A study of the effects of paclobutrazol on post-harvest behaviour of apple and tomato fruit

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A STUDY OF THE EFFECTS OF PACLOBUTRAZOL ON POST-HARVEST BEHAVIOUR OF APPLE AND TOMATO FRUIT

submitted by Yunbo Luo

for the degree of Ph.D.

of the University of Bath

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To:

My Motherland.

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SUMMARY

Foliage applications of paclobutrazol at 1500 $mg.l^{-1}$ were made at different times during the growing season to cropping Bramley Seedling and Golden Delicious apple trees.

Applications made early in the season, at first bloom and petal fall inhibited shoot extension strongly, tended to reduce mature fruit size and increased the calcium and phosphorus contents of the fruit while potassium content was reduced. Fruit from the early treatment was firmer and remained greener after three months storage than the control. The fruit from early treated trees showed a delay in respiration climacteric of three days and a reduction in the production of ethylene by the fruit tissue.

Later season applications (fruitlet and preharvest stages) were comparatively ineffective in modifying extension growth or fruit post-harvest characteristics.

The application of GA_3 and GA_{4+7}(150μm) either alone or in combination with paclobutrazol(1000 $mg.l^{-1}$) at near fruitlet stage showed that some of the effects of paclobutrazol could be reversed by gibberellin and vice versa. For example, the respiration climacteric of apple fruit after storage for three months was earlier from the gibberellin treatment than the control or the fruit from gibberellin plus paclobutrazol. Ethylene production of the fruit was increased by the application of gibberellins and paclobutrazol tended to reverse this effect. The calcium and phosphorus content of fruit from gibberellin treatment was decreased whereas the potassium content was increased, however, paclobutrazol did not completely reverse these effects.

In tomato fruit, the surge of ethylene production during post-harvest ripening was delayed, when the fruit were taken from plants which had been treated with paclobutrazol as a soil drench of 4 mg ai $plant^{-1}$ at various fruit development stages, and the calcium content of these fruit was higher. No influence of paclobutrazol treatment

was found on fruit magnesium, phosphorus and potassium contents. The total soluble solids content of the tomato fruit increased during the ripening period, but the increase in fruit from plants treated with paclobutrazol was smaller than the control. The colour changes of tomato fruit during the ripening period were not influenced by paclobutrazol application.

The mechanism whereby paclobutrazol influences fruit post-harvest behaviour and the potential application of the technique for controlling fruit storage disorders are discussed.

1 INTRODUCTION

The work reported in this thesis discusses the changes in fruit post-harvest behaviour with the use of a new growth regulator, paclobutrazol. Two apple cultivars, the CV Golden Delicious, CV Bramley Seedling, and tomato (CV Turbo) were chosen as research materials for this study.

The apple (Malus domestica L.) is the main tree fruit crop grown in Britain, and also occupies an important position in world fruit production. The CV Golden Delicious is a popular cultivar which has a long history, is widely cultivated around the world, genetically stable and was used in this study for these reasons. The CV Bramley is a culinary cultivar, which is of important commercial significance in the U.K. but not extensively grown in the rest of the world. Another reason for choosing Bramley is that it is known to respond to paclobutrazol (Quinlan 1981). A comparison of the apple (a tree fruit) with tomato (Lycopersicon esculentum, a herbaceous annual) may also give further insight into this area of research. In addition, the tomato is one of the most important vegetables in the western world with the study of its post-harvest physiology being directed towards improved shelf life and quality (see Table 1, world apple and tomato production),

Table 1 Apple and tomato production in the world (figures are in 1000 metric tonnes)

	Apples	Tomatoes
China	3815	5260
USA	3542	7836
USSR	7000	6900
UK	318	117
World	37922	60825

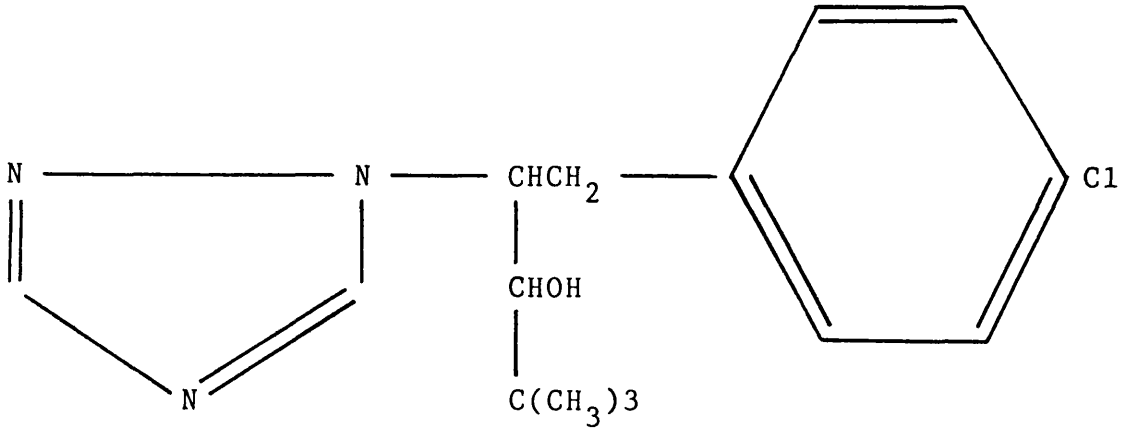
Source: FAO Production Yearbook, Vol.39,1985

by using paclobutrazol, a known plant growth retardant, a better understanding may be obtained of the interaction between vegetative growth and the post-harvest physiology of fruits. In the following introduction, paclobutrazol is described then a review of the relevant biological literature and the general aspects associated with the fruit post-harvest physiology.

1.1 The Knowledge of Paclobutrazol

1.1.1 Structure and mode of action Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4.4-dimethyl-2-(1,2,4-triazol-1-1-yl)pentan-3-ol] (code PP333) is a plant growth regulator first synthesized by the Imperial Chemical Industry P.L.C.(ICI),Plant Protection Division, at Jealott's Hill Research Station U.K. in 1976 (Froggatt et al 1981) and now commercially available in the U.K. The structure of this compound is shown in Figure 1.

Paclobutrazol is a growth retardant which inhibits plant vegetative growth and plant vigour . Structurally, paclobutrazol is a substituted triazole with two asymmetric carbon atoms, and is produced as a mixture of the 2R,3R and 2S,3S enantiomer. Other N-containing heterocyclic growth retardants such as ancymidol, and tetcyclacis have been shown to inhibit gibberellin (GA) biosynthesis by blocking specifically the three steps in the oxidation of ent-kaurene to ent-kaurenoic acid (Coolbaugh et al 1978, Rademacher et al 1984). Paclobutrazol was therefore expected to have a similar mechanism of action. This expectation was proved by Dalziel and Lawrence (1984) where paclobutrazol inhibited gibberellin biosynthesis by blocking the oxidation of kaurene to kaurenoic acid in the gibberellin biosynthesis pathway in a cell-free system from pea apices. This



Chemical name : (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol

Empirical formula : $C_{15}H_{20}ClN_3O$

Molecular weight : 293.5

Figure 1 Chemical Structure of Paclobutrazol

finding has been supported by Hedden and Graebe (1985) when they reported that paclobutrazol inhibited specifically the three steps in the oxidation of gibberellin-precursor ent-kaurene to ent-kaurenoic acid in a cell-free system from Cucurbita maxima endosperm. The gibberellins biosynthesis pathway is shown in Figure2.

The reduction of gibberellin level within the plant reduces rates of cell division and expansion. The direct morphological consequence is a reduction in vegetative growth. Paclobutrazol does not inhibit the activity of either existing endogenous or exogenous gibberellin. This has been illustrated by experiments using exogenous application of GA_3 to reverse a range of paclobutrazol-induced symptoms both under glasshouse and field conditions (Curry et al 1983).

Paclobutrazol main biological effect is to inhibit the vegetative growth of plant, but many other side effects have been associated with this inhibition such as an alteration in sink strength within the plant, allowing a greater proportion of assimilates to contribute to reproductive growth including flower bud formation, fruit formation and fruit growth. This redistribution of assimilates within the plant has influenced the behaviour of either preharvest and post-harvest fruit.

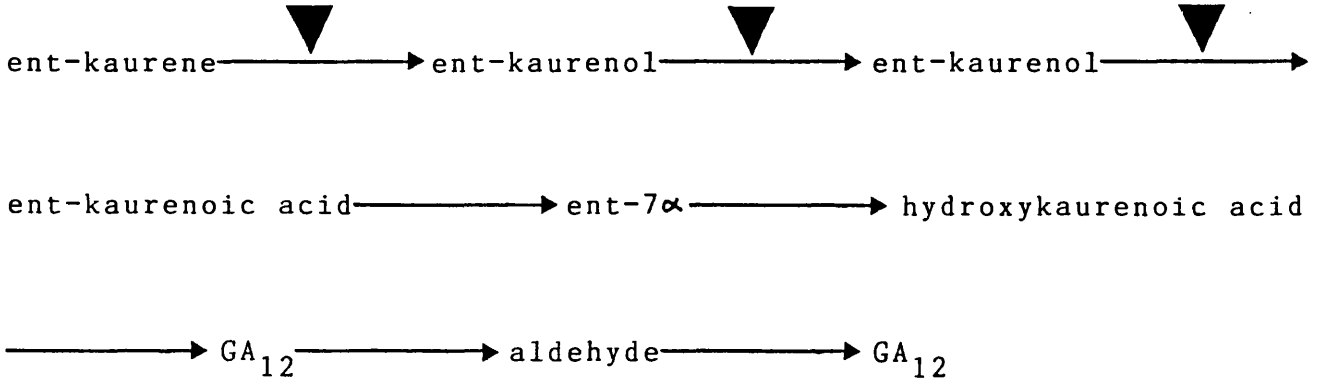


Figure 2 Gibberellic acid biosynthetic pathway

1.1.2 Mobilization and translocation Current studies have shown that paclobutrazol may be taken up passively through roots, stem tissue and foliage. Movement within plant is acropetal, moving almost exclusively in the xylem to leaves and buds (Lever et al 1986). Paclobutrazol was also believed to move relatively slowly to active sites in sub-apical meristems from reservoirs of paclobutrazol in soil or stem tissue. The chemical can also be taken up through young sub-apical shoot tissue following foliage sprays (Richardson et al 1985, Quinlan et al 1986). It is not very clear whether paclobutrazol moves in phloem. Studies with C^{14} labeled paclobutrazol (Richardson et al 1985) have shown no problem of mobility, which Sterrett(1985) reported, when C^{14}-paclobutrazol was injected in one year old apple trees.

1.1.3 The use of paclobutrazol As a growth regulator, paclobutrazol has been widely shown to be active on many fruit crops. A great amount of research work including the uses of paclobutrazol on control of vegetative growth and other physiological process has been conducted in last ten years (Steffens et al 1985, 1986, Webster and Quinlan 1986). An early report which associated with the inhibition of shoot extension growth of fruit tree was published by Quinlan (1980), when paclobutrazol was applied at a concentration of 5000 ppm on four year old CV Bramley Seedling giving

large reductions in extension growth. These results have been supported by Williams (1983), when a similar experiment was done in U.S.A. In Williams' work, paclobutrazol applied either to the foliage of fruit trees or to the soil near the tree base was shown to have the same result. The comparison of several growth retardants with paclobutrazol had been investigated (Quinlan 1981). It was shown that paclobutrazol was more effective on inhibition of fruit trees' vegetative growth than M.B.25-105, PP528 and Alar (Daminozide). Paclobutrazol was also reported to inhibit the vegetative growth of several other fruit crops including plum, pear, cherry (Webster and Quinlan 1984, 1986), strawberry (Atkinson and Crisp 1986) and apricot (Shearing and Jones 1986). In recent years, more results which connected with the use of paclobutrazol on fruit trees have been obtained, throughout a large number of experiments which were investigating various aspects of fruit physiology including flowering, fruit setting, water loss and cropping either direct effects or indirect effects which resulted from this inhibition. In 1984, Quinlan and Richardson demonstrated that paclobutrazol gave a long-lasting control of tree growth with little or no adverse effect on fruit yield or quality. In their experiment, paclobutrazol was applied to apple shoots. The effect on shoot elongation was influenced by the treatment site, such that application to the

shoot stem and/or shoot tip caused a greater reduction in shoot leaves or to the woody stem. The best control of tree growth was found when paclobutrazol was applied as a foliar treatment sequentially during early summer.

Studies with apple seedlings (Steffens et al 1983, Steffens and Wang 1984, Wang et al 1985) have shown that paclobutrazol has a greater inhibiting effect on shoot elongation and leaf area expansion than on leaf number. When CV Golden Delicious seedlings were treated with paclobutrazol, root growth was affected (Steffens et al 1985, 1983, Wang et al 1985). There were increased numbers of shoot roots with an enlarged diameter on treated plants, and GA applied to the foliage tended to reverse this effect.

Besides the monphological changes resulting from the application of paclobutrazol, various physiological changes also take place in fruit trees after paclobutrazol treatment, such as uptake and redistribution of mineral nutrients, the efficiency of photosynthesis and partitioning of assimilates. Wang et al (1985) have reported that paclobutrazol had a profound effect on the metabolism of apple seedlings by regulating the partitioning and utilization of carbon assimilates. They found that paclobutrazol increased the concentration of several carbohydrates including sorbitol, fructose, glucose, sucrose and starch in

all parts of the apple seedlings, and shifted the partitioning of assimilates from the leaves to root. The paclobutrazol increased the chlorophyll content per unit area of leaf to give an improvement in photosynthetic efficiency. In their work, an increase in the soluble protein content in leaves, an increase in the concentration of mineral elements in leaf tissue including N, P, K, Ca, Mg, Cu, B, Zn and Sr, resulted in more efficient use of mineral nutrients and increased the activity of root respiration. Similar results have been obtained by Steffens et al (1985) when they demonstrated that the partitioning of carbohydrate within a fruit tree was influenced by paclobutrazol application. In apple trees of CV Spartan, nonstructural carbohydrate content was significantly greater in both paclobutrazol-treated shoot and spur leaves. Following this result, Steffens et al (1985) proposed that because of the increase in carbohydrate content that may be related to a reduction of vegetative growth, the number of fruit buds will increase as well as a series of physiological changes including leaf photosynthetic capacity, leaf shading and light penetration, bud development, fruit number and water utilization. Such a view has been supported by number of workers when they found that fruit cropping was increased (Greene 1986) and fruit number was increased (Stinchcombe et al 1984, Williams 1984) due to the paclobutrazol application. It is worth mentioning that

paclobutrazol can reduce plant water consumption. It was reported (Steffens et al 1983) that under non-water stress conditions, paclobutrazol-treated seedlings took up less water than control plants. Also the rate of water loss was much slower from paclobutrazol-treated leaves compared to controls (Wang and Steffens 1985), similar results have been obtained by Swietlik and Miller (1983). This information suggests major physiological changes take place in the water relations of paclobutrazol-treated plants which could result in better tolerance to water-stress condition. As it was demonstrated that water-stress caused progressive increase in ethylene production (Yang and Hoffman 1984, Wang and Steffens 1985) and paclobutrazol treatment suppressed the evolution in ethylene production.

Paclobutrazol has significantly influenced the physiological process by inhibition of endogenous GA biosynthesis. Numerous beneficial effects have been obtained as reviewed above. However, most of this research work has concentrated on the influence of paclobutrazol on field behaviour of the tree and not on the equally important post-harvest period of the crop. Since paclobutrazol has radically altered the physiological processes of the plant, it is predictable that paclobutrazol may influence post-harvest behaviour through physiological changes in the fruit during its preharvest period. So far, little work has been done on this aspect.

In a review, Sharples and Johnson (1986) mentioned that with the apple CV Cox's Orange Pippin, paclobutrazol delayed the onset of climacteric in harvested fruit by three days , but no experimental detail was given, and with CV Delicious apples, ripening was also retarded, but by an unspecified amount when paclobutrazol was made as foliar application at the concentrations both 1500 ppm and 3000 ppm during the earlier grown season. In contrast, the ripening behaviour of CV Granney Smith and CV Top-red Delicious apples showed no difference with or without paclobutrazol treatment when they applied paclobutrazol in October at rate of 0.6 g ai per cm trunk diameter in 0.5 m bands (Curry and Williams (1986). With strawberries the harvest date was delayed by as much as five days when 20 kg ha^{-1} of paclobutrazol was applied to soil in spring (Atkinson and Crisp 1986).

Paclobutrazol was found to increase the fruit calcium content (Greene 1986) which is related to an increase in fruit quality and storage potential. In addition, paclobutrazol was also found to suppress ethylene evolution from root tissues through preventation of water loss of plant tissue (Wang et al 1985). These points will be reviewed more thoroughly in the following section. Although paclobutrazol was used to a wide range of plant species, relatively little information was available of its effect on tomato. Wainwright and Bithell (1986) reported tomato plant height and total

plant dry weight were reduced when paclobutrazol was applied as soil drench in compost, but no mention at all about tomato fruit in response to the treatment. However, the morphological changes of tomato plants occured after the treatment of paclobutrazol. It could be predicted that the influence of paclobutrazol may also reach tomato fruit, which associated with the change of fruit nutrients condition and fruit ripening process.

There are several alternative methods that can be used for paclobutrazol application. Dosage, timing and method of application can be very critical. Steffens and Wang (1986) reported root growth can be either increased or decreased depending upon paclobutrazol dosage. Soil application has been reported to result in a good response in United States (Williams 1983, Miller and Swietlik 1986), while a poor response has been noted in U.K. (Quinlan 1981). It was suggested that foliage application may achieve a best response in this country. However, sequential applications have been found to be more effective than single high dose applications in reducing apple shoot growth (Quinlan and Richardson 1984), and it was also thought that sequential sprays could offer advantage of fitting the chemical application to the growth pattern of tree in each year of treatment (Shearing and Jones 1986). In peach, where single foliar application was made at

the same rate at 1 kg ai ha^{-1}, but at different times in the growing season, a greater reduction in shoot growth was found the earlier the application in the growth season (Shearing and Jones 1986). After investigation of various trial in several countries, Shearing and Jones (1986) recommended that the best time and dosage to control apple extension growth were at 3-4 weeks after petal fall with rates of 125 to 500 g ai ha^{-1} with application of sequential foliar spray at two week intervals. Though there are several alternative application methods for paclobutrazol, it is important to fit the application technique to the particular growing system and environmental conditions, and to the sensitivity and responsiveness of the crops.

1.2 Respiration and Respiratory Climacteric

The understanding of fruit respiration is an important aspect in the study of post-harvest physiology. Most of the biochemical changes occuring in harvested fruits are related to oxidative metabolism (Hulme et al 1969) including respiration which provides the energy for the ripening process. Respiration is intimately linked to studies of quality changes, physiological disorders, storage life, maturity, commodity handling and many post-harvest treatments (Bial and Young 1962). Thus, the rate of respiration has become an index of post-harvest longevity of fruit. Respiration rate is considered a measure of the rate at which metabolism is proceeding and as such is often considered an indication of the potential storage life of the fruit. A high rate of respiration is usually associated with a short storage life. It would also indicate the rate at which the fruit is deteriorating in quality and in food value (Phan et al 1973).

The early study of the ripening processes stems from investigation of the physiology of the apple fruit undertaken by Kidd and West in 1920's. They studied the changes in respiratory activity of Bramley Seedling apples picked at various stages in the growth season and subsequently stored at various temperatures. It was found that the CO_2 production of fruit after picking

fell to a minimum value and then rose more or less rapidly, depending on the temperature (Kidd and West 1922, 1923, 1924). Subsequently the respiration declined, visible changes in ripening occuring shortly after the peak in rate of respiration. (Kidd and West 1924) considered that the relatively sudden alternation in the level of respiration marked the transition from the growth to the senescence phase in the life of the fruit. It had been recognized that the increase in respiration was a critical phase in the fruit life and hence it was termed the 'Climacteric'. A similar phenomenon was investigated in a later experiment (Kidd and West 1945) during the maturation of apples still attached to the tree, and later confirmed by Hulme and his co-workers (1963). Kidd and West (1945) showed that once fruit either on or off the tree entered this critical phase and the climacteric rise was under way, the ripening of fruit was an irreversible process which could be slowed but not halted by the application of external factors. The climacteric to which Kidd and West were referring specifically was a pattern of respiratory changes which has been called the respiration climacteric.

In view of the importance of the mitochondria in respiration, they have been widely studied in relation to the respiration climacteric. Pearson and Robertson (1954) were the first to obtain a crude mitochondria

fraction from apples. Their results established that there was a marked increase in mitochondrial activity as the fruit passed through the climacteric. This work of Pearson and Robertson together with later work by Lieberman (1958) and by Hatch et al (1959) established that a krebs tricarboxylic acid cycle (TCA cycle) involving the cytochrome oxidase system, operated in apples, and that the activity of the enzymes concerned increased over the climacteric. It has been shown that the mitochondrial oxidation of succinate becomes more susceptible to inhibition by oxaloacetic acid (Lehninger 1964) as the climacteric develops (Hulme et al 1967) so that there could be a change in the balance of enzyme activity within the mitochondria during the climacteric period.

The respiratory activity of tomato towards the end of maturation is a distinctive and peculiar feature of their physiology. As the tomato is a climacteric fruit, there is, under normal conditions, a rapid rise in respiration coincident with the first signs of red colour (Winsor et al 1962, Pratt et al 1965) accompanied by increased respiratory activity, rapid changes in a wide range of chemical constituents take place in this period. Lycopene and carotene progressively replace chlorophyll and often make their first appearance in semi-liquid material surrounding the seeds. When this occurs, the fruit is close to the respiration climacteric

(Hobson and Davies 1979). It was also found that mitochondria appears to be the most active when obtained from fruit shortly before the climacteric peak (Hobson 1964). Once the fruit is fully ripe, tissue disorganization becomes increasingly dominant. Cell walls become very thin and the organized cytoplasmic units largely disintegrate. The degration of cellulose, as well as most of the pectic components, leads to a progressive loss of tissue cohesion. Respiration continues to fall slowly concurrently with ethylene production. Mitochondrial protein as a percentage of cytoplasmic protein decreases markedly during ripening (Dickiuson and Hanson 1965), and this continues into over-ripeness.

The understanding of fruit respiration and the respiration climacteric suggested that the major task to increase the fruit storage ability, to control post-harvest senescence and to decrease post-harvest disorder is to slow and control the fruit post-harvest respiration so that the onset of the respiration climacteric can be delayed. This principle is still a guide to today's modern post-harvest studies. There are two major methods.

I. Physical methods involve creating or modifying an artificial storage condition in which respiration rate can be restricted to a certain level and the longevity

of the fruit can be increased. This method includes:

a) Controlled-atmosphere (CA) storage which the respiration rate is restricted at a low level by means of a gas mixture in which an increased concentration of CO_2 or a reduced concentration of O_2 or both (relative to the concentrations in air) are maintained within narrow limits.

b) Modified-atmosphere (MA) storage implies an initial adjustment of gaseous concentrations with little or no subsequent control.

c) Hypobaric storage by which the fruit is kept under condition of a removal of gases to lower atmosphere pressure.

All these physical storage methods are normally combined with the use of low temperature condition, generally 1-4° C.

II. Chemical methods use a plant growth regulator applied as a pre- or post-harvest treatment to improve the fruit storage capability. There are several plant growth regulators currently being used in fruit storage such as Alar, CCC, AVG, and these will be discussed later.

To summarize, physical methods have been widely applied commercially for many decades. However, these methods are of over-simplification and only partially improve storage ability since post-harvest deterioration of fruit can be caused by many factors other than the post-harvest environment (Kader 1986) in addition to high respiration rate including metabolic changes (biochemical changes associated with respiratory metabolism, ethylene biosynthesis and action, and compositional changes), growth and development changes, water loss, physiological disorder etc. Furthermore, low temperature storage needs a great amount of energy, specifically electricity and an expensive insulated building that will greatly increase the cost of fruit storage. The cost and ability to low temperature store is a problem, specially in many developing countries as well as some developed countries. Therefore, interest has increased in the use of plant growth regulators to improve the fruit's own storage capability. This method was thought to be an economical, convenient and effective way comparing with creating gg a physical storage environment.

1.3 Ethylene and Fruit Senescence

Ethylene, as a natural plant growth regulator has been long recognized to play a major role in fruit senescence and ripening (Lieberman 1979, Kader 1985). The earliest discoveries that many ripening fruits produce ethylene and that this gas would itself elicit ripening were quickly followed by the suggestion that ethylene accumulation during storage was deleterious. Thus, it became clear that ripe and unripe apples should not be stored together (Kidd and West 1938) and that the removal of ethylene from the storage atmosphere could prolong storage life (Smock 1943). Later research has shown that ethylene introduced into the atmosphere surrounding unripe apples will cause them to ripen and endogenous production of ethylene immediately precedes the climacteric rise in respiration and onset of ripening (Meigh et al 1967). This endogenous ethylene production initiates an increase in many of enzyme process associated with ripening (Hulme et al 1968).

However, for many years, the role of ethylene in post-harvest physiology has been vigorously debated. Some workers have argued that ethylene is a by-product of the fruit ripening process rather than the cause of, or trigger for ripening (Biale et al 1954, Lieberman 1979). The main obstacle in the early studies of ethylene was lack of advanced equipment and precise

techniques to detect small amounts of ethylene (Knee 1985, Pantastico et al 1975). Until the development of gas chromatography, only climacteric fruits were thought to produce ethylene, even in a few climacteric fruits, like mango, no ethylene was measurable using the manometric procedure (Biale et al 1954) but by chromatographic analysis, Mattoo et al (1968) determined the ethylene concentration in the gases involved from ripening 'Atphonso' mango slices and reported that they produced ethylene in the range of 0.02-0.18 ppm. With modern techniques there is no longer any doubt that ethylene is a ripening hormone. Recent research which shows the retardation of fruit ripening by inhibitors of ethylene synthesis (Bangerth 1978), ethylene action (Janes and Frenkel 1978) and by hypobaric storage (Burg and Burg 1966) has confirmed the natural role of ethylene in regulation of ripening in climacteric fruit. Furthermore, in hypobaric storage, ripening and senescence of many fruits were also significantly retarded when ethylene was removed (Bangerth 1975), therefore ethylene appears to play a major role in ripening even at low temperature and low oxygen tensions. Ethylene deeply affects harvested fruit in increasing respiration activity, increasing the activity of enzymes such as polygalacturonase, peroxidase lipoxidase, alphaamylase, polyphenol oxidase and phenylalanine - ammonialyase, increasing permeability and loss of cell compartmentalization and altering

auxin transport or metabolism (Pratt and Goeschl 1969).

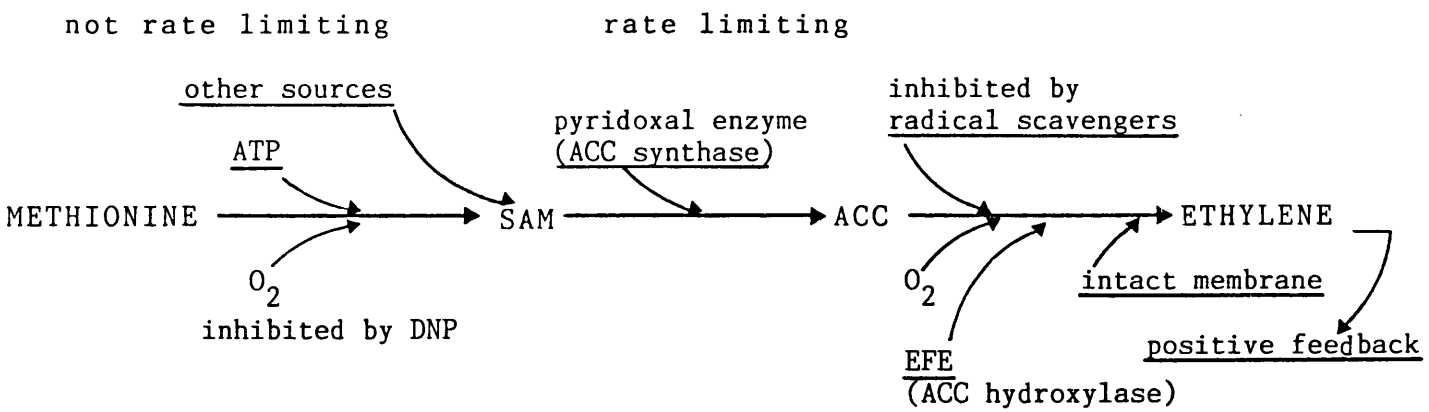
Ethylene as a ripening hormone was also supported by the tomato experiment. Pratt and Workman (1962) reported that in normal circumstances, exogenous supplies of ethylene enable tomatoes to ripen more quickly and with more uniformity by initiating an earlier climacteric rise. Burg and Burg (1962, 1965) presented evidence that amounts of ethylene sufficient to stimulate ripening were present in several fruits prior to the climacteric respiration rise and that the concentration increased towards the respiration peak. A report by Lieberman and Mapson (1962) indicates that ethylene oxide delayed the ripening of tomatoes, and subsequent work (Lieberman et al 1964) demonstrated an antagonism between ethylene oxide and ethylene in their respective effects upon green tomatoes. Dostal and Leopold (1967) reported that ethylene stimulated the pigment changes associated with the normal ripening of tomatoes, but prior application of gibberellin eliminated this effect.

Since ethylene induces ripening, it is important to know what initiates ethylene production, how it is synthesized and what is the mode of ethylene action in promoting ripening. In last decade, researches have concentrated on this area and new approaches have been made specially on the establishment of the pathway of

ethylene biosynthesis. Yang and Hoffman (1984) have reported the ethylene biosynthesis pathway (see Figure 3). This finding provided a better understanding of the physiological effects of ethylene and a more rational basis on which to interpret the effects of exogenously applied plant hormones including those that inhibit ethylene biosynthesis. However, the role of ethylene in fruit ripening and senescence is still far from fully understood. Interest and activity in ethylene research have been increasing year by year with the realization that influence of ethylene is of considerable importance in understanding fruit ripening and senescence. Reviews by Lieberman (1979) and Yang and Hoffman (1984) have considered various aspects of ethylene as a hormone and its effect on plant development including its mode of action in plant metabolism, the pathway of its biosynthesis and regulatory controls.

Good methods for the control of ethylene production in post-harvest fruit would enable fruit storage life to be increased, and this still is a challenge to post-harvest physiologists.

Besides ethylene stimulating the onset of the climacteric as reviewed above, it has been reported also that ethylene may cause some post-harvest physiological disorders and diseases. Arpaia et al (1982) found that



ACC = 1-aminocylopropane-1-carboxylic acid

DNP = 2,4-dinitrophenol

EFE = Ethylene-forming enzyme

SAM = S-adenosylmthionine

Figure 3 Ethylene biosynthesis in apple tissue
(Modified from S.F.Yang and N.F.Hoffman 1984)

the presence of ethylene as low as 50 ppm under CA condition can negate the benefits of CA storage at 0°C on kiwifruit (Actinidic chinensis) by enhancing flesh softening and altering the internal appearance of fruit. Ethylene was also found to stimulate rot development by some fungi, e.g. diplodia stem-end rot (Diplodia natalensis Pole-Evans) on citrus fruit. Barmore and Brown (1983), Brown and Barmore (1977) and El-kazzaz et al (1983) reported that the presence of 20 ppm ethylene in the storage atmosphere enhanced growth of Botrytis cinerea Pehs. ex. Fr. and disease development on strawberries. Exposure of 10 post-harvest fruit infecting fungi, in vitro to ethylene at 1, 10, 100 and 1000 ppm stimulated growth of Botrytis cinerea Pers. ex. Fr. and Penicillium italicum Webmer, in vitro and in vitro on strawberries and oranges respectively (El-kazzal et al 1983). It is, therefore very important to protect fruit from ethylene. The strategies for controlling exposure to ethylene can be placed into 3 major categories: avoidance, removal and inhibition.

I. Avoidance: Circumvention of undesirable product exposure to ethylene begins with careful harvesting, grading and packing which includes selecting fruit of the desired maturity, avoiding mechanical injury and using proper temperature management.

II. Removal: Undesirable levels of ethylene in crop storage areas can be removed by ventilation with fresh air, and ethylene can also be scrubbed from the atmosphere by trapping and/or by conversion to other products when ventilation can not be used for removal. A large number of reagents and techniques have been tested for many years (Abeles 1973, Blanpied et al 1982), but only potassium permanganate is presently in common commercial use.

III. Inhibition: Chemical or plant growth regulator treatment could be used to protect horticultural products by inhibiting ethylene synthesis and/or action. Yang and Hoffman (1984) reviewed many of the known inhibitors of ethylene biosynthesis. Some inhibitors appear to be very effective in regulating fruit ripening (Bangerth 1978, Wang and Mellenthim 1977). However, so far, none of these inhibitors appears likely to be developed for commercial use due either to the high manufacturing costs or their lack of suitability as food additives (Sharples and Johnson 1986).

1.4 Mineral Composition and Fruit Post-harvest Physiology

Fruit mineral composition has been understood to be a very important aspect in fruit post-harvest storage and quality. During the last 30 years, a large volume of literature has established that the mineral composition of apple fruit as well as tomato fruit is related to post-harvest development of physiological disorders and decay, and realization of the importance of fruit mineral composition has enabled it to be used to predict the post-harvest storage potential (Weis *et al* 1985, Perring 1968, Marmo *et al* 1985). Because the physiology of post-harvest fruit is profoundly influenced by their mineral composition, an understanding of their role in fruit post-harvest physiology is very important.

Calcium has received the most attention among the mineral elements. This is because of its desirable effects in delaying senescence and controlling physiological disorders in fruit. Studies on fruit ripening (Tingwa and Yang 1974, Poovaiah 1979, Suwwan and Poovaiah 1978) have indicated that the rate of fruit senescence often depends on the calcium status of the tissue and that by increasing calcium levels, various parameters of senescence such as respiration (Faust and Shear 1972, Bangerth *et al* 1972), the

decline in protein and chlorophyll content (Poovaiah and Leopold 1973) and changes in membrane fluidity (Paliyath et al 1984) are altered. Therefore, calcium plays an important role in maintaining the quality of fruits (Shear 1975, Hopfinger and Poovaiah 1979, Huber 1983). In apple, calcium treatments have been shown to help retain fruit firmness, increase vitamin C content, reduce respiration rate (Faust and Shear 1972, Bangerth et al 1972, Irving 1985) and ethylene production (Irving 1985, Poovaiah 1986, Lieberman and Wang 1982) and decrease various physiological disorders such as bitter pit, cork spot, internal breakdown (Faust and Shear 1968, Perring 1968, Weis et al 1980, Bangerth et al 1972), lenticel breakdown (Shear 1971), watercore and Jonathon spot (Bangerth 1979).

In tomato fruit, calcium was thought to be a very important to keep fruit free from blossom-end rot (Geraldson 1957, Wiersum 1966). Calcium levels in fruit excess of about 0.12% dry matter are essential.

Adequate potassium appears to be necessary to ensure red colour development (Walter 1967) and good flavour (Hill 1952, Kidd and West 1939) of the fruit. However, high potassium levels in fruit are usually associated with storage disorders such as bitter pit and internal breakdown (Bangerth 1972, Williams et al 1985).
It was reported that the incidence of bitter pit is

markedly increased by the excessive application of potassium to the soil (Anon 1970, Nyhlen 1954). The positive correlations between the potassium status of leaves or fruits and bitter pit incidence have been found (Oberly and Kenworthy 1961, Sharples 1968, Van der Boon 1968). Potassium applications have also been found to lead to increase in core flush in several varieties of apples (Anon 1965, 1967, Wallace 1953) and in brownheart as well. Soil applications of potassium have been shown to reduce the incidence of low temperature injury in Cox's Orange Pippin (Anon 1967, 1968, 1970, Montgomery and Walkinson 1962). In contrast, inconclusive results have also been obtained (Kidd and West 1939, Weeks et al (1965). Excessive application of potassium may depress calcium uptake, Van der Boon et al (1966) have reported a negative correlation between fruit potassium and calcium. Thus, many of the storage disorder effects of applied potassium such as the increase in bitter pit and rotting may be associated more with the depressive effect on calcium uptake than any direct effects due to promotion of potassium level in the fruit.

Phosphorus was thought to be another important element for maintaining apple postharvest quality and storage. Previous research suggested a certain level of phosphorus in apple fruit is necessary in

apple storage (Perring 1968). It was reported (Anon 1968) that as a response to phosphate, incidence of breakdown was reduced. The level of phosphorus in fruit has been shown to be positively correlated with resistance to breakdown (Perring 1968, Sharples 1968). Martin et al (1965) have also indicated that breakdown incidence may be reduced by foliar application of potassium dilydrogen phosphate in the early summer. A more direct role for phosphorus has been suggested by Letham (1969) who has shown that cell size, respiration rate per cell and susceptibility to breakdown are all positively correlated and that these fruit characteristics are negatively correlated with phosphorus content.

Comparing with other mineral elements, relatively little information is available on the effects of magnesium on fruit storage quality. However, it was reported that application of magnesium led to an increase in the incidence of bitter pit (Rose et al 1951) and a negative correlation between scald and fruit magnesium content has been established for cleopotra apples (Martin et al 1969). In contrast, it was been reported that magnesium reduced ethylene production when it was applied directly to the apple tissue (Lieberman and Wang 1982). However, to understand the role of magnesium on fruit post-harvest physiology, more data is still required. Magnesium was

reported to have a beneficial effect on tomato ripening disorder, particularly at low levels of potassium (Winsor et al 1965, Winsor and Long 1967).

In general, mineral composition plays an important role in fruit physiology. The minerals reviewed here are some of most important elements which are associated with fruit post-harvest life. Present knowledge suggests that adjustment of the mineral composition of fruit either by direct application of certain mineral elements (Bangerth et al 1972, Sharples 1971, Weis et al 1980) or by the use of growth regulators to alter fruit mineral composition (Fisher and Tooney 1967, Ashby and Looney 1968, Martin et al 1968) could achieve desirable changes in storage life and fruit quality. In this study, the four mineral elements have also been investigated in both apple and tomato fruit after the application of the growth regulator, paclobutrazol.

1.5 Paclobutrazol and other Growth Regulators on Post-harvest Physiology

The significance of the chemical control of fruit senescence has been realized in recent years. But so far, none of the growth regulators is being applied as a special agent for storage and little information is available on their possible effects on fruit post-harvest behaviour, despite the fact that growth regulators are now widely used to regulate fruit growth and development.

Daminozide (Alar) has been employed in apples to prevent preharvest drop and as a growth retardant. It was also reported that daminozide delays the onset and reduces the intensity of the respiration climacteric (Blanpied et al 1967, Clijsters 1971, Dilley and Austin 1967, Looney 1967, 1968, Rhodes et al 1969), when applied in mid-July or later, it may also reduce the rate of respiration during storage at 0°C (Looney 1967). Ethylene production is also reduced, and so too is the rate at which it increases during the climacteric (Rhodes et al 1969, Sharples 1971). Daminozide has variable effects on apple storage disorders. Scald has been found to be reduced in Boskoop (Wertheim 1967), Cortland (Dilley and Austin 1967, Shutak et al 1966, Southwick and Lord 1970) and Delicious (Williams et al 1964). Recently Sharples (1986) reported that daminozide

delayed the rate of respiration by suppression of ethylene synthesis and, where preharvest spray application of daminozide is supplemented combined by efficient ethylene removal in the store. However, by contrast, more consistent effects of daminozide have been obtained with core flush (Sharples 1967, 1971). And application made in late July have been found to increase bitter pit incidence in Cox's Orange Pippin (Sharples 1971).

The effects of cycocel (2-chloroethyltrimethylamnonium chloride ccc) in controlling plant vegetative growth and promoting fruit bud formation have been widely investigated. However, it appears to have relatively little effect on fruit composition or ripening rate. But it was suggested that application of cycocel at the rate of one percent, made four weeks after bloom might improve the storage quality of conference, comice and other pear cultivars (Buchloh 1960, Sharples and Johnson 1986).

Aminoethoxyvinylglycine [2-amino-4-(2'-aminoethoxy) -trans-3-butenoic acid, A.V.G.] has recently received most attention on pome fruit (Sharples and Johnson 1986). Bramlage et al (1980) and Child et al (1984) have demonstrated that A.V.G. will delay ripening in apples while similar effects have been reported for pears (Romani et al 1982). A.V.G. inhibits the action of ACC

synthesis (Yang and Hoffman 1984) and it has been shown that its effects can be completely overcome by treatment with exogenous ethylene (Bufler 1984). Hence, if A.V.G. were ever to be used effectively to extend the storage life of fruit, supplementary measures would be essential to maintain an ethylene-free environment in the storage chamber. At present, although A.V.G. provides the scientist with a valuable experimental tool, it is unlikely to be developed for commercial use due to the high manifacturing costs (Sharples and Johnson 1986).

It was reported that paclobutrazol delayed the onset of the respiration climacteric in harvested fruit by about three days when it was applied on Cox's Orange Pippin (Sharples and Johnson 1986). Paclobutrazol has also shown to improve fruit firmness and to increase fruit calcium content (Greene and Murray 1983, Greene 1986). However, paclobutrazol acts as an anti-gibberellin biosynthesis hormone. It was reported that GA applied at petal fall (100 ppm GA_3/ 10 ppm 2,4,5-TP) to promote fruit set leads to the production of low calcium apples at harvest (Jackson et al 1982). Bramley Seedling apples treated similarly with GA set higher proportions of low-seeded fruits with particularly low calcium concentration (Sharples 1986). Similar results have been obtained by Looney (1979) and Greene et al (1982). It was understood that low

calcium increased quantity of fruit as reviewed before. It was also reported that increased breakdown in parthenocarpic Golden Delicious apples induced by treatment with GA_3 and GA_{4+7} (Bangerth 1976). In a series of trials on Bramley Seedling (Sharples and Johnson 1986) fruit from the GA-sprayed trees ripened seven days earlier, as evidenced by starch hydrolysis, the onset of the respiration climacteric, lower firmness and more yellow ground colour at harvest. Following storage, treated fruit were more severely affected by superficial scald, internal breakdown, core flush and bitter pit. Mathur (1965) reported that gibberellic acid increased the incidence of decay of tomato.

The use of paclobutrazol to look at its effects on fruit post-harvest behaviour is still primary, although the number of research mentioned about the work has been conducted in this area. At the beginning of this study in October 1984, there were no reports on the effect paclobutrazol had on the post-harvest behaviour of fruit, though since that data a limited number have been published and have been reviewed earlier. The mechanism by which paclobutrazol influences the fruit post-harvest physiology is still far from fully understood, and more fundamental work is required on this subject.

2 MATERIAL AND METHODS

2.1 The Study of Effect of Paclobutrazol on Apple

2.1.1 Field trial and experiment design Foliar applicationsof paclobutrazol were applied to six year old CV Golden Delicious apple tree grown on M9 rootstocks and five year old Bramley Seedling apple tree grown on MM106 rootstocks in the Long Ashton Research Station experimental orchard. Spraying times were at first bloom (26-28 April), petal fall (30 May-1 June), fruitlet stage (8-10 July) or preharvest stage (9-11 August) and the control received no spray. Trees were sprayed on three successive days with a concentration of 500 $mg.l^{-1}$ of paclobutrazol with wetter on each day. Application was made with a hand held sprayer and sprayed until incipient run off. Each spray treatment was applied to separate single tree plots arranged in three randomised blocks, each block containing five trees. The investigation of shoot growth, fruit setting, fruit swelling was made during the period of fruit development after paclobutrazol application. The fruit was harvested on 2 October 1985 (Bramley Seedling) or 16 October 1985 (Golden Delicious) and size graded for each tree. Bramley Seedling fruit of 65-70 mm size (4-5 apples per kg) and Golden Delicious fruit of 60-65 mm size (7-8 apples per kg) were retained. The measurements of respiration rate (CO_2

production), ethylene production, mineral composition, fruit firmness, total soluble solids and colour composition were made on random sample taken from the size grade either immediately, or after storage at 4°C for 89 days (Golden Delicious) or 117 days (Bramley Seedling).

2.1.2 Respiration rate determination A fruit sample of approximately 1 kg from each tree in size grades described above was placed in 4 litre respiration chamber. The use of similar sized apples for analysis of fruit ripening was adopted so that any effect of treatment on ripening due to differences in fruit size would be eliminated (small apples being known to ripen later than large fruit). Each treatment comprised three replicate chambers, each containing fruit from one tree. The respiration chambers were maintained in a constant temperature room at 25°C. This temperature was adopted in order to accelerate the ripening process and shorten the period required to measure the respiration climacteric.

Carbon dioxide-free air was pumped through each chamber at a rate of 24 litre / 1 hour (Air was purified by pumping through 10% NaOH and 10% KOH), and the CO_2 production during a one hour period was measured every 12 hours by absorbing CO_2 from the chamber outflow into 50 mls of 0.1 N NaOH contained in a

250 ml flask, which was then determined by titration with 0.1 N oxalic acid.

2.1.3 Ethylene production determination The ethylene production by apple tissue slices was determined using four slices taken from the same fruit, and 6 apples from each replicate of each treatment. Slices 4 mm thick and 15 mm in diameter were cut with a corkborer. The discs were washed in 600 mM sorbitol and 10 mM Mes buffer, then 5 mls of a similar solution were placed in 50 ml flasks with four slices (approximately 3 g) from each apple (Lieberman and Wang 1982). The possibility that microbial contamination would influence ethylene production was avoided by addition of 100 mg chloramphenicol (antibiotic) in each flask. The flasks were sealed and incubated in a shaking water bath at 25°C for 6 hours, then a 1 ml sample of flask atmosphere was taken with a gastight hypodermic syringe and was analysed by means of a Pye GCD gas chromatograph with flame ionisation detector, using an alumina column at 120°C and N_2 carrier gas.

The direct influence of paclobutrazol on ethylene production from apples untreated in the orchard was tested by including different concentration of paclobutrazol in the incubation solution, the procedure being otherwise similar to the above.

2.1.4 Mineral composition determination A sample of 10 apples was taken randomly from each replicate of each treatment. The sample apple was washed in distilled water and dried with tissue paper. The calyx and stalk were removed and the apple cut into four pieces, the core removed and the remainder dried in a oven with a temperature of 100°C for 24 hours. The dried samples were ground up to a powder. A 1 g of each sample powder was ashed in a muffle furnace at a temperature of 450°C for approximately 5 hours until the ash became white and the carbon had been burnt off completely. The cooled ash was dissolved by adding 5 ml 6M HCl and then the sample solution was evaporated to driness on a bench heater. Then, 2 ml 36% W/W HCl was added, boiled gently for about 2 minutes and then approximate 5 ml deionised water was added and this solution was boiled again for 2 minutes. The sample solution was allowed to cool and transfered into a 25 ml volumetric flask and diluted up to 25 mls with deionised water. The sample solution was filtered (Watman NO. 541 filter paper 0.22 mm) and the Ca, Mg, P, K concentration of this solution was determined by means of a SP9 Phillips Atomic Absorption Spectrophotometer.

2.1.5 Fruit firmness and total soluble solids

Firmness of apple fruit was determined by means of an electrical penetrometer with a 9 mm probe in diameter. Nine apples of 65 mm in size for Bramley Seedling and 60 mm in size for Golden Delicious from each replicate of each treatment were selected randomly from each tree. The measurements were made at two opposite sides of each apple mid way between the stalk and calyx i.e. by pushing the probe into apple for 10 mm in depth, and the mean of two values of firmness was obtained from each apple.

The determination of total soluble solids was made by the use of an Abbe Refractometer immediately after the measurement of fruit firmness. The fruit juice was collected from various parts of each apple for this measurement. The mean value of total soluble solids was obtained.

2.1.6 Fruit colour assessment The fruit colour assessment was made by the use of an XL10 CDM colour meter, which was from Grandner Laboratory inc. Bethesda. Six size graded apples from each replicate of each treatment were selected at random from each tree. The measurements were made at three areas on the apple cheek separated by 120 degree and 6 values (Y,X,Z,L,a and b, explanation see later) of each area were obtained (Wright 1958, Mackinney and Little 1962,

Hunter 1975).

2.1.7 Fruit setting investigation The fruit setting investigation started after first bloom treatment (8 May 1985). Flowers (200 per tree) were randomly selected from each tree of each treatment and were labelled, and the number of flowers on fruit tree was counted. Counting was repeated on 20 May, 10 June and on 3 July. The final counting was made at preharvest time (8 September).

2.1.8 Shoot extension growth investigation
Nine new shoots randomly selected from different faces (N, S, E and W) of each tree of each treatment of control, first bloom and petal fall were labelled for this investigation. The shoot length measurements began soon after petal fall spray, and continued every week until the shoots stopped growth (9 August 1985). Besides investigation of the shoot length, the number of the leaves per shoot was recorded when shoot growth had stopped. (No investigation was made on the treatment of fruitlet and preharvest stage because the shoots stopped making extension growth when those two applications were made.)

2.1.9 Fruit size investigation The fruit size of the control, first bloom and petal fall treatments was investigated during early stages of fruit development, and later investigations were for all treatments.

Thirty fruits were randomly selected from each tree at four different faces (N,S,E,W) and were measured in diameter by means of micrometer. The investigation was made every week. After harvest, the fruit size was determined for each tree of all treatments by a commercial mechanical fruit size grader.

2.2 Investigation between Paclobutrazol and Gibberellic Acid

2.2.1 Field trial Foliar applications of gibberellic acid (GA_3, GA_4+7) and/or paclobutrazol were applied to six year old Golden Delicious apple trees grown on M9 rootstock, and five year old Bramley Seedling apple trees grown on MM106 rootstocks. The treatments were GA_3 at a concentration of 150 µm, GA_{4+7} at a concentration of 150 µm, GA_3 plus paclobutrazol (150 µm for GA, 1000 $mg.l^{-1}$ for paclobutrazol) and GA_{4+7} plus paclobutrazol (again 150 µm for GA, 1000 $mg.l^{-1}$ for paclobutrazol). The control was sprayed with water. The treatments were made on the 11 June and all solutions had a wetter added. Application was made with a hand held sprayer and sprayed until incipient run off. Each spray treatment was applied to separate single tree plots arranged in three randomised blocks, each block containing five trees. The fruit was harvested on 2 October 1985 (Bramley Seedling or 16 October 1985 (Golden Delicious)

and size graded for each tree. Bramley Seedling fruit of 65-70 mm size (4-5 apple per 1 kg) and Golden Delicious fruit of 60-65 mm size (7-8 apple per 1 kg) were retained. The measurements of respiration rate (CO_2 production) ethylene production, fruit mineral composition, fruit firmness, soluble solids and fruit colour composition were made either immediately or after storage at 4°C for 102 days (Golden Delicious) or 134 days (Bramley Seedling) as previously described. This experiment intended to study the interaction between paclobutrazol and gibberellic acid on the fruit post-harvest aspects, so the morphological changes of tree after chemical application were not investigated.

2.3 The Study of Effect of Paclobutrazol on Tomato

Tomato is an important crop with commercial significance in the world. It can be used as a kind of fruit although more popularly it is used as a vegetable. Although paclobutrazol has been used on a wide range of plant species, its effects on tomato do not appear to have been investigated. This trial intends to study the effect of paclobutrazol on the post-harvest physiology of tomatoes. A comparison of a herbaceous annual fruit crop with the woody perennial apple was considered to be especially interesting. Particularly as it was known from the previous study that paclobutrazol could benefit apple

fruit storage (Luo et al 1987). The trial included two parts:

I Tomato grown in normal greenhouse condition where paclobutrazol was applied.
II The various determinations of harvested tomato fruit associated with the post-harvest physiology.

2.3.1 General experimental design The seeds of tomato CV Turbo were obtained from Rijk Zwaan Varieties seed company in the Netherland. They were sown in Levington Universal compost and two-week-old seedlings were transfered to plastic boxes containing Levington Potting compost and moved to a growing greenhouse with a mean temperature of 23°C. The first flower truss appeared, the seedlings were transfered to the final growing pots (12 litre) containing both compost and compound fertilizers as recommended by Kingham (1973). All plants were trained to a single stem and all flowers on the first truss had assisted pollination by daily vibration.

The experiment was arranged with single plant plots in 7 randomised blocks, each block containing five plants. The paclobutrazol was applied as a soil drench with a concentration of 4 mg ai per pot. There were five treatments in each block, the first treatment being given at full bloom in the first truss and the other

treatments applied at two-week intervals after the first treatment. The control received no paclobutrazol treatment. The five treatments were termed control, treatment 1, treatment 2, treatment 3 and treatment 4 respectively. The plate 1, 2, 3 and 4 show the tomato fruit of the first truss at the time of treatment.

Fruit were only taken from the first truss and these were picked at mature green stage according to the ATB* tomato ripening colour charts. Fruit on the other trusses were not used, but were allowed to develop normally on the plant.

* ATB tomato colour chart issued by the Agricultural Training Board.

2.3.2 Colour assessment Two tomato fruit from the first truss of each plant were randomly placed in a ripening room with a constant temperature of 20°C. The changes of fruit colour associated with fruit ripening were assessed by the means of XL10CDM tristimulus colorimeter every two days till the fruit became red. Four positions of cross section's surface of each tomato fruit were taken for colour assessment.

2.3.3 Ethylene production The ethylene produced by tomato was determined at each ripening stage: Mature-Green(MG), Green-Orange(GO), Orange-Green(OG),

Plate 1 The stage of tomato fruit development when paclobutrazol was applied as treatment 1.

Plate 2 The stage of tomato fruit development when paclobutrazol was applied as treatment 2.

Plate 3 The stage of tomato fruit development when paclobutrazol was applied as treatment 3.

Plate 4 The stage of tomato fruit development when paclobutrazol was applied as treatment 4.

Orange (O) and Red (R). The same tomato fruit that were used for colour assessment were used for this analysis. The single fruit was placed in a glass container and sealed for one hour in the ripening room, then 1 ml of the container atmosphere was sampled by a syringe and analysed by means of a Pye GCD gas chromatograph with flame ionisation detector, using an alumina column at 120°C and N_2 carrier gas.

2.3.4 Mineral composition Two fruit from the first truss of each plant were taken for mineral composition analysis at mature green stage. The fruit was washed with deionised water and sliced, then dried in a electric oven at a temperature of 100°C for 48 hours. The dry tomato tissues were finally ground and the minerals of calcium, magnesium, phosphorus and potassium were analysed by the means of an atomic absorption spectrophotometer as described earlier.

2.3.5 Total soluble solids The fruit juice obtained from tomato fruit that was to be used for mineral analysis at green mature stage. One fruit from each plant for the rest of ripening stages (GO, OG, O and R) was taken from the same analysis. The total soluble solids of fruit were measured by the means of an Abbe Refractometer.

3 RESULTS

3.1 The Application of Paclobutrazol on Apples

3.1.1 The influence of paclobutrazol on fruit respiration rate

The time of paclobutrazol application during the various preharvest stages to cultivars Bramley Seedling and Golden Delicious influenced the time of the peak in CO_2 production and therefore the respiration climacteric. Fruit from trees sprayed with paclobutrazol at first bloom and petal fall stages produced a climacteric peak significantly later (2 - 3 days) than controls or trees sprayed at fruitlet or preharvest stages for both cultivars (Figures 4,5), when fruit respiration was measured soon after harvest. A similar but slightly smaller delay in the peak in respiration rate (1.5 - 2 days) was seen after fruit of both Bramley Seedling and Golden Delicious had been stored at 4°C for either 89 or 117 days (Figures 6,7). There was no significant difference in the height of the peaks in CO_2 production between treatments or cultivars.

The respiration rate immediately after harvest did initially rise for both cultivars, but after five days this had stabilised. This stable respiration rate preceding the peak in CO_2 production was also influenced by the time of paclobutrazol application.

Figure 4

The production of CO_2 showing the respiration climacteric of Golden Delicious apple at 25°C. Respiration was measured immediately after harvest. Trees were either untreated(■) or treated with paclobutrazol at first bloom(○), petal fall(△), fruitlet (▲) or preharvest stage(□). The bar shows the L.S.D.($p = 0.05$, $n = 3$) between the time of the peaks in CO_2 production.

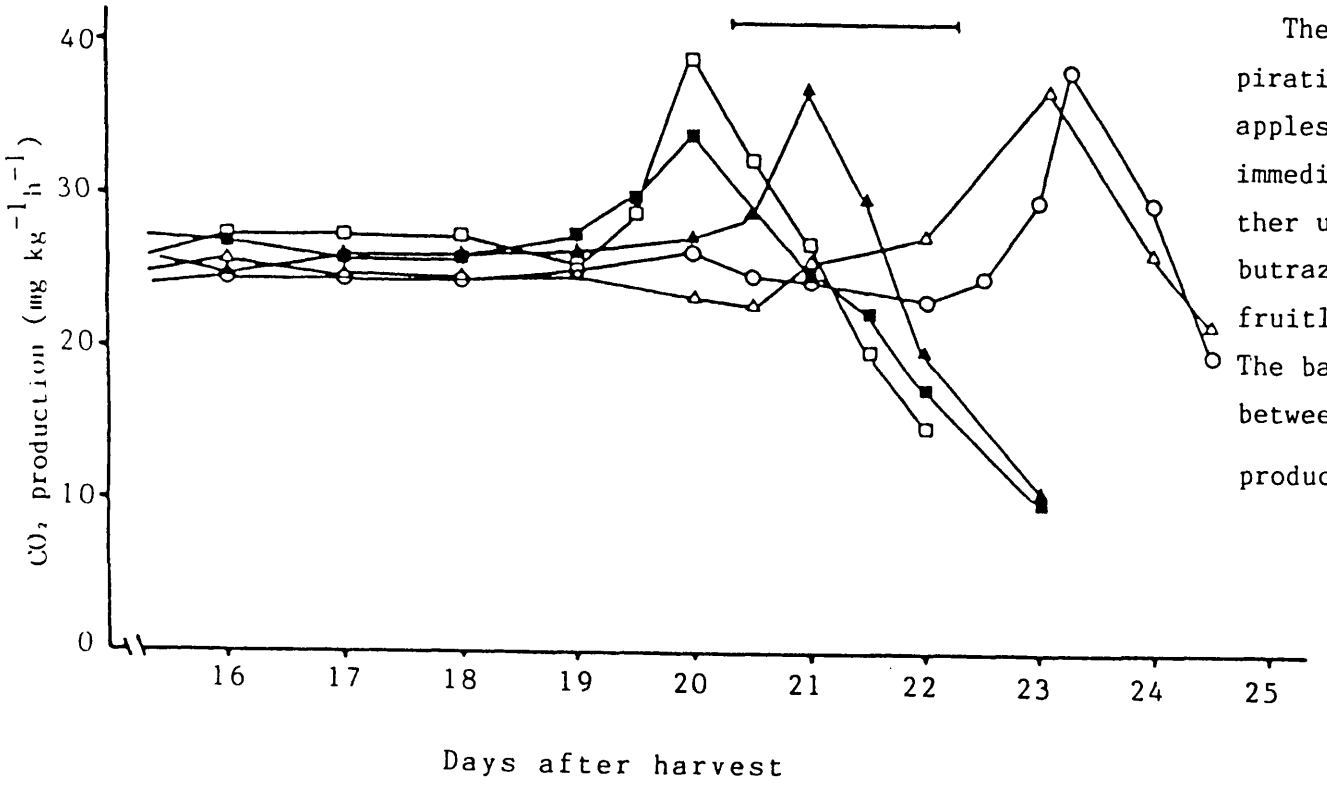


Figure 5

The production of CO_2 showing the respiration climacteric of Bramley Seedling apples at 25°C. Respiration was measured immediately after harvest. Trees were either untreated(■) or treated with paclobutrazol at first bloom(○), petal fall(△), fruitlet(▲) or preharvest stage(□). The bar shows the LSD($p = 0.05$, $n = 3$) between the time of the peaks in CO_2 production.

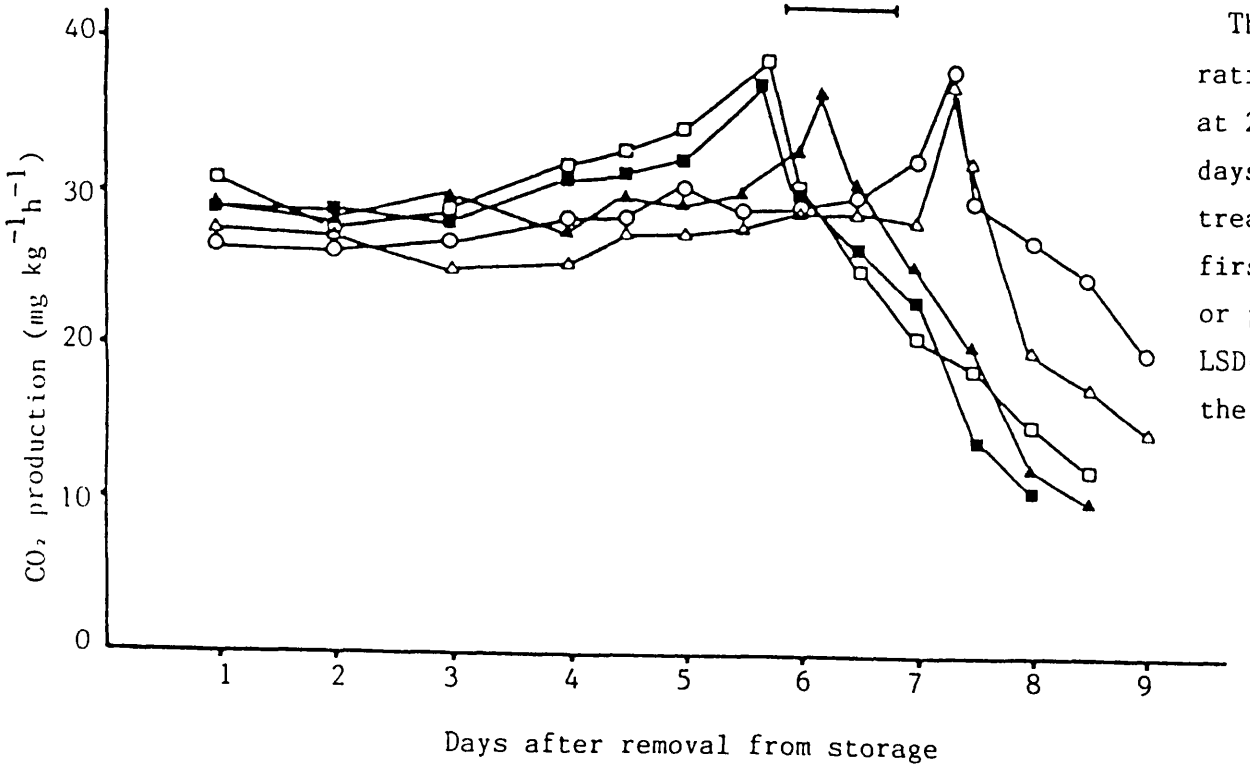


Figure 6

The production of CO_2 showing the respiration climacteric of Golden Delicious apple at 25°C. Respiration was measured after 89 days' storage. Fruit trees were either untreated(■) or treated with paclobutrazol at first bloom(○), petal fall(△), fruitlet(▲) or preharvest stage(□). The bar shows the LSD($p = 0.05$, $n = 3$) between the time of the peaks in CO_2 production.

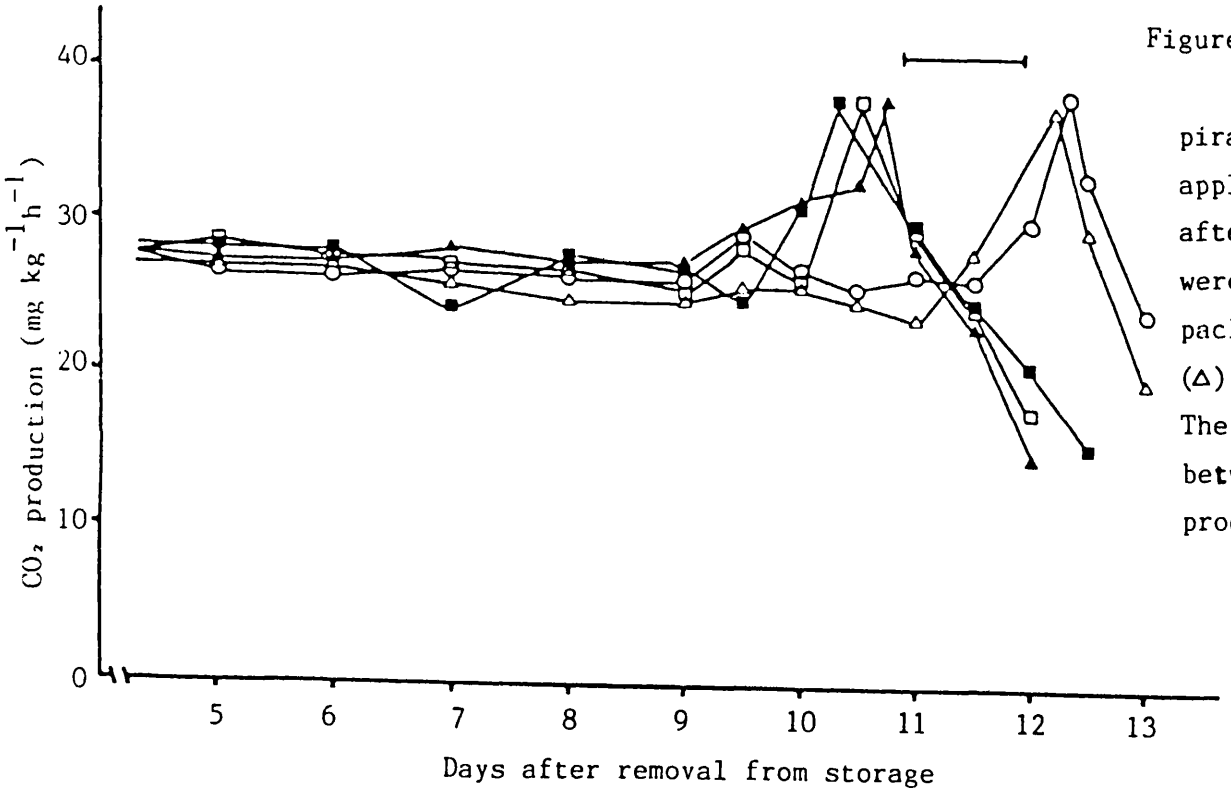


Figure 7

The production of CO_2 showing the respiration climacteric of Bramley Seedling apples at 25°C. Respiration was measured after 117 days storage when fruit trees were either untreated(■) or treated with paclobutrazol at first bloom(O), petal fall (△), fruitlet(▲) or preharvest stage(□). The bar shows the LSD ($p = 0.05$, $n = 3$) between the time of the peaks in CO_2 production.

The mean value of CO_2 production between day 6 and day 18 after harvest (Table 2) shows that the earlier in the growing season the trees were treated with paclobutrazol, the lower was the stable rate of respiration prior to the onset of the respiration climacteric, though for Golden Delicious, there was no difference between the two earlier sprays or between the two later sprays and the control.

3.1.2 Discussion for the influence of paclobutrazol on respiration rate The respiration of fruit is one of the most important aspects both of fruit postharvest physiology and in relation to fruit storage problems as reviewed before. Little is known about the influence of paclobutrazol on plant respiration. Although a large number of investigations have been carried out on behaviour of fruit in orchards where paclobutrazol had been used as a plant growth regulator, these have not usually included a study of respiration, despite its great importance in postharvest physiology.

Steffens et al (1983) reported that apple shoots of paclobutrazol-treated seedlings produced less CO_2 on a unit weight basis compared to the control. This effect was found just a few days after the treatment and the differences continued throughout the 18 day experimental period. They also investigated the respiration of the roots after treatment of the

Table 2 The mean of the daily respiration rate (mg. $CO_2 kg^{-1}$. hr^{-1}) at 25°C of fruit between day 6 and 18 after harvest having previously been treated with paclobutrazol in the orchard.

	Time of treatment					
Cultivar	Control	First bloom	Petal fall	Fruitlet	Preharvest	Mean
Bramley Seedling	28.0	24.8	25.9	26.7	28.1	26.7
Golden Delicious	27.7	25.2	25.1	27.2	27.2	26.5
Mean	27.9	25.0	25.5	26.9	27.6	

L.S.D. ($p = 0.05$) for column means = 0.6, $n = 26$.

seedlings with paclobutrazol via leaves and stems, however no differences in respiration were found between the roots of treated and control plants. Later, Wang et al (1985) demonstrated that new white fibrous roots of apple seedling grown in nutrient solution containing paclobutrazol tended to show higher respiration rates and GA_3 application appeared not to reduce the stimulated respiration. It seems that gibberellins were irrelevant to the mechanism of respiration. Recently Wang and Steffens (1987) reported that the respiration rate and respiration climacteric of post-harvest 'Spartan' apples were not influenced by pre-harvest paclobutrazol treatment. However instead of foliar application, in their experiment, the 'Spartan' apple trees were treated by means of a uniform soil drench with concentration of 1 g ai/tree and the respiration determinations were made only after the fruit had been stored for 7 months.

The results presented in this study demonstrate that the respiration climacteric is influenced by paclobutrazol application. The figures 4, 5, 6 and 7 show that paclobutrazol delayed the onset of the respiration climacteric of harvested fruit provided that the applications were made at an early stage (first bloom and petal fall). Similar results were obtained when the respiration measurements were made

either soon after harvest, where the respiration climacteric appearances were delayed by 3 days, or after three months storage where the onset of respiration climacteric was delayed by approximately 1.5 days. Similar results were obtained in both cultivars investigated: Golden Delicious and Bramley Seedling. Our results also show that fruit from the earlier paclobutrazol treatment had a lower respiration rate during the day 6 to day 18 period after picking, although this was not significant for the Golden Delicious fruit (Table 2).

The studies of the respiration climacteric and ethylene evolution of postharvest fruit and the influence of paclobutrazol on them were the major parts of this project. Care was taken throughout the experiment including field trial and laboratory work. During spray application, each apple tree was isolated by a plastic film screen ensuring the foliar sprays would not influence adjacent trees. In laboratory analysis, fruit of uniform size were taken for respiration measurements as it is known that the larger fruit tend to give higher rates of respiration. The respiration measurements were carried out in dark condition to prevent any photosynthetic consumption of the respiratory CO_2 produced during the experimental period. The temperature was also well controlled, because respiration is known to be influenced by

temperature variation (Fidler et al 1973).

The mechanism by which paclobutrazol influences the plant or fruit respiration is unknown. Steffens and Wang (1983) presumed that the energy requirements of treated tissues are less than for controls, which would be expected for less metabolically active plants. A lower rate of respiration which utilizes less carbohydrate, may also partially account for the increased carbohydrate content in treated tissues (Steffens and Wang 1986, 1984, Steffens et al 1985 and Wang et al 1985).

The anti-gibberellins biosynthesis is the major property of paclobutrazol. The results of Wang et al (1985) implied that gibberellins do not affect respiration in plant tissue. They demonstrated that new white fibrous roots of apple seedling grown in nutrient solution containing paclobutrazol tended to show higher respiration and GA_3 application appeared not to reduce the stimulated respiration. In contrast, Sharples and Johnson (1986) reported that in series of trials on Bramley's Seedling fruit from the GA-treated trees ripened 7 days earlier as evidenced by starch hydrolysis and the onset of respiration climacteric. These results suggested that the delay in the respiration climacteric resulting from paclobutrazol treatment may be due to the inhibition of endogenous gibberellic

acid biosynthesis in the plant.

The results obtained in our study clearly show that preharvest paclobutrazol treatment resulted in a delay of the onset of the respiration climacteric in fruit of both Bramley Seedling and Golden Delicious either at harvest time or after storage. However, the time of paclobutrazol application appeared to be very critical, only the two early season applications (first bloom and petal fall stages) influencing the surge of the CO_2 production, while the two later applications failed to produce any responses. The reason for this may be that later applications (fruitlet and preharvest stages) had less influence on the current seasons vegetative growth of the trees and therefore had less effect on nutrients distribution, and the balance of internal hormones and enzyme activities within the tree. Indirect changes of this kind may influence the fruit post-harvest behaviour. It is also possible that the later applications may have failed to enter the site of activity within the fruit, whilst early treatments allowed paclobutrazol enter the fruit and directly influence respiration. It is known that the mobilization of paclobutrazol is rather slow in xylem (Quinlan and Richardson 1986).

The delay in the onset of the respiration climacteric was shorter in both cultivars when the fruit had been

stored for three months. This may be because the respiratory activity of the fruit declined after having been stored for long period, and the fruit might have already passed through almost all the ripening stages. This would account for why the significance of difference in respiration between treated and untreated apple fruit also declined, and why there are no differences in the time of onset of the respiration climacteric between treated and untreated fruit in Wang and Steffens' work (1987).

To summarize to the above discussion, two explanations can be drawn for paclobutrazol benefiting the storage of the fruit through reducing the respiration rate and delaying the onset of respiration climacteric.

I The profound effects of paclobutrazol on vegetative growth of apple trees may cause a great change in the overall physiology of the tree and indirectly affect the post-harvest behaviour of the fruit. For example, paclobutrazol increased fruit carbohydrate content (Steffens and Wang 1985) which could be provided as substances for respiration.

II Paclobutrazol may directly inhibit the formation of some growth hormones in the fruits which enhance the process of fruit ripening, for instance reduced production of the ripening hormone ethylene,

inhibition of gibberellins production. However, to understand the mechanism of paclobutrazol on fruit respiration, more work is required.

3.1.3 The influence of paclobutrazol on fruit ethylene production The ethylene production from fruit that had been previously treated at different time during the growing season in the orchard was determined both at harvest and after storage. When paclobutrazol was applied either at first bloom or petal fall then less ethylene was produced from the fruit either at harvest or after storage than in controls or when treatment was at fruitlet or preharvest stages (Table 3). There were only small differences between ethylene produced by fruit from trees sprayed at first bloom and petal fall stages, a significant difference only occurring for Golden Delicious after storage. Similarly with the later treated trees, there was a significant difference between Golden Delicious trees treated at fruitlet and preharvest stages, only when ethylene was measured using fruit immediately after harvest.

Fruit slices treated with paclobutrazol during incubation were obtained from fresh fruit purchased locally. With Bramley Seedling apples, ethylene production decreased with increasing concentration of paclobutrazol, but with Cox's Orange Pippin although paclobutrazol treated fruit produced less ethylene

Table 3 The mean ethylene produced ($nl.g^{-1}.h^{-1}$) by apple tissue slices incubated for 6 hours at 25°C, the fruit having previously been treated with paclobutrazol in the orchard.

Cultivar	Control	Time of treatment: First bloom	Petal fall	Fruitlet	Preharvest
		Ethylene produced at harvest			
Bramley Seedling	34.02	24.88	25.45	30.43	30.90
Golden Delicious	36.68	22.79	23.37	34.49	39.46
Mean	35.35	23.84	24.41	32.46	35.18
		Ethylene produced after storage			
Bramley Seedling	38.99	28.35	28.23	37.95	40.84
Golden Delicious	36.22	21.06	26.96	38.99	38.18
Mean	37.61	24.71	27.59	38.47	29.51

L.S.D. ($p = 0.05$) for column means = 5.11, $n = 12$.

than untreated fruit, the response to increasing paclobutrazol concentration is not evident(Table 4).

Table 4 The mean ethylene produced($nl.g^{-1}.h^{-1}$) by apple tissue slices incubated for 6 hours at 25° C in different concentrations of paclobutrazol.

	Paclobutrazol concentration($mg.l^{-1}$)					
Cultivar	0	1	10	100	1,000	L.S.D. (p=0.05) (n=6)
Bramley Seedling	29.1	26.8	24.3	21.6	17.9	2.1
Cox's Orange Pippin	27.3	24.7	22.7	23.8	24.8	2.0

3.1.4 Discussion for the influence of paclobutrazol on ethylene production The technique for measurement of ethylene production was primarily adopted from Lieberman and Wang (1982), when they used apple tissue slices to determine fruit ethylene production. However, some improvements in this technique were made in this study. The production of ethylene from different parts of the apple fruit was found to be different. The table 5 shows that the production of ethylene by apple tissue taken from near the peel (epidermis) was higher than that of apple tissues taken from near the core. It is therefore, essential to obtain apple tissue slices from the same part of the apple pulp to ensure obtaining an accurate comparison between each treatment.

Table 5 The ethylene production ($nl.g^{-1}.h^{-1}$) of apple fruit tissues taken from different area of the pulp.

	Area		
Cultivar	Near peel	Middle	Near core
	Ethylene production		
Bramley Seedling	32.8	29.1	28.8
Golden Delicious	43.5	31.2	29.8

L.S.D. ($p = 0.05$, $n = 6$) for table means = 2.1

Variation in ethylene production by different samples of apple tissue was also found to be caused by the bacterial contamination during the isolation and incubation. This was proved because the variation of ethylene production vanished when the choramphnicol was included in the incubation medium.

The results in table 3, 4 show that treatment of apple slices with paclobutrazol reduces ethylene production and a similar effect on the production of stress-induces ethylene has been reported by Wang and Steffens (1985). In this work, the reduction in ethylene production was not due to an effect on 1-aminocyclopropanearboxylic acid (ACC) conversion to ethylene, but due to a reduction in ACC synthesis. It is possible that the effect of paclobutrazol on apple fruit ripening, resulting from application to the orchard tree might also be due to a direct effect of this kind, but indirect effects may also be involved. For instance, paclobutrazol has been reported to increase the calcium content of fruit (Greene 1986) and calcium has been reported to retard senescence and senescence-dependant ethylene production (Lieberman 1979).

The latest report, published by Wang and Steffens (1987) showed that the ethylene production of post-harvest apple fruit was not affected by treatment of the tree with paclobutrazol as a soil drench.

However, since the determination of ethylene production was made after the fruit had been stored for seven months, it is possible that differences in ethylene production of the fruit may have become indistinguishable between each treatment after such a long storage period. In addition, it is possible that different cultivars and different paclobutrazol application methods may be responsible for the different results from this work.

The interaction of internal growth hormones is known to profoundly influence ethylene evolution within plant organs (Lieberman 1979, Yang and Hoffman 1984) and thus the results obtained in this study may help understand the role of paclobutrazol in ethylene biosynthesis and the interaction with other growth hormones associated with ethylene production.

The later season application was not shown to influence ethylene production to a significant extent. There are various possible reasons for the differences in effectiveness of early and later applications. For instance, paclobutrazol may enter the fruit , but the responsiveness of tissue may decline with the season. Alternatively paclobutrazol may only enter the fruit in the early part of the season whilst during the later part of the season and during fruit swelling, little paclobutrazol moves into the fruit. Finally it

may be that paclobutrazol does not enter the fruit in significant quantities at any time of the year. The effects of sprays at different time in the season may be by a selective influence on other growth processes which in turn affect fruit quality.

3.1.5 The influence of paclobutrazol on fruit mineral composition The fruit mineral composition of cultivars Bramley Seedling and Golden Delicious was influenced by the time of paclobutrazol application during the growing season. The major four mineral elements (Ca^{++}, Mg^{++}, P^{++} and K^{+}) which are thought to influence the post-harvest quality of fruit were determined either soon after harvest or after storage.

The calcium content of fruit soon after harvest or three months storage of both Bramley Seedling and Golden Delicious from trees treated at first bloom and petal fall was significantly higher than control or fruit from trees sprayed at fruitlet and preharvest stages (Table 6). There were no differences in calcium content between control and the treatments of fruitlet and preharvest stage for both cultivars. There was no difference in calcium content between fruit analysed at harvest or after storage for Bramley Seedling while the fruit calcium content after storage did decline in Golden Delicious. In addition, fruit of Bramley Seedling had a higher calcium status than that of

Table 6 Calcium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with paclobutrazol at various preharvest stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	238.2	234.2	167.5	167.1	201.8
First bloom	288.7	286.7	237.5	214.7	256.9
Petal fall	309.5	291.8	226.0	224.7	263.0
Fruitlet	236.2	234.5	181.8	169.1	205.4
Preharvest	240.7	234.5	170.0	163.5	202.2
Mean	262.7	256.3	196.6	187.8	

Column means L.S.D. = 7.71 (n=15)

Row means L.S.D. = 8.87 (n=12)

Table means L.S.D. = 25.7 (n=3)

P = 0.05

Golden Delicious (Table 6).

The fruits of both Bramley Seedling and Golden Delicious when treated at first bloom or petal fall had a slightly higher magnesium status than the control or fruit from later treatments, there were no statistically significant differences between control and each treatment (Table 7). However, when the data analysis was made by combining the data obtained from both cultivars and both measurements (at harvest, after storage), the magnesium concentrations of fruit from trees which had been treated with paclobutrazol at first bloom or petal fall were sinificantly higher than control or fruit from treatment of fruitlet and preharvest stages. There was still no difference between fruit from control and fruit from treatments of fruitlet and preharvest (Table 7). There were no differences in magnesium concentration between fruit tested at harvest and fruit tested after storage for both cultivars. However, Bramley Seedling had a higher magnesium content than cultivar Golden Delicious.

The phosphorus concentrations of fruit from paclobutrazol treatments at first bloom and petal fall were increased in both Bramley Seedling and Golden Delicious, soon after harvest or after storage. However, no difference was found in phosphorus content between fruit from control and fruit from paclobutrazol treatments at

Table 7 Magnesium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with paclobutrazol at various pre-harvest stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	240.9	240.9	200.8	199.8	220.6
First bloom	248.3	246.3	215.5	218.0	232.0
Petal fall	265.2	253.5	207.6	208.8	233.8
Fruitlet	238.9	228.7	206.4	202.9	219.2
Preharvest	238.5	239.7	194.6	194.5	216.8
Mean	246.4	241.8	205.0	204.6	

Column means L.S.D. = 7.9 (n=15)

Row means L.S.D. =9.06 (n=12)

Table means L.S.D. = 26 (n=3)

P = 0.05

fruitlet or preharvest stage (Table 8). In addition, there was no difference between fruit tested at harvest and fruit tested after storage. Golden Delicious had a higher phosphorus concentration than Bramley Seedling (Table 8).

In Bramley Seedling, the potassium content of fruit from trees treated at petal fall stage was significantly lower than the control, when the determination was made soon after harvest. Though fruit from other treatments had a somewhat lower potassium concentration than the control, there was no statistical difference between them (Table 9). After storage, the potassium content of fruit from trees sprayed at first bloom and petal fall was significantly lower than the control, while no difference was found between fruit from the control and fruit treated at fruitlet and preharvest stages (Table 9). In Golden Delicious, the fruits from petal fall and fruitlet treatments had a lower potassium concentration than the control, and fruits from the later treatment were not different from the control when the determinations were made soon after harvest. After storage, the potassium concentration of fruit from treatments of first bloom and petal fall was lower than control and the rest of treatments had no difference from control. Again there were no diffrences in potassium concentration in fruit at harvest or after storage for both cultivars. The

Table 8 Phosphorus content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with paclobutrazol at various pre-harvest stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	494.4	488.7	510.8	517.5	502.9
First bloom	559.5	556.8	581.3	586.0	570.9
Petal fall	546.0	548.7	578.2	582.8	563.9
Fruitlet	493.3	492.0	509.8	501.8	499.2
Preharvest	504.7	494.2	511.3	507.3	504.4
Mean	519.6	516.1	538.3	539.1	

Column means L.S.D. = 11.14 (n=15)

Row means L.S.D. = 12.73 (n=12)

Table means L.S.D. = 37.2 (n=3)

P = 0.05

Table 9 Potassium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with paclobutrazol at various pre-harvest stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	6250.0	6305.8	6860.8	7000.0	6604.2
First bloom	5472.2	5110.8	6139.2	5972.5	5673.7
Petal fall	5250.0	5166.7	5860.8	5916.7	5548.6
Fruitlet	5556.0	5944.2	5694.2	6250.0	5861.1
Preharvest	5860.8	6277.5	6833.3	6860.8	6458.1
Mean	5677.8	5761.0	6277.7	6400.0	

Column means L.S.D.= 371 (n=15)

Row means L.S.D. = 424 (n=12)

Table means L.S.D. = 972 (n=3)

P = 0.05

Golden Delicious had a higher potassium content than the Bramley Seedling.

3.1.6 Discussion for influence of paclobutrazol on mineral composition Mineral composition plays very important role in fruit post-harvest physiology, especially the calcium concentration of the fruit, which is positively related to the fruit storage life (Poovaiah 1986) and a lot of storage disorders are associated with the deficiency of calcium content. The results reported in this work demonstrated that the calcium content of fruit was remarkably increased when the paclobutrazol was applied earlier in the growing season (first bloom and petal fall treatments) for both Bramley Seedling and Golden Delicious. These results were supported by numbers of workers who also found that fruit calcium concentration was increased by paclobutrazol treatments (Greene and Murray 1983, Sharples and Johnson 1986, Steffens and Wang 1986, Greene 1986). In those studies, similar results to this study were reported by Greene (1986), where the calcium concentration of fruit of 'Delicious' apples was increased when 1500 or 3000 ppm paclobutrazol was applied as a foliar spray after 21 days of full bloom. The final fruit size was also reduced by this application. Sharples and Johnson (1986) presumed that the increase of calcium concentration of fruit may be due to the lower dilution of calcium which occurs in more slowly

growing fruits. This may partly explain the increase of fruit calcium content, however, in this study, the size of fruit from earlier stages of paclobutrazol treated trees was not found to be reduced at harvest time, although fruit calcium concentrations were still increased. However, just one exception did occur, where the application at first bloom to Golden Delicious was found to reduce the fruit final size (Table 6). The change in fruit mineral status by paclobutrazol on vegetative growth of apple trees, which may indirectly influence the fruit by shifting assimilate partitioning from one part to another part within the plant, and thereby changing the mineral composition of fruits.

The phosphorus content of the fruit was also found to be increased for both cultivars again when paclobutrazol was applied at earlier growing seasons. Letham (1969) showed that a phosphorus content above a critical threshold was required to avoid fruit storage disorders, and that higher phosphorus levels were beneficial. The present results suggest that paclobutrazol could increase fruit storage capability in this way, although little is known about the direct metabolic association of phosphorus levels with post-harvest physiology.

Potassium content was also found to be changed by paclobutrazol application (Table 9), fruit from trees treated at the early growth stages have decreased

potassium concentration. Since high potassium content causes internal breakdown of apple fruit (Nyhlen 1960, Weeks et al 1965) and various post-harvest disorders, the effect of paclobutrazol would be consistent with improved storage capability. The comparison of fruit mineral content soon after harvest and after storage showed that no changes in mineral element concentration of fruit took place. This indicates that no redistribution of fruit flesh minerals for example towards the seed took place over the three months storage period.

No earlier reports were available on the influence of paclobutrazol on fruit P, K, Mg content, and the mechanism by which paclobutrazol influences the fruit microelements content is not fully understood, more research work is still largely required.

During this experiment, apple fruit of equal size were selected for microelements analysis in order to eliminate any difference in mineral elements caused by the variation of fruit size.

The mineral nutrients tested here are just four basic elements associated with post-harvest physiology. However the fruit mineral constitution comprised far more than these four elements. Some of them are very important factors in fruit ripening and senescence e.g. nitrogen, but unfortunately this work did not

test them.

Neverthless, this work demonstrated that paclobutrazol changed fruit mineral concentration and these changes tend to benefit the fruit post-harvest storage and quality when it was applied early in the growing season.

Steffens and Wang (1985) tested the change of mineral constitution of apple leaves after application of palcobutrazol. They found that all the N, P, K, Ca and Mg were increased after 66 days of application. These results indicated that paclobutrazol could increase the rate of mineral elements uptake by the roots of apple seedling and enhance the efficient exploitation of soil minerals. Other hormones were also reported to affect mineral uptake by roots (Treharne 1982), this may suggest that the influence of paclobutrazol on root mineral uptake could also associated with interaction with other growth hormones which may affect mineral uptake.

With the realization of the role of microelements in the fruit physiology, the mineral composition of fruit becomes more and more important in fruit post-harvest storage and the control of post-harvest disorders. Calcium, phosphorus and magnesium are thought to be particularly important in fruit storage, and increasing

the contents of these minerals to improve fruit storage capability, is still a task for fruit growers.This work suggests that paclobutrazol may be a ideal agent to improve fruit quality and storage ability through the changes of fruit mineral concentration.

3.1.7 The influence of paclobutrazol on fruit firmness and total soluble solids The fruit firmness was influenced by the time of paclobutrazol application. The firmness of fruit that had previously been treated with paclobutrazol at different times during the growing season was measured soon after harvest and after three months storage.

For Golden Delicious, fruits from trees treated either at first bloom or petal fall were firmer than the control or when treatment was at fruitlet or preharvest stages. Fruit from treatment at petal fall had a higher firmness than fruit from treatment at first bloom when the measurements were made at harvest. There were no differences between fruit from control and fruit from treatments at fruitlet and preharvest stages (Table10). After storage, similarly the firmness of fruit from treatments at first bloom and petal fall was higher than control and/or the fruit from treatments at fruitlet and preharvest stages. There was no difference in fruit firmness between treatments of first bloom and petal fall after storage.

Table 10 Firmness (kg.cm-2) of apple fruit having been treated with paclobutrazol at various preharvest stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	11.77	5.96	8.92	5.11	7.94
First bloom	11.91	7.51	10.23	7.51	9.29
Petal fall	12.00	7.35	10.74	7.25	9.33
Fruitlet	11.75	5.73	8.85	5.44	7.94
Preharvest	11.58	5.99	8.65	5.28	7.87
Mean	11.80	6.51	9.48	6.12	

Column means L.S.D. = 0.19 (n=120)

Row means L.S.D. = 0.21 (n=96)

Table means L.S.D. = 0.45 (n=24)

P = 0.05

For Bramley Seedling, there were no statistical differences between control and each treatment, although fruit from two earlier treatments had a slightly higher firmness when the measurements were made at harvest (Table 10). After storage, the firmness of Bramley Seedling fruit from trees treated at first bloom and petal fall was higher than control or than fruit from treatments at fruitlet and preharvest stages. The fruit firmness declined with storage for both Bramley Seedling and Golden Delicious.

The fruit total soluble solids content was determined either soon after harvest or after three months storage and was influenced by the time of application of paclobutrazol. The fruits of both Bramley Seedling and Golden Delicious from trees having been sprayed at first bloom and petal fall had a lower total soluble solids content than fruits from control or when treatments were at fruitlet and preharvest stages (Table 11), when the determinations were made either at harvest or after storage. The total soluble solids content of fruit of Bramley Seedling from trees treated at fruitlet stage was also lower than control when the measurement was made soon after harvest. However no differences at the time of harvest or after storage were found for both cultivars between control and preharvest treatment. The fruit total soluble solids content was increased after storage for both cultivars, and the

Table 11 Total soluble solids (%) of fruit having been treated with paclobutrazol at various preharvest stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	11.93	12.38	10.27	11.67	11.56
First bloom	10.37	10.74	9.18	9.67	9.99
Petal fall	10.48	10.46	9.13	9.85	9.98
Fruitlet	11.12	12.03	10.45	11.56	11.29
Preharvest	11.85	12.15	10.32	11.38	11.42
Mean	11.15	11.55	9.87	10.83	

Column means L.S.D. =0.16 (n=120, p=0.05)

Row means L.S.D. = 0.18 (n=96, p=0.05)

Table means L.S.D. = 0.39 (n=24, p=0.05)

Bramley Seedling had a higher total soluble solids content than the Golden Delicious.

3.1.8 Discussion for influence of paclobutrazol on firmness and total soluble solids Fruit firmness was found to be improved after application of paclobutrazol by many studies (Greene and Murray 1983, Sharples and Johnson 1986, Greene 1986, Curry and Williams 1986, Wang and Steffens 1987). The results obtained in the present study confirmed that paclobutrazol application could increase fruit firmness (Table10). A similar experiment, reported by Greene, showed that fruit firmness was increased by foliar paclobutrazol at 1500 or 3000 ppm, when the firmness test was made soon after harvest. However, in his study, the number of seeds and fruit size were also reduced. In our study, fruit firmness was increased in most cases without reduction of fruit size.
It is possible that the increase of fruit firmness may result from the increase of fruit calcium concentration, as calcium is thought to be important in maintaining cell structure (Poovaiah 1986) and paclobutrazol provides fruits of a higher total calcium status. Many reports have shown that increases in fruit firmness resulting from paclobutrazol treatment are accompanied by an increase in the calcium concentration of the fruit (Greene and Murray 1983, Sharples and Johnson 1986, Greene 1986, Curry and Williams 1986,

Wang and Steffens 1987). In the present work, fruit firmness was tested either soon after harvest or after storage. Stored fruit of both cultivars showed significantly greater firmness in the two early paclobutrazol treatment comparing with controls (or later paclobutrazol treatment). Golden Delicious fruit showed similar differences immediately after harvest, whereas Bramley Seedling fruit showed no differences in firmness at this stage.

The difference between two cultivars at harvest may be due to different variety of apple in response of paclobutrazol. Similar to the present results with Bramley, Sharples and Johnson (1986) reported that in a Cox's Orange Pippin trial at East Malling, no differences between treated and untreated fruits in the instrumental firmness were found during the first few months after harvest, although until late March or early April taste panelists found the treated fruit to be less mealy, the calcium level in fruit from trees treated with paclobutrazol was higher.

Our results clearly showed that the calcium content of fruit of both Bramley Seedling and Golden Delicious was increased by earlier growing season application of paclobutrazol. After storage the firmness of fruit from earlier application was higher by 2 $kg.cm^{-2}$ for both cultivars, and this may bring a marketing

benefit.

The fruit total soluble solids content could be an indicator of fruit ripening (Hulme 1979) and higher total soluble solids are usually associated with a shorter storage life. However, in this study, the fruit total soluble solid contents were found to be reduced by the paclobutrazol application. Greene(1986) reported that the total soluble solids contents of fruit treated with paclobutrazol at earlier growing season were lower when determination was made soon after harvest. Similar results were obtained in this study (Table 11) where the total soluble solids of fruit were found to be reduced for both Bramley Seedling and Golden Delicious, when paclobutrazol applications were made at earlier growth season. Greene and Murray (1983) explained that paclobutrazol reduced total soluble solids of apple fruit because paclobutrazol reduced leaf area per spur. It has been shown that a reduction in the leaf area around a fruit will lower soluble solids at harvest time (Greene and Lord 1983). In this study, we also found that the total soluble solids of fruit from the earlier growing season treatment changed only slightly during three months storage while the total soluble solids of fruit from all the other treatments had increased considerably over this period. This may imply that paclobutrazol slowed the fruit metabolism (and particularly hydrolytic processes) during the

post-harvest period, thereby elongating the fruit storage life. Such an effect could be due to a direct effect of paclobutrazol which inhibited the activity of catalytic enzymes within the fruit, or by an indirect effect of paclobutrazol via the inhibition of respiration activity and ethylene production which could in turn be responsible for slowing down the metabolism of the fruit.

3.1.9 The influence of paclobutrazol on fruit colour

Fruit colour can be measured in terms of 6 values obtainable from colour analysis instrument. The 6 values are Y, X, Z, L, a and b. The Y, X and Z values are in a group which stands for three original colours green, red and yellow respectively as shown in Figure 8. The L, a and b in another group which stands for the range of darkness to lightness, green to red, and blue to yellow respectively (Figure 8). As fruit colour changes during ripening, this will cause a change in these 6 components. In normal circumstances, the colour will change from green to red, blue to yellow and dark to light.

The changes of fruit colour components of both CV Bramley Seedling and Golden Delicious during the storage were influenced by the time of paclobutrazol application during the growing season. The fruit colour was assessed soon after harvest and after storage.

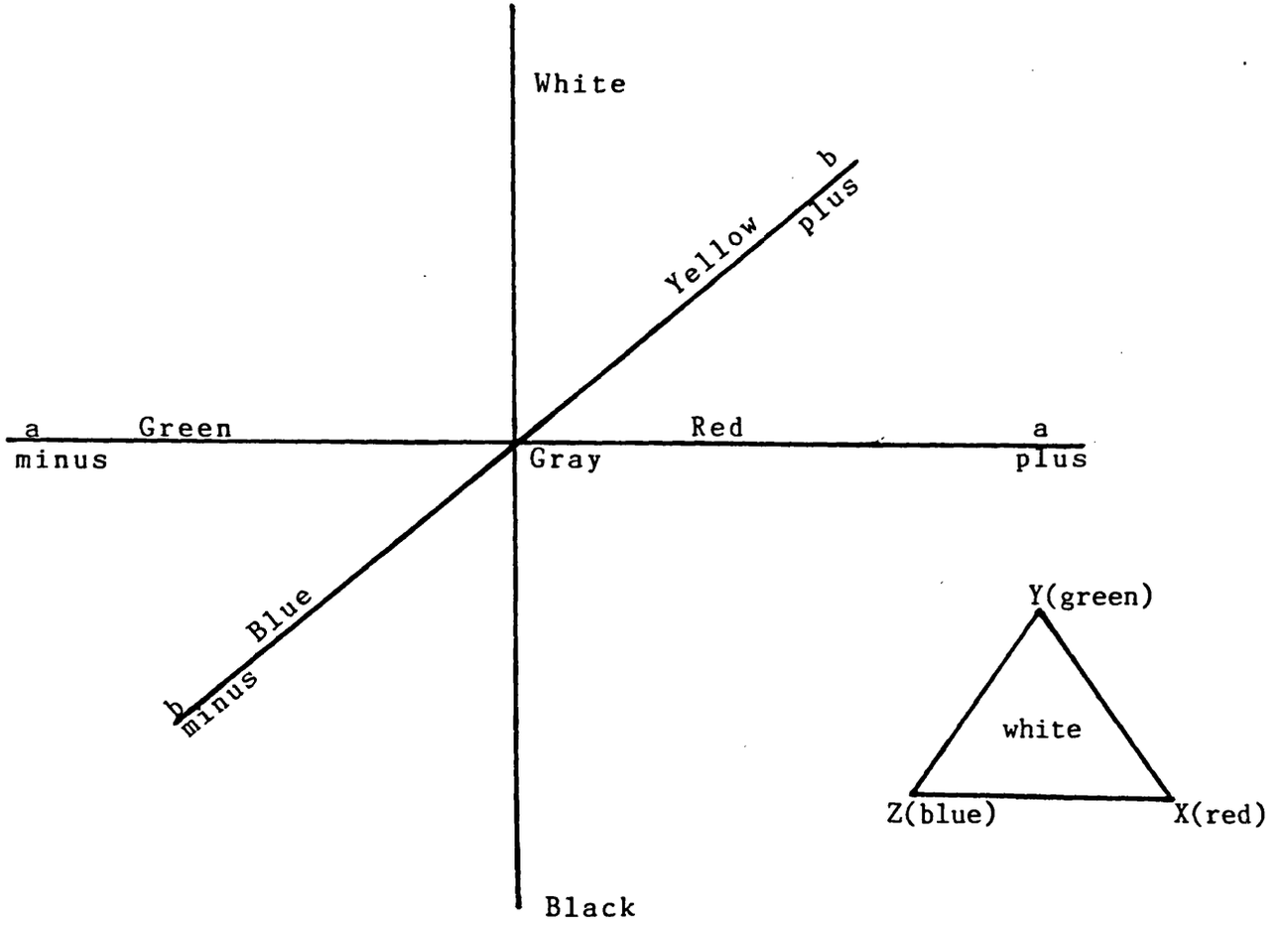


Figure 8 The principle of tristimulus colorimetery.

Source: The measurement of appearance by Hunter R.S.(1975).

There were no differences in fruit colour component Y value between control and each treatment for both Bramley Seedling and Golden Delicious when the assessment was made both at harvest and after storage. For Bramley Seedling the Y value of fruit after storage was higher than fruit at harvest, but no difference was found between the fruit at harvest and after storage for Golden Delicious (Table 12).

The X value was negatively related to the red colour, therefore lower X value is associated with a redder fruit colour. The X value of fruit of both cultivars had no difference between control and all the treatments when the determinations were made soon after harvest. However, after three months storage, the X values of fruit from trees having been sprayed at first bloom and petal fall were higher than those of fruit from control and from the treatments of fruitlet and preharvest stages for both Bramley Seedling and Golden Delicious. There were no differences between control and the treatments of fruitlet and preharvest stages. The X values of fruit at harvest were higher than fruit after storage for both cultivars Bramley Seedling and Golden Delicious (Table 13).

The Z value of fruit colour had no differences between control and all the treatments for both Bramley Seedling and Golden Delicious when determinations were made

Table 12 Fruit colour component Y value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	28.96	37.80	37.99	41.25	36.50
First bloom	28.53	31.80	38.80	41.60	35.08
Petal fall	29.00	32.33	37.83	37.11	34.07
Fruitlet	28.20	33.69	36.32	38.89	34.28
Preharvest	29.11	36.88	38.34	40.01	36.09
Mean	28.76	34.44	37.86	39.77	

L.S.D. (p= 0.05) for column means = 4.37 n=15

for table means = N.S.* n=3

for row means = N.S.* n=12

* not significant

Table 13 Fruit colour component X value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	32.26	25.10	31.70	30.24	29.83
First bloom	35.11	32.81	35.60	35.47	34.74
Petal fall	36.04	30.55	35.70	36.39	34.67
Fruitlet	35.27	25.16	34.97	31.84	31.81
Preharvest	33.40	26.33	32.70	29.70	30.53
Mean	34.42	27.99	34.13	32.73	

L.S.D. (p = 0.05) for column means = 1.47 n=15
table means = 4.92 n=3
row means = 1.68 n=12

either at harvest or after storage. The Z value of Bramley Seedling fruit after storage was higher than fruit at harvest, while no difference was found between the fruit at harvest and after storage for Golden Delicious. In addition, the cultivar Golden Delicious had a higher Z value than Bramley Seedling (Table 14).

The colour component L value was not influenced by the paclobutrazol treatment. The fruit from trees which had received paclobutrazol treatment had no difference in L value from control for both Bramley Seedling and Golden Delicious, when determinations were made either soon after harvest or after storage. The L values for both cultivars declined after storage, however the Golden Delicious had a higher L value than the Bramley Seedling (Table 15).

The a value was influenced by the time of paclobutrazol application. Although there were no differences in the a value for both cultivars between control and all the treatments when assessments were made at harvest. The measurement after storage clearly shows that the a values of fruit from treatments of first bloom and petal fall were significantly lower than control and than those of fruit from treatments of fruitlet and preharvest stages for both cultivars. There were no differences between control and the treatments of fruitlet and preharvest stages for both Bramley Seedling

Table 14 Fruit colour component Z value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	10.15	13.06	13.52	14.24	12.74
First bloom	10.00	11.67	14.99	13.16	12.46
Petal fall	9.79	11.70	13.72	12.62	11.96
Fruitlet	10.02	11.88	13.30	13.90	12.28
Preharvest	9.67	12.60	13.80	14.23	12.58
Mean	9.93	12.18	13.87	13.63	

L.S.D. (p=0.05) for column means = 0.8 n=15

table means = N.S.* n=3

row means = N.S.* n=12

* not significant

Table 15 Fruit colour component L value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	54.66	56.55	62.75	64.11	60.77
First bloom	53.31	56.32	62.23	63.75	58.90
Petal fall	53.36	54.41	61.52	60.92	57.55
Fruitlet	53.35	56.06	60.21	62.21	57.96
Preharvest	52.50	54.69	62.38	63.10	58.17
Mean	53.44	56.61	61.82	62.82	

L.S.D. (p=0.05) for column means = 1.04 n=15
table means = N.S.* n=3
row means = 1.19 n=12

* not significant

and Golden Delicious (Table 16). The table 16 also shows that the a value of fruit of both cultivars from treatments of first bloom and petal fall had no difference between values determined at harvest time and after storage, while the control and the rest of treatments had an increased a value after three months storage. In general the a value was increased for both cultivars after storage and the cultivar Golden Delicious had a higher a value than the Bramley Seedling either at harvest or after storage.

Paclobutrazol treatment had no influence on the b value. Results from table 17 show that there were no differences in b value for both cultivars between control and each treatment when either determined at harvest or after storage. The b vlaue of fruit was increased after storage for both cultivars (Table 17).

Two photographs of the Bramley Seedling and the Golden Delicious fruit were taken after having been removed from three months storage (Plate 5, 6).

3.1.10 Discussion for influence of paclobutrazol on fruit colour The change of colour is an important manifestation of ripening for many fruits including some apple cultivars, although it is not a major character in the ripening of CV Golden Delicious and

Table 16 Fruit colour component a value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	-15.10	-8.87	-13.57	-4.65	-10.55
First bloom	-15.52	-15.02	-12.65	-12.43	-13.91
Petal fall	-16.26	-15.72	-13.02	-12.55	-14.39
Fruitlet	-15.69	-7.60	-13.69	-3.75	-10.18
Preharvest	-15.69	-8.11	-14.09	-4.63	-10.63
Mean	-15.65	-11.06	-13.40	-7.60	-11.93

L.S.D. (p=0.05) for column means = 1.6 n=15

table means = 5.6 n=3

row means = 1.8 n=12

Table 17 Fruit colour component b value of Bramley Seedling and Golden Delicious having been treated with paclobutrazol at different stages during the growing season.

	Bramley Seedling		Golden Delicious		
Time of treatment	At harvest	After storage	At harvest	After storage	Mean
Control	26.42	30.44	29.32	32.55	29.68
First bloom	26.11	29.89	29.23	32.33	29.39
Petal fall	26.57	29.44	29.78	31.27	29.27
Fruitlet	26.15	28.85	28.97	32.20	29.04
Preharvest	26.09	29.61	28.03	32.80	29.13
Mean	26.27	29.65	29.07	32.23	

L.S.D. (p=0.05) for column means = 1.0 n=15
table means =3.34 n=3
row means =1.14 n=12

Plate 5

Golden Delicious apples after three months storage after fruit trees were either untreated (CON) or treated with paclobutrazol at first bloom (FB), petal fall (PF), fruitlet stage (FS) or preharvest(PH). Note the increasing ripeness (yellow) with the later applications and control.

Plate 6

Bramley Seedling apples after three months storage after fruit trees were either untreated (CON) or treated with paclobutrazol at first bloom (FB), petal fall (PF), fruitlet stage (FS) or preharvest (PH). Note the increasing ripeness (yellow) with later applications and control.

Bramley Seedling. The investigation of colour change during apple ripening may still give us some useful knowledge to understand apple ripening behaviour after application of paclobutrazol.

Judgement of colour varies from person to person and is highly subjective. Vague non-quantifiable terms are used to describe colour, such as red, bluish-green or yellowish. The technique of colour assessment in this study was adopted by the use of tristimulus colorimetry which offers a precise, objective method for describing the colour of the fruits. The value Y, X and Z represented three primary colours: green, blue and red respectively. It was understood that variation of colour was caused by changes in the proportion of these three primaries which could be quantified by the tristimulus colorimetry. The L, a and b values are the supplementary scale for describing the changes of colour. They quantify changes from dark to light, from green to red and from blue to yellow respectively. This scale is considered to be more useful than the X, Y, Z scale for colour assessment, because it is relatively easy to appreciate and interpret the L, a and b colour dimensions.

The results showed that paclobutrazol application in addition to changing fruit respiration and ethylene production, also influenced fruit colour change.

The colour of fruits from trees treated with paclobutrazol at earlier growing season was greener as shown in table 13, 16 and plate 5, 6 for both Bramley Seedling and Golden Delicious, when the colour assessment was made after three months storage. No differences in colour were found between each treatment when the assessment was made soon after harvest (Table 13,16), but during the three months storage period differences in fruit ripening speed could be detected by the change in colour values.

The change in colour during ripening in these cultivars can be attributed largely to chlorophyll breakdown (loss of green colour) (Holden 1976), although carotene and anthocyanin formation may also take place. The speed of colour change during fruit ripening depends on the speed of chlorophyll breakdown. However the mechanism of chlorophyll breakdown of ripening fruit is not fully understood (Seymour et al 1987 ab). The influence of paclobutrazol on fruit colour may be due to direct inhibition of the enzyme which triggers the chlorophyll breakdown through its inhibition of fruit ripening. Alternatively, paclobutrazol may influence fruit colour by its inhibition of GA biosynthesis, as some workers reported that gibberellins may increase fruit reddening (Greene et al 1982) as well as enhancing the fruit ripening process by increasing respiration (Sharples and Johnson 1986).

However, the influence of gibberellin on fruit colour was far from fully understood as some worker found that gibberellin inhibited chlorophyll breakdown in banana degreening (Blackbourn 1987, personal communication). In addition, paclobutrazol has been frequently found to increase leaf chlorophyll content (Wang et al 1985, Steffens and Wang 1984, 1986), and it could be presumed that paclobutrazol treated fruit had more green colour also resulting from the increase of chlorophyll content of fruit peel.

Little quantitative information appears to have been published on colour change in apples during storage. The colour of fruit of Golden Delicious and Bramley Seedling was basically green to yellow and little change occurred during ripening, although it was enough to allow fruit ripening to be monitored. The results in this work showed that the Y and b values of the fruit did not differ between each treatment either at harvest or after storage. It may be that since the basic colour of the fruit was green, a change of the value for green colour was not detectable during storage.

3.1.11 The influence of paclobutrazol on fruit setting The fruit setting investigation was carried out through the growing season. The fruit setting was influenced by the time of paclobutrazol application.

However different responses were obtained from both Bramley Seedling and Golden Delicious.

For Bramley Seedling, the final fruit setting of treatments of first bloom and petal fall was higher than control or the trees treated at fruitlet and preharvest stages. This observation suggests that paclobutrazol treatment improves the fruit setting when applied during the earlier season (Table 18). The investigation of cultivar Golden Delicious showed rather different results where the final fruit setting from trees sprayed at first bloom was lower than control or all the other treatments, and the treatment at petal fall had a higher fruit setting than control or all the other treatments (Table 18). There were no differences between control and treatments of fruitlet and preharvest stage for both Bramley Seedling and Golden Delicious (Table 18).

The fruit drop pattern for both cultivars was obtained by repeated fruit counts on pre-selected flowers through the growing season (Figure 9, 10). Both cultivars had a light fruit drop during the earlier growing season when the first evaluation was made on 20 May 1985. Approximately 50% of the fruit counted for investigation were still retained in this time, and there were no differences between control and each treatment for both Bramley Seedling and Golden Delicious.

Table 18 Fruit setting (% of flowers developing as fruits) of two apple cultivars having been treated with paclobutrazol at various growing stages

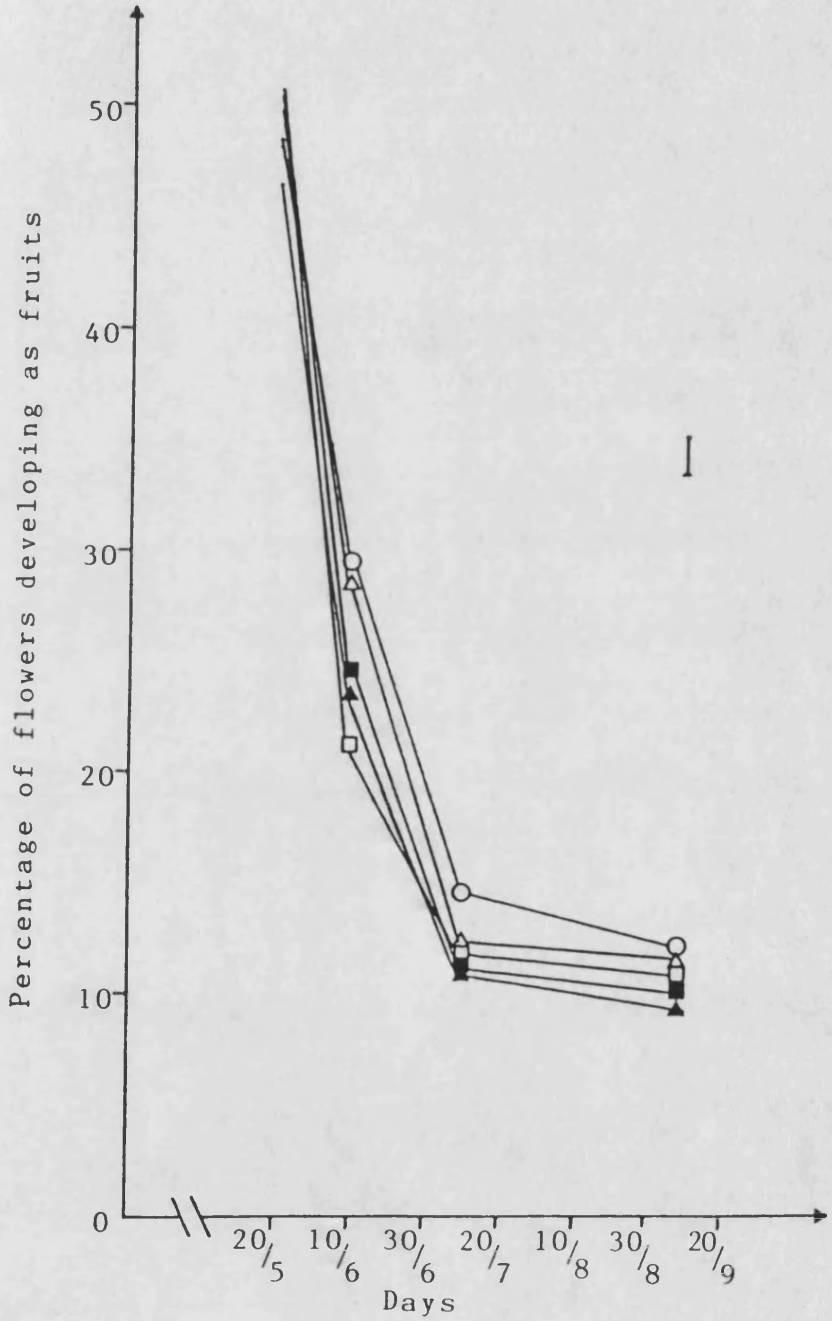
Fruit setting (%)

Cultivar	Control	First bloom	Petal fall	Fruitlet	Preharvest	Mean
Bramley Seedling	10	12	11.8	9.8	10.3	10.68
Golden Delicious	8.3	6.3	10.7	8.6	8. 3	8.4
Mean	9.15	9.15	11.3	9.2	9.3	

L.S.D. ($p=0.05$) for table means = 1.49 n = 3

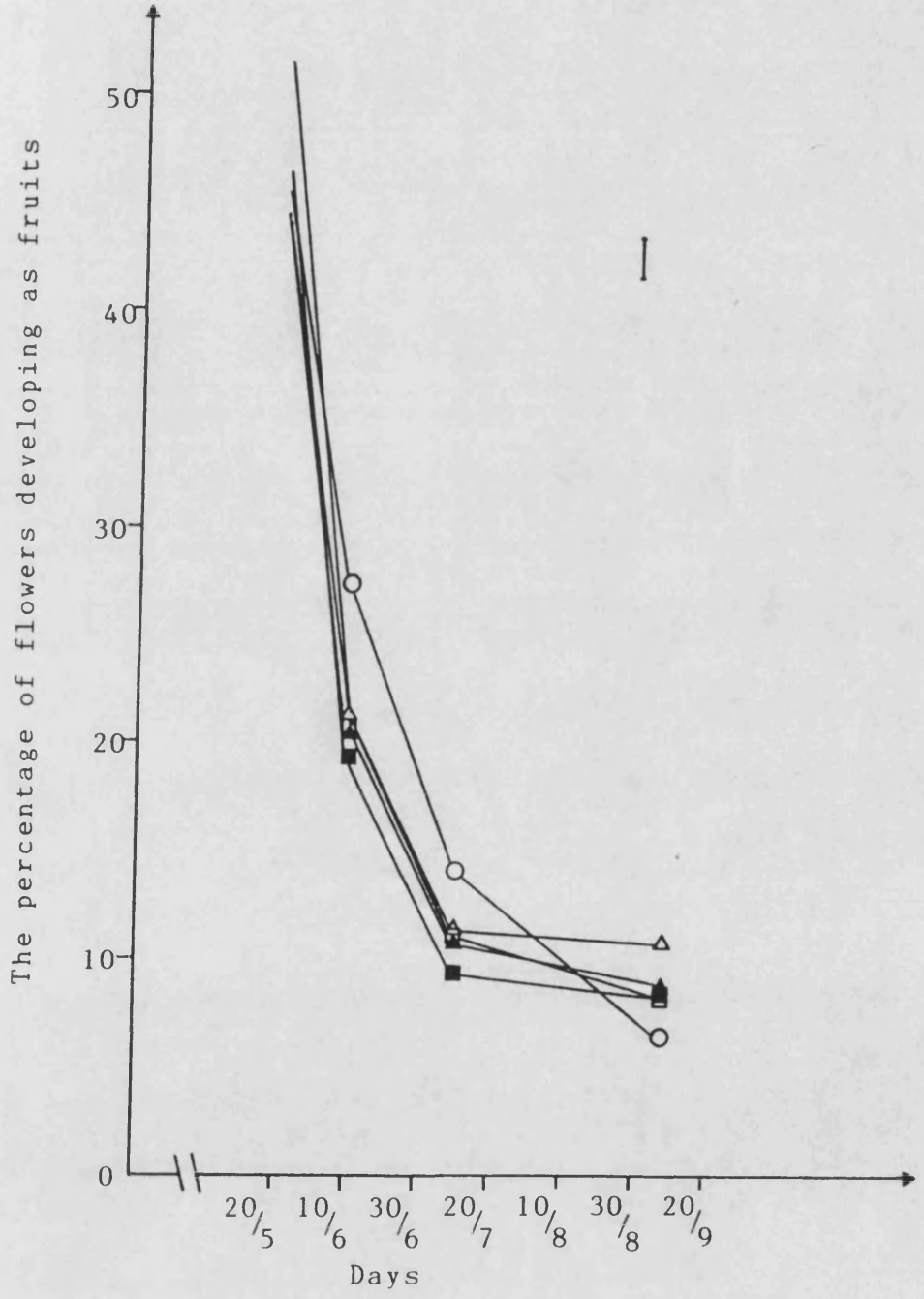
column means = 0.81 n = 6

Figure 9 The preharvest fruit drop pattern of Bramley Seedling having either been treated with paclobutrazol at first bloom(○), petal fall(△), fruitlet(▲), preharvest stage(□) or been untreated(■).



The bar shows(L.S.D. p=0.05, n=3) the percentage of fruit setting.

Figure 10 The preharvest fruit drop pattern of C.V. Golden Delicious having either been treated with paclobutrazol at first bloom(○), petal fall(△), fruitlet(▲), preharvest stage(□) or been untreated (■).



The bar shows(L.S.D. p=0.05, n=3) the percentage of fruit setting.

The second evaluation was made on 10 June 1985. For Bramley Seedling, more fruit was retained on the trees treated at first bloom and at petal fall than the control and later sprays. However for Golden Delicious, more fruit was retained on trees treated at first bloom comparing with control and all the other treatments (Figure 10) and there were no differences between control and treatments of petal fall, fruitlet or preharvest stage. After June drop, when the third evaluation was made, about 11% of the fruit were retained on the tree, but the fruit setting of trees treated at first bloom was still higher than in the control or in all the other treatments for both Bramley Seedling and Golden Delicious. However the preharvest evaluation (8 September 1985) shows that the fruit from the treatment at first bloom of Golden Delicious had a relatively large drop. All the evaluations indicated that only the earlier growing season applications affected fruit setting, whilst later applications of paclobutrazol at fruitlet or preharvest stage had no influence on fruit setting.

3.1.12 Discussion for influence of paclobutrazol on fruit setting The influence of paclobutrazol on fruit setting has been demonstrated in a number of reports (Williams 1984, Webster and Andrew 1985, Miller and Swietlk 1986, Edgerton 1986, Blanco 1987).

The responses were various when paclobutrazol treated different fruit species. Webster and Andrew (1985) reported that paclobutrazol could be used as a fruit thinning agent when applied to Victoria plums (Prunus domestica L.) at a concentration of 1000 and 2000 mg l^{-1} as a foliar sprays either at full bloom or in early mid-May. It was also reported that paclobutrazol thinned peach fruit (Prunus persica L. Batsch) when sprayed at petal fall and shuck off stages (Blanco 1987). However, in most of the apple studies, paclobutrazol was found to increase fruit setting and cropping probably by its growth retardant action resulting in inhibition of vegetative growth and shifting more nutrition to reproductive growth. For instance, Miller and Swietlik(1986) reported that fruit setting was significantly increased on 4 year old Golden Delicious apple trees treated at concentration of either 5, 10 or 20 g ai $tree^{-1}$. Similarly, Williams (1984) reported that yield increased on treated trees and treated trees were more consistent in annual flower production and cropping when paclobutrazol applied to soil at the base of CV Delicious and Golden Delicious apple trees.

In our study, however, the time of paclobutrazol application was found to be critical to fruit setting, and the responses varied on different varieties.

For Bramley Seedling, although the earlier applications

(first bloom and petal fall) increased final fruit setting, fruit counts before the June drop showed (Figure 9) there had been no differences in fruit setting between each treatment. The differences in fruit numbers occurred only during and after the June drop. It could be explained that earlier growing season applications effectively inhibited the vegetative growth which results in more nutrition such as carbohydrates, micronutrients shifting for generative growth and caused a lower June drop and preharvest drop.

For Golden Delicious, however, the fruit setting of trees treated at first bloom was generally decreased compared to all the other treatments, although the treatment at petal fall resulted in an increased fruit setting. This may be due to a similar effect as in Bramley Seedling that earlier applications allowed more nutrition to generative growth. However, for application at first bloom, when investigation was made during June drop or after June drop, the fruit setting was largely higher than the control and the two later applications (fruitlet and preharvest stages). This high fruit setting may be beyond the tree carrying capability. During the preharvest stage, the fruit swelled rapidly and a large amount of nutrition would be demanded by the fruit. Many of the fruit on trees treated at first bloom dropped at this stage, possibly due to the shortage of nutrition (Figure 10).

An interesting observation was also reported by Blanco (1987) where he applied paclobutrazol as a fruit thinning agent on peach. The trees were sprayed at petal fall stage, but the majority of fruit drop occurred at the preharvest stage together with a reduction in fruit size. This observation was similar to the effect on Golden Delicious when palcobutrazol was applied at first bloom in this study, which was also found to induce a smaller fruit size. This matter will be discussed in following section.

In general, earlier growing season application of paclobutrazol tend to increase apple fruiting effficiency. However, the care should be taken for different varieties when paclobutrazol is applied for this purpose. The dosage may also alter fruit setting when paclobutrazol is applied at earlier growing season.

3.1.13 The influence of paclobutrazol on shoot extension growth The results obtained in this study confirmed that paclobutrazol effectively inhibits plant vegetative growth. The shoots of both Bramley Seedling and Golden Delicious from trees treated either at first bloom or petal fall were shorter than the control (Table 19, 20). However, the time of paclobutrazol application also influenced extension growth. The earlier application (first bloom) gave greater inhibition of extension growth than later application (petal fall)

Table 19 Shoot extension growth (cm) of Golden Delicious having previously been treated with paclobutrazol in orchard

Treatment	Extension growth	
	Mean of shoot growth	Mean No.of leaves per shoot
Control	25.94	11.03
First bloom	19.73	10.50
Petal fall	22.76	11.53

L.S.D. (p=0.05) for table means = 2.3 n=9

Table 20 Shoot extension growth (cm) of Bramley Seedling having previously been treated with paclobutrazol in orchard

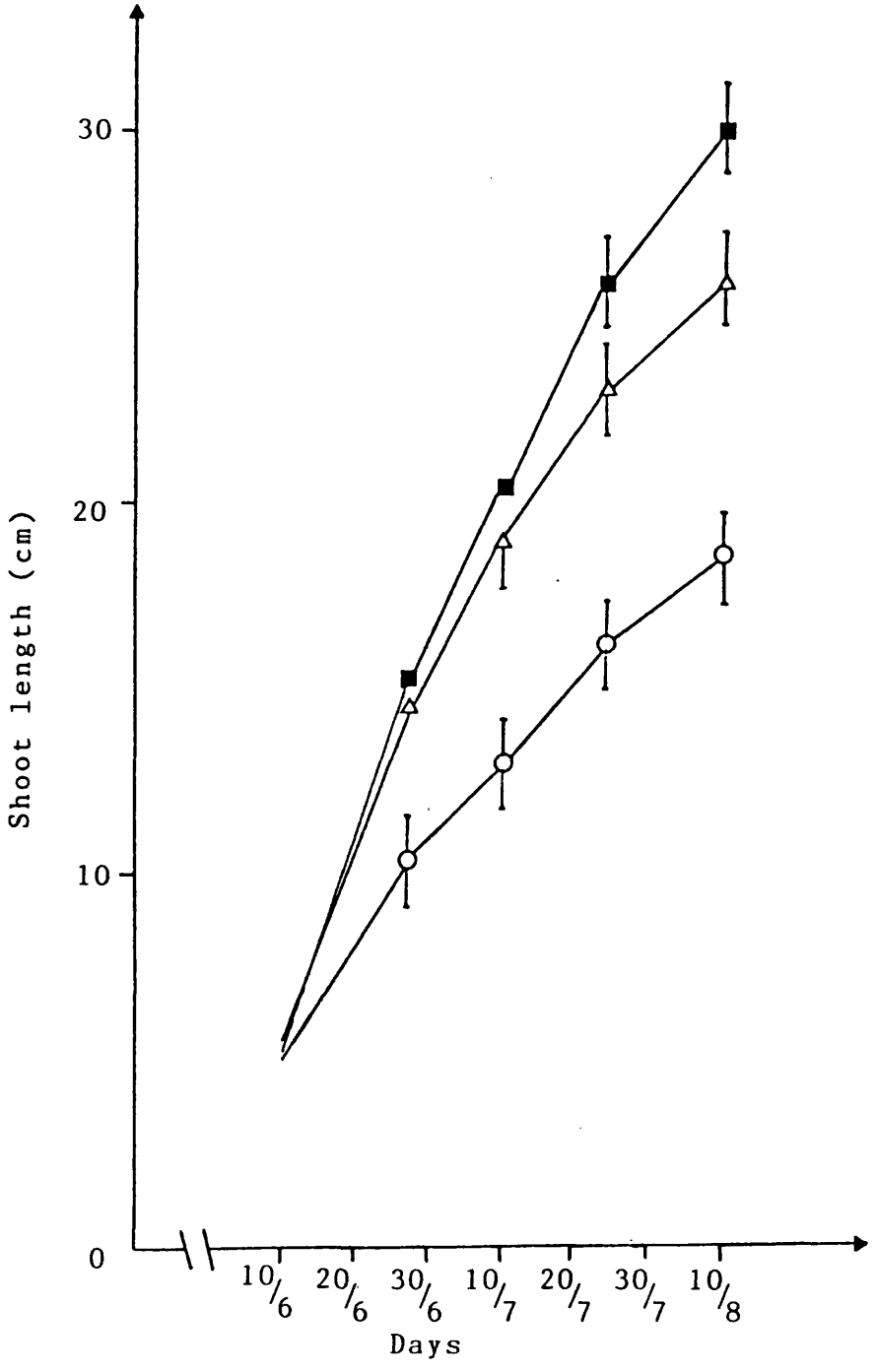
Treatment	Extension growth	
	Mean of shoot growth	Mean No.of leaves per shoot
Control	29.73	13.14
First bloom	18.23	12.33
Petal fall	25.76	16.72

L.S.D. (p=0.05) for table means = 3.7 n=9

in both Bramley Seedling and Golden Delicious (Table 19, 20). The numbers of leaves per shoot were also counted. Although the shoot growth was inhibited, the average number of leaves per shoot was not reduced. This indicated that paclobutrazol inhibited extension growth by shortening the shoot internals. The shoot extension growth during early growing season after treatment with paclobutrazol was shown in figure 11, 12. The shoot extension growth period from begining to end is about 100 days, however in the first 50 days, the shoots were growing rapidly and then the rate of extension growth declined. The results suggest that paclobutrazol treatment inhibited the shoot growth particularly during the period of rapid growth.

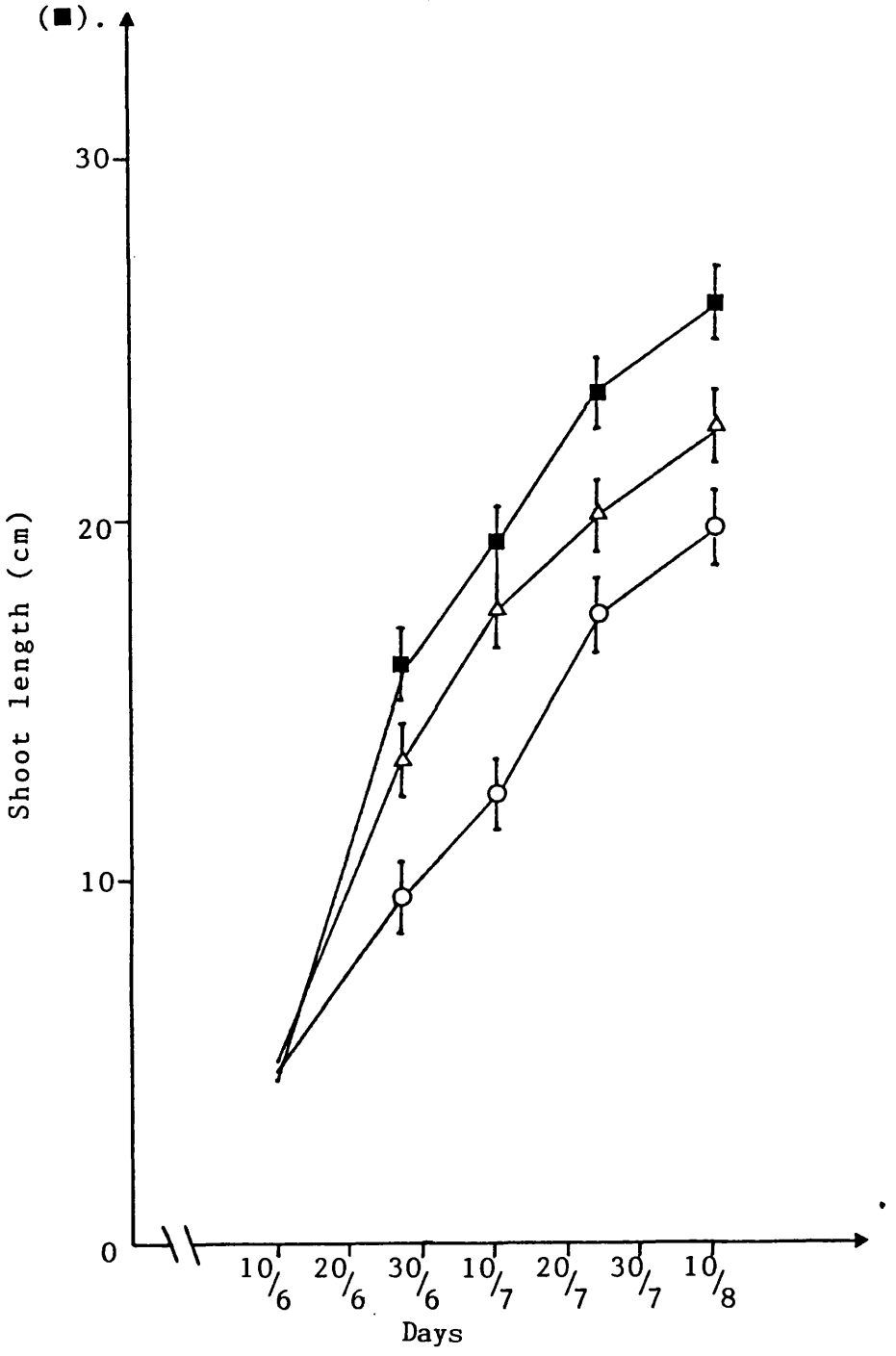
3.1.14 Discussion for influence of paclobutrazol on extension growth The plant growth regulator paclobutrazol was developed for the control of fruit tree vegetative growth. A great amount of work has been done during the last 10 years to investigate the control, by paclobutrazol, of fruit tree form and size including shoot growth. This has been reviewed in the introduction. In the current study, the terminal growth in year 1985 was investigated when paclobutrazol had been applied during the various growing stages. The results were similar to most of the studies conducted previously by the others, showing that paclobutrazol is an effective agent to inhibit vegetative growth and that

Figure 11 The shoot growth pattern of C.V. Bramley Seedling having been either treated with paclobutrazol at first bloom(○), petal fall(△), or untreated(■).



The bar shows the LSD(p=0.05,n=9)the shoot extension growth between each treatment.

Figure 12 The shoot growth pattern of C.V. Golden Delicious having been either treated with paclobutrazol at first bloom(○), petal fall(△) or untreated (■).



The bar shows the LSD(p=0.05,n=9)the shoot extension growth between each treatment.

shoot growth was remarkably reduced when paclobutrazol was applied as foliar spray at earlier growth season. The results also showed that the earlier the paclobutrazol application, the more effective it was in reducing shoot growth. The application at first bloom gave a bigger reduction of shoot growth than the application made at petal fall (Figure 11, 12) for both Bramley Seedling and Golden Delicious. The mechanism by which paclobutrazol inhibits plant vegetative growth is thought to be by an inhibition of endogenous gibberellin biosynthesis, because it was found that application of gibberellins reversed the effects of paclobutrazol (Williams 1983). It is now clear that paclobutrazol inhibits the three steps in oxidation of ent-kaurene to ent-kaurenoic acid through the inhibition of activity of ent-kaurene oxidase in gibberellin biosynthesis (Coolbaugh et al 1978, Rademacher et al 1984).

3.1.15 The influence of paclobutrazol on fruit swelling The investigation of fruit size was carried out during the growing season. The fruit size was influenced by the time of paclobutrazol application. However, only fruits of Golden Delicious from trees treated with paclobutrazol at first bloom were significantly smaller than control or than fruit from all the other treatments at final measurement (Table 21). Though the fruit of Golden Delicious from trees treated at petal fall and the fruit of Bramley Seedling from trees treated

Table 21 The fruit swelling (cm) of Golden Delicious having previously been treated with paclobutrazol at various growing stages.

	Date of investigation					
Treatment	18/6	27/6	10/7	24/7	9/8	30/8
Control	1.90	2.20	3.03	3.75	4.60	5.20
First bloom	1.30**	1.60**	2.60**	3.20**	4.00**	4.60**
Petal fall			2.90	3.72	4.50	5.00
Fruitlet					4.60	5.10
Preharvest						5.20

L.S.D. p = 0.05 n = 27

** very significantly different

at first bloom appeared smaller, there were no statistically significant differences from control fruit (Table 21). The pattern of fruit swelling was obtained by recording fruit size repeatedly during the growing season (Table 21, 22). The pattern shows that the swelling of both Bramley Seedling and Golden Delicious fruit was inhibited by the earlier application of paclobutrazol. However in Bramley Seedling, the fruit from trees treated at first bloom were significantly smaller than control fruit when the measurements were made in June (18 June 85, 27 June 85), but after June, the fruit from trees treated at first bloom quickly caught up in size, although they were still smaller than control at the preharvest stage (Table 22). The later applications (fruitlet, preharvest) of paclobutrazol had no influence on fruit swelling. There were no significant differences in fruit size between control and treatments at petal fall, fruitlet and preharvest stage for both Bramley Seedling and Golden Delicious although fruit of Golden Delicious from treatment at petal fall appeared slightly samller.

The fruit had final grading after harvest. The percentage of fruit above 65 mm in diameter for Bramley Seedling and fruit above 60 mm in diameter for Golden Delicious are shown in table 23. For Bramley Seedling there was no significant difference between the control and each treatment, but the percentage of

Table 22 The fruit swelling (cm) of Bramley Seedling having previously been treated with paclobutrazol at various growing stages.

	Date of investigation					
Treatment	18/6	27/6	10/7	24/7	9/8	30/8
Control	2.82	3.50	4.29	5.06	6.00	6.10
First bloom	2.40*	3.00**	4.12	5.02	5.90	5.90
Petal fall			4.30	5.14	5.90	6.10
Fruitlet					6.00	6.13
Preharvest						6.20

L.S.D. $p = 0.05$ $n = 27$

* significantly different

** very significantly different

Table 23 Percentage of fruits ≥ 65 mm in size for Bramley Seedling or ≥ 60 mm in size for Golden Delicious from trees having been treated with paclobutrazol at various growing stages in orchard.

Treatment	65 mm Bramley Seedling	60 mm Golden Delicious
Control	62.5	51.67
First bloom	50.2	24.43
Petal fall	61.6	57.00
Fruitlet	53.3	48.33
Preharvest	62.7	53.80

L.S.D. (p = 0.05) for table means = 17.46, n = 3.

fruit above 65 mm in the treatment at first bloom was smaller. For Golden Delicious, the percentage of the fruit above 60 mm from treatment at first bloom was significantly smaller than that of the control or the three other treatments. There were no differences between the control and treatments at petal fall, fruitlet and preharvest stage.

In summary, paclobutrazol inhibited the fruit development when it was applied at first bloom stage, later application had less influence on fruit size, however, this effect can be different with the difference of cultivars. In this study, fruit of Golden Delicious was more influenced by paclobutrazol than Bramley Seedling due to there was no difference in fruit size of Bramley Seedling between control and each treatment at final assessment (Table 22).

3.1.16 Discussion for influence of paclobutrazol on fruit swelling Fruit size is considered to be an important component of fruit quality and is of considerably commercial significance. Paclobutrazol treatment tends to result in a reduction in fruit size. Several reports (Curry and Williams 1983, Greene and Murray 1983, Stinchcombe et al 1984, Greene 1986) have indicated that paclobutrazol reduces apple fruit size, although some of them found that the reduction of fruit size was very small and may not be

reflected in reduced yield since the numbers of fruit per tree usually increase (Stinchcombe et al 1984, Williams 1984). The study here also found that paclobutrazol tends to reduce fruit size (Table 21, 22), however the time of paclobutrazol application may be critical and the response was also different from variety to variety. In this study, the fruit size of only Golden Delicious was significantly reduced when paclobutrazol was applied at first bloom. Similar results were obtained by Greene (1986) where the individual fruit weight was reduced for 'Delicious' apple when paclobutrazol was applied with concentration either 1500 ppm or 3000 ppm after 21 days full bloom. Interestingly, in our study, when paclobutrazol was applied at first bloom and petal fall on Bramley Seedling, the fruit development was slow and reduced during the earlier fruit swelling stage. However this retardation of fruit development did not influence the final fruit size, because it was observed that fruit size had quickly caught up during the later growing stages. Although the mean final fruit size was slightly smaller than the control, this difference was not significant (Table 22). A similar phenomenon was also found in Golden Delicious fruit treated with paclobutrazol at petal fall stage (Table 22). A possible explanation of this transient effect on fruit enlargement may be that paclobutrazol inhibits fruit swelling at the earlier fruit development stage

by the inhibition of cell extension without affecting cell division. Fruit size may have caught up at harvest time by delayed cell expansion. It is known that gibberellic acid can stimulate cell extension (Moore 1979), and if paclobutrazol can directly enter the fruit at the earlier growing stage and inhibit the synthesis of gibberellin within the fruit, this would explain the reduction in early fruit enlargement. This inhibition would be released at the later fruit growing stage as more gibberellins being constantly produced by the seeds overcome the effect caused by the limited amount of paclobutrazol which entered at the earlier stage.

The results obtained here show that the earlier the application, the greater the influence on fruit size, whilst later season applications did not influence fruit size in this study. It may be that the earlier application of paclobutrazol results in greater penetration into the fruit. There are other possible reasons, and these will be discussed more fully later.

In many studies, the paclobutrazol has been found to increase fruit setting as discussed before. The increased fruit number resulting from paclobutrazol treatment will also increase the competition between fruits which is another possible reason for the smaller fruit size. However, when paclobutrazol is

used as a fruit thinning agent, it tends to increase fruit size. Edgerton(1986), for example, demonstrated that peach size was increased after application of paclobutrazol. However, the pear size at harvest was found to be slightly reduced by paclobutrazol (Williams 1984) due to the significant increase in fruit load. Therefore, the effects of paclobutrazol on fruit size may be different from species to species. More work is still required to characterize the effects of paclobutrazol on fruit development in different species and varieties.

3.2 The Effect of Interaction between Paclobutrazol and Gibberellins on Apples

Paclobutrazol is a plant growth regulator believed to prevent gibberellin biosynthesis. the trial undertaken here was to study the interaction between paclobutrazol and gibberellic acid associated with their influence on the fruit post-harvest physiology

3.2.1 The influence of interaction between paclobutrazol and gibberellins on fruit respiration rate

. The fruit respiration rate of the cultivars Bramley Seedling and Golden Delicious having previously been treated at pre-harvest with gibberellic acid and gibberellic acid plus paclobutrazol, was determined either soon after harvest or after three months storage. The time of the peak

in CO_2 production (respiration climacteric) of both Bramley Seedling and Golden Delicious showed no difference between each treatment when the determinations were made soon after harvest (Figure 13, 14). However, after storage, the time of the peak in CO_2 production of Bramley Seedling fruit from trees sprayed with GA_3 or GA_{4+7}, moved approximately one day ahead compared with the control (Figure 15), and 1.5 day ahead compared with fruit from trees treated with GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol. However, there were no significant differences between control and treatments of GA_3 plus paclobutrazol or GA_{4+7} plus paclobutrazol. In Golden Delicious, the time of the peak in CO_2 production in fruit from trees which had been treated only with GA_3 moved approximately 1 day in advance compared with the control or all the other treatments (Figure 16). The time of the peak in CO_2 production of fruit from trees treated with GA_{4+7} showed no difference either from the control or from fruit from trees which had been treated with GA_3 plus paclobutrazol or GA_{4+7} plus paclobutrazol (Figure 16).

The mean of the daily respiration rate (in CO_2 production of fruit of both Bramley Seedling and Golden Delicious was also obtained. The mean value of CO_2 production of fruit from day 3 to day 18 after harvest (Table 24) shows that fruit from the trees

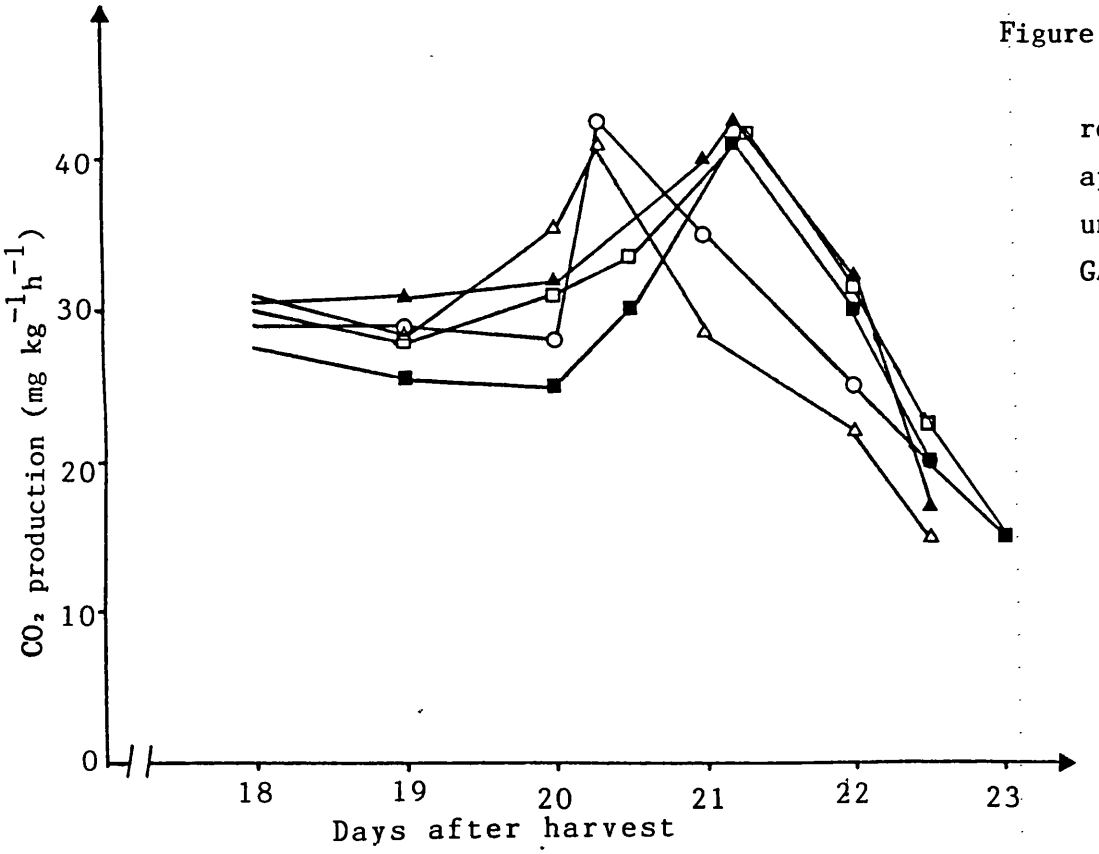


Figure 13

The production of carbon dioxide showing the respiration climacteric of Bramley Seedling apples at harvest when fruit trees were either untreated(■) or treated with GA_3(○), GA_{4+7}(△), GA_3+paclobutrazol(▲) or GA_{4+7}+paclobutrazol(□).

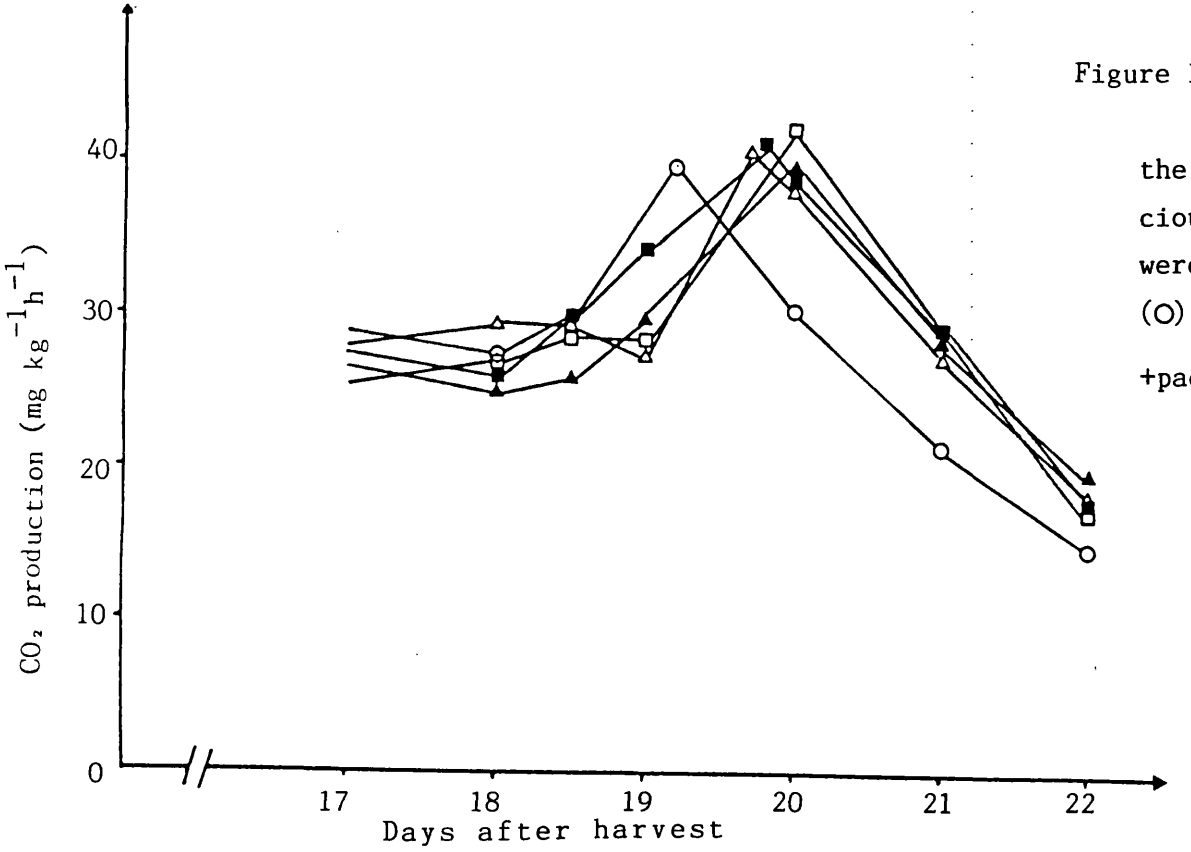


Figure 14

The production of carbon dioxide showing the respiration climacteric of Golden Delicious apples at harvest when fruit trees were either untreated(■) or treated with GA_3 (○), GA_{4+7}(△), GA_3+paclobutrazol(▲) or GA_{4+7} +paclobutrazol(□).

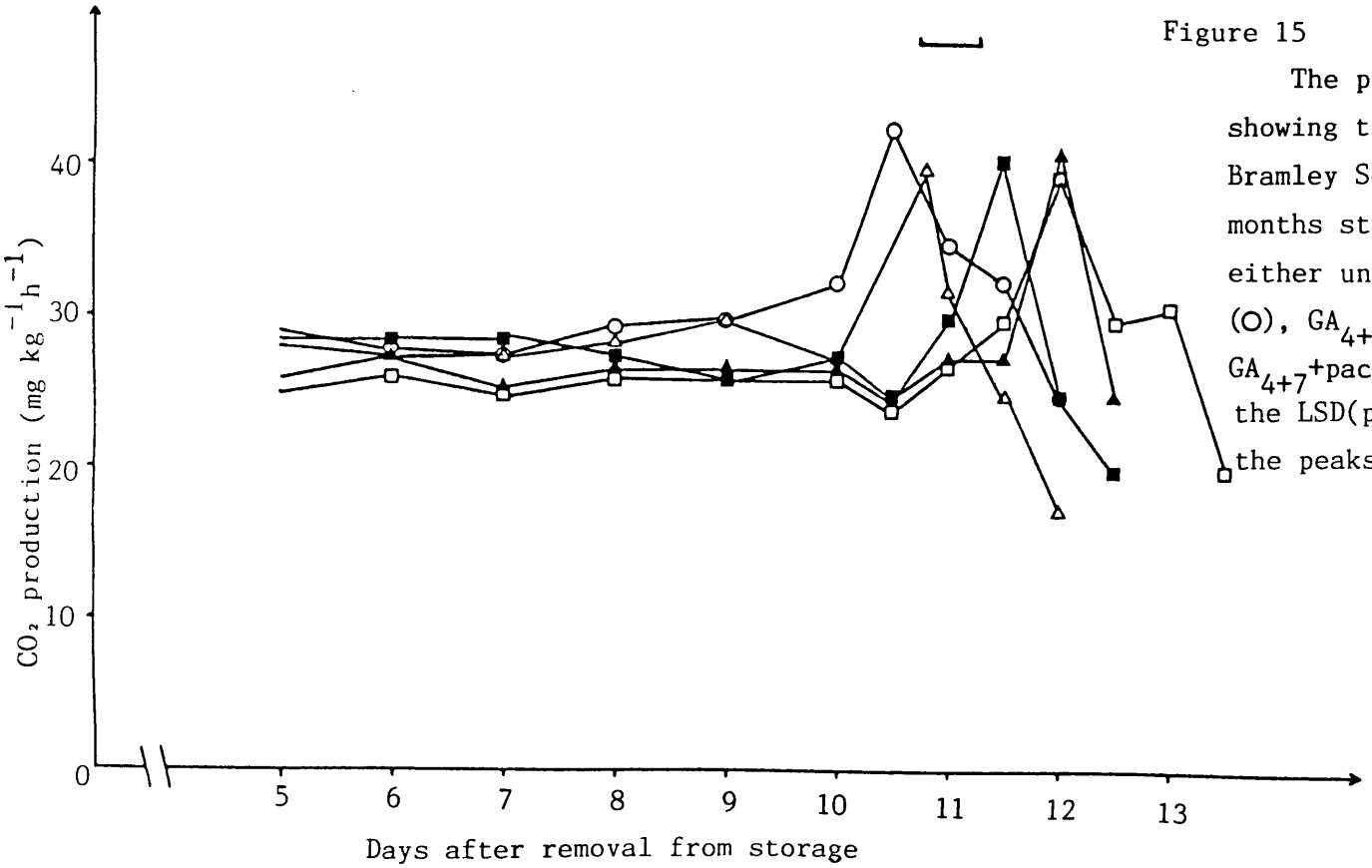


Figure 15

The production of carbon dioxide showing the respiration climacteric of Bramley Seedling apples after three months storage when fruit trees were either untreated(■)or treated with GA_3 (○), GA_{4+7}(△), GA_3+paclobutrazol(▲)or GA_{4+7}+paclobutrazol(□). The bar shows the LSD(p=0.05,n=3) between the time of the peaks in CO_2 production.

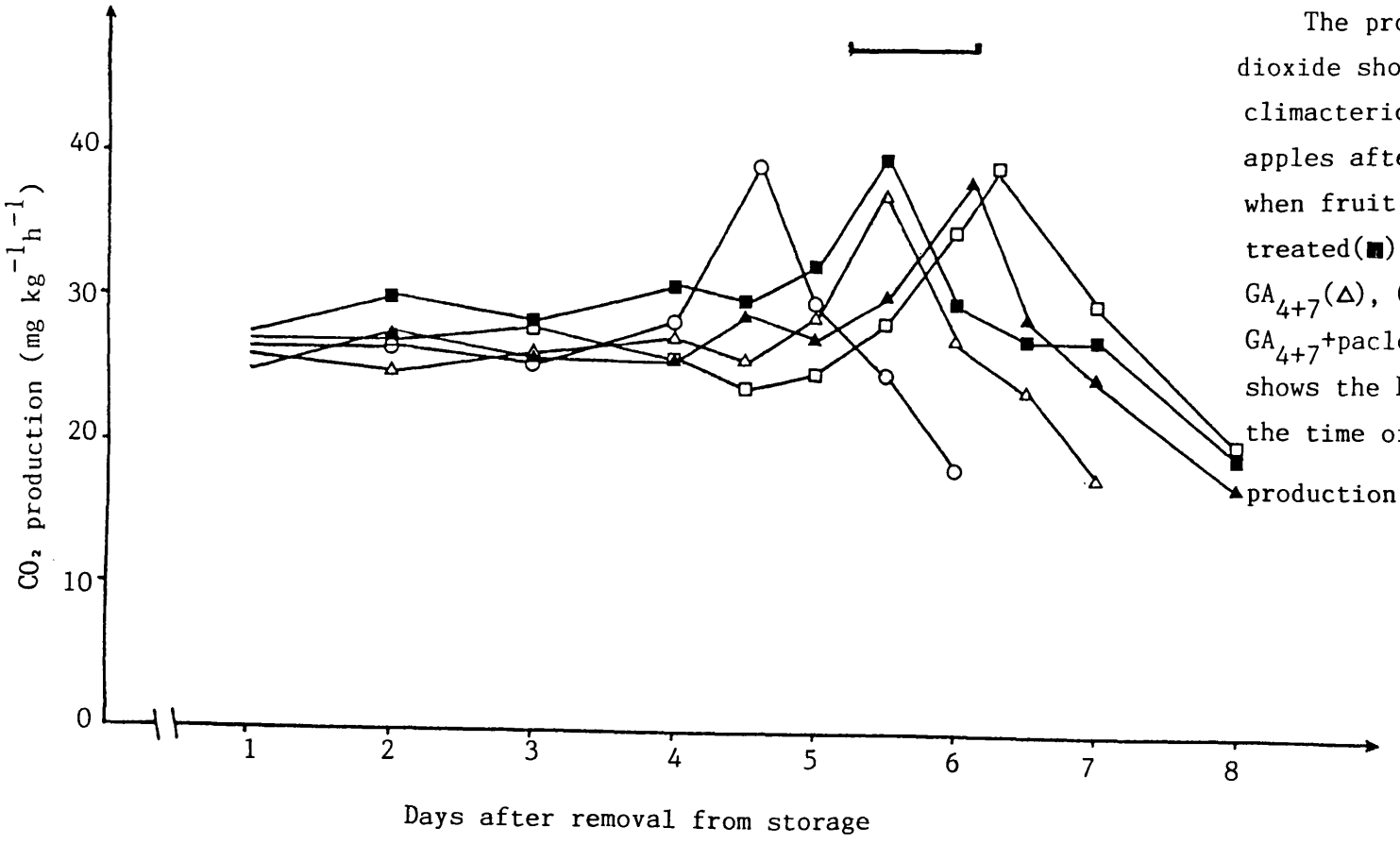


Figure 16

The production of carbon dioxide showing the respiration climacteric of Golden Delicious apples after three months storage when fruit trees were either untreated(■) or treated with GA_3(○), GA_{4+7}(△), GA_3+paclobutrazol(▲) or GA_{4+7}+paclobutrazol(□). The bar shows the LSD(p=0.05,n=3) between the time of the peaks in CO_2 production.

Table 24 The mean of the daily respiration rate (mg. $CO_2 kg^{-1}$. hr^{-1}) at 25°C of fruit between day 3 and 18 after harvest having previously been treated with GA_3, GA_{4+7}, GA_3 plus paclobutrazol or GA_{4+7} plus paclobutrazol in the orchard.

	Treatment					
Cultivar	Control	GA_3	GA_{4+7}	GA_3+ paclobutrazol	GA_{4+7}+ paclobutrazol	Mean
Bramley Seedling	27.5	29.3	29.7	27.8	28.3	28.5
Golden Delicious	27.7	28.8	28.9	28.1	28.5	28.4
Mean	27.6	29.1	29.3	28.0	28.4	

L.S.D. ($p = 0.05$) for column means = 0.8, n = 32.

treated with GA_3, GA_{4+7} or GA_{4+7} plus paclobutrazol had a higher respiration rate prior to the onset of the respiration climacteric (the peak in CO_2 production) in both Bramley Seedling and Golden Delicious. There was no difference between the treatments of GA_3 plus paclobutrazol and control for both cultivars. In addition, the fruit of Bramley Seedling had a higher daily respiration than that of cultivar Golden Delicious (Table 24).

3.2.2 Discussion for influence of interaction between gibberellins and paclobutrazol on fruit respiration rate Paclobutrazol was developed to inhibit plant vegetative growth, and its major biological property is to inhibit gibberellin biosyhthesis. It is therefore important to study the interaction between paclobutrazol and gibberellin on fruit, so that the mechanism of paclobutrazol on post-harvest behaviour can be more fully understood.

The role of gibberellin in fruit respiration is not clear. In this study, the application of GA_3 or GA_{4+7} did not affect the onset of the respiration climacteric in fruit of either cultivar immediately after harvest. However, the preclimacteric respiration rate from day 3 to day 18 of fruit treated with GA_3 or GA_{4+7} was higher than in the control. This suggests that GA treated fruit had a higher metabolic activity

after harvest, possibly as a result of the general growth stimulatory activity of gibberellins.

When the respiration determinations were made after the fruit had been stored for 3 months, the onset of the respiration climacteric of fruit treated with GA_3 or GA_{4+7} was influenced. The preharvest application of GA_3 or GA_{4+7} alone advanced the onset of the climacteric, while the combination of gibberellin and paclobutrazol showed no differnce from the control. It could simply be explained that the influence of gibberellin was reversed by the paclobutrazol application. However the reason why the onset of the climacteric was not influenced by gibberellin application when the respiration measurement was made soon after harvest, still requires explanation. One possibility is that the fruit after storage may have entered the final stage of ripening and to be sensitive even to very small amount of growth hormones within the fruit. If the endogenous gibberellin levels within the fruit normally decline during storage, the endogenous GA would be relatively more important in fruit after storage, whereas in recently harvested fruit, the endogenous gibberellin levels may be too high for the fruit to be affected by exogenous GA.

Little is known about the influence of gibberellic acid on the respiration of post-harvest fruit.

In apple fruit, Clijsters (1971) found that six applications of GA at 200 ppm applied from early June until late July reduced the rate of respiration of Golden Delicious apple and led to less wastage in stored Jonathan apples. Sharples and Johnson (1986), however, have recently reported that fruit from GA-sprayed trees ripened seven days earlier as evidenced by the onset of the respiration climacteric in a series of trials on Bramley Seedling at East Malling. In this work, the GA applications were made at the petal fall stage. These results were broadly similar to those of the current study although the onset of the respiration climacteric was advanced to a lesser extent in the present study (Figure 15, 16). In view of the conflicting results of gibberellin application on the fruit respiration shown in these studies, it would appear that the time of application may be particularly important. Earlier growing season applications may enhance respiration, demonstrated by the present study and as Sharples and Johnson (1986) showed recently. In contrast, later growing season applications may inhibit respiration. Sharples (1973) has also found that six sprays of GA_3 at 150 ppm, applied at weekly intervals from early August until harvest, led to a marked reduction in respiration rate. If this is the case, it could understand that GA promoted respiration in this study due to relatively earlier application.

3.2.3 The influence of interaction between paclobutrazol and gibberellins on fruit ethylene production

The ethylene production of fruit was determined either immediately after harvest or after three months storage.

For Bramley Seedling, when the determinations were made at harvest, the ethylene production of fruit from all treatments was higher than that of fruit from the control, and the ethylene production of fruit from treatment of GA_{4+7} was higher than those of fruit from the rest of treatments. However, after storage, the difference in ethylene production between each treatment no longer existed (Table 25).

In Golden Delicious, the ethylene production of fruit from treatments of GA_{4+7} and GA_3 plus paclobutrazol was higher than control or fruit from trees treated with GA_3 or GA_{4+7} plus paclobutrazol when measurement was made at harvest time. However, after storage, a higher ethylene production occurred on the fruit from trees treated with GA_{4+7} and GA_{4+7} plus paclobutrazol. There was no difference in ethylene production between treatments of GA_{4+7} and GA_{4+7} plus paclobutrazol nor between control, GA_3 and GA_3 plus paclobutrazol (Table 25).

Table 25 The mean ethylene produced ($nl.g^{-1}.h^{-1}$) by apple tissue slices incubated for 6 hours at 25°C, the fruit having previously been treated with gibberellic acid or gibberellic acid plus paclobutrazol in orchard.

	Treatment				
Cultivar	Control	GA_3	GA_{4+7}	GA_3+ paclobutrazol	GA_{4+7}+ paclobutrazol
			Ethylene produced at harvest		
Bramley Seedling	26.01	35.83	42.89	32.89	34.89
Golden Delicious	27.72	31.28	32.44	37.50	27.97
Mean	26.87	33.56	37.67	32.50	31.43
			Ethylene produced after storage		
Bramley Seedling	29.73	33.78	32.39	32.17	33.11
Golden Delicious	31.52	30.07	36.23	32.46	29.82
Mean	30.62	31.93	34.31	32.32	31.47

L.S.D ($p = 0.05$) for table means = 4.39 n = 12

for column means = 2.94 n = 24

3.2.4 Discussion for influence of interaction between gibberellins and paclobutrazol on fruit ethylene production The role of endogenous gibberellin in ethylene biosynthesis is still not fully understood (Lieberman 1979) and little information of gibberellin on ethylene production was available. Scott and Leopold (1967) observed opposing effects of GA_3 and ethylene in the lettuce hypocotyl elongation assay for GA, in the induction of invertase formation by GA in sugar beet tissue, and in the α-amylase induction assay for GA. There are also several growth and development functions in which ethylene and gibberellins act synergistically. These include reversal of induced dormancy in lettuce seed (Dunlap and Morgan 1977a,b), induced leaf abscission in cotton plant (Morgan and Durham 1975) and stem elongation in rice (Suge 1974) and the fresh water plant Callitriche platycarpa (Musgrave et al 1972), where ethylene acts as a promoter of growth, CO_2 and GA enhance ethylene action as does auxin (Osborne 1977). However, no report was found on the role of GA associated with the ethylene production of post-harvest fruit.

The results in our study failed to contribute a clear picture of the role of gibberellin on ethylene production of post-harvest fruit. However, it could still be seen that GA tends to increase the ethylene production of post-harvest apple. For example, Bramley

Seedling fruit from trees treated with GA_{4+7} and fruit from Golden Delicious trees treated with GA_3 had a relatively higher ethylene production after harvest, whilst fruit from Golden Delicious trees treated with GA_{4+7} had a higher ethylene production after storage than the controls.

Treatment of the trees with paclobutrazol did not reverse the enhancement of ethylene production resulting from gibberellin application, because ethylene production from treatment of GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol for Bramley apple at harvest, and GA_3plus paclobutrazol at harvest and GA_{4+7} plus paclobutrazol after storage for Golden Delicious, were all higher than control. It is possible that the time of paclobutrazol application may have been too late as it is known the mobilization of paclobutrazol is slow (Richardson and Quinlan 1986), and applications after petal fall normally fail to produce a response in the current year as results shown previously. If this is the case, application of gibberellins plus paclobutrazol at this stage would be expected to be effectively the same as a single gibberellin application. It could be predicted that earlier applications (either at first bloom or at petal fall) would ensure both hormones adequate time to affect the plant and the results may give more interesting aspects to discuss.

Gibberellin has also been reported to increase apple fruit internal breakdown and coreflesh (Sharples and Johnson 1986) during storage. Although it was clear that wounded fruit was stimulated to produce more ethylene, the fruit internal breakdown or coreflesh was not investigated in this study.

The direct influence of gibberellin on ethylene production was tested by addition of gibberellin in the incubation medium. The ethylene production of apple tissue was determined over a 4 hours period. When the apple tissue was young, the presence of GA in the incubation medium increased the tissue ethylene production (Table 26) while in old tissue, the presence of GA resulted in less ethylene evolution than in the control.

Table 26 The mean ethylene production ($nl.g^{-1}.h^{-1}$) by apple tissue slices incubated for 4 hours at 25°C in different concentration of gibberellin.

Bramley Seedling	Gibberellin concentration($mg.l^{-1}$) 0	1	10	100	LSD (p=0.05,n=6)
Young tissue	31.2	32	39.1	38.7	
Old tissue	35.4	30.7	31.4	29.2	

These results may be explained by the suggestion that when the tissue is young, exogenous GA, acting as a growth promoter increased the activity of tissue metabolism, including ethylene biosynthesis. When the tissue is old, however the application of GA may tend to inhibit the senescence processes, and thereby reduce and delay the rapidly increasing ethylene production associated with the stage of fruit development.

3.2.5 The influence of interaction between paclobutrazol and gibberellins on fruit mineral composition

The mineral composition of fruit which had been previously treated with gibberellic acid or gibberellic acid plus paclobutrazol at pre-harvest was tested soon after harvest and again after three months storage. The results showed that the mineral composition of fruit was influenced by the plant growth regulator applications.

The calcium content of fruit from all the treatments was decreased and was lower than the control for both Bramley Seedling and Golden Delicious when the mineral concentration determinations were made either at harvest or after storage (Table 27). The calcium content of fruit from trees treated with GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol for both cultivars was slightly higher than those of fruit from trees treated with pure GA3 or GA_{4+7}, however

Table 27 Calcium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with gibberellic acid and gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	241.5	243.1	175.3	173.5	208.4
GA_3	196.2	194.6	140.7	147.5	169.8
GA_{4+7}	197.1	195.3	137.5	142.0	168.0
GA_3+paclobutrazol	209.3	190.8	154.0	147.8	175.5
GA_{4+7}+paclobutrazol	208.8	211.1	144.1	144.8	177.2
Mean	210.5	207.0	150.3	151.1	179.8

L.S.D. (p = 0.05) for column means = 7.92 n = 15

table means = 26.44 n = 3

row means = 9.05 n = 12

there were no significant differences between treatments of GA_3 plus paclobutrazol, GA_{4+7} plus paclobutrazol and treatments of GA_3, GA_{4+7}. There were also no differences in calcium content between determination made soon after harvest and determination made after storage for both Bramley Seedling and Golden Delicious. However, Bramley Seedling had a higher calcium status than cultivar Golden Delicious (Table 27).

The magnesium content of fruit was less influenced by the treatments compared with calcium concentration. In Bramley Seedling, there were no differences in magnesium content between control and each treatment with either the determination made at harvest or after storage (Table 28). For Golden Delicious, though fruit from treatment of GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol had a slightly higher magnesium content, there was no significant difference from the control fruit when the test was made at harvest. However, the magnesium content of fruit from treatments of GA_3 plus paclobutrazol and/or GA_{4+7} plus paclobutrazol was significantly higher than fruit from treatments of GA_3 and/or GA_{4+7} when the determination was made at harvest. Similarly, after storage, there was no difference in magnesium content between the control and all the other treatments. The fruit from treatment of GA_{4+7} plus paclobutrazol had a

Table 28 Magnesium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with gibberellic acid and gibberellic acid plus paclobutrazol in orchard.

Treatment	Bramley Seedling		Golden Delicious		Mean
	At harvest	After storage	At harvest	After storage	
Control	237.9	236.6	234.0	232.8	235.3
GA_3	239.1	235.8	226.2	226.3	231.9
GA_{4+7}	242.5	242.0	227.6	229.2	235.4
GA_3+paclobutrazol	236.2	238.4	241.2	228.4	236.1
GA_{4+7} +paclobutrazol	241.7	240.7	243.3	240.8	241.7
Mean	239.5	238.7	234.5	231.5	236.0

L.S.D. (p = 0.05) for column means = 2.94 n = 15

table means = 9.83 n = 3

row means = 3.36 n = 12

higher magnesium content than the fruit from treatments of GA_3, GA_{4+7} and GA_3 plus paclobutrazol. In addition, the fruit tested at harvest had a higher magnesium content than the fruit when the test was made after storage (Table 28).

The phosphorus concentration of fruit was influenced by the applications of gibberellins or gibberellins plus paclobutrazol. For Bramley Seedling, when the determinations were made soon after harvest, the phosphorus content of fruit from the trees treated with GA_3, GA_{4+7} or GA_{4+7} plus paclobutrazol was lower than fruit from control. Although the phosphorus content of fruit from treatment of GA_3 plus paclobutrazol was also reduced, there was no statistical difference from control (Table 29). After storage, only fruit from treatment of GA_{4+7} had a lower phosphorus content than control. Although the phosphorus concentrations of the rest of treatments were also decreased, there were no significant difference from control. For Golden Delicious when determination was made soon after harvest, the phosphorus content of fruit from all treatments were reduced and lower than that of fruit from control. However, phosphorus content of fruit from treatment of GA_3 plus paclobutrazol and/or GA_{4+7} plus paclobutrazol were significantly higher than those of fruit from treatment of GA_3 and/or GA_{4+7} (Table 29). When determinations were made after storage,

Table 29 Phosphorus content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with gibberellic acid and/or gibberellic acid plus-paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	495.7	495.9	553.7	551.2	524.1
GA_3	463.2	479.7	471.7	490.1	476.2
GA_{4+7}	457.0	463.7	490.4	501.7	478.2
GA_3+paclobutrazol	475.9	495.4	520.1	504.8	499.1
GA_{4+7}+paclobutrazol	467.7	482.1	529.9	530.1	502.4
Mean	471.9	483.4	513.2	515.6	496.0

L.S.D. (p = 0.05) for column means = 7.75 n = 15

table means = 25.90 n = 3

row means = 8.86 n = 12

the phosphorus concentrations of fruit from all the treatments except GA_{4+7} plus paclobutrazol were significantly lower than that of fruit from control. There was no statistical difference between treatments of GA_3, GA_{4+7} and GA_3 plus paclobutrazol, as well as no difference between control and treatment of GA_{4+7} plus paclobutrazol. In general, there was no difference in the phosphorus content between harvest and after storage for both Bramley Seedling and Golden Delicious, however the Golden Delicious had a higher phosphorus status than Bramley Seedling.

The potassium content of fruit was changed by the plant growth regulator treatments. When the determinations were made soon after harvest, the potassium content of fruit from treatments of GA_3, GA_{4+7} and/or GA_{4+7} plus paclobutrazol was higher than that of fruit from control for both Bramley Seedling and Golden Delicious. There was no significant difference in potassium content between control and treatment of GA_3 plus paclobutrazol although the potassium concentration in fruit from the GA_3 plus paclobutrazol treatment was slightlyhigher than that of control fruit of both cultivars. After storage, the potassium content of fruit from all the treatments was higher than that of control fruit of both Bramley Seedling and Golden Delicious, and there was no difference between each treatment. In general there was no difference in

potassium content between fruit tested at harvest and fruit after storage for both Bramley Seedling and Golden Delicious (Table 30).

3.2.6 Discussion for influence of interaction between paclobutrazol and gibberellins on fruit mineral composition There is some evidence for the current studies which indicate that gibberellic acid may influence fruit mineral concentration. Looney (1979) found that orchard applications of GA_{4+7} plus benzyladenine (BA) reduced the fruit calcium content of Spartan apple and remarkably increased the fruit internal breakdown (6—12 fold) during storage. Similarly Greene et al (1982) demonstrated that after application of daminozide with gibberellin, the calcium content of McIntosh apple was decreased through the increase of the concentration of gibberellin, and percentage of senescent breakdown was increased during the storage under normal Control Atmosphere (CA) condition. They also analysed fruit calcium concentration in fruit treated with 0, 25 and 100 mg l^{-1} GA_{4+7} plus BA plus daminozide and found that there was a highly significant linear reduction of fruit calcium concentration as GA_{4+7} concentration increased, and as a result the treated fruit had increased incidence of bitterpit.

The results above which show that gibberellic acid can reduce the fruit calcium content were supported

Table 30 Potassium content ($\mu g.g^{-1}$ dry matter) of fruit having been treated with gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	6222.5	6305.8	7110.8	6750.1	6597.5
GA_3	7277.5	7333.3	8166.7	7805.8	7645.8
GA_{4+7}	7500.0	7194.2	8174.2	7444.2	7583.3
GA_3+paclobutrazol	6805.8	7139.2	7694.2	7860.8	7375.0
GA_{4+7}+paclobutrazol	7583.3	7500.0	7916.7	7722.5	7680.8
Mean	7077.5	7094.2	7816.7	7516.7	7376.7

L.S.D. (p = 0.05) for column means = 200.6, n = 15

for table means = 669.7, n = 3

for row means = 229.3, n = 12

by the results obtained in our study, where the calcium contents of fruit from GA_3 or GA_{4+7} with or without paclobutrazol were reduced for both Bramley Seedling and Golden Delicious. The paclobutrazol failed to overcome the GA effect on fruit calcium status. However the calcium contents of fruit from GA plus paclobutrazol were slightly higher than the fruit treated with GA_3 or GA_{4+7} alone, although these differences in calcium content were not statistically significant. The failure of paclobutrazol to completely reverse the decrease of calcium due to GA treatment may be due to the time of application being too late for effective mobilization of paclobutrazol. The concentration of GA used in this study was also slightly higher compared with Looney and Greene's work, and this may also have been important.

In this study, the magnesium content of both Bramley Seedling and Golden Delicious fruit were unaffected by the gibberellic acid treatment and no reports of gibberellic acid affecting fruit magnesium content were found in the literature.As discussed previously, paclobutrazol was found to increase fruit magnesium content, however, the application of paclobutrazol in this trial was somewhat late, and perhaps for this reason, magnesium was not increased significantly in fruit treated with GA plus paclobutrazol, even though the magnesium concentration of Golden Delicious apple

from the GA_3 plus paclobutrazol or GA_{4+7} plus paclobutrazol treatments was slightly higher thsn the control.

The phosphorus concentrations were decreased and potassium concentrations were increased by gibberellic acid. Such changes have been associated with an increase in physiologycal disorders of fruit during storage, as reviewed before. The fact that these changes are directly opposite to those resulting from paclobutrazol treatment is consistent with the suggestion that the effects of paclobutrazol on fruit phosphorus and potassium content are due to the inhibition of gibberellin biosynthesis.

The mechanism by which gibberellic acid influences fruit mineral composition is not fully understood. Looney (1979) stated that reduction of mineral concentration within the fruit may be due to the dilution of fruit volume resulting from the increase of fruit size by the gibberellin application. If this is the case, however, it is difficult to explain the increase in potassium concentration in the fruit resulting from GA treatment in the present study. Interestingly, Steffens et al (1985) tested various mineral elements of leaves of apple trees after application of paclobutrazol or paclobutrazol plus gibberellins, and found that gibberellin completely reversed the effects of paclobutrazol, and resulted in a reduction in leaf

calcium, magnesium, phosphorus concentrations whilst the potassium content of the leaf was increased. These authers suggested that both paclobutrazol and gibberellin could influence the uptake of nutrients by apple seedlings by a physiological change in the root system. If this is true, a similar explanation could account the results in the present study. However in our view, the balance of growth hormones may also affect the partitioning of nutrients within the plant. For example, since gibberellic acid enhances the plant vegetative growth, nutrients may have been shifted for this purpose and this would affect the availability of mineral elements and carbohydrates for fruit.

3.2.7 The influence of interaction between paclobutrazol and gibberellins on fruit firmness and total soluble solids The fruit firmness of cultivars Bramley Seedling and Golden Delicious was tested either soon after harvest or after three months storage. For Bramley Seedling, the firmness of fruit from treatments of GA_3, GA_{4+7}, GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol was higher than that of fruit from control, and there were no differences between treatments of GA_3, GA_{4+7}, GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol when the tests were made soon after harvest (Table 31). After storage, no differences were found between control and all the treatments, although the fruit from treatment of GA_4 and/or GA_{4+7} had a

Table 31 Firmness ($kg.cm^{-2}$) of apple fruit having been treated with gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	10.81	5.84	9.47	5.28	7.85
GA_3	11.44	5.40	9.45	4.61	7.73
GA_{4+7}	11.64	5.15	9.50	4.78	7.77
GA_3+paclobutrazol	11·58	5.72	9.43	5.25	8.00
GA_{4+7} +paclobutrazol	11.67	5.74	9.63	5.25	8.07
Mean	11.43	5.57	9.50	5.03	

L.S.D. (p = 0.05) for column means =0.51 n = 120

for table means =0.73 n = 24

for row means =0.58 n = 96

lower firmness than the others (Table 31). For Golden Delicious, there were no differences in fruit firmness between control and each treatment when the tests were made either at harvest or after storage though the firmness of fruit from treatments of GA_3 and/or GA_{4+7} was slightly lower than that of fruit from control and from the rest of treatments when tests were made after storage (Table 31). In general the firmness of fruit at harvest time was higher than that of fruit after storage for both Bramley Seedling and Golden Delicious and Bramley Seedling had a higher fruit firmness than Golden Delicious (Table 31).

The fruit total soluble solids was also tested either soon after harvest or after storage. For Bramley Seedling, when the measurements were made at harvest, the total soluble solids of fruit from treatment of GA_3 plus paclobutrazol was lower than that of fruit from control (Table 32). The total soluble solids of fruit from treatment of GA_{4+7} plus paclobutrazol was also lower, but there was no significant difference from the fruit from control. However, after storage, although the total soluble solids of fruit from trees treated with GA_3 plus paclobutrazol and/or GA_{4+7} plus paclobutrazol were lower comparing with the others, there were no statistical differences from control (Table 32). For Golden Delicious, when the measurements were made at harvest, the total soluble solids of fruit from

Table 32 Total soluble solids (%) of fruit having been treated with gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	11.12	12.39	11.40	11.29	11.30
GA_3	11.45	12.40	11.03	11.94	11.71
GA_{4+7}	11.33	12.36	11.04	11.83	11.64
GA_3+paclobutrazol	10.36	11.83	11.04	10.44	10.92
GA_{4+7} +paclobutrazol	10.90	11.84	10.53	10.49	11.19
Mean	11.03	12.16	11.01	11.20	

L.S.D.(p = 0.05) for column means = 0.27 n = 120

for table means = 0.64 n =24

for row means = 0·31 n = 96

treatment of GA_{4+7} plus paclobutrazol were lower than control and there were no significant differences between control and all the other treatments (Table 32). After storage, the total soluble solids of fruit from treatment of GA_3 were higher than control while total soluble solids of fruit from the treatment of GA_3 plus paclobutrazol and GA_{4+7} plus paclobutrazol were lower than that of fruit from control and fruit from treatments of GA_3 and/or GA_{4+7} (Table 32). Although fruit from treatment of GA_{4+7} had a higher total soluble solids content, there was no statistical difference from the control fruit. The fruit total soluble solids content of Bramley Seedling were higher after storage than at harvest. However, no changes in total soluble solids were found between fruit at harvest and fruit after storage for Golden Delicious (Table 32).

3.2.8 Discussion for influence of interaction between paclobutrazol and gibberellins on fruit firmness and total soluble solids Calcium is believed to play a major role in membrane permeability and the maintenance of cell integrity, and therefore is an important factor in fruit firmness and tends to reduce metabolic disorders in fruit (Mengel and Kirkby 1978). As discussed earlier, the application of gibberellic acid induces a reduction in fruit firmness. In this study, fruit from Bramley Seedling trees treated with GA_3 or GA_{4+7} had slightly higher values of firmness than the control, and fruit

from trees treated with GA plus paclobutrazol had also a slightly higher firmness than control when measured after storage although these numerical differences in firmness between each treatment had no statistical significance. However, Greene et al (1982) reported that fruit of 'McIntosh' apple sprayed with GA_{4+7} plus BA had softened more during storage, with increasing softening as concentration increased. They stated that neither GA_{4+7} nor BA alone produced this effect. But the results do provide evidence that gibberellin can reduce fruit quality by reduction of fruit firmness. The absence of such an effect in the present study cannot be explained without future investigation.

Few studies have investigated the effect of gibberellin on fruit soluble solids, although Greene et al (1982) found no effect of gibberellic acid on this component. The results recorded in the present study showed that GA alone had no effect, although fruit treated with GA plus paclobutrazol had slightly lower total soluble solids than controls. This effect might have been a response to the paclobutrazol component, since treatment with paclobutrazol alone was found to reduce fruit total soluble solids as reported before.

3.2.9 The influence of interaction between paclobutrazol and gibberellins on fruit colour The fruit colour components were assessed either soon after harvest or

after three months storage for both CV Bramley Seedling and Golden Delicious. The fruit colour was influenced by the treatments of gibberellic acid or gibberellic acid plus paclobutrazol.

There was no statistical difference in colour component Y value between each treatment for both Bramley Seedling and Golden Delicious when the determinations were made either at harvest or after storage, however, when the determination was made after storage, the Y values of fruit were higher than those of fruit which had been determined soon after harvest for both cultivars. In addition, fruit Y values of CV Golden Delicious were higher than those of Bramley Seedling (Table 33).

The fruit colour component X values were influenced by treatment with gibberellins or gibberellins plus paclobutrazol. For Bramley Seedling, the X values of fruit from treatment of GA_3, GA_{4+7} and GA_3 plus paclobutrazol were lower than those of fruit from control, when the determinations were made either at harvest or after storage (Table 34). Although the X values of fruit from treatment with GA_{4+7} plus paclobutrazol were also lower, there was no statistical difference from the control fruit. For Golden Delicious, similarly, the X values of fruit from treatments of GA_3, GA_{4+7} and GA_{4+7} plus paclobutrazol were lower than those of fruit from the control, when the assessments were made either soon after harvest

Table 33 Fruit colour component Y value of Bramley Seedling and Golden Delicious having been treated with gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	27.07	32.43	38.53	41.16	34.80
GA_3	29.00	33.21	35.64	43.49	35.34
GA_{4+7}	27.92	34.59	38.79	41.10	35.60
GA_3+paclobutrazol	26.67	34.87	36.98	40.66	34.80
GA_{4+7} +paclobutrazol	31.09	34.53	36.28	42.84	36.21
Mean	28.35	33.93	37.27	41.85	35.35

L.S.D. (p = 0.05) for column means = 2.25 n = 15

for table means = N.S* n = 3

for row means = N.S.* n = 12

* not significant

Table 34 Fruit colour component X value of Bramley Seedling and Golden Delicious having been treated with Gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	28.33	25.31	33.69	37.95	31.30
GA_3	26.28	21.69	30.08	34.56	28.15
GA_{4+7}	25.61	21.49	31.02	34.53	28.16
GA_3+paclobutrazol	26.57	22.55	32.50	36.90	29.63
GA_{4+7} +paclobutrazol	27.95	24.74	31.61	35.69	29.99
Mean	26.95	23.16	35.93	31.78	

L.S.D. (p = 0.05) for column means = 0.84 n = 15

for table means = 1.5 n = 3

for row means = 0.96 n = 12

or after storage (Table 34). There was no difference between treatment of GA_3 plus paclobutrazol and control, although the X values of treatment of GA_3 plus paclobutrazol were slightly lower. The X values were reduced after storage for both Bramley Seedling and Golden Delicious, and Golden Delicious had a higher X value than Bramley Seedling (Table 34).

The colour component Z values of fruit were not influenced by the treatment. There were no differences between each treatment for both Bramley Seedling and Golden Delicious either the determinations made at harvest or made after storage. And there was also no difference in Z value between at harvest and after storage for both cultivars, however cultivar Golden Delicious had a higher Z value than Bramley Seedling (Table 35).

Determining the L value soon after harvest, there were no differences between each treatment for both Bramley Seedling and Golden Delicious. After storage, however, the fruit from Bramley Seedling, which had been treated with GA_3, GA_{4+7} and/or GA_{4+7} plus paclobutrazol had a higher L value than fruit from the control. There was no difference between control and treatment of GA_3 plus paclobutrazol. In Golden Delicious, only the fruit from the GA_{4+7} treatment gave a lower L value than the control. There were no differences between control and all the other treatments. The fruit of both cultivars had a higher

Table 35 Fruit colour component Z value of Bramley Seedling and Golden Delicious having been treated with gibberellic acid and/or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	9.16	10.23	14.09	13.00	11.87
GA_3	10.49	10.24	13.38	14.35	12.12
GA_{4+7}	9.31	9.18	14.01	13.08	11.40
GA_3+ paclobutrazol	9.52	9.33	13.36	12.94	11.29
GA_{4+7}+ paclobutrazol	10.61	9.92	13.31	13.42	11.82
Mean	9.82	9.78	13.63	13.36	11.70

L.S.D. (p = 0.05) for column means = 0.49, n = 15.

for table means = N.S.* n = 3

for row means = 0.56, n = 12

* not significant

L value after storage than fruit at harvest and the cultivar Golden Delicious had a higher L value than Bramley Seedling (Table 36).

The colour component a value is very important in fruit colour assessment because it indicates fruit redness during ripening. When the colour determinations were made soon after harvest, there were no differences in a value between the treatments in either cultivar (Table 37). However, after three months storage, the Bramley Seedling fruit from the GA_3 and GA_{4+7} treatments had a significantly higher a value than from the control fruit. Although fruit from the GA_3 plus paclobutrazol and/or GA_{4+7} plus paclobutrazol treatment had also slightly higher a values than the control fruit, there were no significant differences between them (Table 37). For Golden Delicious the same results had been obtained where fruit from the GA_3 and GA_{4+7} treatments had a higher a value than the control fruit. In addition, the fruit colour a value increased significantly after storage in both Bramley Seedling and Golden Delicious fruits, and there were no differences in a value between these two cultivars (Table 37).

The colour component b value of fruit was not affected by the treatment. There were no differences in b value between control and each treatment, neither at harvest nor after storage for both cultivars (Table 38). The measurements only show that Golden Delicious fruit had a higher b value than Bramley Seedling fruit.

Table 36 Fruit colour component L value of Bramley Seedling and Golden Delicious having been treated with gibberellic acid and / or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	51.96	52.13	61.85	64.49	57.53
GA_3	54.62	57.32	60.16	65.71	59.45
GA_{4+7}	52.69	57.59	62.14	61.70	58.53
GA_3+ paclobutrazol	52.53	53.18	60.61	63.74	57.52
GA_{4+7} + paclobutrazol	53.87	56.41	60.24	65.32	58.96
Mean	53.13	55.33	61.00	64.20	

L.S.D. ($p = 0.05$) for column means = 1.55, n = 15.
for table means = 3.45, n = 3.
for row means = 1.77, n = 12.

Table 37 Fruit colour component a value of Bramley Seedling and Golden Delicious having been treated with gibberellic acid and / or gibberellic acid plus paclobutrazol in orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	-16.04	-12.30	-13.35	-15.02	-14.18
GA_3	-16.54	-7.24	-14.02	-9.36	-11.79
GA_{4+7}	-16.24	-8.73	-15.06	-5.70	-11.43
GA_3+ paclobutrazol	-16.06	-9.95	-15.41	-14.82	-14.06
GA_{4+7} + paclobutrazol	-14.13	-11.92	-13.99	-14.16	-13.55
Mean	-15.80	-10.03	-14.37	-11.81	

L.S.D. ($p = 0.05$) for column means =-1.34, n = 15.

for table means =-2.58, n = 3.

for row means = -1.53, n = 12.

Table 38 Fruit colour component b value of Bramley Seedling and Golden Delicious having been treated with gibberellic acid and gibberellic acid plus paclobutrazol in the orchard.

	Bramley Seedling		Golden Delicious		
Treatment	At harvest	After storage	At harvest	After storage	Mean
Control	25.83	24.35	28.94	29.63	27.19
GA_3	26.79	26.72	29.18	32.71	28.85
GA_{4+7}	26.73	25.63	30.17	30.62	28.29
GA_3+ paclobutrazol	25.05	24.33	29.41	32.54	27.83
GA_{4+7}+ paclobutrazol	26.93	23.78	28.94	33.59	28.31
Mean	26.27	24.96	29.33	31.82	

L.S.D. ($p = 0.05$) for column means = 2.65, n = 15.
for table means = N.S.* n = 3.
for row means = N.S.* n = 12.

* not significant

3.2.10 Discussion for influence of interaction between paclobutrazol and gibberellins on fruit colour

In this study, the fruit from trees treated with gibberellin were redder as indicated by the decrease of the X value and increase of the a value. The loss of green colour during ripening is mainly due to chlorophyll breakdown and the reddening in these cultivars results from this loss of green colour rather than from any increase in red pigmentation.

The results suggest that the gibberellin treatment reduced the fruit chlorophyll content. Wang et al (1985) reported that application of GA_3 to paclobutrazol-treated plants decreased chlorophyll content in the leaves. They suggested that decreased leaf chlorophyll content by GA_3 application was probably due in some way to regrowth of shoot and leaves. Possibly the chlorophyll of the fruit peel may have been reduced in a similar way. The direct colour assessment for apple fruit was mentioned by Greene et al (1982) where red colouration of the fruit was significantly increased by the application of GA plus BA and daminozide. However, to what extent this increase of red colour was caused by gibberellic acid needs to be distinguished because it had been known for many years that daminozide alone can improve fruit red colour and prevent preharvest drop (Sharples and Johnson 1986).

3.3. The Application of Paclobutrazol on Tomato

3.3.1 The influence of paclobutrazol on tomato fruit colour

Fruit colour was assessed in terms of three colour parameters L, a and b as described previously. However no differences were found in either L value, a value or b value for each treatment during the process of ripening (Table 39, 40, 41). Thus, the colour changes which occurred during tomato ripening were not affected by the paclobutrazol application. The table 40 (a value) shows that the fruit from harvest mature-green stage turned steadily to red stage for each treatment while table 41 shows that the b value of the fruit steadily turned from blue to yellow as the b value declined throughout ripening.

3.3.2 The influence of paclobutrazol on tomato fruit ethylene production

The ethylene production of intact tomato fruit was determined during each ripening stage, and the tomato ethylene evolution was influenced by the time of paclobutrazol application (Figure 17).
The ethylene production of fruit from plants treated at the fourth time (treatment 4) was higher than that of fruit from control or those of fruit from all the other treatments, when the determination was made at mature-green stage. In contrast, when the determination was made at green-orange stage, the fruit from plants treated at the fourth time, produced less ethylene than that

Table 39 The changes of L value of tomato during the ripening.

	Treatment				
Time	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
7June	44.14	43.60	43.59	44.79	44.32
12June	41.84	42.27	43.14	42.77	43.10
15June	39.57	40.02	39.63	40.52	40.83
17June	37.61	36.48	39.10	38.70	38.85
19June	36.57	36.34	36.59	37.57	38.12
24June	33.91	34.45	34.23	35.52	35.12
Mean	38.94	38.86	39.38	39.97	40.05

L.S.D. ($p = 0.05$) for column mean = N.S.* n = 42

* not significant

Table 40 The changes of colour parameter a value of tomato fruit during the ripening.

	Treatment				
Time	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
7June	-11.41	-11.91	-11.82	-11.94	-11.83
12June	-7.31	-9.87	-9.09	-8.82	-9.69
15June	0.85	-1.76	-2.03	0.24	-2.55
17June	7.27	6.08	5.77	5.82	3.44
19June	12.39	10.70	12.47	10.55	10.71
24June	16.08	14.23	16.12	15.06	16.22
Mean	2.98	1.24	1.90	1.82	1.05

L.S.D. ($p = 0.05$) for column means = 1.02 $n = 42$.

Table 41 The changes of colour parameter b value of tomato fruit during the ripening.

	Treatment				
Time	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
7June	19.26	18.80	19.11	19.72	19.70
12June	17.48	17.84	18.09	18.20	18.17
15June	15.88	16.26	16.09	16.70	17.10
17June	15.24	15.20	15.39	15.88	16.08
19June	13.65	13.99	14.06	14.45	14.78
24June	15.88	16.26	16.09	16.70	17.10
Mean	16.23	16.39	16.47	16.94	17.15

L.S.D. ($p = 0.05$) for column means = N.S.* n = 42.

* not significant

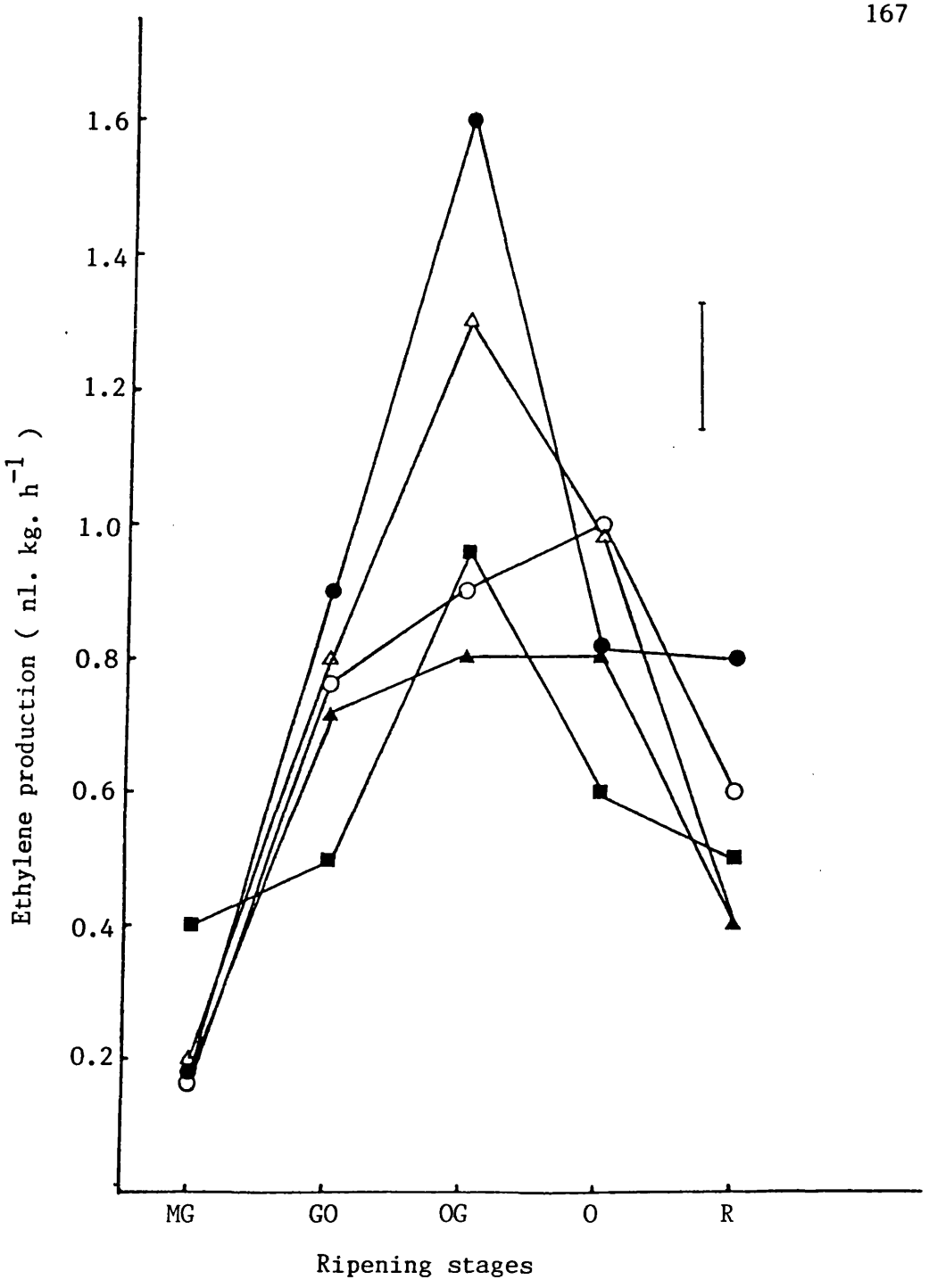


Figure 17 The ethylene production of tomato fruit CV Turbo at different ripening stages when tomato plant were either untreated(●) or treated with paclobutrazol at time 1(O), time 2 (▲), time 3(△) and time 4(■).

The bar shows the LSD (p=0.05, n=7) between each treatment in ethylene production.

of fruit from control or those of fruit from all the other treatments. The ethylene production of fruit from the control was the highest, although there were no statistically significant differences between control and the treatment 1, treatment 2, treatment3. In orange-green stage, the most of the treatments had a surge in ethylene production, however the peak in ethylene production of fruit from control was higher than those fruit from all the other treatments. The peak in ethylene production of fruit from plant treated at the third time (treatment 3) was lower than control but higher than the rest of the treatments (Figure 17). There were no differences between the treatment 1, treatment 2 and treatment 4 in this stage. The surge in ethylene production of fruit from treatment 1 appeared at the orange stage. However, there were no differences in ethylene production between treatment 1, treatment 2, treatment 3 and control, where the ethylene production of fruit from treatment 4 was lower than those of fruit from treatment 1 and treatment 3 at this stage. At the final ripening stage, red stage, the ethylene production of fruit from all the treatments declined, however the fruit from the control still produced more ethylene than fruit from all the other treatments although there was no difference in ethylene production between treatment 1 and the control (Figure 17).

In general the ethylene production of fruit from the

control was higher than all the fruit from plants having been treated with paclobutrazol, at most of the tomato ripening stages referred by the fruit colour. Fruit from control also maintained a higher rate of ethylene production.

3.3.3 The influence of paclobutrazol on tomato fruit mineral composition The mineral composition of calcium, magnesium, phosphorus and potassium was measured soon after harvest, and the content of some elements was influenced by the treatment of paclobutrazol.

Calcium concentration of tomato fruit was found to be increased in all the fruit from plants which had been treated with paclobutrazol (Table 42), although the calcium content of fruit from treatment 3 was not significantly different from that of fruit from the control. Influence was also found in potassium content where the fruit from plants having been treated with paclobutrazol had a lower potassium content although this difference had no statistical significance. No difference was found in magnesium and phosphorus concentration between control and each treatment (Table 42).

3.3.4 The influence of paclobutrazol on tomato fruit total soluble solids The tomato total soluble solids contents were determined at each ripening stage defined by the colour changes. The total soluble solids contents

Table 42 Tomato fruit mineral content (mg/100g dry matter) after application of paclobutrazol at various preharvest stages.

	Minerals			
Treatment	Ca	Mg	P	K
Control	16.5	25.5	42.1	250.3
Treatment 1	22.7	26.1	42.4	242.9
Treatment 2	20.3	25.3	43.2	235.4
Treatment 3	19.8	24.9	42.9	245.7
Treatment 4	21.9	24.4	39.2	250.1
L.S.D.	3.4	N.S.*	N.S.*	N.S.*

($p = 0.05$, $n = 7$)

* not significant

of fruit were influenced by the time of paclobutrazol treatment.

At mature-green stage, there were no differences in total soluble solids content between control and each treatment. Five days later, when tomato fruit entered green-orange stage, the total soluble solids contents of fruit from treatment 1 and treatment 2 were lower than that of fruit from control (Table 43) while no differences were found between treatment1, treatment2, treatment3 and treatment4. There were also no differences between the control and treatment3, treatment4. Similarly, at the orange-green stage, the total soluble solids contents of fruit from not only treatment1 or treatment2, but also treatment3 were lower than that of fruit from control, however, fruit from treatment4 had no differences in total soluble solids content either from control or from treatment1, treatment2 and treatment3. When the determinations were made at either orange stage or red stage, the total soluble solids contents of fruit from all the paclobutrazol treated plants were lower than that of fruit from control. There were no differences between each paclobutrazol treatment at either stage (Table 43). In general, the tomato fruit total soluble solids content was increased through each ripening stage defined by fruit colour, however the increase for the control reached up to 1.4 per cent, compared with 1.1 per cent for treatment 1, 0.9 per cent for treatment 2, treatment 3 and treatment4 (Table 44).

Table 43 Total soluble solids(%) of tomato fruit having been treated with paclobutrazol at various preharvest stages.

	Ripening stage					
Treatment	MG	GO	OG	O	R	Mean
Control	5.4	5.9	6.2	6.5	6.8	6.16
Treatment 1	5.2	5.4	5.7	6.1	6.3	5.74
Treatment 2	5.3	5.5	5.7	6.0	6.2	5.74
Treatment 3	5.5	5.7	5.8	6.0	6.4	5.88
Treatment 4	5.4	5.7	6.0	6.1	6.3	5.9

L.S.D. (p=0.05, n=7) for table means=0.34, row means=0.13.

Table 44 The changes of total soluble solids of tomato fruit having previously been treated with paclobutrazol.

	Treatment				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Increased T.S.S.* from MG to R	1.4	1.1	0.9	0.9	0.9

L.S.D.(p=0.05, n=35) for table means=0.15

* total soluble solids

3.3.5 Discussion for application of paclobutrazol on tomato Commercially, the maturity and ripening of tomato fruit are defined by the changes in fruit colour during ripening (Hunter 1976). Since tomato has a rather short storage life, tomatoes are normally harvested at the green stage (termed the mature-green stage) and marketed when the colour becomes fully red. The change of colour during ripening is an important indicator of ripening stage in tomato and colour changes provide an index of tomato maturation, ripening and senescence. Although the assessment of the ripening stage from the colour change is highly subjective, tomatoes can still be roughly divided by eye into five different ripening stages. These are: mature-green (MG), green-orange (GO), orange-green (OG), orange(O) and red(R) stages (Besford and Hobson 1973). Recently, Hobson (1983) demonstrated that the ripening stage can be more accurately defined by the use of tristimulus colorimetry which could provide an objective way to measure the fruit colour.

In this study, although the five ripening stages were divided subjectively, the tristimulus colour measurement technique was adopted for determination of the influence of paclobutrazol on colour change of the tomato fruit so that a quantitative colour assessment between each treatment could be obtained.

In our experiment, paclobutrazol treatment did not show

any significant influence on the fruit colour change (Table 39, 40, 41). The mean a value of fruit from paclobutrazol treated plants was lower than that of the controls, however, and this may indicate some small influence of paclobutrazol in slowing down the colour change process, but the difference was not significant.

Ethylene production increased during ripening in controls and in the treated fruit, generally falling at the fully ripe (red) stage. The peak in ethylene production appeared at the orange ripening stage in the fruit from early-treated plants (treatment 1 and 2) whereas controls and fruit from later-treated plants (treatment 3 and 4) showed a peak in ethylene production at the earlier orange-green stage and the orange-green stage, fruit from paclobutrazol-treated plants produced significantly less ethylene than the control.

It would seem that the earlier application of paclobutrazol gives a more effective control of fruit ethylene production because the peak of ethylene production was either delayed (treatment 1) or lower than control. The reasons may be similar to those discussed fully for apple fruit and it is interesting that both types have shown a similar response. The results suggest that factors operating early in fruit development can be important in affecting fruit ripening.

Both mineral composition and total soluble solids of the

fruit were influenced by paclobutrazol application, as shown in the results. The increase in the calcium content of the fruit from paclobutrazol treated plants implied that such fruit might have a longer shelf life and show less physiological disorders after harvesting. The application of paclobutrazol was expected to change the partitioning of assimilates in the fruiting tomato plant. Other workers have shown that once the fruit starts to swell, the rate of vegetative growth decreases to a minimum (Salter 1958). The growth of roots decreases to zero 4 weeks after first anthesis and the growth of leaves falls substantially when the total fruit growth rate reaches a maximum (Hurd et al 1979). Apparently, the high fruit growth rate is at the expense of vegetative growth. However, in terms of assimilate supply, reproductive growth is not always favoured more than vegetative growth (Ho 1984). In fact, when assimilate supply is low, a initiating truss only obtains assimilate after the demand by the apex and young leaves has been met (Kinet 1977). Furthermore the development of a truss is delayed if the plant already has a heavy load of fruit in earlier trusses (Hurd et al 1979). Therefore, the partitioning of dry matter in tomato is regulated by the competition between vegetative and reproductive growth and by the competition among fruit for leaf assimilates. Clearly, effective inhibition of vegetative growth in the plant by paclobutrazol would be expected to result in a shift towards reproductive growth both of assimilates, as well as other

nutrients taken up via the roots. The results of the experiment showed that vegetative growth was strongly inhibited 1 weeks after paclobutrazol application (plate 7). It would seem likely that this inhibition of vegetative growth would make more nutrients available to enter the inflorescence for fruiting and this would be expected to influence fruit physiology both during the preharvest and post-harvest period.

Paclobutrazol has also been reported to increase the chlorophyll content of the leaves as well as to increase the efficiency of photosynthesis in apple trees (Wang et al 1985) which could also increase the supply of assimilates. Similar effects might occur in tomato, however, the chlorophyll content and photosynthetic capability of tomato leaves after the application of paclobutrazol was not investigated in the present study.

The results of the change of total soluble solids content of tomato fruit could indicate that the rate of hydrolysis during storage was reduced in fruit from paclobutrazol treated plants. The total soluble solids of fruit from untreated plants increased more than the fruit from treated plants (Table 44) during the period after harvesting. This suggests that paclobutrazol may directly or indirectly influence the post-harvest metabolism of the tomato fruit, for instance by inhibiting the production of hydrolase.

Plate 7

The vegetative growth of tomato plant was inhibited comparing the treated plant (left) to the untreated plant (right).
The picture was taken one week after paclobutrazol application.

The metabolism of tomato fruit either before harvest or after harvest may be regulated by various endogenous growth hormones. It was suggested (Ho 1984) that the import of assimilate into the inflorescence may be regulated by the level of endogenous hormones in the inflorescence. In his work it was shown that under adverse light conditions in which flowers failed to open and may abort, the endogenous cytokinin level in the inflorescence decreased 11-fold whereas the endogenous GA level increase 9-fold. Thus the reduction of assimilate import was in paralled with the reduction of endogenous cytokinin, and the increase of gibberellic acid. Although this would be more likely to affect fruit set than fruit development, it does provide a basis for the suggestion that paclobutrazol application may help the import of assimilates into the inflorescence, and this could influence later stages of fruit development.

It was also reported (Kinet et al 1978) that flowering in tomato is attained by a higher import of dry matter into the inflorescence at the expense of that into young leaves above the first truss. The increase in leaf assimilate import into the inflorescence has been observed within a day after cytokinin treatment. The fact that the growth substance treatment only altered the import into the inflorenscence and the apical shoot rather than into other organs suggests that the competition between inflorescence and apical shoot may be mediated by hormones (Leonard et al

1983). Clearly, growth hormones have an important role in regulating the growth of tomato and this influence would also either directly or indirectly affect the physiology of the tomato fruit either before or after harvest. However more work is still required to understand the role of growth hormones in the metabolism of tomato, and particularly the effects of paclobutrazol on the tomato's physiology.

4 GENERAL DISCUSSION AND CONCLUSION

Paclobutrazol has been commercially available for many years. A great amount of work has been conducted by many horticulturists to characterize the properties of paclobutrazol and to exploit its uses. However, knowledge of the effects of paclobutrazol on fruit post-harvest physiology is still limited. Until Wang and Steffens (1987) recently reported the influence of paclobutrazol on the CO_2 production and ethylene evolution of post-harvest Spartan apple fruit, there was no report available dealing specially with its effects on fruit post-harvest physiology.

Our study has established that paclobutrazol can improve fruit storage capability through regulating various components of post-harvest physiology. The reduction of respiration rate and delay in the onset of the respiration climacteric of apple fruit and the reduction in ethylene production of apple and tomato fruit showed that paclobutrazol radically changes the post-harvest behaviour of fruit and could delay their ripening. However, the mechanism of this effect is not clear. There are two major possibilities. Either the paclobutrazol enters the fruit (by translocation within the plant or by uptake through the fruit surface) and directly affects fruit physiology, or it may not enter the fruit, but have an effect by inhibiting vegetative growth and indirectly

affecting fruit composition and development. However, the question of whether paclobutrazol does enter the fruit cannot be fully answered due to the lack of complete understand of how paclobutrazol moves within the plant. Although it has been reported that the paclobutrazol can move via either the xylem or phloem system within apple shoot (Sterrett 1985), Quinlan and Richardson (1986) demonstrated that the mobilization of paclobutrazol occurred only in the xylem system, and it is said that fruit from trees treated with C^{14}-paclobutrazol do not normally contain detectable quantities of paclobutrazol (Richardson 1985, personal communication.)

If paclobutrazol does move only in the xylem, the time of application may be an important factor for paclobutrazol entering the fruit. The earlier applications (at first bloom and petal fall) delayed the CO_2 climacteric, reduced ethylene production, increased calcium, magnesium, phosphorus content and fruit firmness and showed the colour change etc, whereas application made later in the season (fruitlet and preharvest stages) had little or no effect. One possible explanation of this difference in effectiveness may be that paclobutrazol may only enter the fruit in the early part of the growth season. It is known to be transported virtually exclusively in the xylem system (Quinlan and Richardson 1986) and during swelling the fruit has little or no transpirational demand. The possibility that paclobutrazol

might enter the fruit uptake via the fruit surface seems highly unlikely since the late season applications, which would have a larger fruit surface for absorption, and a high chance of being carried away after harvest were apparently quite ineffective.

If the paclobutrazol effects on fruit post-harvest physiology are due to direct effects of the substance within the tissue, it is not clear how these effects are brought about. The post-harvest formation of ethylene, for example, was found to be affected by paclobutrazol treatment. However, although the study of ethylene biosynthesis has received considerable attention over recent years and the pathway of ethylene biosynthesis is now well understood (Yang and Hoffman 1984), the way in which endogenous or exogenous growth substances and other factors such as mineral content and environment regulate the formation of ethylene is still far from fully understood.

It is possible that paclobutrazol might directly affect ethylene biosynthesis in the way shown by incubation of apple tissue with paclobutrazol (chapter 3). However, for this type of effect to be responsible for the post-harvest changes in fruit from paclobutrazol-treated trees, rather large quantities of the substance would have to have entered the fruit.

However, it is possible that more complex interactions with endogenous growth hormones might be involved. Hormonal regulation of ethylene biosynthesis is known to occur, for instance, auxin was reported to stimulate ethylene evolution by tomato plants (Zimmerman and Wilcoxon 1935) and Kinetin was found to stimulate ethylene production in 3-4 day old etiolated pea seedlings and enhance IAA-induced ethylene production synergistically (Fanchs and Lieberman 1968). As one of the triazole series of growth regulators, paclobutrazol was expected to have similar biological properties to known triazole series growth regulators having an anti-gibberellin biosynthesis function (Hedden and Graebe 1985), but apart from that, little is known about the interaction of paclobutraozl with other growth hormones which might influence plant metabolism such as auxin and cytokinin. Therefore, it is of importance to consider the possibility that paclobutrazol might be interacting with other internal hormones in order to understand the mechanism which paclobutraozl could change the fruit post-harvest physiology.

However, paclobutrazol may not enter the fruit in significant quantities with any path or at any time of the year. If this is the case, any differential effect of sprays at different times in the season may be by a selective influence on other growth processes which in turn effect fruit post-harvest behaviour. For instance

vegetative shoot growth in apple and peach has been shown to be reduced more when paclobutrazol sprays are applied earlier in the season (present study, Shearing and Jones 1986). An effect of this kind would reduce the demand for nutrients e.g. calcium by the shoots thus allowing more to be diverted to the fruit, and this in turn could reduce the production of ethylene during ripening as outlined earlier. A similar mechanism is thought to result from summer pruning of apple trees which completely removes the vegetative shoots and extends the storage life of fruit by, among other factors, increasing fruit calcium content (Preston and Perring 1974).

Although late application of paclobutrazol clearly has little or no influence on the fruit in the current year, it would be interesting to investigate the post-harvest behaviour of fruit from trees having had a later season application in the preceding year, since paclobutrazol is thought to be a long-lasting growth regulator (Quinlan and Richardson 1984). Some workers have found that paclobutrazol is even more effective in the second year (John 1985, personal communication). However, this study could not include any investigation in following year due to the limited period of the project.

If the effects of paclobutrazol on fruit post-harvest physiology are an indirect result of the strong inhibition of vegetative growth, this has important practical

implications for the use of paclobutrazol on different fruit crops. For example, it would be expected that the relationship between vegetative growth and fruit development would be critical. Therefore in a fruit crop in which vegetative growth occurs at a different time in relation to fruit development, the effects may be different. For instance, in tomato vegetative growth and fruiting continue concurrently. In present study, it was found that the height of treated plant was reduced considerably one week after application (Plate 7), but the final plant height showed no differences between the treated and untreated plants. It would seem that the dosage was too low to produce a permanent inhibition of elongation and the effect paclobutrazol was eventually overcome by internal gibberellins. The dosage used in this study for tomato plants was adopted from a study of chrysanthemum (Barrett 1982) where paclobutrazol was applied as a soil drench, because little information was available about the applicaiton of paclobutrazol on tomato. It may be necessary to use a higher dosage or multiple application for this kind of plant which vegetative growth and fruit development occur at the same time.

Paclobutrazol was shown to be a versatile growth hormone which is now active in the horticulture industry. The study here has opened up a new area for the use of this growth regulator. However, only two species of

fruit (apple and tomato) have been tested in the present study and there were some differences in response of paclobutrazol application for these two species. Before paclobutrazol could be used as a ripening retardant agent, more fruits such as pear, peach and citrus should be tested in order to evaluate its potential and to establish the appropriate dosage and time of application for the best response.

An important aspect which deserves a more extensive study is the possible value of paclobutrazol treatment in relation to physiological disorders of fruit during storage. It would be necessary to investigate the effects of paclobutrazol on fruit physiological disorders during storage such as bitter pit, coreflesh and internal breakdown in any future work since paclobutrazol improves fruit storage capability by change of fruit post-harvest physiology. Although this study obtained some valuable results, to understand the role of paclobutrazol on fruit post-harvest physiology, more work is still required.

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Effects of orchard application of paclobutrazol on the post-harvest ripening of apples

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SUMMARY

Foliage applications of paclobutrazol at 1500 mg l^{-1} were made at different times during the growing season to cropping Bramley Seedling and Golden Delicious apple trees. When applications were made early in the season, at first bloom and petal fall, the respiratory climacteric of the harvested fruit was delayed by three days and the amount of ethylene produced by discs from these fruit was reduced compared with fruit from trees which were either untreated or treated at the fruitlet or preharvest stage. After storage for three months at 4°C, differences in the time of the respiration climacteric and amount of ethylene produced were still apparent. The stable respiration rate of fruit prior to the onset of the climacteric was also lower for trees treated earlier in the growing season. Using untreated fruit, tissue slices of Bramley Seedling and Cox's Orange Pippin were incubated in different concentrations of paclobutrazol for 6 h and ethylene production was lower in all paclobutrazol-treated tissue. Possible mechanisms whereby paclobutrazol influences ethylene production and the time of fruit ripening are discussed.

THE plant growth regulator, paclobutrazol, has been widely shown to be active on many fruit crops. Numerous beneficial effects have been recorded on apple including reduced vegetative shoot growth (Quinlan and Richardson, 1984), increased yield (Greene, 1986), enhanced fruit set (Stinchcombe *et al.*, 1984) and reduced water loss (Wang and Steffans, 1985). Most reports have concentrated on the influence of paclobutrazol on the field behaviour of the tree and not on the equally important post-harvest period of the crop. In a review Sharples and Johnson (1986) mention that with the apple cv Cox's Orange Pippin, paclobutrazol delayed the onset of the climacteric in harvested fruit by three days though no experimental detail was given, and with cv Delicious apples ripening was also retarded but by an unspecified amount (Greene, 1986). In contrast, the ripening behaviour of cvs Granny Smith and Top-Red Delicious showed no difference with or without paclobutrazol treatment (Curry and Williams, 1986). With strawberries the harvest date was delayed by as much as five days when 20 kg ha^{-1} of paclobutrazol was applied to the soil in the spring (Atkinson and Crisp, 1986).

There are several alternative methods that can be used for paclobutrazol application to the foliage. Sequential applications have been found to be more effective than single high doses in reducing apple shoot growth (Quinlan and Richardson, 1984). With peach, where single foliar applications were made at the same rate but at different times in the growing season, a greater reduction in shoot growth was found the earlier the application was made in the growing season (Shearing and Jones, 1986). In the current work the influence of the time of single foliar applications of paclobutrazol is being investigated, and in this paper the effect of such treatment on post-harvest ripening of cvs Bramley Seedling and Golden Delicious apples is reported. As a means of determining the time of ripening the production of CO_2 and ethylene by the fruit were measured, as their rise is coincident with the ripening climacteric (Reid, Rhodes and Hulme, 1973).

MATERIALS AND METHODS

Foliar applications of paclobutrazol were applied to six-year-old cv Golden Delicious apple trees grown on M.9 rootstocks and five-year-old cv Bramley Seedling apple trees grown on MM.106 rootstocks. Spraying times were at

the first bloom (26–28 April), petal fall (30 May–1 June), fruitlet (8–10 July) or preharvest stage (9–11 August), and the control trees received no spray. Trees were given three paclobutrazol sprays each of 500 mg l^{-1} with wetter on three successive days. Application was made with a hand-held sprayer and sprayed until incipient run off. Each spray treatment was applied to separate single-tree plots arranged in three randomized blocks, each block containing five trees. The fruit was harvested on 2 October 1985 (Bramley Seedling) or 16 October 1985 (Golden Delicious) and size graded for each tree. Bramley Seedling fruit of 65–70 mm size (4–5 apples kg^{-1}) and Golden Delicious fruit of 60–65 mm size (7–8 apples kg^{-1}) were retained. Measurements of respiration rate (CO_2 production) and ethylene production were made on random samples taken from the size grade either immediately or after storage at 4°C for 89 days (Golden Delicious) or 117 days (Bramley). The use of similar sized apples for analysis of fruit ripening was adopted so that any effects of treatment on ripening due to a change in fruit size (small apples being known to ripen later than large fruit) would be eliminated.

Respiration rate

A fruit sample of *c.* 1 kg from each tree in the size grades described above was placed in a 4-l respiration chamber. Each treatment therefore comprised three replicate chambers, each containing fruit from one tree. The respiration chambers were maintained in a constant temperature room at 25°C. This temperature was adopted in order to accelerate ripening and shorten the period required to measure the respiratory climacteric.

Carbon dioxide-free air was pumped through each chamber at 24 l h^{-1} and the CO_2 production during a 1-h period was measured every 12 h by absorbing the CO_2 from the chamber outflow into 50 ml of 0.1 N NaOH contained in a 250 ml flask, which was then determined by titration with 0.1 N oxalic acid.

Ethylene production

The ethylene production by apple tissue slices was determined using four slices taken from the same fruit, and six apples from each replicate of each treatment. Slices 4 mm thick and 15 mm in diameter were washed in 600 mM sorbitol and 10 mM Mes buffer, then 5 ml of a similar solution were placed in 50-ml flasks with four slices (*c.* 3 g) from each apple. (Lieberman and Wang, 1982). The flasks were sealed and incubated in a shaking water bath at 25°C for 6 h, then a 1 ml sample of flask atmosphere was analysed by means of a Pye GCD gas chromatograph with flame ionization detector, using an alumina column at 120°C and N_2 carrier gas. The direct influence of paclobutrazol on ethylene production was tested by including different concentrations of paclobutrazol in the incubation medium, the procedure being otherwise similar to the above. The production of wound ethylene when using apple slices to determine the relative amounts of ethylene produced by ripening apples was not considered a problem as Lieberman and Wang (1982) had previously demonstrated that apple fruit already producing ethylene do not form wound ethylene on slicing.

RESULTS

Respiration rate

The time of paclobutrazol application during the growing season to the Bramley Seedling and Golden Delicious apples influenced the time of the peak in CO_2 production and therefore the ripening climacteric. When fruit respiration was measured soon after harvest, fruit from trees sprayed at first bloom and petal fall produced a climacteric peak significantly later (2–3 days) than controls or trees sprayed at fruitlet or preharvest stage for both cultivars (Figures 1 and 2). A similar but slightly smaller delay in the peak in respiration rate (1.5–2 days) was seen after fruit of both cultivars had been stored at 4°C for either 89 or 117 days (Figures 3 and 4). There was no significant difference in the height of the peaks in CO_2 production between treatments or cultivars.

The respiration rate immediately after harvest did initially rise for both cultivars, but after five days this had stabilized. This stable respiration rate preceding the peak in CO_2 production was also influenced by the time of paclobutrazol application. The mean value of CO_2 produced between day 6 and day 18 after harvest (Table I) shows that the earlier in the growing season the trees were sprayed with paclobutrazol, the lower was the stable rate of respiration prior to

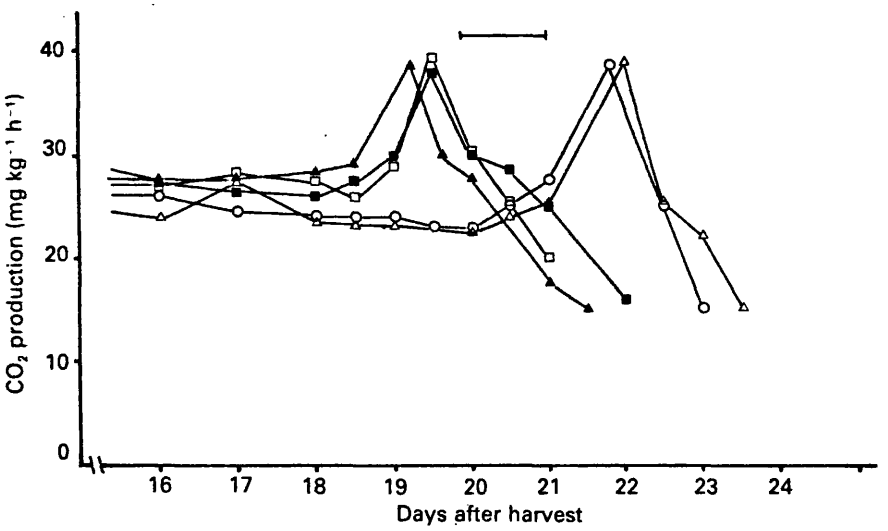

FIG. 1
The production of CO_2 showing the respiration climacteric of Golden Delicious apples at 25°C. Respiration was measured immediately after harvest. Trees were either untreated (■) or treated with paclobutrazol at first bloom (○), petal fall (△), fruitlet (▲) or preharvest stage (□). The bar shows the LSD ($P = 0.05$, n = 3) between the time of the peaks in CO_2 production.

the onset of the peak in CO_2 production, though for Golden Delicious there was no difference between the two earlier sprays or between the two later sprays and the control.

All the respiration values recorded in this study were somewhat higher than those reported previously (e.g. Hulme, 1954) on fruit maintained at 12°C, probably as a result of the higher temperature (25°C) at which our measurements were made.

Ethylene production

The ethylene production from fruit that had been treated at different times in the orchard

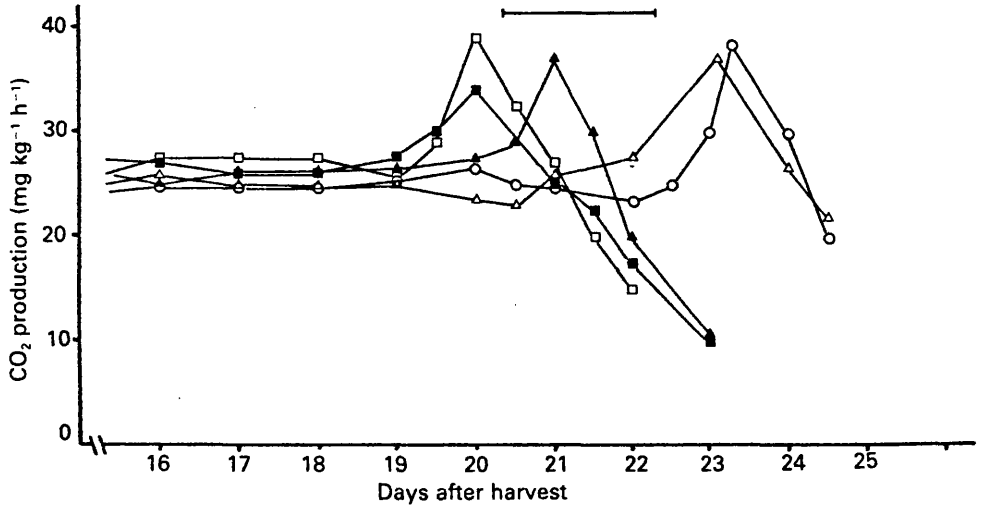

FIG. 2
The production of CO_2 showing the respiration climacteric of Bramley Seedling apples at 25°C. Respiration was measured immediately after harvest. Trees were either untreated (■) or treated with paclobutrazol at first bloom (○), petal fall (△), fruitlet (▲) or preharvest stage (□). The bar shows the LSD ($P = 0.05$, n = 3) between the time of the peaks in CO_2 production.

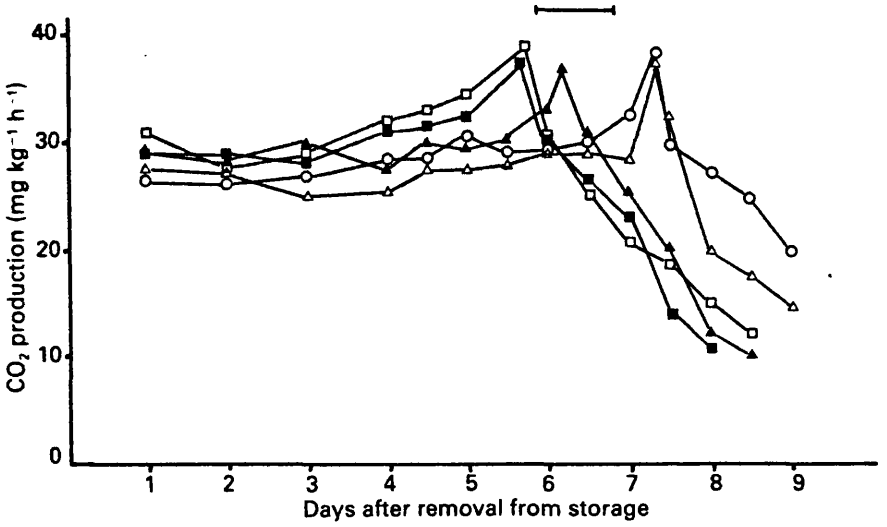

FIG. 3
The production of CO_2 showing the respiration climacteric of Golden Delicious apples at 25°C. Respiration was measured after 89 days' storage. Fruit trees were either untreated (■) or treated with paclobutrazol at first bloom (○), petal fall (△), fruitlet (▲) or preharvest stage (□). The bar shows the LSD (P = 0.05, n = 3) between the time of the peaks in CO_2 production.

was determined at harvest and after storage. When paclobutrazol was applied either at first bloom or petal fall then less ethylene was produced from the fruit either at harvest or after storage than in controls or when treatment was at the fruitlet or preharvest stages (Table II). There were only small differences between ethylene produced by fruit from trees sprayed at first bloom and the petal fall stage, a significant difference only occuring for Golden Delicious after storage. Similarly with the later-treated trees there was a significant difference

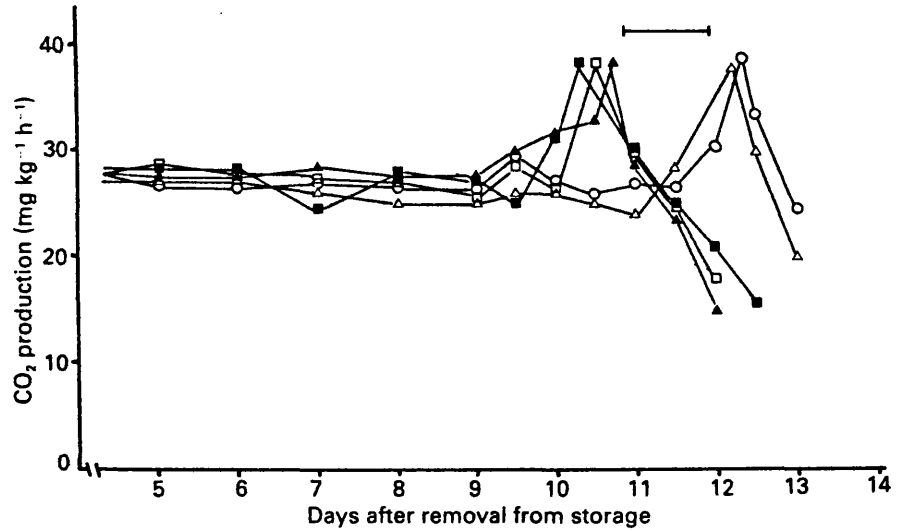

FIG. 4
The production of CO_2 showing the respiration climacteric of Bramley Seedling apples at 25°C. Respiration was measured after 117 days storage when fruit trees were either untreated (■) or treated with paclobutrazol at first bloom (○), petal fall (△), fruitlet (▲) or preharvest stage (□). The bar shows the LSD (P = 0.05, n = 3) between the time of the peaks in CO_2 production.

TABLE I
The mean of the daily respiration rate (mg CO_2 kg^{-1} h^{-1}) at 25°C of fruit between day 6 and 18 after harvest having previously been treated with paclobutrazol in the orchard

Cultivar	Control	Time of treatment				
		First bloom	Petal fall	Fruitlet	Preharvest	Mean
Bramley Seedling	28.0	24.8	25.9	26.7	28.1	26.7
Golden Delicious	27.7	25.2	25.1	27.2	27.2	26.5
Mean	27.9	25.0	25.5	26.9	27.6	

LSD ($P = 0.05$) for column means = 0.6, n = 26.

between Golden Delicious trees treated at fruitlet and the preharvest stage only when ethylene was measured using fruit immediately after harvest.

Fruit slices treated with paclobutrazol during incubation were obtained from fresh fruit purchased locally. With Bramley Seedling apples ethylene production decreased with increasing concentration of paclobutrazol, but with Cox's Orange Pippin, though paclobutrazol-treated fruit produced less ethylene than the untreated fruit, the response to increasing paclobutrazol concentration was not evident (Table III).

DISCUSSION

The results presented in this paper establish that paclobutrazol can delay the ripening of Bramley Seedling and Golden Delicious apples, as shown by a reduction in the production of ethylene and a delay in the climacteric of CO_2 production in the fruit from treated trees. This finding is supported by the results of Sharples and Johnson (1986) and Greene (1986) with other apple cultivars and of Atkinson and Crisp (1986) for strawberries.

Care was taken in the present study to carry out the measurements of CO_2 and ethylene production on fruit of similar size within each cultivar, irrespective of paclobutrazol treatment, and this has enabled us to show that paclobutrazol has effects on the timing of apple ripening itself, quite apart from any indirect effects due to a change in fruit size in treated trees.

The time of paclobutrazol application has been shown in our results to have a particularly important influence on the ripening time. The earlier applications (at first bloom and petal fall) delaying the CO_2 climacterics (Figures 1–4) and reducing ethylene production (Table II) whereas applications later in the season have little effect.

There are various possible reasons for the differences in the effectiveness of early and later applications. For example, paclobutrazol may enter the fruit but the responsiveness of the tissue may decline with the season. Or possibly paclobutrazol may only enter the fruit in the early part of the season as it is known to be transported virtually exclusively in the xylem system (Quinlan and Richardson, 1986) and during swelling the fruit has little or no transpirational demand. Finally it may be that paclobutrazol does not enter the fruit in significant quantities at any time of the year, the effects of sprays at different times in the season

TABLE II
The mean ethylene produced (nl g^{-1} h^{-1}) by apple tissue slices incubated for 6 h at 25°C, the fruit having previously been treated with paclobutrazol in the orchard

Cultivar	Control	Time of treatment			
		First flower	Petal fall	Fruitlet	Preharvest
		Ethylene produced at harvest			
Bramley Seedling	34.02	24.88	25.45	30.43	30.90
Golden Delicious	36.68	22.79	23.37	34.49	39.46
Mean	35.35	23.84	24.41	32.46	35.18
		Ethylene produced after storage			
Bramley Seedling	38.99	28.35	28.23	37.95	40.84
Golden Delicious	36.22	21.06	26.96	38.99	38.18
Mean	37.61	24.71	27.59	38.47	39.51

LSD ($P = 0.05$) for column means = 5.11, n = 12.

TABLE III
The mean ethylene produced (nl g^{-1} h^{-1}) by apple tissue slices incubated for 6 h at 25°C in different concentrations of paclobutrazol

Cultivar	Paclobutrazol concentration (mg l^{-1})					
	0	1	10	100	1000	LSD ($P = 0.05$) ($n = 6$)
Bramley Seedling	29.1	26.8	24.3	21.6	17.9	2.1
Cox's Orange Pippin	27.3	24.7	22.7	23.8	24.8	2.0

may be by a selective influence on other growth processes, which in turn affect fruit quality. For instance, vegetative shoot growth in peach has been shown to be reduced more when paclobutrazol sprays are applied earlier in the season (Shearing and Jones, 1986). An effect of this kind would reduce the demand for nutrients, e.g. calcium, by the shoots thus allowing more to be diverted to the fruit, and this in turn could reduce the production of ethylene during ripening. A similar mechanism is thought to result from summer pruning of apple trees which completely removes the vegetative shoots and extends the storage life of the fruit by, among other factors, increasing the fruit calcium content (Preston and Perring, 1974).

The mechanism by which paclobutrazol influences the time of fruit ripening is not known. The results in Table III showed that treatment of apple slices with paclobutrazol reduces ethylene production, and a similar effect on the production of stress-induced ethylene has been reported by Wang and Steffans, (1985). In this work, the reduction in ethylene production was not due to an effect on 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene, but due to a reduction in ACC synthesis. It is possible that the effects of paclobutrazol on apple fruit ripening, resulting from application to the orchard tree, might also be due to a direct effect of this kind, but indirect effects may also be involved. For instance, paclobutrazol has been reported to increase the calcium content of fruit (Greene, 1986) and calcium has been reported to retard senescence and senescence-dependant ethylene production (Lieberman, 1979). Paclobutrazol is known to inhibit gibberellic acid biosynthesis through the inhibition of kaurene oxidase (Hedden and Graebe, 1985), though since reports are somewhat in conflict, no clear conclusions can be drawn about the effects of gibberellins on the endogenous levels of ethylene in fruit (McGlasson, Wade and Adato, 1978). Interestingly, when gibberellin-containing sprays were applied to Bramley Seedling trees on or at just after flowering, the fruit ripened earlier and contained less calcium than unsprayed trees (Johnson and Wickendon, 1984) which is the reverse of the effect of a paclobutrazol spray applied at the same stage (this work and unpub. results).

Paclobutrazol has been shown to be an important and powerful plant growth regulator for use on apple trees. Results reported here show that the time of fruit ripening is delayed by the application of paclobutrazol at certain times, but this delay was small (max. 3 days) considering the relatively high rate of application used. This change in the time of ripening is not seen as a barrier to the widespread horticultural use of paclobutrazol because of the other agronomic advantages which result from the use of this agent.

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