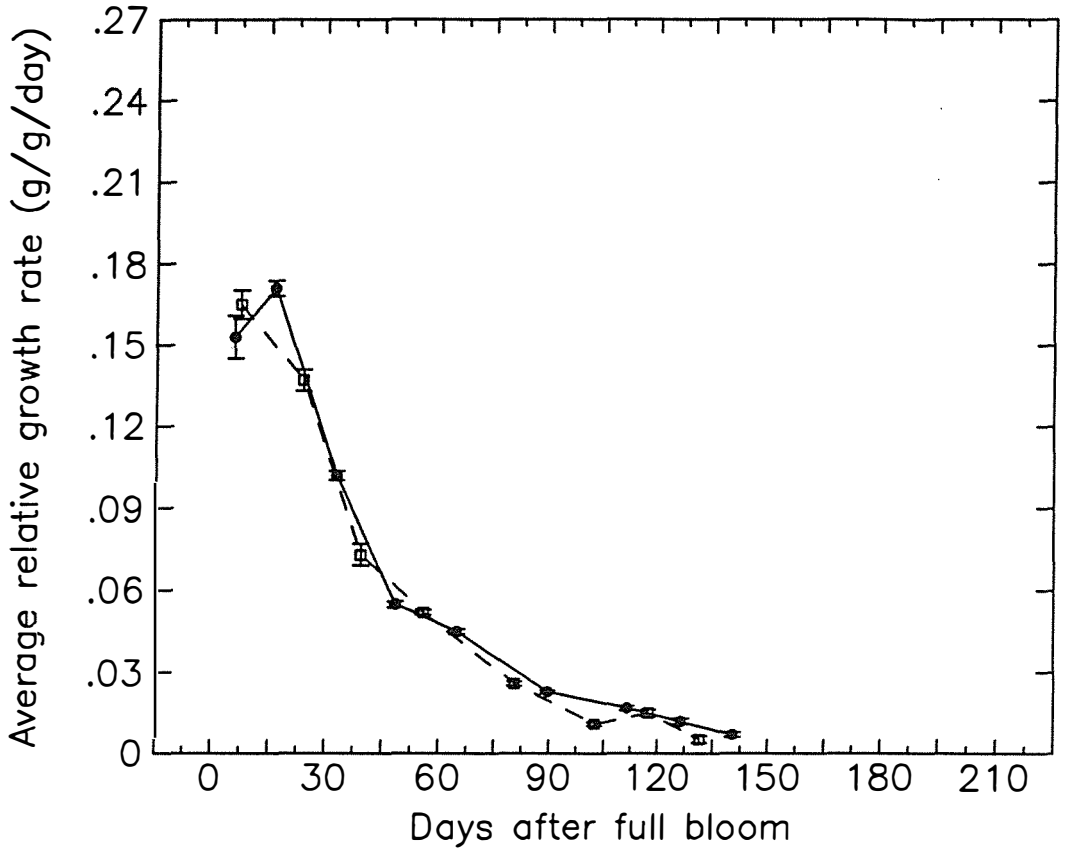


Figure 4.8 Average relative growth rates of apple fruit (cv. 'Braeburn') from two-year spur buds (●) and one-year lateral buds (□), expressed as a function of days after full bloom, for each bud type. Bars indicate ± SE.

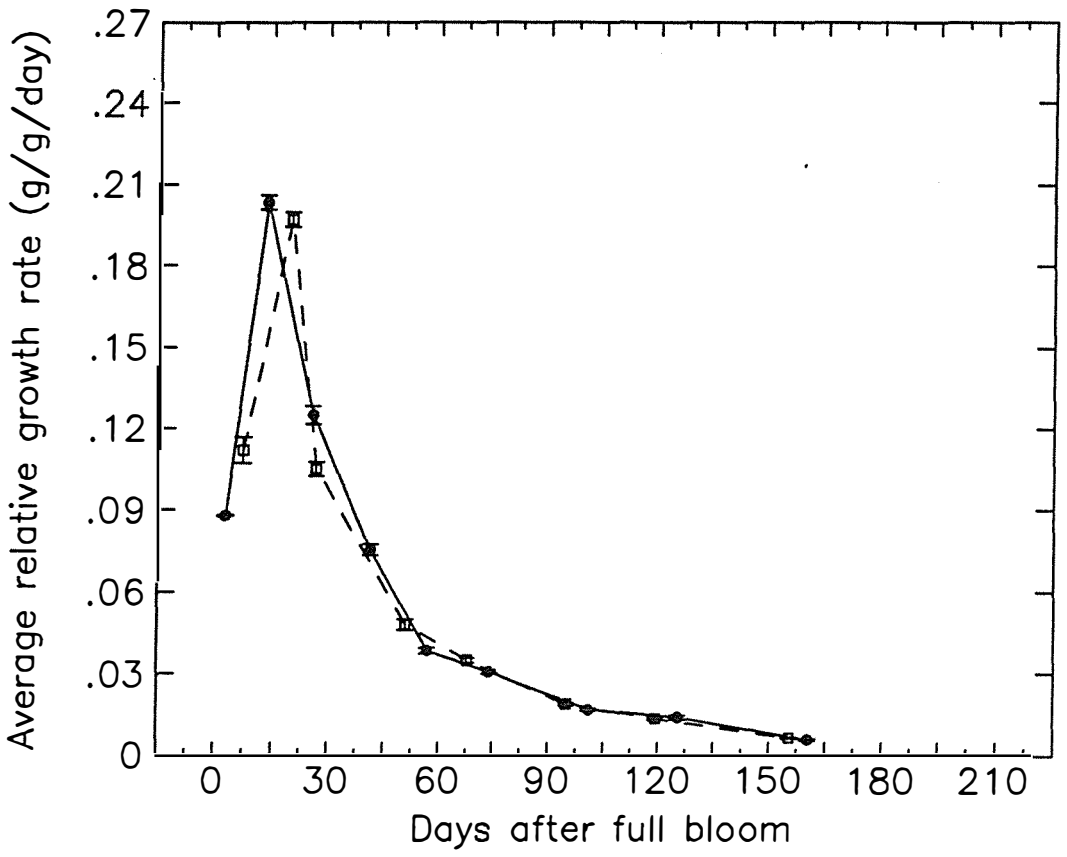
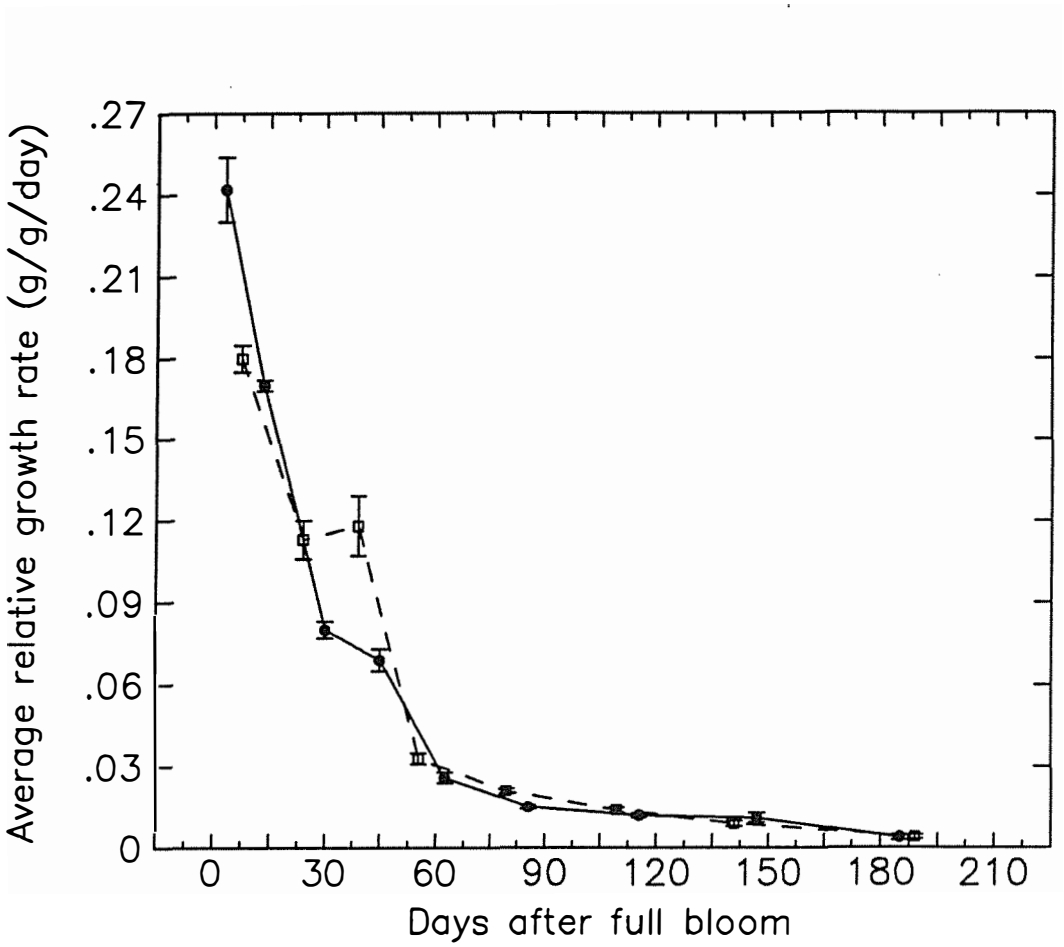


Figure 4.9 Average relative growth rates of apple fruit (cv. 'Granny Smith') from two-year spur buds (●) and one-year lateral buds (□), expressed as a function of days after full bloom for each bud type. Bars indicate ± SE.



4.6 Discussion

4.6.1 Fruit Size at Commercial Harvest

Two-year spur buds old produced larger fruit at harvest than did one-year lateral buds in all cultivars evaluated. This occurred even when fruit numbers per bud were higher on two-year spur buds than one-year lateral buds on unthinned 'Granny Smith' and 'Golden Delicious'.

Lespinasse (1977) noted that 'Golden Delicious' fruit on one-year lateral buds were smaller than fruit from older spurs. He suggested this difference was due to the delay in flowering on one-year lateral buds and that if harvesting was delayed by 6-10 days then fruit on such buds would "develop adequately". In the present study, fruit size differences between two-year spur and one-year lateral bud types were still apparent at the final harvest, even when fruit growth curves were corrected for the delay in flowering time (Figures 4.4B, 4.5B, 4.6B).

Nevertheless, early flowers have been shown to produce larger fruit compared with later flowers for apple (Denne, 1963; Sullivan, 1965), kiwifruit (Lai et al., 1990), sweet cherry (Patten et al., 1986), apricot (Jackson and Coombe, 1966), and tomato (Bangerth and Ho, 1984). Indeed, in the present study, for 'Royal Gala', for any one bud type, fruits which had developed from early opening flowers were larger than those from later blooming flowers (Table 4.6). It would therefore appear that some attribute of late flowering

one-year lateral buds, other than a delay in flowering time, is causing the smaller fruit size. Possible attributes will be explored in the following sections.

4.6.2 Flower Receptacle Size

One-year lateral buds had smaller receptacles at bloom than two-year spur buds. This difference occurred whether measurements were made from harvested flower samples (Table 4.4), or on flowers which, as fruit, were retained on the tree until maturity (Table 4.6). Further, after removing seed effects on fruit size, those flower receptacles which were small at bloom produced small fruit at harvest, especially for 'Granny Smith' (Figure 4.3).

It must be noted however, that relationships between receptacle and final fruit size were poor, albeit significant (r^2=0.02-0.30) (Table 4.9). Further, the very large differences in receptacle size (from 15-99mg) were reflected in relatively small differences in final fruit size. This result may have occurred because flowers at each bud type were tagged at various stages of development on one day at 50% full bloom (Table 4.5). A more accurate measure of receptacle size would have occurred if all flowers on selected replacement branches were measured when they had individually reached king bloom. Successful pollination and fertilisation of a flower can occur up to 7 days after first flower opening in suitable conditions (Williams, 1970). Calculated receptacle sizes may also have varied for flowers at the same developmental stage depending upon their time of pollination and fertilisation. Supplementary

hand pollination of all tagged flowers immediately after they opened may also have improved correlations between receptacle size and final fruit size.

Nevertheless, results from this experiment indicate that the smaller receptacle flower size for those borne on late flowering one-year lateral buds determines its smaller fruit size at harvest compared with receptacles and fruit on early flowering two-year spur buds. Denne (1963) also found that later-opening 'Cox's Orange Pippin' and 'Dougherty' flowers were lighter than early-opening flowers but there was no consistent effect of spur age, although buds on one-year wood were not considered. Early flowers of kiwifruit have heavier ovaries than later flowers on the same age of wood (Lai et. al., 1990). In sweet cherry, flower and ovary weight at anthesis decreased at late bloom times (Patten et al., 1986).

Flower number is lower and primary leaf area at bloom are also less for one-year lateral spurs than for two-year spurs (Chapter 3). This suggests that positional factors controlling development of the flower bud may also regulate growth of individual flower receptacles within the bud. Timing of bud initiation and/or nutrient availability to the bud before anthesis may be important in controlling flower receptacle growth as well as growth of other parts of the flower bud (Chapter 3). These factors may be important in determining cell number in the receptacle as is thought to occur in tomato flowers at different positions on a truss (Ho, 1988). Bergh (1985b) found that growth of 'Granny Smith' flower receptacles in the autumn was reduced on heavy cropping trees compared with lighter cropping trees, so reducing fruit

growth in the following season. This further indicates regulation of flower receptacle size during development may be an important factor in determining final fruit size in apple.

4.6.3 Fruit Growth Rate

Cumulative and fruit growth rates curves for 'Royal Gala' and 'Braeburn' in this study were typically sigmoidal and parabolic respectively, agreeing with other studies on apple (Bain and Robertson, 1951; Denne, 1960). For 'Granny Smith', however, there was an indication that fruit growth rate decreases 45 DAFB, before slowly increasing again several weeks later. This result supports an earlier finding for 'Cox's Orange Pippin' and 'Golden Delicious' in a Belgian study over five seasons, where fruit growth slowed for one week 40-50 DAFB (Magein, 1989). Fruit growth rates in the published study were measured weekly. The two-three weekly measurements employed in the present study may not have allowed more subtle changes in growth rates of 'Royal Gala' and 'Braeburn' to be detected. A reduction in growth rate at this time, for 'Granny Smith' at least, coincides with final fruit drop (Chapter 3). It may indicate that overall resources on the tree are limiting growth of fruit which remain on the tree.

There has been no published relative growth rate curves for apple fruit. The fruit relative growth rate curves calculated in the present study for 'Braeburn' and for those from two-year spurs of 'Royal Gala' followed a characteristic pattern, and were similar for that of tomato fruit

(Monselise et al., 1978). Relative growth rates rose sharply to a maximum shortly after bloom, before decreasing continuously in a concave shape. The lack of a constant relative growth rate indicates that true exponential growth did not occur. Rather, there may be a super-exponential growth phase following an initial lag phase after fertilisation. This is followed by a sub-exponential phase. Such changes in relative growth rate occurring during the first few weeks after bloom may depend upon the extent to which cells are dividing and enlarging. In apple (Smith, 1950), and many other fruits (Bollard, 1970), cell division ceases at anthesis before increasing rapidly after fertilisation. Both cell division and enlargement contribute to fruit growth during the first few weeks after anthesis (Smith, 1950). The lack of any upwards rise in the relative growth rate curve for 'Granny Smith' fruit may be due to an initiation of cell division, immediately following king flower opening and fertilisation and before the first measuring date.

'Royal Gala' fruit had a slightly higher absolute and relative growth rate during the 15-70 days after bloom than 'Granny Smith'. However the length of this phase was shorter. This may be an indication of the greater rate of cell division for any early maturing cultivar. Denne (1963), in a more detailed study, showed that rate of cell division was faster and was completed sooner for the early maturing 'Cox's Orange Pippin' compared with the late maturing cultivar, 'Dougherty'.

Environmental and cultural factors may also influence duration and rate of cell division. Early crop thinning (Quinlan and Preston, 1968) and high fruit

phosphorus levels (Letham, 1961) have increased the length of the cell division phase while small increases in temperature can increase rate of cell division (Jackson and Coombe, 1966). This indicates that site-dependent factors may have contributed to the difference in growth rates between the cultivars.

Fruit on two-year spurs had a greater cumulative growth rate, absolute growth rate and therefore apparent sink strength throughout the growing season than fruit on one-year laterals, from 40 ('Granny Smith') or 70 ('Royal Gala', 'Braeburn') DAFB. In other words, the capacity of developing fruits on two-year spurs to attract carbon from leaves during the season would have been greater than that for fruit on one-year laterals. This probably resulted from flowers on two-year old spurs having a larger receptacle size (sink size) than one-year lateral buds. Similar results have been found for tomato fruits borne on different positions on a truss (Bangerth and Ho, 1984). Fruit borne in the apical position on a truss have a reduced growth rate because of smaller flower size at bloom compared with fruit in the more basal zone.

What of differences in metabolic activity (sink activity) between fruit from different bud types? In fact sink activity would seem to be similar as indicated by similar relative growth rate curves. This is perhaps a surprising result given that early-set fruit for other species such as tomato have a higher assimilate import rate than later-set fruit (Ho, 1988).

Fruit abscission is greater on one-year lateral than two-year spur buds (Chapter 3). It is possible that the "competitive" ability of fruit on one-year lateral buds was lower than that for fruit on two-year spur buds, before fruit

drop. The resultant reduction in the capacity of many fruits on one-year lateral buds to compete for assimilate may have invoked the abscission process to a greater degree compared with fruits on two-year spurs. This may have allowed balancing of assimilate demand with supply, whilst growth of the remaining fruit was maintained, being limited only by their initial (sink) size. Other workers have suggested that dominant sinks may inhibit other organs directly, even in a situation where carbohydrate is freely available (Bangerth, 1989; Lai et al., 1990). Following this hypothesis, fruit drop on one-year lateral buds may be invoked through production and transport of inhibitory hormones from two-year spur buds.

There are several examples of fruit set being reduced by experimental treatments without fruit sizing being influenced (eg. a reduction in leaf area or ringing on individual spurs, Ferree and Palmer, 1982). Unfortunately, there is no published study which reports on the converse. That is, the effect of a treatment on early fruit sizing where fruit set is increased to an intermediate level (eg. 50% of total flower blossoms). In such a case, one might expect fruit growth not to be affected by treatment, as assimilate would be used to support additional fruit set on the tree. However when treatments have been imposed which have increased fruit set to very high levels then additional resources would seem to have been used in stimulating fruit growth. For instance, when early fruit set was increased from 10 to 100% at 4 weeks after full bloom by shoot (tip) removal at anthesis, fruit growth was also increased at this time (Abbott, 1960). Quinlan and Preston (1968) increased resources

to remaining flowers/fruits on apple trees by thinning at pink bud. By 4 weeks after full bloom, fruit set as a proportion of total flower numbers present at bloom increased from 20 to 90% for thinned compared with unthinned trees. Fruit size at this time was also greater by 35% for the thinned trees.

Initial assimilate supply to fruits on one-year lateral buds may also have been less than that to fruits on the other bud type. Two-year spurs have considerably greater primary and bourse leaf area during the period immediately after bloom compared with one-year laterals (Chapter 3). Assimilate is supplied to apple fruit during the first 3-4 weeks after bloom from these leaves (Hansen, 1971; Tustin and Lai, 1990), after which time assimilate from other leaves also contribute to fruit growth (Hansen and Christenson, 1974). Source strength should have been greater for two-year spur fruit. Reductions in source strength have been shown to have large effects on fruit abscission in apple. For instance artificial reduction in primary and/or bourse leaf area on a whole tree (Llewelyn, 1968; Quinlan and Preston, 1971) or an individual spur basis (Ferree and Palmer, 1982) increased fruit drop. Partial or total shading of trees following bloom reduced initial and final fruit set (Dennis, 1986). Growth measurements of surviving fruits were not made. However in the present study it would seem that source strength was not limiting fruit growth on one-year lateral buds as there was no difference in relative growth rates between fruit from the two buds types.

In summary, final fruit size and fruit growth rates were larger on two-year apple spurs than on one-year lateral buds. Sink strength for fruit on two-year

spurs would seem to be greater because of larger flower receptacles at bloom and not to differences in "metabolic activity" of fruit during the season. It is suggested that sink strength, and particularly initial sink size, rather than source strength may limit apple fruit growth, at least for those fruit borne from buds on the replacement branch.

CHAPTER FIVE

FRUIT MATURATION AND RIPENING

5.1 Introduction

Considerable variation in fruit productivity has been shown to exist within the tree canopy and within apple replacement branches. Variation in the visual appearance and eating quality of fruit within replacement branches is also important, particularly from the view of the consumer.

Much research has been conducted on fruit quality differences between different parts of the tree. For instance, incidence of storage disorders such as superficial scald and core flush is greater for fruit harvested from the inside of trees than those on the outside of the canopy (Jackson, 1967). Fruit located inside the canopy may have a lower soluble sugar level, less red colour, and more intense green colour, and be of a smaller size, compared with fruit on the outside of the canopy (Jackson et al., 1971; Seeley et al., 1980; Barritt et al., 1987; Tustin et al., 1988). The amount of light which penetrates the canopy probably plays an important role in determining many of these differences in apple fruit quality within the tree (Jackson, 1980).

The stage of maturity when a fruit is picked may also influence fruit quality. Many sensory characteristics of apple fruit and occurrence of some fruit disorders after storage, are determined in part, by the stage of fruit

maturity at harvest. For instance, less mature apple fruit can be firmer, greener and higher in acid, but have less red colour, and have poorer flavour, than more mature fruit (Smock, 1948; Lau, 1985). Early harvested fruit held in storage tend to be susceptible to physiological disorders, such as bitter pit and superficial scald (Padfield, 1969; Reid et al., 1978). Later harvested fruit can develop senescent breakdown (Padfield, 1969). Therefore, it is important to understand positional factors affecting fruit maturity, and relationships between maturity and fruit quality characteristics such as colour, flavour and texture.

Fruit ripening is defined as the set of measurable processes which change the structure, composition and other sensory characteristics so that the fruit becomes acceptable to eat (Rhodes, 1980). Maturity is an ambiguous term however, as it can refer to both time of harvest and to a distinct physiological stage (Watada et al., 1984). Watada et al. (1984) defined physiological maturity as the stage of development where a plant (fruit) continues ontogeny through to ripening even if detached. In contrast ripeness is the stage of development where a plant (fruit) becomes acceptable for the consumer, in terms of visual appearance and eating quality.

It is important to recognise that many New Zealand apple cultivars when harvested are often physiologically mature and have acceptable colour development but are unripe in terms of eating quality. Thus at harvest fruit often have very high acid and starch levels and flavour is poor. However, after storage at low temperatures, fruit ripen to optimum eating quality with a

minimum of senescence-related fruit disorders. Commercial harvesting of some cultivars cannot begin too early, otherwise fruit may not ripen properly and fruit disorders such as bitter pit and superficial scald may develop.

In New Zealand, apple fruit maturity needs to be assessed so that growers know when to begin and finish harvesting fruit from their orchards. Fruit pickers in the orchard and fruit sorters in the packhouse may also need to assess maturity during harvesting operations. Indeed, selective picking of mature fruit is current practice for many new cultivars, such as 'Braeburn', 'Royal Gala' and 'Fuji'.

Apple fruit maturity can be objectively assessed by measuring biochemical and physiological changes which may occur during the latter stages of fruit development on the tree. Many of these changes are also important "quality" components of the fruit and can be influenced by factors other than fruit maturation.

5.1.1 Hormonal Regulation

The apple is a climacteric fruit. Immediately before ripening occurs, fruit respiration rate reaches a pre-climacteric minimum before increasing dramatically to a climacteric peak then subsequently declining (Rhodes, 1980). This rise is accompanied by changes in fruit hormonal balance (in particular ethylene production and sensitivity) and sensory characteristics. Ethylene production by apple fruit parallels the change in respiration rate, the rise in production of both ethylene and CO_2 occurring simultaneously (Reid et al.,

1973 for 'Cox's Orange Pippin'). Early studies suggested that an increase in ethylene played a major part in the initiation of ripening (Burg and Burg, 1965). However, now it appears that this increase in ethylene synthesis is more a consequence of the initiation of ripening. This autocatalytic burst of ethylene production would seem to play a major role in the coordination of ripening changes rather than being the key regulator of the ripening event (Rhodes, 1980).

Two systems of ethylene production in climacteric fruit (such as apple) have been distinguished; System I representing the low level of ethylene present in unripe preclimacteric fruit, and System II representing the autocatalytic burst. Nevertheless, these two systems are interlinked. The presence of low levels of ethylene before the climacteric is required for fruit ripening to occur. Reducing the ethylene level chemically (eg. with aminoethoxyvinyl glycine (AVG) for instance) or under hypobaric conditions can retard ripening (Yang and Hoffmann, 1984).

Recent evidence indicates that the initiation of ripening may involve changing tissue sensitivity to ethylene rather than to actual ethylene production *per se* (Firn, 1986). Exogenous ethylene applications to preclimacteric apple fruit shorten the time to the onset of ripening (Harkett et al., 1971). Further, as fruit approach natural onset of ripening there is an increased fruit response to ethylene application (Knee et al., 1987). The nature of the tissue sensitivity is unknown. It may involve the concentration, activity and/or affinity of ethylene binding sites and/or the rate of response reactions resulting from

ethylene/receptor binding (Firn, 1986). Yang (1987) proposed that tissue sensitivity to ethylene may be related to the disappearance of a "ripening inhibitor" during development. Ripening is faster if fruit are detached from the tree indicating some tree-inhibitor limits this process (Yang et al., 1986). Knee et al. (1987) suggested that a binding system present throughout fruit development may increase in its affinity for ethylene before ripening.

The effect of fruit position within the tree canopy on fruit maturity as measured by changes in ethylene production has rarely been explored. Farhoomand et al. (1977) showed that ethylene levels were higher in 'Delicious' apple fruit located on the "inside" of the tree canopy than for those fruit located on the outside. These results suggest that fruit on the outside of the canopy may appear more mature, in terms of red colour and sugar levels, while physiologically they may be less advanced. However, recently Meir and Bramlage (1988) found that 'Cortland' apples located inside a mature canopy had much lower internal ethylene concentrations than those harvested on the outside.

The role of other hormones in apple ripening is not clear. Fruit ripening has been associated with an increase in abscisic acid (ABA) levels in apple (Rhodes, 1980) and pear (Leshem et al., 1986). Application of ABA accelerates ripening in preclimacteric apples, as well as other fruits (Brady, 1987), although some exceptions do occur. Mousdale and Knee (1981) also associated a peak in auxin levels within apple fruit as System II ethylene was initiated. Both ABA and auxin have been shown to stimulate ethylene

production through enhancement of ACC synthesis (Yang and Hoffmann, 1984; Riov et al., 1990).

The presence of leaves near fruit may also regulate the ripening process, by mediating ethylene production in the fruit. Sfakiotakis and Dilley (1973), isolated individual fruiting apple spurs from the rest of the tree by ring-barking late in the season. Internal fruit ethylene concentrations were greater when leaves were removed from the spurs, compared with those where leaves were present. This indicates that the presence of leaves on the spur may suppress ripening, possibly by the production and transport of a ripening inhibitor to the fruit.

5.1.2 Fruit Quality Changes During Maturation

Carbohydrates, Organic Acids and Volatile Compounds

Levels of carbohydrates, organic acids and volatile components show characteristic changes during apple fruit maturation and ripening, which sometimes may relate to physiological maturity (Hulme, 1958). Their levels at harvest and after storage are also important in determining flavour of the ripe fruit.

Sorbitol is the major photosynthetically derived carbohydrate translocated from leaf to fruit *via* the phloem in apple trees (Priestley, 1980). In the fruit conversion of sorbitol to sucrose and other free sugars occurs readily in the cytosol (Beruter and Kalberer, 1983) or across the tonoplast

(Yamaki, 1984). During mid-summer, carbohydrate is accumulated in the fruit as starch (Hulme, 1958). Starch synthesis can occur from assimilates imported into the fruits over a long period of time as well as from "recently" translocated carbohydrates (Hansen, 1979). Starch breakdown to sucrose occurs during fruit maturation although contributions to sucrose levels can also be made from recently imported sorbitol. This brings about a rapid rise in sucrose concentration during maturation. At the climacteric, soluble sugar levels are characterised mainly by fructose, with lesser amounts of glucose and sucrose (Brown and Harvey, 1971; Chan et al., 1972).

In apple fruits, malic acid is the dominant organic acid at harvest. There is a marked increase in malic acid utilisation in apple during fruit ripening, particularly at the climacteric (Hulme, 1958). Consequently the level of organic acids and malic acid decreases during this phase.

A wide range of volatile compounds have been isolated from ripe apple fruits - these include acids, alcohols, esters, ketones and aldehydes. Production of these compounds increases many fold during apple ripening (Rhodes, 1980). Many studies in this area have concentrated upon describing specific "impactor" volatile compounds - that is the compounds contributing to the characteristic aroma of the apple (Williams and Knee, 1977; Paillard, 1979).

Williams and Knee (1977) described the importance of the low boiling point esters, hexyl and butyl acetate as linked with "Cox-like" character for 'Cox's Orange Pippin' and the banana-like flavour which developed late in senescence for this cultivar being associated with 2,3 methyl butyl acetate.

Possibly the increase in one or several of these compounds during fruit maturation may be useful as measures of fruit maturity.

Fruit Softening

Fruit softening is another characteristic process of maturing apple fruit. In addition, the texture of the ripe fruit after storage is often determined by the fruit's firmness at harvest.

Morphological changes in fruit tissue structure such as a breakdown of the middle lamella between fruit cells have been described during apple softening (Ben-Arie et al., 1979). Biochemically, softening is associated with changes in the walls of fruit cells. For instance, soluble pectic polysaccharides and neutral sugars, such as galactose, which partly make up cell walls, increase during ripening (Bartley and Knee, 1982; Gross and Sams, 1984). The concentration of cell wall (pectin)-cleaving enzymes, such as exopolygalacturonase, (PG) also rise during apple softening (Bartley, 1974, 1978). Cell wall modification before degradation may occur before PG attack. Knee (1978) suggested that synthesis of a less branched methylated pectin polymer occurred before cell wall breakdown in ripening 'Cox's Orange Pippin' fruit. Later he proposed that an exchange of protons for calcium ions which might aid the bridging of pectin polymers in the cell wall, was a major event in apple softening (Knee, 1982). However apple fruit swelling also occurs during maturation and ripening as a result of cell expansion (Bain and Robertson, 1951; Denne, 1963). The extent to which cell expansion

contributes to softer fruit during the maturation period, independent of cell wall/middle lamella breakdown, is not known.

Skin Colour

Apple background skin colour changes from green to yellow during fruit maturation and ripening. The green skin colour of pipfruit is derived from two chlorophyll pigments, (*chlorophyll a* and *b*) which are found in the chloroplast (Rhodes, 1980). Chlorophyll breakdown occurs in fruit skin during ripening, as it does in the leaf during senescence (Knee, 1972; Gorski and Creasy, 1977). The destruction of chlorophyll during ripening is associated with an increase in activity of the chlorophyllase enzyme (Rhodes and Wooltorton, 1967).

Some yellow pigments known as chloroplast carotenoids are found in unripe fruits, together with chlorophyll, but these decrease during fruit development (Gross, 1987). During ripening, chloroplasts undergo ultrastructural changes to form chromoplasts, whereupon rapid production of several other types of carotenoids can occur. In apple, carotenoids can increase (Knee, 1972), remain steady (Gorski and Creasy, 1977) or decrease (Mussini et al., 1985) during ripening. The relative changes in concentration of individual carotenoids during ripening may be more important than changes in gross carotenoid level in determining amount of yellow pigment present. In several apple cultivars, variation in a chromoplast carotenoid, violaxanthin, has

been found to be a suitable index of maturity to determine optimal harvest date (Gross, 1987).

Red skin colour is an important quality characteristic of many commercial apple cultivars which increases in area and intensity during maturation and ripening. Red anthocyanin pigments are stored in the epidermal and sub-epidermal cell vacuoles in the skin (Gross, 1987). The anthocyanin which predominates in apple is the cyanidin galactoside, idaein, its direct precursors being shikimic acid (a cinnamic acid), acetate and galactose (Faust, 1965).

Phenylalanine ammonia-lyase (PAL) is thought to be the controlling enzyme of anthocyanin synthesis in apple skin, catalysing the reaction 1-phenylalanine to cinnamic acid. Its activity, synthesis or degradation is regulated by several factors, including light, temperature and ethylene (Gross, 1987; Saure, 1990).

Several factors promoting anthocyanin synthesis in apple skin (eg. wounding, UV light and maturity) have been correlated with levels of tissue ethylene and PAL activity (Chalmers and Faragher, 1977; Faragher and Chalmers, 1977). Exogenous ethylene applications also increase the rate of anthocyanin synthesis and levels of PAL in apple fruit. This suggests that ethylene may regulate anthocyanin production by influencing PAL activity.

Two photoreactions control red colouration (Downes, 1965). The apple fruit has one action spectrum similar to that found important for photosynthesis, whilst the second activates the phytochrome system. Stimulation of PAL by

light is thought to be mediated by the phytochrome system (Saure, 1990). On the other hand, PAL in apple skin discs in the dark may increase, without corresponding anthocyanin synthesis (Faragher and Chalmers, 1977). Several studies have correlated low temperatures with promotion of red colour development in apple, low night temperatures being particularly important (Uota, 1952; Blankenship, 1987). Cold temperatures stimulated accumulation of both PAL and anthocyanin in apple skin (Tan, 1979). However, Tan (1980) later concluded that high temperatures might increase levels of a PAL-inactivity system.

As already indicated, regulation of many of the above fruit quality changes are mediated by positional factors within the canopy. Fruit inside the canopy have lower soluble solids concentration and anthocyanin levels at commercial harvest than fruit on the outside of the canopy. Fruit sugar and skin anthocyanin production are both regulated by the amount of light received by the fruit or leaves surrounding the fruit. Therefore it is not surprising that levels of intercepted light within the apple tree canopy have been positively correlated with red colour and fruit soluble solids (Palmer, 1989). Positional factors influencing other aspects of fruit quality have rarely been explored. Tustin et al. (1988) indicated that 'Granny Smith' fruit on the inside of the tree are greener than those on the outside. This may have been caused by high levels of chlorophyll and/or low levels of yellow pigment in the skin.

Clearly fruit maturity has a major influence on fruit quality at harvest. However little work has been conducted which explores variation in fruit

maturity on the tree and its possible role in influencing fruit quality variation within the tree and specifically on replacement branches.

5.2 Experimental Objectives

Since little is understood of variation in fruit ripening/maturation within the apple tree, an experiment was undertaken to examine the maturation patterns of fruit from two bud types on the replacement branch system. These fruit were compared with those borne on the inside of the tree canopy, on older spur buds. Sensory characteristics of fruit at harvest were also measured so that relationships between fruit maturity and quality could be examined.

5.3 Influence of Bud Type on Fruit Maturation and Ripening

5.3.1 Materials and Methods

Experimental

Ten uniform heavy cropping 'Royal Gala', 'Braeburn' and 'Granny Smith' trees, were selected. Fruit were sampled from one-year lateral buds and two-year spur buds located on replacement branches growing on the outside of the canopy. In addition, fruit were harvested from older spur buds (>three years) borne on pendant laterals within each tree.

Fruit Sampling

Fruit samples were harvested three times during the commercial harvest

season from ten unpicked trees for each cultivar (Table 5.1). At each harvest, one fruit from each bud type was harvested at random from the north, east and west sectors of each tree. Thus, for each cultivar, a total of thirty fruit from each bud type was picked at each harvest.

Table 5.1 Commercial and experimental harvesting dates for apple in Hawkes Bay (1988)

Cultivar	Commercial harvest date		Experimental harvest date		
	Opening	Closing	Early	Middle	Final
Royal Gala	15/2	16/3	10/2	24/2	14/3
Braeburn	21/3	19/4	21/3	5/4	18/4
Granny Smith	11/4	13/5	11/4	2/5	23/5

Fruit were transported immediately after harvest to Massey University, Palmerston North (2 hour trip). As temperature can have a significant effect on ethylene evolution, fruit were allowed to equilibrate in a constant temperature (20°) room for 12 hours before fruit were weighed and assessed for several maturity and sensory characteristics.

Measurements

Internal ethylene concentration (IEC)

An internal atmosphere sample was extracted from the core of each apple (Saltveit, 1982).

1) A length (3cm) of fuse wire (5 amp) was inserted into the tip of a syringe needle (18 gauge-40mm length). This ensured that the needle tip was not blocked by apple flesh tissue when it was pushed into the core cavity (Saltveit, 1982).

2) A plastic syringe (1 ml) was attached to the needle which was pushed through the calyx into the core of the fruit which was held under water. This ensured that air in the syringe was from inside the fruit. Water contamination occurred in several fruit in each bud type at each harvest date (maximum = 5 fruit). These readings were regarded as missing plots.

3) A sample of internal gas was withdrawn from the apple into the syringe. After removal from the fruit, the 18-gauge needle was replaced quickly by another needle (23 gauge-25mm length).

4) Each sample was injected into a Pye Unicam 104 Gas Chromatogram fitted with a flame ionisation detector and an "Alltech" alumina column 3/8 in. diameter, 6' length. The carrier gas was N_2 at a flow rate of 30 ml/min. The minimum detectable level of ethylene was 0.05ppm. All measurements which recorded a non-detectable level were assumed to be 0.05ppm.

Internal ethylene concentrations (IEC) were log-transformed to stabilise the variance before formal analysis of variance of the results (Steel and Torrie, 1986).

Fruit Skin Colour

1) Ripening Index ('Royal Gala'): A commercial maturity chart provided by the New Zealand Apple and Pear Marketing Board was used to visually grade each fruit from immature (1) to over-mature (12). This chart shows several photographs of 'Royal Gala' fruit with various background and red skin colours, ranging from dull red/green (immature) to bright red/yellow (overmature).

2) Red Blush ('Royal Gala' and 'Braeburn'): The amount of striped red blush on an apple was visually determined as a % of total skin cover. Data were transformed to stabilise the variance of the means using arcsine (sqrt(x*0.01)) where x = % red blush (Steel and Torrie, 1986).

3) Skin Colour: Blush over-colour of 'Royal Gala' and 'Braeburn' apples increases in intensity with maturity and colour changes from a dull bronze to a bright red. This change is used commercially to some extent as a maturity guide for select harvesting fruit (see above). Green ground skin colour is an important quality attribute for 'Granny Smith' apples, with a deep green colour being preferred by the market. The Judd-Hunter L* a* b* tristimulus colour notation system (Hunter, 1975) was used to measure these changes in the total colour (chromaticity) of the skin. Although a* or b* can be used separately to represent the chromaticity of an object, Hunter (1975) points out that these two measurements are not independent and should be combined. Francis (1980) suggests that chromaticity can be obtained by comparing the appropriate Judd-Hunter a*:b* ratios. In the present experiment, chromaticity of red blush over-colour is represented by +a*/+b* whilst that of green-yellow ground skin or flesh colour is represented by -a*/+b*.

A Minolta Chromameter II (Reflectance) was calibrated to a white standard illuminant (6774 k) condition, and was used to measure a* and b* values. One red-blush colour measurement using the Chromameter was made at the equator of each fruit where the red-blush colour appeared heaviest. Measurement of red-blush colour of 'Royal Gala' fruit harvested from the first harvest date was not carried out. Care was taken to ensure that each measurement was taken over areas where no ground colour was visible or no blemishes such as sun-burn, branch-rub marking and bruising occurred. Colour spot measurements on 'Granny Smith' fruit were made using the Chromameter at the equator and on opposite sides of each fruit. Ground skin colour could not be measured on 'Royal Gala' and 'Braeburn' fruits as they were often completely covered in red stripes, especially at the second and third harvests.

4) Flesh Colour: Objective measurement of flesh colour has been used as a measure of peach quality and maturity (Sims and Comin, 1963; Dann and Jerie, 1988). Ground flesh colour changes from green to yellow during apple ripening. Therefore changes in flesh colour on 'Royal Gala' (second and third harvests only) and 'Braeburn' fruits were determined using the Chromameter. Spot measurement of flesh colour was made directly underneath the skin at the equator on opposite sides of the fruit.

Fruit Fresh Weight

Each fruit was weighed using a Mettler balance to the nearest 0.1g.

Fruit Flesh Firmness

Flesh firmness was measured on peeled surfaces from both sides of each apple using a Effegi penetrometer (11.1mm diam. probe). Both blushed and non-blushed sides of each fruit were tested for 'Royal Gala' and 'Braeburn' cultivars. For 'Granny Smith', opposite sides of each fruit were sampled. Care was taken to avoid sunburnt patches on the skin as these can give abnormally high readings.

Soluble Solids Concentration (SSC)

SSC(%) was determined at room temperature using juice expressed from two opposite sides of the fruit and measured using an Atago L-20 hand-held refractometer.

Starch Pattern Index

The pattern of starch hydrolysis to soluble sugars was determined using the starch iodine test (Beattie and Wild, 1973; Reid et al., 1982). Each apple was transversely cut at the equator and one half was placed, cut surface down, for 3 mins in a solution (100ml) of KI (1g) and I_2 (0.25g). The resulting pattern on the cut surface, indicating relative amounts of starch and soluble sugar, was scored on a scale from 0-6, where 0 indicates the least and 6 the most starch to sugar conversion.

Fruit from inside the tree canopy have considerably lower absolute levels of starch compared to those fruit on the outside (Robinson et al., 1983). In some instances these levels are so low that index patterns do not show up. The starch index test was therefore not carried out on these fruit.

Statistical Analyses

The experiment was organised as a split plot design, each tree being a block, each bud type the main plot and each harvest date the sub plot. There were three individual fruit replicates in each block.

Correlation coefficients (r) were calculated between variables using the Minitab statistical package.

5.3.2 Results

Analyses of variance showed significant differences between harvest dates and between bud types for all measured fruit characteristics, for all three cultivars (Table 5.2). Comparisons between bud types or harvest dates are therefore presented in tabular form. Results are also presented graphically where a significant interaction was found between harvest date and bud type.

Internal Ethylene Concentration (IEC)

IEC in the core cavity of 'Royal Gala' and 'Braeburn' fruits increased steadily with time across all bud types (Table 5.3). The greatest increase occurred between the last two harvest dates. IECs for all 'Granny Smith' fruit from the first and second harvests were at very low levels ($< .08$ppm) (Figure 5.1), but a substantial increase occurred at the last harvest date. IEC levels were much higher at this later harvest date for 'Granny Smith' compared with the other two cultivars.

Table 5.2 Summary of the significance of F values from the balanced split plot ANOVA.

Independent variable	Royal Gala			Braeburn			Granny Smith		
	B.T.	H.D.	I	B.T.	H.D.	I	B.T.	H.D.	I
Fruit weight	***	***	NS	***	***	NS	***	NS	NS
Starch index	***	***	**	***	***	*	NS	***	NS
IEC	***	***	NS	***	***	NS	**	***	***
Flesh firmness	***	***	**	***	***	NS	***	***	NS
SSC	***	***	NS	***	***	NS	***	***	NS
Red blush coverage	***	***	*	***	***	***	-	-	-
Red blush colour (a*:b*)	***	***	NS	***	***	NS	-	-	-
Flesh colour (-a*:b*)	***	***	NS	***	***	NS	-	-	-
Background colour (-a*:b*)	-	-	-	-	-	-	***	***	NS

B.T. = Bud type, H.D. = Harvest date, I = Interaction between bud type and harvest date

IEC = Internal ethylene concentration

SSC = Soluble solids concentration

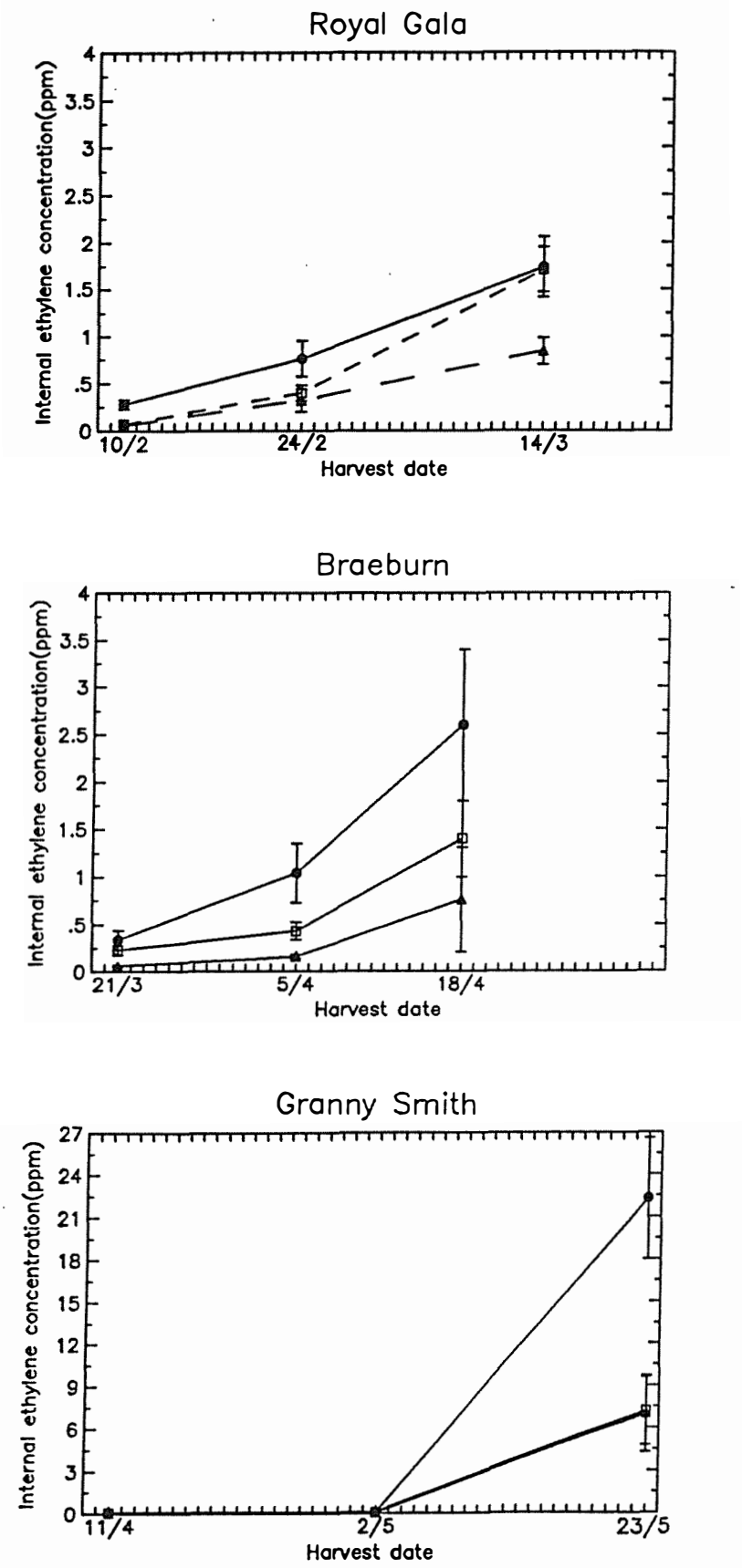
*, **, *** Significant F value at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 \geq P$ respectively

NS = Not significant

Table 5.3 Internal ethylene concentrations for apple fruit for all bud types at different harvest dates (1988). All data were log transformed, amalgamated for all bud types and LSD calculated.

Cultivar	Harvest date	IEC Transformed data	Untransformed data (ppm)
Royal Gala	10/2	-2.50	0.20
	24/2	-1.40	0.76
	14/3	0.03	1.74
	LSD (P=0.05)	0.31	
Braeburn	21/3	-2.08	0.21
	5/4	-1.23	0.55
	18/4	-0.74	1.56
	LSD (P=0.05)	0.28	
Granny Smith	11/4	-2.70	0.07
	2/5	-2.72	0.07
	23/5	0.94	12.90
	LSD (P=0.05)	0.35	

Figure 5.1 Increase in internal ethylene concentration over the commercial harvest period for apple for three bud types (1988). Bars = ± SE. [two-year spur (●), one-year lateral (□), >three-year spur (▲)]



IECs across all three harvest dates were greatest for fruit from two-year spur buds, this difference being significant for 'Braeburn' and 'Granny Smith' (Table 5.4). Fruit from the oldest spur buds had a significantly lower IEC than fruit from both other bud types for 'Royal Gala' and 'Braeburn'. Rate of IEC increase over the harvest period tended to be lower for fruit from the oldest spur buds for 'Royal Gala' and 'Braeburn' than fruit from other bud types (Figure 5.1).

There was a wide range in IEC values, from at least 0.05 - 12.0ppm for any one bud type at any one harvest date (Figures 5.2-5.4). This variation tended to increase with increasing harvest date for the two red cultivars although at the third harvest date, 'Granny Smith' had the greatest variation, with IECs ranging from 0.1 - 65ppm.

Differences in these IEC distributions between harvest dates and bud types usually reflected differences in average IECs (seen in Figure 5.1). Generally, two-year spurs had the highest proportion of fruit in the moderate to high IEC classes and lowest proportions of fruit in the lower IEC classes. These trends were reversed for inner spurs, as they had the lowest proportions of fruit in the higher IEC classes and the highest proportions of fruit in the lower IEC classes. Fruit from one-year laterals were intermediate in this pattern. One notable exception occurred for 'Granny Smith' (Figure 5.4). At the third harvest date, one-year lateral buds had 84% of fruits producing IECs greater than 0.5ppm. In comparison, the oldest spur buds had 66% of fruits in this same category, yet the average IEC was 7ppm for both bud types. This occurred because four fruit from the oldest spurs had very high

Table 5.4 Internal ethylene concentration for apple fruit from all harvest dates picked from different bud types (1988). All data were log transformed, amalgamated for all harvest dates and LSD values calculated.

Cultivar	Bud type	IEC Transformed data	IEC Untransformed data (ppm)
Royal Gala	2 yr spur	-0.90	0.93
	1 yr lateral	-1.31	0.75
	>3 yr spur	-1.86	0.41
	LSD (P=0.05)	0.42	
Braeburn	2 yr spur	-0.72	1.33
	1 yr lateral	-1.12	0.67
	>3 yr spur	-2.20	0.33
	LSD (P=0.05)	0.32	
Granny Smith	2 yr spur	-1.14	7.48
	1 yr lateral	-1.78	2.13
	>3 yr spur	-1.71	2.43
	LSD (P=0.05)	0.39	

Figure 5.2 Proportion of fruit in each of five internal ethylene concentration classes, for three bud types, at three harvests for apple cv. 'Royal Gala' (1988).

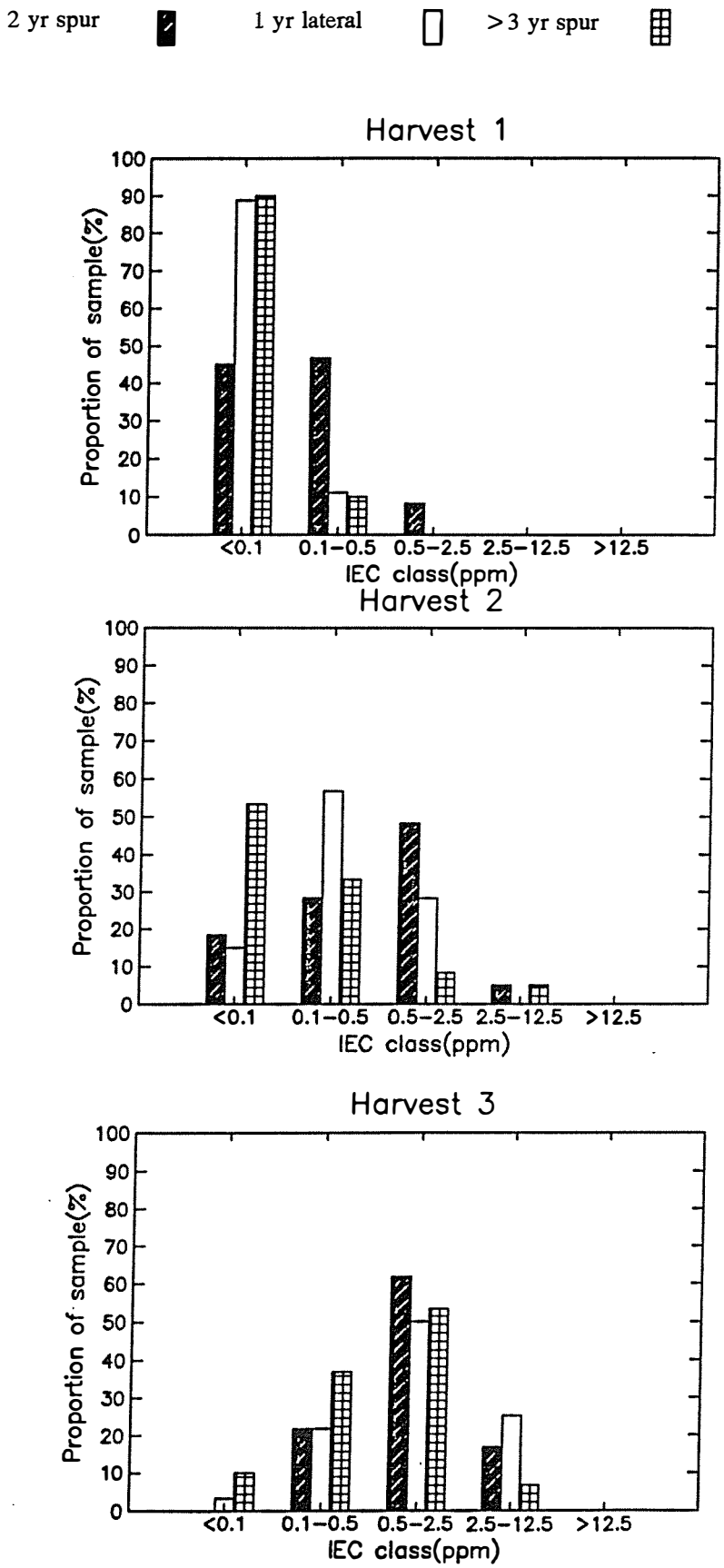


Figure 5.3 Proportion of fruit in each of five internal ethylene concentration classes, for three bud types, at three harvests for apple cv. 'Braeburn' (1988).

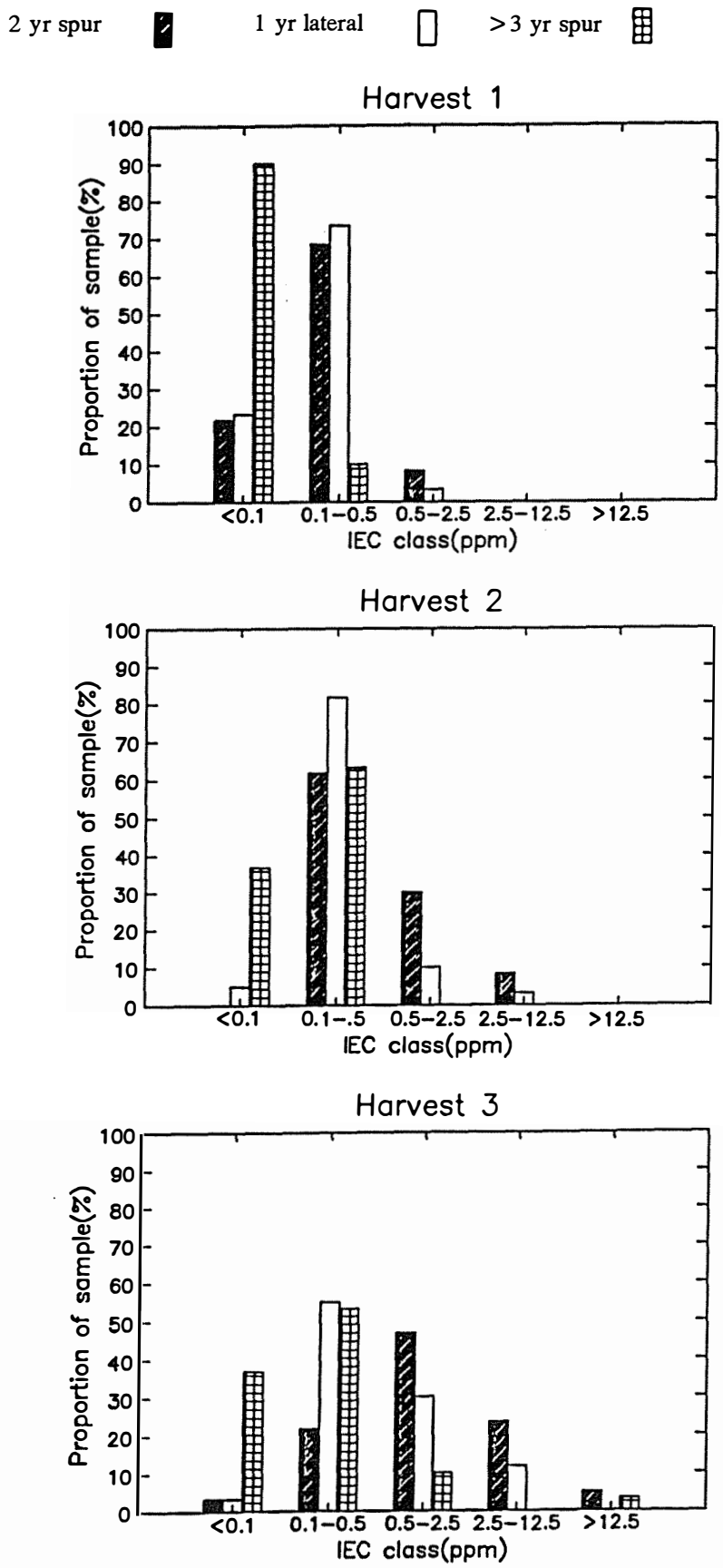


Figure 5.4 Proportion of fruit in each of five internal ethylene concentration classes, for three bud types for apple cv. 'Granny Smith'. (Harvest date = 23/5/88)

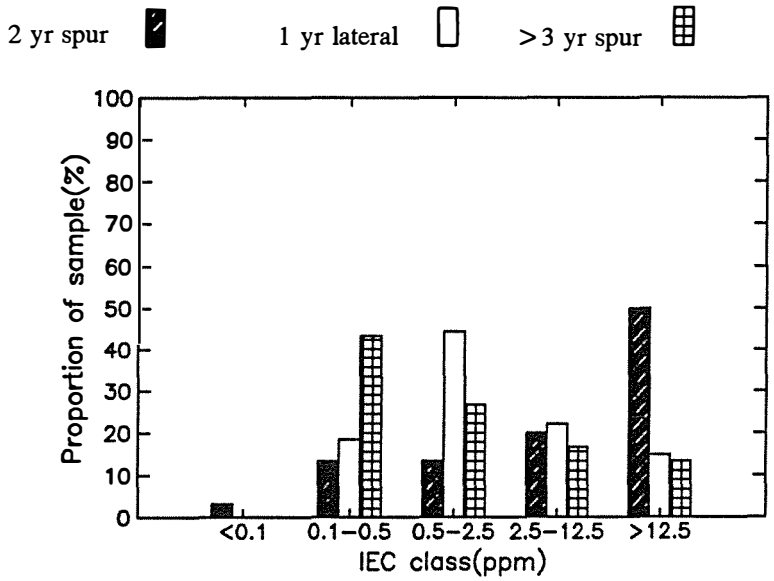


Table 5.5 Correlation coefficients (r) across all bud types and harvest dates for the relationships between log internal ethylene concentration and other maturity indices for apple (1988).

Dependent variable	Cultivar		
	Royal Gala	Braeburn	Granny Smith
Red blush	0.93 ***	0.85 ***	-
SSC	0.74 ***	0.90 ***	0 NS
Starch index[z]	0.93 ***	0.91 ***	0.54 ***
Flesh firmness	-0.74 ***	-0.65 ***	0.17 **
Blush colour	0.84 ***	0.90 ***	-
Flesh colour	0.98 ***	0.93 ***	-
Ripening index	0.98 ***	-	-
Background colour	-	-	0.40 ***

NS, **, *** Not significant, significant at $0.01 \geq P > 0.001$, $0.001 \geq P$ respectively.

SSC = Soluble solids concentration

[z] Relationships between IEC and starch index do not include measurements from fruit from > 3-year spur buds.

concentrations of 35-55ppm, whilst the highest IEC from the youngest bud type was only 18ppm.

While IECs increase during the harvest period for 'Royal Gala' and 'Braeburn' paralleled similar changes in all other ripening characteristics this was not the case for 'Granny Smith' (Table 5.5). IEC was significantly correlated with these characteristics for 'Royal Gala' and 'Braeburn' fruit. Correlation coefficients (r) were between 0.65 and 0.98 for these two cultivars, whilst those for 'Granny Smith' ranged between 0 and 0.54. Also IEC was strongly correlated with fruit fresh weight for all three cultivars (Table 5.6).

Table 5.6 Correlation coefficients (r) across all bud types and harvest dates for the relationships between fruit weight and log internal ethylene concentration for apple (1988).

Cultivar	Correlation coefficient
Royal Gala	0.89 ***
Braeburn	0.75 ***
Granny Smith	0.72 ***

*** Significant at $0.001 \geq P$

The relationship between IEC and fruit weight was further explored by correlating these two variables for individual apples for each bud type at each harvest date. However, these relationships were not significant ($P > 0.05$).

Fruit Colour

Ripening Index for 'Royal Gala' fruit increased significantly over the harvest period (Table 5.7A). Fruit from two-year spur buds had a significantly higher index than that of one-year lateral buds (Table 5.7B). Fruit from the oldest spur bud type had a significantly lower index compared to that of fruit from one and two-year spur buds.

Total percentage red blush covering the surface of the apple (Table 5.8) and blush chromaticity (a*/b*) (Table 5.9) increased significantly with time during the harvest period for 'Royal Gala' and 'Braeburn'. The increase in a*/b* indicated that blush colour became brighter and of a more intense red. These two characteristics were similar for fruit from one-year lateral and two-year spur buds, but were both significantly lower for fruit from the oldest bud type (Table 5.10, 5.11). However rate of increase of red blush coverage on fruit over the harvest period was greatest for fruit from the oldest spur type (Figure 5.5).

Flesh chromaticity (-a*:b*) decreased with harvest date for 'Royal Gala' and 'Braeburn' (Table 5.9). This indicated that fruit flesh became less green as the fruit matured. Flesh of fruit from two-year spur buds had less green flesh during the harvest period, than fruit from both other spur types, while fruit from one-year lateral buds was less green than fruit from the oldest spur buds (Table 5.11).

The chromaticity of the skin of 'Granny Smith' fruit, (-a*:b*), generally decreased over the harvest period (Table 5.12A), indicating that the fruit became less green and more yellow. Fruit from two-year spur buds were generally less green and more yellow than fruit from both other buds types (Table 5.12B). However, the

Table 5.7 'Ripening Index' of 'Royal Gala' apple fruit for all bud types from different harvest dates (A) and from all harvest dates for different bud types (B) (1988). 'Ripening Index' is expressed on a scale of 1 (immature) to 12 (overmature) using the NZAPMB colour charts.

A

Harvest date	Ripening Index
10/2	2.1
24/2	3.9
14/3	6.4
LSD (P=0.05)	0.4

B

Bud type	Ripening Index
2 yr spur	5.0
1 yr lateral	4.2
>3 yr spur	3.3
LSD (P=0.05)	0.4

Table 5.8 Red blush coverage for apple fruit for all bud types from different harvest dates (1988). All data were transformed (arcsin [sqrt (% blush x 0.01)]) and LSD values were used to separate harvest date means.

Cultivar	Harvest date	Red blush Transformed data	Red blush Untransformed data (%)
Royal Gala	10/2	0.66	41.3
	24/2	1.07	71.9
	14/3	1.47	95.3
	LSD (P=0.05)	0.09	
Braeburn	21/3	0.74	50.4
	5/4	0.90	61.7
	18/4	1.07	75.1
	LSD (P=0.05)	0.06	

Table 5.9 Blushed skin colour and flesh colour for apple fruit for all buds types from different harvest dates (1988). Blushed skin colour derived from the ratio of red (a*) to yellow (b*) colour of the total chromaticity of the fruit skin. Flesh colour derived from the ratio of green (-a*) to yellow (b*) colour of the total chromaticity of the flesh.

Cultivar	Harvest date	Blushed skin colour	Flesh colour
Royal Gala	24/2	1.20	0.397
	14/3	1.45	0.293
	LSD (P=0.05)	0.10	0.022
Braeburn	21/3	0.70	0.414
	5/4	0.89	0.354
	18/4	1.24	0.311
	LSD (P=0.05)	0.10	0.011

Table 5.10 Red blush coverage for apple fruit from all harvest dates for different bud types (1988). All data were transformed (arcsin [sqrt (% blush x 0.01)]) and LSD values were used to separate harvest bud type means.

Cultivar	Bud type	Red blush	
		Transformed data	Untransformed data (%)
Royal Gala	2 yr spur	1.17	77.1
	1 yr lateral	1.12	73.7
	>3 yr spur	0.91	57.8
	LSD (P=0.05)	0.09	
Braeburn	2 yr spur	1.09	76.9
	1 yr lateral	1.10	78.0
	>3 yr spur	0.53	32.3
	LSD (P=0.05)	0.08	

Table 5.11 Red blush coverage for apple fruit from all harvest dates for different bud types (1988). Blushed skin colour derived from the ratio of red (a*) to yellow (b*) colour of the total chromaticity of the fruit skin. Flesh colour derived from the ratio of green (-a*) to yellow (b*) colour of the total chromaticity of the flesh.

Cultivar	Bud type	Blushed skin colour	Flesh colour
Royal Gala	2 yr spur	1.44	.318
	1 yr lateral	1.40	.340
	>3 yr spur	1.13	.377
	LSD (P=0.05)	0.13	.029
Braeburn	2 yr spur	1.06	.334
	1 yr lateral	1.08	.349
	>3 yr spur	0.69	.397
	LSD (P=0.05)	0.14	.012

Figure 5.5 Red blush on apple fruit over the harvest period for different bud types (1988). Bars = ± SE. [two-year spur (●), one-year lateral (□), >three-year spur (▲)]

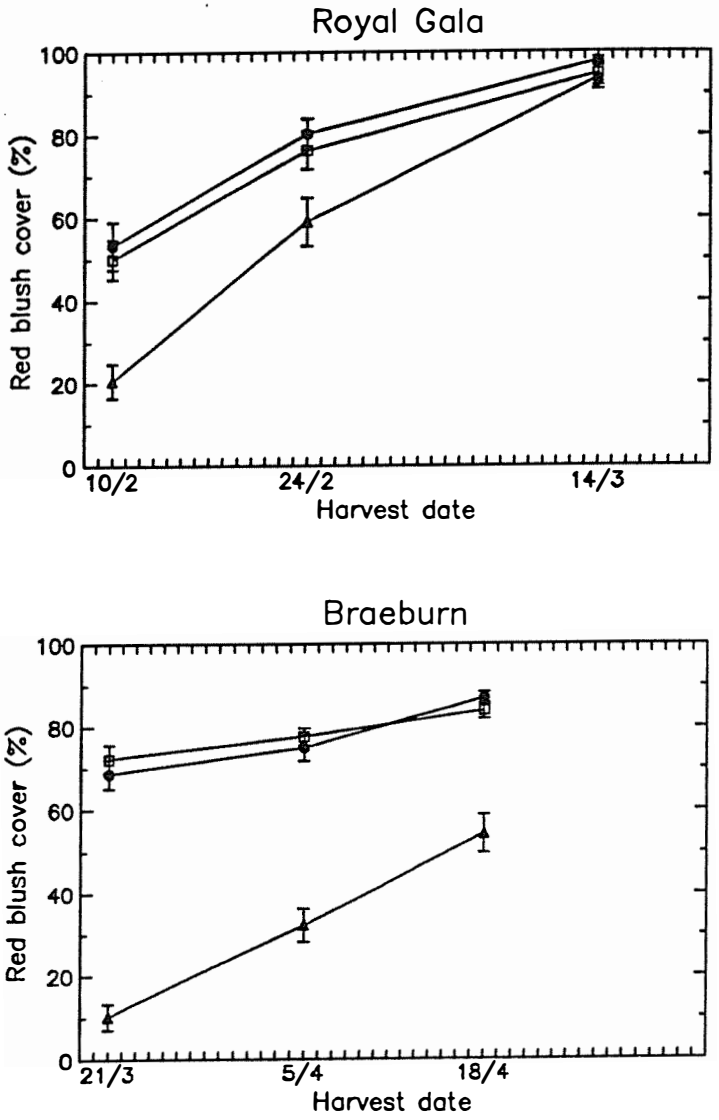


Table 5.12 Skin colour of 'Granny Smith' apple for all bud types from different harvest dates (A) and from all harvest dates for different bud types (B) (1988). Background skin colour derived from the ratio of green (-a*) to yellow (b*) colour of the total chromaticity of the skin.

A

Harvest date	Background colour
11/4	0.493
2/5	0.486
23/5	0.454
LSD (P=0.05)	0.006

B

Bud type	Background colour
1 yr lateral	0.485
2 yr spur	0.465
>3 yr spur	0.486
LSD (P=0.05)	0.013

extent of these differences between bud types was dependent on harvest date (Figure 5.6). Fruit from buds on old spurs (>three years) were consistently greener than fruit from two-year spur buds, across the harvest period. There was no difference in background colour between fruit from one-year lateral and two-year spurs on 11/4. There was no colour change for fruit from one-year lateral buds between 11/4 and 2/5, whereas fruit from two-year spur buds became less green. After the middle harvest date, fruit from both these bud types became less green at a similar rate, so that by 23/5 fruit from one-year lateral buds remained greener than those from two-year spur buds.

Fruit Weight

Fruit fresh weight increased with harvest date, with the rate of increase being greatest for 'Braeburn' and least for 'Granny Smith' (Table 5.13). Fruit from two-year spur buds were heavier than fruit from both the other spur types (Table 5.14). However differences between fruit from one-year and the oldest bud type were cultivar dependent. There was no consistent difference between fruit from the different spur bud types in rate of fresh weight increase over the harvest period.

Flesh Firmness

Flesh firmness generally decreased with time during the harvest period, except between the middle and last harvest dates for 'Granny Smith' (Table 5.13). Fruit from one-year lateral bud spurs were consistently and significantly firmer than fruit from two-year spur buds for all three cultivars, whilst fruit from the oldest spur

Figure 5.6 Change in skin colour over the harvest period for different bud types for 'Granny Smith' apple (1988). Bars = ± SE. [two-year spur (●), one-year lateral (□), >three-year spur (▲)]

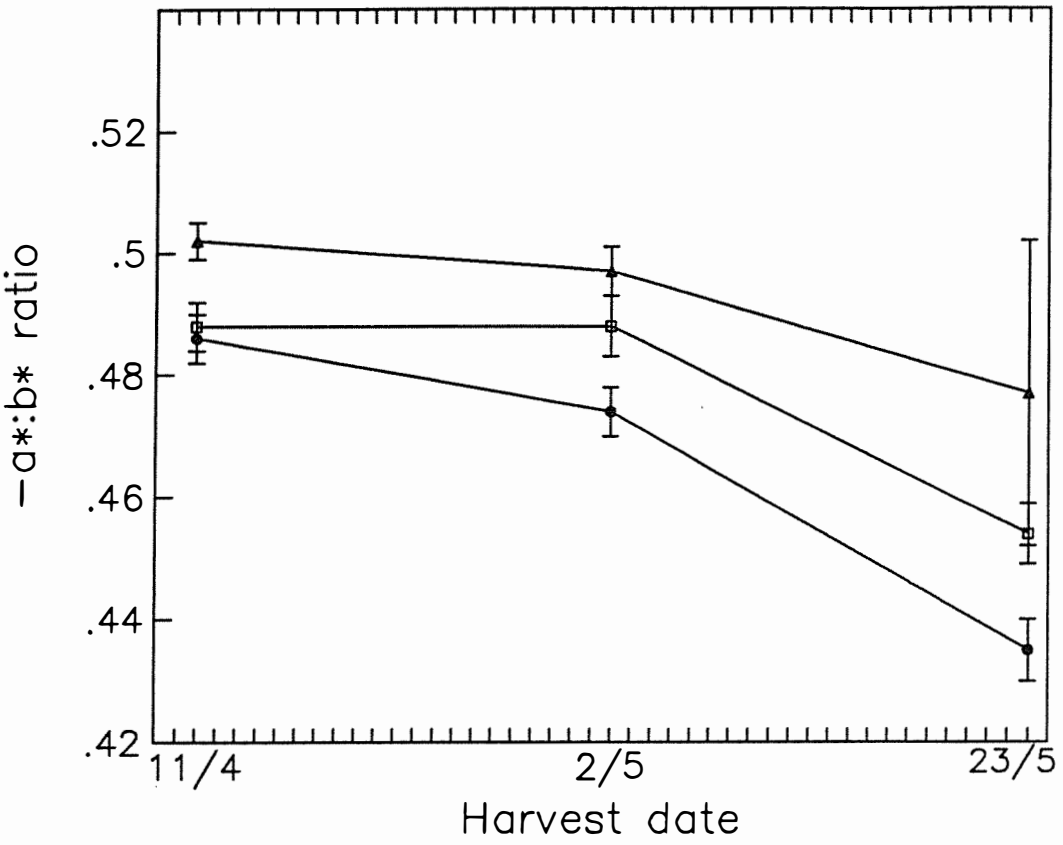


Table 5.13 Fruit fresh weight, flesh firmness, soluble solids concentration and starch index pattern for apple for all bud types from different harvest dates (1988). Starch index pattern is expressed on a scale of 1 (immature) to 6 (mature) (see text).

Cultivar	Harvest date	Fruit Weight (g)	Flesh Firmness (kg)	SSC (%)	Starch Index
Royal Gala	10/2	95.7	10.1	10.4	1.3
	24/2	107.3	9.4	10.4	2.6
	14/3	120.0	8.3	11.1	5.2
	LSD (P=0.05)	5.2	0.2	0.3	0.4
Braeburn	21/3	157.8	8.9	11.0	2.1
	5/4	168.5	8.3	11.3	2.8
	18/4	183.4	8.0	12.0	3.9
	LSD (P=0.05)	8.9	0.1	0.2	0.3
Granny Smith	11/4	147.3	8.3	12.2	3.4
	2/5	153.3	7.6	11.8	4.9
	23/5	155.8	7.7	11.9	6.0
	LSD (P=0.05)	10.5	0.1	0.3	0.3

Table 5.14 Fruit fresh weight, flesh firmness, soluble solids concentration and starch index pattern for apple from all harvest dates for different bud types (1988). Starch index pattern is expressed on a scale of 1 (immature) to 6 (mature) (see text).

Cultivar	Bud type	Fruit Weight (g)	Flesh Firmness (kg)	SSC (%)	Starch Index
Royal Gala	2 yr spur	127.4	8.7	11.0	3.8
	1 yr lateral	98.1	10.1	10.9	2.3
	>3 yr spur	97.6	9.1	10.0	---
	LSD (P=0.05)	5.2	0.2	0.4	0.2
Braeburn	2 yr spur	188.2	8.1	11.8	3.4
	1 yr lateral	171.8	8.6	11.9	2.4
	>3 yr spur	149.7	8.5	10.6	---
	LSD (P=0.05)	6.7	0.1	0.3	0.4
Granny Smith	2 yr spur	177.0	7.5	12.1	4.7
	1 yr lateral	130.7	8.3	12.2	4.7
	>3 yr spur	144.5	7.9	11.5	---
	LSD (P=0.05)	14.5	0.2	0.4	0.2

buds were intermediate (Table 5.14). For 'Royal Gala', rate of flesh firmness reduction over the harvest period was greater for fruit from one-year lateral buds, than for fruit from two-year and old (> three-year) spur buds (Figure 5.7), however this did not occur for 'Granny Smith' or 'Braeburn'.

Starch Index and SSC

SSC and the starch pattern index increased with harvest date, except for 'Granny Smith', where SSC decreased from 12.2% to 11.9% and 'Royal Gala' between 10/2 and 24/2 (Table 5.13). There was no difference in SSC between fruit from one-year lateral and two-year spur buds. However SSC in fruit from the oldest spur bud type was significantly lower than in fruit from younger spur types, for all three cultivars. Starch index was greater for fruit from two-year spur buds than for fruit from one-year lateral spur buds, across all three harvest dates for both 'Royal Gala' and 'Braeburn'. However the rate of starch index change over the harvest period was greatest for fruit from the youngest bud type (Figure 5.8). Similar Starch index patterns were similar for fruit of both bud types in 'Granny Smith'

5.4 Discussion

5.4.1 Fruit Size

Fruit found on two-year spurs on the outside of the canopy were larger than fruit on the inside. Other studies have also indicated that fruit on the outside of the

Figure 5.7 Reduction in flesh firmness over the harvest period for different bud types for 'Royal Gala' apple (1988). Bars = ± SE. [two-year spur (●), one-year lateral (□), >three-year spur (▲)]

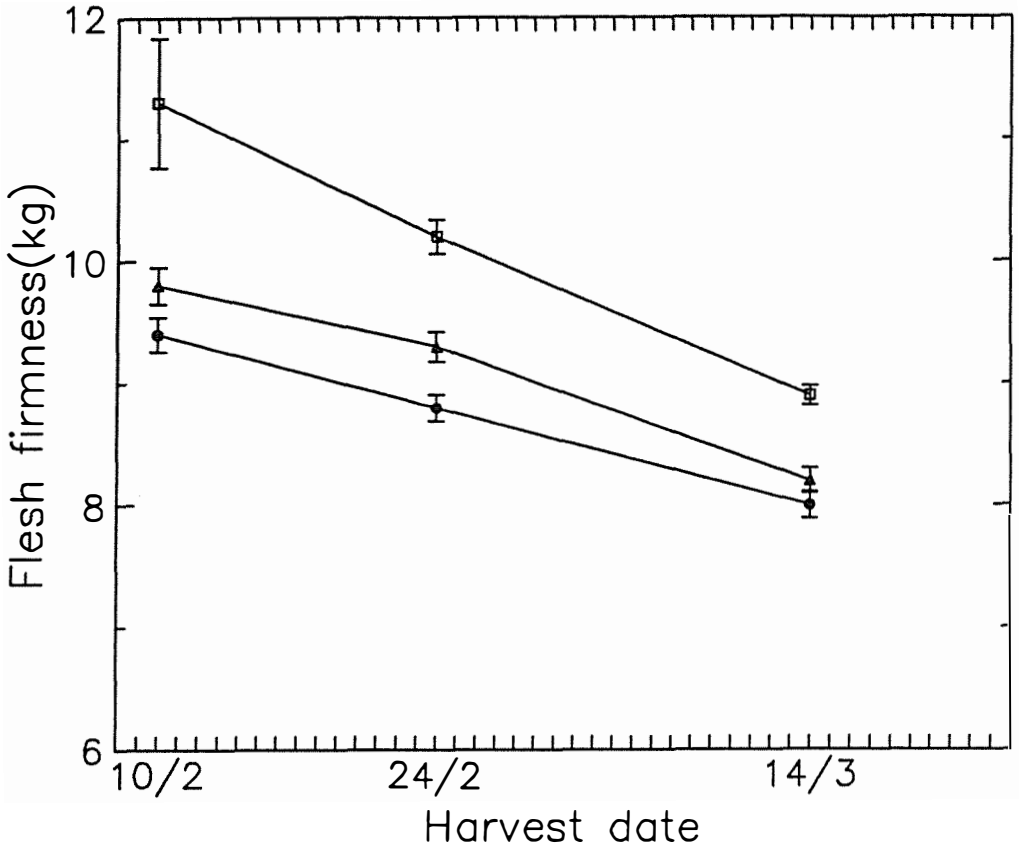
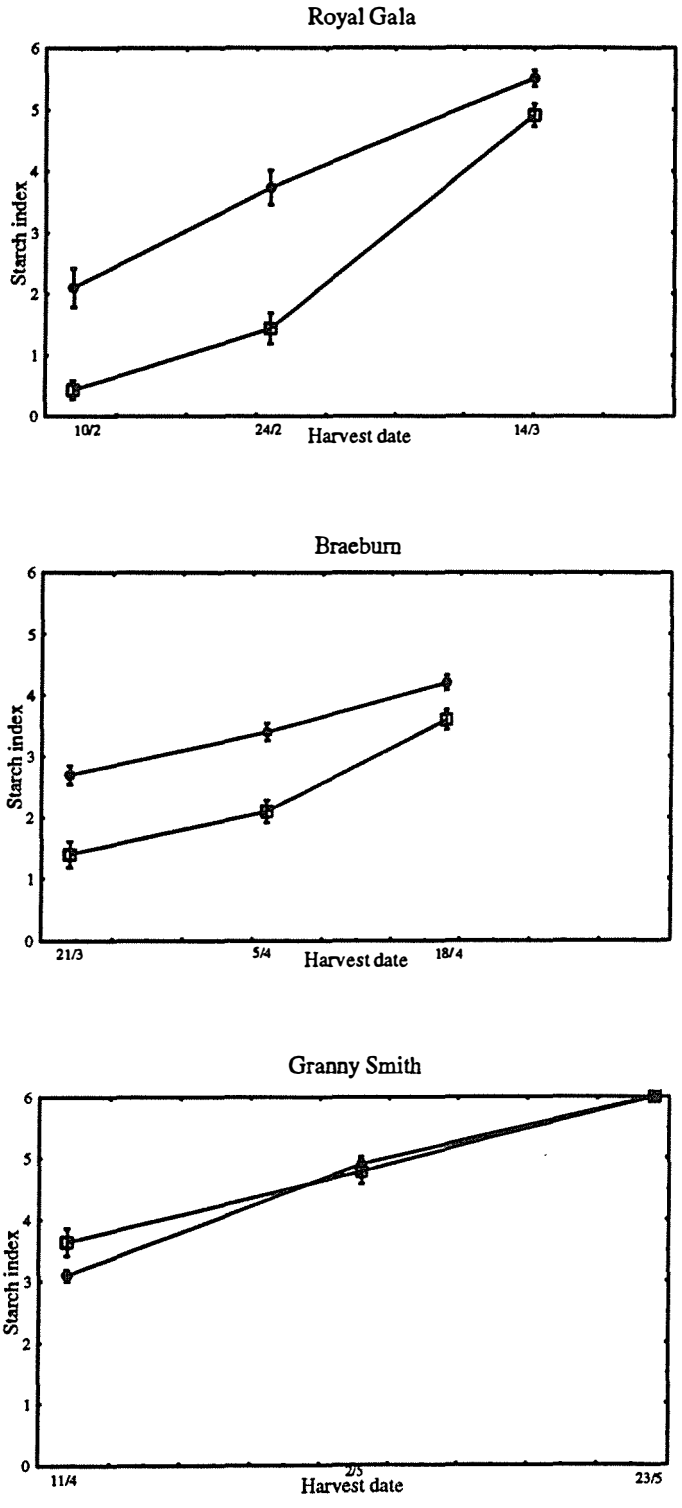


Figure 5.8 Increase in starch index over the harvest period for different bud types for apple (1988). Bars = ± SE. [two-year spur (●), one-year lateral (□)]



tree are larger than those inside the tree canopy (Morgan et al., 1984; Tustin et al., 1988). Fruit on two-year spur buds were also larger than fruit on the one-year lateral buds. Discussion of such differences has been presented in Chapter 4.

5.4.2 Influence of Harvest Date on Physiological Maturity

The logarithmic increase in internal ethylene concentration found within the fruit over the harvest period in this experiment is known to occur in apple (Reid et al., 1973; Walsh, 1977a). However in the present study, differences between cultivars were apparent. Both 'Royal Gala' and 'Braeburn' fruit showed a moderate increase in the rate of IEC change over their four week harvest period as indicated by an increasing percentage of individual fruits, from all the bud types, with IECs >0.1ppm. These results agree with published studies which assessed ethylene production for these two cultivars over the harvest season (Watkins et al., 1989a; Walsh and Volz, 1990). In contrast, 'Granny Smith' fruit had a much higher rate of IEC increase between the final two harvest dates, where the percentage of fruit with high IECs (>2.5ppm) increased dramatically. Watkins et al. (1982a) have commented that the end of the commercial harvest season coincided with the climacteric for 'Granny Smith'.

Substantial differences in the time of the onset of the ethylene rise and rate of increase have been noted between cultivars (Chu, 1988; Watkins et al., 1989a). Differences between cultivars in the timing of the increase, rates of change and maximum IEC may represent inherent physiological differences in the capacity of apples to produce autocatalytic ethylene. However they may also indicate differences

in ripening characteristics of the population of sampled fruits. For instance 'Delicious' (Farhoomand et al., 1977) and 'Cox's Orange Pippin' (Reid et al., 1973) can produce "a few" high-ethylene producing fruit early during the harvest period. This was not found for any of the three cultivars tested in the present study.

It is however, unwise to make comparisons between cultivars based on results from one season and at different orchard sites. The pattern of average IEC levels over the harvest period for any one cultivar can vary significantly in different years (Chu, 1988). Rootstock (Fallahi et al., 1985), and crop load (Sharples, 1964) may also affect the timing of the climacteric.

5.4.3 Influence of Bud Type on Physiological Maturity

For all three cultivars a considerable difference occurred in average IEC between fruit from different bud types (Table 5.4). This was mainly due to differences in the percentage of fruit with IEC's below 2.5ppm for 'Royal Gala' and 'Braeburn' (Figures, 5.2, 5.3). For these two cultivars, apples borne on the three bud types would seem to have different capacities to initiate the ethylene rise and/or different sensitivities to endogenous ethylene which affects autocatalysis. Once autocatalysis has been initiated, fruits would seem to have similar ethylene production rates and maxima.

Fruit located on old spurs within the tree canopy had a lower average IEC than fruit from two-year spur buds on all three cultivars and also fruit from one-year lateral spur buds of 'Royal Gala' and 'Braeburn'. There was also a tendency for fruit from old spurs to ripen at a slower rate than fruit on the replacement branch for

these two cultivars (Figure 5.1). The delay in the ethylene rise also occurred after that from fruit from one-year spur buds. Flowering on old spur buds occurred between the two other bud types, indicating that their low IECs could not be explained by simple fruit ageing.

Most other studies have compared maturity characteristics of fruit from lower and upper parts of the tree canopy making direct comparisons with the present study difficult. Robinson et al. (1983) quoted work by Chu (1980), who found greater IECs in fruits from the upper canopy position compared to those in a lower canopy position. In contrast, Farhoomand et al. (1977) found a higher ethylene production in fruit harvested from the base of the tree compared to fruit at the top of the tree. However that study did not account for fruit size differences between the two within-tree locations. Small fruit naturally produce higher ethylene for any given IEC, compared to a larger fruit because of a greater surface area to volume ratio. If Farhoomand et al. (1977) had expressed ethylene production as a function of fruit fresh weight they may well have shown a different trend.

Differences in timing of the increase in IEC for fruit from inner and outer parts of the canopy are probably not related to differences in the light environment. Shading whole 'Cox's Orange Pippin' trees during the growing season was shown to reduce maximum ethylene production rate, but had little effect on the timing of increase (Jackson et al., 1977). In the present study, there was a tendency for 'Royal Gala' and 'Braeburn' fruit from the old spurs to have a lower rate of IEC increase, which agrees with results from an earlier study on 'Cortland' apple (Meir

and Bramlage, 1988). However the main difference between these fruit and fruit on the younger spurs was the percentage of fruit with low IECs.

Of perhaps more importance than shade in explaining variation in fruit ripening times between those located on the inside and outside of a tree is the variation in temperature which exists between different parts of a tree. Leaves and fruit exposed to the sun can be up to 10-14°C warmer than those organs located in the shade (Thorpe and Butler, 1977; Robinson et al., 1983). It is well known that ethylene production by tissues is greatly influenced by temperature. For apple, fruit picked when air temperatures were low had a lower ethylene production rates (Reid et al., 1973) or a lower internal ethylene concentration (Fallahi et al., 1985), relative to fruit picked at higher temperatures. Ethylene production from woody tissue would seem to respond in a similar fashion to temperature as to that of fruit (Walsh and Kender, 1980). In the present study harvested fruit were left to equilibrate at a constant temperature 12 hrs before IEC sampling. If within-tree differences in temperature were having some influence on the variation in the timing of fruit ripening, then this would not simply be a short-term response.

However, there is some evidence that accumulated temperature over a long period influences apple fruit ripening. From a number of weather variables, including length of growing season, accumulated temperature from June to September was shown to correlate best with the "recommended" harvest date for 'Cox's Orange Pippin' apple in England (Luton and Hamer, 1983). They concluded that a 1°C decrease in mean daily ambient temperature would delay ripening by 4 days, and

suggested that such a decrease would also cause a reduction in growth rate of the fruit. Certainly, fruit on old spurs were smaller than fruit on (two-year) outer-located spurs in the present study (Table 5.14). Smaller fruit at harvest were less advanced in ripening than larger fruit for all cultivars (Table 5.6). Cultural practices such as thinning increase the rate of development of fruit and advance fruit ripening (Sharples, 1964). Indeed, it is well known that the duration of a developmental process is simply the reciprocal of its rate of development (Squire, 1990). Thus, the lower temperature accumulated over time (degree days) by fruit located inside the canopy, particularly in the latter part of the growing season, may well explain their delayed ripening compared with those fruit located on the outside of the canopy.

Another possible explanation for these differences in ripening patterns is that maturity of inner-located fruit is inhibited by growth substances originating from the roots, as suggested by Dann and Jerie (1988). These workers found that peach fruit within the canopy were smaller and physiologically less mature than fruit in the upper part of the canopy at harvest and also when measured earlier in the season. These differences could not easily be explained by differences in light levels or the time of flowering. Although it appears that tree trunk cambial growth may indeed be regulated by hormones produced from roots (and shoots) (Chalmers, 1985), the mechanism by which plant hormones from roots and shoots control fruit growth and ripening remains very speculative. Further discussion on this subject occurs in Chapter 8.

The delay in IEC rise in fruit from one-year lateral buds compared with those fruit from two-year spurs, was approximately one week for both 'Royal Gala' and

'Braeburn' cultivars. This difference may well reflect the age of the fruits. Fruit age is a major factor affecting the initiation of fruit ripening (Rhodes, 1980). Differences in blossom date between fruit from both bud types on replacement branches were also approximately one week (Chapter 3). 'Delicious' apples harvested from early flowering spurs ripen earlier than those from later flowering spurs (Sullivan, 1965). In sweet cherry, bloom date was shown to be a causative factor in variability of fruit maturity and quality (Patten et al., 1986). However, differences in fruit age may also reflect different rates of fruit growth, as suggested in the preceding discussion on inner and outer located fruit. Fruit on one-year laterals have a lower growth rate in the latter part of the season, than fruit on two-year spurs (Chapter 4). Thus fruit age, as determined by a fruit's date of anthesis and its rate of development during the season would seem to be important in influencing the timing of fruit ripening.

On the other hand, leaf area associated with one-year lateral buds is considerably less than that of two-year spur buds (Chapter 3). Deleafing and ring-barking spurs advances fruit ripening suggesting that leaves or a substance produced from the leaves may inhibit this process (Sfakiotakis and Dilley, 1973). If the amount of subtending spur leaf area is important in inhibiting fruit ripening, then it might have been expected that fruit from two-year spur buds would have been somewhat delayed in maturity compared to those from the one-year lateral buds. This was not the case, and this study provides little evidence that spur leaf area plays a major role in determining differences in the timing of fruit ripening between bud types. It would have been interesting to deleaf spurs from both bud types at mid-season to investigate possible leaf effects on fruit ripening.

5.4.4 Influences of Harvest Date and Bud Type on Fruit Colour

Ethylene is probably a major regulator of fruit colour for the three apple cultivars tested in this experiment. For 'Granny Smith', the relatively large decrease in green skin colour between the middle and final harvest dates was associated with a substantial increase in IEC at this time. There also was a general trend for flesh colour of 'Royal Gala' and 'Braeburn' fruit to become more yellow and less green during maturation. These changes in flesh colour probably reflected similar changes in background skin colour, as has been found on peaches (Sims and Comin, 1965). Red colour coverage over the skin and red blush colour increased over the harvest period for 'Royal Gala' and 'Braeburn' (Table 5.8, 5.9). For the two red cultivars, both red colour and flesh colour changes correlated well with changes in IECs (Table 5.5).

Skin colour changes during apple fruit maturation have often been associated with the action of ethylene. Preharvest applications of ethylene-releasing compounds to apple trees promote anthocyanin formation in the fruit skin of many cultivars (Saure, 1990), while also advancing background colour at harvest (Smith et al., 1985; Watkins et al., 1989b). An increase in the activity of the critical enzyme for anthocyanin formation (PAL) and anthocyanin formation has been linked to the beginning of natural ethylene production in several cultivars (Kubo et al., 1988). The yellowing/degreening associated with apple fruit ripening seems to be mainly due to chlorophyll degradation (Knee, 1972; Gorski and Creasy, 1977; Mussini et al., 1985). Ethylene control of this process may occur *via* regulation of chlorophyllase, the major enzyme involved in chlorophyll breakdown (Looney and Paterson, 1967)

and/or disruption of chloroplast membranes (Saure, 1990). Yellow pigments can increase during apple ripening (Knee, 1972) and carotenoid biosynthesis may be enhanced by exogenous ethylene applications, at least for citrus (Gross, 1987). However, most other studies on apple have not associated carotenoid biosynthesis with apple fruit ripening (Gorski and Creasy, 1977; Mussini et al., 1985).

Some slight skin degreening/yellowing on 'Granny Smith' fruit did occur before the early and middle harvest dates, before the ethylene upsurge. This colour change has been noted throughout the season for 'Granny Smith' in a previous study (Hirst et al., 1990). It may be linked to a reduction in skin chlorophyll concentration as the fruit expands during development (Mussini et al., 1985), so unmasking yellow pigments present. Knee et al. (1989) found that peel chlorophyll concentration in 'Cox's Orange Pippin' apples decreased before ripening occurred. They showed that this was due to a dilution of chlorophyll content in the skin of fruit rather than to any changes in chlorophyll metabolism. However, it is also possible that carotenoid biosynthesis may also occur before fruit ripening (Gross, 1987). Clearly, green/yellow skin colour changes during maturation can be independent of ethylene action, but in many cases is accentuated by this hormone.

In the present study, fruit from the oldest spur buds (> three years) growing inside the tree canopy, had a greener/less yellow skin colour than fruit on two-year spurs (Table 5.13B) ('Granny Smith'), or had poorer red colour development and greener flesh colour than fruit on replacement branches growing on the outside of the canopy ('Royal Gala' and 'Braeburn') (Table 5.11, Figure 5.5). These results confirm other studies for apple canopies (Jackson et al., 1971; Jackson, 1980;

Seeley et al., 1980) which showed that reduced light penetration inside a tree canopy is a major cause of variation in skin colour found for apple fruit.

However it can be argued that differences in the ground skin or flesh colours between "inner" and "outer" located fruits were due, at least in part, to differences in fruit maturity. In conditions where light levels were similar, fruit from one-year lateral and two-year spur buds showed different patterns of green skin colour change ('Granny Smith') (Figure 5.6) or different flesh colours ('Royal Gala' and 'Braeburn') (Table 5.11) over the harvest period. These were accompanied by similar differences in IECs. Morgan et al. (1984) and Tustin et al. (1988) working on 'Gala' and 'Granny Smith' respectively, found that fruit inside the tree were greener than fruit on the outside, but this could not be correlated with light levels.

Stage of fruit maturity may have a large bearing on the development of red colour for fruit inside the tree canopy. 'Royal Gala' and 'Braeburn' fruit from the oldest bud type had a more rapid rate of blush increase than fruit on the outside of the canopy. On the other hand, differences in blush coverage or blush intensity for fruit grown in a similar light environment seemed little influenced by fruit maturity differences. There were no differences in these two colour characteristics between fruit borne on two-year spur and one-year lateral buds for 'Braeburn' or 'Royal Gala', despite these fruit being of different physiological maturities.

These results indicate that in "high" light conditions anthocyanin synthesis and/or degradation is independent of ethylene action. However, where light becomes limiting, ethylene concentrations may regulate anthocyanin formation, as suggested

by Chalmers et al. (1973). High light or high ethylene levels may independently increase PAL enzyme, thereby stimulating anthocyanin formation (Saure, 1990).

The Ripening Index Chart, developed by the New Zealand Apple and Pear Marketing Board for 'Royal Gala', employs a combination of red and ground colour chromaticity combinations to describe maturity changes for this cultivar. Indeed, this index was the best measure of fruit maturity for 'Royal Gala' on the basis of correlation with IEC and the associated differences between bud types and harvest dates. However, select picking fruit commercially on the basis of % red blush (for 'Braeburn'), red colour chromaticity or in some cases 'green' skin colour (for 'Granny Smith'), may lead to harvesting fruit with different physiological maturities.

5.4.5 Influence of Harvest Date and Bud Type on Internal Fruit Quality

The general reduction in fruit flesh firmness and increase in soluble solids concentration observed in this study (except for 'Granny Smith') and starch pattern index during maturation has been noted for several apple cultivars (Smock, 1948; Reid et al., 1982; Lau, 1985). However substantial differences in these parameters between fruit from the different bud types were maintained throughout the harvest season (Table 5.14).

The starch index pattern was higher for fruit from two-year spur buds than fruit from one-year lateral buds for 'Royal Gala' and 'Braeburn'. It is unlikely that this difference occurred as a result of higher overall levels of starch in fruit from one-year lateral buds at the beginning of maturation, although this, of course, is not certain. Growth rates are greater for fruit from two-year spur buds than for fruit

from one-year lateral buds (Chapter 4) indicating that amount of carbohydrate imported into fruit is likely to be greater (Ho, 1988) and starch levels higher for fruit from two-year spur buds.

Differences in starch index pattern are more likely to indicate that starch conversion to soluble sugars began earlier for fruit from two-year spur buds than fruit from one-year lateral buds for 'Royal Gala' and 'Braeburn'. This result and the very good correlation between starch index and IEC (Table 5.5) suggests that for some apple cultivars, starch hydrolysis may be regulated by ethylene. Exogenous ethylene applied to attached apple fruit advances the beginning and/or rate of starch hydrolysis (Watkins et al., 1989b) while the initiation of starch hydrolysis for some other apple cultivars has been correlated with ethylene concentration (Lau, 1985; Lau, 1988). A critical ethylene level in apple may be required to activate a starch hydrolysing enzyme, such as an amylase. However, there was a greater rate of change of the starch index pattern over the harvest period for fruit from one-year lateral buds compared with fruit from two-year spur buds, even though rates of change of IEC with time was similar. This indicates that other factors may also be important in controlling starch degradation.

However some other results do not support this hypothesis. For some cultivars, changes in starch index pattern occur well before the ethylene increase [for instance 'Granny Smith' in the present study and the work of Watkins et al. (1982a) and for 'Golden Delicious' Lau et al. (1986)]. Also in the present study, there was no difference in starch index between 'Granny Smith' fruit from two-year spur and one-year lateral buds, despite large differences in their IECs. Different relationships

for starch hydrolysis and ethylene production during fruit maturation or different cultivars may simply reflect genetic differences in response. However this is not likely as there is a relatively high degree of genetic uniformity in most of the apple cultivars quoted above, compared with cultivar variation in many other crop species (despite significant differences in some characteristics which occur).

One possibility in explaining differences is the influence of very low ethylene levels on enzyme production/activation. Recently, significant increases in ethylene below 0.1 ppm have been measured using laser detection techniques in several cut flower species, before the autocatalytic surge in ethylene production (Woltering and Harren, 1989). These authors suggested that low basal changes in ethylene production may trigger senescent processes. Adapting this hypothesis to apple fruit, one might therefore speculate that for some cultivars very low increases in IEC not detected using conventional gas chromatogram methods, may stimulate ripening-associated changes in fruit quality (eg. those changes associated with starch hydrolytic enzymes).

Larger fruit are generally softer than smaller sized fruit at harvest (Marmo et al., 1985) but the physiological reasons for this are not clear. In the present experiment, the larger size of fruit from two-year spur buds could account for their lower flesh firmness, compared with fruit from one-year or the oldest "inner" spur buds. However for 'Royal Gala', fruit from one-year buds were firmer than fruit from the oldest bud type, yet fruit size was similar. Similarly, fruit size was markedly reduced on the oldest spur buds for 'Braeburn' yet flesh firmness was similar to that of fruit from the one-year lateral buds. Clearly these differences do

not relate to maturity as fruit from one-year lateral buds had greater IECs than fruit from the oldest spurs. In some other studies where weight of fruit from inside the tree canopy was significantly reduced, flesh firmness did not show an increase Heinicke 1966; Seeley et al., 1980). The nature of factors influencing variation in flesh firmness of apple fruit within the tree canopy, independent of fruit size, are not known.

Soluble solids concentrations were considerably reduced in fruit from spur buds located inside the canopy compared with fruits on the outside of the canopy, confirming other studies (Morgan et al., 1984; Tustin et al., 1988). There was little difference in the soluble solids concentration between fruit from the two bud types located on the outside of the canopy, where light conditions were similar, despite large differences in IEC. As the starch index pattern was also more advanced in fruit from two-year spur buds for 'Royal Gala' and 'Braeburn', a higher concentration of sucrose and therefore soluble solids derived from starch might have been expected in these fruits. This was not the case. Possibly utilization of sugars for respiration may be higher for fruit from two-year spur buds. Alternatively total carbon content in fruit from one-year lateral may have been greater than those fruit from two-year spur buds.

In summary, apple fruits from two-year spur buds were larger, had higher IECs, and were softer with less green/more yellow flesh or skin colour than fruit from one-year lateral buds. However red blush development and sugar levels were little influenced by either bud type. Fruit on older spurs (>three years), inside the tree canopy, were less mature based on IEC values than fruit from both other bud

types on the replacement branch. They also had poorer red blush development, greener/less yellow skin or flesh colour and lower sugar levels.

It is suggested that within-canopy variation in fruit "age" has a major bearing on the variation in the timing of fruit ripening between bud types. Differences in fruit "age" may be caused by differences in the date of anthesis and rate of fruit development during the later part of the growing, as influenced by flower receptacle size at bloom (Chapter 4) and within-canopy variation in temperature.

CHAPTER SIX

FRUIT MINERAL NUTRITION

6.1 Introduction

Considerable variation in fruit size and quality occurs within apple replacement branches. Mineral concentrations of individual apples are also known to vary within a population of fruit on a tree - this variation often may be associated with a number of factors, particularly fruit size (Perring and Jackson, 1975). As fruit size increases, calcium concentration often decreases, while potassium and magnesium concentrations increase.

Several within-tree positional factors affect growth and final size of fruit (Chapter 4) and indirectly influence mineral concentration. However some positional factors are also known to affect uptake of minerals by developing fruitlets. For instance, fruit inside the tree canopy may have a higher calcium but a lower magnesium, nitrogen, and potassium content compared with fruit of the same size in upper parts of the canopy (Jackson et al., 1971; Haynes and Goh, 1980). It therefore might be expected that some variation in mineral concentration and accumulation might occur in fruit from different bud types.

6.1.1 Influence of Fruit Mineral Levels on Apple Quality

Why is it important to know of sources of variation in apple fruit mineral concentrations at harvest? Mineral concentrations in apple fruit are important in determining fruit quality at harvest and after storage. Delay of ripening and senescence of many fruits, including apple, have also been associated with high fruit calcium concentrations (Faust and Shear, 1972; Bramlage et al., 1974). Many apple storage disorders such as bitter pit, senescent breakdown, lenticel blotch and water core may be influenced by the contents of calcium, potassium, magnesium and nitrogen in fruit (Shear, 1975; Perring, 1984). For instance, high levels of bitter pit have been related to low fruit calcium concentrations. This relationship has been quantified and used as a method of predicting the incidence of this disorder in various lines of fruit (Perring and Sharples, 1975; Ferguson et al., 1979; Marmo et al., 1985). The New Zealand Apple and Pear Marketing Board does not allow 'Cox's Orange Pippin' fruit to be exported unless the concentration of calcium in fruit at harvest is above 2.0mg/100g FW. Despite the strength of the above relationship, situations do occur where low or high levels of pit are associated with low or high fruit calcium concentrations respectively. Bitter pit can also occur in other apple cultivars such as 'Braeburn' and 'Granny Smith'. However mineral concentrations critical to bitter pit occurrence have not been determined in these cultivars. Other workers have suggested that high concentrations of magnesium and potassium ions, in association with low calcium, influence incidence of bitter pit in apples (Bangerth, 1973). Van der Boon (1980a, b) has used fruit Ca:K

and/or Ca:Mg ratios to predict bitter pit. Application of Mg and K salts onto fruit can cause bitter pit (Ferguson and Watkins, 1989).

Senescent breakdown of several apple cultivars has been correlated with low fruit calcium concentrations and over-maturity (Perring, 1968), and low fruit phosphorus concentrations (Letham and McGrath, 1969). Bramlage et al. (1985) used calcium concentration in cortical flesh of 'McIntosh' fruit at harvest to predict the length of time fruit should be held in storage before senescent breakdown occurred.

Further evidence for a major role of calcium in the expression of disorders comes from the success of calcium sprays during fruit development, and of dips and vacuum-infiltration of fruit with calcium immediately after harvest, in alleviating these storage disorders (Sharples and Little, 1970; van Goor, 1971; Mason, 1979). Infiltration of calcium into apples at harvest also reduces respiration rate and ethylene production of fruit and the rate of reduction in flesh firmness (Scott and Wills, 1977; Watkins et al., 1982b). Fruit sprayed with calcium during the growing season were greener at harvest and firmer following storage, compared with unsprayed fruit (Watkins et al., 1989b).

6.1.2 Mineral Transport into Fruit

Minerals move into fruit *via* both phloem and xylem transport systems. Studies indicate that water movement into fruit occurs *via* the xylem during the first few weeks after anthesis whereupon phloem transport dominates

(Wiersum, 1966; Lang, 1990). Many nutrients, including nitrogen, potassium and magnesium move in both xylem and phloem. During the first part of the growing season nitrogen stored in the bark of stems and shoots is translocated *via* the phloem while nitrogen from the roots is moved in the xylem (Tromp and Oövaa, 1990). Movement of these nutrients within the plant is regulated by mechanisms similar to those controlling assimilate transport (Chapter 4). That is, *via* strength of the nutrient "source", (usually determined by the concentration of minerals at the root surface or within the reserves of the plant) and strength of the fruit "sink" (Shear, 1979). Thus the content of potassium, magnesium and nitrogen in fruit increase exponentially immediately after bloom and this is followed by a steady linear increase over the rest of the season (Ferguson et al., 1987).

In contrast, calcium content of many developing fruit, including apple, usually shows a characteristic linear increase after bloom, followed by a levelling off midway through the growing season (Jones and Samuelson, 1983; Ferguson et al., 1987). A progressive increase in fruit size dilutes the calcium concentration in the fruit during the season. Calcium movement in the symplast (and therefore the phloem) seems very restricted (Ferguson, 1979) and therefore calcium transport is likely to be solely *via* the xylem and only early in the season. It is worth noting, however, that a number of workers have noted a linear trend in the uptake of calcium during the entire season (Wilkinson, 1968; Haynes and Goh, 1980). The reasons for these differences are not known. Some workers have attributed these different trends to different

transpirational demands imposed by leaves during the latter half of the growing season, which remove water and therefore calcium from the fruit (Tromp, 1979).

Nevertheless factors controlling calcium accumulation by the fruit during the early part of the season would seem to be important in determining final calcium content and therefore concentration in the fruit.

Calcium transport into fruit is thought to be controlled by factors influencing mass flow (Ferguson and Watkins, 1989). Transpiration in young developing fruitlets is relatively high, as there is a high fruit surface to volume ratio relative to when the fruit is large. Mass flow and calcium uptake into the fruit is therefore high early in the season. As the fruit grows the surface to volume ratio falls, the cuticle thickens, lenticels disperse per unit area and transpiration, mass flow and calcium uptake is reduced.

On the other hand, vigorous shoot growth may compete with fruit for calcium. Summer pruning may increase calcium supply to fruits (Perring and Preston, 1974). Low fruit calcium levels have been associated with vigorous shoot growth on the tree top (Schumacher et al., 1979).

There is some suggestion that spur leaves may have a positive influence on calcium movement into fruit. A large total area of spur leaves associated with the growing apple has been related to high levels of fruit calcium (Jones and Samuelson, 1983). Partial removal of primary spur leaves reduced fruit content at harvest (Ferree and Palmer, 1982; Jones and Samuelson, 1983). Spur leaves may aid transpiration flow into the spur so increasing calcium

movement into the fruit. Positional factors which influence spur leaf area might also influence fruit calcium uptake.

Fruit calcium uptake into fruit may also move in the xylem cylinder *via* a cation exchange mechanism. Calcium ions are absorbed on negatively charged sites on vessel walls and move upwards in a series of exchange reactions (van de Geign et al., 1979). More recently, a positive effect of high seed number on calcium uptake in apple fruit has been indicated (Bramlage et al., 1990). Seeds may influence fruit calcium accumulation *via* auxin production and transport across the fruit stalk. Basipetal auxin transport has been linked to acropetal calcium transport into fruit (Banuelos et al., 1987). Positional factors influencing auxin flows from seed to spur might also affect calcium accumulation in the fruit.

6.2 Experimental Objectives

The type of bud from which fruits and leaves originate on an apple replacement branch influences both fruit, shoot and leaf growth (Chapters 3 and 4). As fruit and leaf growth can effect fruit mineral content it is quite possible that the origin of the fruit bud may have an important effect on mineral status of fruits at harvest and therefore susceptibility of fruit to storage disorders.

The objective of the following experiment was therefore to determine the effect of bud type on mineral content, concentration and ratios in individual fruit of the apple cultivars, 'Braeburn' and 'Granny Smith'.

6.3 Influence of Bud Type on Fruit Mineral Nutrition

6.3.1 Materials and Methods

Fruit and Tissue Sampling

Two fruit were randomly taken from the three fruit sampled from each bud type per tree harvested from the experiment detailed in Chapter 5 ('Braeburn' - harvest date = 5/4 and 'Granny Smith' - harvest date = 2/5). For 'Braeburn', these bud types were two-year spur buds, one-year laterals and spur buds older than three years located within the tree canopy. In addition, two fruit per tree from one-year terminal buds were harvested from sample branches from each of the ten 'Granny Smith' trees. In all cases this gave a total of twenty fruit for each bud type per cultivar. As such, a wide range of fruit sizes for each bud type was harvested.

Each fruit was individually analysed by the methods of Turner et al. (1977). After weighing, each fruit was cut in half and a full equatorial slice (- 5mm thick) was taken from the cut surface. Apple cortical tissue (3-4g) close to the skin was removed from each slice using a corkborer (12mm diam.), six plugs being taken from all points of the circumference. After the fresh weight of the plugs was measured, samples were stored at -20°C.

Mineral Analyses

Mineral analysis of the apple tissue was carried out at the New Zealand Apple and Pear Marketing Board Laboratory in Hastings using a procedure developed by Ferguson et al. (1979). The frozen tissue from each fruit was

placed in Kjeldahl flasks (50ml) and conc HNO_3 (5ml) was added. Flasks were heated gradually over an electric element for 1.5-2 hrs until complete tissue digestion occurred. $HClO_4$ (50%) (1ml) was added and the solution reheated for 2 hrs until all the HNO_3 had boiled off. $LaCl_3$ (26.7g) and $CsCl_3$ (8g) were dissolved together in distilled water (5l). This solution (48.5ml) was added to the concentrate in each of the Kjeldahl flasks.

Calcium, potassium and magnesium determinations were performed on an Instrumentation Atomic Absorption Spectrophotometer. Standard solutions of 2.5 and 5.0ppm (Ca and Mg) and 50 and 100ppm (K) were made up using AA Atomic Absorption Spectroscopy Reagents (1000+/-5ppm). Atomic Absorption readings of samples taken from individual fruit were subsequently determined.

Individual fruit mineral concentrations were calculated from AA readings using the following equation:

$$A = (5 \times C)/B$$

where A = mineral concentration (mg/100g fruit fresh weight)

B = fresh cortical tissue weight (g)

C = AA reading

Mineral content was calculated for each individual fruit by multiplying fruit mineral concentration by fruit weight. However, it must be noted that this

is only an estimate of the total mineral content of the fruit but allows variable fruit size to be taken into account. The original tissue sample was taken from cortex tissues only. Skin, seed and core also contribute significantly to total mineral content of the fruit and may have higher mineral concentrations than cortical tissues (Ferguson and Watkins, 1983).

Fruit mineral ratios were also calculated for Ca:Mg and Ca:K, based on mineral concentrations.

Statistical Analyses

The experiment was organised in a randomised block design, each tree being a block and each bud type a treatment with two treatment replicates per block.

Coefficients of determination (r^2) and lines of best fit were developed between fruit mineral concentrations or fruit mineral ratios with fruit fresh weight, for each bud type and cultivar, *via* linear regression analysis using the 'Reg' procedure on SAS.

6.3.2 Results

Two-year spur buds produced significantly larger fruit than one-year lateral buds for both 'Braeburn' and 'Granny Smith' cultivars (Tables 6.1, 6.2). Fruit on the oldest spur buds were intermediate in size on 'Granny Smith', but were significantly smaller than fruit on one-year lateral buds for 'Braeburn'. One-year terminal buds on 'Granny Smith' had significantly larger fruit than

one-year lateral buds, but tended to have smaller fruit than two-year spur buds. They also were larger than those from the oldest spur bud type. However these differences were not significant.

Concentrations of potassium were substantially higher than magnesium and calcium concentrations for both cultivars (Tables 6.1, 6.2). Calcium, potassium and magnesium concentrations were generally slightly higher in 'Braeburn' fruit than in 'Granny Smith' fruit.

For 'Granny Smith', fruit from one-year terminal buds had the highest fruit calcium concentration. For both cultivars, there was no significant difference in calcium concentration between fruit from two-year spur and one-year lateral buds. However, calcium concentration was higher in fruit from the oldest spur bud type, compared with both these other bud types, this being significant for 'Braeburn'.

Potassium and magnesium concentrations generally showed little difference between bud types for either cultivar. An exception was on 'Granny Smith' where fruit from one-year terminal buds had a significantly higher concentration of both potassium and magnesium compared with that from other bud types.

'Granny Smith' fruit sampled from one-year terminal buds had significantly higher fruit mineral content than those of fruit from one-year lateral buds or the oldest spur bud type. Fruit from one-year terminal buds had a significantly higher calcium content and similar magnesium and potassium contents compared with those in fruit from two-year spur buds.

Table 6.1 Fruit fresh weight, fruit mineral concentration, fruit mineral content and fruit mineral ratios for different buds types for apple cv. 'Braeburn' (1988).

Bud type	Fruit wt(g)	Fruit mineral conc. (mg/100gFW)			Fruit mineral content (mg/fruit)			Ratios	
		Ca	K	Mg	Ca	K	Mg	Ca/K	Ca/Mg
2 yr spur	194.4	3.9	93.7	3.8	7.5	184	7.3	0.044	1.00
1yr lateral	168.7	3.6	93.5	3.6	6.1	160	6.2	0.041	1.00
>3 yr spur	147.6	4.4	88.7	3.4	6.4	130	5.0	0.051	1.30
LSD (P=0.05)	19.6	0.4	13.5	0.4	1.2	33	1.2	0.009	0.04

Table 6.2 Fruit fresh weight, fruit mineral concentration, fruit mineral content and fruit mineral ratios for different bud types for apple cv. 'Granny Smith' (1988).

Bud type	Fruit wt(g)	Fruit mineral conc. (mg/100gFW)			Fruit mineral content (mg/fruit)			Ratios	
		Ca	K	Mg	Ca	K	Mg	Ca/K	Ca/Mg
2 yr spur	177.2	3.0	90.9	3.1	5.2	160	5.6	0.034	1.02
1 yr lateral	129.4	3.1	97.7	3.1	3.5	121	4.1	0.031	1.03
1yr terminal	164.0	4.1	116.6	3.8	6.5	190	6.3	0.036	1.10
>3 yr spur	146.9	3.3	92.4	3.0	4.8	137	4.4	0.037	1.15
LSD (P=0.05)	26.8	0.5	10.0	0.4	0.9	31	1.2	0.006	0.16

For 'Braeburn', fruit from the oldest bud type had the lowest potassium and magnesium content - the difference between this bud type and two-year spur buds being significant. However, one-year lateral buds had the lowest fruit calcium content, that from two-year spur buds being the highest, and that from the oldest spur bud type being intermediate.

The Ca:K ratio was significantly higher in fruit harvested from the oldest spur bud type compared to one-year lateral buds for both cultivars. For 'Braeburn' a significantly higher Ca:Mg ratio occurred in fruit from the oldest spur bud type compared with that from both other bud types. A similar trend was noted for 'Granny Smith' although these differences were not significant.

Calcium concentration (Figure 6.1) and Ca:K and Ca:Mg ratios were all inversely related to fruit weight of individual 'Granny Smith' apples for all bud types, except for the Ca:K ratio on two-year spur buds on 'Granny Smith' (Table 6.3). The estimated linear regressions were all significant at $P < 0.05$ however coefficients of determination (r^2) were low, ranging from 18-55%. Fruit mineral concentrations and ratios generally showed poor relationships with fruit weight for 'Braeburn' (Table 6.3). However, fruit weight was negatively related to calcium concentration and both mineral ratios for fruit from two-year spur buds. There was no significant relationship between potassium or magnesium concentration and fruit weight, for any of the bud types, for either cultivar. Possibly concentration expressed on a dry weight basis may have given more meaningful results, as this would have possibly reduced variation in % water content between fruits.

Figure 6.1 Relationship between fruit fresh weight and calcium concentration for each of four different bud types cv. 'Granny Smith' (1988). A comparison of lines of best fit between bud types is presented in Figure 6.2.

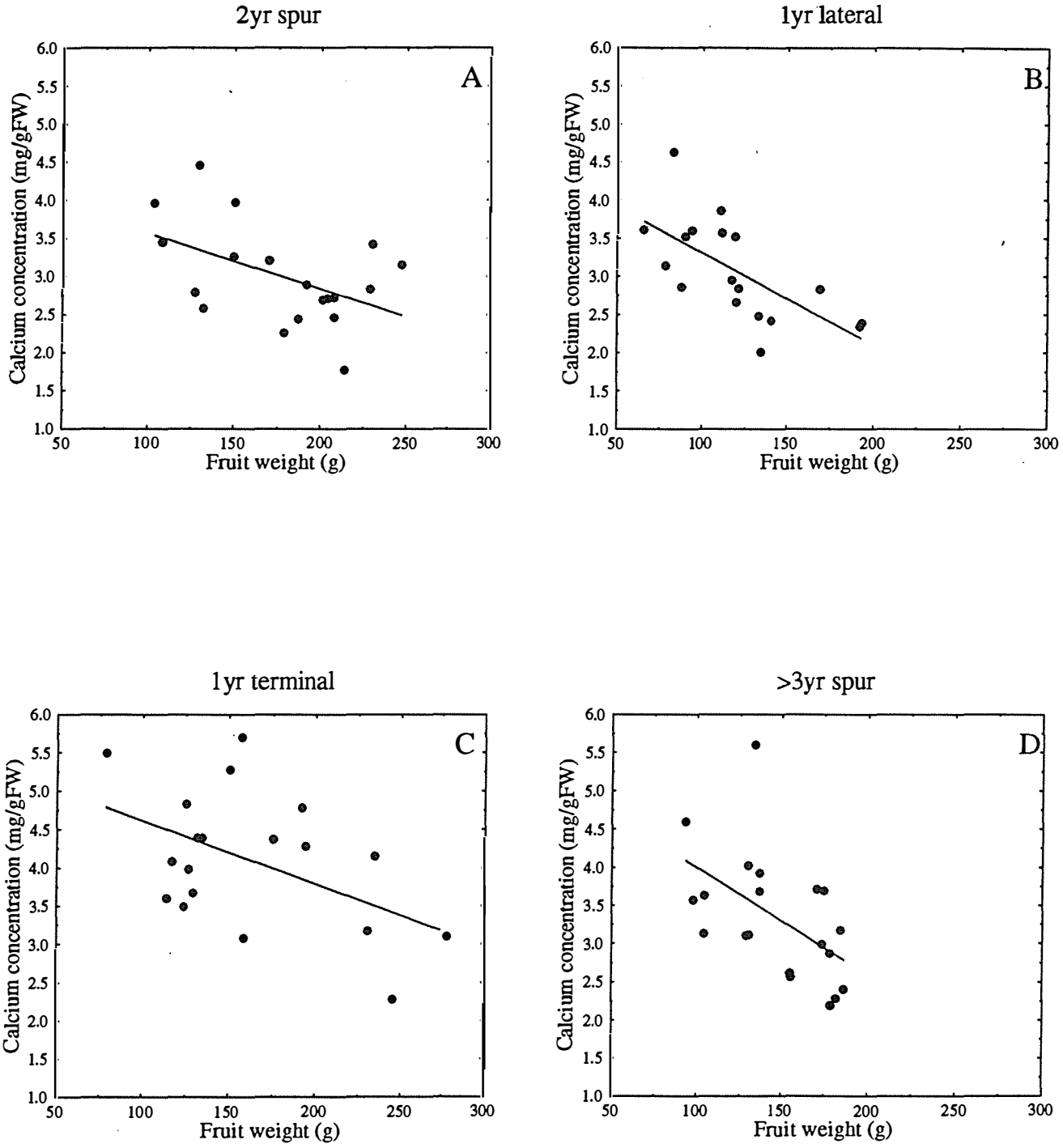


Table 6.3 Coefficients of determination (r^2) for linear regression lines relating fruit mineral concentration and ratios to fruit fresh weight by bud type and cultivar.

Cultivar	Bud type	Fruit mineral conc.			Ratio	
		Ca	K	Mg	Ca/K	Ca/Mg
Braeburn	2 yr spur	0.33*	0.07	0.0	0.22*	0.53***
	1 yr lateral	0.0	0.01	0.0	0.0	0.02
	>3 yr spur	0.0	0.0	0.06	0.0	0.10
Granny Smith	2 yr spur	0.19*	0.0	0.04	0.0	0.41**
	1 yr lateral	0.40**	0.0	0.0	0.35**	0.53***
	1 yr terminal	0.19*	0.0	0.0	0.22*	0.39**
	>3 yr spur	0.24*	0.0	0.14	0.20*	0.56***

*,**,*** Significant at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 \geq P$ respectively.

For 'Granny Smith', the relationship between fruit weight and calcium concentration was often different for each bud type, although not necessarily in slope. Thus fruit from one-year terminal buds had an estimated calcium concentration 1.4-1.9mg/100gFW higher than fruit from one-year lateral buds (a 41-83% increase), across a range of fruit weights from 100-200g (Figure 6.2). Fruit from the other two-bud types had fruit Ca-weight relationships intermediate between one-year terminal and lateral buds.

The extent to which different bud types influenced fruit Ca:Mg and Ca:K ratio seemed to be more dependent upon fruit weight, than was calcium concentration (Figures 6.3, 6.4). Two-year spur and one-year terminal buds had similar ratios and the highest Ca:Mg ratios for fruit above 150g. Below 150g, fruit from the oldest spurs had the highest Ca:Mg ratio. Fruit from one-year lateral buds had the lowest ratios over all fruit weights.

6.4 Discussion

6.4.1 Fruit Size

Fruit size differences between bud types followed the same trend as those observed in Chapter 4. Such differences will be discussed further in Chapter 8.

Figure 6.2 A comparison of the relationship between fruit fresh weight and calcium concentration, as influenced by bud type cv. 'Granny Smith' (1988). (Lines of best fit: two-year spur, y = 4.3 -.0073(x); one-year lateral, y = 4.5 -.012(x); one-year terminal, y = 5.4 -.0081(x); > three-year spur, y = 5.4 -.014(x))

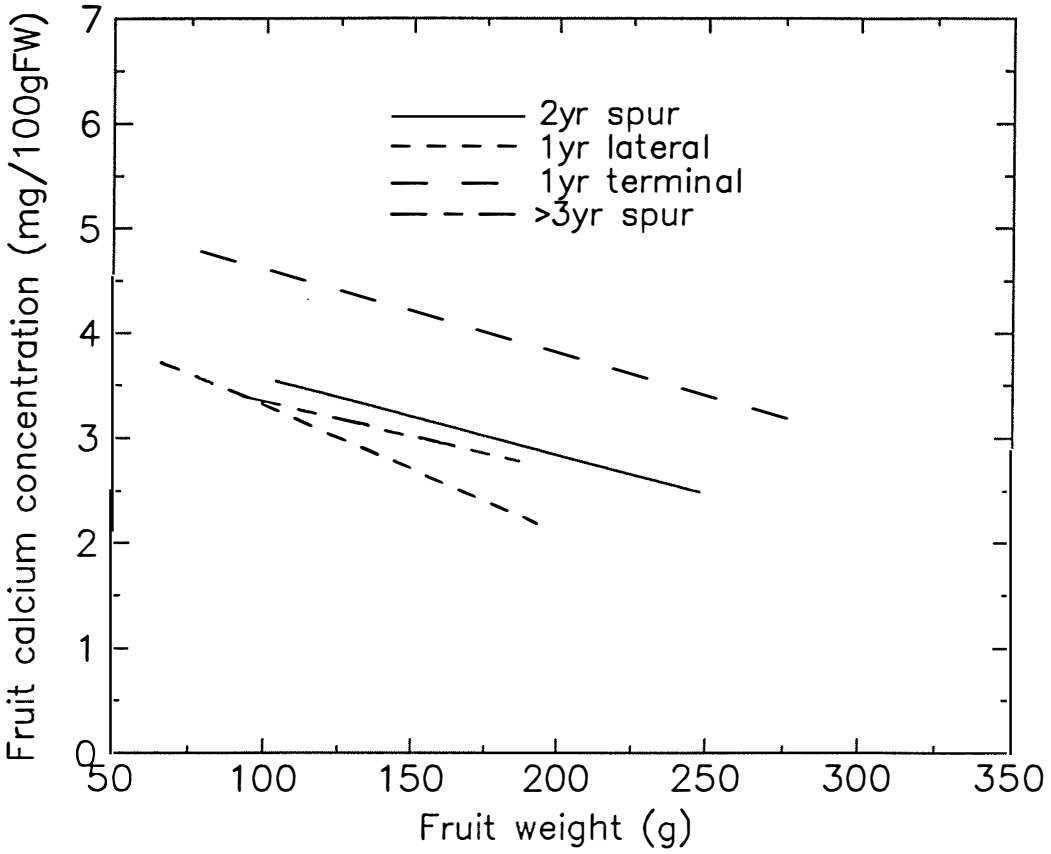


Figure 6.3 A comparison of the relationship between fruit fresh weight and Ca:Mg ratio, as influenced by bud type cv. 'Granny Smith' (1988). (Lines of best fit: two-year spur, y = 1.6 -.0033(x); one-year lateral, y = 1.6 -.001(x); one-year terminal, y = 1.6 -.0033(x); >three-year spur, y = 2.2 -.007(x))

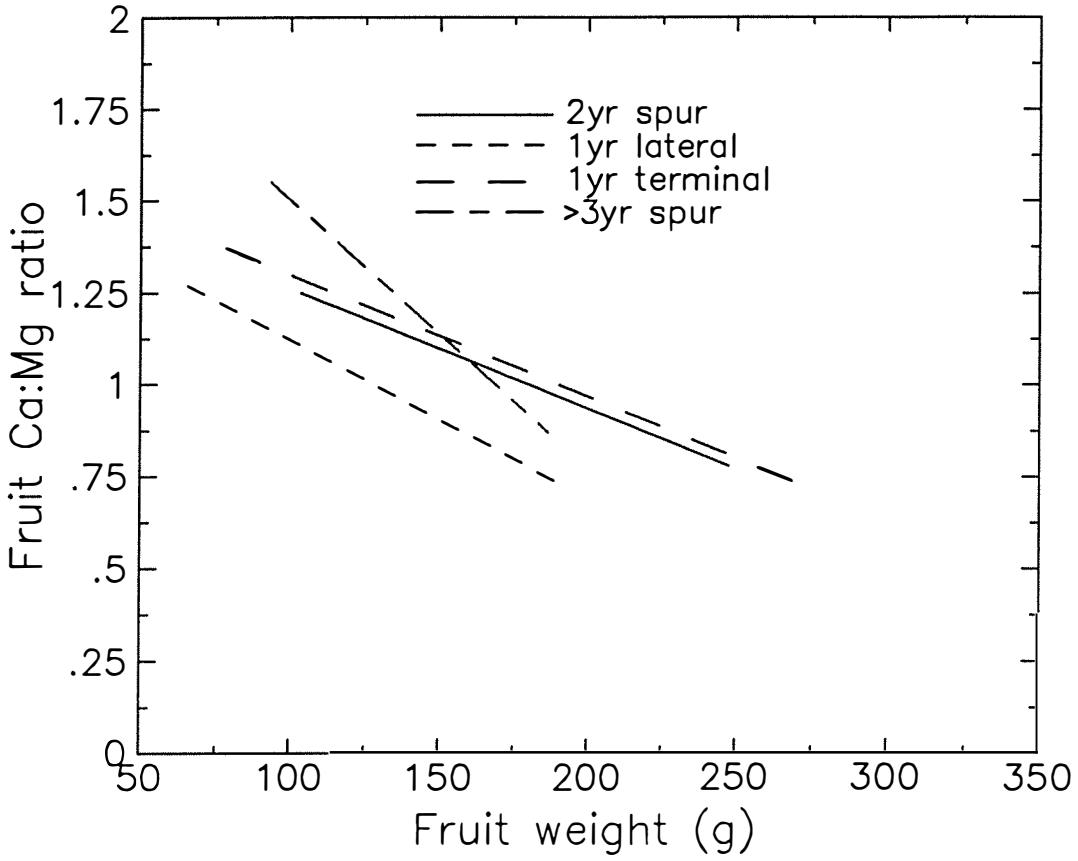
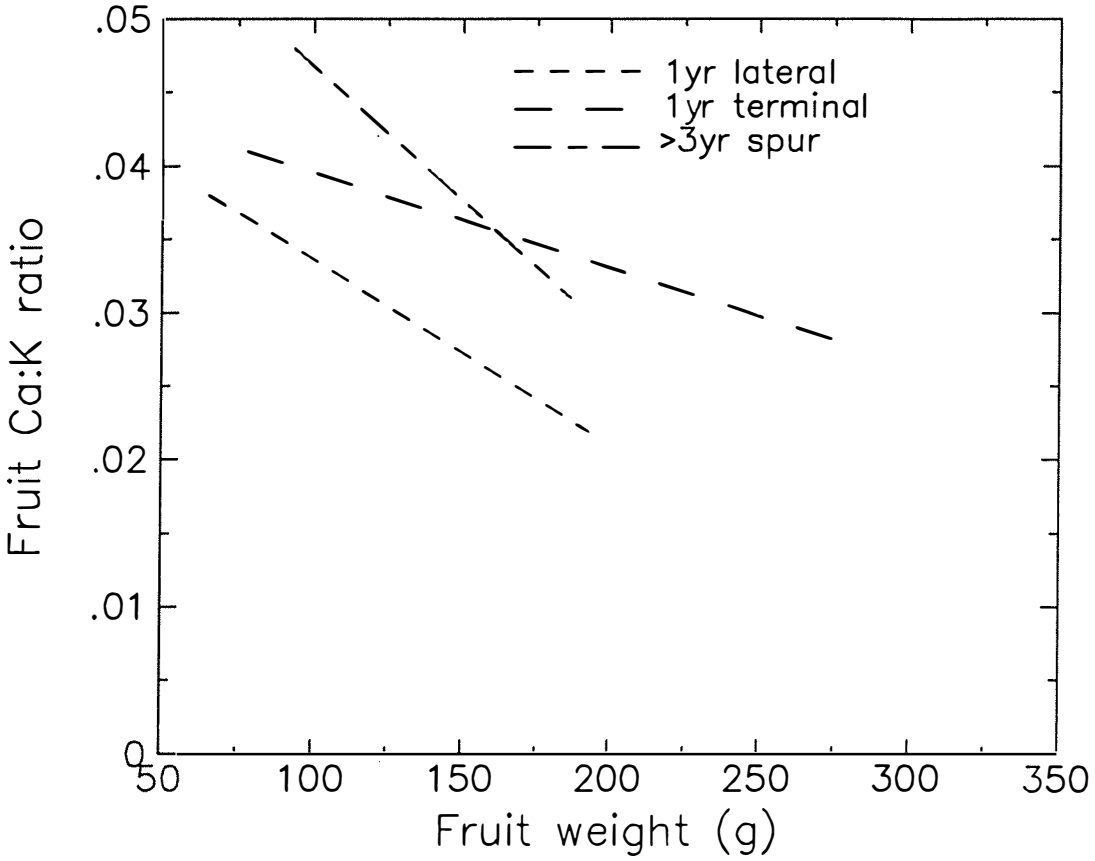


Figure 6.4 A comparison of the relationship between fruit fresh weight and Ca:K ratio, as influenced by bud type cv. 'Granny Smith' (1988). (Lines of best fit: one-year lateral, y = .047 -.00013(x); one-year terminal, y = .047 -.00007(x); >three-year spur, y = .064 -.00018(x))



6.4.2 Fruit Mineral Concentrations and their Relationships with Fruit Size

Fruit on two-year spur buds had a slightly lower calcium concentration, Ca:K and Ca:Mg ratio than fruit from the oldest spur buds. The "diluting" effect of the increased fruit size on the younger buds was only partially offset by the increase in the content of fruit calcium (Tables 6.1, 6.2). This was only significant for 'Braeburn'. Fruit on two-year spur buds were 32% larger than fruit on old spurs while calcium content was higher by only 17%. For Granny Smith, fruit size was 21% greater for two-year spur buds while calcium content was higher by only 8% compared with fruit on old spurs. Jackson et al. (1977), using 'Cox's Orange Pippin', showed that the increase in fruit calcium concentration and Ca:K ratio of fruit harvested from trees artificially shaded throughout the season was entirely due to the reduction in fruit size. Lower calcium concentrations for fruits on the outside of the tree compared with those on the inside indicated that fruit on the outside may be more prone to calcium-related storage disorders such as bitter pit and internal breakdown, as has been reported in previous studies (Wallace, 1953; Jackson, 1967; Jackson et al., 1971).

Prediction of storage disorders based on mineral analyses of fruit is only reliable for fruit of the same size (Ferguson and Watkins, 1989). Therefore a more appropriate comparison of mineral concentrations and ratios between bud types is to compare fruit of a similar size. Negative linear relationships of individual fruit weight with calcium concentration (Figure 6.1, 6.2), and fruit mineral ratios (Figure 6.3, 6.4) for 'Granny Smith' for each bud type were

shown to be significant. Across a range of sizes, fruit on one-year lateral buds had the lowest calcium concentrations, Ca:K, and Ca:Mg ratios whilst fruit on one-year terminal buds had the highest calcium concentration. These relationships also allow a comparison of calcium concentration for any one fruit size. For example, fruit of the premium size count 113 (175g) borne on one-year lateral buds had a calcium concentration of 2.2mg/100gFW, those borne on terminal buds had a calcium concentration at 4.0mg/100gFW, whilst those of the other bud types were intermediate.

However, for any one fruit size, relative differences between bud types in calcium concentration were not always reflected by similar differences in Ca:Mg or Ca:K ratios. For instance, although fruit from terminal buds had the highest calcium concentration (across all fruit sizes), Ca:Mg ratios were similar to those of fruit from two-year spur buds. Below 150g, both fruit Ca:Mg and Ca:K ratios from terminal buds were lower than that of fruit from old spur buds. It is difficult to understand these different patterns, particularly as fruit magnesium and potassium concentrations were not related to fruit size (Table 6.3). Nevertheless, these patterns may be important from a fruit quality viewpoint. In some circumstances, Ca:Mg and/or Ca:K ratio relate better to incidence of storage disorders than fruit calcium concentration (Ferguson and Watkins, 1989). High Ca:K or Ca:Mg ratios might also be better associated with anti-senescent properties of the fruit than calcium concentrations, although little published information is available concerning this point.

These results indicate that fruit on one-year lateral buds may be more prone to fruit storage disorders and senesce more quickly than fruit from the other bud types. Low fruit calcium concentrations (and low Ca:Mg or Ca:K ratios) are associated with these poor fruit quality characteristics (Bramlage et al., 1974; Ferguson et al., 1979). In fact threshold concentrations of calcium have been used to predict the incidence of bitter pit in 'Cox's Orange Pippin' (Ferguson et al., 1979), and senescent breakdown for 'McIntosh' (Bramlage et al., 1985). The extent to which the above figures relate to storage disorders which might occur in 'Granny Smith' has not been determined.

Coefficients of determination less than 50% for most of these weight/calcium concentration or weight/mineral ratio regression lines for 'Granny Smith' confirm that factors other than fruit weight and bud type are also important in influencing fruit mineral concentrations and ratios within a tree. Indeed, for 'Braeburn', there was no relationship found for fruit on one-year lateral or inner canopy spur buds. An inverse logarithmic relationship between calcium concentration and fruit weight has been described for 4000 replicates of bulked orchard samples (Perring and Jackson, 1975). However they noted a positive relationship between fruit size and calcium concentration for individual fruit samples taken from trees within a single orchard block, and did not consider within-tree fruit positions as a variant. Recently, Bramlage et al. (1990) reported that large 'Delicious' fruit with high seed numbers had accumulated more calcium than smaller fruit. Thus it is clear that seed number should also be considered when determining sources of within-tree variation in

fruit calcium concentration. Unfortunately, seed number per fruit was not assessed in the present experiment.

The number of fruit harvested per bud type was only 20 and perhaps with greater fruit numbers and a wider/equal range of fruit sizes, more accurate relationships would have been established. Nevertheless, these results of the present study indicate that growers should encourage large numbers of fruit on terminally borne buds while discouraging fruits on one-year lateral buds. This may be particularly important for cultivars which are prone to calcium-related disorders (eg. 'Cox's Orange Pippin'), and where fruit is required to be stored for a long period. For cultivars less susceptible to calcium-related storage orders such as bitter pit (eg. 'Gala', Ferguson and Watkins, 1989), fruit with high calcium concentrations may maintain firmness, texture and green background colour better than fruit with low calcium concentrations. The differing mineral concentration/fruit weight relationships for different bud types may also explain why young trees produce fruit of poorer storage quality, compared with fruit from mature trees (Sharples, 1973). Young trees often produce the majority of fruit of a large size and on one-year lateral buds.

Magnesium and potassium concentrations (unlike that of calcium) have been correlated to growth rate of apple fruit (Tromp, 1975) and dry matter production (Lewis, 1980). Both magnesium and potassium are available at high concentrations in the phloem and are thus transported to meristematic tissue throughout the whole season in response to demand of the growing tissue

(Mengel and Kirkby, 1982). However in the present study, neither magnesium nor potassium concentrations were related to fruit size for any bud type.

6.4.3 Comparison of Different Minerals

Overall potassium content of the fruit from both apple cultivars was much higher than magnesium or calcium contents, a result in agreement with other studies (Jackson et al., 1977; Haynes and Goh, 1980; Jones et al., 1983; Ferguson et al., 1987). To some extent the amount of each mineral reaching the fruit is determined by the relative importance of xylem/phloem pathways for the influx of water into the fruit. Potassium and magnesium are both supplied to the growing fruit throughout the whole growing season *via* both phloem and xylem (Mengel and Kirkby, 1982). By comparison, calcium moves into fruit predominantly *via* the xylem during the first few weeks after anthesis (Wiersum, 1966). Calcium movement into fruit after this time is variable and often restricted (Wilkinson, 1968).

6.4.4 Influence of Bud Type on Mineral Content

Mineral content per fruit is a more useful expression of mineral input than mineral concentration when trying to understand transport factors influencing input, as mineral uptake (especially calcium) can be quite independent of fruit growth (Ferguson and Watkins, 1989). Many authors have expressed mineral accumulation in apple fruit as fruit mineral content (Wilkinson, 1968; Haynes and Goh, 1980; Jones et al., 1983).

Uptake of each mineral into apple fruit was clearly dependent upon bud type for both cultivars. Fruit from one-year terminal and/or two-year spur buds located on the outside of the tree canopy had a higher content of all three minerals compared than fruit from spur buds older than three years harvested from inside the tree canopy. Two similar studies have shown that fruit from the upper parts of trees can have higher magnesium and potassium contents but lower calcium content than fruit from the bottom part of the tree (Jackson et al., 1971; Haynes and Goh, 1980).

These authors suggested that competition for calcium during the growing season may occur between growing fruits and the vigorous shoots borne in upper parts of the tree. In the present study shoots in the outer part of the tree canopy were greater in number and longer than those on the inside. Calcium content was greater for fruit borne on spurs located on the outside of the canopy than fruit inside the canopy (except for fruit on one-year lateral buds). These results tend to conflict with the suggestions of Jackson et al. (1971) and Haynes and Goh (1980). The upper parts of the trees used in their experiments may have had extreme shoot vigour, compared with the outer part of trees in the present study. Their trees were grown on vigorous M 4 and M 793 rootstocks. In comparison, the trees used in the present study were grown on MM 106, a semi-dwarfing rootstock.

Results from whole tree shading studies suggest that shade has little effect on fruit calcium uptake *per se* (Jackson et al., 1977). Rather, the outer located two-year spur may have actually benefitted from having a larger leaf area

subtending the fruit. Further discussion of the benefits of leaves on calcium uptake is presented in the following section.

Large differences in fruit calcium content occurred between buds located on replacement branches borne on the outside of the tree canopy. One-year terminal buds had higher contents of fruit calcium compared with fruit from either of the other two bud types on 'Granny Smith' (Table 6.2). On both cultivars, two-year spur buds had higher fruit calcium content compared with that for one-year old lateral buds.

Jones and Samuelson (1983) compared the mineral concentration of fruit from the basal and tip ends of a branch. Recalculation of their fruit mineral concentration data showed that calcium content, in fact, tended to be higher at the basal end. However neither a description of the branch type used in their experiments nor details of relevant bud types were given. Leaf calcium concentration has been shown to decline with increasing distance from the trunk (Preuschoff, 1968). This may have related to an increase in leaf dry weight with distance from the trunk. Leaves on the outside of the tree have a higher specific leaf weight than those leaves on the inside (Ferree, 1989). Nevertheless, variation in the leaf characteristics of the spur or bud at any position may be of importance in determining input of minerals into the fruit.

6.4.5 Effect of Leaves on Mineral Uptake

Whilst there have been no published reports of comparisons of fruit mineral composition from different bud types borne on an apple tree, individual

spur studies suggest that spur leaf area during the first few weeks after blossom is very important in directing calcium (and possibly other minerals) into the fruit. Removal of primary leaves at pink bud reduced total calcium and magnesium content (but not potassium) in the fruit (Ferree and Palmer, 1982). Furthermore, spurs associated with longer bourse shoots had higher levels of fruit calcium and magnesium than those on shorter bourses (Jones and Samuelson, 1983). Jones and Samuelson (1983) argued that transpiration from spur leaves directed calcium *via* mass flow to these leaves through the xylem. The close connection between these leaves and associated fruitlets would allow a proportion of the calcium to be diverted to fruitlets. Thus the smaller the spur leaf area the lower the calcium influx into fruit.

Total spur leaf area can be substantially lower on old fruiting spurs located on the inside of the tree canopy (Barritt et al., 1987; Ferree and Forshey, 1988). This could explain the lower fruit calcium levels achieved on these inner spur buds compared with those from buds on the outer canopy (two-year spur and one-year terminal) in the present study.

It is also likely that wind flow around leaves and leaf temperatures would be greater for "outer" located buds compared with those spur buds on the inside of the tree canopy. This might induce higher transpiration for those "outer" located leaves, leading to an increased water and calcium supply to the leaves and thereby to the subtending fruits. Indeed, where transpiration was reduced by bagging spurs soon after bloom for several weeks, fruit calcium content at harvest was significantly reduced (Jones and Samuelson, 1983).

Leaf area for the three "outer" bud types was measured during the seven weeks after blossom on the same ten 'Granny Smith' trees used in the present experiment (Chapter 3). Primary spur leaf area was similar for the two-year spur and one-year lateral buds yet fruit calcium contents differed considerably (Table 6.4). However bourse leaf area can be related to differences in final fruit calcium content across all three bud types. One-year terminal buds had the highest bourse leaf area and fruit calcium content, one-year lateral buds had the lowest leaf area and fruit calcium content whilst values for two-year spur buds were intermediary (although actual differences are greatly out of proportion). This suggests that bourse leaf area may be more important than primary leaf area in determining differences in final calcium content between fruit from the different bud types. On the other hand, Ferree and Palmer (1982) found little difference in fruit calcium concentration between deboursed and untreated spurs.

Bud type differences in final fruit potassium and magnesium contents also followed a pattern similar to total leaf area per bud for 'Granny Smith'. Removal of some spur leaves from fruiting spurs also reduced magnesium uptake into fruit (Ferree and Palmer, 1982). Magnesium and potassium are both transported in the xylem (and phloem), and as for calcium, transpirational flux as effected by spur leaves, may be important in determining input of the minerals into the growing fruit.

In summary, for 'Granny Smith' it has been shown that fruit on one-year terminal buds had a significantly greater calcium concentration compared with

Table 6.4 Fruit calcium content at harvest and primary, bourse and total leaf area (from Chapter 3) on 27/11/88 for different bud types for apple cv. 'Granny Smith'.

Bud type	Fruit calcium content (mg/fruit)	Spur leaf area (cm^2)		
		Primary	Bourse	Total
2 yr spur	5.2	26.0	151.9	177.9
1 yr lateral	3.5	24.1	30.6	54.7
1 yr terminal	6.5	78.5	202.2	280.7

fruit on one-year lateral buds, with fruit on two-year spurs and old spurs intermediate across a range of fruit sizes. These trends were similar for 'Braeburn', although one-year terminal buds were not considered and concentrations of fruit from the different bud type could not be compared for the same sized fruit.

These differences in calcium concentration are due to a variation in calcium uptake by fruits and fruit growth during the growing season, reflected by final calcium content in the fruit and final fruit size at harvest. A large part of the calcium uptake into the apple fruit occurs during the first few weeks after bloom (Jones et al., 1983) and is coincident with leaf emergence from developing bourse shoots subtending the fruit. It is proposed that differences in bourse leaf area between the three bud types may explain the differences in fruit calcium uptake. Total bourse leaf area in mid November for the three 'Granny Smith' bud types on the replacement branch was associated with differences in calcium content at harvest. Following this, bud types with low bourse leaf areas (such as one-year lateral buds) may produce fruit of poor quality after storage, depending upon the mineral "diluting" effects of fruit sizing, fruit maturity at harvest and fruit storage conditions.

CHAPTER SEVEN

LEAF EFFECTS ON FRUIT GROWTH AND MINERAL UPTAKE

7.1 Introduction

Leaves and shoots associated with the fruiting spur can strongly influence apple fruit growth and development. C-14 radioactive labelling studies have shown that nearby spur leaves contribute all the carbohydrate to the flowers and young fruit (Hansen, 1971). However later in the season, carbohydrate can be transported from leaves to fruit over large distances (Hansen and Christenson, 1974). Bourse shoots may compete with spur leaves for carbohydrates immediately after bloom before exporting to fruit later in the season (Quinlan and Preston, 1971; Tustin and Lai, 1990). Partial leaf defoliation and/or shoot removal from individual spurs before bloom (Ferree and Palmer, 1982), and at petal fall (Quinlan and Preston, 1971), and from whole trees before and after flowering (Llewelyn, 1968), reduces fruit set. Growth of fruit remaining on trees may increase (Llewelyn, 1968) or be little influenced by such treatments (Quinlan and Preston, 1971; Ferree and Palmer, 1982). On the other hand, Lakso et al. (1990) reported that high fruit number per spur during several weeks after bloom at a constant leaf area may limit fruit growth.

Leaves and shoots can also influence mineral transport to the fruit. Mineral uptake by the fruit is affected by leaf area, shoot and fruit growth, fruit position within the tree and type of transport system supplying nutrients to the fruit (Ferguson and Watkins, 1989). Partial removal of primary spur leaves from individual spurs at bloom reduces final fruit calcium and magnesium concentrations (Ferree and Palmer, 1982). The role of bourse shoots is not so clear. Ferree and Palmer (1982) showed that bourse shoot removal reduced final fruit magnesium concentration but not calcium concentrations. However fruit on spurs with long bourses had higher calcium concentrations than those on spurs with short bourses (Jones et al., 1983). Differences in final fruit calcium content between different bud types for 'Granny Smith' were also associated with differences in bourse leaf area measured in November (Chapter 6). Unfortunately these published studies were all conducted on spurs with variable fruit number and raises the question of how this may have influenced mineral uptake into fruit.

7.2 Experimental Objectives

The objective of the following experiments was to further compare the effects of primary leaf and bourse shoot removal on fruit growth and fruit mineral accumulation during the season on spurs with similar fruit number.

7.3 Leaf Removal Effects on Fruit Growth and Mineral Uptake

7.3.1 Materials and Methods

Experimental Procedure

Experiment A (1988) Primary Leaf Effects

Ten 'Gala' and 15 'Golden Delicious' trees were selected at the DSIR Research Orchard, Appleby. For 'Gala', 30 flowering two-year spurs on each tree were tagged at the pink bud stage. For 'Golden Delicious', 24 flowering two-year spurs on each tree were tagged at king full bloom stage. All tagged spurs were well exposed and approximately 1.5m from the ground. All lateral flowers on each spur were removed leaving the king flower. On each tree, 50% of the tagged spurs were randomly allocated to one of two treatments as follows:

1) Bourse shoot/bud removed.
2) Bourse shoot/bud removed. 50% primary leaves removed.

Treatments were applied immediately after lateral flower removal. In this first experiment, the investigation was confined solely to the effect of primary leaves on mineral uptake and fruit growth immediately after bloom. Therefore there was no "untouched" control treatment included.

Experiment B (1989) Interaction of Primary and Bourse Leaves

Thirty 'Royal Gala' were selected at the DSIR Research Orchard, Appleby. Twenty-four flowering two-year spurs on the outside of each tree, 1-2m from the ground, were tagged at the king full bloom stage. All lateral flowers were removed from tagged spurs leaving the king flower. On each tree, six spurs were randomly allocated to one of four leaf removal treatments as follows:

1) Bourse shoot/bud removed.
2) Bourse shoot/bud removed. 50% primary leaves removed.
3) 50% primary leaves removed.
4) No primary leaf/shoot removal (control).

As in the previous experiment, treatments were applied immediately after lateral flower removal.

For treatments involving partial removal of primary leaves in both experiments, the total primary leaf number was counted for each spur and half the leaves removed at random. Spurs had 6-8 primary leaves present for all three cultivars before treatments were imposed. All bourses were actively growing immediately before treatments. For 'Gala' in 1988 and 'Royal Gala' in 1989, bourses were 0.5-2cm in length with 0-3 small unfolded leaves present. For 'Golden Delicious' in 1988, bourse lengths were 1-5cm with 0-5 small unfolded leaves present. Bourse regrowths were removed weekly if

necessary from appropriate treatment spurs. Bourse regrowth length was always less than 2cm.

Fruit Sampling

Fruit were harvested at 6-15 day intervals over the following 40 days in both experiments. In 1989, two further samples were made at 63 and 131 days (commercial harvest) after anthesis. In 1988, the first 'Gala' sample was taken at king full bloom (at anthesis) and for 'Golden Delicious', and 'Royal Gala' in 1989, the first sample was at petal fall (5-6 days after anthesis). At each harvest date in 1988, 20 'Gala' and 15 'Golden Delicious' tagged spurs for each treatment were selected at random from throughout the block. In 1989, the 30 trees were divided into five six tree plots. At each harvest date three tagged spurs per treatment were selected at random from each plot.

Measurements

Fresh fruit weight was immediately determined at each harvest in both experiments. Fruit were subsequently sent to the DSIR Fruit and Trees laboratory, Mt Albert Research Centre, Auckland. Each fruit was acid-digested individually and calcium, magnesium and potassium for each fruit were analysed using atomic absorption spectrophotometry (see Chapter 6 for details). In 1989, individual fruit were too large to digest at the final harvest date. Therefore two sample wedges (5% of total weight) were cut from each fruit.

The wedges were digested and minerals determined as above. Mineral analyses of whole fruit or wedges therefore included skin, core, seeds as well as cortex tissues (in contrast to the experiment in Chapter 6).

Leaves from sampled spurs were divided into primary and bourse types. They were counted and total primary and bourse leaf areas per spur determined using a Delta T Area Meter (Mark 2).

Statistical Analyses

Experiment A (1988) was organised in a completely randomised design for both cultivars, there being 20 ('Gala') and 15 ('Golden Delicious') spur replicates per treatment per harvest. Experiment B (1989) was organised in a randomised block design, each block composed of six trees with three spur replicates per treatment per harvest. In this second experiment changes in rate of mineral uptake into the fruit over time was calculated between each harvest date from the average fruit mineral content for each block.

Correlation coefficients (r), coefficients of determination (r^2) and multiple linear regression equations were developed relating fruit mineral content to fruit weight, and primary and/or bourse leaf areas for individual fruit using the 'Stepwise' regression procedure of SAS.

7.3.2 Results

Experiment A (1988) Primary Leaf Effects

Partial removal of primary leaves from deboursed spurs at bloom resulted in a 45-48% reduction in primary leaf area at the first harvest date (0 or 6 days after anthesis for 'Gala' and 'Golden Delicious' respectively) (Figure 7.1). This occurred for both cultivars and similar differences were apparent at all other harvest dates. 'Gala' spurs had slightly greater primary leaf area than 'Golden Delicious' spurs over the assessment period.

Cumulative fruit weight increased exponentially with time 5-8 days after anthesis for both cultivars (Figure 7.2). There was a slow sub-exponential growth phase for 'Gala' immediately after anthesis. Fruit weight increased at a faster rate for 'Golden Delicious' than 'Gala'. There was no significant difference in fruit weight between treatments at any assessment date for the two cultivars.

Fruit calcium content increased steadily after anthesis for both treatments and cultivars (Figure 7.3 A,B). 'Gala' spurs with 100% primary leaves had significantly higher fruit calcium content than that in the other treatment at anthesis and 34 days after anthesis. This same trend was apparent for 'Golden Delicious' with significant differences occurring at the last two

Figure 7.1 Change in primary leaf area during the early growing season for apple following two leaf removal treatments (Experiment A, 1988). [100% primary leaves 'Gala' (■), 50% primary leaves 'Gala' (○), 100% primary leaves 'Golden Delicious' (♦), 50% primary leaves 'Golden Delicious' (△)].

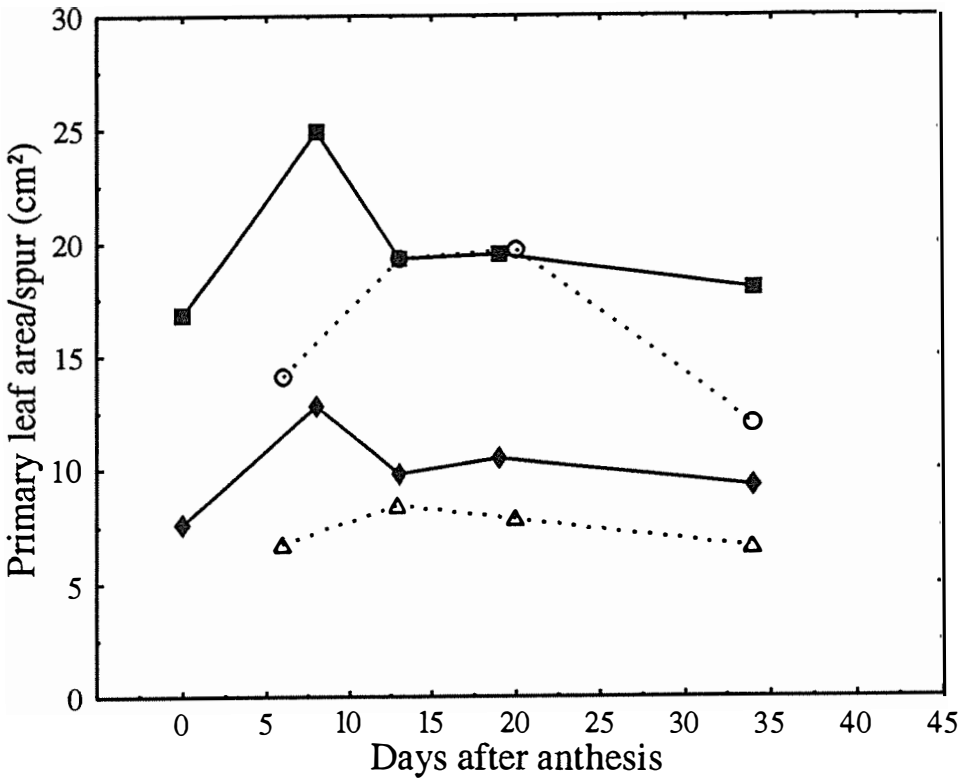


Figure 7.2 Change in fruit fresh weight during the early growing season expressed on a logarithmic scale for apple following two leaf removal treatments (Experiment A, 1988). [100% primary leaves (■), 50% primary leaves (○)].

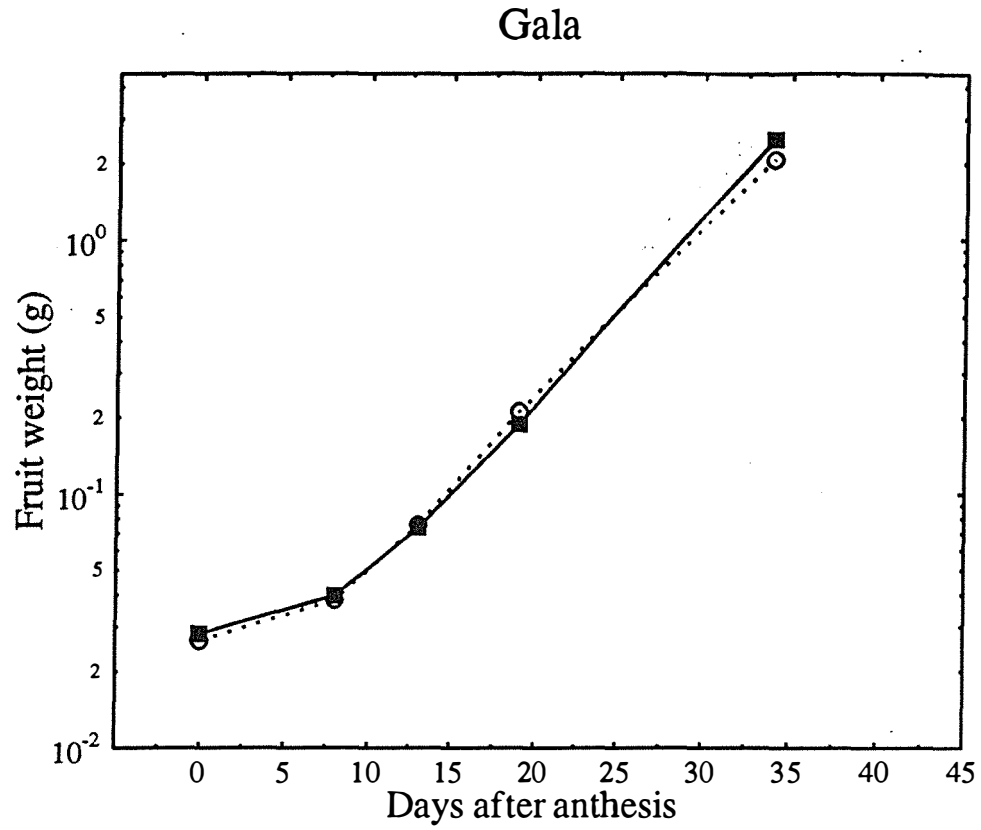
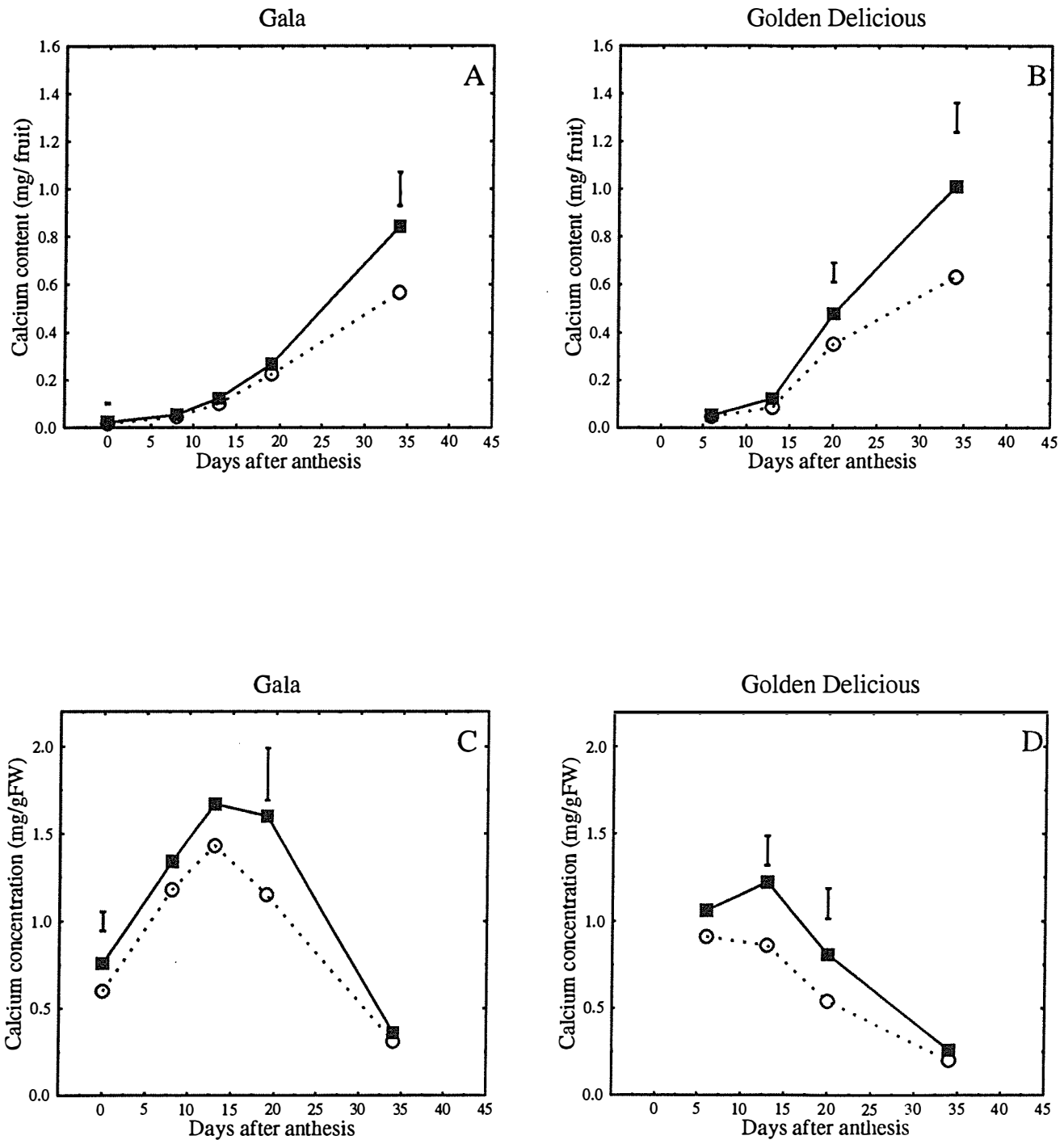


Figure 7.3 Change in fruit calcium content (A,B) and concentration (C,D) during the early growing season for apple following two leaf removal treatments (Experiment A, 1988). [100% primary leaves (■), 50% primary leaves (○)]. Bars = LSD (P=0.05).



harvest dates. Differences between treatments in calcium content increased with advancing harvest date. By 34 days after anthesis, between treatment differences in fruit content were 33 and 37% for 'Gala' and 'Golden Delicious' respectively.

Calcium concentration increased up to 12 days after anthesis to 1.6 and 1.25mg/gFW for 'Gala' and 'Golden Delicious' respectively, before declining (Figure 7.3 C,D). Generally, calcium concentration was greater for the 100% primary leaf treatment over the entire assessment period for both cultivars. Significant differences between the treatments were apparent 0 and 19 days after anthesis for 'Gala' and 12 and 19 days after anthesis for 'Golden Delicious'.

Fruit magnesium content increased steadily over the growing season from 12 days after anthesis (Figure 7.4 A,B). There was little difference in magnesium content between two cultivars or between treatments. An exception occurred at 34 days after anthesis when magnesium content was significantly lower for the partial primary leaf removal treatment for 'Gala'.

Magnesium concentration generally declined after anthesis for both cultivars (Figure 7.4 C,D). Removing half primary leaves significantly reduced magnesium concentration 19 days after anthesis for 'Gala'. Otherwise there was no difference in magnesium concentration at any date for either cultivar.

Fruit potassium content also increased approximately exponentially after anthesis for both cultivars (Figure 7.5 A,B). There was no significant

Figure 7.4 Change in fruit magnesium content (A,B) and concentration (C,D) during the early growing season for apple following two leaf removal treatments (Experiment A, 1988). [100% primary leaves (■), 50% primary leaves (○)]. Bars = LSD (P=0.05).

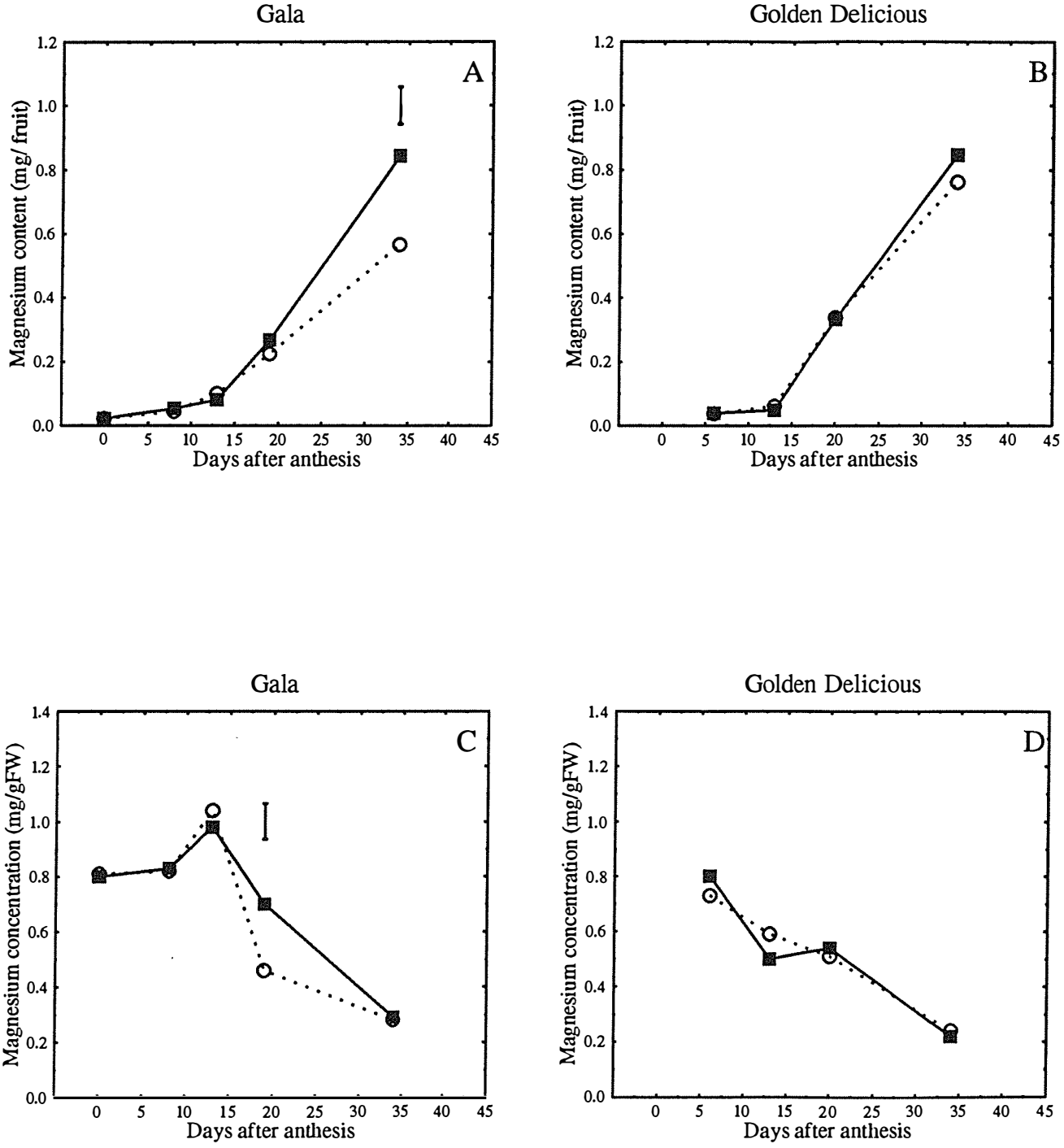


Figure 7.5 Change in fruit potassium content (A,B) and concentration (C,D) during the early growing season for apple following two leaf removal treatments (Experiment A, 1988). [100% primary leaves (■), 50% primary leaves (○)].

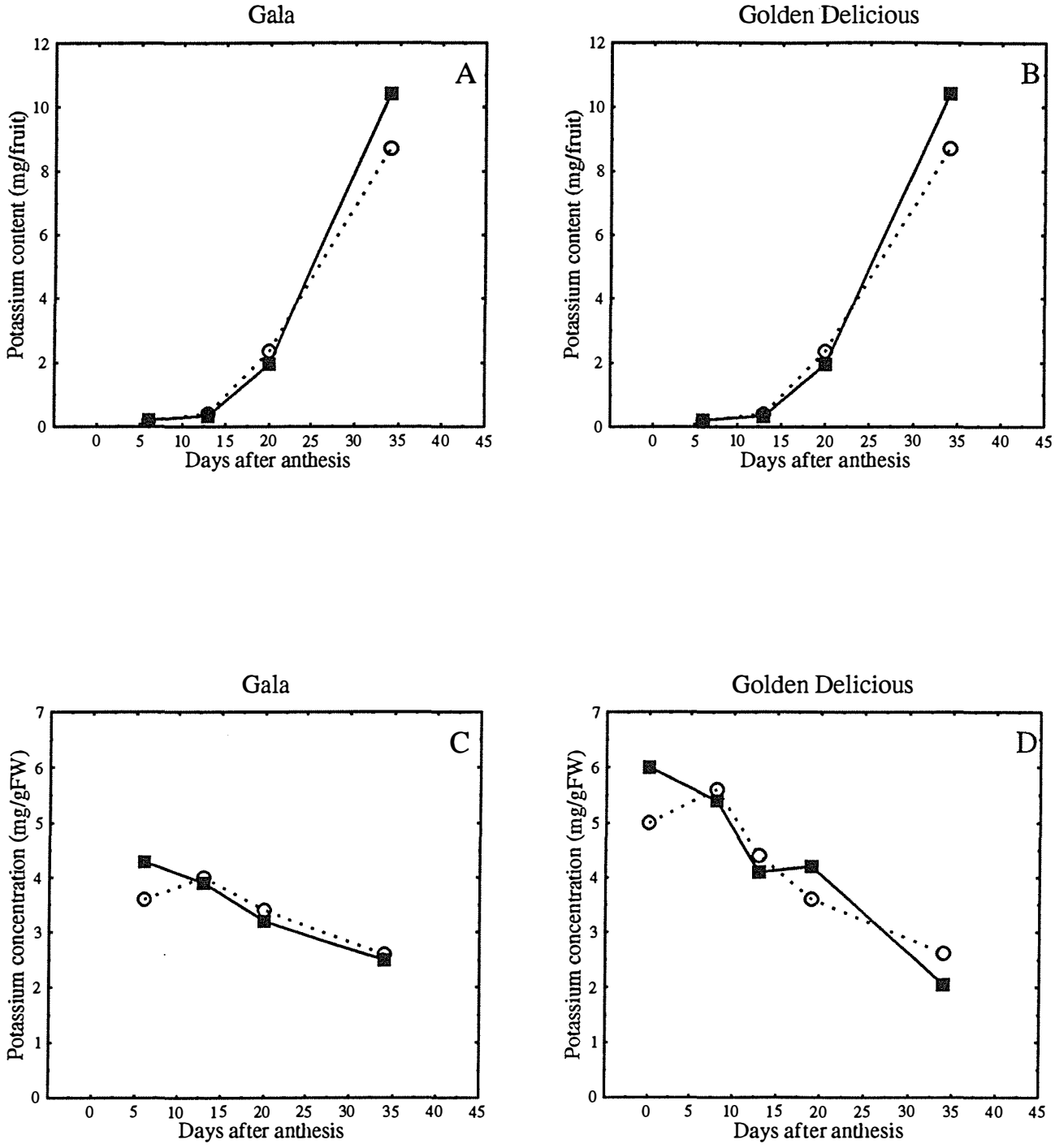


Table 7.1 Correlation coefficients (r) between fruit mineral content, primary leaf area and fruit weight during the early growing season for individual apple fruit (Experiment A, 1988).

'Gala'

Mineral	Variable	Days after anthesis 0	8	12	19	34
Calcium	Primary leaf area	0.70^{**}	0.48^{**}	NS	0.57^{***}	0.88^{***}
	Fruit weight	0.61^{**}	0.54^{**}	0.78^{***}	0.64^{***}	0.63^{***}
Magnesium	Primary leaf area	NS	NS	NS	0.54^{***}	0.71^{***}
	Fruit weight	0.56^{*}	0.76^{***}	0.88^{***}	0.67^{***}	0.79^{***}
Potassium	Primary leaf area	0.60^{***}	NS	NS	NS	NS
	Fruit weight	0.56^{*}	0.82^{***}	0.88^{***}	0.83^{***}	0.63^{***}

'Golden Delicious'

Mineral	Variable	Days after anthesis 6	13	20	34
Calcium	Primary leaf area	0.58^{***}	0.69^{***}	0.63^{***}	0.79^{***}
	Fruit weight	0.72^{***}	0.83^{***}	NS	0.69^{***}
Magnesium	Primary leaf area	0.44^{*}	NS	NS	0.59^{**}
	Fruit weight	0.87^{***}	0.96^{***}	0.51^{**}	0.91^{***}
Potassium	Primary leaf area	NS	NS	-0.51^{**}	0.43^{*}
	Fruit weight	0.66^{***}	0.96^{***}	0.90^{***}	0.95^{***}

*, **, *** = $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $P < 0.001$ respectively

NS = Not significant

difference in potassium content or concentration (Figure 7.5 C,D) between the two treatments for either cultivar.

Calcium contents of individual fruit were highly correlated with primary leaf area and fruit weight for both cultivars at all but two harvest dates (Table 7.1). Average correlation coefficients across both cultivars were 0.59 and 0.60 for primary leaf area and fruit weight respectively. In contrast, magnesium and potassium contents were strongly related to fruit weight at all harvest dates, but generally not with primary leaf area. Fruit weight and primary leaf area were not generally correlated together (Table 7.2).

Table 7.2 Correlation coefficients (r) between fruit weight and primary spur leaf area during the early growing season for apple (Experiment A, 1988).

'Gala'

	Days after anthesis				
	0	8	13	19	34
r value	0.46^{**}	NS	NS	NS	0.46^{**}

'Golden Delicious'

	Days after anthesis			
	6	13	20	34
r value	NS	NS	-0.41^{*}	NS

*, **, *** = $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $P < 0.001$ respectively
NS = Not significant

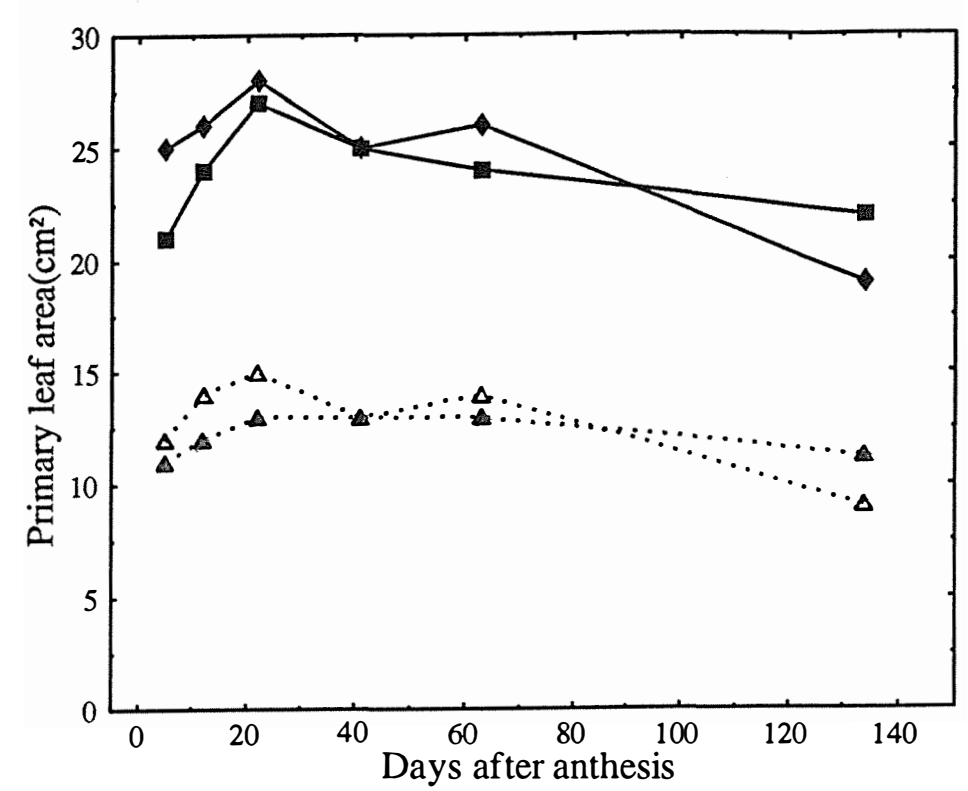
Experiment B (1989) Interaction of Primary and Bourse Leaves

Partial removal of 'Royal Gala' primary spur leaves at king full bloom successfully reduced primary spur leaf area by approximately 50% (Figure 7.6). The reduction was found at all harvest dates and was not influenced by bourse shoot removal. Primary leaf area peaked at 22 days after anthesis after which time leaf area decreased. Bourse leaf area increased steadily after anthesis until reaching a maximum of over 200cm^2 41 days after anthesis. There was no influence of primary leaf area removal on bourse leaf area development.

Cumulative fruit weight increased in a curvilinear fashion up to 63 days after anthesis before increasing in a linear fashion to final harvest (Figure 7.7). The curvilinear phase was exponential from 13 to 42 days after anthesis. There were no significant differences between treatments except at the final harvest date. At this time, spurs without a bourse shoot and 50% of the primary leaves removed at bloom had a significantly lower fruit weight ($P<0.05$) than the other three treatments (by 20%).

Fruit calcium content during the growing season was affected by bourse shoot and primary leaf removal treatments. There was no interaction between these two factors except at 22 days after anthesis (Table 7.3). Therefore, changes in calcium content and concentration over the growing season are presented as main factor averages for each harvest date.

Figure 7.6 Changes in primary and bourse leaf area throughout the growing season for apple cv. 'Royal Gala' following several different leaf removal treatments (Experiment B, 1989). [100% primary leaves + bourse (♦), 100% primary leaves - bourse (■), 50% primary leaves + bourse (△), 50% primary leaves - bourse (▲)].



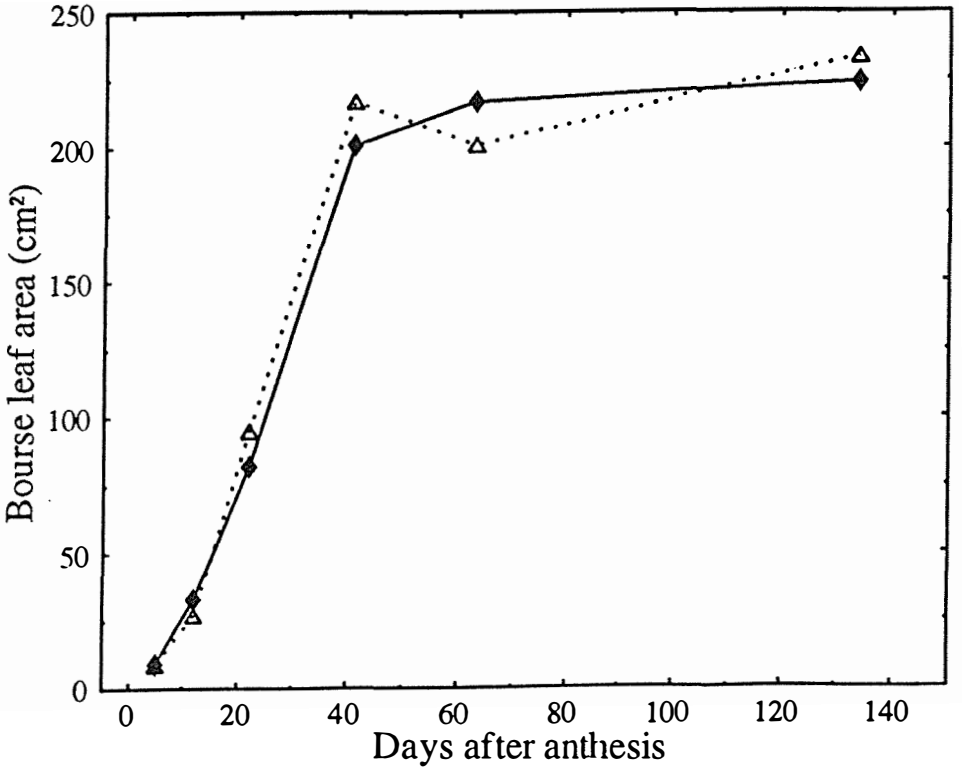
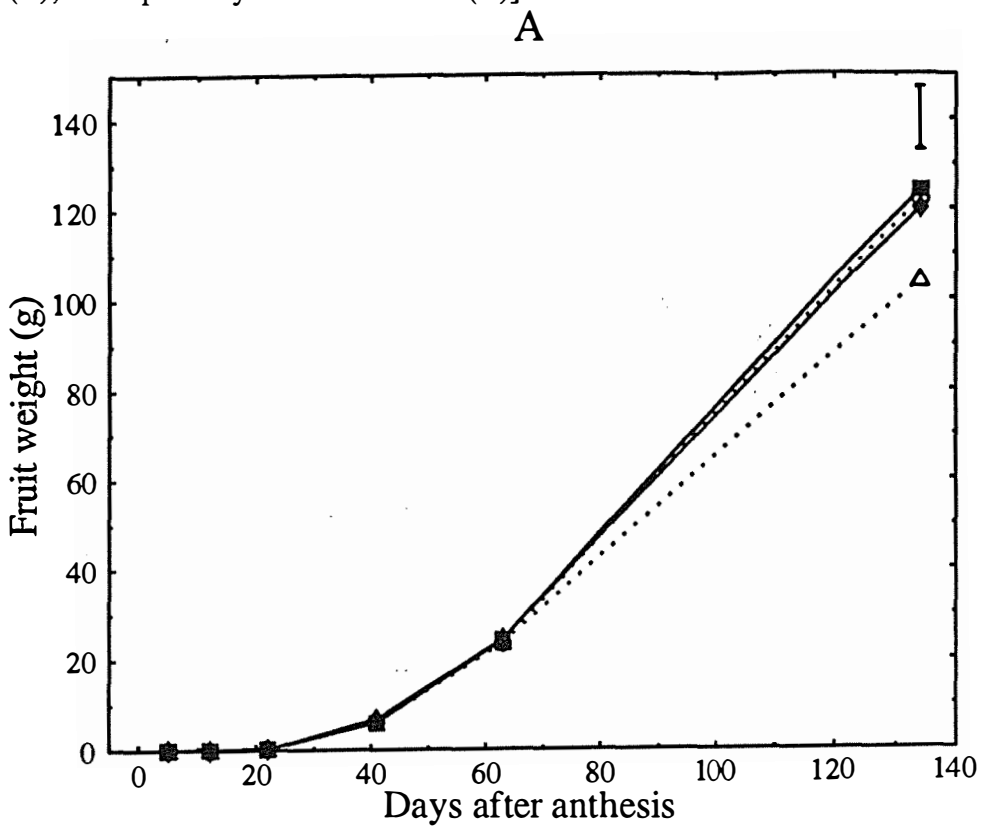


Figure 7.7 Change in fruit fresh weight during the growing season, expressed on a linear (A) or logarithmic scale (B), for apple cv. 'Royal Gala' following several leaf removal treatments (Experiment B, 1989). Bars = LSD (P=0.05) [100% primary leaves + bourse (♦), 100% primary leaves - bourse (■), 50% primary leaves + bourse (○), 50% primary leaves - bourse (△)].



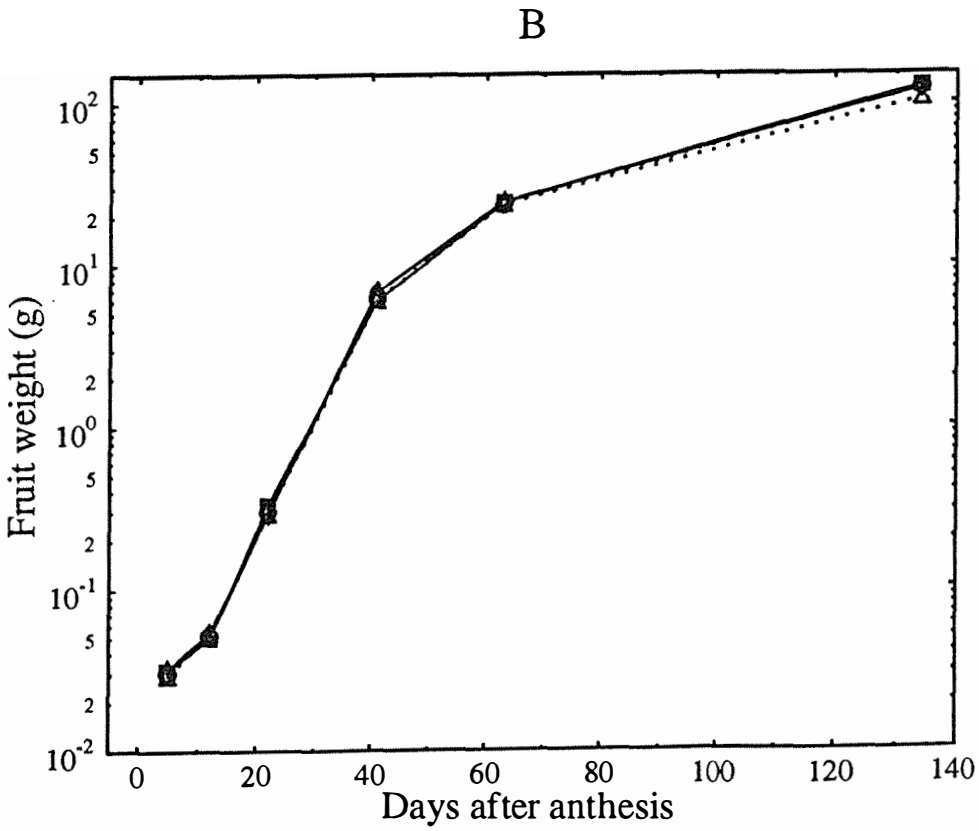


Table 7.3 Summary of the significance of F values from the factorial ANOVA in Experiment B (1989), for fruit calcium content, concentration and rate of calcium uptake.

Days after anthesis	Independent variable	Primary leaf treatment	Bourse shoot treatment	Interaction
5	Content	*	NS	NS
	Concentration	*	NS	NS
9	Rate	NS	NS	NS
12	Content	NS	NS	NS
	Concentration	NS	NS	NS
17	Rate	**	NS	*
22	Content	**	NS	*
	Concentration	*	NS	NS
32	Rate	*	***	NS
41	Content	**	***	NS
	Concentration	*	***	NS
52	Rate	NS	NS	NS
63	Content	*	**	NS
	Concentration	*	***	NS
99	Rate	NS	***	NS
134	Content	***	***	NS
	Concentration	*	***	NS

*, **, *** Significant F value at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 > P$ respectively

NS = Not significant

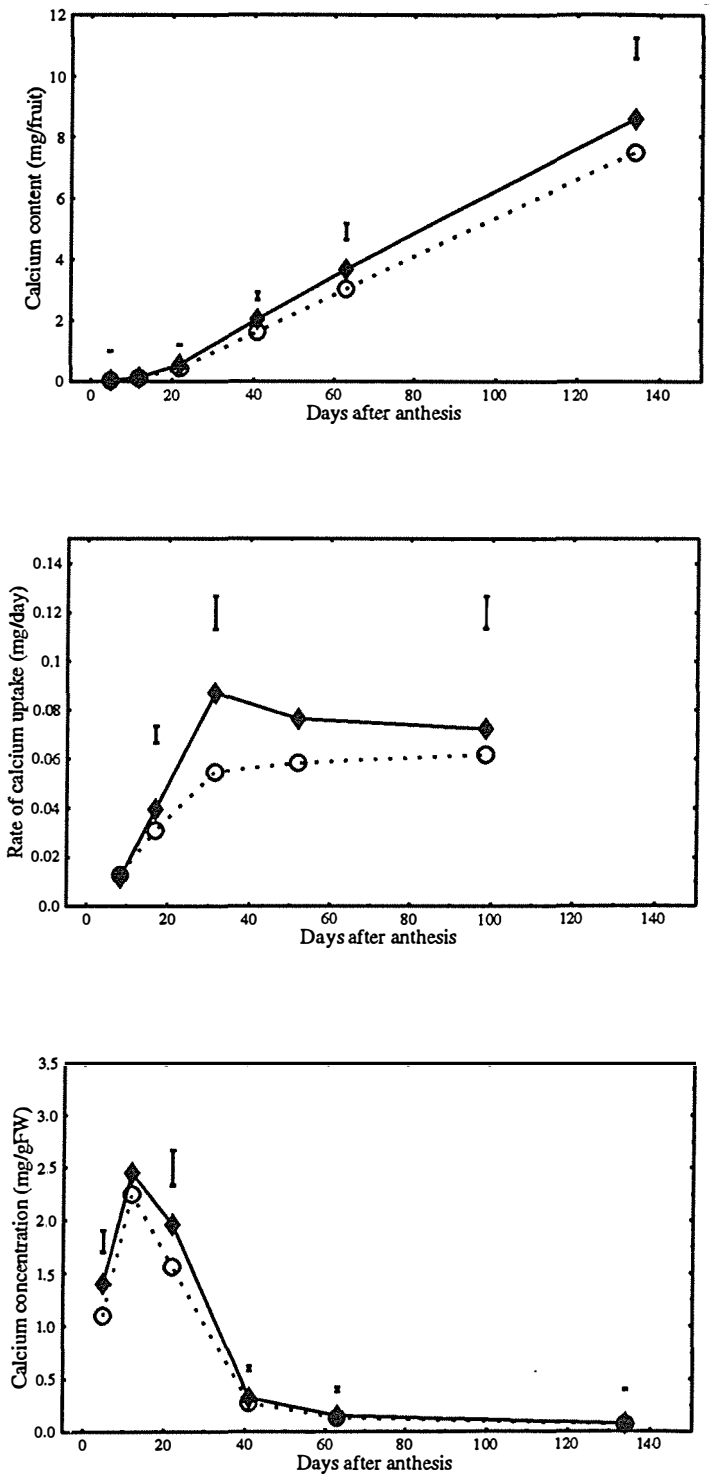
Fruit calcium content showed a curvilinear increase up to 22 days after anthesis. This was reflected by the steady increase in the rate of calcium uptake (Figure 7.8). After this time calcium content per fruit increased at a constant rate up to final harvest.

Removal of 50% of primary spur leaves at bloom significantly reduced fruit calcium content at all harvest dates, except at 12 days after anthesis (Figure 7.8). However at 22 days after anthesis, spurs which had 50% of primary spur leaves removed but retaining a bourse shoot, had a similar calcium content to spurs with a full complement of primary spur leaves (Table 7.4).

Table 7.4 Effect of leaf removal at bloom on calcium content 22 days after anthesis for apple cv. 'Royal Gala' (Experiment B, 1989).

Treatment	Calcium content (mg/fruit)
100% Primary, + Bourse	0.54
100% Primary, - Bourse	0.57
50% Primary, + Bourse	0.50
50% Primary, - Bourse	0.38
LSD (P=0.05)	0.10

Figure 7.8 Changes in fruit calcium content, rate of calcium uptake into fruit and fruit calcium concentration for apple cv. 'Royal Gala' following partial removal of primary leaves (Experiment B, 1989). Bars = LSD (P=0.05) [100% primary leaves (♦), 50% primary leaves (○)].



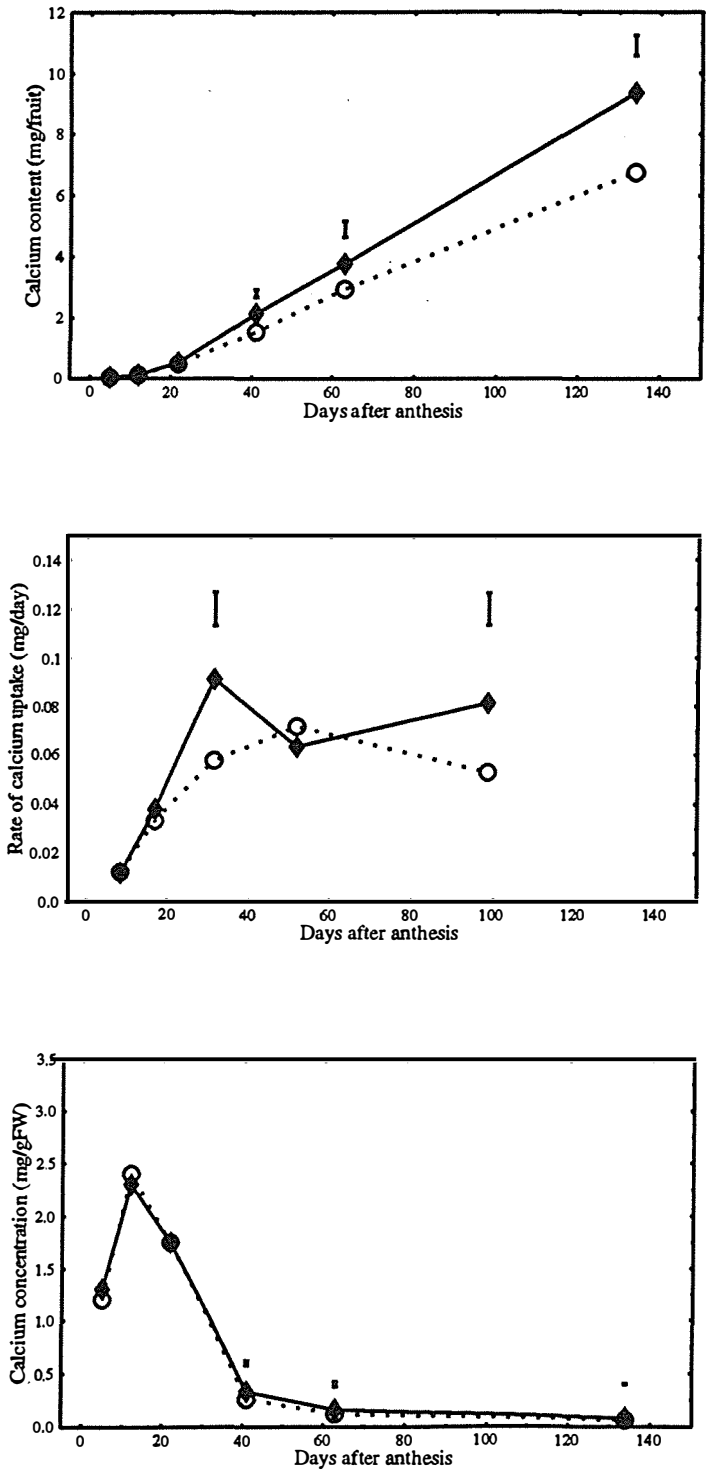
Spurs with 50% of primary spur leaves removed and without a bourse shoot had a significantly lower calcium content than both 100% primary spur treatments at this time ($P < 0.05$). There was a 13% difference in fruit calcium content between 100% and 50% primary leaf treatment at the final harvest. Rate of calcium uptake was significantly lower for the 50% primary spur treatment at all harvest dates except at 5 and 56 days after anthesis ($P < 0.05$).

Fruit calcium concentration increased rapidly after anthesis peaking at 2.5mg/gFW (Figure 7.8). This was followed by a rapid decrease to 41 days after anthesis and a slower rate of decline after this time. Removal of 50% of primary spur leaves significantly reduced fruit calcium concentrations at all dates except 12 days after anthesis. Differences in calcium concentration ranged from 0.1mg/gFW 8 days after anthesis to 0.02mg/gFW at the final harvest (a difference of 7 and 25% respectively).

The specific effect of bourse shoot alone on calcium content and concentration is shown in Figure 7.9. Fruit continued to accumulate calcium throughout the growing season. Removal of the bourse shoot from spurs reduced both content and concentration significantly from 41 days after anthesis. At the final harvest there was a 28% difference in fruit calcium content between spurs with and without bourses, whilst the difference in calcium concentration was 25%. Rate of calcium uptake was significantly lower for spurs without bourses at 32 and 99 days after anthesis.

Fruit magnesium and potassium content increased in a curvilinear fashion up to 63 days after full bloom after which time a linear increase in content

Figure 7.9 Changes in fruit calcium content, rate of calcium uptake into fruit and fruit calcium concentration for apple cv. 'Royal Gala' following removal of bourse shoots (Experiment B, 1989). Bars = LSD (P=0.05) [+ bourse (♦), - bourse (○)].



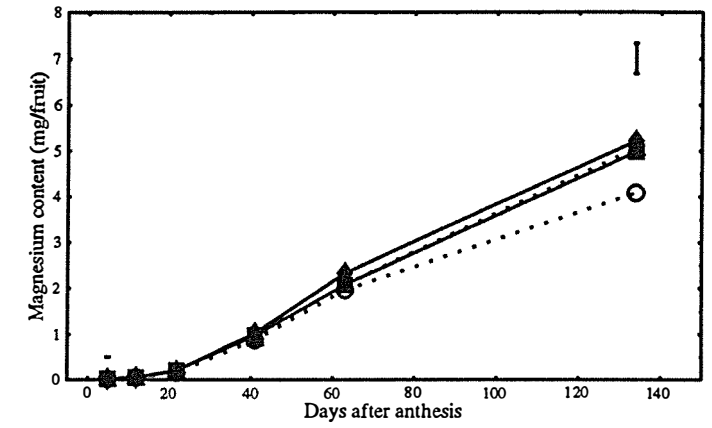
occurred (Figures 7.10, 7.11). This was reflected in the rapid increase in rate of uptake of both minerals after anthesis in rates peaking at 0.06mg/day (magnesium) and 1.8mg/day (potassium) at 52 days after anthesis.

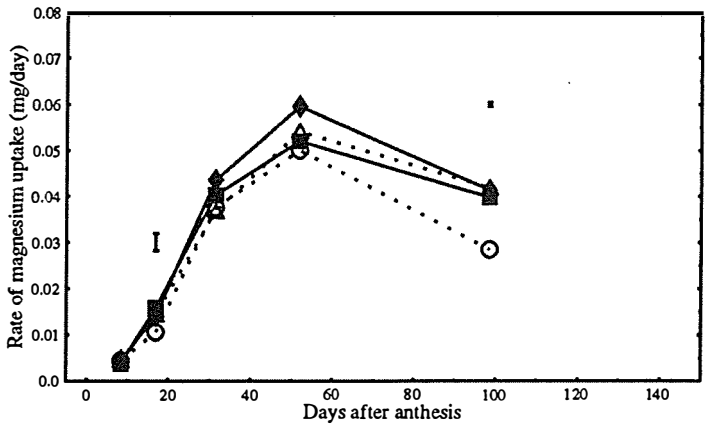
Spurs which had 50% of primary leaves and bourse shoots removed had significantly lower magnesium and potassium content than the other three treatments at final harvest. Rate of mineral uptake was also significantly lower for this treatment at 99 days after anthesis. Otherwise there was no difference in content or concentration of either mineral. An exception occurred 5 days after anthesis when magnesium content was significantly lower for the partial primary and leaf bourse shoot removal treatment.

Fruit magnesium and potassium concentrations both increased marginally after anthesis before declining in a curvilinear fashion to final harvest. Concentration of both nutrients peaked 12 days after anthesis (0.98mg/gFW magnesium; 17mg/gFW potassium).

Calcium content of individual fruit was significantly correlated with primary leaf area and fruit weight at each harvest date (Table 7.5). Correlations of calcium content with bourse leaf area were also significant, albeit weaker compared with the other two variables. Fruit magnesium and potassium contents also showed weak correlations with primary and bourse leaf areas at some harvest dates but were strongly correlated with fruit weight at all harvest dates. Fruit weight was weakly correlated with primary or bourse leaf area at only a few harvest dates as were primary and bourse leaf areas (Table 7.6).

Figure 7.10 Changes in fruit magnesium content, rate of magnesium uptake into fruit and fruit magnesium concentration for apple cv. 'Royal Gala' for several leaf removal treatments (Experiment B, 1989). Bars = LSD (P=0.05) [100% primary leaves + bourse (♦), 100% primary leaves - bourse (■), 50% primary leaves + bourse (△), 50% primary leaves - bourse (○)].





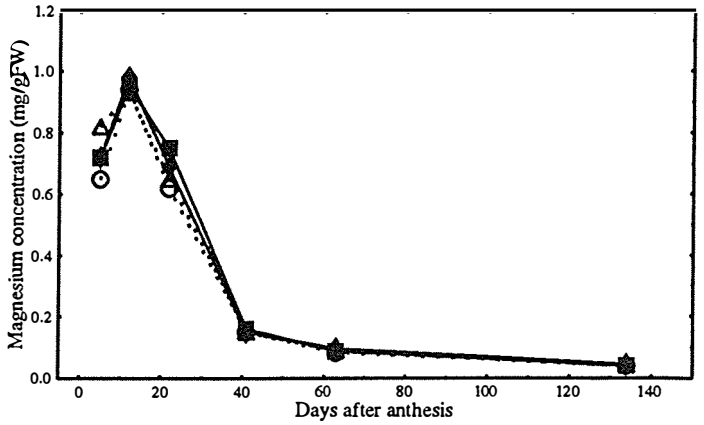
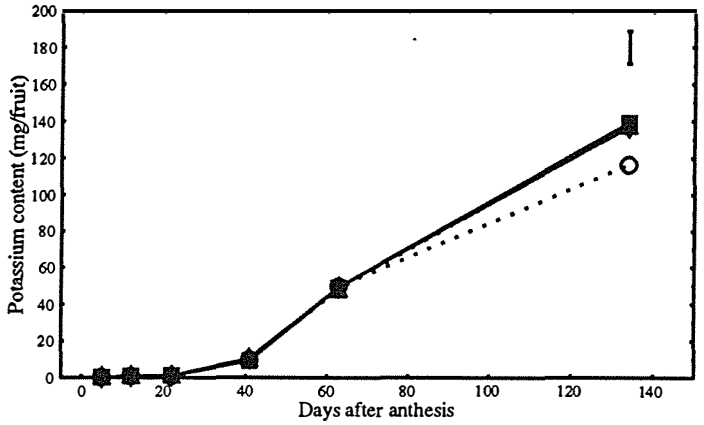
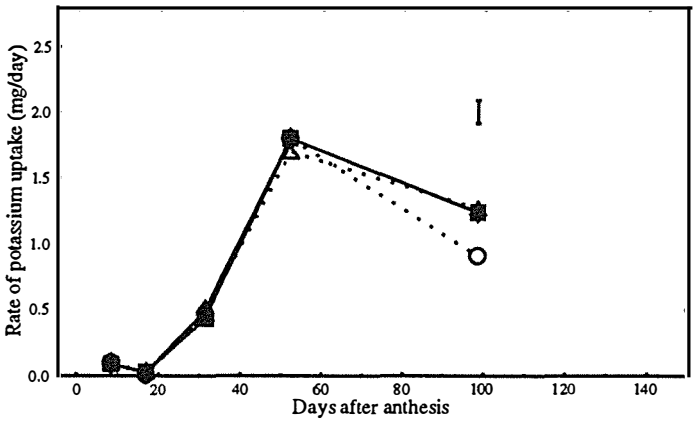


Figure 7.11 Changes in fruit potassium content, rate of potassium uptake into fruit and fruit potassium concentration for apple cv. 'Royal Gala' for several leaf removal treatments (Experiment B, 1989). Bars = LSD (P=0.05) [100% primary leaves + bourse (◆), 100% primary leaves - bourse (■), 50% primary leaves + bourse (△), 50% primary leaves - bourse (○)].





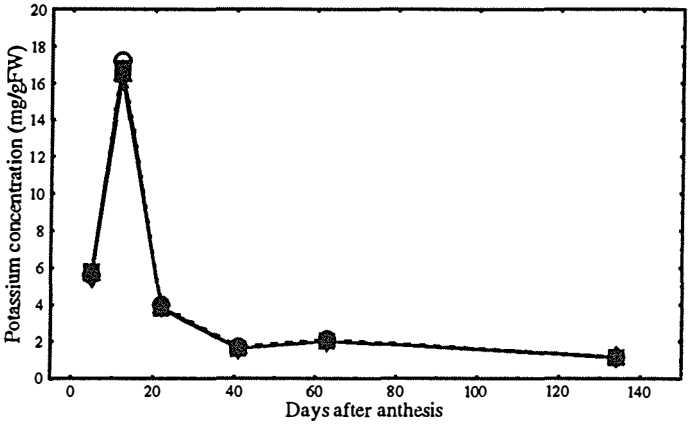


Table 7.5 Correlation coefficients (r) between fruit mineral content, primary and bourse leaf area and fruit weight during the growing season for apple cv. 'Royal Gala' (Experiment B, 1989).

Mineral	Variable	Days after anthesis						Average
		5	12	22	41	63	134	
Calcium	Primary leaf area	0.69***	0.45***	0.51***	0.56***	0.54***	0.57***	0.51
	Bourse leaf area	0.34**	NS	0.28*	0.65***	0.40**	0.46***	0.36
	Fruit weight	0.60***	0.82***	0.50***	0.48***	0.47***	0.57***	0.57
Magnesium	Primary leaf area	0.36**	0.40**	NS	0.47***	0.54***	0.31***	0.35
	Bourse leaf area	0.37**	NS	NS	NS	0.37**	0.28***	0.17
	Fruit weight	0.44***	0.90***	0.30*	0.66***	0.68***	0.91***	0.65
Potassium	Primary leaf area	0.28*	NS	NS	NS	0.38***	0.22**	0.15
	Bourse leaf area	NS	NS	NS	NS	NS	0.20**	0.05
	Fruit weight	0.67***	0.83***	0.44***	0.56***	0.86***	0.92***	0.71

*, **, *** Significant at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 > P$ respectively

NS = Not significant

Table 7.6 Correlation coefficients (r) between primary and bourse leaf area and fruit weight during the growing season for apple cv. 'Royal Gala' (Experiment B, 1989).

Variable(1)	Variable(2)	Days after anthesis						Average
		5	12	22	41	63	134	
Fruit weight	Primary leaf area	0.54***	0.27*	NS	0.38**	0.38**	0.26**	0.31
	Bourse leaf area	0.34**	0.27*	NS	NS	NS	0.20**	0.14
Primary leaf area	Bourse leaf area	0.29*	0.27*	NS	NS	0.35**	NS	0.15

*, **, *** Significant at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.001$, $0.001 > P$ respectively

The causes of variation in final fruit calcium content from individual 'Royal Gala' spurs were explored in terms of final fruit weight, primary and bourse leaf areas. A mathematical model for each of the four leaf/shoot removal treatments was created from the individual spur data using stepwise multilinear regression analysis (Table 7.7). The use of higher order terms (quadratic, cubic) did not improve r^2 or F values over those found for the linear relationships.

Variation in fruit weight and primary leaf area both contributed significantly ($P < 0.001$) to variation in individual fruit calcium content for all leaf/shoot removal treatments except the most drastic treatment (Table 7.8). In this case, the addition of primary leaf area in the model was not significant. Bourse leaf areas also contributed significantly to variation in fruit calcium content for the '+ bourse' treatments ($P < 0.001$). Each model explained between 35-79% of the total variation in fruit calcium content within any one treatment. Further, each treatment model accurately predicted the actual fruit calcium content except for the most drastic leaf/shoot removal treatment (Table 7.9). A 20% difference in predicted and observed calcium content was found for this treatment.

When the most drastic treatment was not considered, the change in fruit calcium content with primary or bourse leaf areas (for any one fruit weight) was similar for each treatment (Table 7.7). However the response of individual fruit calcium content to changes in primary leaf area was 20 times as great as to changes in bourse leaf area. For every 10 cm^2 increase in primary or bourse

Table 7.7 Equations developed to describe relationships between final fruit calcium content and fruit weight, bourse and primary leaf areas for individual 'Royal Gala' apple spurs at commercial harvest after four bloom leaf removal treatments (Experiment B, 1989).

Treatment	Equation	r^2	P value
100% primary leaves, + bourse	$y = 1.45 + 0.044_{(x1)} + 0.1_{(x2)} + 0.005_{(x3)}$	0.79	0.0001
100% primary leaves, - bourse	$y = 2.79 + 0.021_{(x1)} + 0.094_{(x2)}$	0.52	0.0001
50% primary leaves, + bourse	$y = 2.63 + 0.034_{(x1)} + 0.097_{(x2)} + 0.005_{(x3)}$	0.54	0.0001
50% primary leaves, - bourse	$y = 0.52 + 0.044_{(x1)}$ [1]	0.35	0.0001

y = fruit calcium content (mg/fruit)
x_1 = fruit weight (g)
x_2 = primary leaf area (cm^2)
x_3 = bourse leaf area (cm^2)

[1] Relationship between fruit calcium content and primary leaf area not significant

Table 7.8 Summary of stepwise regression procedure relating individual final fruit calcium contents to bourse and primary leaf areas and fruit weights for each leaf removal treatment for apple cv. 'Royal Gala' (Experiment B, 1989).

Treatment	Variable	Partial r^2	Model r^2	P value
100% primary, + bourse	Bourse leaf area	0.06	0.06	0.002
	Primary leaf area	0.14	0.20	0.001
	Fruit weight	0.59	0.79	0.001
100% primary, - bourse	Primary leaf area	0.42	0.42	0.001
	Fruit weight	0.10	0.52	0.004
50% primary, + bourse	Bourse leaf area	0.13	0.13	0.005
	Primary leaf area	0.10	0.23	0.008
	Fruit weight	0.31	0.54	0.001
50% primary, - bourse	Primary leaf area	0.06	-	NS
	Fruit weight	0.35	0.35	0.001

NS = Not significant

Table 7.9 Observed and predicted final fruit calcium content for four leaf removal treatments for apple cv. 'Royal Gala' (Experiment B, 1989).

Treatment	Average fruit wgt (g)	Average primary leaf area (cm^2)	Average bourse leaf area (cm^2)	Calcium content observed	predicted	% Difference
100% primary leaf, + bourse	119.8	18.9	224	9.7	9.7	0
100% primary leaf, - bourse	123.9	22.3	0	7.5	7.5	0
50% primary leaf, + bourse	122.1	9.4	234	9.0	8.9	-1
50% primary leaf, - bourse	103.7	11.3	0	6.1	7.3	+20

NB: Predicted calcium contents calculated using individual treatment models (see Table 7.7).

leaf area there was a 0.94-1.0mg or 0.05mg increase in fruit calcium content respectively. For common primary and bourse leaf areas a 10 g increase in fruit weight was associated with an increase in fruit calcium content which varied from 0.21-0.44mg depending upon treatment.

7.4 Discussion

7.4.1 Effects of Leaves on Fruit Growth

Partial removal of spur leaves from individual spurs before bloom (and during the season) has been previously shown not to effect fruit growth (Ferree and Palmer, 1982; Jones and Samuelson, 1983; Proctor and Palmer, 1991). Only complete spur defoliation reduced fruit size significantly. However in these published experiments, flower/fruit thinning was not carried out and spurs with large leaf areas set greater numbers of fruit than spurs with smaller leaf areas. High fruit numbers on individual spurs can limit growth of fruit remaining (after fruit drop) on spurs (Lakso et al., 1990). Therefore, leaf area limitations on growth of fruit remaining on spurs in the published studies may have been confounded with effects of variable fruit number per spur.

In the present study flower thinning was carried out so that only spurs with one fruit were sampled. Fruit growth was not influenced by partial primary leaf and/or bourse shoot removal in either experiment, (except in the most drastic treatment in Experiment B at the final harvest date) (Figures 7.2,

7.7). The absence of effect on fruit growth occurred despite 90% of the potential total spur leaf area being removed in some cases. Also, in both experiments fruit weight was only weakly correlated with primary or bourse leaf areas or not correlated at all (Tables 7.2, 7.3). Thus results from the present study support the hypothesis that individual spur leaf area does not limit growth of fruit which remain on trees, after drop, unless spurs are at extremely low leaf areas throughout the season (Chapter 4).

C-14 studies have indicated that carbon is mobile throughout the apple tree from mid-season onwards (Hansen and Christenson, 1974). However, their studies also showed that only very small amounts of carbon from distant leaves could be transported to the fruit at 8 weeks after anthesis. Later studies have indicated that carbohydrate may flow from non-fruiting spurs to nearby fruitlets less than 4-5 weeks after anthesis (Lakso et al., 1989). Therefore, any shortfall in local carbohydrate supply for partially deleafed spurs may have been made up by nearby leaves from other spur or shoot systems.

On the other hand, isolation of spurs from the rest of the tree by ringing before bloom does not influence final fruit size although it reduces leaf photosynthetic capacity (Ferree and Palmer, 1982). This indicates that carbohydrate demand by fruit which remain on the tree can be met by local leaf supply. This concept was looked at in more detail by calculating final leaf:fruit ratios for various spur leaf:shoot removal treatments from the data of Ferree and Palmer (1982) (no flower thinning) and compared with those from Experiment B (1989) (flower thinned). Primary and bourse leaf areas were not

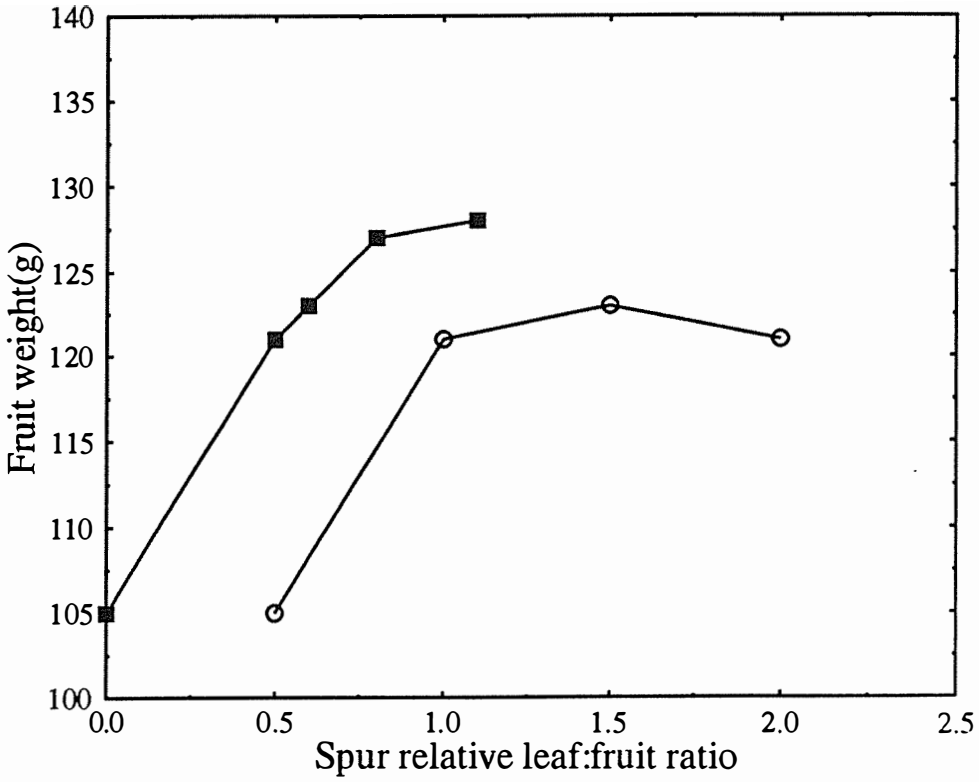
available from the published study, therefore primary and bourse leaf areas were assumed to be the same and each equal to 1 "unit" (following the same procedure used by Jones and Samuelson (1983) when relating effects of variable primary and bourse leaf areas on mineral concentrations in apple fruit) (Table 7.10). That is, where there was only 1 fruit per spur present, a treatment which removed 50% of primary leaves equates to a relative leaf:fruit ratio of 1.5, a treatment with no leaves removed equates to a ratio of 2 and a treatment with 50% of primary leaves and bourse removed equates to a ratio of 0.5. If there were 2 fruit on the spur, relative leaf:fruit ratios would be 0.75, 1 and 0.25 respectively for each of the treatments described above. Quite clearly bourse leaf area in Experiment B (1988) is, in fact, not equal to primary leaf area (225cm^2 compared with 25cm^2 for bourse and primary respectively) (Figure 7.6). Nevertheless, this method provides a basis for comparing results from both studies.

In both studies, a similar threshold relative leaf:fruit ratio on the spur was reached (0.8-1.0), after which fruit weight did not increase (Figure 7.12). A relative leaf:fruit ratio of 1 equates to a primary spur leaf area for 'Royal Gala' of 25cm^2 (Figure 7.6). For spurs on the outside of the tree, under relatively "high" light conditions, it would appear that this spur leaf area is the minimum required to support maximum fruit growth. Further studies are required to analyse minimum threshold spur leaf areas, above which fruit growth is not limited by spur leaf area.

Table 7.10 Final fruit size for apple from spurs with different leaf:fruit ratios. Primary and bourse leaf areas are assumed to be the same and equal to 1 unit.

Study	Relative leaf unit area		Leaf:fruit ratio	Fruit no/ spur	Average fruit wt (g)
	Primary	Bourse			
Ferree and Palmer (1982)	1	1	1.1	1.9	128
	0	1	0.8	1.2	127
	1	0	0.6	1.6	123
	0.5	0	0.5	1.1	121
	0	0	0	0.4	105
Experiment B	1	1	2.0	1.0	121
	0.5	1	1.5	1.0	123
	1	0	1.0	1.0	121
	0.5	0	0.5	1.0	105

Figure 7.12 Average fruit weight for different spur leaf:fruit ratios following leaf removal treatments [Experiment B, 1989 (○), from Ferree and Palmer (1982 (■)]. Spur relative leaf:fruit ratio calculated from the relative amount of primary and bourse leaf areas and fruit number present on a spur at harvest (see text and Table 7.10 for details).



There are few other studies relating spur leaf area to fruit sizing for apple. Fruit growth on spurs is often reduced inside tree canopies where light levels are low (Jackson, 1980; Tustin et al., 1988). Total leaf area of spurs located in the upper part of a canopy can be greater than those in the shaded lower canopy (Ferree and Forshey, 1988). However, other studies indicate little correlation between total leaf area and final fruit size (Barritt et al., 1987). Within-tree canopy variation in fruit size may be more related to light interception and photosynthetic capacity of associated spur leaves, rather than spur leaf area *per se*, especially during the first 5 weeks after anthesis. Carbohydrate partitioning between and within spur units may also be altered by shading (Lakso et al., 1989). Where shading reduces early relative fruit growth rates, fruit sizing can be affected over the whole season (Lakso et al., 1989).

Fruit set was not measured in either experiment in the present study, but it is likely that set differences between partial leaf/shoot removal treatments would have occurred. Apple fruit set can be reduced by slight reduction in individual spur leaf area (Ferree and Palmer, 1982) and total tree leaf area (Llewelyn, 1968). Variation in total spur leaf area for eight apple cultivars was also associated with variation in their productivity (Rom and Ferree, 1984a). These results indicate that fruit abscission is more sensitive to reductions in local carbohydrate supply than is growth of remaining fruit on the spur.

7.4.2 Seasonal Trends in Mineral Uptake

Calcium moved into fruit at an increasing rate at the beginning of the season (all experiments) followed by a constant rate 32 days after anthesis (Experiment B, 1989) (Figures 7.3, 7.8, 7.9). This high constant rate of fruit calcium measured late in the season is consistent with several other reports (Tromp, 1975, 1979; Haynes and Goh, 1980). However in other work, rate of calcium accumulation in fruit was substantially reduced late in the season (Quinlan, 1969; Tromp and Oele, 1972; Ferguson et al., 1987). Differences in fruit calcium content curves between laboratories might have been explained by different tissues sampled. Seeds, skin and core tissues have higher calcium concentrations than that of the outer cortex (Ferguson and Watkins, 1989). Removal of the former tissues from fruit samples might influence the fruit calcium curve during the season. However, all published studies cited also analysed whole fruit samples, except for Haynes and Goh (1980). In Experiment B, fruit were not sampled between 63 and 132 days after anthesis (Figures 7.8, 7.9). It is possible that accumulation of fruit calcium content accelerated immediately after the penultimate sample before slowing prior to final harvest. However, this is unlikely as in all other studies calcium accumulation rate was constant or decreased mid-late season (Wilkinson, 1968; Quinlan, 1969; Tromp, 1975, 1979; Haynes and Goh, 1980; Ferguson et al., 1987).

Indeed, Wilkinson (1968) compared seasonal changes in total fruit calcium (minus seeds) for several orchards over several years. He found that

fruit calcium accumulation increased at a constant rate for all blocks until mid-summer, whereupon it continued at a reduced or constant rate, or levelled off and in one case, decreased. Greater variation occurred between orchard blocks than between years. This indicates that local cultural or soil differences might be important in determining the amount of calcium taken up by fruit late in the season.

At the physiological level, the disparity between workers in calcium uptake patterns late in the season has been explained by late-season phloem supply of calcium to fruit (Tromp, 1975). However, there is little evidence for calcium movement in phloem. Redistribution of calcium from older to young tissue does not occur and calcium movement is always upwards rather than displaying the downward movement of phloem-transported nutrients (Kirkby and Pilbeam, 1984). Calcium concentration in phloem sap is extremely low and it is probable that calcium is actively excluded from sieve tubes (as well as cytosol of other living cells) (Kirkby and Pilbeam, 1984; Ferguson and Drobak, 1988). Backflow of water from fruit to leaves during the daytime under conditions of high leaf water potential might also decrease net calcium uptake into the fruit late in the season (Ferguson and Watkins, 1989). Although there is some recent evidence for backflow of water occurring from fruit to tree, calcium concentrations in this daytime xylem backflow is considerably less than that measured in sap moving into the fruit at night (A. Lang, pers. comm., DSIR Fruit and Trees, Palmerston North, New Zealand).

Differences between workers in the pattern of calcium accumulation by fruit may rather reflect differences in the amount of water supplied by the xylem to fruit late in the season. Wiersum (1966) provided indirect evidence for a sharp reduction in xylem supply to fruit from several weeks after bloom. However, recently Lang (1990) showed that xylem transport to 'Royal Gala' fruit continued throughout growth while that of 'Cox's Orange Pippin' was insignificant in late season. Environmental, cultural and genetic factors influencing late season calcium supply to fruits *via* shifts in xylem:phloem balance may be critical in regulating final fruit calcium content.

Rates of potassium and magnesium influx into fruit also showed a curvilinear increase with time after anthesis in all experiments (Figures 7.4, 7.5, 7.10, 7.11). However in Experiment B the increase lasted for a longer time than that of calcium, until 43 days after anthesis, before decreasing slightly. Slight differences in patterns between calcium and potassium or magnesium accumulation in apple fruit have been noted by other workers (Tromp and Oele, 1972; Himelrick and Walker, 1982). They probably reflect differences in the mechanism of supply. Potassium and magnesium move in the phloem and showed an accumulation pattern similar to that of fruit weight gain. These two minerals appear to be more responsive to fruit "sink" demands than calcium. Calcium moves in the xylem and its accumulation in fruit is probably regulated more by leaf than by fruit factors.

7.4.3 Effect of Leaves on Mineral Uptake

Partial removal of primary leaves at bloom reduced fruit calcium content, this effect occurring whether bourse shoots were removed at bloom (all experiments) and/or were present on spurs (Experiment B) (Figures 7.3, 7.8). Similarly the absence of bourse shoots also reduced fruit calcium content for spurs with variable primary leaf number (Experiment B) (Figure 7.9). Calcium content of individual fruit throughout the season was usually highly correlated to primary and bourse leaf areas across all treatments for all three cultivars (Tables 7.1, 7.5). Natural variation in calcium content of individual 'Royal Gala' fruit at commercial harvest was partially explained by differences in both primary and bourse leaf area (Tables 7.7, 7.8). This leaf effect on fruit calcium accumulation was independent of fruit growth as fruit size was only influenced by the most drastic leaf removal treatment for 'Royal Gala' at the final harvest date. In contrast, neither potassium nor magnesium contents were generally influenced by leaf/bourse shoot removal unless fruit weight was affected. Natural variation in potassium/magnesium content of individual fruit was weakly correlated with primary and bourse leaf area or not related at all throughout the growing season.

Removal of primary leaves from individual spurs at pink bud (Ferree and Palmer, 1982) or complete spur defoliation during the season (Jones and Samuelson, 1983) also reduced final calcium content, most often with little effects on fruit size. However, Ferree and Palmer (1982) measured only a non-significant 9% reduction in fruit calcium concentration in response to

bourse shoot removal at pink bud. In contrast, a 21% reduction was achieved for the similar deboursed treatment in the present study (Experiment B) (7.5 mg/fruit cf 9.5 mg/fruit). The disparity in results may have occurred because there was no thinning to a uniform flower number per spur in the work of Ferree and Palmer (1982), as took place in Experiment B. Unthinned deboursed spurs set significantly lower fruit number per spur than unthinned control spurs (Ferree and Palmer, 1982). Although calcium flow to the spur may have been reduced for deboursed compared with untreated spurs, calcium influx into individual fruit would have been higher presuming equal calcium partitioning between fruit. Evidence for a reduction in fruit calcium content with increasing fruit number per spur is lacking, as no comparison of fruit mineral nutrition for thinned and unthinned spurs has been published. Generally, early season flower/fruit thinning whole trees decreases final calcium concentration in fruit because of a resultant increase in fruit size (Sharples, 1968; Ferguson and Watkins, 1989). However, Quinlan (1969) showed that thinning 'Laxton's Fortune' apple could increase fruit calcium concentrations despite an increase in average fruit size. Further studies are required to test the effects of variable fruit number per spur on fruit calcium uptake.

Trees used in the study of Ferree and Palmer (1982) were on low vigour M 9 rootstocks, whereas those in the present study were on more vigorous M 793. Therefore bourse shoots from spurs in Experiment B may have been

considerably longer with greater leaf area having a greater impact on fruit calcium status, compared with those used in the published study.

The positive effect of primary leaves on fruit calcium status was only measured at or shortly after anthesis (for 'Gala' and 'Royal Gala') and again from 20-34 days after anthesis until the final harvest date for all three cultivars. Complete defoliation of 'Golden Delicious' spurs 3 weeks after bloom also reduced fruit calcium content at 10 weeks but not 2 weeks after treatment (Jones and Samuelson, 1983). The delayed response of fruit calcium status to leaf defoliation at bloom observed in the present work may indicate that factors other than spur leaf area are regulating calcium uptake up to 3 weeks after bloom. The role of fruit growth in regulating calcium uptake at this time will be explored in Section 7.4.5. On the other hand, it may also represent a real time delay between defoliation and resultant effects on fruit calcium accumulation.

A positive effect of bourse shoots on calcium input (in Experiment B) was consistently observed somewhat later than the effect of primary leaves (41 days after anthesis) (Figure 7.8 cf Figure 7.9). This bourse effect coincided with shoot growth termination, and may indicate that bourse leaves contribute to fruit calcium uptake once demand for calcium from the (growing) bourse shoot tip is reduced. Removal of bourse shoot tips in November may temporarily increase calcium flow into fruit (I.B. Ferguson, pers. comm., DSIR Fruit and Trees, Auckland, New Zealand). Competition for calcium between vigorous shoots and fruit may decrease fruit calcium concentrations and thus

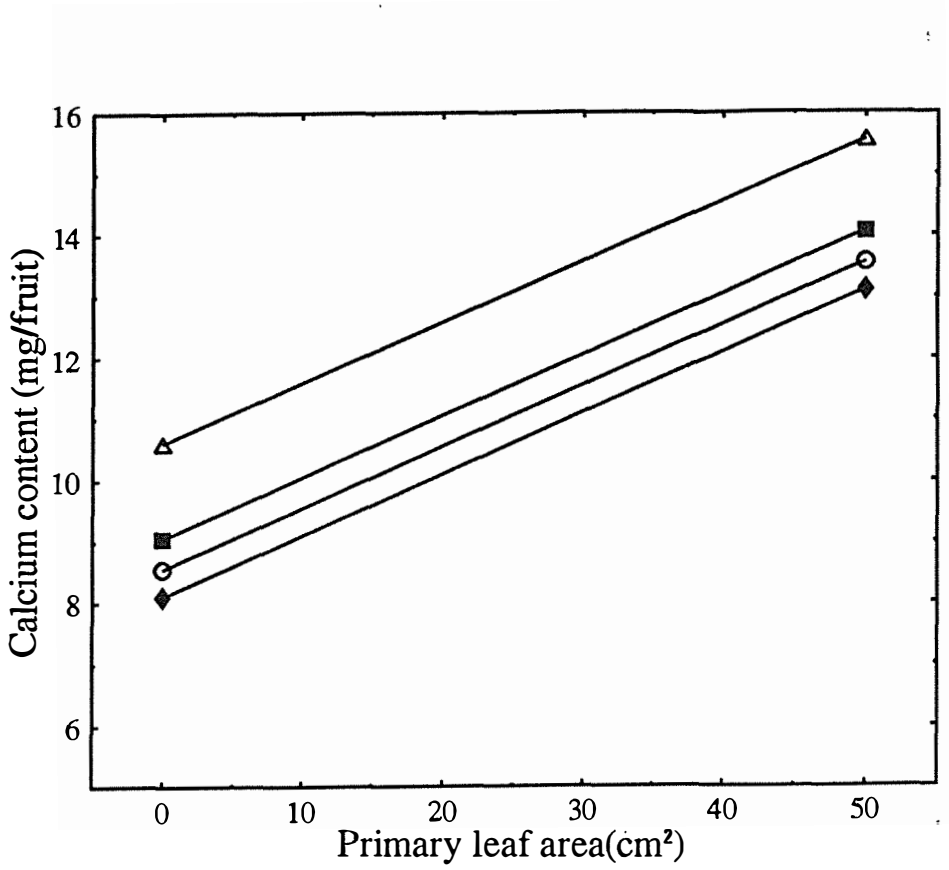
increase bitter pit incidence (Schumacher et al., 1979), while summer pruning of (bourse) shoots can also increase final fruit calcium concentrations (Preston and Perring, 1974).

In the present work the effect of partial removal of primary leaves was half that of complete bourse shoot removal at final harvest (Experiment B). Final fruit calcium was reduced 28% by bourse removal across treatments (Figure 7.8) while 50% primary leaf removal decreased uptake by 13% across treatments (Figure 7.9). However, on a per leaf basis, it is clear that calcium uptake is influenced more by primary than bourse leaves, as primary leaf area was 90% less than bourse leaf area 41 days after anthesis. For individual 'Royal Gala' spurs at final harvest fruit calcium content responded 20 times more to increases in primary leaf area than increases in bourse leaf area.

This concept is illustrated in Figure 7.13. The estimated linear relationship between final fruit calcium content and primary leaf area for a 150g 'Royal Gala' fruit and four bourse leaf areas uses the equation developed for untreated spurs presented in Table 7.6. Primary leaf area was not related to bourse leaf area at final harvest for this cultivar. Therefore calcium content would be dependent upon the relative leaf areas of the two leaf types.

This model offers a basis for comparing the influence of leaf types and fruit size on calcium content for different spurs within the tree, on different trees, orchards and between seasons. It also allows some estimate of the leaf area requirements for attaining "safe" levels of calcium in the fruit. A critical minimum calcium concentration for New Zealand 'Cox's Orange Pippin' apple

Figure 7.13 Influence of primary leaf area on final fruit calcium content for spurs with different bourse leaf areas for a 150g 'Royal Gala' apple fruit. Figure derived from Equation 1 (100% primary leaves + bourse) in Table 7.7. [Bourse leaf area = 500cm² (△), 200cm² (■), 100cm² (○), 20cm² (♦)].



is 2.5mg/100gFW of cortical pulp tissue, which equates to a "whole" apple calcium concentration of 6.5mg/100gFW. For a premium sized 150g fruit, a minimum of 10mg total calcium is therefore required to be taken up by the fruit during the growing season. This could be achieved with a $10cm^2$ of primary leaf area and $200cm^2$ bourse leaf area, or $20cm^2$ of primary leaf area and $20cm^2$ bourse leaf area (Figure 7.13).

In the present study, there was no indication that curvilinear relationships between final fruit calcium content and primary or bourse leaf areas over a wide range of values for individual spurs existed for any deleafing treatment. However other studies have reported a non-linear relationship between final calcium content and (total) leaf area per spur for 'Golden Delicious' (Ferree and Palmer, 1982; Jones and Samuelson, 1983). The latter authors concluded that increases in spur leaf area above a critical threshold level would not increase fruit calcium content. This was based upon a comparison of spurs with long and short bourses. However the 'Royal Gala' model predicts that only very large differences in bourse leaf area will be reflected by large differences in final calcium contents. Further, precise leaf data was not obtained in either published study and different quantitative effects of bourse and primary leaf types were not considered. It would be interesting to manipulate bourse and primary leaves such that the effect of a range of leaf areas on final fruit calcium concentration could be explored further.

7.4.4 Mechanism of Leaf Effect

The mechanism by which leaves exert an effect on fruit calcium accumulation during the season is not clear. Calcium inflow into the fruit is liable to occur in the xylem rather than the phloem (Ferguson and Watkins, 1989), thus reducing spur leaf area may decrease transpiration and mass flow of water and calcium into the spur and fruit. Indeed, calculations of fruit calcium content based on xylem sap concentration and mass flow of water into developing fruit have correctly estimated fruit calcium content for the middle of the growing season but not at the season's beginning or end (Jones et al., 1983). Also a reduction in leaf transpiration rate by bagging bourse and primary leaves from several weeks after petal fall reduces final fruit calcium content considerably (Jones and Samuelson, 1983).

The transpirational flow of water through the plant is driven by evaporation which lowers the leaf water potential which in turn sets up a water potential (pressure) gradient between soil and leaf (Jones, 1985). The force driving mass flow of water and therefore rate of water flow within capillaries such as xylem vessel tubes, is determined by this pressure gradient. Thus a reduction in the area of leaves subtending a spur should reduce the water deficit of the spur and subsequently the rate at which water (and therefore calcium) moves into the spur. Of course, this does not take into account any possible changes in the biological regulation of water movement, particularly stomatal control. Certainly if a significant proportion of leaves were removed from a tree then one might expect an increase in transpiration of the remaining leaves

as a result of an increase in leaf photosynthesis, less within-tree shade and improved air-flow through the canopy. However, leaf photosynthesis was shown not to change after some leaves were removed from individual spurs at pink bud (transpiration was not measured) (Ferree and Palmer, 1982).

Calcium flux into the spur will also depend upon its concentration within xylem sap. Concentration in xylem sap of many nutrients shows a decrease during the season. This is coincident with an increased water flow in the xylem, as tree leaf area increases and temperatures become warmer. However it is difficult to envisage calcium concentration in the xylem changing in response to a reduction in transpiration of an individual spur system.

This proposed reduction of water flow into the fruit due to deleafing is not evidenced by a reduction in fruit fresh weight or % water content (Ferree and Palmer, 1982) and it seems unlikely that fruit transpiration would have been adversely affected by such treatments. However, an explanation for such an observation comes from the knowledge that the contribution of xylem to water influx into fruit decreases substantially during the season in favour of phloem supply (Lang, 1990). A defoliation-induced fruit weight change as a result of reduced water uptake supplied by the xylem may have been too small to detect with the sample sizes used in the present experiment.

The question remains as to the means by which additional water (and calcium) attracted by the spur leaves into the spur is taken up by the fruit. As vascular differentiation is controlled by local auxin, cytokinins and gibberellin

concentration in many plants (Aloni, 1987), spur leaf area may regulate the growth regulator balance and thereby control secondary xylem formation within apple spur tissue. If the number and size of xylem conduits in the spur limits fruit water uptake, then their formation may also limit calcium inflow into the fruit. Tomato fruit calcium concentrations were lower for plants grown in high saline solutions compared with plants grown in solutions of lower conductivity, although fruit weight was unchanged (Evert and Ho, 1986). This reduction in fruit calcium accumulation was associated with a smaller xylem cross-sectional area in the fruit pedicel. The delayed response of fruit calcium to primary leaf removal observed in this and other studies (Jones and Samuelson, 1983) might be indicative of the time required for treatment differences in xylem differentiation within the spur to occur.

An alternative hypothesis is that calcium moves into fruit *via* a non-vascular route, from leaves or spur tissue. Leaf calcium content increases in primary and bourse leaves during the season (Jones and Samuelson, 1983). These authors suggested that diffusion of calcium from leaves to fruit might occur at night, as distances between primary leaves, basal bourse leaves and fruit are small. If this was the case, it might also explain the finding that primary leaves are more efficient than bourse leaves in assisting calcium inflow into fruit (Experiment B). However, it is difficult to envisage how bourse leaves situated some distance (up to 30cm) from the fruit, could influence fruit calcium content (as predicted in the 'Royal Gala' model), if diffusion was the sole means of leaf-fruit calcium transport.

There is good evidence that apple fruit tissue is a calcium "sink" *via* diffusion and also cation-exchange transport mechanisms (Kirkby and Pilbeam, 1984). The degree to which a particular type of transport predominates is dependent upon the concentration of the external calcium solution and the stage of fruit development. Thus fruit disc tissue affinity for calcium *via* cation exchange is greatest at low external calcium concentrations early in the growing season. Diffusion forces are greatest in discs cut from older fruit at high external concentrations (Harker and Ferguson, 1988).

However there is little evidence that such mechanisms are important with regards the leaf effect on fruit calcium accumulation. Jones et al. (1986), determined external "equilibrium" calcium concentrations for apple cortical tissue discs, whereby there was no return movement of calcium into or out of the discs. As these equilibrium concentrations were similar to that of previously measured xylem sap concentrations (Jones and Samuelson, 1983), they concluded that non-vascular movement of calcium into apple fruit was insignificant. However xylem saps were extracted from long one-year old shoots. It may have been more critical to determine the calcium concentration at the fruit pedicel/leaf base interface within the spur. In this way, a more accurate assessment of the roles of diffusion and cation exchange calcium transport mechanism could have been made. Further studies are required to determine the roles of diffusion and cation exchange transport systems in relation to the beneficial effects of primary and bourse leaves on fruit calcium accumulation.

7.4.5 Effect of Fruit Growth on Mineral Uptake

Potassium and magnesium inflows into the fruit during the season would seem to be regulated by those factors influencing carbohydrate movement into fruit. Potassium inflows into fruit followed that of cumulative fruit growth changes in most cases, both increasing in a curvilinear fashion up to mid season before continuing linearly to commercial harvest (Figures 7.5, 7.11). Magnesium fruit content followed a similar pattern to that of fresh weight for 'Royal Gala' (Figure 7.10) (but not for 'Gala' or 'Golden Delicious' in Experiment A) (Figure 7.4). Individual fruit potassium and magnesium contents were strongly related to fruit weight at all times in all three experiments (Figures 7.1, 7.5). There were no differences in potassium and magnesium concentrations between treatments. These results confirm conclusions from other studies which indicate that fruit sink strength determines potassium and magnesium inflow (*via* the phloem) into fruit (Tromp and Oele, 1972; Tromp, 1979).

Primary and bourse leaves may provide a significant pool of magnesium and potassium ions available to the fruit. Further, the movement of magnesium from these leaves to the fruit may not always be linked with carbohydrate movement. Removal of bourses at bloom reduced fruit magnesium content without concurrent reduction in fruit growth (for 'Gala' in Experiment A and Ferree and Palmer, 1982).

By contrast, calcium inflows into fruit were not strongly related to fruit size. Fruit calcium content did not follow fresh weight change during the

season. Nevertheless, calcium content of individual fruit was related to fruit weight throughout the growing season in both experiments. This is not likely to be confounded by leaf effects on calcium uptake since primary or bourse leaf areas were either weakly correlated or not correlated at all with fruit weight during the season. At final harvest for 'Royal Gala', fruit weight explained between 10 and 59% of the natural variation in individual fruit calcium content depending upon leaf removal treatment. Calcium accumulation followed that of fresh weight gain early in the growing season, and from 0-16 days after anthesis was not influenced by defoliation treatment in any experiment.

Jones and Samuelson (1983) calculated calcium uptake into fruit based on mass flow estimates into spurs. They underestimated the observed calcium inflow, especially early in the season. The influence of fruit growth at this time on calcium uptake may be important. An increase in fruit growth might influence fruit calcium content through effects on intensive cell division acting as a "sink" for calcium. Certainly where fruit cell division has been stimulated (by an increase in air temperature), fruit calcium accumulation also increased (Tromp, 1975). Ion-exchange process may be an important mechanism for directing calcium to active sinks, such as fruit. That is, the cation exchange sites in newly synthesised fruit cell walls are "filled" with calcium ions from the xylem (Clarkson, 1984). Another mechanism whereby fruit growth might directly stimulate calcium uptake is *via* production of the plant hormone, IAA. There is a considerable body of evidence which indicates that basipetal movement of IAA (eg from developing fruit) stimulates acropetal movement of

calcium (Banuelos et al., 1987). Seeds produce large amounts of IAA immediately after anthesis (Luckwill, 1953), coincident with the time when fruit growth is having its most significant effect on calcium uptake. Apple fruit with high seed numbers also have high fruit calcium contents relative to fruit with low seed numbers (Bramlage et al., 1990). It would seem that factors controlling the rate of fruit growth immediately after anthesis are more important than those controlling later fruit growth, for the purpose of stimulating calcium uptake into the fruit.

In summary, partial defoliation of primary leaves and/or bourse shoot removal at bloom of spurs thinned to one flower reduced fruit calcium accumulation throughout most of the growing season. Partial removal of primary leaves influenced calcium uptake earlier than bourse shoot removal and on a per leaf basis was more effective in reducing calcium uptake. Fruit size, potassium and magnesium levels were not influenced by deleafing except at commercial harvest where defoliation was drastic. Nevertheless, fruit size was highly correlated with fruit calcium content, particularly 1-3 weeks after bloom when leaf effects on calcium were minimal.

The most likely mechanism by which spur leaves influence fruit calcium uptake is through leaf control of xylem differentiation within the spur, thereby altering the flow of water and calcium to the fruit, although there is little evidence for this in apple.

CHAPTER EIGHT

GENERAL DISCUSSION

8.1 On-Tree Variation in Fruit Quality and Productivity - Shading Effects Within the Canopy

This study confirms the benefit of producing fruit on replacement branches located on the outside of the tree. Fruit on the outside of the tree canopy are generally larger, have higher soluble solids and have a more intense and increased skin coverage of red blush colour than fruit located inside the canopy (Chapter 5). This has been observed previously for apple (Jackson et al., 1971; Barritt et al., 1987) and for other fruits (Patten and Proebsting, 1986; Dann and Jerie, 1988). Susceptibility to some apple storage disorders, such as brown core (Smock, 1946), shrivelling and core flush (Jackson, 1967; Jackson et al., 1971) is also higher for fruit inside the canopy. Conversely, some quality characteristics can also be poorer for fruit located outside the canopy compared with those on the inside. Outer-located fruit may be more susceptible to storage disorders such as bitter pit and fungal rots (Jackson, 1967; Jackson et al., 1971) and for 'Granny Smith', have a yellower skin colour (Chapter 5; Tustin et al., 1988). Fruit productivity is also greater for buds borne on the outside of the canopy than for those inside. Flower bud

initiation and fruit set are lower for buds inside the canopy (Cain, 1971; Jackson, 1980).

Further variation in some of these characteristics can also be found within a sector of the tree canopy. This is often dependent upon orientation of the branch. Pendant branches growing on the outside of the tree have a greater fruit drop and produce smaller fruit with lower soluble solids concentration at harvest than fruit from replacement branches growing horizontally or vertically (Tustin et al., 1988).

Role of Light

The basis of many of these fruit quality and productivity differences is probably the influence of light. Within (and between) tree shading results in differential levels of light being received by fruit and fruiting sites within the canopy (Jackson, 1980). Light radiation across the tree canopy has often been positively related to fruit size and soluble sugar levels (Barritt et al., 1987; Tustin et al., 1988), and red colour (Jackson, 1970b; Seeley et al., 1980; Morgan et al., 1984). Shade treatments applied throughout the season to previously well-exposed apple trees reduce fruit set, size, yield and fruit colour while reducing flowering in the following season (Jackson and Palmer, 1977; Doud and Ferree, 1980). On the other hand a reduction in fruit size due to shading may lead to an increase in flesh firmness (Heineke, 1966; Robinson et al., 1983) and a higher concentration of fruit calcium (Jackson et al., 1977),

the latter contributing to a decrease in fruit susceptibility to bitter pit (Jackson et al., 1971).

There can be little doubt that shading of fruit within the apple tree during the growing season inhibits skin anthocyanin production for red-skinned cultivars (Saure, 1990), while stimulating green colour development for green-skinned cultivars such as 'Granny Smith' (Chapter 5; Tustin et al., 1988; Hirst et al., 1990). The former effect is likely to occur through inhibition of PAL enzyme synthesis *via* stimulation of the inactive form of phytochrome (Saure, 1990). In dark and at low red to infrared ratios (as occurs inside tree canopies) the inactive form of phytochrome dominates. Other fruit quality characteristics influenced by shade would seem to occur as a result of, or connected with shade-induced inhibition of fruit growth (eg. flesh firmness, mineral concentrations, soluble solids concentrations). However, the physiological mechanism/s by which within-tree shade affects fruit growth are not well understood.

Timing of Fruit Growth Inhibition by Shade

Development of flower parts (Bergh, 1985a) and primary and basal bourse leaves (Pratt, 1990) within the flower bud occur after flower bud initiation during the previous season. Therefore one might expect the growth of the fruit and/or the characteristics of the leaves supplying much of the carbohydrate to the fruit early in the growing season (Hansen, 1971) to be also influenced by the light environment in the previous year. Total previous

accumulation of photosynthetically active radiation (PAR) determines leaf structure and leaf photosynthetic capacity rather than light illumination at any one time (Barden, 1977). Flower bud diameter measured at the end of the growing season has been positively correlated with canopy light level (Barritt et al., 1987) and with fruit set in the following season (Dennis, 1986).

Whilst a negative carry-over effect of heavy shade on fruit set of whole trees has been previously noted (Jackson and Palmer, 1977), moderate or light shade levels had no such effect. Further, artificial shading whole trees early in the season (Rom and Ferree, 1984b) or of individual spurs during middle-late season (Rom and Ferree, 1986b) had no effect on fruit set or growth of fruit or spur leaf characteristics in the following season. The main effect of shading on fruit set and fruit growth therefore would seem to occur during the current growing season.

As leaves and shoots develop on the tree canopy after budbreak, light levels within the tree fall to a minimum between 1 and 4 weeks after anthesis (Ferree, 1989). During this time, growth of shaded fruit which remain on the tree until harvest as well as those shaded fruit which later abscise is inhibited, compared with exposed fruit (Rom and Ferree, 1984b; Lakso et al., 1989). Further, Lakso et al. (1989) concluded from relative growth rate studies on exposed and naturally shaded fruit that the major effect of shading on final fruit size was during this early phase of growth. Heavy fruit abscission following 3 days of artificial shading of whole trees occurs only between 2 and 4 weeks after bloom (Byers et al., 1991). This critical period of growth occurs before

the maximum rate of fruit growth and at the time of final fruit drop measured in the present study (Chapters 3 and 4). Growth inhibition is likely to occur through effects on cell division. Artificial shading of whole trees reduces final cell number in fruit (Jackson et al., 1977), and fruit cell division occurs up to 6-8 weeks after anthesis (Denne, 1963).

Shade Effects on Carbohydrate Supply to Fruit

There is a significant body of evidence which indicates that such an effect of shading on fruit growth 1-4 weeks after bloom is due to limitations of carbohydrate supply to fruit clusters. Shading has large effects on both assimilate production and distribution of that assimilate within the tree. Total dry matter production of apple trees is linearly related to light interception accumulated by the canopy during the season (Palmer, 1989). Natural shade within the canopy reduces the photosynthetic rate of leaves surrounding nearby fruit (Chalmers et al., 1975; Lakso, 1980). Artificial shading of whole trees reduces the photosynthetic rate of previously well exposed leaves (Barden, 1977) and probably of whole tree canopies. Shade within the apple canopy may also directly decrease the rate of assimilate loading at the source leaf/phloem sieve element boundary and rate of carbon flow through the phloem, as has been shown to occur for other plants (Hartt, 1965; Geiger and Bateley, 1967). Moderate shading of apple extension shoots 3-5 weeks after bloom was reported to eliminate carbon export from these shoots to nearby fruit (Lakso et al., 1989). Further, Johnson and Lakso (1986) predicted from

a carbon balance model of a growing 'Jonamac' shoot, that time to first net carbohydrate export of a long (50cm) shoot grown in a low light environment would be delayed compared with that of an exposed shoot (18 cf 26 days after bud break respectively). Johnson and Lakso (1986) also predicted that total assimilate export production, after 30 days, would be 70% lower for shaded compared with exposed shoots.

Carbohydrate supply to fruitlets on shaded spurs may be limiting at the "local spur" level. Hansen (1971) predicted from early 14-C studies that assimilates were provided to fruits entirely from subtending bourse and primary spur leaves during the first 3 weeks after bloom. Partial defoliation and ring-barking of individual spurs at pink bud and up to 2 weeks after bloom reduces fruit set (Ferree and Palmer, 1982; Proctor and Palmer, 1991), and final fruit size, although to a much lesser extent (Chapter 7; Ferree and Palmer, 1982; Proctor and Palmer, 1991). Further, on an individual spur, the bourse shoot tip may actively compete with developing fruitlets for carbohydrate from primary spur leaves 1 week after bloom and from "mature" bourse leaves 3-8 weeks after bloom (Tustin and Lai, 1990). Reduction of this competition through tipping increases carbohydrate flow into the fruit (Quinlan and Preston, 1971). However, recent work indicates that carbohydrate may be available to fruiting spurs from other leaves much earlier than that predicted by Hansen's (1971) study. Lakso et al. (1989) reported that for the first 2-3 weeks after bloom non-fruiting spur leaves can supply carbohydrate to nearby fruitlets.

After this time, extension shoots begin to export carbohydrate to developing fruit. Hansen and Christenson (1974) had earlier concluded that carbohydrates could only move in limited amounts over large distances from one side of the tree to the other 8 weeks after bloom. However their 14-C experiments did not include the possibility of short-distance travel from spur-spur or shoot-spur on the same branch. Slight reductions in spur leaf area reduces fruit set, but only if conducted up to two weeks after anthesis (Proctor and Palmer, 1991). After this time there is no effect of defoliation - additional evidence that assimilate may become available to fruit from outside the spur much earlier than first envisaged.

If assimilate is available throughout the tree to fruiting spurs soon after anthesis, then it could be argued that the efficiency of the total tree canopy in capturing light and producing assimilate is a major determinant of early fruit growth and final fruit set.

With a knowledge of fruit growth rates, leaf areas and leaf photosynthetic rates at 4 weeks after full bloom, one can model an approximate carbon balance of whole trees at this time. Leaf carbon supply can be estimated and a prediction made as to whether supply might limit growth of fruit on the tree, thereby stimulating fruit abscission. Unfortunately data on fruit number, fruit growth rates, leaf area and photosynthetic rates measured on the same tree at this critical time do not exist. Some information has been provided on total tree leaf area and flower/fruit number per tree for 'Golden Delicious' trained as slender spindles (Ferree, 1980). This data has been included with fruit

growth rates (Chapter 4) and leaf photosynthetic rates (Ferree and Palmer, 1982) in calculating carbon demand and supply for these trees (Table 8.1). Notwithstanding the assumptions that carbon demand has been under estimated (as tree respiration and demand by other sinks were not taken into account), carbon demand would seem to be in excess of supply at this critical time.

Table 8.1 Carbon balance model for an apple tree.

		Assumptions
Carbohydrate supply		
Leaf area/tree	= 12.9m^2	(1)
Leaf Pn rate	= 2 $gCO_2m^{-2}hr^{-1}$	(2)
Therefore tree Pn rate	= 206 $gCO_2tree^{-1}day^{-1}$	(2)
	= 56 $gCtree^{-1}day^{-1}$	
Carbohydrate demand		
No fruit/tree	= 5900	(3)
Individual fruit growth rate	= 0.3 $gFWday^{-1}$	
	= 0.03 $gDWday^{-1}$	(4)
Total fruit growth rate	= 177 $gDWday^{-1}$	
	= 73 $gCday^{-1}$	

A number of assumptions have been made in the model. They are detailed as follows:

1. It was assumed that full canopy development had been reached by 4 weeks after blossom, as trees had reached 90% full leaf area 2 weeks after bloom (data from Ferree, 1980).

2. The leaf Pn rate was provided from average rates of individual 'Golden Delicious' leaves 4 weeks after bloom (Ferree and Palmer, 1982). All leaves were from well-exposed spurs and does not take into account Pn rates of shaded leaves. As such the Pn rate calculated is likely to be a maximum rate only. Also assumes that tree respiration rates are negligible.

3. Fruit no per tree 4 weeks after bloom was assumed to be equal to flower no per tree. This assumed that all flowers successfully set during the preceding 4 weeks. Carbon demand was also assumed to include only fruit at this time and not other sinks such as cambium, vegetation and roots.

4. Fresh weight growth rate of fruit was assumed to be equal to those described in Chapter 4. Fruit dry weight was assumed to be 10% of fruit fresh dry weight at this time.

While assimilate supply may limit fruit growth/set for all fruiting clusters (and fruit) on the tree at this critical time, it does not explain in itself localised effects of shading inside the canopy. Tymoszuck et al. (1984) conducted several C14 and dye experiments tracing the pattern of assimilate movement from extension (water) shoot to fruiting spur. The authors suggested that carbohydrate transport from shoots to fruiting spurs took place only where direct vascular connections existed between them. The extent of phloem differentiation between shoots and spurs may limit carbohydrate supply from elsewhere in the tree. If carbohydrate resources from subtending leaves are also limiting (such as within a shaded tree canopy) then a reduction in fruit set and growth might occur. The extent to which shading influences development of phloem is unknown, and so this hypothesis remains speculative. Length of the pathway from source to sink may also contribute a significant limitation to assimilate movement (Patrick, 1988). Thus in the apple tree, length of the vascular system pathway from exposed leaves to shaded fruiting clusters may also limit carbohydrate supply to the shaded fruiting clusters.

The idea that the vascular connection and distance between source and sink have a major influence on partitioning is not new. Cook and Evans (1983), on wheat, showed that a small sink (spikelet) could receive more assimilate if located on the same side as the source (awn), relative to another larger spikelet which was closer to the awn, simply because of stronger vascular connections. It would seem that carbohydrate partitioning to fruit positioned in different parts of the canopy may be influenced by an interaction

between distance and strength of the vascular connections between fruit and (sun) leaves. However whether these two factors indeed limit fruit sizing inside the canopy is not known.

Within-Canopy Temperature Effects on Fruit Growth

Temperature differences between inside and outside the tree canopy may also contribute to differences in source and sink strengths. For instance, Thorpe (1974) concluded that effects of sunlight on apple fruit size could be simply due to heating. Leaves and fruit exposed to the sun can be up to 10-14 °C warmer than those organs located in the shade (Thorpe and Butler, 1977; Robinson et al., 1983). Whilst having a direct stimulatory influence on fruit growth [as suggested by Thorpe (1974)], and other growth processes (eg. ripening, Chapter 5), such temperature gradients may also have a major influence on carbon balance of shoots. Johnson and Lakso (1986) using a carbon model of long shoots, predicted that a 10°C reduction in maximum temperature (25 to 15°C, as might occur between leaves on the inside and outside of the canopy) would result in an 80% reduction in carbon export from those shoots 30 days after budbreak. As little information is available on leaf and fruit temperature gradients in the tree during the early season, further studies are required to assess their influence on fruit growth and development within the canopy.

Shade Effects on Fruit Sink Strength

Demand for assimilate may be relatively lower for fruit inside the canopy compared with fruit on the outside. Relative sink (fruit) strength just before fruit drop and rapid growth may be crucial in determining different rates of sizing and final fruit set of fruits borne in different locations on the tree.

In this regard light may have a direct influence on sink strength of apple fruits. Shading grape (Quinlan and Weaver, 1970) and rose (Mor and Halevy, 1980) shoot tips reduces sink mobilizing capacity. This effect may occur through the action of light on hormone metabolism (Vonk et al., 1986).

Apple fruit sink activity may also be influenced by the quality of light. In several plant species changes in ratios have been shown to have major photomorphogenic effects on plant growth (Vince et al., 1983) as well as sink strength (Wareing and Patrick, 1975). For instance, inside the soybean canopy a reduction in the red:infrared ratio decreases sucrose accumulation in fruit so increasing fruit abscission. Such regulation probably occurs *via* the phytoreceptor, phytochrome (Myers et al., 1987).

Light radiation is active for apple leaf photosynthesis from 400-700nm. These wavelengths are absorbed by leaves so that inside the apple tree canopy their levels are reduced (Jackson, 1980). However infrared radiation (730nm) is transmitted through apple leaves (Palmer, 1977). Infrared wavelengths are subsequently found at relatively high levels inside the apple canopy and the ratio of red to infrared is low.

The influence of different red to infrared ratios on apple fruit growth, abscission and sugar levels at harvest is not known. Recently however, Greene et al. (1986) reported that fruit abscission on apple trees could be inhibited by short illumination bursts of red light at night. Effects if they occur may be mediated *via* phytochrome.

A shade-induced reduction in fruit sink strength may not result in a reduction in fruit growth and abscission by way of a direct reduction in demand for carbohydrate *per se.* Assimilate levels in the tree or in the fruit do not change under conditions which invoke abscission such as low light or heavy cropping (Avery et al., 1979; Beruter, 1985). For a high crop loaded tree, Beruter (1985) suggested that "hormonal signals" emanating from successfully competing fruit may inhibit the competitive capacities and sink activities of other fruit on the tree. Bangerth (1989) cites evidence from various studies on bean, apple and tomato where high indoleacetic acid (IAA) concentrations in, and transport from fruit have correlated with a high set. In apple fruit high auxin levels in the seed are correlated with a reduced tendency to abscise (Ebert and Bangerth, 1981; Andrews et al., 1985). Situations where inter-fruit inhibition have been manipulated experimentally in apple resulted in significant changes in diffusible IAA from remaining fruit (Gruber and Bangerth, 1990). Andrews et al. (1985) concluded for pear that fruitlet abscission was based upon a reduction in the capacity of a fruitlet to transport auxin across the pedicel rather than loss of auxin synthesis capability *per se.*

An alternative mechanism (other than competition for assimilate) whereby fruit situated on the outside of the canopy might directly inhibit those on located on the inside might involve them:

(1) becoming a more "dominant" sink through being supplied with more assimilate during the early stages of fruit growth from subtending sun leaves and/or through light quality/temperature effects

(2) producing a higher level of diffusible IAA which is transported to "inner" located fruit, so inhibiting fruit growth and stimulating abscission. However until such experimental evidence becomes available determining interactions between shaded and exposed fruit during 1-4 weeks after bloom, this hypothesis remains speculative.

In summary, the role of shade in influencing fruit quality and productivity within the apple tree is clearly very important. There is a direct effect of low light on skin colouring. Most other effects on fruit quality are connected with an inhibition of fruit growth which occurs during an early phase of fruit development, 1-4 weeks after full bloom. This inhibition results in an increase in fruit abscission and a reduction in final size of fruit which remain on the tree. The evidence to date suggests that it is probably mediated by several interacting factors involving both light and temperature effects which include:

1. Carbohydrate supply to fruit from subtending spur/bourse leaves.

2. Differentiation of secondary phloem linking fruiting spurs to other spur and shoot complexes in a more "exposed environment".

3. Direct fruit sink activity regulation *via* hormonal and/or phytochrome influences.

8.2 On-Tree Variation in Fruit Quality and Productivity - Influence of Fruit Position Within the Canopy

In the present study, differences in the physiological maturity of apple fruit located on the inside and outside of the tree canopy were also apparent (Chapter 5). Quality differences between fruits borne at different locations on the tree may occur if fruit are not harvested at the same stage of physiological maturity. Green ground skin ('Granny Smith'), flesh and probably background skin colour of red cultivars may be strongly influenced by such maturity differences within the tree (Chapter 5). Susceptibility to core flush and shrivelling during storage increases (Wilkinson and Sharples, 1967) while susceptibility to scald (Watkins et al., 1982a) and bitter pit (Padfield, 1969) decreases with increasing fruit maturity. One can speculate that in some instances, differences in the maturity of fruit harvested at the same time from different locations on the tree may lead to variation in the keeping quality of these fruits during storage.

Delayed maturity of fruit inside the tree may also interact with shade to influence fruit quality. The extent to which fruit inside the tree canopy have a lower red coverage compared to fruit on the outside would seem to be dependent on the fruit's stage of maturity (Chapter 5).

The delay in maturation for fruit on the inside of the tree was associated with a delay in the timing of ethylene evolution (Chapter 5). Such a delay is probably not related to differences in light level *per se* as shade has little effect on the timing of the climacteric (Jackson et al., 1977). Fruit position with respect to distance from the tree trunk may play an important role in determining apple fruit maturity and quality, this role being independent of environmental influences. Recently, on single branched trees of peach, fruit located near the branch tip grew faster and matured earlier than those at the basal end of the branch (Dann et al., 1990). In contrast, there was more vegetative growth and flowers opened earlier at the branch base.

Relative (fruit) sink activity may be strongly influenced by positional signals emanating from growing shoots and roots (Chalmers, 1985). Apical buds and young leaves produce abscisic acid and auxins and export auxins basipetally (Goodwin, 1978; Goodwin and Ernee, 1983). Mature leaves contain and export auxins, cytokinins, and gibberellins. Cytokinins and gibberellins are formed in roots and move acropetally towards the top of the plant, being found in large quantities in root exudate and xylem sap (Skene, 1975; Tromp and Oovaa, 1990). Chalmers (1985) suggested that growth of cambial meristems at any one point on the tree may be determined by the

balance of all these hormones. Further, competition between fruit sink and cambium would determine fruit growth. The growth increment of apple trees usually tapers towards the top. Thus one might expect that growth of cambium in branches located inside the tree canopy would be greater than that of branches located on the outside. Following the above hypothesis, inner-located fruit would compete less favourably with non-fruit sinks for assimilate. It is interesting to note that maximum cambium growth in apple occurs immediately after bloom (Evert, 1963) which is when "inner" located fruit exhibit a reduction in sink activity (1-4 weeks after bloom, Lakso et al., 1989).

The nature of the hormones which might influence ripening patterns of fruit located on the tree is less clear. Greater extension shoot growth and leaf dry weight per fruiting spur occurs on the outside compared with inside the apple tree canopy (Barritt et al., 1987; Ferree and Forshey, 1988; Ferree, 1989). One therefore might expect higher concentrations of leaf-derived hormones to be found in leaves located near "outer" positioned fruit. Although Mousdale and Knee (1981) associated a peak in auxin level with the beginning of apple ripening, exogenous auxin applications inhibits ripening in pear (Frenkel and Dyck, 1973). ABA has been associated with promotion of fruit ripening, both in terms of exogenous application to fruit and reported increases in concentration within fruit tissues occurring at the same time as fruit ripening (Rhodes, 1980; Brady, 1987). However, recently Tsay et al. (1984) (in Brady, 1987) could find no relationship between ABA level and ripening for several fruit. It is also commonly observed that environmental stresses can increase

ethylene production in plant tissue and this can be associated with increased ABA levels, particularly in young leaves and stems (Goodwin and Ernee, 1983).

Evidence against the role of leaves in promoting apple fruit ripening (*via* hormonal effects) comes from work by Sfakiotakis and Dilley (1973). They isolated apple fruit from the rest of the tree by bark ringing spurs and/or removed leaves subtending the fruit and showed that leaves tended to inhibit fruit ripening. It can be argued however that these drastic treatments may have resulted in fruits being "stressed" so that ABA may have directly induced ripening. Inner-canopy located fruit are also closer to the roots from which significant amounts of hormones are produced. Gibberellins have been reported to inhibit ripening of some fruits (Ben-Arie et al., 1986 in Saure, 1990). However, the role of roots and leaves in modifying the ripening patterns of apple fruit within trees remains speculative.

8.3 On-Tree Variation in Fruit Quality and Productivity - Influence of Bud-Type on the Replacement Branch

Bud-type Effects on Fruit Quality and Productivity

A significant amount of variation in fruit quality and fruit productivity is also apparent within a replacement branch growing on the outside of the tree where light levels are similar across the branch. Such differences can be attributed directly to the type of bud from which the fruit is borne. Thus fruit

size from two-year spurs is greater than fruits from lateral buds on long extension shoots, whilst that for fruit on one-year terminal buds is intermediate (Chapters 4 and 5). The proportion of flower buds which set fruit and number of fruit per fruiting site at harvest are also lowest for the one-year lateral bud type (Chapter 3).

Fruit maturity is delayed on one-year lateral buds compared with fruit from two-year spur buds (Chapter 5). There is little influence of such delayed maturity on red colour development or soluble sugar concentration in fruit at harvest. However 'Granny Smith' fruit from two-year spur buds may show a greater loss of green colour over the harvest period than fruit from one-year lateral buds (Chapter 5).

Fruit from one-year terminal buds have very high calcium concentrations, while fruit harvested from one-year lateral buds have lower calcium concentrations and lower Ca:Mg and Ca:K ratios than fruit from older spur buds (Chapter 4). Fruit from two-year spur buds are intermediate. For 'Granny Smith' these differences between fruit of different bud types were apparent when fruit of the same size were selected. This indicates that fruit from one-year lateral buds may be more susceptible to bitter pit and senesce more quickly after harvest than fruit from older spur buds. In contrast, fruit from one-year terminal buds may be less susceptible to bitter pit and senesce more slowly.

Differences in fruit size, set and maturity within the replacement branch may be directly attributable to differences in bud characteristics at bloom.

Flower receptacle size is smaller for one-year lateral buds compared to those from two-year spur buds (Chapter 4). Early flowering buds have been shown to produce larger flowers and fruit at harvest for apple (Denne, 1963) and kiwifruit (Lai, et al., 1990) and to produce fruit of higher quality for sweet cherry (Patten et al., 1986) than later flowering buds. The delay of approximately one week in fruit maturity from lateral buds at harvest is similar to the difference in the time of anthesis between these two bud types. Differences in fruit abscission between two-year spur, one-year terminal and one-year lateral buds was also associated with the timing of flower bud opening at bloom. Fruits on the later bud type open later and had the highest fruit drop, while fruit on two-year spurs opened earliest and had the lowest fruit drop (Chapter 6).

Factors Influencing Bud Characteristics

In apple, both timing of flower bud initiation and rate of flower bud development may affect bud characteristics at flowering (Buban and Faust, 1982). Factors which influence these two processes may ultimately be important in determining fruit quality at harvest and after storage, as well as the distribution of fruits on the replacement branch and on the tree as a whole. In sour (Roberts, 1917) and sweet cherry (Patten et al., 1986) a large leaf area subtending the bud during the previous season was associated with early flowering during bloom. Differences in flower characteristics at bloom between bud types on apple replacement branches may also be related to

differences in the number or area of leaves subtending buds in the previous season - a low leaf number per bud associated with delayed bloom and reduced king flower receptacle size. Only one leaf subtends one-year lateral buds in the previous season while at least seven leaves can be associated with potential two-year spur buds (one-year laterals in the previous season, Chapter 6). One-year terminal buds have 2-3 leaves subtending them in the previous year. This may well indicate that the rate of flower bud development on the replacement branch is limited by nutrients and/or hormones supplied by the subtending leaf in the previous year.

Large differences in total spur leaf number and area were also found for one, two and three-year old spur buds on spur-type 'Red Delicious' trees (Rom and Barritt, 1990). However, these differences were not reflected by differences in flower number per bud in the following spring. Further, they reported that partial removal of leaves from spurs in the summer reduced flower bud initiation, but had little influence on king receptacle diameter or other bloom characteristics of those spurs which did flower. Crop loading on the tree (Bergh, 1985b) and possibly on individual spurs, and shading (Auchter et al., 1926) have both been shown to affect rates of flower bud development. Rom and Ferree (1984b) indicated that early season shading trees from tight cluster to fruit set onwards could delay bloom in the season of shading and reduce flower number per cluster in the following year. Crop loading and shading in the previous season may be more important in determining bud characteristics at bloom than simple leaf number or area subtending the bud.

Further studies in this area of factors influencing bud development are warranted.

8.4 Influence of Leaves on Mineral Uptake

This study clearly shows that large within-tree differences in fruit calcium accumulation is induced by variation in subtending primary and bourse leaf area. Removal of bourse shoots at bloom reduces calcium content (Chapter 7), calcium uptake into the fruit being inhibited from six weeks after bloom (Chapter 7). Differences in fruit calcium content between bud types on the replacement branch would seem to be associated with differences in bourse leaf area (Chapter 6). Primary leaves are clearly also very important since differences in fruit calcium content in fruit are also associated with differences in primary leaf area (Chapter 7). Their removal at flowering reduces calcium content from 21 days after bloom (Chapter 7) and on a per leaf basis they would seem to be more efficient in assisting calcium into the fruit than bourse leaves (Chapter 7, Ferree and Palmer, 1982).

Subtending spur and bourse leaves also supply carbohydrate to fruit soon after anthesis (Hansen, 1971; Tustin and Lai, 1990). However, the role of spur leaves in directly limiting fruit set and fruit growth though limitations of such supply is debatable. Additional carbohydrate would seem to be available to fruit at this time from other shoot and spur complexes on the tree soon after anthesis. Inter-sink inhibitory effects may also be important (see Section 8.1).

Nevertheless, the dual role of limiting carbohydrate and calcium supply to fruit suggests a common "leaf control" mechanism regulating fruit growth and fruit calcium status. Such a mechanism may involve the development of vascular tissues within the spur connecting fruit to other leaves on the branch (phloem-carbohydrate) and to water conducting tissue in the branch (xylem-calcium). Such development may be mediated by the subtending leaves themselves.

Leaves and Vasculature of the Spur

Within new spur tissue it is likely that differentiation of secondary xylem and phloem occurs after anthesis, the extent of vascular differentiation depending upon growth of local spur leaves. At bloom, flowers are connected to older branch tissue through the central axis of the bud. This axis develops during the season into the new "spur". In new apple extension shoot tips, differentiation of primary phloem and xylem occurs at the beginning of leaf primordial differentiation (Pratt, 1990). Within the developing flower bud, primary vascular tissue is therefore likely to develop synchronously with flower formation in the bud. However in the following season, differentiation of secondary xylem formation begins 2-3 weeks after full bloom, at which time two-thirds of secondary phloem tissue has differentiated (Evert, 1963). Within the fruit pedicel (of Asian pear), secondary xylem and phloem begins differentiation 1-2 weeks after anthesis continuing throughout the season (Nii, 1980).

Apple spur leaves may regulate differentiation of vascular tissues through alteration of endogenous hormone levels within the spur. Spur defoliation decreases ABA, while increasing GA and cytokinin concentrations in the subtending closed buds (Edwards, 1985). Auxin levels have not been measured in apple bud or spur tissue following deleafing. However removal of mature and young leaves from several plant species can reduce flow of polar and non-polar transported auxin within the nearby vascular transport system. This has been associated with a concurrent reduction in xylem formation (Jacobs, 1984).

The mechanism whereby spur leaves might regulate vascular differentiation in the spur may be one involving xylem-borne hormones (eg. cytokinins or gibberellins) (Tromp and Oovaa, 1990). These hormones are produced from root tips or stem tissue and may be directed into the spur *via* the transpiration stream tissue. Various cytokinins promote xylem formation (Aloni, 1987). Increasing humidity around *Helianthus* leaves reduced root-originated cytokinin supply and at the same time secondary xylem formation in the stem (Saks et al., 1984). Quite clearly, however, this hypothesis remains speculative until some experimental evidence is available linking the leaf effect on calcium supply to the fruit to vasculature of the spur.

In summarising the "leaf-control" hypothesis therefore, apple spurs with low leaf area would (1) directly supply less assimilate to developing fruits, (2) indirectly reduce vascular differentiation within the spur, thereby leading to lower calcium and carbohydrate (from external spur/shoot sources) flow to developing fruitlets.

Regulation of Spur Leaf Area

Factors influencing the development of both primary and bourse leaves would therefore seem to be important in determining fruit calcium uptake. Partial leaf removal from vegetative spurs in mid-summer reduced bourse leaf area of spurs which flowered in the following season, although neither primary leaf area nor leaf dry weight were affected (Rom and Barritt, 1990). Thus bourse shoot growth seems to be particularly sensitive to changes in leaf area in the previous season. Up to 9-10 bourse leaf primordia can initiate and develop at the base of 1 or 2 primary leaf initials within the developing flower bud (Pratt, 1990). The nutritional/hormonal requirements of basal bourse leaves in flower buds may only be met once that of other vegetative/flower components within the bud has been satisfied.

Spurs within the canopy also tend to have a smaller total leaf area compared with more exposed spurs (Barritt et al., 1987; Ferree and Forshey, 1988; Ferree, 1989), although in these studies primary/bourse leaves were not distinguished. Artificial shading of spurs early (Rom and Ferree, 1984b) or late in the season (Rom and Ferree, 1986b) did not influence leaf characteristics in the following season. This indicates that hormonal control rather than carbohydrate availability may limit leaf area development for spurs within the mature canopy.

The bourse growing tip may actively compete with developing fruitlets for calcium (Schumacher et al., 1979) as well as carbohydrate (Quinlan and Preston, 1971; Tustin and Lai, 1990). This indicates that the growth dynamics

of the bourse shoot may play an important role in determining mineral and carbohydrate uptake by the fruiting cluster. Interaction of different growing shoots borne on the replacement branch may occur through correlative inhibition and apical dominance (Brown et al., 1967; Mika, 1986; Bangerth, 1989). Such interactions may be important in determining calcium and carbohydrate flow into fruit borne in different locations. The extent to which pruning operations can affect such shoot interactions and thus mineral and carbohydrate inputs warrants further study.

8.5 Influence of Bud-Type Sink Strength on Fruit Growth and Abscission

The evidence in this study suggests that the capacity of fruit on one-year lateral buds to import carbohydrate is reduced because of their smaller receptacle size (initial sink size) compared with fruits on two-year spur buds (Chapter 4). This can be viewed as a physical limitation on carbon import (Ho, 1988). In contrast there appears to be little physiological difference in growth between fruit from these two bud types. Their sink activities were similar as indicated by their similar relative growth rates during the growing season (Chapter 4). This is surprising since fruits from the former bud type blossomed later (Chapter 3). Fruit on one-year laterals should have been at a competitive disadvantage (for assimilate) and subsequently relative sink activity should have been lower compared with fruits from earlier opening flowers, as has been shown for a number of other crops (Stephenson, 1981).

One could argue that there may not have been a shortage of assimilate for fruit from both bud types. However, fruit abscission was greater for fruit on one-year lateral buds. This may indicate that assimilate was limiting (for set at least) on these buds. Certainly, the whole-tree carbon balance model provided in section 8.1 supports the hypothesis that assimilate limits fruit development at this time.

Alternatively, direct inter-sink dominance effects (see Section 8.1) may be involved in causing these differences in set (Bangerth, 1989; Gruber and Bangerth, 1990). High IAA "export" from better developed fruit on two-year spurs might have inhibited IAA "export" from fruit on later flowering one-year lateral buds. Possibly what is of importance in determining fruit set on the replacement branch is the concentration of IAA produced by two-year spur fruit which is transported to the base of the pedicel of one-year lateral bud fruit. However there is little evidence for this effect occurring on apple, at least on apple replacement branches.

It is therefore proposed that on apple replacement branches fruit abscission occurs so that assimilate demand is balanced with supply. The heavier fruit drop on one-year laterals would allow those fruits remaining to compete successfully for assimilates from nearby leaves. Partial or complete defoliation of spurs at bloom reduces fruit set drastically but size of fruit remaining is little affected by such treatments unless at very low leaf:fruit ratios (Chapter 7; Ferree and Palmer, 1982; Proctor and Palmer, 1991).

This mechanism does not seem to operate for shaded fruiting spurs inside the tree canopy, as shading increases fruit abscission, as well as reducing growth of fruit remaining on the tree. Possibly the mechanism by which shade influences fruit growth is different to that which influences fruit abscission. Shade may directly effect cell division within the fruit, by influencing light quality, thereby influencing fruit growth (see Section 8.1). A shortage of carbohydrate imported into the spur from shaded primary and bourse leaves would result in increased abscission as the spur alters assimilate demand to match supply.

It is clear that one of several factors may explain the many differences in fruit quality and productivity between fruit located on various bud types on the replacement branch (and indeed the tree). For instance greater fruit abscission on one-year laterals may be related to the fruit's later developmental stage or lower bourse leaf area. Clearly it would have been beneficial to clarify the role of each of these factors so as to assess which was more important. This could have been achieved by removing bourse leaves at bloom (and afterwards) on two-year spurs (and one-year terminals), so that their total leaf areas were similar to that of one-year lateral buds. Conversely a method of advancing bloom on one-year lateral buds so that full bloom coincided with that of two-year spur buds may have lead to a better understanding of which factor/s limit fruit growth, abscission and mineral uptake in apple.

8.6 Conclusions: Implications for Tree Management

The art of growing fruit is based partly upon growers applying a set of scientific principles at any one time to a given situation. This allows growers to maximise profit within their own set of individual circumstances. This study has shown that a wide range of quality exists in fruit produced from the standard centre-leader pyramid tree grown in New Zealand orchards. Light and fruit position within the canopy, as well as bud type on the replacement branch, appear to be dominating factors influencing fruit skin colour, eating quality, physiological maturity (Chapter 5), fruit mineral concentrations (Chapter 6) and fruit size (Chapter 4). A major objective of growers in tree management must therefore be to reduce the number of reject low quality fruits while maximising the number of high quality marketable fruits on the tree. In future, growers must be prepared to accept changes in the definitions of "marketable" and "reject" as specified by the market and adapt management practices accordingly. Nevertheless results from this study have a number of important implications for present day apple tree management.

Planting Systems

This study confirms the detrimental effect of bearing large numbers of fruit on the "inside" of tree canopies. At present, most New Zealand orchards are composed of trees arranged in single hedgerows on semi-dwarfing rootstocks (MM 106 or M 793). Trees are usually conically shaped and at

maturity, approximately 4m high with a maximum diameter of 3m. These dimensions obviously vary with soil, climate and scion cultivar. Nevertheless, many fruiting sites within such trees intercept low levels of light because of shading (Morgan et al., 1984; Tustin et al., 1988). This directly contributes to reduced productivity and quality of the fruiting site as well as producing an inefficient canopy for harvesting light. Such canopies produce fruit with a wide variation in skin colour, eating quality and size (Chapter 5; Morgan et al., 1984; Tustin et al., 1988). Corelli and Sansivini (1989) also concluded that light distribution within the canopy rather than the total amount of light intercepted by the canopy was the major limitation imposed by orchard design and training systems in Bologna, Italy.

Many European apple training systems have been developed which have much smaller shallow canopies than those grown in New Zealand. For instance the single hedgerow slender spindle developed in Holland, uses dwarfing rootstocks, such as M 9, and trees are grown as conically-shaped centre-leaders. Three to five permanent horizontal branches are inserted on the centre-leader, with fruiting laterals borne on these (similar to replacement branches). Trees are approximately 2m in height and width, depending on cultivar, soil type etc. In the North European environment, such systems seem to yield higher quality fruit than more extensive systems (Palmer, 1989). They have been suggested as suitable growing system alternatives for New Zealand growers.

Total solar radiation accumulated over the growing season by tree canopies is likely to be substantially higher in New Zealand than in Northern Europe, because of higher sunshine hours and higher solar elevations during spring and autumn (Monteith and Unsworth, 1990). Fruit dry matter production increases with an increase in seasonally intercepted PAR (photosynthetically active radiation) (Palmer, 1989). Light interception is determined by leaf area index, among a number of other factors. Thus smaller volume canopies in New Zealand may not perform as well as large tree volumes because of a lack of canopy density and leaf area. Optimum production of export fruit in the future may well come from orchards with canopy volumes and forms somewhere between the present day New Zealand system and those shallow canopy designs of Northern Europe. Clearly further studies are required to assess the overall performance of smaller volume canopies in New Zealand.

Winter and Summer Pruning

Management practices carried out every season on centre-leader trees can also be fine-tuned to reduce variation in fruit quality. Pruning of trees in winter enables growers to regulate the number and position of potential fruiting buds in the canopy. Removal of pendant branches (Tustin, 1989) and old spur buds must occur so that potential sites of poor quality fruit are removed. Dormant pruning of mature trees also should remove large structural branches from the tree so that light penetration into the tree interior is increased.

Such dormant pruning will also encourage new vertical and horizontal extension shoots to grow as replacement branches for the following years (Mika, 1986). Ideally these new shoots should be distributed evenly over each tier branch on the tree. This will optimise the number of potential replacement branches within the canopy while not clumping them together on the canopy exterior.

The number, position and length of these new shoots is determined by the overall vigour status of the tree and vigour balance or equilibrium within the tree (Lespinasse, 1977). This vigour status and balance is influenced not only by the amount and type of winter pruning but also crop load and tree shape. Tree vigour management is a vital part of replacement branch development.

New shoots which grow in the previous season should not be headed even though this would remove one-year lateral buds, a source of poor quality fruits. On vigorous trees (such as those in New Zealand orchards) heading of upward growing shoots in winter often encourages new strongly growing shoots to develop from the cuts (Mika, 1986). The remaining part of the shoot stiffens on the tree and will not bend downwards in later years where required. New shoots should be left unpruned for at least two or three seasons so that they become replacement branches bearing large numbers of two (and three) year old spur buds.

A further refinement could be to remove some extension shoots during winter pruning so that in later years, replacement branches are evenly

distributed along each tier branch. This might allow better penetration of light into the canopy. Generally, such shoot thinning is less invigorating than heading shoots (Mika et al., 1983), although many factors seem to regulate overall shoot growth responses to dormant pruning (eg. cultivar, rootstock, crop load, soil type etc) (Mika, 1986). Summer pruning may also offer some possibilities in reducing the density of new shoots without involving vigorous regrowth. Summer pruning can increase light penetration into the tree canopy (Morgan et al., 1984) although in some circumstances, it can reduce light interception, presumably by reducing leaf area index and spread of the canopy (Palmer, 1989). Summer pruning can also have the additional advantage of increasing red skin colour coverage of some cultivars, without affecting other fruit quality characteristics (Morgan et al., 1984; Mika, 1986).

Pollination

Production of fruit on two-year spur buds and one-year terminal buds in replacement branches should be maximised so that large fruit with high calcium concentrations are produced, with even (and early) maturity at harvest. Two and three-year spur buds are the earliest flowers to open on the tree. Flowering of suitable pollinizers must overlap with these spurs so that adequate cross-pollination at bloom occurs on these spurs. 'Braeburn' is an early flowering cultivar and is often surrounded by cultivars which bloom late. Consideration might be given to planting early flowering cultivars or different *Malus* species within or nearby 'Braeburn' blocks. These pollinizers must

produce viable pollen and overlap with the entire 'Braeburn' flowering period. This should ensure adequate fertilisation of flowers and seed production in fruit on two-year spurs.

Thinning

Fruit set on one-year lateral buds should be minimised. This occurs naturally to a limited extent as unsuccessful competition with (or domination by) fruit on other bud types on the replacement branch often occurs after flowering. This results in a relatively low fruit set on one-year lateral buds. This competition/domination can be reinforced by ensuring that heavy fruiting occurs on two-year spur and one-year terminal buds. Individual spurs often have a biennial bearing tendency. Flowering (and fruiting) on one-year lateral buds in one season may not allow flower initiation to occur on the same bud site in the following year. This not only reduces the number of high quality fruits on the branch but also tends to increase fruit set on one-year lateral buds.

Elimination of fruit on one-year lateral buds can simply take place by selective hand thinning after final fruit drop. However, there is some advantage in thinning at or shortly after bloom, as this can increase the growth rate of fruit which remain on the tree (Quinlan and Preston, 1968). Chemical thinning of flowers or young fruit offers growers an alternative method of reducing fruit number on one-year lateral buds. Traditionally, chemical thinning strategies have aimed to reduce crop load across the tree thereby increasing overall fruit size (Williams, 1979). However growers could also

selectively remove fruit on one-year lateral buds by taking advantage of the late flowering of this bud type.

DNOC (sodium 4,6-dinitro-ortho-cresylate) is a blossom thinner which damages the exposed flower parts (Williams, 1979). Application of this chemical to trees at a time when one-year lateral flowers are open and when fertilisation on other bud types has been completed should reduce fruit set specifically on these buds. Other post-blossom chemical thinners which induce fruit abscission at a specific stage of fruit development include carbaryl (1-naphythylCN-) methyl carbamate and NAA (Napthalenacetic acid). Carbaryl is the most common fruitlet thinner used in New Zealand apple orchards and for some cultivars thins maximally at a fruitlet diameter of 12mm (Knight, 1980). Looney and Knight (1985) found that 'Greensleeves' fruit on one-year lateral buds were thinned more easily than spur fruit when carbaryl was applied to each 7 days after petal fall. NAA has also been shown to thin optimally at petal fall and also when fruit reach 15-18mm in length (Donoho, 1967), although different cultivars may show "optimum" sensitivities at different fruitlet sizes (Leuty, 1973). Benzyladenine (BA) also offers some potential as a selective fruitlet thinner for apple. Although this chemical has been tested experimentally on just a few cultivars, it also thinned maximally when fruit size was 10mm for 'McIntosh' (Greene and Autio, 1989). Thus, specific thinning of potentially low quality fruit on replacement branches might occur if one of these chemicals was applied at a timing which coincided with (1) high thinning

sensitivity for fruit on one-year lateral buds, (2) low thinning sensitivity for fruit on spur and one-year terminal buds.

Harvest Management

At harvest, care should be taken to select pick fruit which are physiologically mature and of the correct colour. Ethylene probably plays a major role in co-ordinating red and background skin colour change for 'Royal Gala' and 'Braeburn'. This indicates that skin colour is a useful measure of physiological maturity for these two cultivars. It confirms present harvesting advice that skin colour can be used as a maturity index for select picking individual fruit on the tree. Although colour charts are available for 'Royal Gala' fruit background colour charts for 'Braeburn' could possibly be developed to aid selective picking.

For cultivars such as 'Granny Smith' the timing of the climacteric does not occur simultaneously with other ripening changes. This cultivar is strip-picked. Theoretically it is possible that fruit of various physiological maturities may result in an increased incidence of storage disorders and variation in skin colour and eating quality after storage. It may be of benefit to harvest fruit from buds on the outside of the canopy (excluding fruit from one-year laterals) separately from those on the inside of the canopy. This should somewhat reduce fruits of a wide range of maturity being harvested from the tree at any one time.

In summary, a knowledge of variation in fruit quality and productivity within apple trees and replacement branches would seem to be of major benefit to growers. With such knowledge, growers will be able to refine tree management practices so that efficient export production and maximum profit is achieved today and sustained into the future.

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