THE ROLE OF ETHYLENE IN FRUIT AND PETAL ABSCISSION IN THE RED RASPBERRY (*RUBUS IDAEUS* L. cv. GLEN CLOVA)

Jeremy N. Burdon

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ABBREVIATIONS

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
AEC	1-amino-2-ethylcyclopropane-1-carboxylic acid
AOA	Aminooxyacetic acid
AOAC	Association of Official Agricultural Chemists
AVG	Aminoethoxyvinylglycine
CEPA	Chloroethylphosphonic acid
CHI	Cycloheximide
DNPH	Dinitrophenylhydrazine
DTT	Dithiothreitol
EFE	Ethylene forming enzyme
EPR	Ethylene production rate
ER	Endoplasmic reticulum
FRS	Fruit retention strength
GA	Gibberellin, gibberellic acid
GC	Gas chromatograph
Hepps	4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid
IAA	Indoleacetic acid
IRGA	Infra red gas analyser
Kd	Dissociation constant
KMB	α -Keto- γ -methylthiobutyrate
MACC	1-(malonylamino)-cyclopropane-1-carboxylic acid
mRNA	Messenger ribonucleic acid
MTA	5'-methylthioadenosine
MTR	5-methylthioribose
NAA	1-Napththaleneacetic acid

PAS	Periodic Acid Schiffs
PCA	Perchloric acid
pI	Isoelectric point
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic acid
RQ	Respiratory quotient
SAM	S-adenosylmethionine
SCRI	Scottish Crop Research Institute
SEM	Scanning electron microscope
STS	Silver thiosulphate
TCA	Trichloroacetic acid

The following abbreviations have been used in describing Rubus fruit and the characteristics of each category are detailed in Section 3.3.1 for raspberries and Section 3.1.14 for blackberry.

In raspberry:

HG	Hard green fruit
SG	Soft green fruit
M	Mottled fruit
R	Ripe fruit
PR	Purple ripe fruit

In blackberry:

SG	Small green fruit
LG	Large green fruit
BR	Brown fruit
BL	Black fruit

ABSTRACT

Weakening of fruit and petal abscission zones in Rubus idaeus L. cv. Glen Clova was accompanied by increased rates of ethylene production. Both processes were accelerated by a supply of exogenous ethylene. In the ripe fruit natural ethylene levels were saturating. The rise in ethylene production clearly preceded petal abscission but in fruit the increase virtually coincided with the start of weakening. Raspberry fruit of other varieties and blackberries clearly showed the abscission zone weakening could precede any increase in ethylene production.

The internal ethylene concentrations of Glen Clova fruit at the mottled stage reached those levels which had to be added to stimulate abscission (ie 0.25 to 0.5 ppm). This is the very stage at which abscission zone weakening was first noticeable.

Both fruit and petal abscission was retarded by the application of inhibitors of ethylene production (AVG, Co^{2+}) or action (Ag^{+}). Likewise a reduction in the internal ethylene under hypobaric pressure also retarded fruit abscission. None of these treatments were totally capable of preventing abscission.

In fruit abscission the receptacle appears to have an important role. The increase in receptacle ethylene production precedes that of the drupelets. The enlargement and swelling of the receptacle tissues are important in both abscission zone weakening and ethylene production. This receptacle development may in turn be controlled by the development of fertilised drupelets.

The ethylene production in both fruit and petal abscission is limited initially by the supply of ACC. In both cases endogenous ACC levels increase in step with ethylene production.

Ethylene's role as a coordinating/accelerating agent in fruit and petal abscission is discussed.

CHAPTER ONE

INTRODUCTION

The term abscission is derived from Latin: ab - from, scindere - to cut or sunder, and in its strictest sense covers the shedding of any organ from the parent plant irrespective of the mechanism involved. Some authors use abscission in this broad sense and would include the shedding of structures like bark by desiccation and subsequent fracture of the dead cells (Addicott, 1982). The majority of workers however regard abscission in higher plants to involve a highly coordinated sequence of events, the principal of which being the dissolution of the middle lamella and the degradation of cell walls (Sexton et al., 1984). These changes occur around living cells which control the separation through the production of wall degrading enzymes (Horton and Osborne, 1967; Morré, 1968; Lewis and Varner, 1970, Riov, 1974). This allows neighbouring cells to separate rather than being mechanically fractured, although a certain amount of fracture may occur in the epidermis and stele (Jankiewicz, 1985). The process is widespread with examples of virtually all aerial portions of plants being abscised including leaves, fruit, flower buds, sepals, cotyledons and bud scales (for complete list see Kozlowski, 1973).

Under normal conditions the point of separation is predictable and known as an abscission zone (Sexton and Roberts, 1982). Generally separation occurs along a plate of cells within the abscission zone known as the separation layer. Recent immunological evidence suggests the position of this separation layer to be determined by the presence of cryptically differentiated cells which are preprogrammed to respond to an abscission signal (Osborne and McManus, 1984). An alternative hypothesis, although

now less favoured, is that all cells are capable of separation if provided with the precise inductive conditions required, and that these conditions only occur at the separation layer (Sexton, 1979).

Abscission is generally achieved by the dissolution of the wall between adjacent cells along the line of separation, coupled with the generation of mechanical forces to facilitate separation (Sexton et al., 1984). The degree to which cell wall degradation or mechanical fracture are important varies between systems. It is possible that the mass of the organ being shed, and the stress it places on the abscission zone, could influence the amount of degradation required prior to mechanical fracture occurring. An abscission zone, however, is not an inherently weak area of cells prior to cell wall dissolution (Morré, 1968). Cell wall hydrolysis commences at one or two loci and spreads across the whole separation layer (Sexton, 1979). It is not a contagious phenomenon since individual abscission zones may be dissected into as many as 30 pieces with the majority retaining the capacity to abscise (Kendall, 1918; Sexton, 1979).

There have recently been several thorough reviews on various aspects of abscission (Addicott, 1982; Sexton and Roberts, 1982; Jankiewicz, 1985; Reid, 1985; Sexton et al., 1985). The literature reviewed here is that concerned directly with the experimental programme outlined in Section 1.6.

1.1 Anatomy and cell biology of abscission

Separation occurring during abscission is limited to an area of 1-5 layers of cells in a flat plane traversing the whole base of the structure to be shed (Sexton and Roberts, 1982). This separation layer is itself a small part of a larger area of 5-50 layers of small cells which constitutes the abscission zone. This separates the organ to be shed from the main body of the plant (Sexton and Roberts, 1982).

The cells of the abscission zone are often small and isodiametric (Kendal, 1918; Hodgson, 1918; Webster, 1973a) and von Mohl (1860) considered cell division within the abscission zone to be an essential feature of abscission, this idea surviving unquestioned for the best part of a century. In 1950 the idea was refuted by the demonstration of ethylene induced abscission prior to any cell division (Gawadi and Avery, 1950). The meristematic activity may however play a role in maintaining the activity of older abscission zones which may be required to remain responsive for a long time. Abscission zone cells can also be recognised by their dense cytoplasm (Yampolsky, 1934; Facey, 1950; Webster, 1973a), small intercellular spaces (Addicott, 1965; Osborne and Sargent, 1976a; MacKenzie, 1979), large starch deposits (Gilliland et al., 1976; MacKenzie, 1979), highly branched plasmodesmata (Valdovinos et al., 1972; Webster, 1973b; Osborne and Sargent, 1976a) or a lack of phloem fibre lignification in the stele coupled with branching of the vascular bundles (Addicott, 1982). While the whole abscission zone is easily distinguishable the cells of the separation layer are not (Sexton and Roberts, 1982). In Ecballium however they can be distinguished because they are endopolyploid

(Wong and Osborne, 1978).

In the separation layer cell separation is a result of cell wall modification, the separation being between whole cells (Sexton et al., 1984). This was first observed by Inman (1848) and von Mohl (1860) and confirmed chemically by Lloyd in 1916. An early observation on cell wall modification was the reduction in calcium levels within the middle lamella (Sampson, 1918), an observation repeated more recently by Stösser et al. (1969). Cell wall degradation can be more extensive (Hodgson, 1918; Lloyd, 1927; Huberman et al., 1983) and may involve the rest of the primary cell wall. Cell separation may be the result not only of wall hydrolysis but also mechanical stresses caused by differential growth across the abscission zone (MacKenzie, 1979). The turgor theory envisaged that mechanical separation was achieved through the removal of the restricting intercellular cement from parenchyma cells allowing the cells to expand osmotically and become spherical. This theory was mainly proposed as a result of observations made by Kubart (1906) and Fitting (1911) and extended more recently by Sexton and Redshaw (1981).

Changes in the cell wall integrity are supported by ultrastructural studies showing the middle lamella to swell, fenestrate and finally disappear during abscission (Bornman, 1967; Valdovinos and Jensen, 1974; Iwahori and van Steveninck, 1976; Sexton, 1976, Sexton et al., 1977; Sexton et al., 1984). Cell wall breakdown has been observed around all the classes of living cells in the separation layer, xylem fracture however appears to be largely mechanical (Sexton, 1976; Sexton and Redshaw, 1981) although some vessel wall degeneration has been noted (Lloyd, 1927; Brown and

Addicott, 1950; Bornman et al., 1967, MacKenzie, 1979). The dense cytoplasm present prior to abscission is an indication of the metabolic activity of the cells producing the enzymes responsible for cell separation. There is an increase in the cells' organelles including starch grains (Tison, 1900; Webster, 1973a; Gilliland et al., 1976), rough endoplasmic reticulum (Valdovinos et al., 1972; Iwahori and van Steveninck, 1976; Sexton et al., 1977. Lieberman et al., 1983) dictyosomes (Gilliland et al., 1976, Iwahori and van Steveninck, 1976; Koehler et al., 1976, Sexton et al., 1977) and mitochondria (Gilliland et al., 1976). Likewise there is an increase in the respiratory rate and low oxygen tensions and respiratory inhibitors delay abscission (Addicott, 1965).

During the period prior to separation the application of protein synthesis inhibitors, especially cycloheximide, retards abscission (listed in Sexton and Roberts, 1982) suggesting newly synthesised proteins are essential for cell separation. Also increased RNA levels (Webster, 1968; Goodenough, 1986) and rates of RNA synthesis (Abeles, 1968; Abeles et al., 1971) have been demonstrated during abscission. As with protein synthesis, inhibitors of RNA synthesis are generally effective at reducing weakening (Abeles and Holm, 1966, 1967; Holm and Abeles, 1967; Ratner et al., 1969) although not in the faster floral systems (Henry et al., 1974; Hänisch ten Cate et al., 1975). Very recently Kelly et al. (1987) have confirmed these inhibitor studies using 'in vitro' translation techniques to show the appearance of new mRNA species in the abscission zone during separation.

The proteins synthesised and secreted by the separation zone cells include the pectin degrading polygalacturonase (Morré, 1968;

Rogers and Hurley, 1971; Riov, 1974; Greenberg et al., 1975; Berger and Reid, 1979; Huberman and Goren, 1979; Sexton et al., 1984). Pectin methylesterase usually decreases allowing an increase in conversion to the soluble methylated pectin (Facey, 1950; Osborne, 1968). In 1978 Knee demonstrated 'in vitro' methylation of cell walls to cause separation. There are however several reports of inconclusive findings on pectin methylesterase activity during abscission (Yager, 1960; Ratner et al., 1969; Abeles et al., 1971; Hänisch ten Cate, 1975).

The majority of the work on the enzymology of wall degradation in abscission zones has involved an endo-ß-1:4-glucan-4-glucan hydrolase (cellulase) which was initially shown to increase during cell separation by Horton and Osborne in 1967. The accumulation of cellulase during abscission has now been shown to occur in numerous abscission systems, these have been reviewed by Sexton and Roberts (1982). Much of the work has been done on bean leaf explants where there are two forms of cellulase present (Lewis and Varner, 1970), one with an acid isoelectric point (pI = 4.5, 4.5 cellulase), the other basic (pI = 9.5, 9.5 cellulase - Lewis et al., 1974). Only the 9.5 cellulase increases during abscission and has been purified with antibodies raised against it (Koehler et al., 1980). These antibodies did not precipitate the 4.5 cellulase (Durbin et al., 1981) and could be used to inhibit bean abscission (Sexton et al., 1980). The 9.5 cellulase from bean is synthesised 'de novo' (Lewis and Varner, 1970; Durbin et al., 1981) and secreted into the cell walls of the abscission zone (Reid et al., 1974; Lieberman et al., 1981) under the control of the abscission accelerant ethylene (Abeles and Leather, 1971).

Lewis et al. (1974) showed a mixture of 9.5 cellulase and pectinase to be capable of causing cell separation with neither being effective alone. The target of the cellulase is uncertain, both cellulose and xyloglucan being proposed, although Sexton et al. (1984) were unable to find any xyloglucanase activity associated with the 9.5 cellulase.

The enzymatically mediated cell wall degradation allows the rounding up of turgid cells along the fracture line thereby assisting separation by pushing the two surfaces apart. This also provides the force required to stretch and ultimately rupture the more chemically resistant xylem vessels which may appear on separation as drawn out springs (Sexton and Redshaw, 1981). In addition to these localised forces there may be more general tensions created within the abscission zone by the differential expansion of tissues either side of it (Lloyd, 1927; Horton and Osborne, 1967; Leopold, 1967; MacKenzie, 1979). As cells become free of the intercellular cement the tension produced is loaded onto less cells thereby aiding separation or even the tearing of those cells still joined. The presence of rounded cells at the fracture surface is considered to be indicative of the abscission process rather than a totally mechanical tearing of tissues. The rounded cells retain their selective permeability (Morré, 1968; Abeles and Leather, 1971; Sexton et al., 1977) and initially contain very active looking cytoplasm (Valdovinos et al., 1972; Iwahori and van Steveninck, 1976; Sexton, 1976; Sexton et al., 1977).

Whilst the involvement of enzymes has been demonstrated other factors can influence cell separation. One of the earliest noted was that of changing calcium levels (Sampson, 1918). Stösser

et al. (1969) also showed calcium to be lost from cell walls during cell separation, an effect which if carried out artificially using calcium chelators results in tissue maceration (Ginzburg, 1961). Also the addition of calcium to the abscission zone has been shown to inhibit cell separation (Poovaiah and Leopold, 1973; Dwelle, 1975; Iwahori and Oohata, 1980).

The above evidence all confirms the process of abscission to be an active, rather than passive, shedding of plant organs which involves numerous metabolic processes. It therefore leads to the question of how the process is controlled naturally and can it be manipulated through artificial treatments.

1.2 General features of abscission control

The process of abscission can be envisaged as being subject to control at three different levels, i) the acquisition of cellular competence to respond to the abscission signal, ii) the expression of the separation programme, iii) the modulation of the rate of weakening (Sexton and Trewavas, 1987).

1.2.1 The acquisition of competence

The initial prerequisite for abscission to occur appears to be the appearance of a positionally differentiated class of target cells (Osborne, 1977; Osborne, 1982; Politio and Stallman, 1982; Osborne and McManus, 1984). These cells are capable of responding to a signal inducing abscission. In some situations this competence is lost at a later stage of development (Durieux et al., 1983).

1.2.2 The expression of the separation programme

Having achieved a state of competence abscission zone target cells may remain quiescent for a long period, possibly maintaining their competence through several cell division cycles. For abscission to occur there must be some form of internal or external signal to which the target cells can respond. The induction of abscission has been recognised for a long time and many stimuli have been noted (Addicott, 1982). In 1844 Gartner considered mineral deficiencies, drought and low light, as well as the possibility of internal factors within the plant, to cause the shedding of fruit and flowers. The concept of plant hormones being involved was initiated by Weisner (1871) who observed that removal of a leaf blade caused rapid petiole abscission. Kuster (1916) followed this

up by showing that if a small piece of lamina was left, abscission was inhibited and thereby concluded that a chemical from the leaf inhibited the petiole abscission. The lamina could also be replaced by auxin rich orchid pollinia (Mai, 1934) again preventing abscission. Eventually in 1936 La Rue showed purified IAA had the same effect.

As a result of these and many other subsequent observations (Addicott, 1970) the auxin status of the abscission zone is considered crucial, with reduced auxin levels contributing to separation. Shoji et al. (1951) showed the concentration of auxin to decline during bean leaf abscission whilst Jacobs (1979) reported a reduction in auxin transport as leaves aged, thereby reducing its level at the abscission zone. A simple control system through auxin concentration was confounded by the demonstration that proximal (stem end) applications of auxin accelerated abscission whilst distal (lamina end) applications would inhibit (Addicott and Lynch, 1951; Chatterjee and Leopold, 1963). In 1950 Barlow showed that if auxin additions were delayed after excision it slowly lost its inhibitory effect. This was repeated by Rubinstein and Leopold (1963) who also noted that auxin accelerated abscission if application was delayed for 12 hours. Consequently abscission was considered to be a two stage process, inhibited by auxin in Stage I and accelerated by auxin in Stage II, the duration of Stage I being dependent on the time taken for auxin levels to decline sufficiently for abscission to occur (Leopold, 1971; Jackson and Osborne, 1972). In 1967 Abeles showed the different effect of proximal and distal applications could be explained by differences in the speed with which the auxin reached the zone. Distal applications moved rapidly

by diffusion and polar transport whilst auxin applied proximally moved slowly by diffusion opposed by polar transport. Hence if applied distally auxin reached the zone in Stage I whereas proximally applied auxin only arrived during Stage II. The accelerating effect of proximal applications was lost if the auxin was applied in high concentrations or very close to the zone.

In 1913 (Harvey) and 1917 (Doubt) demonstrated that very low levels of ethylene were capable of causing abscission. It had been known earlier that an unidentified component of gas used for lighting could cause this effect (Girardin, 1864; Kny, 1871; Fitting, 1911). The production of ethylene by fruits was shown by Gane (1934) a fact extended to many other plant tissues by Crocker (1948). By 1940 apples were being used to produce ethylene for the commercial defoliation of roses (Milbrath et al., 1940). Despite the early knowledge of ethylene's capabilities and the ability of auxin to stimulate ethylene producton (Zimmerman and Wilcoxon, 1935) the prospect of an auxin/ethylene interaction to control abscission was not examined until the 1950s. Gawadi and Avery (1950) found ethylene chlorohydrin to be effective at accelerating the ageing of immature leaves. They suggested the ethylene produced by senescing tissues had a similar role in natural abscission and that the balance of auxin and ethylene controlled abscission. Hall (1952) tested this theory and showed petiole base abscission to be dependent on the relative concentrations of auxin and ethylene applied to them.

Abeles and Rubinstein (1964) used this interactive effect along with the ability of auxin to increase ethylene production (Morgan and Hall, 1964) to explain Stages I and II in terms of

ethylene sensitivity. The sensitivity is to some extent dependent on the auxin status of the abscission zone. Application of auxin during Stage I maintains the ethylene insensitive state. Later additions (during Stage II) are to a tissue insensitive to auxin yet responsive to the ethylene produced as a result of that auxin.

Ethylene also plays a role in plants auxin metabolism. It is an inhibitor of auxin transport (Beyer and Morgan, 1971; Morgan and Durham, 1972; Beyer, 1973; Riov and Goren, 1979) and reduced its level through enhanced auxin destruction or conjugation (Gaspar et al., 1978; Riov and Goren, 1979) and a reduction in synthesis (Ernest and Valdovinos, 1971). Ethylene therefore has an effect which enhances the tissues sensitivity to it.

Morgan et al. (1972) demonstrated that the ethylene production rates occurring naturally whilst being capable of accelerating abscission also inhibited auxin transport. It was therefore suggested that ethylene might have its effect by the reduction of auxin at the abscission zone. Subsequent experimentation with auxin transport inhibitors and exposure of only the leaf blade to ethylene failed to induce abscission, thereby indicating a direct role for ethylene in the abscising tissue itself (Morgan and Durham, 1972; Beyer, 1975).

Other compounds have been examined with variable results. Cytokinins are capable of extending Stage I thereby inhibiting abscission (Gorter, 1964; Chatterjee and Leopold, 1964). A reduced sensitivity to ethylene was found in cytokinin treated tissues (Abeles et al., 1967) although Hänisch ten Cate and Bruinsma (1973) could find no effect of cytokinins. More recently Suttle (1986) has shown a range of cytokinins to stimulate ethylene production and

presumably through this they could enhance abscission.

Whilst the role of gibberellins in plant development has been examined in depth there have been few reports of effects on abscission (Addicott, 1982). Addicott (1982) takes this as indicative that GA is not a limiting factor under field conditions. In fruit GA applications stimulate development thereby reducing young fruit drops in tomato (Rappaport, 1957) cotton (Walhood, 1957) apples (Hield et al., 1958) and citrus (Crane, 1969). Generally the application of GA to explants accelerates abscission (Chatterjee and Leopold, 1964, Jacobs and Kirk, 1966, Lyon and Smith, 1966, Trippi and Boninsegna, 1966), but to a lesser extent than ethylene. GA can also counteract the inhibition caused by large IAA applications (Greenblatt, 1965), low IAA additions in conjunction with GA accelerated abscission further than that possible from either substance alone. Lewis and Bakhshi (1968b) indicated GA to stimulate protein synthesis related to the production of hydrolytic enzymes in Citrus abscission zones. GA has variable effects on ethylene production rates, having no effect on pea seedlings (Fuchs and Lieberman, 1968) or rape seed (Takayanagi and Harrington, 1971) yet the majority of cases having a small promotive effect (Abeles, 1973). Hence any acceleration of abscission attributed to GA may be a result of increased ethylene production.

The effect of abscisic acid (ABA) on abscission is controversial in spite of its name. The first positive correlation between ABA levels and abscission was shown in cotton fruit by Davis and Addicott in 1972 (and repeated by Varma in 1976 and Rodgers in 1980) and extended to flower abscission (Bentley et al., 1975; Porter, 1977). There are cases however where there is no clear

correlation, in leaves (Perry and Hellmers, 1973; Peterson et al., 1980) flower buds (Ramina and Masia, 1980) and fruit (Takeda and Crane, 1980).

ABA has been shown to stimulate abscission in a number of systems (Addicott, 1982). It has also been shown to increase ethylene production leading to the suggestion that ABAs effect may be mediated via ethylene (Edgerton, 1971). This hypothesis is supported by work showing that ABA has no effect on abscission in the presence of saturating levels of ethylene (Jackson and Osborne, 1972) or the inhibitor of ethylene production AVG (Sagee et al., 1980). The hypothesis is contradicted by results showing ABA to have an effect on abscission under conditions (hypobaric pressures) which would normally prevent ethylene stimulated abscission (Cooper and Horanic, 1973; Rasmussen, 1974). Also ABA has not always been shown to promote ethylene production (Mayak and Halevy, 1972; Marynick, 1977; Dörffling et al., 1978). Recently Sargent et al. (1984) have shown ABA to be effective at promoting fruit abscission in wild oats, a system in which ethylene has no effect. Addicott (1982) concluded that ABA does have a direct role to play in abscission in spite of the evidence against this and a lack of effect of ABA additions to many abscission systems (reviewed by Milborrow, 1974; Porter, 1977).

Hence the five plant growth regulators discussed so far interact to regulate abscission through their relative concentrations. It is difficult however to ascribe effects to changes in the levels of individual regulators. It must also be borne in mind that the growth regulator probably has its effect through complexing with a receptor, which must itself be available

for there to be a response (Trewavas, 1982). The ability of a tissue to respond to any given concentration of growth substance (its sensitivity) is thus dependent on the concentration and affinity of its receptor. The concept of sensitivity is important in that it can account for an effect occurring in the absence of increased ethylene. An increase in sensitivity and a steady ethylene production can thus result in a response. This can also account for the shedding of immature fruit at specific abscission zones which later become non-functional with the mature fruit being shed at a different abscission zone (eg Peach-Nelson et al., 1984; Rascio et al., 1985). Sexton et al. (1985) have put forward the view that abscission may not be under the control of a single receptor but is under multifactoral control. This would include the levels of several regulators and the sensitivity of the tissue to them.

The use of inhibitors has revealed different levels of control in different abscission systems. Generally applications of protein sythesis inhibitors (in particular cylcoheximide) have been successful at delaying abscission suggesting that abscission control is at least translational (listed in Sexton and Roberts, 1982). The evidence from RNA synthesis inhibitors is less conclusive. Transcriptional inhibitors such as actinomycin D may delay weakening, but not always to the same extent as cycloheximide (Abeles, 1968; Ratner et al., 1969; Abeles et al., 1971) and in some cases have little effect (Pollard and Biggs, 1970; Henry et al., 1974; Riov, 1974; Hänisch ten Cate et al., 1975). Thus the role of transcriptional regulation is more contentious. The results of 5-fluorouracil and actinomycin D application led to the conclusion

that it was the synthesis of specific mRNA rather than general RNA synthesis which was essential (Abeles, 1968; Abeles et al., 1971). It therefore appears that in the slower (greater than 18 hours) abscission systems both transcriptional and translational control are involved. In those systems showing a quicker (less than 18 hours) response control is at the post transcriptional or translational level, there being no requirement for new mRNA synthesis.

More recently Kelly et al. (1984) have shown by 'in vitro' translation the appearance of new mRNA species in bean explant abscission zones. These mRNA species appear within 6 hours of adding ethylene, occur more slowly in air and don't appear in hypobaric treated explants. Transfer of ethylene treated explants to hypobaric treatment caused 2 of the new mRNA to disappear, a change prevented by maintaining ethylene in the hypobaric system. Ethylene has also been shown to cause the synthesis of new mRNAs during both tomato (Grierson et al., 1985) and avocado (Tucker et al., 1985) fruit ripening.

1.2.3 Modulation of the rate of weakening

It is possible to alter the rate of weakening and even reverse it once underway. Application of additional ethylene may accelerate weakening, whereas to maintain the weakening the continual presence of ethylene is necessary (dela Fuente and Leopold, 1969; Abeles and Leather, 1971; Stead and Moore, 1983). Withdrawal of ethylene has also been shown to reverse the decline in breakstrength (Abeles et al., 1971). Abeles and Leather (1971) showed ethylene to function by controlling hydrolytic enzyme

secretion, the control of the wall degrading enzymes therefore occurs at transcriptional, translational and post-translational levels. Increased respiratory and protein synthetic capacity, cellular expansion and decline in cell wall synthesis and repair are also probably controlled at this time (Sexton and Trewavas, 1987).

1.3 Evidence for ethylene's involvement in abscission

The early experiments which led to the idea that ethylene might be involved in natural abscission have been reviewed in Section 1.2. A large amount of evidence has now accumulated implicating naturally produced ethylene in abscission control (reviewed recently by Morgan, 1984; Reid, 1985; Sexton et al., 1985) although its exact role is still debated (Addicott, 1982). Even amongst those who accept ethylene's involvement in abscission uncertainty arises from a possible role either as the inductive agent or simply in coordinating and accelerating the process. The case for a role for ethylene in natural abscission has been supported by several types of evidence. These are discussed below.

1.3.1 The effect of exogenous ethylene on abscission

Since the early reports of Gawadi and Avery (1950) and Hall (1952) there have been a large number of reports where exogenous additions of ethylene have accelerated abscission. Sexton et al. 1985 list approximately 50 such examples and others as a result of more recent work can be added (Nagao and Sakai, 1985; Wood, 1985; Burdon et al., 1986; Nunez-Elisea and Davenport, 1986).

It may well be that this is the most universally demonstrated growth substance response. The ethylene can be administered either by enclosure in an ethylene containing atmosphere, or by the application of an ethylene releasing chemical (see Sections 2.13 and 2.15). Abscission zones from fruit, leaves, stems, buds, bud scales and floral structures from virtually every plant family have shown to be similarly effected. The abscission of oat seeds (Sargent et al., 1984), oil palm fruit (Chan et al., 1972)

and the leaves of oak, beech, pine and ivy (Abeles, 1973) represent the only reported exceptions to this generalisation.

The use of ethylene releasing sprays is a very imprecise method of applying ethylene in that exact ethylene levels are difficult to predetermine. The use of atmospheres containing known concentrations of ethylene allows the determination of threshold and saturating concentrations for the abscission response. Usually concentrations of 0.1 ppm (100 nl l^{-1}) are necessary to accelerate abscission and 10 ppm (10 µl l^{-1}) to saturate the response (listed in Sexton et al., 1985).

1.3.2 The correlation between abscission and increased ethylene production rates

The prospect that ethylene could induce abscission naturally was not examined in depth until the mid 1960's. On the basis of the relationship between the internal ethylene and ethylene production rates, Burg (1968) suggested that ethylene production rates had to exceed 3-5 µl/kg/h in order to produce internal ethylene levels capable of enhancing abscission. These levels could readily be found in senescing plant tissues. As a result of work on the role of RNA and protein synthesis in abscission, Abeles (1968) proposed that ethylene actually induced abscission through the induction of the new mRNA species necessary for the production of cell wall degrading enzymes.

Since these initial experiments numerous positive correlations between increased ethylene production rates and abscission have been reported (listed in Sexton et al., 1985). Several authors have claimed to have established ethylene to be the

natural regulator of abscission on the basis of these correlations. Whilst such parallels add weight to this hypothesis, the correlations on their own do not provide proof of ethylene involvement.

There are also abscission systems reported (Hänisch ten Cate et al., 1975b; Witzum and Keren, 1978) where there is no correlation between abscission and increased ethylene production rates. In these systems the involvement of ethylene in abscission cannot be ruled out because of the possibility of a change in ethylene sensitivity (Durieux et al., 1983; Halevy et al., 1984). For instance Durieux et al. (1983) showed Lilium buds to have a 2 week responsive period between weeks 4 and 6 of development. Outside this period the buds are insensitive to ethylene. In general the sensitivity of specific organs to ethylene is dependent on auxin supply (Addicott, 1970), is increased by ageing (Abeles, 1973) and water stress (Jordan et al., 1972) and decreased by calcium sprays (Poovaiah and Leopold, 1973; Martin et al., 1981). The same organ in different varieties may also show differences in sensitivity (Craker and Abeles, 1969).

1.3.3 The actual level of ethylene within tissues

To substantiate a role for ethylene on the basis of correlative data it is necessary to establish that the ethylene concentration in the abscission zone is at least equal to that level which must be applied exogenously to stimulate the process. The determination of ethylene levels within plant material depends on the ability to extract the internal gases. For large organs such as apples internal gases can be removed using a syringe (Blanpied,

1986) and the gas analysed directly for ethylene. For smaller samples such as leaves and petioles it is not possible to extract internal gases in this way and vacuum extraction under liquid is necessary (Yeang and Hillman, 1981). Unfortunately most abscission zones are so small that it is not possible to extract their internal gases. The best that can be done is to extrapolate an ethylene concentration from a knowledge of ethylene production rates and the relationship between the two (Burg, 1968). In several systems ethylene levels above the threshold have been calculated implying that natural ethylene levels are high enough to be effective in abscission (Beyer and Morgan, 1971; Morgan et al., 1972; Lipe and Morgan, 1973; Ben Yehoshua and Aloni, 1974; Swanson et al., 1975; Hänisch ten Cate et al., 1975; Morgan and Durham, 1980).

This type of evidence is rather unsatisfactory for two reasons. Firstly the abscission zone itself cannot be assumed to be identical to the tissues used to establish the internal ethylene and ethylene production rate relationship. Secondly it is difficult to measure ethylene production rates of abscission zones accurately. Excising the zone introduces the complication of large amounts of wound ethylene production, whilst using larger pieces of tissue leaves unresolved the contribution of any contaminating adjacent tissue. A solution to this dilemma was devised by Jackson et al. (1973). They added the ethylene releasing chemical chloroethylphosphonic acid to petioles at concentrations which produced ethylene at rates found to occur naturally during leaf abscission. The treatment stimulated abscission.

1.3.4 Influencing abscission by ethylene removal

More substantial evidence of ethylene involvement in abscission is available than that already described. If ethylene is controlling abscission it should be possible to stop or retard abscission by removing ethylene. The simplest method to reduce internal ethylene levels is by increasing the diffusion gradient out of the tissue through the use of ethylene absorbing chemicals such as potassium permanganate or mercuric perchlorate (Jackson and Osborne, 1970; Young and Meredith, 1971). In both cases abscission was delayed.

A more effective technique for lowering internal ethylene levels is the use of hypobaric pressures. Since Burg and Burg (1966) first showed the technique to be successful at prolonging fruit storage, pressures of between 100 and 300 mmHg have been used to reduce abscission (Cooper and Horanic, 1973; Lipe and Morgan, 1973; Rasmussen, 1974; Morgan and Durham, 1980; Sipes and Einset, 1982; Kelly et al., 1984). The reduction in internal ethylene levels is a result of a combination of effects: i) an immediate drop in ethylene partial pressure as gases vent the tissue, ii) an increased diffusive loss as the gas phase becomes less dense, iii) reduced retention through pressure effects on ethylene solubility and iv) a reduction in ethylene biosynthesis due to limited oxygen availability (Nilsen and Hodges, 1983).

The control experiment for hypobaric treatments is the inclusion of ethylene in the reduced pressure atmosphere (Cooper and Horanic, 1973). This should reinstate abscission provided the inhibition was solely the result of a lack of ethylene. A technically more difficult control experiment involves the use of

pure oxygen at 0.2 atmospheres, thereby maintaining the normal atmospheric partial pressure of oxygen whilst still applying hypobaric conditions (Nilson and Hodges, 1983). This technique removes the possibility of there being any effect of the hypobaric treatment as a result of a lack of oxygen.

1.3.5 Influencing abscission by inhibiting ethylene production or action

Having shown ethylene production to change in a manner compatible with a role in controlling abscission the prospect arises of affecting abscission through inhibiting ethylene production or action. The most commonly used inhibitors of these two processes are aminoethyoxyvinylglycine (AVG) and silver thiosulphate respectively.

The rhizobitoxine analogue AVG was first shown to prevent ethylene production in 1971 by Owens et al. Since then it has been shown specifically to inhibit a key enzyme (ACC synthase) in the ethylene biosynthetic pathway, (Boller et al., 1979; Yu et al., 1979). AVG has been shown to reduce abscission in numerous systems including citrus leaf explants (Sagee et al., 1980), avocado fruit (Davenport and Manners, 1982), apple fruit (Williams, 1980; Greene, 1983), tomato flower (Roberts et al., 1984), bean explants (Kushad and Poovaiah, 1984) and the corolla of Digitalis (Stead, 1985). One of the problems of using inhibitors is ensuring that they reduce ethylene production to insignificant levels in terms of abscission. It is also necessary to ensure that AVG is functioning through a reduction in ethylene production alone, and not through a general effect on pyridoxal phosphate catalysed reactions. This can easily

be verified by adding AVG in the presence of exogenous ethylene, the ethylene should reinstate AVG inhibited abscission so long as only ethylene production had been inhibited.

The silver ion is nowadays considered a potent inhibitor of ethylene action, based on the findings of Beyer in 1976. It is considered to function by binding to the ethylene binding site (Sisler, 1982). Initially Ag^{+} was applied as the nitrate (Saltveit et al., 1978) but its phytotoxic nature (Cameron and Reid, 1981) made the more mobile thiosulphate complex, STS, (Veen and Van de Geijn, 1978) a better prospect for use in stopping ethylene action. Veen (1983) reported STS to be non toxic yet a highly effective anti-ethylene agent at concentrations of up to 0.2 mM. There are several reports of STS being capable of retarding abscission (Cameron and Reid, 1983; Mor et al., 1984; Agnew et al., 1985) although the majority of work has been on extending the marketable life of cut flowers (reviewed by Halevy and Mayak, 1981; Nichols, 1984). Recently electron microscopy coupled with X-ray microanalysis has shown particles of silver and sulphur to be deposited in the cell wall and middle lamella (Hobson et al., 1984). It is possible that the presence of silver may inhibit the enzymes involved in wall degradation which is required for cell separation rather than interfering with ethylene binding. Baird et al. (1984) however showed STS to prevent the reorganisation of the endomembrane system which is generally associated with abscission, as was also shown by Valdovinos et al. (1981). Hence STS does appear to influence abscission in a way consistent with its anti-ethylene role although there is no simple control experiment to verify its action. Beyer (1978) reported the use of acetylene as a

method of overcoming the antiethylene effects of Ag^+, possibly through a salt formation. This however simply adds another unknown to the system and has not been used to any great extent.

Carbon dioxide is often cited as being a competitive inhibitor of ethylene action (Burg and Burg, 1967; Sexton and Roberts, 1982; Morgan, 1984) and has been shown to delay abscission in some circumstances (listed in Abeles, 1973; Jerie, 1976). To view increased CO_2 levels as simply affecting ethylene action is perhaps an oversimplistic view. The more widespread implications of CO_2 and ethylene interrelationships are now being examined in greater detail (Horton, 1985; Philosoph-Hadas et al., 1986).

Numerous other chemicals have also been used with varying degrees of effectiveness at preventing ethylene production or abscission. These include norbornadiene (Sisler et al., 1985), aminooxyacetic acid (Yu et al., 1979; Nagao and Sakai, 1985) and cobaltous ion (Lau and Yang, 1976; Yu and Yang, 1979). There is thus a very wide range of treatments available affecting ethylene production and abscission thereby providing a basis for implicating ethylene in the control of abscission. Coupled with the correlative evidence of ethylene production rates, and the effect of adding or removing ethylene it should be possible to examine an abscission system in sufficient depth to determine the role of ethylene. There is already a large mass of evidence for ethylene's controlling role in abscission which taken together is quite convincing. However, there are few definitive experiments examining all the possibilities in a single abscission system.

1.4 Ethylene involvement in fruit ripening

Ripening involves changes in colour, texture, taste and aroma of the fruit through anabolic and catabolic processes (Biale, 1975) rendering the fruit fit for consumption. These are a result of changes in those enzymes involved either through their synthesis, degradation, release, activation or inactivation.

The period known as ripening is largely a subjective evaluation (Biale and Young, 1981). In most fruit ripening does not occur until after the majority of growth has occurred although there is no clear cut delimitation of these two processes (Coombe, 1976).

It has long been accepted that in terms of their ripening characteristics fruit are said to be either climacteric or non-climacteric (Biale and Young, 1981). This classification is based on the initial observation of Kidd and West in the 1920s that there was an upsurge in respiration at the time of visible ripening in apples (1924). This characteristic has since been shown in numerous other species such as melon (Pratt and Goeschl, 1969) avocado (Burg and Burg, 1962) and tomato (Lyons and Pratt, 1964). A more complete list is available in the review by Rhodes (1980). Subsequently it was shown that accompanying the increased respiration was an elevated production of ethylene which could precede (banana), follow (apple, tomato) or coincide (mango, pear, avocado) with the respiratory peak (Burg, 1962). Kidd and West (1947) showed that exogenous additions of ethylene caused apples to respond by increasing both respiration and ethylene production rates. By 1973 Abeles declared that there was no exception to the observation that ethylene could promote ripening, so long as the tissue was in a receptive state. Presumably the pomegranates

examined by Elyatem and Kader (1984) could be deemed to be in a non receptive state since ethylene was found not to effect their ripening. Fruit ripening is generally assessed by the changes in pigmentation and texture as well respiratory rate and ethylene production and in these terms ethylene clearly accelerates ripening in numerous species (Abeles, 1973; Rhodes, 1980).

The role for ethylene in regulating fruit ripening has been established on the basis of results showing exogenous ethylene to stimulate ripening in immature fruit and the potentially stimulating levels which accumulate within fruit (reviews: Biale, 1960; Burg, 1962; Hansen, 1966; Pratt and Goeschl, 1969; Sacher, 1973; Coombe, 1976; McGlasson, 1985). The role of ethylene has been examined by preventing its biosynthesis or action (Biale and Young, 1981; Bangerth et al., 1984; Knee, 1985) or by preventing it accumulating within fruit (Burg and Burg, 1966; Burg, 1975). The results clearly indicate a role for ethylene with the inhibition of its production or action and its removal from fruit all delaying ripening in climacteric fruit. This is particularly noticeable with respect to fruit softening and pigmentation changes (Knee, 1985; Goodenough, 1986).

McMurchie et al. (1972) using propylene as an ethylene mimic (Burg and Burg, 1965) showed there to be two ethylene production systems involved in the regulation of ripening in bananas. System I ethylene synthesis is as a result of tissue ageing and accumulates to a level capable of inducing the autocatalytic System II ethylene production. This results in the climacteric peak of ethylene production.

The concept of ethylene as a natural regulator of ripening

which had to exceed a threshold limit to elicit a response was first suggested by Kidd and West in 1945. Burg (1962) reported threshold values of 0.1 ppm ethylene for a range of ethylene dependent responses including fruit ripening with between 1 and 10 ppm being saturating. In mangoes and bananas Burg and Burg (1962) showed there to be physiologically active levels of ethylene present within the tissues at the onset of the climacteric.

Despite the above characteristics being quite clear it was evident by the 1940s that not all fruit showed the characteristics of this typically climacteric type of ripening. Biale (1960) identified a group of fruit which showed neither increased respiration nor autocatalytic ethylene production (non-climacteric fruit). Typical of this category are oranges which if treated with ethylene when green (Biale, 1960) responded by increased respiration, loss of green colour and accumulation of reducing sugars (Goldschmidt et al., 1977). Unlike climacteric fruit however removal of the exogenous ethylene caused a return to the initial respiratory rates. McMurchie et al. (1972) found propylene to increase respiration but not autocatalytic ethylene production in oranges. In their terms the orange possesses System I ethylene production but not System II. This inability to produce ethylene autocatalytically would appear to be characteristic of non-climacteric fruit (McGlasson, 1985). The question of how non-climacteric fruits ripening is controlled in the absence of increased ethylene production rates remains unanswered. In these cases (eg Citrus fruit) it may be that a change in tissue sensitivity to ethylene, or another growth substance initiates ripening (Trewavas, 1982).

There are two main theories regarding the metabolic changes which together are termed ripening. The organisational resistance of the cell has been considered to change as a result of alterations to membrane permeability (discussed by Rhodes, 1980). This results in a leakage of ions and metabolites and a release or activation of hydrolytic enzymes. Alternatively ripening can be considered to involve an initial sequence of biosynthetic changes dependent on gene expression (Grierson et al., 1985). This second theory was examined by Grierson et al. (1985, 1986) in tomato fruit with the aim of identifying genes associated with ripening and the role of ethylene in regulating their expression. In tomato some mRNAs decline during ripening, others persist or increase and new mRNAs appear. One of the new mRNAs coded for a polygalacturonase, a major cell wall softening enzyme (Grierson et al., 1986). Likewise there is a synthesis of new mRNA species during the ripening of avocado (Tucker et al., 1985). One of these new mRNAs gives rise to a product which is immunoprecipitable by antibody raised against avocado cellulase (Tucker and Laties, 1984). Through these more modern techniques it is now becoming possible to identify in detail the changes which ethylene can induce in fruit material during the course of ripening.

1.5 Ethylene biosynthesis and action

The physiological activity of ethylene was first reported by Neljubow in 1901 as the active ingredient of illuminating gas. The ability of the gas used for lighting to have an effect on plants had been well documented prior to this (Girardin, 1864; Kny, 1871). Later, specific reports indicated ethylene to be a very potent plant growth regulator (Crocker and Knight, 1908; Denny, 1924). The capacity of plant material to actually produce ethylene itself was first demonstrated by Gane in 1934, although Cousins in 1910 had reported the ability of gases produced by oranges to ripen bananas prematurely. Further work led Crocker, Hitchcock and Zimmerman (1935) to view ethylene as a ripening hormone in fruit and as a regulator in vegetative organs. In the same year Zimmerman and Wilcoxon discovered the capacity of auxin to increase ethylene production in plant material, a fact which was ignored in work on abscission until approximately 30 years later (Abeles and Rubinstein, 1964).

Ethylene's role in fruit ripening was in doubt even in 1954 (Biale, Young and Olmstead) since their chemical assay suggested there was insufficient ethylene produced by fruits to induce a response. This was amended five years later with the development of gas chromatographic analysis for ethylene (Burg and Stolwijk, 1959; Burg and Thimann, 1959). Nearly all parts of higher plants have now been shown to be capable of producing ethylene (Abeles, 1973).

The elucidation of the biosynthetic pathway for ethylene took a further 14 years from the initial observation by Lieberman et al. (1965) that carbons 3 and 4 of methionine were converted to ethylene. Other model systems for ethylene production had also been

proposed involving ethylene production from linolenic acid, methional or α-keto- γ-methylthiobulyrate (KMB) or propanal (reviewed by Lieberman 1979). The actual pathway from methionine to ethylene was determined by Adams and Yang. In 1977 they demonstrated that the CH_3S-group of methionine was conserved and that the 4-carbon moiety of methionine was replenished from the ribosyl moiety of ATP (fig. 1.1). The CH_3S group was released as methylthioadenosine (MTA) from S-adenosylmethionine (SAM) and rapidly hydrolysed to methylthioribose from which the CH_3S group was returned to methionine. In 1979 Adams and Yang identified the intermediate between SAM and ethylene as 1-aminocyclopropane-1-carboxylic acid (ACC) which accumulated along with MTR under a nitrogen atmosphere. In the presence of oxygen the ACC was rapidly converted to ethylene. Burroughs (1957) had earlier isolated ACC from cider apples and perry pears but had not assigned any role to it although he did note that it appeared only in ripe fruit. In the same year Vahatalo and Virtanen (1957) also extracted ACC but this time from ripe cowberries.

The two enzymes converting SAM to ACC (ACC synthase) and ACC to ethylene (the ethylene forming enzyme - EFE) have been examined. In 1979 Boller et al. and Yu et al. reported the capacity of cell free tomato extracts to convert SAM to ACC and MTA. Since the report of a simple chemical assay for ACC (Lizada and Yang, 1979) the assay of the product (ACC) can now be performed routinely and can also be used to determine the enzymes activity. ACC synthase appears to be a pyridoxal enzyme requiring low concentrations of pyridoxal phosphate for maximal activity and being inhibited by inhibitors of pyridoxal dependent enzymes such as AVG or AOA (Yu et

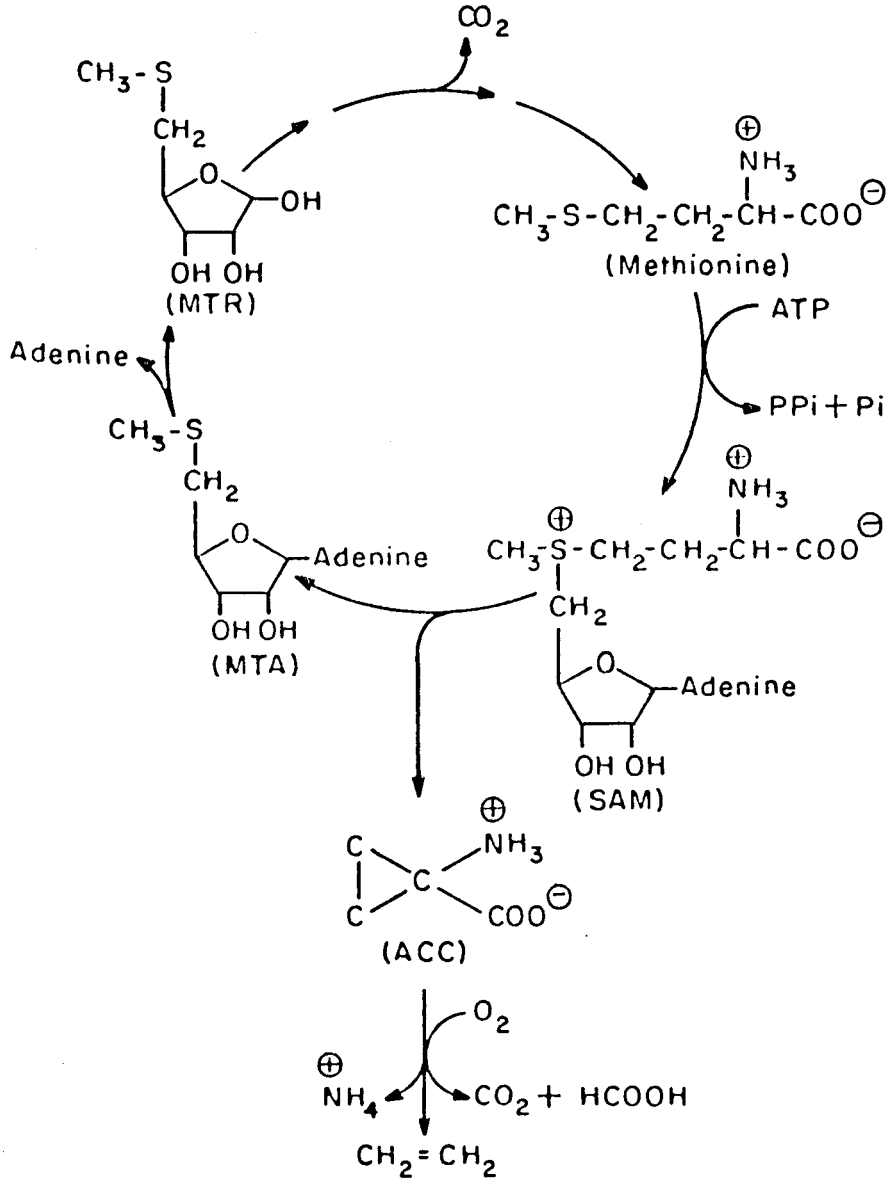


Fig. 1.1 The methionine cycle in relation to ethylene biosynthesis (from Yung et al., 1982).

al., 1979). In plant tissues producing little or no ethylene the supply of ACC has generally been found to be limiting with the ACC synthase activity paralleling the increase in ethylene production during ripening of tomatoes (Boller et al., 1979), during synthesis of wound ethylene (Boller and Kende, 1980; Yu and Yang, 1980) and in response to IAA (Jones and Kende, 1979; Yu and Yang, 1979). Wounding tomato pericarp tissues causes both increased ethylene production and a ten fold increase in ACC synthase activity within 40 minutes of wounding (Boller and Kende, 1980). This increase in ACC synthase activity is eliminated by inclusion of cycloheximide in the incubation medium (Acaster and Kende, 1983). The use of density labelling of ACC synthase also suggests de novo synthesis of enzymes rather than the activation of a precursor in pericarp tissue (Kende, Acaster, and Guy, 1985). Bleecker et al. (1986) have used monoclonal antibodies in the purification and characterisation of ACC synthase, identifying a 50 kDa protein as ACC synthase from wounded tomato pericarp tissue.

The ethylene forming enzyme is largely constitutive since applications of ACC to various plant organs from different species caused marked increases in ethylene production rates (Cameron et al., 1979; Lürssen, Naumann and Schröder, 1979). Preclimacteric fruit and flowers are considered to be exceptions to this with both ACC synthase and EFE being limiting (Yang et al., 1985). The activity of the EFE is dependent on membrane structure and oxygen and can be activated or inhibited by various compounds (Yang, 1984; Yang et al., 1985). Light can influence EFE activity indirectly through CO_2 metabolism, increases in CO_2 promoting ethylene production in leaf tissues (Dhawan et al., 1981; Grodzinski et al.,

1982; Kao and Yang, 1982).

Various systems have recently been proposed for the conversion of ACC to ethylene from various sources. Konze and Kende (1979) reported an enzyme extract from etiolated pea seedlings, also IAA-oxidase and peroxidase (Vioque et al., 1981) carnation microsomes (Mayak et al., 1981) pea microsomes (McRae et al., 1982) and pea mitochondria (Vinkler and Apelbaum, 1983). Whilst being oxygen dependent, heat denaturable and inhibited by radical scavengers, these sytems all had characteristics not in agreement with the *in vivo* system. One important characteristic is the ability to distinguish between the four stereoisomers of 1-amino-2-ethylcyclopropane-1-carboxylic acid (AEC) as suggested by Hoffman et al. (1982). Data obtained by McKean and Yang (1984) indicated a preferential conversion of (4)-allocaronamic acid to 1-butene by intact pea epicotyls, but not the pea epicotyl homogenate. Guy and Kende (1984) however demonstrated regiospecificity in AEC conversion in a vacuolar fraction isolated from pea protoplasts. The vacuoles also resemble the *in vivo* system by being sensitive to both Co^{2+} and membrane disruption, and accounted for 80% of the protoplast ethylene production.

The reaction mechanism of ethylene formation is uncertain although several aspects have been examined. Yang (1981) proposed the formation of ethylene and cyanoformic acid from ACC through a nitrenium intermediate or equivalent. Spontaneous degeneration of cyanoformic acid results in CO_2 and HCN. This pathway is supported by work by Peiser et al. (1984) indicating the carboxyl group of ACC to yield CO_2 and carbon 1 to yield HCN. The capacity to detoxify HCN by ß-cyanoalanine synthase which combines HCN and

cysteine is sufficient to account for even the most rapid production in plant material (Miller and Conn, 1980). The feeding of [cis-2,3-$^{2}H_{2}$]ACC to apple slices resulted in equal amounts of cis and trans [1,2 $^{2}H_{2}$] ethylene, indicating progress through an intermediate between ACC and ethylene allowing the loss of stereochemistry (Adlington et al., 1983). Based on the electrochemical oxidation of [cis-2,3 $^{2}H_{2}$]ACC Pirrung (1983) proposed two sequential one electron oxidation reactions with the initial reaction producing amine radical intermediates with concomitant ring opening and loss of stereochemistry. This indicates the EFE to be a fluorooxidase utilising molecular oxygen as a final electron acceptor. It is still however possible for the elimination reaction to occur through an ionic intermediate, still giving a scrambling of hydrogens after ring opening.

The ethylene biosynthetic pathway from methionine via SAM and ACC is now clear, and the reaction mechanism for ethylene production from ACC is being examined. However, for ethylene to have a physiological role it must bind to some part of a cell. The receptor was suggested to contain a metal by Burg and Burg in 1967 with Cu^{2+} now being considered most likely (Beyer, 1976; Thompson et al., 1983). The subject of ethylene binding sites in plants has recently been reviewed by Venis (1985). In Phaseolus vulgaris binding has been assigned to protein body membranes and ER possibly along with a fraction of the plasma membrane (Evans et al., 1982a, b). Binding sites have recently been solubilised and partially purified from P. vulgaris cotyledons (Thomas et al., 1984, 1985) and mung bean sprouts (Sisler, 1980, 1982). The data from different sources on site concentrations and Kd values of different tissues

show similarities (Venis, 1985) but the possibility of these sites being receptors is still in question. The ability of Ag^{+} to function as an antagonist of ethylene action (Beyer, 1976) is not always reflected in these binding site examinations (Bengochea et al., 1980a). Also CO_2 in Phaseolus inhibited binding in a particulate cotyledon preparation (Bengochea et al., 1980b) but stimulated binding in a solubilised extract (Thomas et al., 1984). It was also without effect on binding to mung bean extracts (Sisler, 1982a). It has recently been suggested that ethylene oxidation is necessary for ethylene action (Beyer, 1981). Smith and Hall (1984) have further suggested that ethylene binding and oxidation activity may be as a result of a common system controlling a plants response to ethylene.

Since response to ethylene probably depends on the formation of a transient receptor-ethylene complex there are thus two possibilities to explain growth regulation. In the past a change in the concentration of a growth substance has been considered to regulate growth. More recently Trewavas (1981, 1982) has drawn attention to the possibility that plant development may be determined as a result of the sensitivity of tissues to growth substances, that sensitivity being equated to the number or affinity of hormone binding sites.

1.6 The development of an experimental programme

The initial broad aim of this PhD was to study the physiology and biochemistry of abscission and several pilot projects on different aspects of the process were undertaken. Of these it was decided to concentrate on the role of ethylene in abscission. Raspberry fruit were used as the experimental material for the reasons outlined below. The results of the other programmes have been published elsewhere (Sexton et al., 1984; Burdon et al., 1986).

Many authors consider ethylene to have a major role in abscission although whether this is as the inductive agent or merely an accelerator of the process is still a matter for debate (see Section 1.3). Much of the uncertainty stems from four fundamental shortcomings of the experimental data.

i) Abscission zones are usually very tiny and thus it is difficult to measure accurately internal ethylene levels, or even ethylene production rates. As a result it has not been possible to definitively establish that ethylene concentrations reach an effective threshold in abscission zones. In this respect it is unlike other ethylene regulated processes where it is possible to actually measure the ethylene within the tissue showing the effect, for example fruit (Blanpied, 1986) and water plant stems (Kende et al., 1984).

ii) The induction of abscission in laboratory experiments often requires unnatural and very disruptive manipulation of the material. The excision of abscission zone explants provides a common example (Addicott, 1982). These treatments disrupt natural ethylene production both via wounding and creating metabolic and hormonal imbalances in the material. As a result of these problems

it can be argued that these experimental systems bear little relationship to those that occur naturally (Trewavas et al., 1984)

iii) A number of crucial experiments reported in the literature which appear to indicate a role for ethylene in abscission have not been accompanied by adequate controls.

a) Both Stead (1985) and Kushad and Poovaiah (1984) showed AVG to inhibit abscission. They did not however carry out the control experiments of adding AVG in the presence of exogenous ethylene to demonstrate the effect of AVG to be specifically via a suppression of ethylene production.

b) Morgan and Durham (1980) reported that hypobaric treatments inhibited abscission in Melia azedarach L. They interpreted the effects of low pressure treatment as being exclusively by a reduction in ethylene levels. This however was in the absence of adequate controls to ensure the inhibition was not due to some other effect of low pressures, such as a reduction in oxygen parital pressure.

c) The widely reported inhibition of abscission by the silver ion (Veen, 1983) is often given as evidence for ethylene's controlling role. Until the exact molecular nature of the silver effect is established this data cannot be used as definitive proof of ethylene involvement.

d) Numerous authors (see Sexton et al., 1985) have claimed that correlations between increased ethylene production rates and the onset of abscission substantiate ethylene's role in the process. These are merely correlations and cannot be used as direct proof of ethylene involvement.

Raspberry fruit abscission was chosen as the experimental

system for two main reasons. Firstly, the structure of the raspberry fruit makes it a favourable system in which to study abscission (fig. 1.2). Each fruit is composed of numerous drupelets (usually 50-100) each of which has its own abscission zone connecting it to the central receptacle (MacKenzie, 1979). This has the advantage of the abscission zones being enclosed in the centre of the fruit rather than being at a pedicel-fruit junction. Whilst still not being able to extract the gases from within the abscission zones themselves it is possible to determine the level of ethylene in the atmosphere surrounding the zones. A knowledge of the ethylene concentration around the zones offers the possibility of investigating whether the levels occurring naturally are capable of accelerating abscission in immature fruit.

Secondly the abscission of raspberry fruit is a commercially important process. The increasing costs of labour to pick the raspberry crop and the unreliability of the labour supply has made the use of mechanical harvesters a viable prospect. Harvesting depends on the abscission of each individual drupelet in a coordinated manner to release the cup of drupelets intact from the central receptacle. The problems associated with machine harvesting generally stem from the fact that the raspberry crop ripens over a three or four week period, resulting in there being fruit at different stages of maturity on the canes. This precludes the type of once over harvesting as used on blackcurrants, where the fruit all ripen in synchrony. In raspberry the ripe fruit must be removed from the canes without disturbing those immature green fruit still developing.

Recently there has been much work done on the prospect of

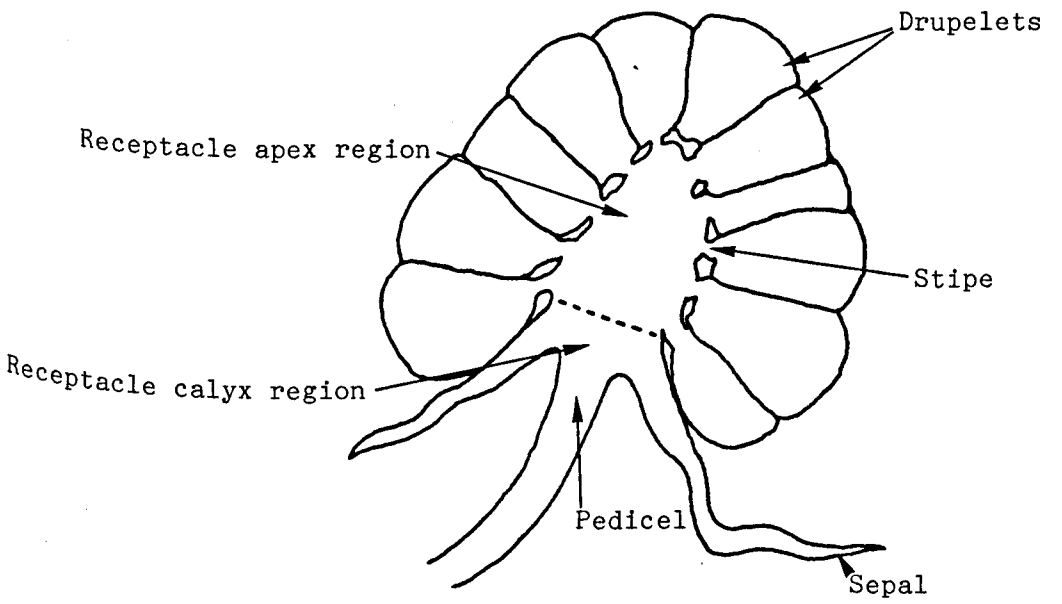


Fig. 1.2 Drawing of a raspberry fruit. The shedding of the cap of drupelets depends on the abscission of each individual drupelet from the receptacle apex. The position of the single abscission zone in blackberries is marked by the dashed line at the base of the receptacle apex.

machine harvesting raspberries. Prototype machines have been available in the USA since the 1950s and in Scotland since 1970 (Waister and Ramsey, 1971). These machines depend on shaking actions to cause fruit separation once abscission is well underway. They do however have the drawback of causing reduced yields. This is as a consequence of the removal of immature fruit, the removal of ripe fruit complete with receptacle and an inability to collect all the fruit dislodged (Waister and Cormack, 1978). There is also the possibility of cane damage reducing the yield in subsequent seasons.

The ability of 2-chlorethylphosphonic acid (as Ethrel or Ethephon) to enhance abscission in other crops (Reid, 1985) through ethylene release was seen as a possible solution to ease the separation of the drupelets from the receptacle. In 1970 Jennings and Carmichael carried out a small scale field trial on the effect of Ethrel on raspberry fruit abscission. Effectiveness was assessed by the proportion of fruit which dropped without their stalks attached. The results indicated Ethrel not to be a ready answer to raspberry harvesting in that it did not simply ease the harvesting of ripe fruit. It also accelerated fruit ripening by several days, caused defoliation and increased leaf yellowing. In some cases it also caused damage to the terminal buds with either tip die-back or the stopping of extension growth resulting in a rosette of leaves at the cane tip.

In the following years there were several field trials of Ethrel using machine harvesters (Zatyko and Sagi, 1973; Jolliffe, 1975b; Knight, 1976), the resuts of which all proved to be inconclusive. So far no laboratory examination of the effects of Ethrel on raspberry fruit has been reported. The main effect noted

as a result of the field trials was an increase in fruit ripeness and an increased removal of ripe fruit at the expense of individual berry weight. Jolliffe (1975b) noted that Ethrel could cause the decreased fruit weight as a premature ripening of fruit and not just an enhanced abscission of ripe fruit.

These field trials were conducted in the virtual absence of data on the ethylene physiology of raspberry fruit during their development. A knowledge of the natural changes in ethylene production rates would have given a background against which the effect of Ethrel could be assessed. For example it could easily have been established as to whether or not natural levels in ripe fruit were saturating, thereby making further ethylene additions ineffective. Knight (1976) did look at the ethylene production rates of fruit with or without Ethrel treatment. However, he only examined 2 categories of fruit (green or ripe) and then only 24 hours or 5 days after treatment. The only other work done on ethylene production in raspberries was by Blanpied (1971, 1972). Whilst showing measurable amounts of ethylene to be present the method involved, and the representation of the results, gives no indication as to either the ethylene level of internal gases or the rate of production as measured by emission.

In retrospect it seems strange to have embarked on field trials without having first carried out adequate experiments to establish the nature of the effect of ethylene on raspberry fruit. Laboratory experiments would have helped to assess whether Ethrel was likely to assist the machine harvesting of raspberry fruit. The unique structural features of the fruit make it capable of providing information on the role of ethylene in abscission not available

through the examination of other structurally different abscission systems such as leaf explants.

On the basis of experience gained in the pilot experiments in 1983 the experimental programme outlined below was conceived:

i) To establish whether there is a correlation between natural abscission in raspberry fruit and elevated ethylene production rates, and hence whether raspberry is a climacteric fruit or not.

ii) To determine whether exposure to ethylene would enhance abscission in fruit at different stages of fruit development and measure a threshold concentration for the effect.

iii) To measure internal ethylene levels during development and relate these to the ethylene threshold concentration.

iv) To determine the point of control of ethylene production in fruit of different maturities.

Since the results of the above observations confirmed the view that ethylene could well be involved in accelerating fruit abscission the study was extended to investigate the effects of AVG, Co^{2+}, STS and hypobaric pressures on the rate of weakening.

During the course of this work incidental observations were made on other aspects of raspberry fruit ripening including changes in pigmentation, acidity, sugar levels, photosynthesis and respiration. The work was also extended to other varieties of raspberry, blackberries and raspberry/blackberry hybrids.

Initial experiments showed high ethylene production rates during flowering. This resulted in the role of ethylene in petal abscission being examined.

CHAPTER TWO

MATERIALS AND METHODS

The materials and methods employed in this research are described below. Where the techniques of other authors have been modified, or where new methods have been devised the developmental programme has been included.

The methods have been presented in the order in which they appear in the results section.

All routine chemicals used were Analytical grade and were obtained from either B. D. H. Ltd (Poole, England) or Sigma Chemical Co., Ltd, (Poole, England) except where specifically stated.

The raspberry variety Glen Clova was the only variety used in both fruit and petal experimentation. In the examination of abscission and ethylene production of the fruit of other raspberry varieties, blackberries and raspberry/blackberry hybrids, measurements were made in the same way as detailed for Glen Clova fruit.

2.1 Plant material

Raspberry canes (Rubus idaeus L. cv Glen Clova) were grown at The Scottish Crop Research Institute, Invergowrie, Dundee and in the gardens at Stirling University. Normal cultural practice was adhered to as outlined by the Scottish Agricultural Colleges (Raspberry Production: Publication No 54, March 1980). At Stirling all primocane growth was allowed to develop providing a few late season fruit.

In order to obtain fruit for exploratory experiments during the spring a planting programme was developed to provide early greenhouse grown fruit. New canes were planted in the late spring of 1984 in 14" x 12" tubs outdoors. These plants were transferred into greenhouses in late December/early January after several nights of frost. Half were kept in an unheated greenhouse, the rest were maintained at 12^{o}C and lit for 16 hour days. This resulted in a staggered bud break and eventually a supply of fruit throughout March, April and May. Those kept heated and lit showed reasonable growth even by late January compared to outdoor canes (plate 2.1). The unheated greenhouse provided an intermediate rate of development.

The use of primocane fruit and indoor canes extended the normal fruiting season but was only extensively developed for use in the final year (1986).

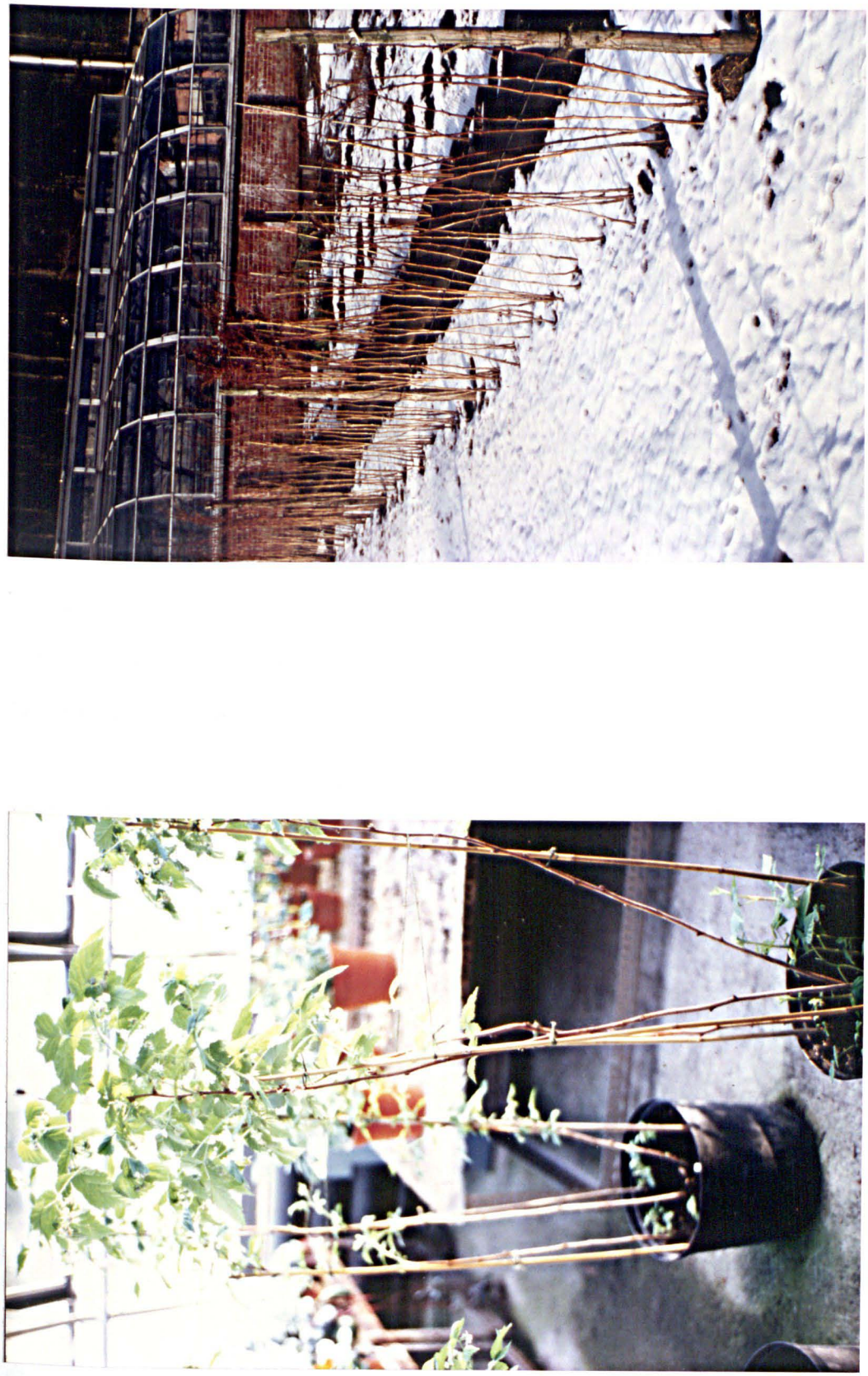


Plate 2.1 Illustration of the earlier bud break achieved by moving canes into a heated greenhouse (12°C, lit for 16 hour day) at the end of the third week of December. Photographs taken on the same day at the end of January.

2.2 The selection of development stages for experimentation

The selection of categories of fruit for examination was based on the necessity to rapidly select fruit of approximately similar physiological age. It was also essential that fruit within each category behaved reproducibly throughout subsequent experimentation. Finally all the major physiological states occurring during fruit maturation had to be examined. These constraints are more fully described in Section 3.1.1.

The stages were selected on the basis of trial experiments, the results of which are shown in Section 3.1.3. These results showed the categories to mark clear transitional states during the maturation of raspberry fruit. The five main categories of hard green, soft green, mottled, ripe and purple ripe were adopted for

Table 2.1 Characteristics of development stages selected for experimentation.

Development stage	Drupelet colour	Average weight (g)	Fruit retention strength (N)
HG	Blueish green	0.8	7.35
SG	Green	1.5	7.35
M	Green and red	2.0	5.0
R	Red	2.5	1.5
PR	Purple red	2.5	0.5

examination and are illustrated in figures 3.1 to 3.5. A brief outline of the characteristics of each category are given in table 2.1.

2.3 Timecourse of berry development

Fifty individual flowers were tagged at the stage where the petals were just visible between the sepals using a thin band of brightly coloured tape around the pedicel. This gave a reasonably synchronised starting point from which to make timings. The development of the whole group was monitored at 2 or 3 day intervals whilst still on the canes and their developmental stages recorded according to the characteristics defined in Section 3.1.1. This method of following a population of fruit and recording their maturation by developmental stage gave an indication of the time taken to progress from stage to stage. It also gave some indication as to the variation in the developmental rates of a population of fruit.

An alternative method was also employed. Sixty individual flowers were tagged as above and at varying intervals eight fruit were removed and their developmental stage, weight and basal diameters were recorded.

2.4 Microscopy

The abscission zones of ripe and unripe fruit were examined by both electron and light microscopy. Observations were made simply to confirm those of MacKenzie (1979) that a typical abscission programme takes place in raspberry, a fact which was not accepted by Reeve (1954).

2.4.1 Scanning electron microscopy

Material for the SEM (ISI 60A) was simply prepared by placing it on a small drop of Tissue Tek II OCT Compound (Lab-Tek Products, Naperville, Illinois) on an insulated stub and freezing in liquid N_2. The sample was then rapidly transfered to the SEM chamber which was evacuated immediately. This material remained frozen in the SEM chamber for approximately 30 minutes (Sexton, 1976) whilst operating with an acceleration voltage of 7kV.

2.4.2 Light microscopy

Material, either individual drupelets or plugs, was initially fixed by immersion in a mixture of 2% glutaraldehyde in 25 mM cacodylate buffer (pH 7.2) and placing under vacuum for 1 hour. After removal from vacuum the material was stored at $4^{o}C$ for 24 hours prior to dehydration in an alcohol series. This consisted of 1 hour in each 10%, 20%, 40% and 60% ethanol followed by standing overnight in 80% ethanol. This was changed to absolute alcohol for 4 hours, changed and left overnight. The absolute alcohol was changed once more and left for a final 24 hours.

The material was finally embedded using a JB4 embedding kit following the makers instructions.

Thin sections (4 μm) were cut using an LKB pyramitone (LKB Ltd, Selsdon, S Croydon, England) and mounted onto slides before staining with PAS according to the method of Feder and O'Brien (1968). The slide was placed for 10 minutes in a DNPH aldehyde blockade (0.5 g to each 100 ml of 15% acetic acid in water, stirred for 1 hour and filtered) and then rinsed in running tap water for 10-30 minutes. To check the blockade sample slides were transferred to Schiffs Reagent (for 10-30 minutes) followed by 3 sodium metabisulphate (0.5%) rinses each of 2 minutes finishing with a 2 minute rinse in running water. A successful blockade prevented any staining.

Having blocked all aldehyde in the material the sugars were oxidised to aldehyde by immersion in 1% Periodic acid for 5-10 minutes followed by a 5-10 minute wash in running water. The slide was then transferred to Schiffs Reagent for up to 30 minutes (until stained pink) and then into 3 successive baths of 0.5% sodium metabisulphate (each for 2 minutes) to stop the Schiffs Reagent reacting. A 5-10 minute rinse in running tap water finished the procedure leaving the preparation ready for a cover slip or counter staining with 1% toluidine blue in 1% aqueous borax before covering.

Photographs were taken using a Zeiss Universal Research Microscope.

2.5 Measurement of abscission

The decrease in strength of the abscission zone was monitored as the force required to cause separation at the abscission zones. Measurements were made by clamping the cut raspberry stalk in a pair of artery clamps. These were suspended from a Chatillon strain guage (John Chatillon and Sons, Kew Gardens, New York, U S A) graduated in 5 g increments. The berry was then held between thumb, forefinger and second finger, to spread the load, and pulled vertically downwards until the drupelets and receptacle separated. This gave a value termed the fruit retention strength (FRS), a measurement of the integrity of the population of abscission zones within the berry.

In accordance with the American Society of Horticultural Science recommendations (Kader, 1982) the FRS data has been expressed as Newtons. However since other work has been presented simply as grams force (Jolliffe, 1975a; Mason 1976) this has been included in the initial results to allow direct comparisons.

Work already published by Fejer and Spangelo (1973) and Mason (1974) advocated the use of a Correx tension guage to measure FRS. This method suffered from the complication that the force is applied in an arc rather than straight along the long axis of the berry. As a consequence the Correx guage had to be carefully turned to ensure the force was always being applied along the long axis of the berry. The Correx guage uses a C ring to pull against the base of the drupelets, the pedicel being held in the hand. This proved awkward since the sepals interfered with its positioning and had to be carefully moved or cut off for each reading, a time consuming process. Hence the Chatillon strain guage was considered much more

convenient and just as accurate and was adopted. It was later found that a similar technique had previously been employed prior to the use of the Correx by Warren and West (1973). Neither Jolliffe (1975a) nor Coulthard (1968) specified their methods.

Mason (1976) has also used an Instron Tensile Testing Machine to determine fruit retention stengths. Whilst giving excellent data on load/time relationships for the fruit retention strengths of individual fruit this method was not suitable for the rapid collection of large amounts of data, especially in the field.

2.6 Measurement of ethylene production rates

All ethylene production rates were measured at 25°C on the basis of allowing ethylene given off by the material to accumulate in a glass container of known volume. Sealing the container for a known time with a suba seal allowed sampling of the headspace which could then be analysed for its ethylene content using a gas chromatograph. The choice of size of container was critical in that material was only sealed for the minimum time necessary to accumulate a measurable level of ethylene. It was also important not to damage the material since this causes the release of wound induced ethylene (Abeles, 1973). Temperature was maintained using a constant temperature room or a water bath at 25°C.

2.6.1 Gas chromatograph optimisation

Initially a Perkin Elmer F11 gas chromatograph was used although later a Pye model 104 G.C. was also employed. Both machines were optimised for the maximum peak height for a known amount of ethylene whilst maintaining its separation from other hydrocarbon peaks (Ward et al., 1978). The major separation problem was between ethylene and ethane, the latter being produced naturally by plant material through the lipid peroxidation occurring in damaged tissues (Riely et al., 1976; Konze and Elstner, 1978; Harber and Fuchigami, 1986). The variables assessed were oven temperature, carrier gas flow rate (N_2) and the hydrogen supply to the flame ionisation detector. Separation was checked using a standard gas mixture of methane, ethane and propane (Phase Separation Ltd, Queensferry, Clwyd, UK) to which known amounts of ethylene were added. Increasing the oven temperature or carrier flow rate

increased the peak height, however it also reduced the degree of separation between the ethylene and ethane peaks. Likewise increasing the hydrogen pressure up to a point increased the peak height, thereafter it declined. These changes are illustrated in fig. 2.1. The maximum peak heights attainable however did not give sufficient separation between ethylene and ethane peaks and hence the optimised settings did not directly equate to the maximal peak height for ethylene. Optimised conditions for the Perkin Elmer F11

Table 2.2 Optimal settings for gas chromatographic determinations of ethylene

Machine	Perkin Elmer F11	Pye 104
Column	Steel	Glass
Column size	200 cm x 3.5 mm	291 cm x 6 mm
Packing	Alumina F_1 (80-100 mesh)	Alumina F_1 (80-100 mesh)
Oven temperature	50°C	80°C
Carrier flow rate	40 ml min^{-1}	40 ml min^{-1}
Hydrogen setting	18 lb in^{-2}	18 lb in^{-2}
Air setting	18 lb in^{-2}	16 lb in^{-2}
Ethylene retention time	110 seconds	120 seconds
Mimimum detectable ethylene	30 nl l^{-1}	30 nl l^{-1}

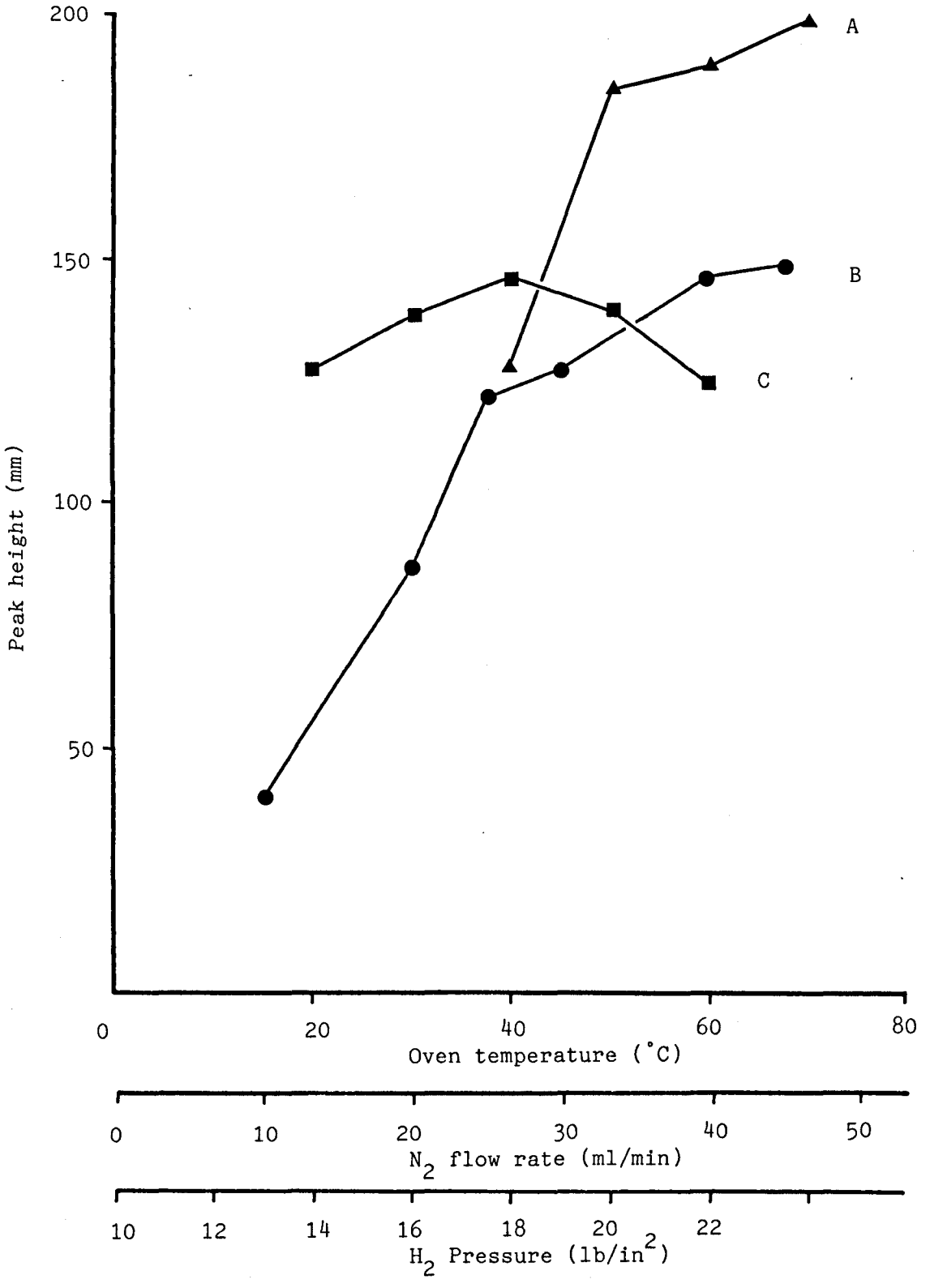


Fig. 2.1 Effect of changing different machine variables in maximising peak height of 1 ml of 5 ppm ethylene injected onto a 200 cm x 3.5 mm Alumina F_1 column attached to a Perkin Elmer F11 G.C.
A - variable oven temperature, N_2 carrier gas 30 ml/min, H_2 16 lb/in^2.
B - variable N_2 carrier, oven temperature 50°C, H_2 16 lb/in^2.
C - variable H_2 pressure, oven temperature 50°C, N_2 carrier gas 40 ml/min.

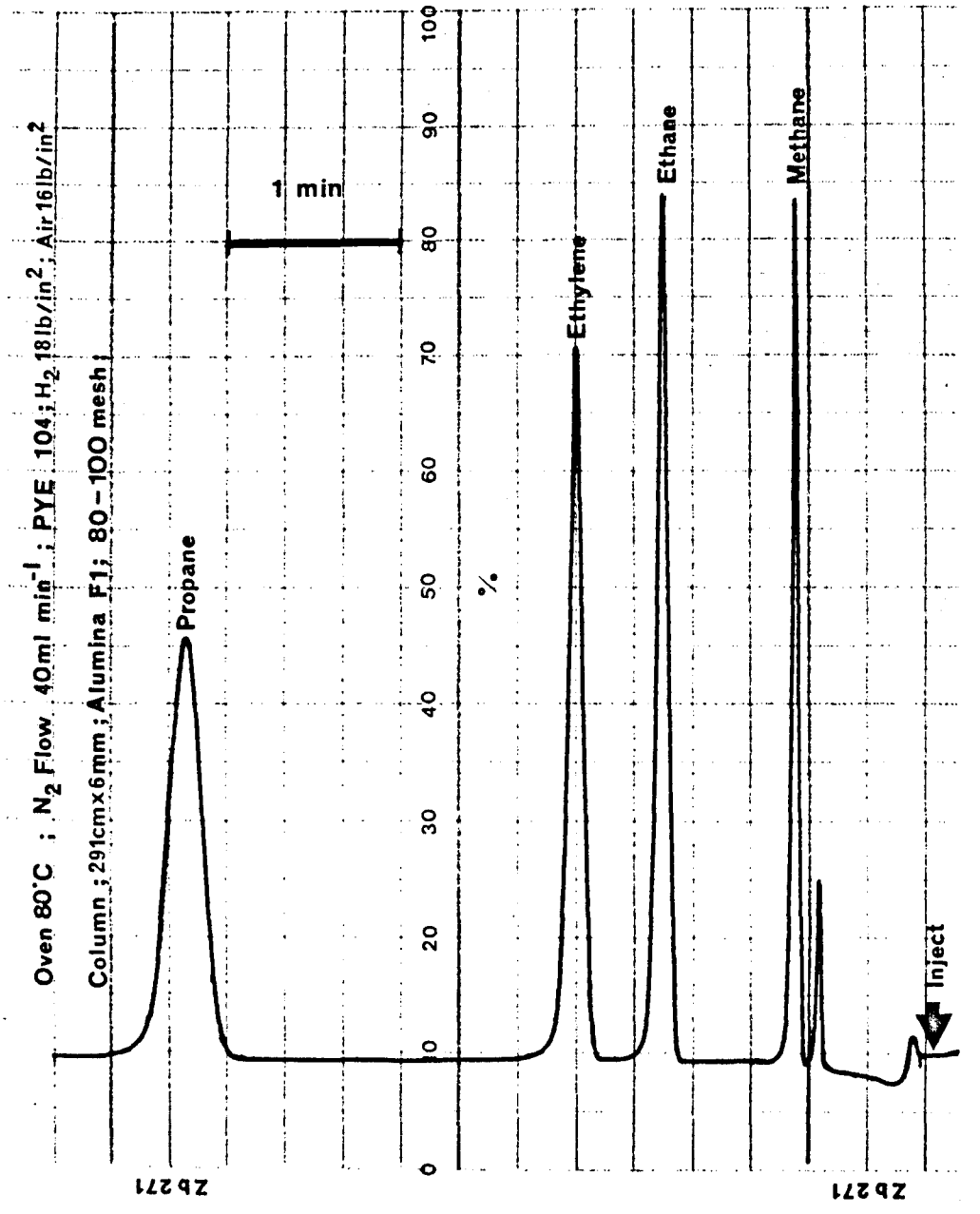


Fig. 2.2 Sample trace of optimum hydrocarbon separation using the Pye 104 Gas Chromatograph. Ethylene peak from 1 ml of 5 ppm ($\mu l\ l^{-1}$) ethylene, the other peaks are from a hydrocarbon mixture, each of approximately 0.1%.

and Pye 104 gas chromatographs are given in table 2.2. Two column packings were examined Poropak R and Alumina F_1 (J J's Chromatography, Kings Lynn, England). The Alumina F_1 column when optimised gave larger ethylene peaks (27% higher) and hence was adopted for future analysis. A sample trace showing hydrocarbon separation by the Pye 104 is shown in fig. 2.2 a 1 ml sample of the gas mixture containing 5 nl ethylene was injected. When removing 1 ml headspace samples for analysis 1 ml of ethylene free air was injected and mixed prior to sampling. This maintained the atmospheric pressure on sampling whilst increasing the total headspace volume by 1 ml.

2.7 Berry fresh and dry weights

Fresh weights were taken for whole fruit with the pedicel removed as completely as possible. In determining separate drupelet and receptacle weights the two could be separated easily at and past the mottled stage of development. In green fruit the receptacles had to be cut out with a scalpel. When receptacles were divided into the apex and calyx regions the point of separation was marked by the lowest abscission zones (fig. 1.2).

Dry weights were obtained by drying material in an oven at $80^{o}C$ until three consecutive daily weighings were identical. Weights were recorded prior to drying to allow the percentage dry matter to be determined.

2.8 Measurement of respiratory and photosynthetic rates

Changes in carbon dioxide production during raspberry development were monitored using a Series 225 Gas Analyser (Analytical Development Co., Ltd, Hoddesden, Herts, England) set for analysis on the absolute mode. The closed circuit was kept as short as possible with the inclusion of a 24 cm x 3 cm piece of silver foil coated glass tubing as a sample chamber. The system was calibrated by injecting 100 µl of pure CO_2 through a suba seal, the response being linear over the range 0-400 µl CO_2 (fig. 2.3). When 10 fruit were placed in the chamber the CO_2 was slowly allowed to accumulate over a 5 minute period and the rate determined. The IRGA was then rezeroed by flushing with air from outside the constant temperature room in which the apparatus was housed and the process repeated several times. Readings were all taken in the dark at 25°C to eliminate photosynthetic and photorespiratory changes.

A crude measurement of photosynthetic activity was obtained by measuring the CO_2 flux in the light and in the dark, the difference being taken as representing photosynthetic fixation. The experiments were carried out in a constant temperature room at 25°C. A Kodak Carousel S-AV projector positioned 2 feet from the sample chamber was used as a light source (160 $\mu E/m^2/s$). This method omitted any consideration of changes in respiratory CO_2 fluxes in the light and dark. It did however provide a crude assessment of changes in the photosynthetic activity of fruit at different stages of development.

Oxygen fluxes in fruit at different stages of development were also examined to back up the respiratory CO_2 data.

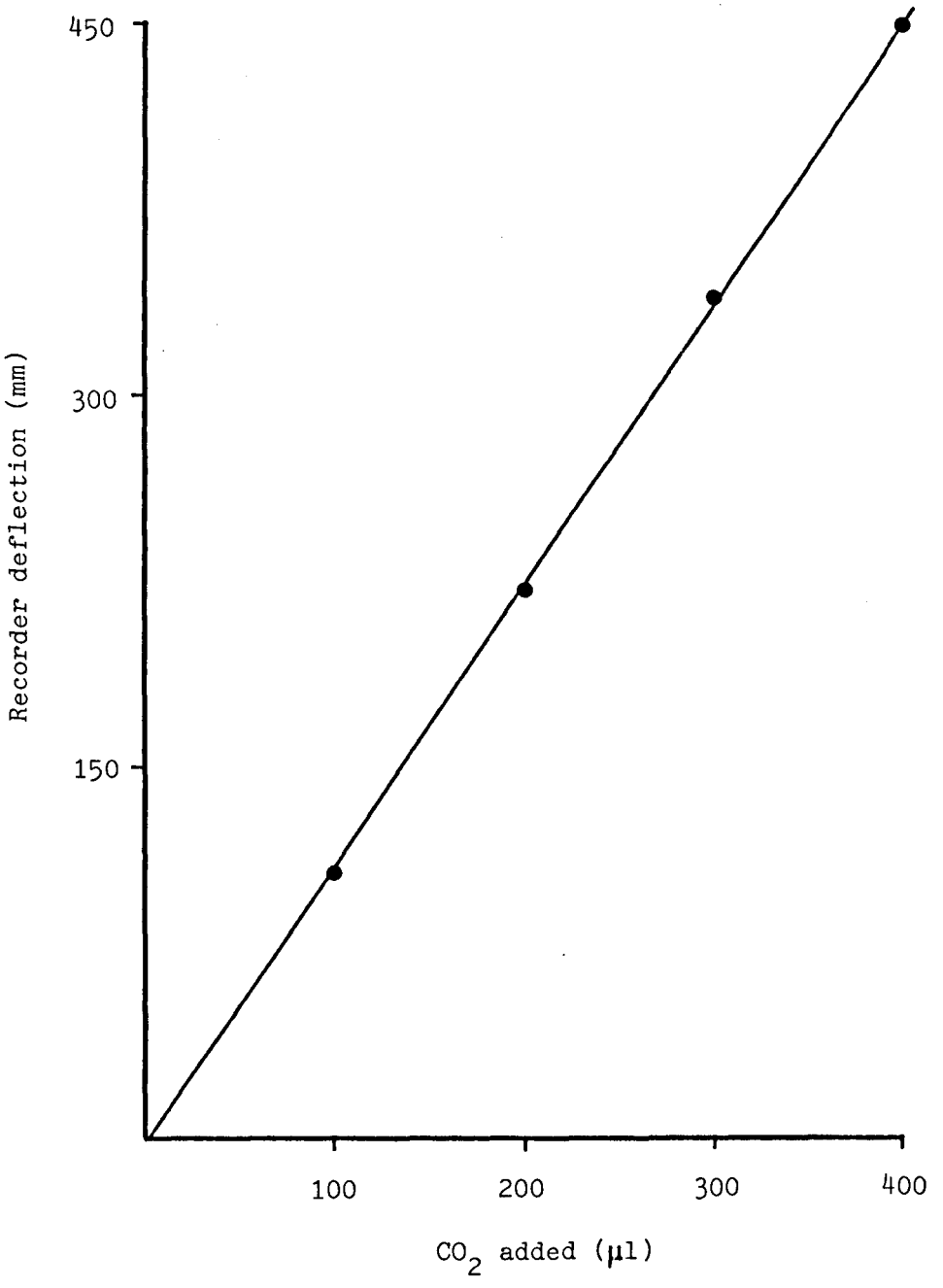


Fig. 2.3 Effect of cumulative additions of 100 µl of carbon dioxide to a sealed atmosphere monitored for carbon dioxide by IRGA.

Approximately 10 fruits were placed into 150 ml Warburg flasks containing 5 ml of carbonate/bicarbonate buffer in a central well to maintain CO_2 levels. The flasks were attached to a Gilson Respirometer (Gilson Medical Electronics Inc., Middleton, Wisconsin, U S A) and the oxygen fluxes determined in the dark at 25°C.

2.9 Extraction and assay of chlorophyll

Chlorophyll levels were determined by the method of Wintermans and de Mots (1965) using 96% ethanol as an extraction medium. Although this may not be the most recent or efficient method (for example Fuke et al. 1985) it was considered sufficient to quickly provide the comparative data required to assess treatments and differences between the stages of development.

Drupelets were extracted by grinding in 96% ethanol at 4°C and filtering under vacuum. The brei was resuspended and repeatedly extracted to remove all green colouration. The extract was then made to a known volume of which a 5 ml sub-sample was centrifuged for 5 mins at 5000 g to clarify. Absorbance was then measured on a Pye Unicam SP 1800 spectrophotometer (Pye Unicam Ltd, Cambridge, England) at 665 nm and 649 nm with 720 nm being used as the reference wavelength.

Chlorophyll levels were calculated as follows (ug/ml)

Chlorophyll a : [(13.7 x OD 665) - (5.76 x OD 649)] x DF

Chlorophyll b : [(25.8 x OD 649) - (7.6 x OD 665)] x DF

Total Chlorophyll : [(6.1 x OD 665) + (20.04 x OD 649)] x DF

$$\text{Dilution factor (DF)} = \frac{\text{total volume of extract}}{\text{weight of sample}}$$

2.10 Extraction and assay of anthocyanins

The measurement of anthocyanins has been well documented (Harborne, 1958, 1963; Markakis, 1975) with much of the work being done on cranberries. Blundstone and Crean (1966) listed the anthocyanins in Rubus idaeus as cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-sophoroside and cyanidin-3-(2-glucosylrutinoside). Lees and Francis (1972) stated that their extraction procedure was suitable for measuring raspberry anthocyanins. This was based on the method of Fuleki and Francis (1968) as was a rapid method developed by Deubert in 1978. Eventually the extraction method of Deubert (1978) was adopted for raspberry drupelets to give a rapid method of comparing treatments and differences between developmental stages.

Drupelets were extracted in an equivalent volume of 95% ethanol:1.5 N HCl (85:15 v/v) by pulping in an MSE homogeniser for 3 minutes at full speed. The resultant slurry was made to a known volume with extraction medium and mixed thoroughly. A 10 ml sub-sample of slurry was filtered under vacuum through Whatman No 1 paper and the pulp was resuspended in a further volume of extraction medium (equal to the inital volume). Afer vacuum filtration the extracts were combined and an aliquot diluted 1:8 with extraction medium. This final extract was clarified by centrifuging at 5000 g for 5 mins before reading the absorbance at 536 nm (absorbance maximum of extract) using a Pye Unicam SP1800 spectrophotometer. The total anthocyanin content was calculated according to Fuleki and Francis (1968) and expressed as ug anthocyanin per gram fresh weight using the equation below. The total OD (TOD) is calculated first so the OD measured on a small aliquot of the diluted extract will apply

to a known quantity of drupelets. The TOD for 100 ml extract is calculated below:

TOD = OD x DV x VF

where OD = absorbancy reading of the diluted sample at 536 nm using a 1 cm cell

DV = volume of the diluted extract prepared for OD measurement (mls)

VF = corrects for the difference in size between 100 ml, the original volume (OV), for which the calculation is made and that of the sample volume (SV), or the volume of extract used for the absorbancy measurement

VF = OV/SV = 100/SV

To express the TOD for 100 g, the TOD calculated for 100 ml extract must be brought up to this level by using the following equation.

TOD per 100 g = TOD for 100 ml extract x TEV/W

where TEV = total balance of extract obtained from the drupelet sample extracted (mls)

W = sample weight of drupelet extracted (g)

The total anthocyanins in mg per 100 g drupelet extract was calculated using the extinction coefficient of 982 established by Fuleki and Francis (1968). The E value of 982 is for a 1% solution (10 mg/ml) whereas an E/10 value was used in the calculation of total anthocyanin:

$$\text{Total anthocyanins mg per 100 g} = \text{TOD} \times \frac{10}{982}$$

The above formulae reduce to

$$\text{Total anthocyanins (mg/100 g)} = \text{OD} \times \text{DV} \times \frac{100}{\text{SV}} \times \frac{\text{TEV}}{\text{W}} \times \frac{1}{98.2}$$

2.11 Measurement of drupelet reducing sugar levels

The method of Nelson and Somogyi (Spiro, 1966) was used to measure changes in reducing sugars during development using the following reagents:

Low alkalinity copper reagent:

12 g potassium sodium tartrate (Rochelle salt) was dissolved along with 24 g anhydrous sodium carbonate in 250 ml distilled water. A solution of 4 g cupric sulphate pentahydrate in 25 ml of distilled water was added with stirring, followed by 16 g sodium hydrogen carbonate. A solution of 180 g anhydrous sodium sulphate in 500 ml distilled water was boiled to expel air. The two solutions were then combined and diluted to 1 litre. The clear supernatant was used after standing for 1 week.

Arsenomolybdate reagent:

25 g ammonium molybdate was dissolved in 450 ml distilled water. To this was added 21 ml of 96% sulphuric acid, followed by 3 g disodium hydrogen arsenate heptahydrate dissolved in 25 ml distilled water. The mixed solution was incubated at 37°C for 24 hours and stored in a glass stoppered brown bottle.

Drupelets from 20 berries were weighed and homogenised in distilled water to a final volume of 100 ml. The pulp was centrifuged at 4°C for 20 minutes at 20000 rpm giving a supernatant for analysis and a pellet to be discarded. The supernatant was diluted by a factor of 50 prior to assay. In the case of ripe drupelets this was found to be insufficient and a

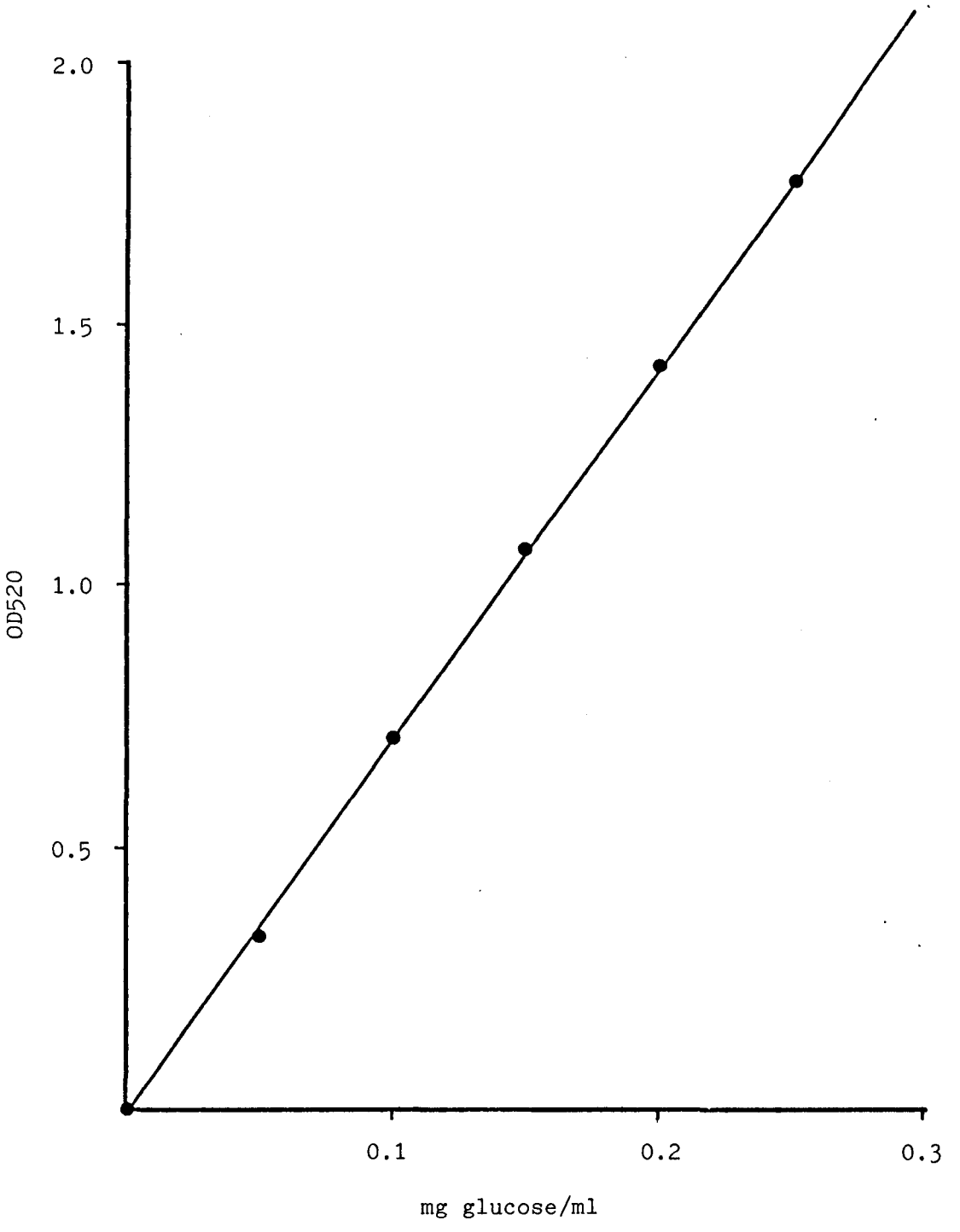


Fig. 2.4 Calibration curve for the OD520 resulting from the reaction of known amounts of glucose in the Nelson-Somogyi reducing sugar assay.

dilution of 100 was used.

For assay 1 ml of the low alkalinity copper reagent was added to 1 ml of the diluted extract and placed in a boiling water bath for 10 minutes. After cooling 2 ml of the arsenomolybdate reagent was added and vortex mixed until gas evolution ceased. Three ml of distilled water was added and the tube allowed to stand for 15 minutes prior to reading the liquids OD at 520 nm.

A standard curve of glucose concentration against OD was shown to be linear over the range zero to 0.25 mg glucose per sample (Fig. 2.4). Results were expressed as equivalent glucose levels per gram fresh weight or per berry.

2.12 Measurement of drupelet titratable acidity

The accepted convention (A.O.A.C. 1960) for expressing titratable acidity is as mls 0.1 N NaOH per 100 mls of pressure expressed juice titrated to pH 8.6 against phenolphthalein indicator. Whilst this is suitable for the ripe end of the spectrum of development (Mason, 1974) it is hardly suitable for the very small green berries. Since there was not going to be an in depth study of acidity, liquid was simply taken immediately after centrifugation in the reducing sugar assay (fruit having been homogenised in distilled water). The acidity was examined without dilution by titrating 1 ml of extract to pH 7 using 20 mM NaOH, results being expressed at acidity per gram fresh weight or per berry.

2.13 Addition of ethylene to fruit in the laboratory

Two methods of ethylene addition were used depending on the type of experiment being undertaken. The effect of ethylene on the time course of abscission was examined by enclosing excised fruit at different stages of development in 22 litre gas tight perspex chambers. Two chambers were used, one with a throughflow of 1.5 $lmin^{-1}$ of ethylene free air, the other with a throughflow of 1.5 $lmin^{-1}$ of air into which ethylene (BOC Special Gases, London, England; 5000 ppm) had been bled to give a final concentration of 40 ppm ($\mu l\ l^{-1}$). The bleed rate of ethylene was controlled by a needle valve (Edwards Vacuum Components, Crawley, England). The air flow was maintained by a pump (Charles Austen Pumps Ltd, Byfleet, England) and regulated by a flow meter (GEC Elliot Process Instruments Ltd, Croydon, England). The level of 40 $\mu l\ l^{-1}$ was considered to saturate any ethylene mediated process whilst being non-toxic (Sexton et al., 1985) and lower levels were found to be difficult to maintain accurately with the available needle valves. The concentration in the chamber was checked off the outlet pipe by withdrawing 1 ml samples for gas chromatographic analysis (as determined in Section 2.6). Both chambers were kept in a constant temperature room at 25°C in constant light (25 $\mu E/m^2/s$).

Throughout the duration of the experiment fruit were maintained transpiring distilled water by placing them on a 15 cm x 10 cm sheet of 1 mm polystyrene through which the cut pedicels protruded. These sheets were floated on distilled water 1 cm deep in 17 cm x 11 cm perspex dishes. This was considered necessary because of the study by Warren and West (1973) indicating the force required for abscission to be dependent on the water

Plate 2.2 Illustration of the method of allowing fruit to transpire water during experimentation. This example shows soft green fruit which have been kept in air or 40 ppm ethylene for 24 hours.

status of the fruit, and the drying effect of the air flow through the cabinets. Samples of fruit (exact numbers given in the results) were removed after 24 hours and 48 hours for FRS and EPR analysis.

To determine the threshold level of ethylene required to influence abscission soft green and hard green fruit were sealed in 6 l desiccators containing known ethylene concentrations. Fruit were floated on water (as described above, see plate 2.2) for the duration of the experiment. A range of ethylene concentrations up to 50 ppm were examined for their effect on these green fruit. The required concentrations were achieved by sealing the fruit in a desiccator and then evacuating it slightly using an Edwards EB3 vacuum pump (Edwards Vacuum Components, Crawley, England) attached via a single necked stopper with a tap. The desiccator was isolated by the tap and the vacuum pump switched off and disconnected. A suba seal was placed over the end of the neck to allow the injection of a known amount of ethylene into the space between the suba seal and the tap. On opening the tap and removing the suba seal the added ethylene was drawn into the desiccator and mixed by the inrushing air. Once set up the desiccators were kept in the constant temperature room at 25^{o}C in constant light (25 $\mu E/m^2/s$). The atmospheres within the desiccators were checked and renewed every 12 hours to ensure no build up of ethylene (none was ever found) and prevent serious fluctuations in carbon dioxide and oxygen.

2.14 Treatment of fruit with hypobaric pressures

Low (hypobaric) pressures were first used by Burg and Burg (1966) to prolong the storage life of fruit. Since then several authors have reported abscission to be reduced or inhibited by hypobaric treatments at pressures between 100 and 200 mmHg (Cooper and Horanic, 1973; Lipe and Morgan, 1973; Rasmussen, 1974; Morgan and Durham, 1980; Sipes and Einset, 1982). The treatment is generally considered to have its effect through a lowering of the ethylene level within the tissues being treated (see Section 1.3).

Fruit at different stages of development were maintained transpiring water (as detailed in Section 2.13) throughout the experiments at reduced pressures. The dishes containing the fruit were kept in 6 l desiccators. Normally two desiccators were connected in series to an Edwards EB3 vacuum pump (Edwards Vacuum Components, Crawley, England) to provide the reduced pressure of 150 mmHg. Air was drawn into the desiccators through a 500 ml conical flask containing 200 ml distilled water. Air entered the flask from a supply line of ethylene free air entering under the surface of the water through a 100 µl Hamilton syringe. Full apparatus is illustrated in fig. 2.5.

The pressure in the desiccators was continually monitored by a vacuum guage inserted into the air supply line between the flask and the first desiccator. The rate of gas flow through the system was regulated by a gate clip the other side of the flask to the guage. Leaks in the system were checked for by reducing the pressure to 150 mmHg and sealing the system before the conical flask and after the second desiccator. If the vacuum was not maintained there were leaks in the system. This may be important because the

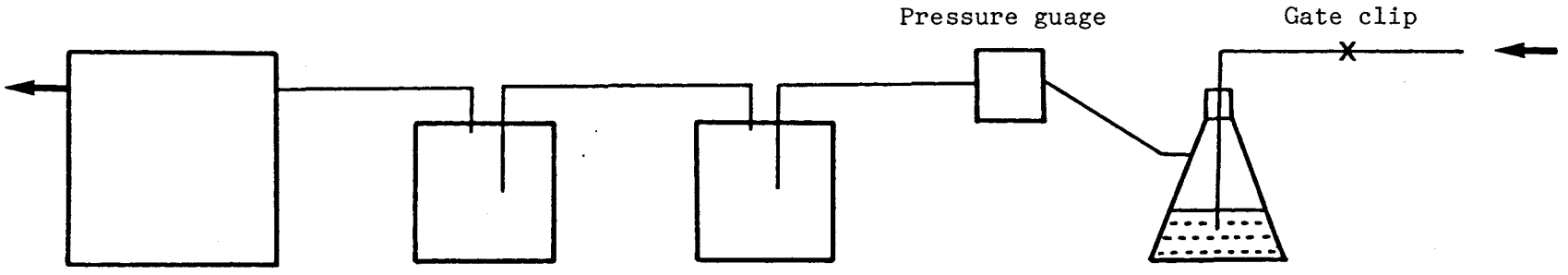


Fig. 2.5 Diagramatic representation of the apparatus used in hypobaric treatments. Reinforced tubing was used to link the conical flask pressure guage and desiccators to the vacuum pump.

pump was capable of maintaining the hypobaric pressure even with air leaking in. The extra air flow would however dilute any ethylene additions in control experiments. The whole apparatus was maintained at 25°C in constant light (25 $\mu E/m^2/s$) with samples being removed for FRS and EPR measurement after 24 hours and 48 hours. Sample sizes are detailed in the individual results.

The use of hypobaric pressures is not a very specific treatment yet is considered to reduce the internal ethylene levels of plant material as a result of the following factors:
i) an immediate drop in ethylene partial pressure as gases leave the tissue, ii) an increase in diffusive loss from the tissue as the gas phase becomes less dense, iii) a reduced retention as a result of low pressure effects on ethylene solubility, iv) a reduced ethylene biosynthesis and metabolism as a result of limited oxygen availability (Nilsen and Hodges, 1983).

A major problem encountered when using hypobaric treatments is that besides specifically effecting ethylene levels the treatment also reduces the oxygen partial pressure. This complication can be overcome by drawing pure oxygen through the apparatus at 150 mmHg thereby maintaining the normal atmospheric partial pressure of oxygen.

Unfortunately the oil free pumps required to do this safely were not available. An alternative control experiment to ensure hypobaric pressures influenced abscission through the ethylene level alone was to add a saturating level of ethylene to the test material under hypobaric conditions. The exogenous ethylene should reinstate any abscission inhibited by the hypobaric treatment assuming there to be no other limiting factors. The addition of ethylene at

150 mmHg was achieved by drawing 250 $\mu l\ l^{-1}$ ethylene in air into the system instead of ethylene free air. This resulted in an ethylene partial pressure of 5×10^{-5} atmospheres at 150 mmHg, a level considered to saturate ethylene mediated processes (Sexton et al., 1985).

2.15 Addition of chemicals to fruit

Chemicals were added to fruit attached to the canes in greenhouses, or detached in the laboratory. Detached fruit could be treated simply by agitating them in the desired solutions without the use of wetting agents. This method supplies the chemical mainly to the drupelets. Supply through the transpiration stream was considered a better method of supplying the whole fruit, assuming the vascular connections to be intact. This was achieved by placing individual fruit on Eppendorf centrifuge tubes full of the test solution into which the cut pedicels dipped. Individual fruit could thus be checked to ensure the solution had been taken up. In experiments where the chemical was to be supplied continuously the fruit were placed on sheets of 1 mm polystyrene with their cut pedicels protruding through into the test solution (see Section 2.13).

After treatment all fruit were maintained transpiring distilled water as described in Section 2.13. The dishes containing the fruit were kept in a constant temperature room at 25°C in constant light (25 $\mu E/m^2/s$).

Fruit treated attached to the canes could not be dipped as above since agitation to cause wetting was impracticable. Wetting agents could not be used since Triton X-100 was found to cause drupelet discoloration and collapse even at 0.005%. Hence fruit were eventually treated by spraying individually using an Aerograph Imp artists spray brush (De Vilbiss, Bournemouth, England). This allowed the accurate selection of those fruit to be treated with the fine aqueous spray being sufficient to cause wetting. The plants used for these experiments were all maintained in a greenhouse

(12°C minimum, 16 hour days) simply to eliminate wash off by rain.

The chemicals examined either inhibited ethylene production (AVG, Co^{2+}), prevented ethylene action (STS), increased ethylene levels in the tissues (Ethrel) or altered the hormonal status of the fruit (auxin). The concentrations of chemicals used and the numbers of fruit treated in individual experiments are detailed in the results.

Aminoethoxyvinylglycine (Fluorochem, Glossop, England) prevents the conversion of SAM to ACC by ACC synthase, a pyridoxal linked enzyme (Boller et al., 1979; Yu et al., 1979), thereby depriving the ethylene forming enzyme of its substrate, ACC. The concentration of AVG used in previous work to slow abscission has varied between 10^{-6} and 10^{-3}M depending on the system being examined (eg Sagee et al., 1980; Kushad and Poovaiah, 1984). Raspberry fruit abscission was examined using 10 mM AVG when applied by dipping or spraying and 100 µM AVG when taken up in the transpiration stream.

Cobaltous ion (Co^{2+}) administered as the chloride has been successfully used over the range 10 µM to 10 mM to prevent ethylene production through inhibiting the ethylene forming enzyme in numerous systems (Lau and Yang, 1976; Yu and Yang, 1979; Samarakoon, 1984). Yu and Yang (1979) found 1 mM Co^{2+} to be saturating with no increase in effectiveness up to 10 mM. Raspberry fruit were treated with 1 mM Co^{2+} when dipped and 100 µM Co^{2+} when allowed to transpire the solution.

The silver ion (Ag^{+}) was shown to be an effective inhibitor of ethylene action by Beyer (1976), probably through an interaction at the ethylene binding site (Sisler, 1982). The use of

Ag^+ as STS has been reviewed recently by Veen (1983) who stated that 0.2 mM Ag^+ was regarded as not being phytotoxic yet was highly active physiologically. Hence this concentration was used in examining the effect of STS on raspberry fruit abscission. The STS complex was prepared by mixing equal volumes of $AgNO_3$ and $Na_2S_2O_3$ $5H_2O$ in a molar ratio of 1:4 to give the desired Ag^+ concentration in the final STS solution.

Ethrel (A. H. Marks & Co., Ltd, Wyke, Bradford) utilising 2-chloroethylphosphonic acid (CEPA) as its active ingredient has been well documented in horticultural journals as a method of increasing tissue ethylene levels where gaseous applications of ethylene are impractical. In the presence of a base CEPA is degraded to release ethylene (Warner and Leopold, 1969). Raspberry fruit were treated with 10 mM Ethrel by both dipping and spraying.

Auxin is generally considered to be antagonistic to ethylene in abscission, its application reducing abscission. Its effect however depends on the timing of the application. If application is delayed it can in fact accelerate abscission through its ability to enhance ethylene production (see Section 1.2). Auxin in the range 10^{-5} to 10^{-2}M is considered to inhibit the accelerating effect of ethylene on abscission (Sexton et al., 1985). Napthaleneacetic acid (NAA) is often used in preference to indoleacetic acid (IAA) since as a synthetic auxin it is considered not to be degraded as rapidly as IAA. Raspberry fruit were treated with 10 mM NAA by spraying and 0.1 mM IAA by dipping and transpiration.

2.16 Measurement of internal levels of ethylene

Sampling the internal atmosphere of plant tissues by vacuum extraction under liquid has been used for a long time (Magness, 1920). Various liquids have been employed including Hg (Marcellin, 1963), saturated NaCl (Maxie et al., 1965), water (Staby and de Hertogh, 1970) and saturated $(NH_4)_2SO_4$ (Beyer and Morgan, 1970; Yeang and Hillman, 1981).

The method employed to measure ethylene in the internal atmospheres of raspberries was essentially the same as that of Yeang and Hillman (1981) which was itself based on that of Beyer and Morgan (1970). Initially whole fruit were examined with a minimum of 20 fruit per sample. The material to be extracted was first swirled in a 0.01% solution of Triton X-100 to help prevent air adhering to the fruit later during vacuum extraction. The fruit was then placed into an upturned 250 ml reagent bottle with its bottom removed. This was then placed right way up in a 500 ml beaker full of saturated $(NH_4)_2SO_4$ and shaken to remove any air adhering to the fruit. A suba seal was inserted into the top of the bottle and any air trapped below it removed using a syringe. The beaker containing the jar and fruit was then put into a 6.5 l desiccator and a vacuum of 100 mmHg applied for 30-45 seconds using an Edwards EB3 vacuum pump. On releasing the vacuum the $(NH_4)_2SO_4$ replaced any gas removed from the fruit leaving the gas trapped under the suba seal. The trapped gas was analysed for ethylene directly by gas chromatography (Section 2.6).

It was later decided to examine the internal ethylene concentrations of the drupelets and receptacles separately. This could only be achieved for fruit more mature than mottled where it

was possible to separate the drupelets and receptacles intact. Immediately on separation both drupelets and receptacles were dropped into a 0.01% Triton X-100 solution and submerged. This served the dual purpose of acting as a wetting agent and trapping the atmosphere within the tissue. The ethylene content of the receptacle was found to decrease by between 30% and 60% when kept in air for 3 or 4 minutes prior to submerging. Internal gases were extracted as quickly as possible since it could be argued that ethylene might accumulate in the submerged tissues if left too long. With 2 people preparing the fruit it was possible to collect enough receptacles and drupelets to complete an extraction and assay of both in 5 minutes.

2.17 The effect of drupelet development on receptacle developement

The dependence of the receptacle on the development of the drupelets was examined by preventing pollination of a portion of the stigmas. In raspberry flowers a tuft of stigmas protrude beyond the anthers and petals whilst the flower is still closed. Approximately half these protruding stigmas were cut off as soon as possible after the sepals had begun to open. The flowers were tagged and allowed to develop on the canes. When the fruit was ripe the drupelets were removed and the development of the receptacle examined.

2.18 The size and development of the receptacle related to fruit retention strength of ripe fruit from six varieties of raspberry

The receptacle dimensions of ripe fruit (as judged by drupelet colour and expansion) from the varieties Glen Clova, Glen Isla, Landmark, St Walfried, Phyllis King and the SCRI trial variety 68/14/106 were examined to see whether there was any relationship between shape and FRS. The receptacles were removed measuring the FRS and then split into apex and calyx regions which were weighed separately. The line between the two parts was marked by the final row of abscission zones (Fig. 1.2). Because of the large numbers of receptacles used (30 each of 6 varieties) the apices for each variety were placed onto a photocopier, covered with a piece of white paper and an enlarged copy taken. From these copies the dimensions of the receptacle apices could be measured later. The measurement of receptacle apex length and basal diameter allowed the shape of the receptacle to be assessed for comparisons with fruit retention strength. Similar measurements have been made before on individual raspberry varieties (Hill, 1958; Jolliffe, 1975a). In these cases the dimensions were not related to FRS and comparative data between varieties was not examined.

2.19 Extraction and assay of 1-aminocyclopropane-1-carboxylic acid

Since the report in 1979 by Lizada and Yang describing a simple and sensitive assay for ACC there have been numerous reports on the endogenous levels of ACC in plant tissues. Several extraction techniques have been used (references below) other than the original method. A variety of extraction techniques were evaluated to find the best method for use with raspberries. Initial experiments were carried out on ripe drupelets which have high EPR's since Hoffman and Yang (1980) showed the EPR to reflect the tissues' ACC content.

2.19.1 Extraction procedures

Extraction media examined included 5% sulphosalicylic acid, 80% ethanol plus 0.05% mercaptoethanol, 80% ethanol plus polyvinylpyrolidone (PVP), 9% trichloroacetic acid (TCA), 6% perchloric acid ($HClO_4$). Detailed procedures are as follows:-

a) Sulphosalicylic acid (as used by Lizada and Yang, 1979; Hoffman and Yang, 1980; Yoshii and Imaseki, 1981; Knee, 1984; Preger and Gepstein, 1984).

Ripe raspberry drupelets were homogenised at 4°C in 5% sulphosalicylic acid at a ratio of 2 ml per gram of drupelets. The resultant slurry was centrifuged at 4°C for 20 minutes at 15000 g and the volume of supernatant measured. A volume equivalent to 5 g of tissue was passed down a Dowex 50 H^+ ion exchange column (6 cm x 1 cm), washed with 3 bed volumes of distilled water and the ACC finally eluted with 20 ml of 2 M NH_4OH.

b) Ethanol plus 2-mercaptoethanol (Boller et al., 1979;

Hyodo et al., 1983).

Ripe raspberry drupelets were homogenised on ice in cold 80% ethanol containing 0.05% 2-mercaptoethanol at a ratio of 5 ml per gram. The resultant slurry was centrifuged at 8000 g for 15 minutes at 2°C and the supernatant volume measured.

c) Ethanol plus PVP (Evensen, 1984 and many others).

PVP was added to ripe raspberry drupelets at a ratio of 10 mg per gram of drupelets. This was sufficient to give the material a thin coating of PVP. The material was then homogenised on ice in cold 80% ethanol at a ratio of 5 ml per gram. The resultant slurry was centrifiged at 8000 g for 15 minutes at 2°C and the supernatant volume measured.

d) Trichloroacetic acid (Brecht and Kader, 1984a, b, c)

Ripe raspberry drupelets were homogenised on ice in cold 9% (w/v) TCA at a ratio of 2 ml per gram. The resultant slurry was centrifuged at 8000 g for 15 minutes at 4°C and the supernatant volume measured.

e) Perchloric acid (Bufler and Bangerth, 1983)

Ripe raspberry drupelets were homogenised on ice in cold 6% (v/v) $HClO_4$ at a ratio of 20 ml per gram. The resultant slurry was centrifuged for 15 minutes at 11000 g at 4°C and the supernatant volume measured.

2.19.2 Concentration of the extract

In all the above extraction procedures the final extract volume was measured so that a volume equivalent to a known weight of drupelets (usually 5 g) could be dried down in a rotary evaporator at 38°C. Drying down times were very different between the

extraction media with the ethanolic extracts being far quicker (well under 1 hour per sample) than the TCA, PCA, or NH_4OH from the sulphosalicyclic acid extraction (in excess of one hour). Once dried down the residue was taken up into distilled water to give a final concentration of 1 g ml^{-1} distilled water.

2.19.3 ACC assay

For the initial comparative work on extraction methods the exact assay method of Lizada and Yang (1979) was followed. Four hundred ul of the aqueous extract and 200 µl of 5 mM $HgCl_2$ were sealed in a 10 ml glass vial with a suba seal and kept on ice. One hundred µl of a cold mixture of 2:1 (v/v) NaOCl:saturated NaOH was injected into the vial. Bleach with 10-14% available chlorine was used as the source of NaOCl. The vial was then vortex mixed for 5 seconds and kept on ice for a further 2.5 minutes. The contents were then vortex mixed for 5 seconds prior to the removal of gas from the headspace for ethylene analysis by gas chromatography as previously described (Section 2.6).

The ethylene liberated was used to determine the samples ACC content using the calibration curve illustrated in fig. 2.6. The percentage conversion of ACC to ethylene was just under 80% with the reaction being complete in under 2 minutes Fig. 2.7. Both these parameters agree with values of 80% yield and 3 minutes reaction time as stated by Lizada and Yang (1979).

2.19.4 Evaluation of extraction procedures

Differences between the extraction techniques were assessed by adding known amounts of ACC (4 nmol per gram) to ripe drupelet

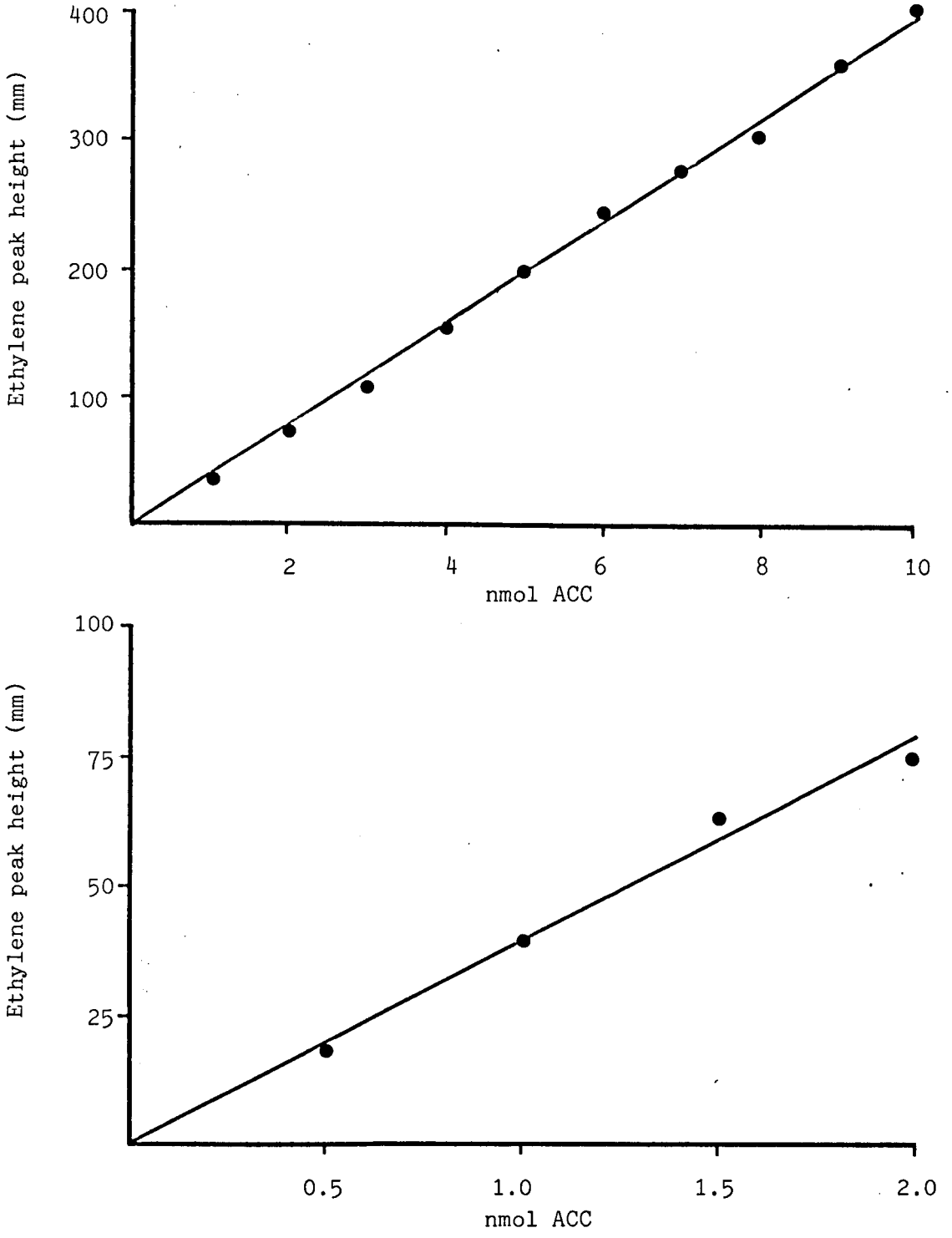


Fig. 2.6 Ethylene release from authentic ACC. Reactions carried out in 10 ml glass vials, final reaction volume of 1 ml containing 400 ul NaOCl/sat.NaOH and 200 ul of 15 mM $HgCl_2$. Headspace (1 ml) analyses for ethylene by G.C. after 3 minutes reaction.

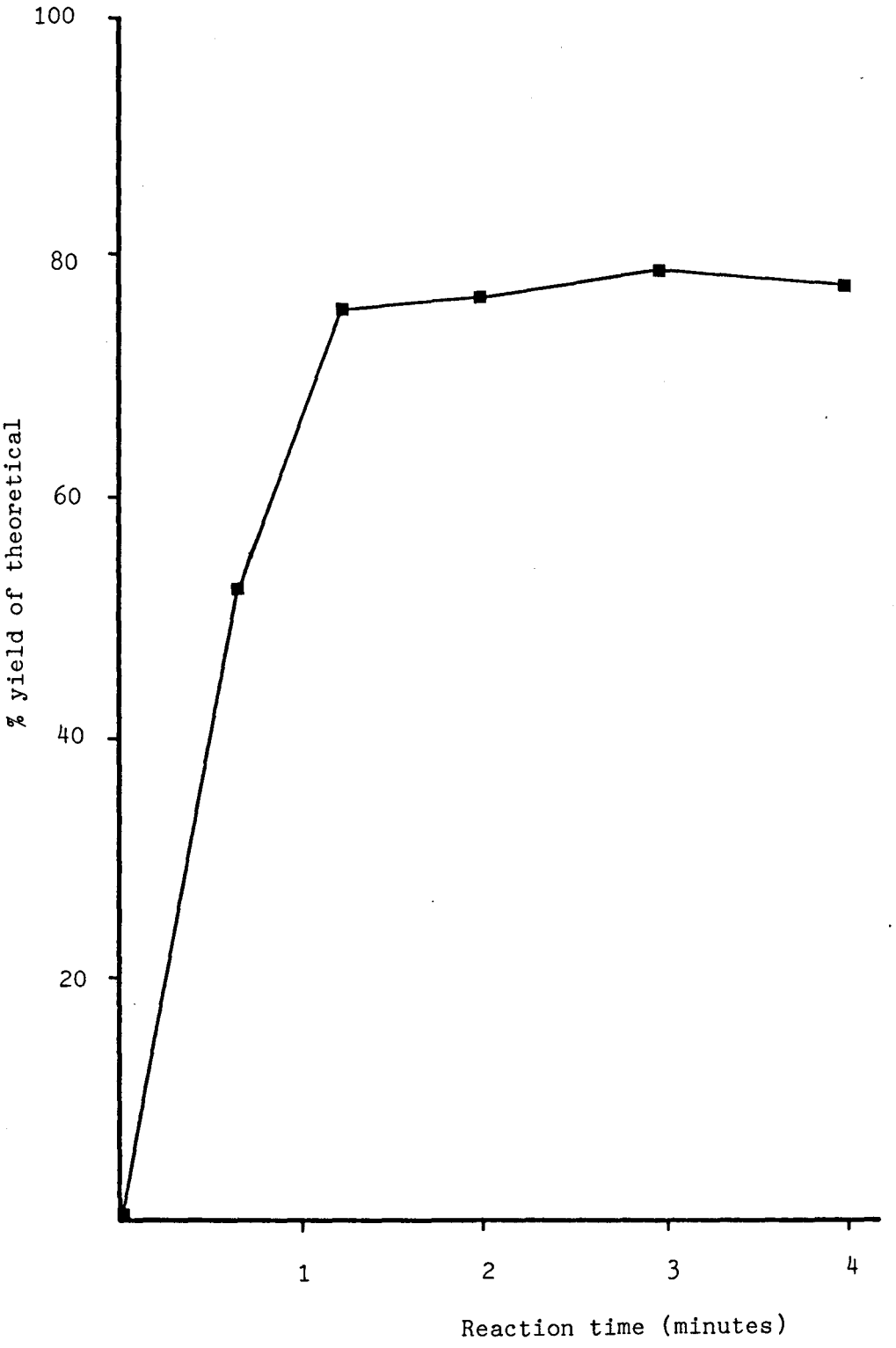


Fig. 2.7 Timecourse of ethylene release from 10 nmol authentic ACC in the presence of 200 µl 15 mM $HgCl_2$ and 400 µl bleach/sat. NaOH. Final reaction volume in a 10^2 ml glass vial was 1 ml using distilled water to balance. Ethylene release was determined by headspace analysis.

samples at the homogenisation stage. Comparison of the ACC levels between samples with or without added ACC were made and the percentage recoveries of the authentic ACC were determined. The results of these trials are presented in table 2.3.

Table 2.3 Evaluation of ACC extraction procedures

Extraction medium	Percentage recovery
Ethanol/PVP	62
TCA	60
Sulphosalicylic acid	48
Ethanol/mercaptoethanol	46
Perchloric acid	55

In their original paper Lizada and Yang (1979) added authentic ACC standards at a later stage, just prior to assay. Hence their percentage recovery being higher at 73%. From this initial examination it was shown that the recovery was highest using the ethanol/PVP extraction medium and the ethanolic extract was the quickest to dry down. As a result of these observations and the fact that the majority of recent papers reporting ACC measurements use simple ethanolic extractions, it was decided to use the ethanol/PVP extraction technique in future work.

2.19.5 Optimisation of the ACC assay

Having decided on an extraction medium it was then necessary to optimise the ACC assay to suit a raspberry extract. In these optimisation experiments 200 µl extract was used to give scope for additions/omissions of chemicals whilst still keeping the final reaction volume at 1 ml, the balance being made up with distilled water.

It soon became apparent that insufficient NaOCl/NaOH was being added to the experimental system, perhaps because the extracts contained large amounts of neutralising fruit acids. The results presented in fig. 2.8 indicated a volume of 400 µl of NaOCl/NaOH was necessary to saturate the raspberry extract system. This increase in ethylene release was a result of both extra NaOCl and NaOH, only some of the increase could be accounted for by NaOH alone.

The amount of Hg^{2+} in the reaction mixture was also reexamined in the presence of raspberry extract. Figures 2.9 and 2.10 show the different Hg^{2+} requirements of authentic ACC and ripe raspberry drupelet extract. Ten nmol authentic ACC required 1.0 µmole Hg^{2+} for maximal ethylene release whereas the ripe raspberry drupelets (containing $\sim$1.5 nmol ACC per aliquot) required 3.0 µmoles Hg^{2+}. Fruit assays were therefore carried out in the presence of 3 µmoles Hg^{2+}.

Having shown that the addition of raspberry extract changes the optimum assay conditions compared to Lizada and Yang (1979) the reaction time course was checked. Ethylene release from authentic ACC was reported to be complete in under 2 minutes by Lizada and Yang (1979) and this was confirmed (fig. 2.7) and found not to change in the presence of fruit extract.

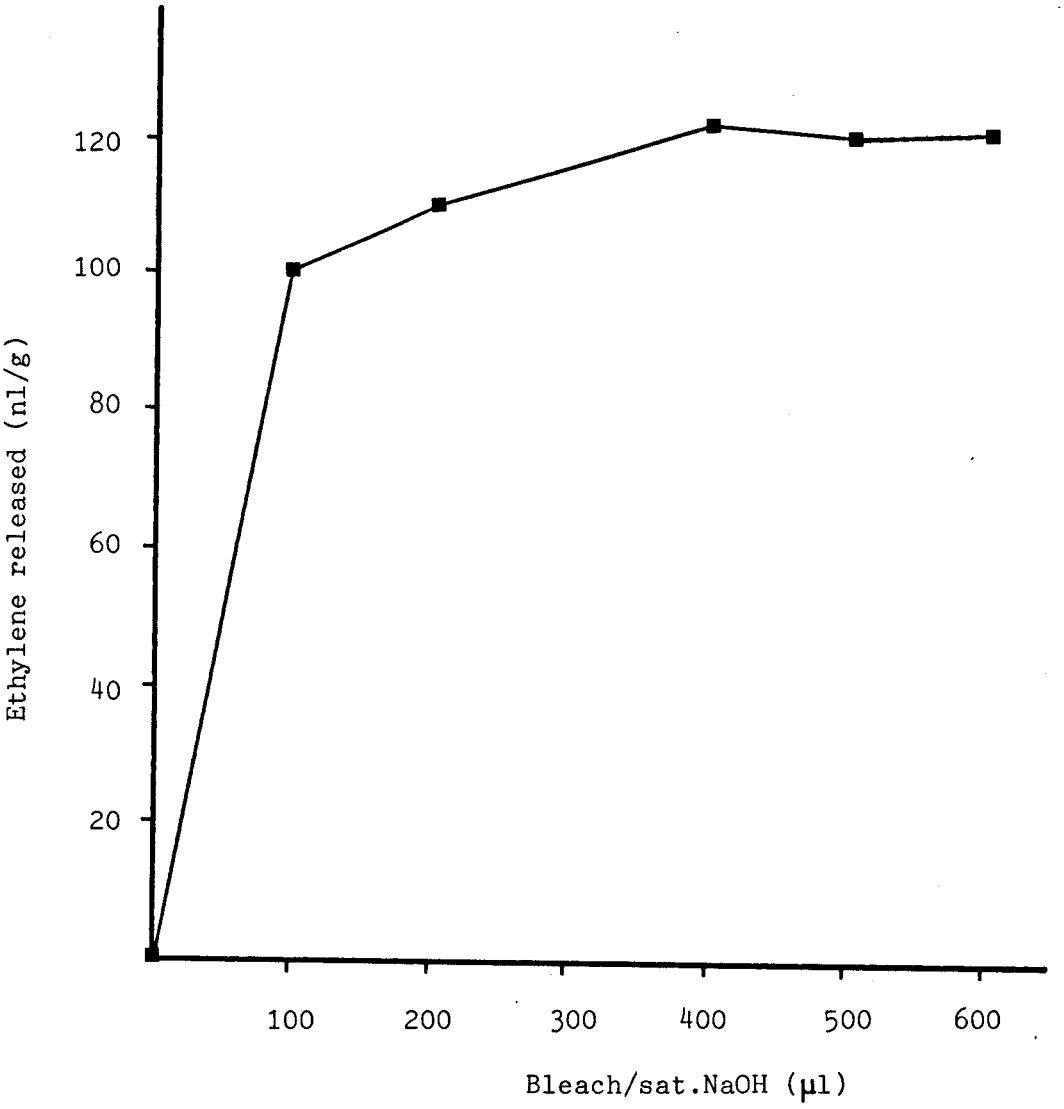


Fig. 2.8 Effect of increased additions of bleach/sat.NaOH to 200 µl ripe drupelet extract and 200 µl 15 mM $HgCl_2$. Final reaction volume in a 10 ml glass vial was maintained at 1 ml using distilled water to balance. Reaction time 3 minutes (after bleach/sat.NaOH addition) prior to headspace analysis for ethylene.

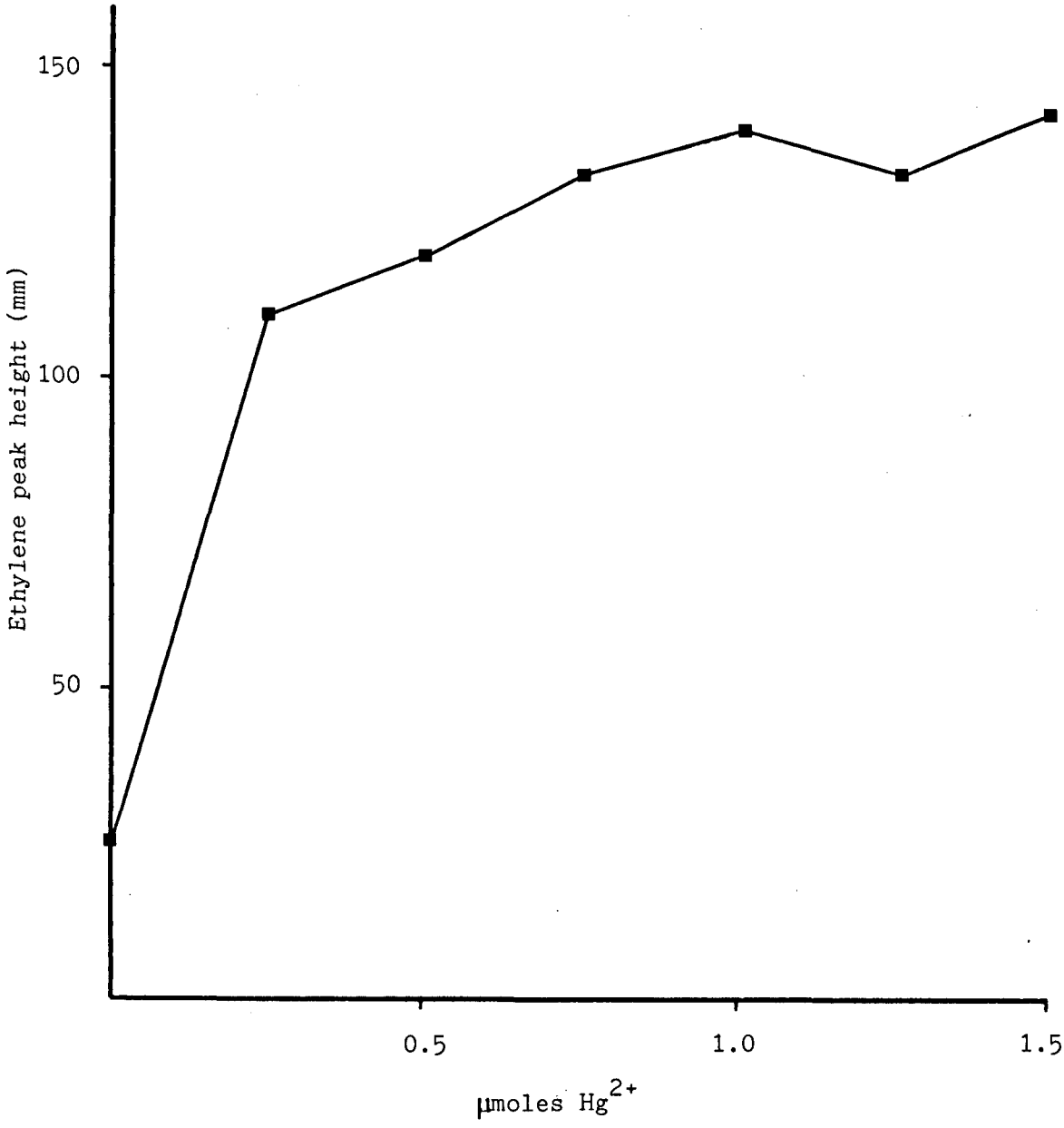


Fig. 2.9 Effect of Hg^{2+} on ethylene release from 2 nmol authentic ACC in a reaction mixture also containing 400 µl bleach/ sat.NaOH in a final reaction volume of 1 ml, the balance being distilled in water. Reaction time was 3 minutes in a 10 ml glass vial prior to headspace analysis for ethylene.

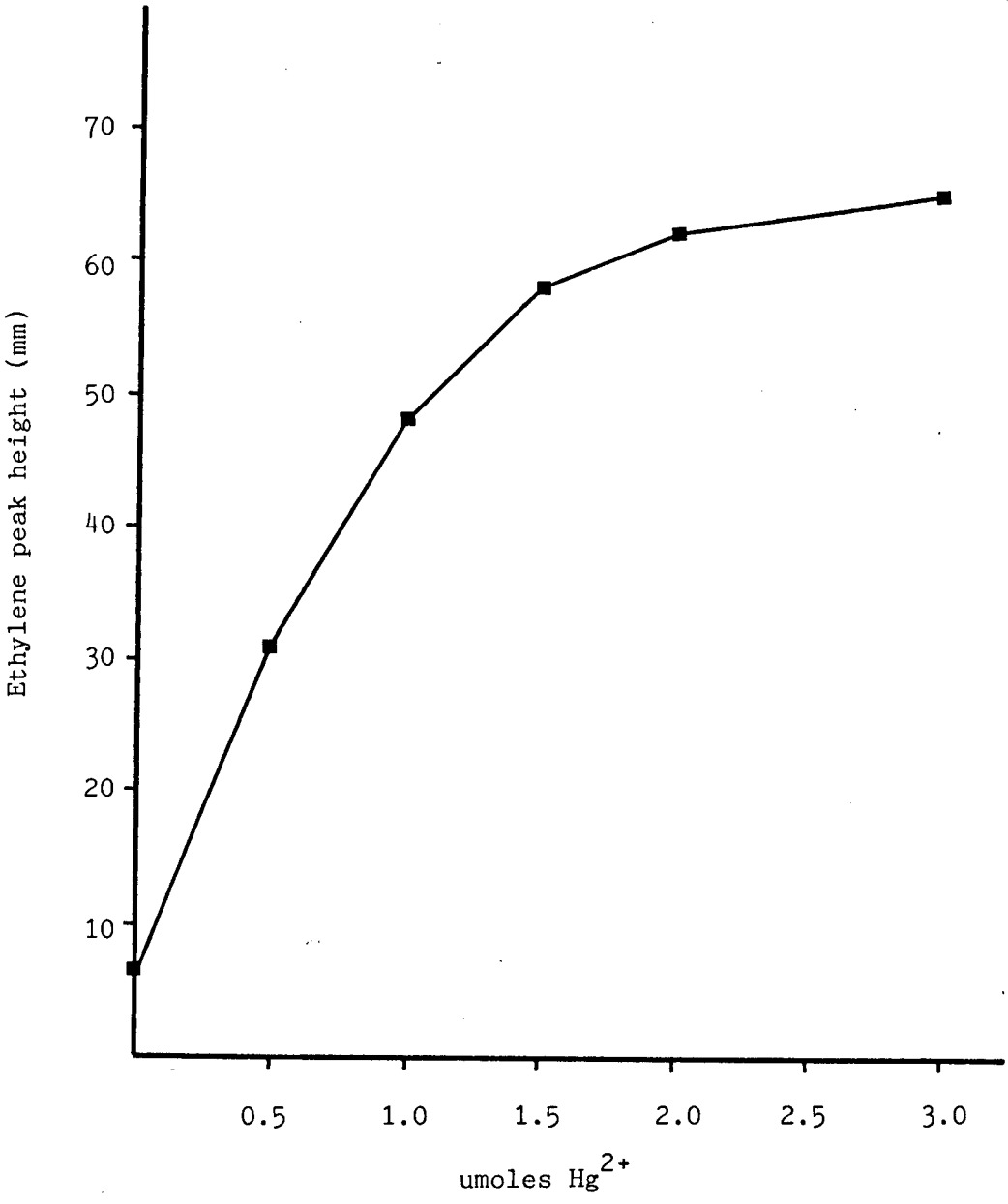


Fig. 2.10 Effect of Hg^{2+} on ethylene release from 400 ul ripe drupelet extract in a reaction mixture also containing 400 ul bleach/sat.NaOH. The final reaction volume was maintained at 1 ml with distilled water. Reaction time 3 minutes in a 10 ml glass vial prior to headspace analysis for ethylene.

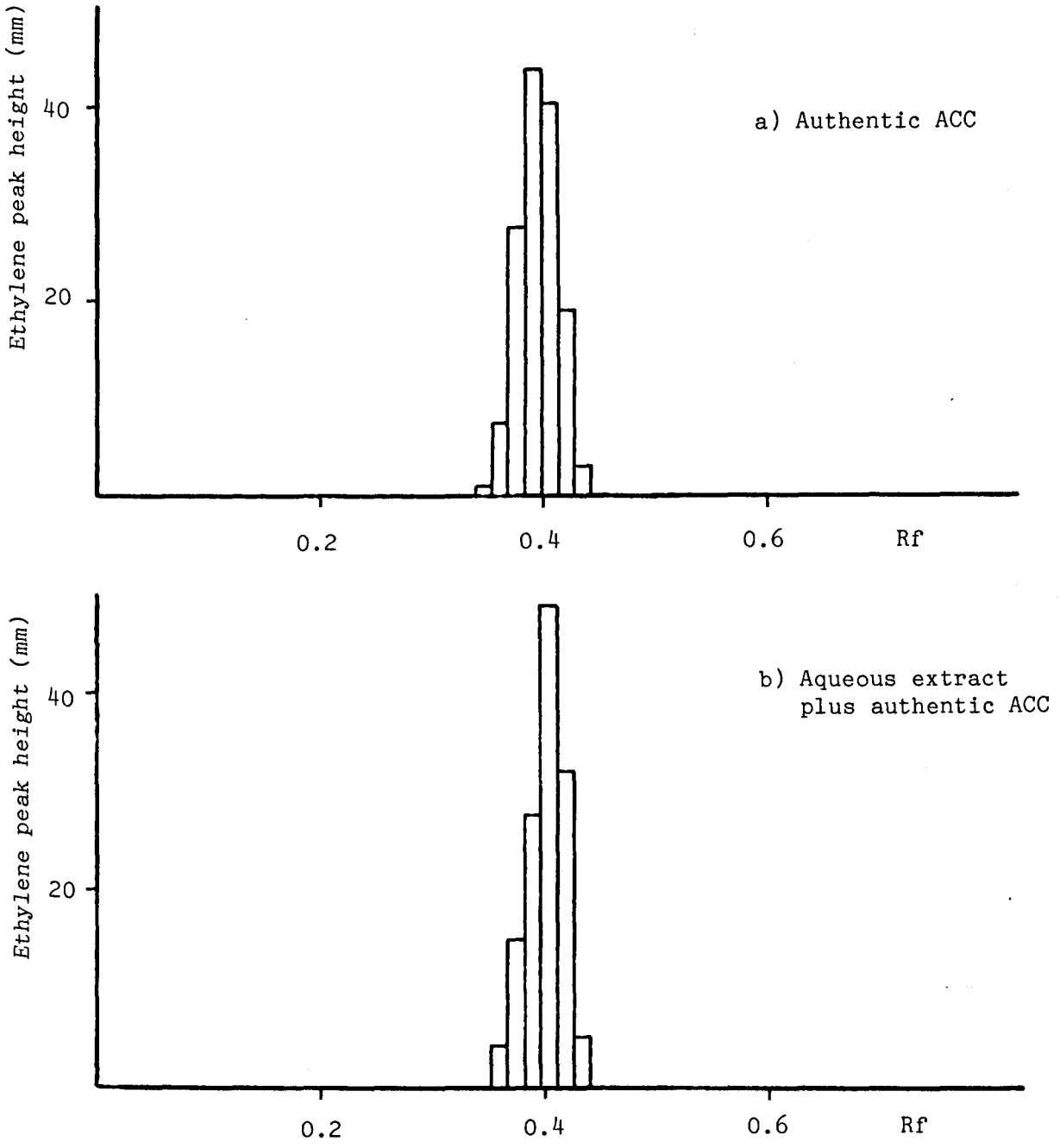


Fig. 2.11 Ethylene release from 1 cm^2 fractions of paper chromatograms of a) 5 nmol authentic ACC or b) 200 µl aqueous ripe drupelet extract plus 2.5 nmol authentic ACC. Individual 1 cm^2 pieces of the chromatogram were assayed directly for ACC in 10 ml glass vials with 200 µl of 15 mM $HgCl_2$, 400 µl distilled water and 400 µl of NaOCl/saturated NaOH. 1 ml samples of the headspace were analysed for ethylene after 3 minutes reaction.

The possibility that ethylene was being liberated by some compound other than ACC was examined using paper chromatography. Whatmann 3MM paper was used in conjunction with butanol-acetic acid-water (4:1:5 v/v/v) according to Lizada and Yang (1979). ACC and other amino acids were visualised by spraying the dry chromatogram with a 0.2% solution of ninhydrin made up in water saturated butanol. The distribution of compounds releasing ethylene on treatment with hypochlorite and Hg^{2+} was examined by directly assaying 1 cm fractions of the dry (but not sprayed with ninhydrin) chromatogram. The validity of this technique was demonstrated by showing an average 86% conversion efficiency for ACC (up to 5 nmol) added to 1 cm^2 pieces of Whatmann 3MM paper when the paper was assayed directly for ACC.

The aqueous extract and authentic ACC were both found to give single peaks with an Rf of approximately 0.4 (fig. 2.11). However, this does not agree with the Rf value of 0.25 demonstrated by Lizada and Yang (1979) but is more in line with work by Knee (1984) and Boller et al. (1979) who showed the Rf for ACC to be 0.46 and 0.47 respectively.

2.19.6 Direct assay of the ethanolic extract

Inconsistent water pump vacuums causing long drying down times in the rotary evaporators led to an investigation of using the ethanolic extract directly for the ACC assay. A close search of the literature revealed two examples of where this appears to have been done previously, Wang et al. (1985) and Larsen et al. (1986). However, when assayed the ethanolic extract showed higher ACC levels per gram of tissue than the aqueous extract obtained by

drying down the ethanolic extract and taking the residue back up into distilled water. This raised the question as to whether simply assaying the ethanolic extract was measuring an artifact, or if there were large losses of ACC being incurred during the drying down process. Several experiments were undertaken to examine these possibilities.

Reacting 80% ethanol with NaOCl/NaOH and Hg^{2+} indicated that it was not simply a chemical reaction with the ethanol since no ethylene was liberated.

Any interaction of ACC and ethanol was examined by making up ACC in 80% ethanol. Subsequent assay of this ACC gave identical liberation as for an equivalent amount of aqueous ACC.

A loss of ACC on drying down was shown not to be the case, there being no difference between the ethanolic extract before drying or when taken back up into 80% ethanol afterwards.

The separation of an ethanolic extract by paper chromatography as described above showed there only to be one peak of activity, coinciding with authentic ACC at Rf 0.4.

All the evidence pointed to an ethanol/extract interaction and at this stage assistance was sought from Professor Yang at the University of California, Davis, USA. He kindly indicated a chemical reaction between amines or ammonia and ethanol yielding ethylene. This observation has now been published (Nieder et al., 1986) and explains the enhanced levels found in the ethanolic extract to be an artifact.

2.19.7 The extraction and assay of ACC from raspberries

The raspberry material (up to 20 g) was thinly coated with

PVP at approximately 10 mg per gram of tissue. The material was then homogenised on ice in cold 80% ethanol at a ratio of 5 ml per gram of tissue. The slurry was divided in two and a known amount of ACC (approximately 4 nmol per gram of tissue) added to one sample to act as a standard. After further homogenisation both samples were centrifiged at 8000 g for 15 minutes at 2°C.

The supernatant volume was measured and a volume equivalent to 4 grams of tissue fresh weight was dried down in a 100 ml pear shaped flask using a rotary evaporator at 38°C. The residue was taken up in 4 ml distilled water to give a final concentration of 1 g/ml distilled water.

The level of ACC in the aqueous extract was determined by placing a 400 µl aliquot of the extract along with 200 µl of 15 mM $HgCl_2$ (3 µmoles) into a 10 ml glass vial on ice. The vial was sealed with a suba seal. Four hundred µl of the 2:1 (v/v) NaOCl/saturated NaOH mixture was injected and vortex mixed for 5 seconds and kept on ice for a further 2.5 minutes. The contents were then vortex mixed for 5 seconds prior to the removal of gas from the headspace for ethylene analysis as described previously (Section 2.6).

At a later date it became necessary to scale down the extraction procedure to deal with individual fruit as the separate drupelets and receptacle. The drupelets or receptacle were weighed minus the calyx, coated thinly with PVP and ground on ice in 5 ml cold 80% ethanol. The resultant slurry was filtered under vacuum directly into a 50 ml pear shaped flask in which it was to be dried down. The mortar and pestle were rinsed with a further 5 ml cold 80% ethanol which was also passed over the dry pulp on the filter

paper into the same 50 ml pear shaped flask under vacuum. The flasks contents were dried down under vacuum at 38°C using a rotary evaporator and taken back up into 2 ml distilled water. This solution was centrifuged for 5 minutes at setting 5 on a B & T Micro Angle centrifuge. The supernatant was assayed for ACC as described above.

2.20 Extraction and assay of 1-(malonylamino)-cyclopropane-1-carboxylic acid (MACC)

ACC is metabolised to MACC in many tissues (Amrhein, 1981; Hoffman et al., 1983) to produce a stable conjugate initially considered to be a biologically unreactive end product (Hoffman et al., 1983; Yang and Hoffman, 1984). The stable conjugate is considered to regulate ethylene production through altering the ACC pool size (Yang and Hoffman, 1984; Philosoph-Hadas et al., 1985). However, ACC malonylation has now been shown to be promoted by ethylene (Liu et al., 1984) and more recently Jiao et al. (1986) have reported that high MACC levels can to some extent induce the capacity to convert MACC to ACC.

MACC is generally assayed from the same extract used for ACC assay by hydrolysing the MACC to ACC and comparing the ACC levels pre and post hydrolysis. The actual hydrolytic conditions vary slightly between workers, but most commonly it is carried out in 3 M HCl at 100°C for 3 hours (Hoffman et al., 1983; Philosoph-Hadas et al., 1985; Jiao et al., 1986; Sitrit et al., 1986) and these conditions were used in assaying raspberry extracts for MACC. Reactions were carried out by mixing equal volumes of the aqueous ACC extract and 6 M HCl to give the final 3 M HCl concentrations. Samples were heated to 100°C using a boiling water bath for 3 hours which was confirmed to be required for maximal conversion. Following neutralisaton of 400 µl aliquots with 200 µl of 2 M NaOH the hydrolysate was assayed for ACC as described in Section 2.19. The ACC level measured before hydrolysis was subtracted from that post hydrolysis, the difference being equivalent to the MACC converted to ACC.

2.21 Extraction and assay of 1-aminocyclopropane-1-carboxylate synthase

Following on from the work on ACC the next stage in examining ethylene production in raspberry fruit was to look at the enzyme producing ACC. Yu et al. (1979) and Boller et al. (1979) both detailed similar techniques to extract and assay ACC synthase from tomato pericarp tissue. Since these reports, work based on them has been reported on mung bean hypocotyl (Yoshii and Imaseki, 1981; Suttle, 1984) apples (Bufler, 1984; Bufler and Bangerth, 1983) winter squash fruit (Hyodo et al., 1983, 1985) and avocado fruit (Sitrit et al., 1986) as well as further work on tomato (Boller and Kende, 1980; Yu and Yang, 1980; Acaster and Kende, 1983).

2.21.1 Extraction of ACC synthase

Initially it was decided to repeat the work done on tomatoes as detailed by Yu et al. (1979) to become familiar with the extraction and assay procedure. Twenty five grams of skinned ripe tomato pericarp was ground on ice with 25 ml homogenisation medium (100 mM Hepps pH 8.5; 4 mM DTT, 2 µM pyridoxal phosphate). The slurry was filtered through 4 layers of muslin and the filtrate spun at 10000 g for 20 minutes at 2°C. Of the 41 ml crude extract obtained after centrifugation, 5 ml was dialysed overnight against 2 mM Hepps pH 8.5, 0.1 mM DTT, 2 µM pyridoxal phosphate. The remainder was fractionated by precipitation with ammonium sulphate. Yu et al. (1979) indicated the fraction precipitated at 60-90% saturation had the highest specific enzymes activity. Hence a 60-90% cut was prepared and allowed to stand overnight at 4°C before being centrifuged at 15000 g for 20 minutes at 2°C. The

pellet was redissolved in 2 ml column buffer (2 mM Hepps, pH 8.5, 0.1 mM DTT, 2 µM pyridoxal phosphate) before being desalted on a 15 cm by 1.5 cm Sephadex G-25 column (Pharmacia Fine Chemicals A.B., Uppsala, Sweden) equilibrated with 2 mM Hepps containing 0.1 mM DTT. Fractions of 35 drops (1.63 ml) were collected on elution with column buffer, up to 100 ml.

2.21.2 Protein determinations

A rough measure of the protein in each sample was obtained by measuring the optical density at 280 nm. Accurate protein determinations were carried out on the peak fractions using the method of Sedmak and Grossberg (1977). One ml of distilled water containing a known amount of protein (as BSA up to 50 ug) was mixed with 1 ml of 0.06% Coomassie Brilliant Blue G250 in 3% perchloric acid. The optical density was then measured at 620 nm and 425 nm and a calibration curve constructed for E620/425 minus the blank E620/425 against known amounts of BSA (fig. 2.12).

2.21.3 Assay of ACC synthase

The method of Yu et al. (1979) was adopted to determine the rate of conversion of SAM to ACC. The reaction mixture consisted of 50 mM Hepps (pH 8.5) containing 50 uM SAM of which 200 µl was incubated with 400 µl of the enzyme extract in a 10 ml glass vial. After 3 hours at 30°C the reaction was stopped by the addition of 100 µl of 15 mM $HgCl_2$ and the vial sealed with a suba seal and placed on ice. The ACC was converted chemically to ethylene by the injection of 100 µl of a 2:1 (v/v) NaOCl:saturated NaOH solution (as described fully in Section 2.19). The ethylene liberated was

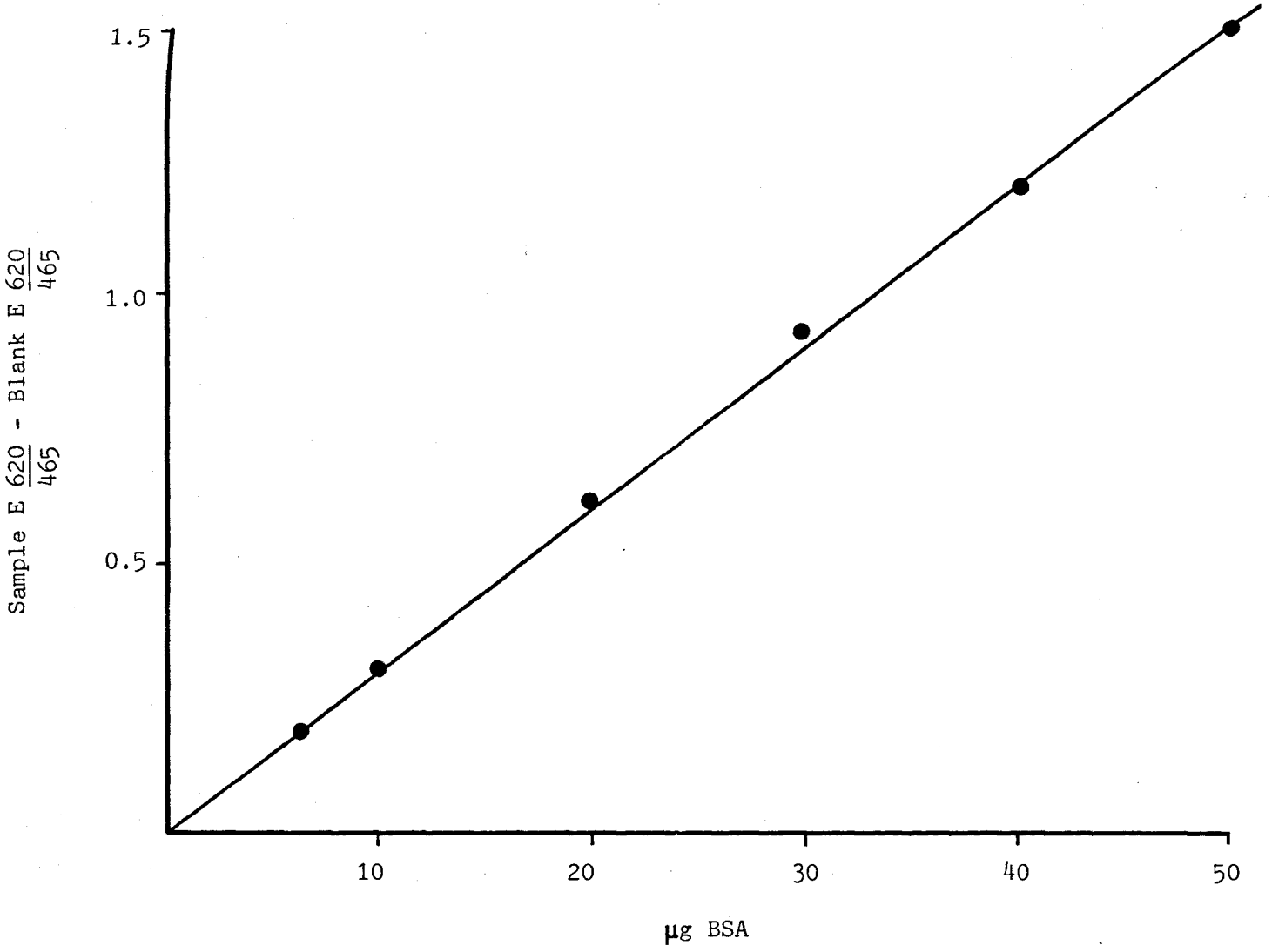


Fig. 2.12 Calibration for the Sedmak Grossberg (1977) protein assay.

measured by headspace analysis as described in Section 2.6.

This initial experiment was simply to see whether the extraction had worked and absolute values were not determined. Having shown ACC synthase activity to be present (fig. 2.13) the extraction was repeated in more detail with smaller fractions and protein being determined accurately. This allowed the enzyme activity to be expressed as nmol ACC produced per mg protein per hour (as was done by Yu et al., 1979; Yu and Yang, 1980; Yoshii and Imesaki, 1981; Acaster and Kende, 1983; Bufler, 1984) whereas other authors express activity on a fresh weight basis (Boller et al., 1979; Hyodo et al., 1983, 1985; Sitrit et al., 1986). The values obtained here (up to 5 nmol ACC/mg protein/hour in the peak fractions, fig. 2.14) are well on line with other results already published on tomato ACC synthase activity. Yu et al. (1979) reported 0.5 nmol ACC/mg protein/h and Yu and Yang (1980) reported 2.1 nmol ACC/mg protein/h in wounded tissue. Acaster and Kende (1983) partially purified the enzyme and reported 35 nmol ACC/mg protein/h in their peak fractions from a Sephadex G100 column.

2.21.4 Extraction and assay of ACC synthase from raspberries

After successfully extracting and assaying the enzyme from tomato the same procedure was applied to ripe raspberry drupelets. Assay of the peak protein fractions however yielded no ACC synthase activity. This was possibly due to a lack of buffering capacity of the extraction medium and the extremely acidic nature of the extract. Increasing the strength of the extraction buffer failed to increase the activity. It was thought that the ripe receptacles might present less extraction problems and these were examined. The

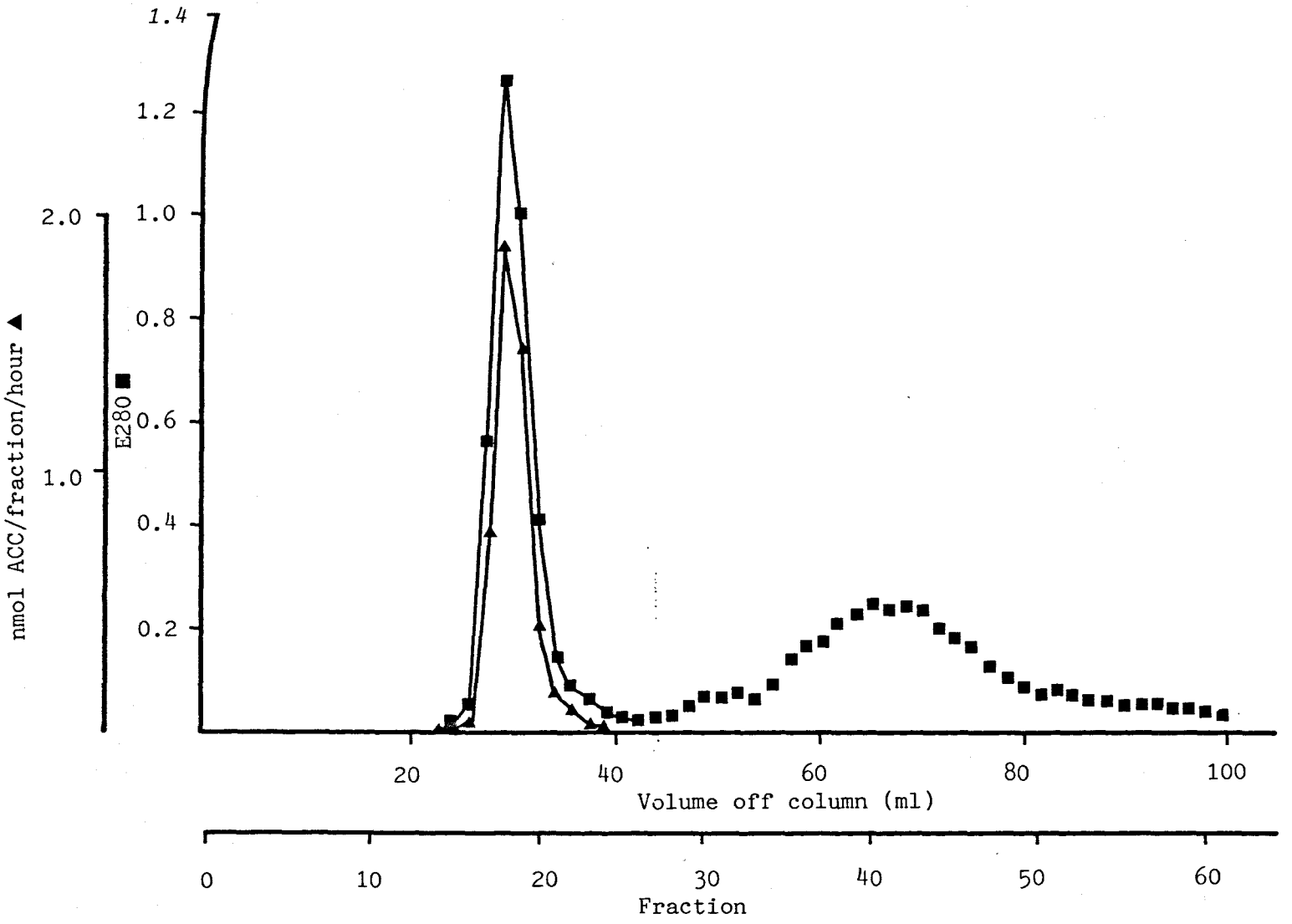


Fig. 2.13 Initial attempt to partially purify and assay ACC synthase from ripe tomato pericarp using the methods of Yu et al. (1979). Each fraction of column eluate was measured for protein and the peak area assayed for ACC synthase.

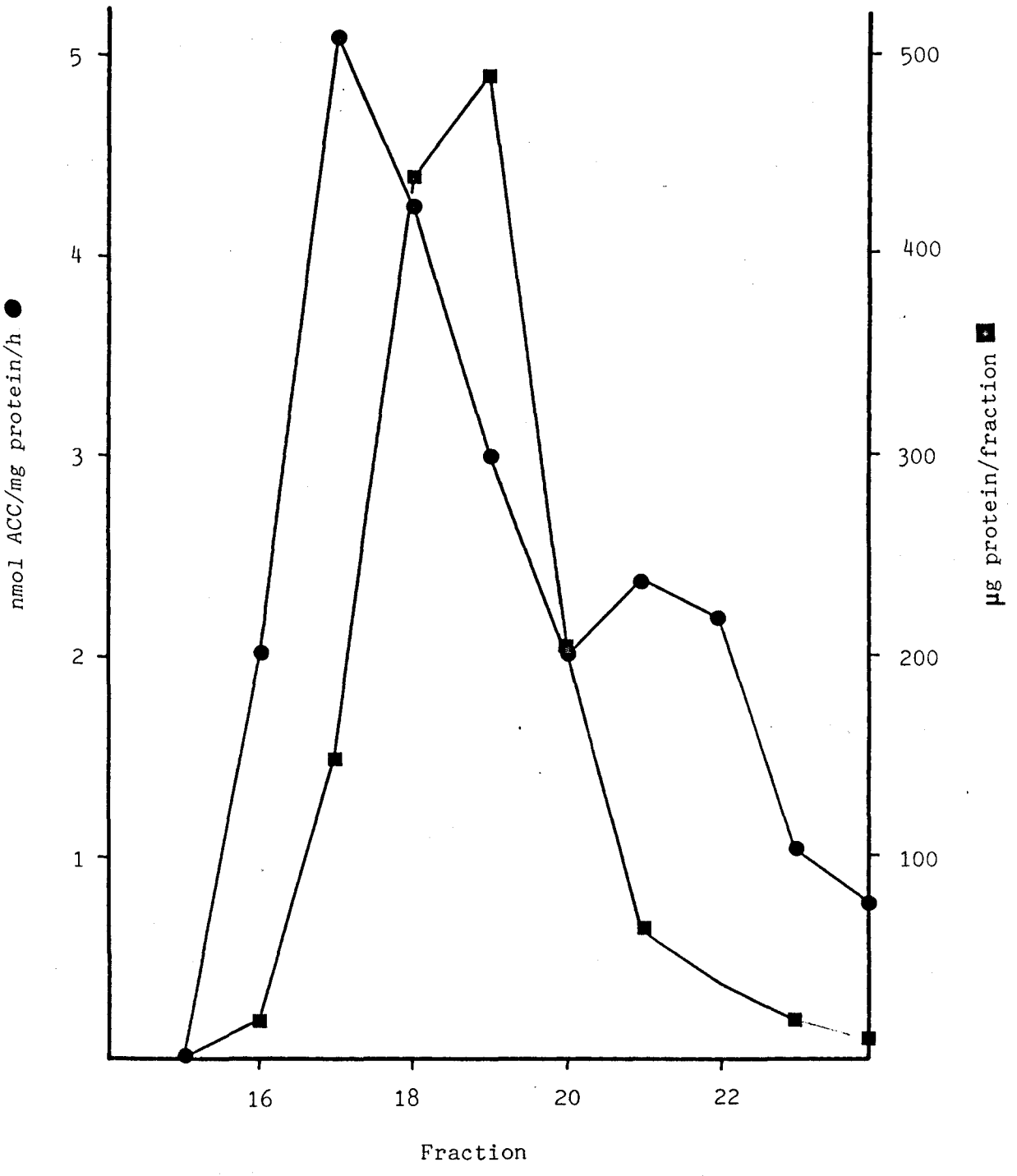


Fig. 2.14 Second attempt at purification of tomato ACC synthase. The specific activity of ACC synthase (nmol ACC/mg protein/h) was determined in the fractions containing the protein peak.

results in fig. 2.15 show similar protein and ACC synthase activity peaks as in tomato. The level of activity however was very low compared to tomato (less than 0.1 nmol ACC/fraction/h compared to 1.9 nmol ACC/fraction/h, equal weights of tissue having been extracted).

To examine whether there were any deactivating substances in the raspberry extracts ripe tomato pericarp tissue and ripe raspberry receptacle tissue were extracted separately and in combination. The results in table 2.4 indicate that there was nothing in the raspberry receptacle extract to interfere with the tomato ACC synthase, in fact the combined sample had greater activity than the sum of the two alone.

Table 2.4 ACC synthase activity of ripe tomato extract and ripe raspberry receptacle apices extracted and assayed individually and combined. (25 g/sample).

Sample	ACC synthase activity nmol ACC/g f.wt/h
Ripe tomato pericarp	0.164
Ripe raspberry receptacle apices	0.076
Tomato + apex - actual	0.348
- theoretical	0.240

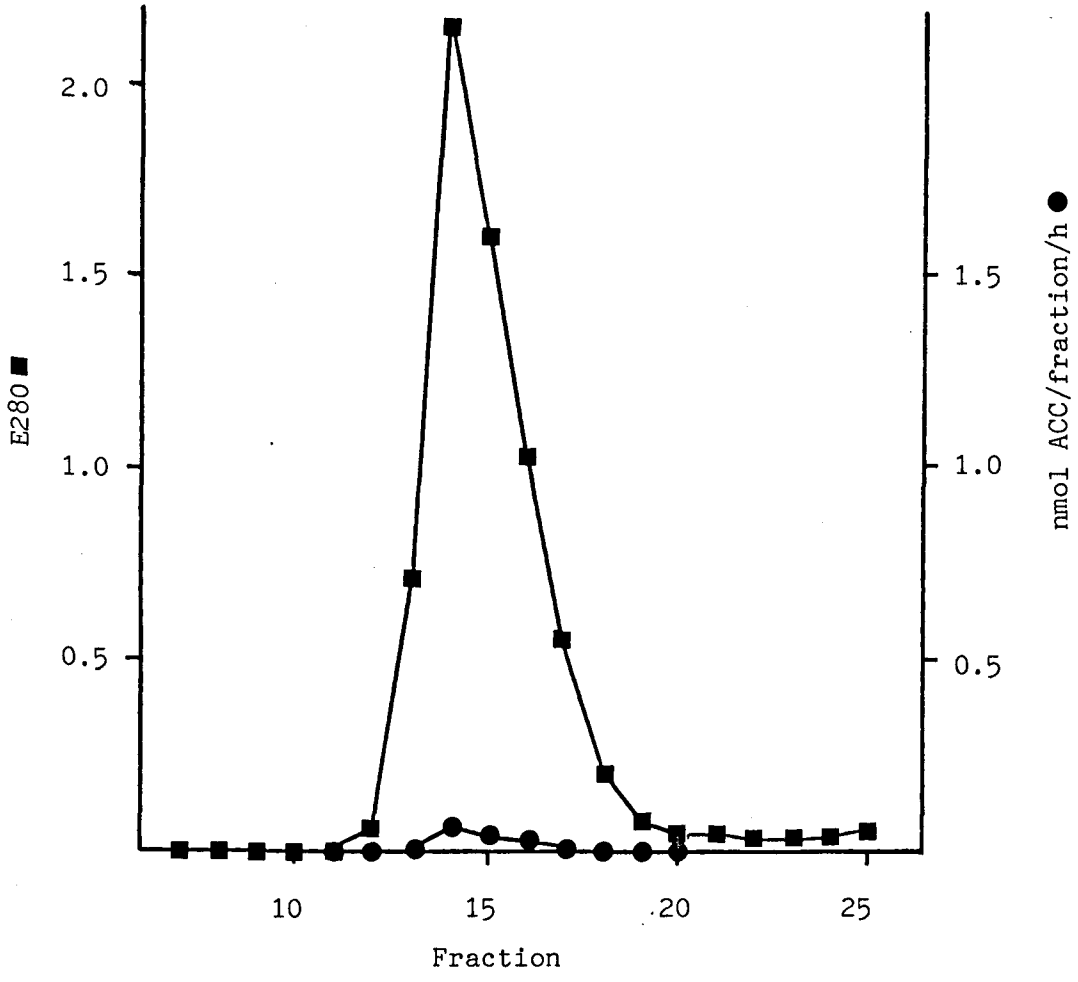


Fig. 2.15 Attempt at partial purification and assay of ACC synthase from ripe raspberry receptacle apices. The specific activity for ACC synthase (nmol ACC/fraction/h) was determined for the fractions containing the protein peak.

2.21.5 Preparation of crude enzyme extracts from raspberry receptacle apices at different stages of development

The receptacles from 115 ripe, mottled or green fruit were weighed minus the calyx. These were ground on ice in the homogenisation medium (detailed above for tomato extractions) at a ratio of 1.5 ml per gram tissue. The pulp was pressed through 4 layers of muslin and the filtrate centrifuged at 10000 g for 20 minutes at 2°C. Each supernatant was dialysed overnight against dialysis medium (see above) and the dialysed extracts were assayed for ACC synthase (see above).

The ACC synthase activity of raspberry flowers was examined in flowers at the peak of ethylene production, when the petals were abscising. In these extracts no enzymatic activity could be found in any of the crude extracts, redissolved pellets or fractions.

2.22 Measurement of ethylene production rates of flowers at different stages of development

Initially the EPR of individual flowers were measured in the same way as fruit (Section 2.6) as part of an experiment on fruit abscission (Section 3.1.6). This was followed up by an examination of flowers at the following stages of development, i) sepals opening, ii) petals opening, iii) petals abscising, iv) petals all abscised. Individual flowers were sealed directly after collection in 10 ml glass vials for between 1 and 2 hours with suba seals for EPR analysis at 25°C. Each sample consisted of 20 flowers with ethylene analysis being determined as described in Section 2.6.

Having repeated the initial observation of elevated EPRs during flowering the actual timing of the increase was examined using a more precise categorisation of development. The way in which the classification was determined is described in Section 2.23. Individual flowers covering the whole spectrum of development were sealed directly after collection in 10 ml glass vials as described above for EPR analysis.

2.23 Timecourse of flower development

The development of flowers from the stage of being swollen buds was examined by allowing the buds to open in the laboratory. Flowers were taken from the canes at the stage where the bud was swollen and the sepals had separated slightly to reveal the petals underneath. Buds were cut with the pedicels as long as possible. This allowed the threading of the pedicel through a hole in the centre of a 1 cm disc of 1 mm expanded polystyrene. Once placed through this floatation collar the pedicel was trimmed to 2-3 mm. This collar had sufficient buoyancy to keep the bud or flower dry when placed on distilled water whilst allowing the pedicel to dip into the water. Twenty buds were observed and notes taken on the sepal and petal movements and the times taken between the stages.

This method of opening flowers whilst transpiring water opened up the possibility of applying chemicals to the flowers though the transpiration stream. It also showed it to be possible to open flowers and get them to go through their normal development, including abscission in the laboratory.

2.24 The effect of pollination on ethylene production

Work on other flowers has shown pollination to cause increased rates of ethylene production (Burg and Dijkman, 1967; Hall and Forsyth, 1967; Nichols, 1977; Halevy et al., 1984; Stead, 1985). This effect in raspberry flowers was examined by opening buds in isolation in the laboratory. Buds were harvested when the petals were just visible between the sepals and kept transpiring water in 33 ml glass vials using floatation collars as described in Section 2.23. This was to prevent pollination until the flowers had opened to the stage where the stigmas were just visible. The flowers were then split into two groups, one of which had pollen from other Glen Clova flowers added to the stigmas, the others being left untreated. This technique should have successfully caused fertilisation since Glen Clova is considered to be highly self-fertile (Redalen, 1977). The EPRs of individual flowers were measured by sealing the vials with suba-seals for between 1 and 2 hours prior to headspace analysis for ethylene by gas chromatography (see Section 2.6).

It soon became obvious that pollination had no effect on EPRs and the flowers were examined using both light microscopy and the SEM. This revealed a ring of basal stigmas pollinated by the adjacent anthers in the supposedly unpollinated control flowers (plate 3.31). Normally pollination experiments in raspberry are conducted on emasculated flowers (Hardy, 1932; Eaton et al., 1968; Redalen, 1977). Unfortunately this technique would have resulted in the production of large amounts of wound ethylene and make observations of flower development impossible.

2.25 Measurement of ethylene production rates of flowers opened in the laboratory

Buds were collected from the field at the stage where they were swollen and the sepals had parted slightly to reveal the petals underneath. The flowers were floated on 5 ml distilled water in individual 10 ml vials at 22°C using the floatation collars described in Section 2.23. As the flowers opened the vials were sealed at intervals for between 30 and 90 minute periods using suba seals and the ethylene content of the headspace determined by gas chromatography (Section 2.6).

Ethylene was determined for the gas phase alone since the maximum possible amount dissolved in the 5 ml water would be insignificant compared to the amounts being measured. This is based on the solubility of ethylene in water being 649 pl l^{-1} at 25°C with a 1 µl l^{-1} (ppm) ethylene atmosphere above the water (M Spencer, pers. comm.). The loss of ethylene into solution depends on the ethylene partial pressure above the water with the surface area available for diffusion effecting the time taken to reach an equilibrium. This surface area is greatly reduced by the floatation collar. These factors show the loss of ethylene into solution not to be significant.

2.26 The effect of aminoethoxyvinylglycine on ethylene production, petal abscission and petal senescence

The capacity of AVG to reduce the ethylene production of flowers opening in the laboratory was demonstrated by allowing the buds to transpire 0.4 mM AVG instead of distilled water as described in Section 2.25. The background to AVG is given in Section 2.15.

To examine this reduction in ethylene production flowers were harvested in the late afternoon at the stage where the buds were swollen and the sepals had just parted slightly to reveal the petals. The buds were floated on a solution of 0.4 mM AVG (Fluorochem, Glossop, England) or distilled water overnight in 17 cm by 11 cm perspex dishes. Next morning at least 50 buds were taken between stages 4 and 6 (see Table 3.23, Section 3.2.1) for the subsequent experiments. Buds outside this range were discarded. In some treatments the flowers were also placed in 40 $\mu l\ l^{-1}$ ethylene or ethylene free air using the chambers described in Section 2.13, at 25^{o}C and in constant light (25 $\mu E/m^2/s$).

Abscission was scored by counting the petals which fell if touched with a needle. The onset of senescence was judged by the presence of brown coloration at the base of the normally white petals.

2.27 The effect of silver thiosulphate on petal abscission and senescence

The effect of STS on ethylene action has been discussed earlier (Section 2.15) and was examined in the same way as AVG. Buds were harvested when the petals were just visible in the late afternoon. These were floated on either distilled water or 0.2 mM STS in 17 cm by 11 cm perspex dishes evernight. The following morning buds outside the stages 4 to 6 (see Table 3.23, Section 3.2.1) were discarded and the remainder allowed to continue development. The developing flowers were maintained at 25°C in continuous light (25 $\mu E/m^2/s$). Abscission was scored by counting the petals which fell if touched with a needle. The onset of senescence was judged by the presence of brown coloration at the base of the normally white petals.

2.28 The effect of adding ACC and the measurement of endogenous ACC levels in flowers at different stages of development

The EFE capacity of flowers at different stages of development was examined by the addition of 1 mM ACC through the transpiration stream. Flowers classed either as i) closed buds, ii) sepals opening, iii) petals just opening or iv) petals open and abscising, were floated on 5 ml of 1 mM ACC in individual 10 ml vials. The vials were ventilated with ethylene free air and sealed 5 hours later with suba seals. Headspace analysis for ethylene was carried out 30 minutes later as described previously (Section 2.6).

The endogenous ACC levels in individual flowers were determined using a method derived from the findings on the extraction of ACC from fruit as detailed in Section 2.19. Individual flowers at different stages of development were first powdered in liquid nitrogen and then extracted twice in 5 ml cold 80% ethanol and vacuum filtered. After drying the combined extract using a rotary evaporator at 38^{o}C the residue was redissolved in 2 ml distilled water. The solution was centrifuged at 5000 g for 5 minutes with 200 µl aliquots of the supernatant being used directly for ACC assay according to Lizada and Yang (1979), see Section 2.19. The percentage recovery of ACC was measured by adding a known amount of authentic ACC (4 nmol) to half of the initial powder. Recovery of this authentic ACC was found to be in the range 72 to 76% at all stages of flower development.

CHAPTER THREE

RESULTS

For the sake of clarity the results have been presented in two sections. Section 3.1 deals with data concerned with fruit abscission whilst that relevant to petal abscission is presented in Section 3.2. Each section has been subdivided according to the experimental programme allowing the different aspects of the role of ethylene in abscission to be examined. Unless specified the red raspberry variety Glen Clova provided the material used. The data is not presented in the chronological order in which the experiments were carried out since many sets of data were obtained from more than one seasons work. All major observations were repeated in more than one season. The data has therefore been presented in a fashion which leads the reader through the rationale of examining the role of ethylene in abscission. The results for all major experiments are representive or averages of at least 3 repetitions. The large climatic differences between seasons (1984 being hot and dry, 1985 very wet) made little difference to those characteristics of raspberry ripening under investigation. Evidence for the lack of variation is presented later in Section 3.1.4. No attempt has therefore been made to define when each set of data was gathered.

To facilitate reading the experimental details for each section are briefly outlined and the main conclusions included to provide the rationale for subsequent experiments. The more general discussion of the results is left to Chapter 4.

SECTION 3.1 FRUIT

3.1.1 The selection of convenient reproducible developmental stages for use in subsequent experimentation

For experimental purposes it is necessary to break up the developmental continuum of the raspberry fruit into a series of defined stages. The stage categories were selected after considering two important factors. Firstly the fruit characteristics chosen to delimit each stage had to allow easy and accurate selection whilst minimising physiological diversity. It was essential that fruit behaved reproducibly throughout subsequent experimentation. Secondly it was necessary to select stages which were likely to be representative of the major physiological stages occurring during fruit maturation.

A series of convenient developmental stages were determined on the basis of experience gained during trial experiments carried out in 1983. Using the visible characteristics of berry size, drupelet size and coloration the following categories were selected for examination (plate 3.1):

i) Hard green fruit (HG). Weighing approximately 0.8 g these fruit have dark green drupelets almost totally filled by the pit, there being very little fleshy (mesocarp) tissue (plate 3.2). The drupelets are tightly attached to the receptacle.

ii) Soft green fruit (SG). Weighing approximately 1.5 g these fruit have green drupelets, having lost any blueish hue present when HG (plate 3.3). There is an increase in mesocarp tissue causing the drupelets to expand and become softer, also forcing the drupelets away from the receptacle. This creates spaces at the drupelet bases

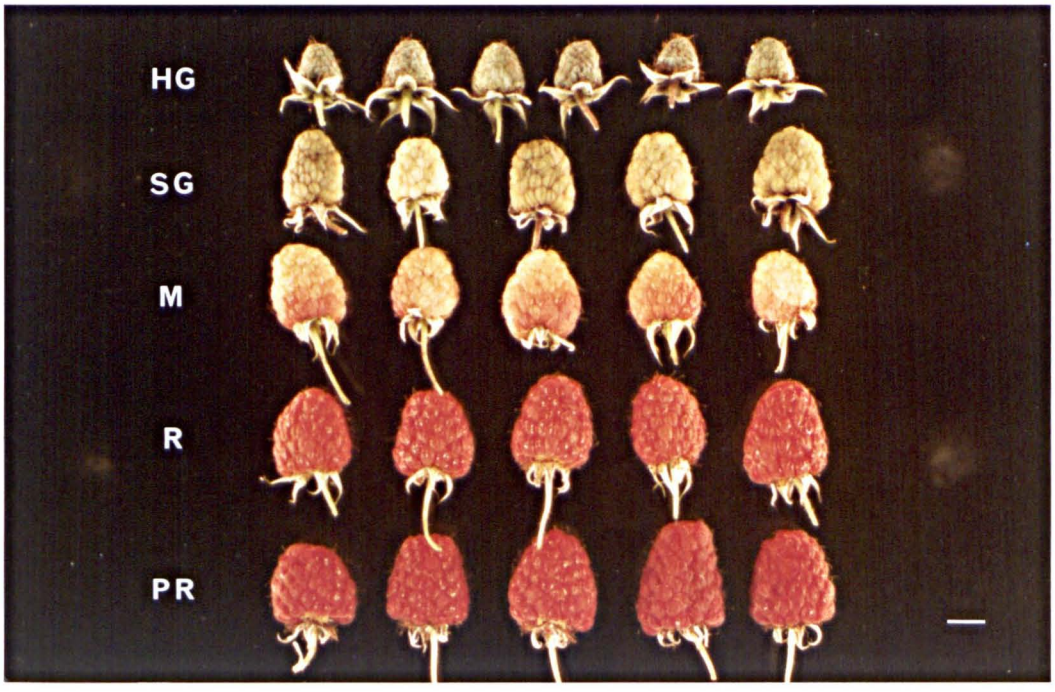


Plate 3.1 Developmental stages of Glen Clova fruit selected for future examination. Categories are: HG, hard green; SG, soft green; M, mottled; R, ripe; PR, purple ripe. Bar = 1 cm.

Plate 3.2 Representative hard green fruit of Glen Clova.
Bar = 2.5 mm.

Plate 3.3 Representative soft green fruit of Glen Clova.
Bar = 4 mm.

leaving the connecting stipe clearly visible.

iii) Mottled fruit (M). Weighing on average just over 2 g, these fruit are composed of a mixture of both green and pink drupelets (plate 3.4). There are still air spaces between the receptacle and the bases of the drupelets although expansion of both reduces their size.

iv) Ripe fruit (R). These can weigh anything over 2.5 g and occasionally less than this. The drupelets are red with expansion of both drupelets and receptacle filling the air spaces so clearly visible in earlier stages (plate 3.5).

v) Purple ripe fruit (PR). As with the ripe fruit these can weigh anything over 2.5 g up to a maximum around 7 g. The drupelets are very fleshly with a purple hue often separating from the receptacle either under their own weight or on touching. Cutting both drupelets and receptacle of PR fruit proved impossible because of their delicate nature and the fact that separation was almost complete. In some fruit the receptacle was found to be starting to dry out and shrivel. Hence PR fruit were only sampled whilst turgid and showing no signs of drupelet collapse, after this point degeneration occurred rapidly and such fruit were not used experimentally.

In addition to these visible characteristics the trial experiments showed the above categories to correspond to major changes in both fruit retention strength (FRS) and ethylene production rates (EPR). These changes are illustrated in fig 3.1. At the HG and SG stages fruit had low EPRs and showed no signs of weakening at the abscission zone. When mottled there was both an increase in EPR and a reduction in FRS. These changes continued

Plate 3.4 Representative mottled fruit of Glen Clova.
Bar = 3 mm.

Plate 3.5 Representative ripe fruit of Glen Clova.
Bar = 4 mm.

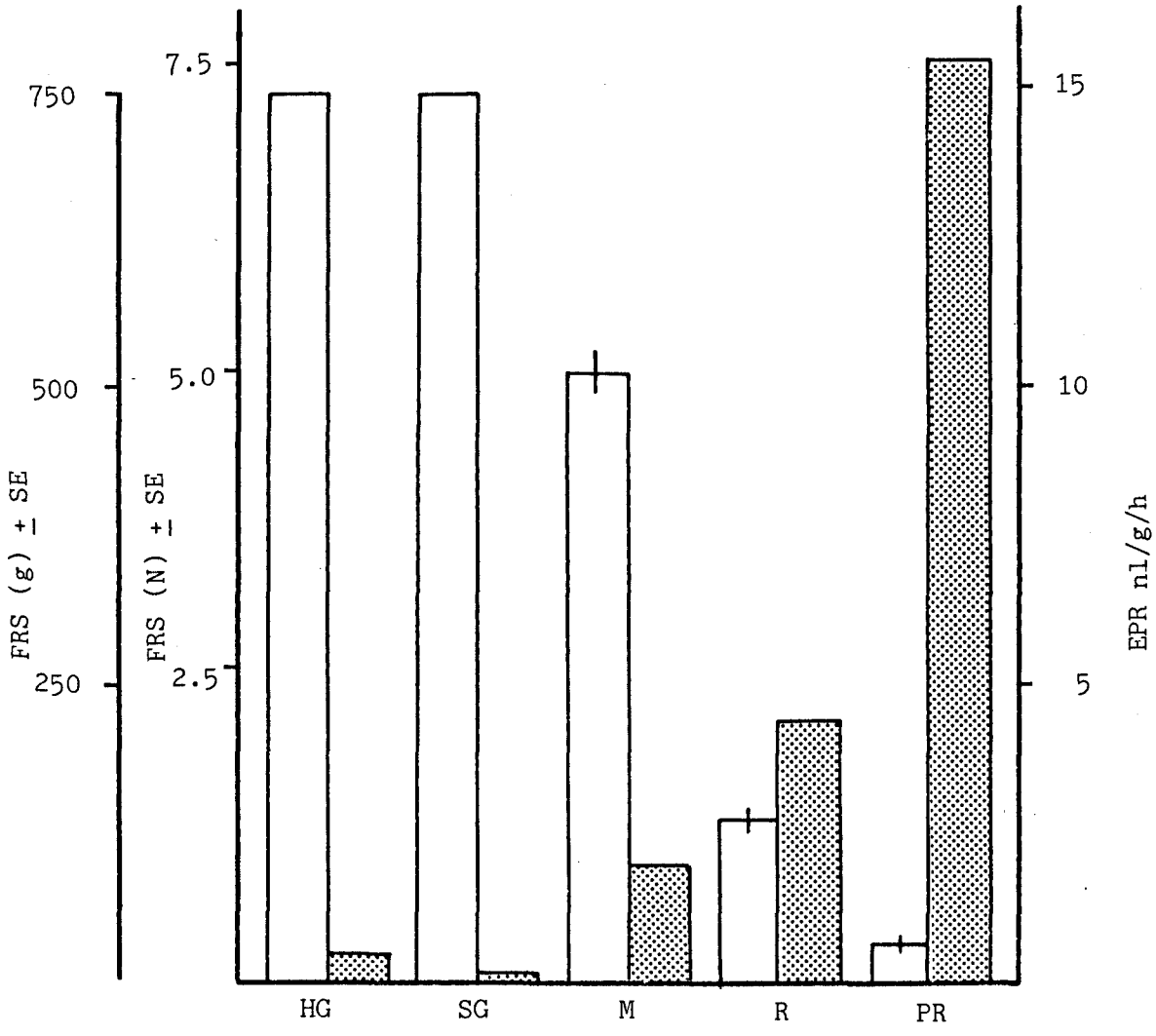


Fig. 3.1 Change in fruit retention strength (☐) and ethylene production rate (▨) during fruit development (30 fruit/ sample).

through the R and PR stages, there being increased EPRs and reduced FRS values.

The ease of identification of these physiologically distinct stages of development, and the link with FRS and EPR changes, resulted in later work being based on these categories.

3.1.2 Temporal sequence of fruit development

Having defined specific categories of fruit for examination (section 3.1.1) the approximate time interval taken to progress from one developmental stage to the next was determined. This was measured by tagging individual flowers just as the sepals were opening to provide a precise starting point from which to time the changes. Initially the 50 tagged flowers were examined at intervals to determine the percentage of each developmental stage present. The HG stage persisted from 7 to 20 days after tagging (fig. 3.2) and together with SG were the only stages present between 20 and 27 days after tagging. In the next 8 days there was a rapid period of change resulting in all fruit being R or PR by the 35th day after tagging. These results give an indication as to the degree of developmental variability within any population of fruit.

An alternative method of examination involved the removal of tagged fruit at intervals and weighing them and measuring their basal diameter, as well as recording the stage of development. The results in fig. 3.3 show the berry weight to increase in a single sigmoidal curve taking approximately 30 days for the first fruit to reach full ripeness. The basal diameters of the fruit however increased at a constant rate for approximately 27 days after tagging, there then being a large increase between 27 and 30. The times taken between stages agree with those in fig. 3.2 with HG fruit persisting between days 12 and 19, and all being SG by day 22. These SG fruit expanded greatly over the next 5 days with all the fruit remaining in the SG or pale soft green category. The final changes in development occur very rapidly. Mottled fruit showing half green and half red drupelets generally turn ripe

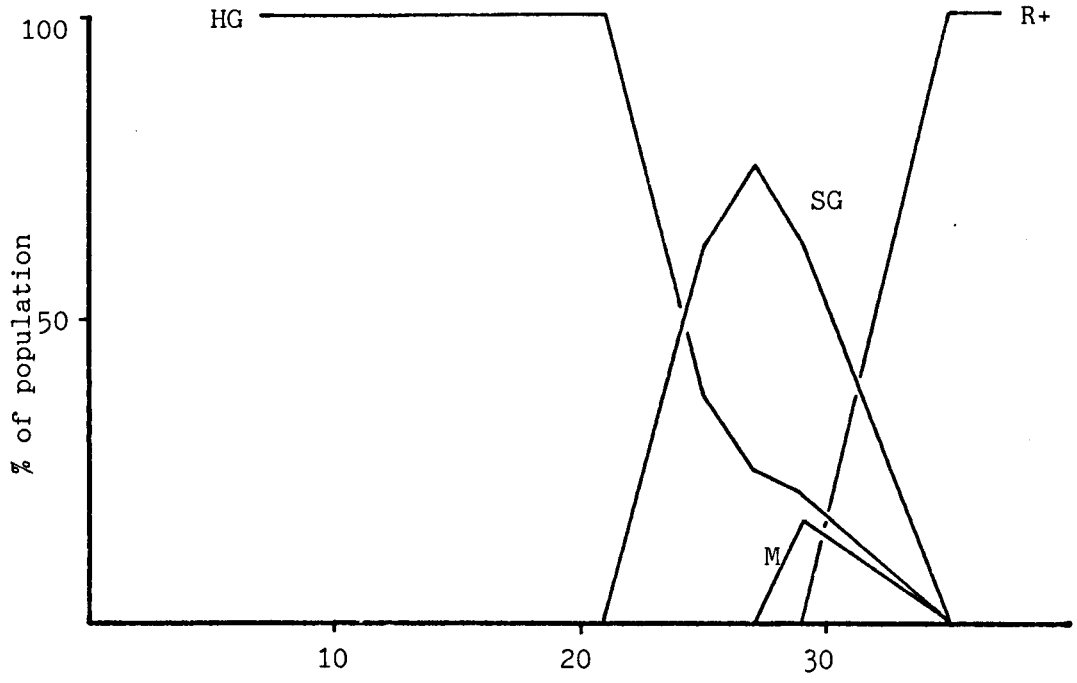


Fig. 3.2 Developmental changes in a population of raspberries of approximately the same age.

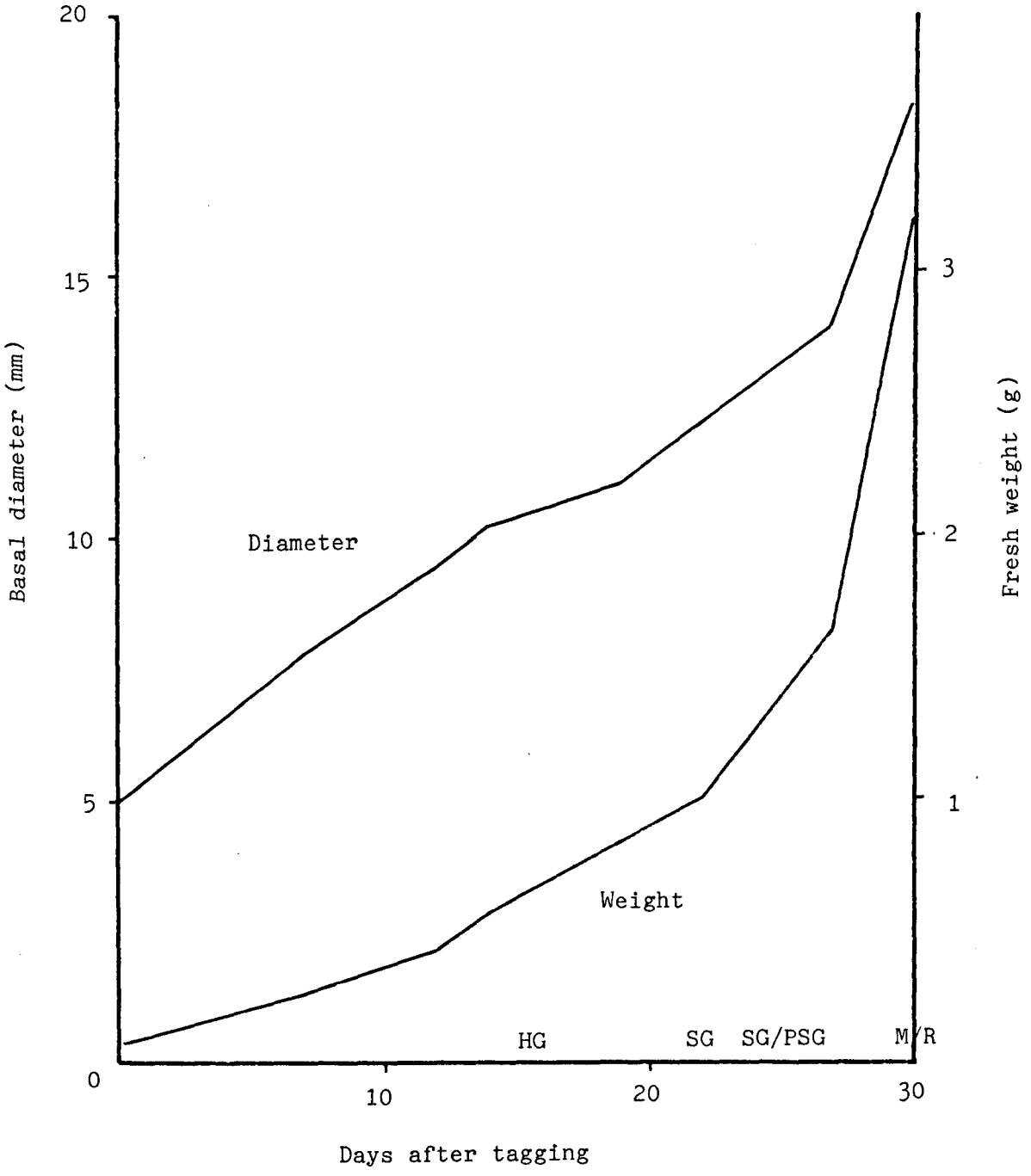


Fig. 3.3 Changes in fruit weight and basal diameter of Glen Clova fruit during development (8 fruit/point).

overnight. This stage persists for a couple of days before the development of the purple hue marking the fruit as PR.

In reviews on fruit development the raspberry is classified as having a double sigmoidal growth curve (Coombe, 1976; Rhodes, 1980). The results presented here (fig. 3.3) show a single sigmoidal curve although there may be a slight inflexion in the basal diameter curve. Other work on raspberry has shown the period of little growth between the periods of rapid growth to be hard, if not impossible to identify when examining berry weight (Hill, 1958; Mason and Topham, 1981a, b). The time interval from anthesis to maturity is also very similar for other varieties of Rubus idaeus. Mason and Topham (1981a, b) showed fruit of the varieties Malling Jewel and Glen Isla to be ripe approximately 37 days after anthesis. In Rubus strigosus (cv Latham) Hill (1958) determined the time from anthesis to maturity as 32 days. Hence the changes observed here for both the rate and duration of growth of Glen Clova fruit are in line with the previously published work on other varieties.

3.1.3 Characteristics of raspberry fruit at various stages of maturation

In comparison to the amount of work which has been carried out on some other fruit, the developmental changes occurring during raspberry maturation have been studied relatively little (Green, 1971). Those features which have been examined are those of importance to horticulturists and include changes in acidity, soluble solids (mainly as a measurement of sugars) and fruit retention strength (FRS). This final characteristic has become more important with the development of machine harvesting (Mason 1974). In addition Blanpied (1971, 1972) has made a cursory examination of ethylene levels in raspberry fruit although the methods used and the means by which the results were expressed make it hard to draw any substantial conclusions.

In order to characterise the fruit stages HG, SG, M, R, and PR a whole range of parameters which change during fruit development were examined. These included fresh and dry weights, acidity and sugar levels, pigment concentrations, respiration rates and photosynthesis. Data on FRS and EPR is also included again since these are the two parameters which will be examined in greater detail later.

Expression of the results has been standardised on a per gram fresh weight basis. Other work on fruit ripening sometimes represents data on a per fruit basis throughout development, regardless of changes in mass. Hence in this section the results have been expressed both on a per gram fresh weight and on a fruit basis where relevant.

The description of fruit ripening changes included in this

section are not meant to be comprehensive in either scope or detail. They have been included simply to define the characteristics of the different categories of fruit examined with respect to abscission. The data does however contribute to the overall understanding of raspberry ripening and is briefly discussed in this context.

3.1.3 i) Fruit retention strength

A reduction in the force required to separate fruit from the parent plant is an important feature in harvesting. Separation is achieved through the weakening of the abscission zone and can be determined as the fruit retention strength (FRS) by measuring the force required to rupture the abscission zone using a strain gauge. In the raspberry the first stages of weakening of the abscission zones occur when the fruit is mottled (FRS = 5 N, fig. 3.1). In earlier stages of development fracture does not necessarily occur at the abscission zone and there may be tearing of either the receptacle or drupelet tissues. Hence only FRS values below 7.35 N were recorded since above this value separation invariably occurred outside the abscission zone. Once weakening had commenced in the mottled fruit the process continued with increasing maturity until PR when the drupelets and receptacle of some fruit were totally separated and fruit would often fall under their own weight if touched (FRS = 0.3 N).

3.1.3 ii) Ethylene production rate

Ethylene production can easily be measured by sealing fruit in a glass container with a suba-seal and allowing any ethylene

given off to accumulate. The amount of ethylene in the headspace can be readily determined with a gas chromatograph (see Section 2.6). The changes in EPR which occur during fruit development indicate the raspberry to be a climacteric fruit with the EPR increasing dramatically with maturity (fig. 3.1). Whilst the fruit is green ethylene is produced at less than 0.5 nl/g/h and increases to around 15 nl/g/h at PR. The first sign of increase occurs at the M stage with the main increase occurring between R and PR (4.5 to 15 nl/g/h). These changes in EPR are examined in greater detail in Section 3.1.6.

3.1.3 iii) Fresh weight

Berry fresh weight has already been shown to increase with development (see Section 3.1.2). The weight approximately doubled whilst green, there being a further three fold increase by PR. Similar results have been obtained for other raspberry varieties (Hill, 1958; Mason and Topham, 1981a, b). Figure 3.4 shows the receptacle to represent approximately 20% of the total berry weight at HG, decreasing to 7 or 8% at R. This change is the result of a more rapid increase in the drupelet weight than the receptacle (8.8x and 3.5x respectively from HG to PR) although both do increase with development.

The receptacle can de divided into two regions, an apical region to which the drupelets are attached and beneath this the calyx region (see fig. 1.2). An increase in size of the receptacle apex accounts for the majority of the increase in receptacle weight during development (fig. 3.5). There is a very slight increase in the calyx segment weight from HG to R but it declines at PR as a

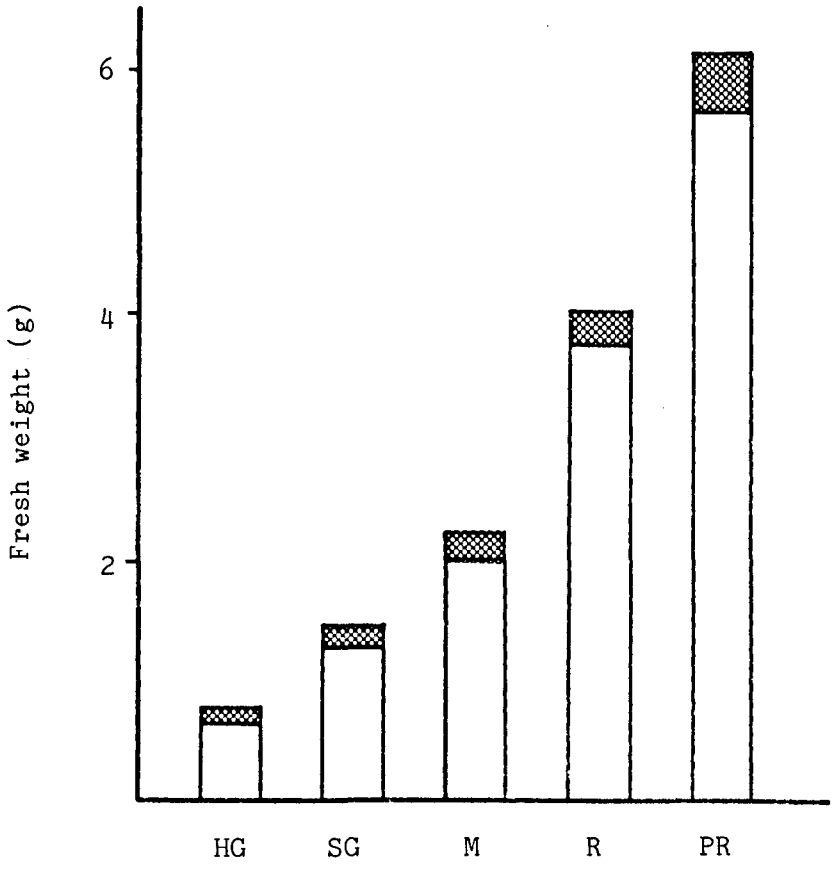


Fig. 3.4 Change in drupelet (□) and receptacle (▩) components of berry fresh weight. (30 fruit/sample).

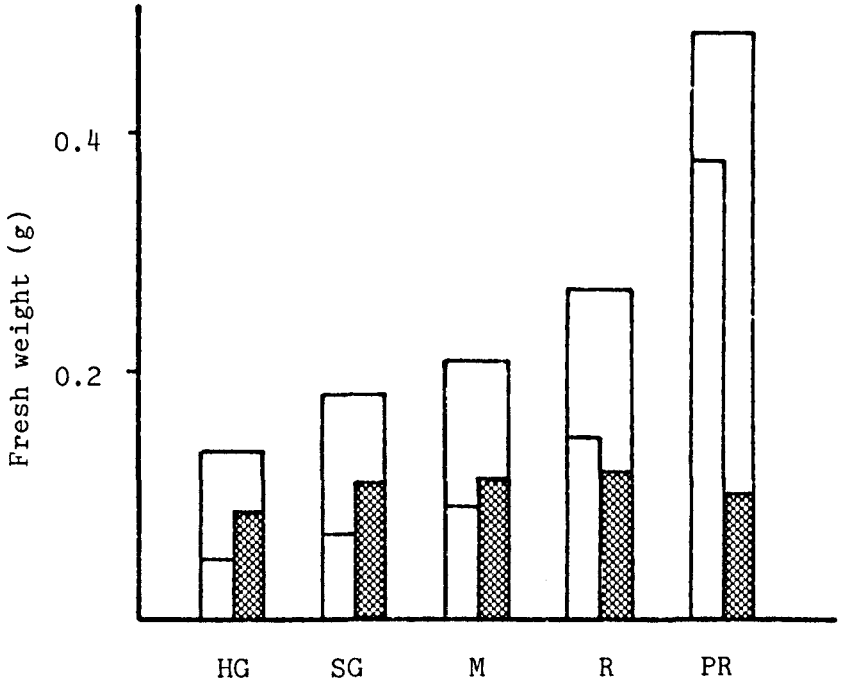


Fig. 3.5 Change in receptacle fresh weight and the apex region (□) and calyx region (▩) separately. (30 fruit/sample).

result of desiccation. This enlargement of the apex of the receptacle may be important in the generation of forces ultimately causing drupelet separation.

3.1.3 iv) Dry weight

An increase in weight with maturity can arise simply through an uptake of water, an increase in cell wall material or increased density of the cytoplasm. Changes in cellular material were examined by determining the dry weight of different tissues (fig. 3.6). These mirrored the fresh weights increasing steadily from HG to PR (0.19 g to 0.76 g per berry) with the major increase between M and PR (0.34 g to 0.76 g). The drupelet dry weight as a percentage of the fresh weight was 24% in HG decreasing to 18% by SG and 15% by M. There was virtually no difference between R and PR at 12.3-12.5%. Hence the percentage of water in the tissues increases with development. This would have been anticipated since drupelets become visibly more fleshy as the fruit matures.

The receptacle dry matter increased slowly from HG to R and by 38% between R and PR, this change mainly being attributed to the swelling of the apical area to which the drupelets are attached, there being little change in the calyx region (fig. 3.7). As a percentage of the fresh weight the receptacle dry weight decreased from 21% at HG to 14 or 15% at R/PR. Examining the receptacle more closely, the apex dry weight as a percentage of its fresh weight decreased steadily from 18% at HG to 10% at PR, coinciding with swelling observed at full maturity. The calyx region dry weight decreased slightly from 25% of the fresh at HG to 20% at R. When PR however the calyx was 31% dry matter as a result of growth and

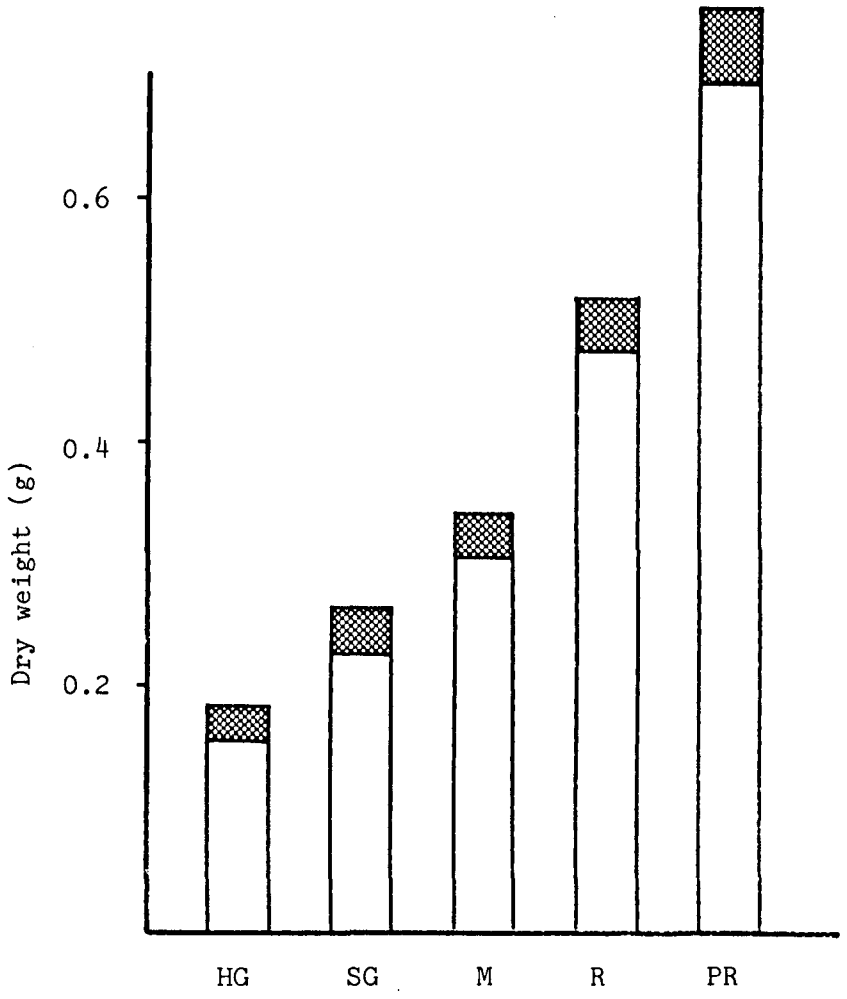


Fig. 3.6 Change in drupelet (□) and receptacle (▩) components of berry dry weight. (30 fruit/sample).

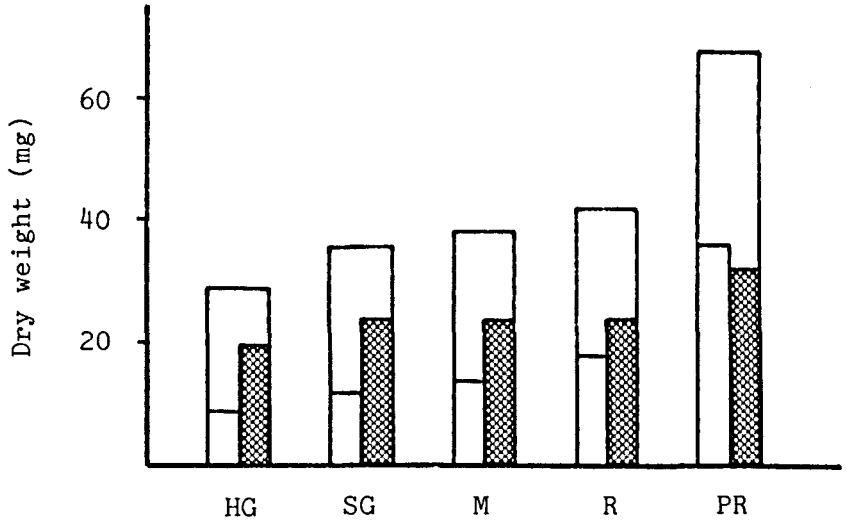


Fig. 3.7 Change in receptacle dry weight and the apex region (□) and calyx region (▩) separately. (30 fruit/sample).

increased material in the area between the apex and the point of sepal insertion. The percentage dry matter increased in the calyx also as a result of the natural desiccation of the sepals.

3.1.3 v) Drupelet titratable acidity

In general fruits decrease in acidity as they ripen although the cell sap of ripe fruit is often acidic. The two most frequently found organic acids are malate and citrate, the latter being predominant in raspberries (Nelson, 1925; Whiting, 1958; Green, 1971). This was confirmed in a preliminary gas chromatographic analysis of the acids in ripe and unripe Glen Clova (results not presented). In some circumstances organic acids can be utilised as respiratory substrates, a fact which may be reflected as an increase in the respiratory quotient (Rhodes, 1980).

The pattern of change in titratable acidity of raspberry depends entirely on the manner by which the results are expressed (fig. 3.8). On a per gram fresh weight basis the acidity declines slightly from HG to R (370 to 305 μEq/g) and is halved in the PR drupelets (155 μEq/g). On the per berry basis there is an increase from HG to R (240 to 1140 μEq/berry), as might have been expected. The decrease from R to PR is still obvious (1140 to 875 μEq/berry).

Other data on raspberry drupelet acidity have tended to examine only the more mature end of the developmental spectrum (Mason, 1974; Topham and Mason, 1981a). These authors used the Association of Official Agricultural Chemists (AOAC, 1960) standard method of expressing titratable acidity as a volume of 0.1 N NaOH/100 ml juice. This method cannot be used with green fruit where juice extraction is difficult and hence a different method had

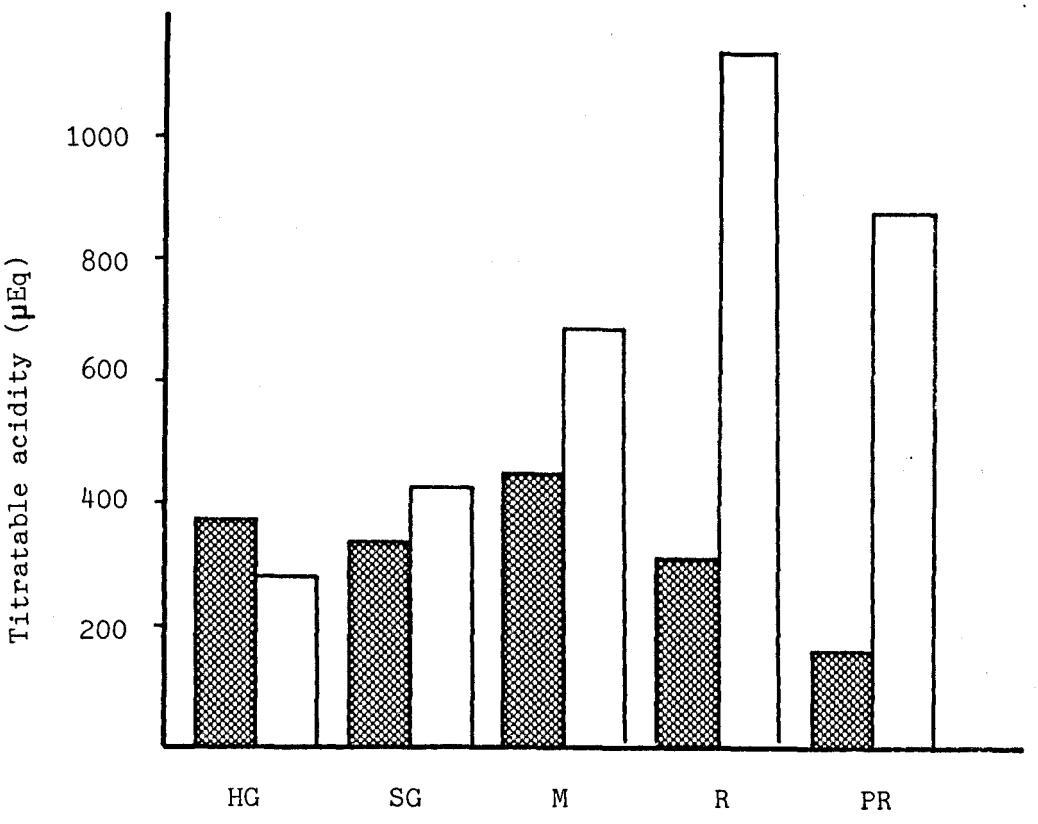


Fig. 3.8 Change in drupelet titratable acidity during development (per gram fresh weight ▩ and per berry □ , 20 fruit/sample).

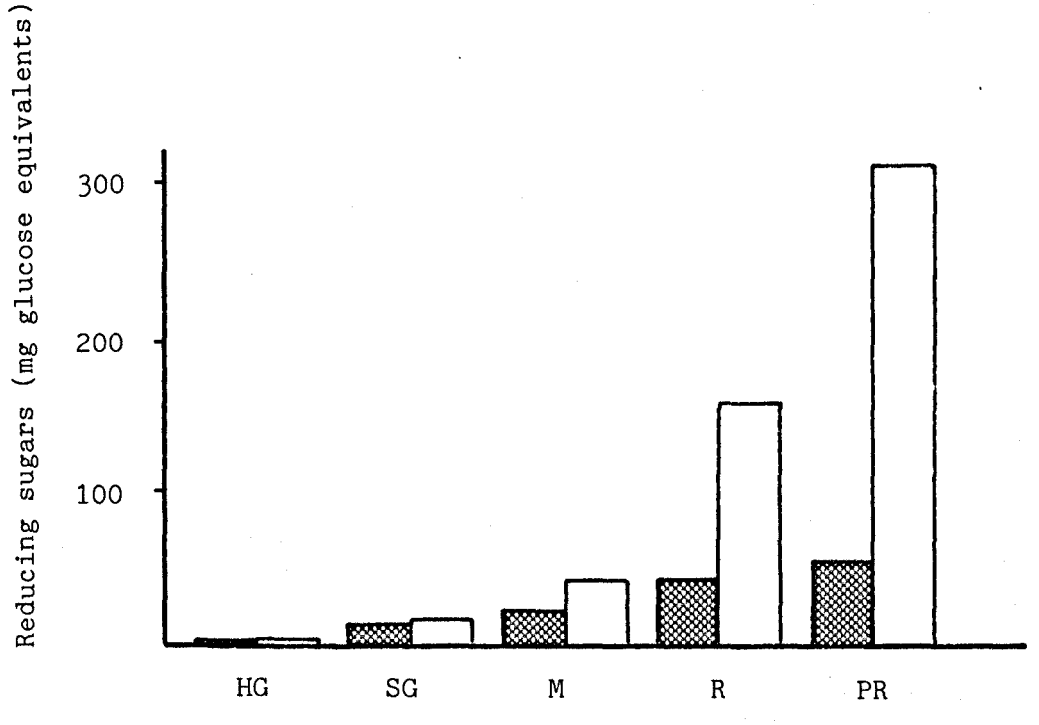


Fig. 3.9 Change in drupelet reducing sugars during development (per gram fresh weight ▩ and per berry □ , 20 fruit/sample).

to be developed whereby drupelets were homogenised in distilled water followed by titration (see Section 2.12). Although the two sets of data cannot be compared directly the values are in the same order of magnitude and show the same trend of decreased acidity at the end of ripening.

3.1.3 vi) Drupelet reducing sugar levels

In most fruits reserve carbohydrates (principally starch) are converted into simple sugars during ripening (Rhodes, 1980). Those fruit lacking these reserves may accumulate sugars by their import from the leaves. The degree of conversion to sugars and the final amount of sugar as a percentage of the dry weight varies between fruit. In bananas 98 to 99% of the starch is converted to simple sugars resulting in 15 to 20% of the dry weight (Palmer, 1971). In grapes sugars may account for up to 80% of the dry weight (Coombe, 1976).

In raspberry drupelets reducing sugar levels rose steadily between the stages examined, increasing from 3 mg glucose equivalents/g at HG to 55 mg glucose equivalents/g at PR (fig. 3.9). It must be borne in mind that the time interval between stages varied and hence if the values were put on a time scale it would appear as a rapid increase during the final few days of development (see Section 3.1.2). On a per berry basis the increase is more marked as would be expected, the main increase being between M and PR (40 to 310 mg glucose equivalents/berry).

As with the titratable acidity data it is difficult to compare these results with those in the literature. Commercially bulk raspberry pulp quality is measured as the percentage of soluble

solids determined with a refractometer, this approximates to the percentage of sucrose in the juice (Macara, 1935; Money and Christian, 1950; Coulthard, 1968; Mason, 1974; Topham and Mason, 1981a). Topham and Mason (1981a) found a similar increase in sugars to that described in Malling Jewel and Glen Isla raspberries, although they also noted a slight decrease in the very over ripe berries, a category not examined in this study. They also pointed out the variability in sugar levels noted by Macara in 1935, indicating environmental changes to have a large influence on drupelet sugar content.

3.1.3 vii) Drupelet anthocyanin content

Anthocyanins are water soluble phenolic pigments occurring in the cell vacuoles usually as sugar conjugates of the anthocyanidins (Markakis, 1975). Frequently more than one anthocyanin is responsible for coloration giving red colours at acid pH and blue at alkaline. Simple changes in pH are not considered a major factor in determining the overall fruit colour (Rhodes, 1980), rather the interaction between anthocyanins with either colourless phenolics or metal ions.

Measurement of drupelet anthocyanins previously has been carried out on ripe fruit (Blundstone and Crean, 1966), often being related to changes in processing or storage (Daravingas and Cain, 1965). The data presented in fig. 3.10 (determined by the method of Deubert, 1978) show the changes which occur during ripening. The low levels present in green fruit are masked by chlorophyll, and only become visible at the mottled stage when there has been a large reduction in chlorophyll. There is a 3 fold increase in levels

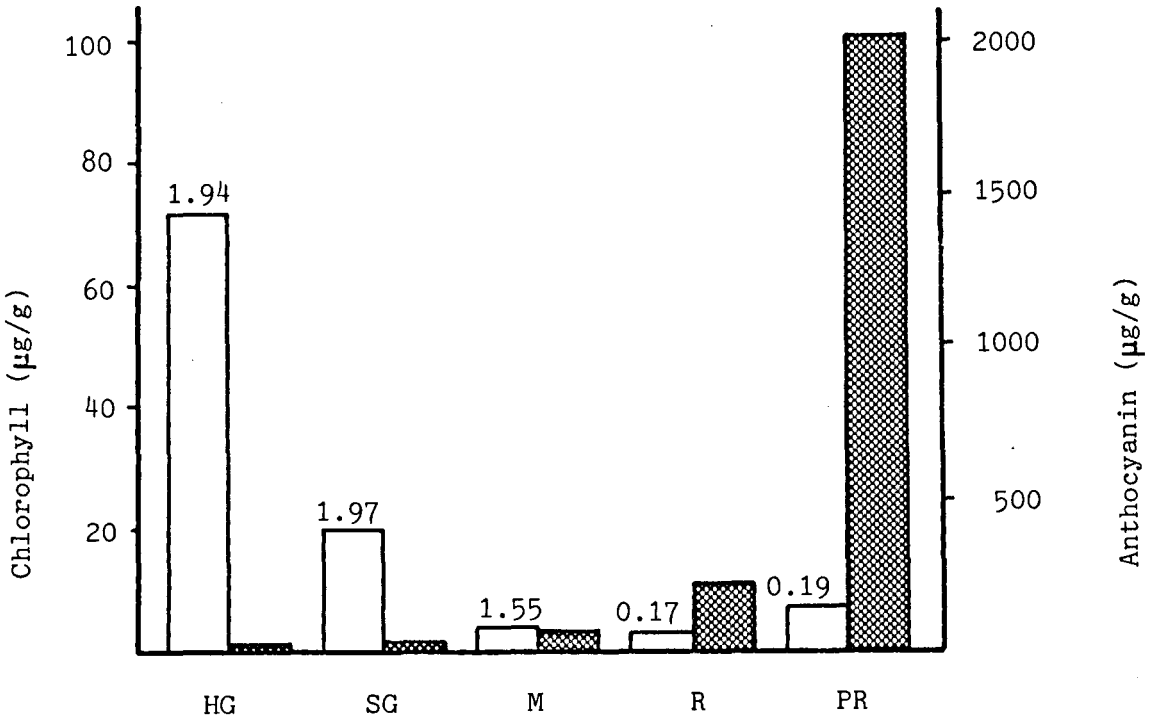


Fig. 3.10 Change in chlorophyll (□) with a:b ratios and anthocyanin (▩) pigment levels during development. (20 fruit/sample).

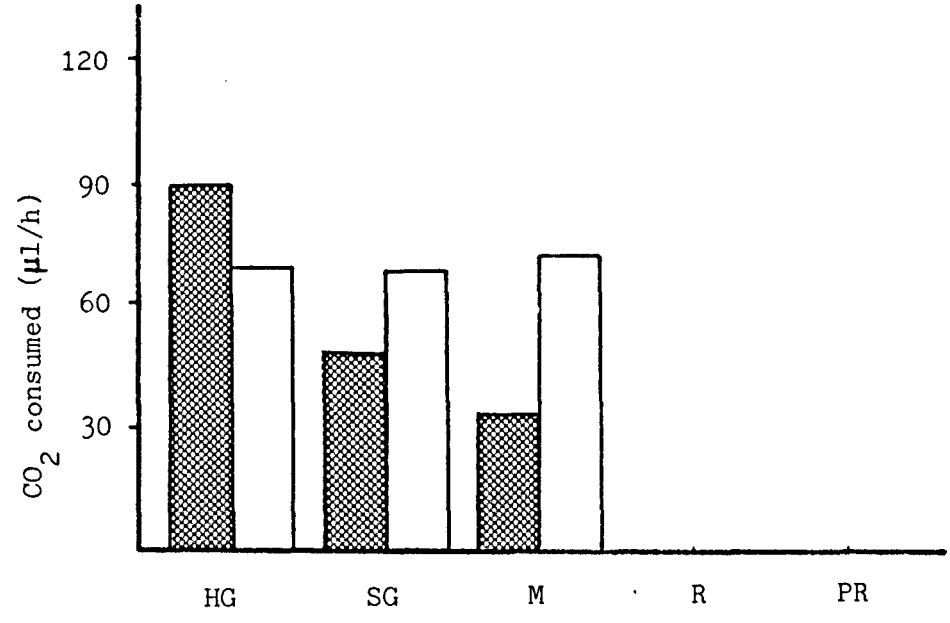


Fig. 3.11 Change in photosynthetic capacity during fruit development (per gram fresh weight (▩), per berry (□), 20 fruit/sample).

between M and R (63 to 200 µg/g) and a further 10 fold increase between R and PR (200 to 2000 µg/g). These values for ripe fruit are of the same order of magnitude as found for canned Williamette berries by Daravingas and Cain (1965). It is possible that the purple hue at PR could be a result of pH changes as illustrated earlier in fig. 3.8 as well as increased anthocyanin and other chemical interactions of the anthocyanins.

3.1.3 viii) Drupelet chlorophyll content

Colour is probably the major characteristic by which fruit are judged to be ripe. This involves a loss of chlorophyll in the majority of cases. Associated with the reduction of green coloration is the loss of the ability to photosynthesise. In apples this has been shown not to be crucial in the overall development of the fruit (Clijsters, 1969) with no direct parallel between chlorophyll levels and photosynthetic activity.

In raspberry drupelets the loss of chlorophyll mainly occurs between the HG and SG stages (71 to 19 µg/g; fig. 3.10). The ratio of chlorophyll a to b remained relatively constant at 1.9 during these changes. The reduction of chlorophyll with further development was a result of the preferential degradation of chlorophyll a resulting in a total chlorophyll level of 5 µg/g and an a:b ratio of 0.1 at PR. Knee (1972) could find no evidence for preferential degradation of chlorophyll in apple peel although he cites two reports where it has been observed - Goodwin (1958) and Zelles (1967).

3.1.3 ix) Photosynthetic activity

Photosynthesis, as the difference between dark and light CO_2 production measured by IRGA, declined rapidly on a per gram basis as berries developed from HG to M (90 to 33 µl/g/h, equivalent to 3.68 to 1.35 µmoles CO_2) and was non existent at R and PR (fig. 3.11). This is what might have been expected considering the changes in chlorophyll which were clearly visible and quantified in Section 3.1.3 viii. Expressed on a per berry basis HG, SG and M berries all had very similar photosynthetic rates around 66 to 72 µl CO_2/berry/h (2.70 to 2.94 µmoles CO_2), indicating the total photosynthetic capacity to remain constant during development from HG to M. The lack of photosynthetic activity in the R and PR fruit and the low levels of CO_2 fixation in the earlier stages point to the significance of the importation of reserve materials from the parent plant into the fruit as discussed by Rhodes (1980).

It should however be noted that the technique of comparing light and dark CO_2 fluxes takes no account of any effects of light on respiration or dark fixation of CO_2. It does however provide a crude measurement of photosynthetic activity sufficient for this study.

3.1.3 x) Respiratory activity

As detailed in the introduction fruit are often classified according to the pattern of changes in respiration during development. Climacteric fruit show an increase in respiration during ripening whilst there is no change in non climacteric fruit. The respiratory rates of raspberries were measured as CO_2 production (Infra Red Gas Analyser) or O_2 consumption (Warburg

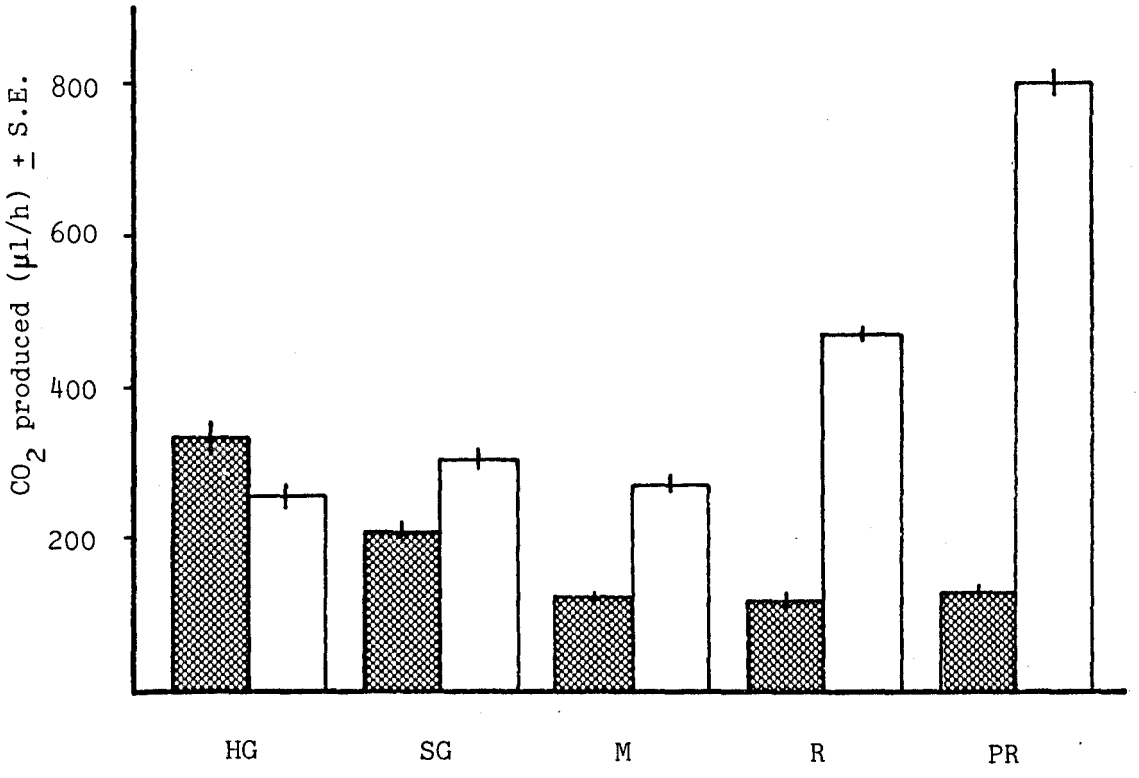


Fig. 3.12 Change in dark CO_2 production during development (per gram ▩, per berry □; means of 5 runs of 10 fruit/sample).

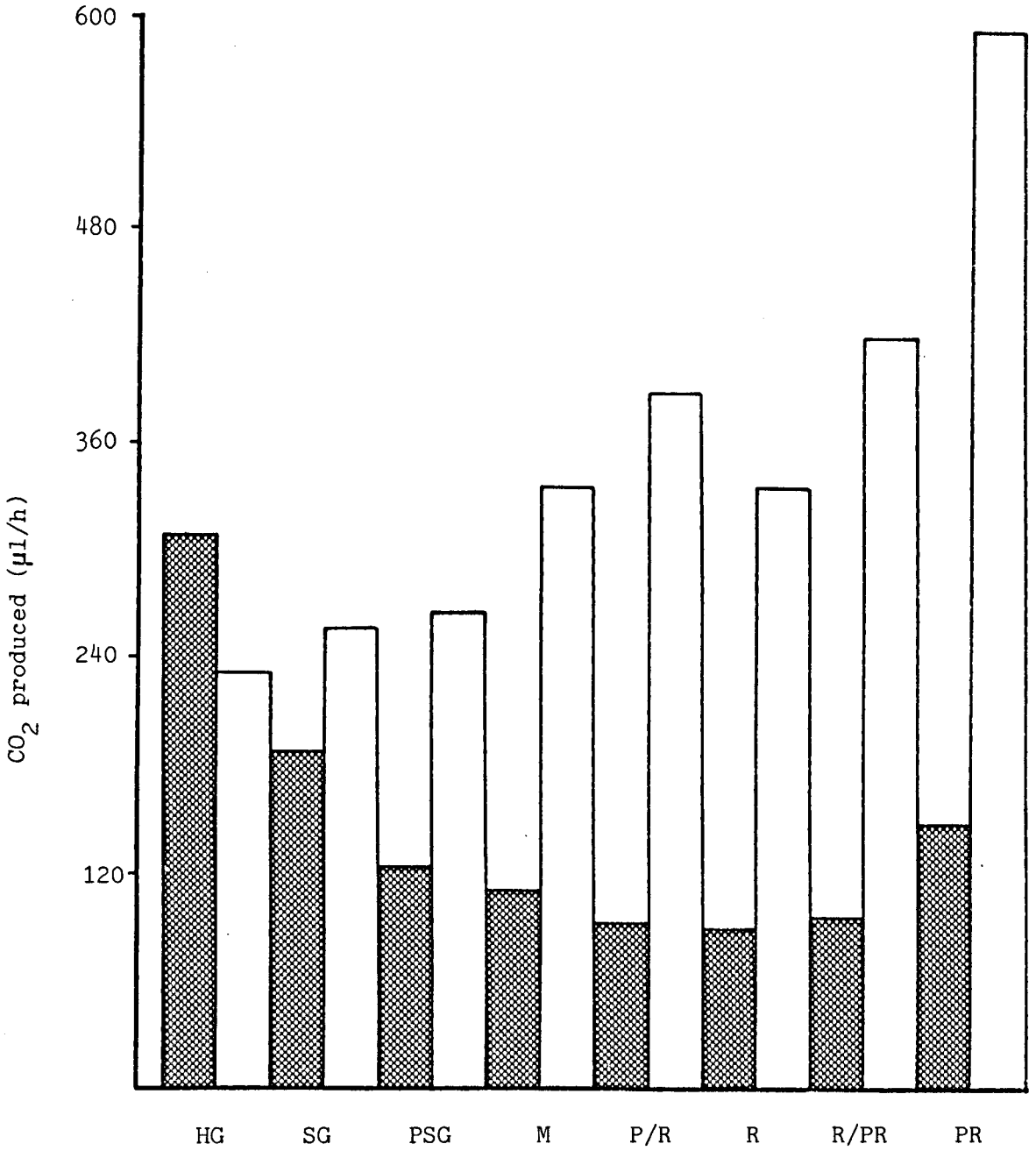


Fig. 3.13 Change in dark CO_2 production at different stages of development (per gram fresh weight ▩ , per berry □ ; 10 fruit/sample). Additional developmental categories have been included; PSG - pale soft green, P/R - intermediate between M and R; R/PR - intermediate between R and PR.

Manometry) in the dark. The use of shaking Warburg manometers attached to a Gilson Respirometer was not suitable for the extremely delicate PR fruit which were susceptible to damage.

The results in fig. 3.12 show the dark CO_2 output to fall steadily from HG to R when expressed on a per gram basis (333 to 118 µl CO_2/g/h; 13.62 to 4.82 µmoles CO_2). The slightly higher average rate for PR fruit (130 µl CO_2/g/h, 5.32 µmoles CO_2) was present in 4 out of 5 of the experimental runs, the fifth being almost equal. Expressed in this way the data suggests raspberry fruit to be non-climacteric. On a per berry basis (fig. 3.12) the dark CO_2 output increased with development from HG to PR 260 to 800 µl CO_2/berry/h, 10.63 to 32.72 µmoles CO_2). Expressed in this way the results give the appearance of a climacteric respiratory response.

In a further experiment the developmental sequence was examined in more detail using 8 categories instead of the usual 5. The results (fig. 3.13) confirmed a decrease in dark CO_2 efflux per gram and an increase on a per berry basis as development proceded. There was again a slight increase in the CO_2 produced by the very ripe fruit on a per gram basis.

Comparable work on raspberries by Jolliffe (1975a) showed the respiratory rate of Williamette raspberries to increase with development. This increase measured on a per gram fresh weight basis occurred between what would be classed as SG as PR fruit in this study. The actual values were considerably higher than for Glen Clova being between 510 and 710 µl CO_2/g/h. The stages examined however omitted the HG category but included a stage past PR. In these very mature berries the CO_2 output fell dramatically

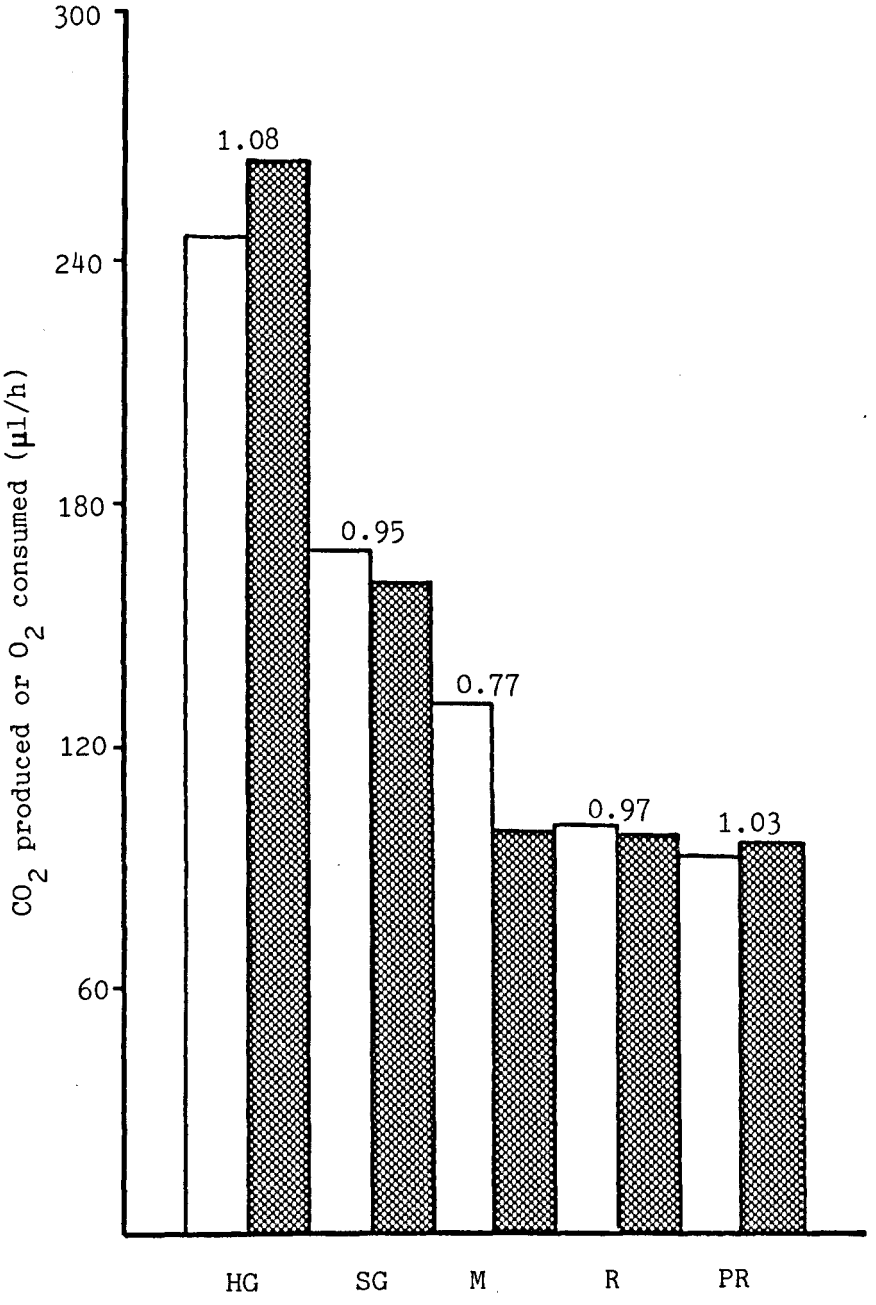


Fig. 3.14 Change in dark CO_2 output (▩) and O_2 uptake (□) with development for the same fruit (10 fruit/sample). Values represent respiratory quotient (RQ).

to around 100 µl CO_2/g/h. In another report examining changes in respiration during storage, detached 'Meeker' raspberries had a CO_2 output of 165 µl/g/h and detached Williamette berries 122 µl CO_2/g/h, both values measured after one days storage at 25°C. Unfortunately no value was presented for freshly harvested raspberries in this report (Varseveld and Richardson, 1980).

As a result of the lack of a detailed study of respiration rates in raspberry fruit, and because of the apparent contradiction of Jolliffe's (1975a) results, oxygen fluxes were also examined. These changes mirrored the fluctuations in CO_2 production rates and both CO_2 and O_2 fluxes in the dark were compared for the same set of berries (fig. 3.14). These show the changes to be almost identical, the difference being expressed as the respiratory quotient (CO_2 produced : O_2 consumed ratio, RQ). The RQ varied during development, there being a decrease at M (0.77), all other stages being close to 1. It is difficult to assess the significance of changes in RQ since in climacteric fruit it may increase eg apple (Kidd and West, 1924) or decrease eg banana (Palmer, 1971) during the respiratory increase. In general an increase in RQ above 1.0 indicates a shift in respiratory substrate towards utilising greater amounts of organic acids than carbohydrate.

It must also be noted that whole fruit included both drupelets and receptacles and their individual contributions cannot be assessed. It is quite likely that the respiratory contributions of the different parts varies depending on their development within the whole fruit. The CO_2 and O_2 flux results corroborate each other suggeting the raspberry to be a non-climacteric fruit in terms of respiration rates.

3.1.3 xi) Summary

The results presented show raspberry fruit to develop in a fashion similar to other fruit with an accumulation of dry matter, sugars and anthocyanins and a decrease in acidity and chlorophyll (and hence photosynthesis). The increasing EPRs indicate raspberry to be a climacteric fruit. This however is not supported by respiration data which shows respiratory activity to decrease on a per gram basis with ripening. The decline in FRS commences when fruit are mottled, the point at which EPR starts to increase and pigment changes are most noticeable.

3.1.4 Reproducibility of sampling from year to year

Having decided on selection of fruit according to colour and drupelet expansion no major inconsistencies were noted for other characteristics between years. For instance, examination of ethylene production rates between 1983 and 1986 (Table 3.1) showed a remarkable consistency in all years in spite of the widely different climatic conditions between years.

Table 3.1 Representative EPR values of different developmental stages from 1983 to 1986. (15 fruit per sample)

Developmental Stage	EPR nl/g/h			
	1983	1984	1985	1986
PR	15.5	13.4	15.4	10.9
R	4.4	5.8	5.6	3.6
M	1.9	1.22	1.11	0.7
SG	0.15	0.10	0.15	0.065
HG	0.45	0.15	0.20	0.20

The data in table 3.1 shows that picking fruit in the classes shown gives fruit of predictable ethylene production rates which along with FRS is the main point of examination. This reproducibility can be seen just as clearly in the results presented in Section 3.1.8 showing the relationship between internal ethylene levels and the production rate as measured by ethylene emission from

fruit (fig. 3.34). The graph is a composite of results from three successive years, with all the data fitting the line quite well, again despite climatic differences between years.

Hence the method of selecting fruit in the different categories is reliable since it provides berries at the same stage of development with respect to EPR (and FRS) from year to year. Values of FRS for the period 1983 to 1986 are shown in table 3.2.

Table 3.2 Representative FRS values of development stages between 1983 and 1986 (50 fruit per sample).

Developmental Stage	FRS + SE (N)			
	1983	1984	1985	1986
PR	0.53 ± 0.06	0.29 ± 0.06	0.54 ± 0.06	0.69 ± 0.08
R	1.52 ± 0.10	1.32 ± 0.12	1.81 ± 0.12	2.14 ± 0.20
M	4.68 ± 0.40	4.68 ± 0.38	4.21 ± 0.35	5.37 ± 0.34
SG	7.35	7.35	7.35	7.35
HG	7.35	7.35	7.35	7.35

3.1.5 Anatomical observation

In a comprehensive examination of raspberry fruit abscission MacKenzie (1979) showed drupelet separation from the receptacle to be true abscission by the nature of the cellular changes. As with other abscission systems cell separation occurred along the middle lamellae with partial cell wall dissolution. MacKenzie also considered tissue tensions caused by the differential growth of drupelet and receptacle to be in part responsible for ultimate separation.

Simple anatomical observations were made using light microscope sections and scanning electron microscopy. These were made to confirm the findings of MacKenzie and not necessarily extend them.

3.1.5 i) Light microscope observations

The hard green material in plate 3.6 shows a single drupelet attached to part of the receptacle. At the base of the drupelet the position of the abscission zone is marked by a band of smaller cells where separation ultimately occurs. Also shown is the receptacle side of a broken abscission zone, clearly showing separation to have occurred through cell wall breakage rather than cell separation.

The detached ripe drupelet in plate 3.7 shows the rounded cortical parenchyma cells typical of abscission at its base where separation has occurred. As well as being rounded the cells are also more loosely connected appearing to be separate and loosely packed together. Abscission doesn't appear to have occurred across a single plane of fracture, rather by a general weakening of pockets of cells in a deeper abscission zone as was observed by MacKenzie

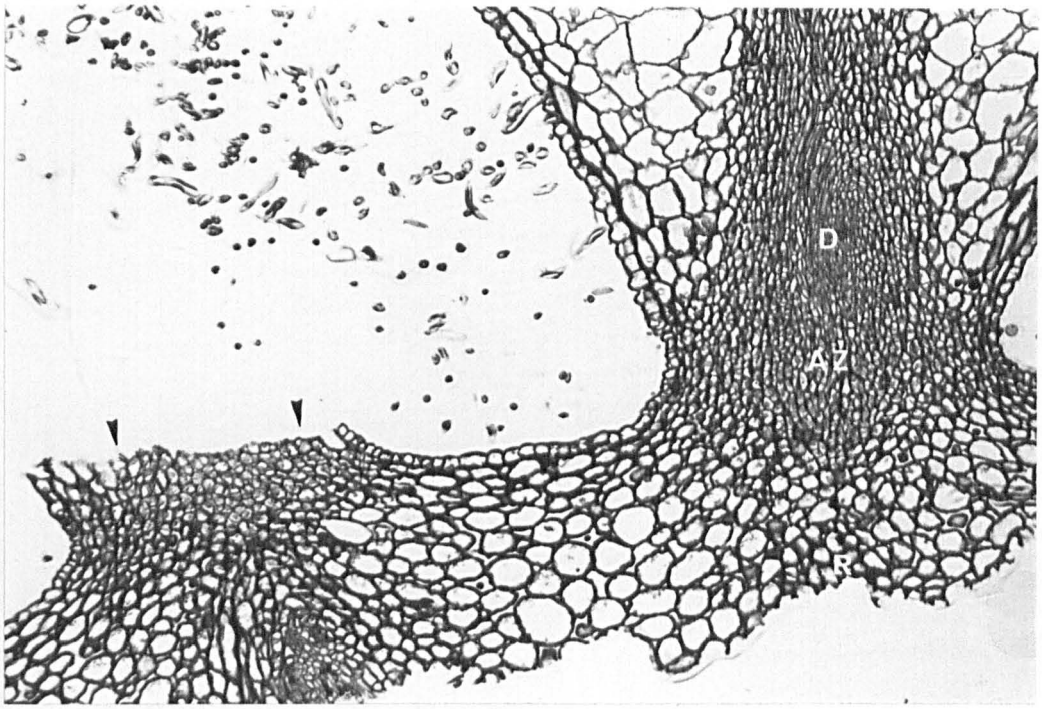


Plate 3.6 Drupelet (D)/receptacle (R) abscission zone (AZ) in hard green Glen Clova fruit (x100). Also showing the broken cells where an abscission zone has been ruptured (►).

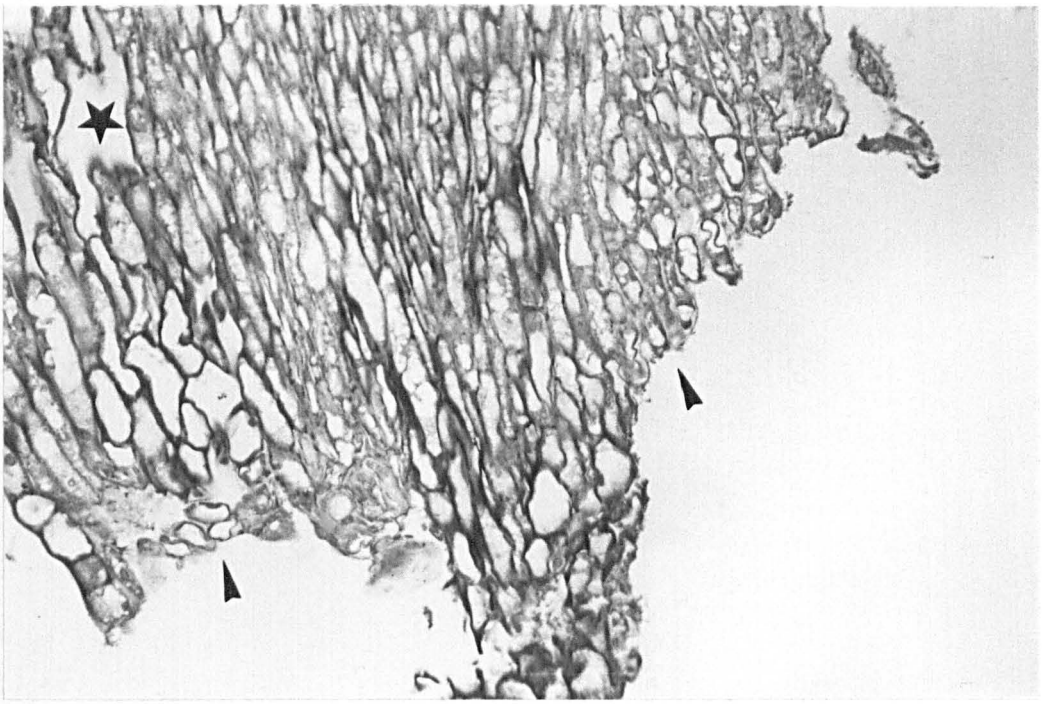


Plate 3.7 Cells at the abscission zone of a ripe drupelet of Glen Clova after separation showing individual rounded cells at the separation surface (►) and cavity formation (*) x250.

(1979). The appearance of cavities in the cortical tissue as a result of the degeneration of pockets of cells is clearly visible and was noted by MacKenzie (1979). The protrusion of a strand of tissue surrounding the vascular trace indicates the abscission process does not weaken the vascular tissues in the abscission zone, their separation being as a result of mechanical rupture outside the zone. Further evidence for this is given in the next section of scanning electron microscope observations.

3.1.5 ii) Scanning electron microscope observations

When viewed with the SEM the scars on the drupelet base (plates 3.8 and 3.9) and receptacle (plates 3.10 and 3.11) after abscission both showed rounded cells at the fracture surface of the cell separation. These plates also clearly show the ruptured vascular material at the centre of the abscission zone. The receptacle after separation (plate 3.10) clearly shows the depth of cell separation and formation of cavities within the tissue. Plate 3.12 shows a whole receptacle after removal of all the drupelets to reveal the extent to which abscission zones cover the surface of the apex region.

These findings confirm MacKenzie's (1979) observations refuting the comment by Reeve (1954) that "There is no abscission tissue at the bases of mature drupelets".

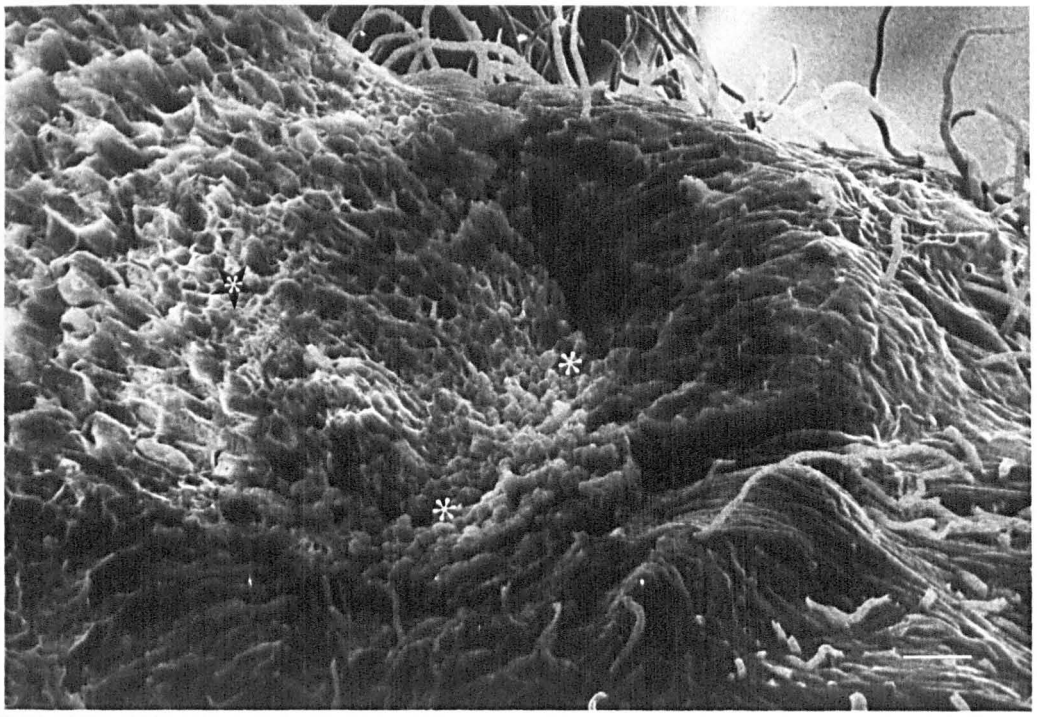


Plate 3.8 SEM of the abscission scar on a ripe Glen Clova drupelet base after abscission, showing rounded cortical cells (*) and fractured vascular strand ( ). Bar = 100 μm.

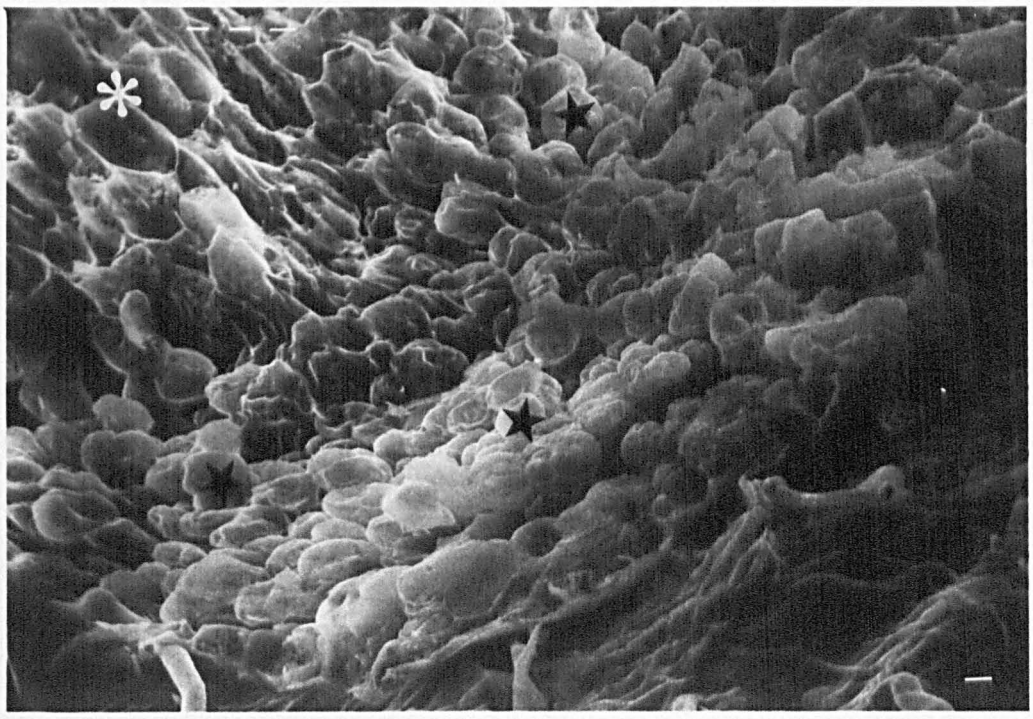


Plate 3.9 Close up SEM of rounded cells ( ) and fractured vascular strand (*) in the tissues at the base of an abscised ripe Glen Clova drupelet. Bar = 10 μm.

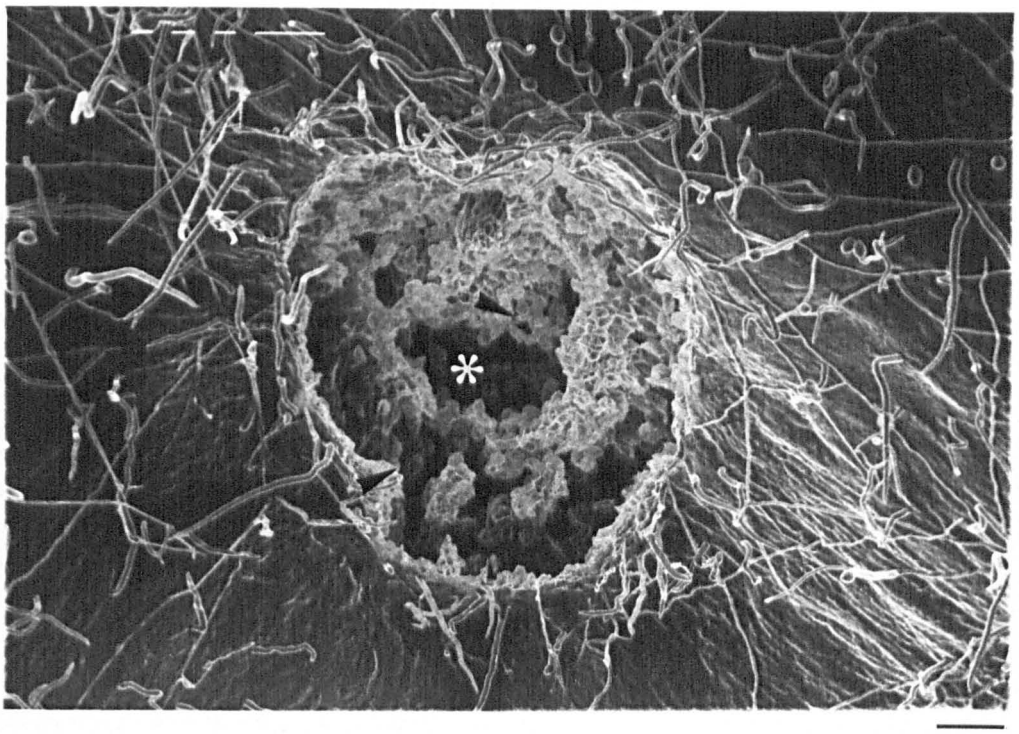


Plate 3.10 SEM of the abscission scar on the receptacle after abscission of a ripe Glen Clova drupelet, showing cell separation (►) and depth of cavitation (*). Bar = 100 μm.

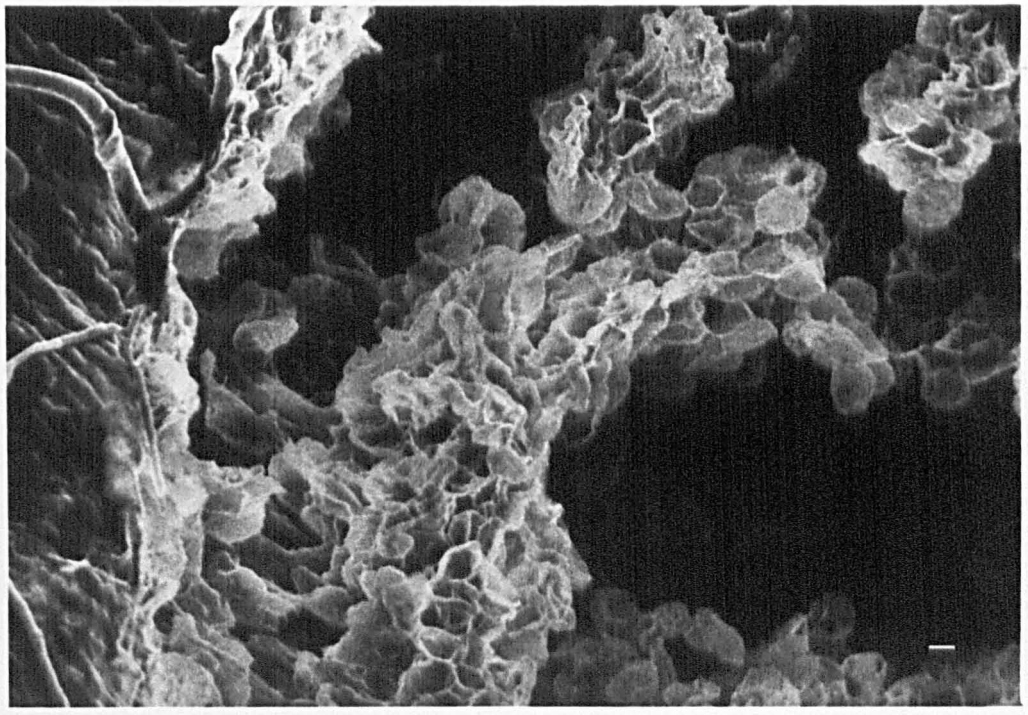


Plate 3.11 Close up SEM of cells and cavitation in a receptacle scar after abscission of a ripe Glen Clova drupelet. Bar = 10 μm.

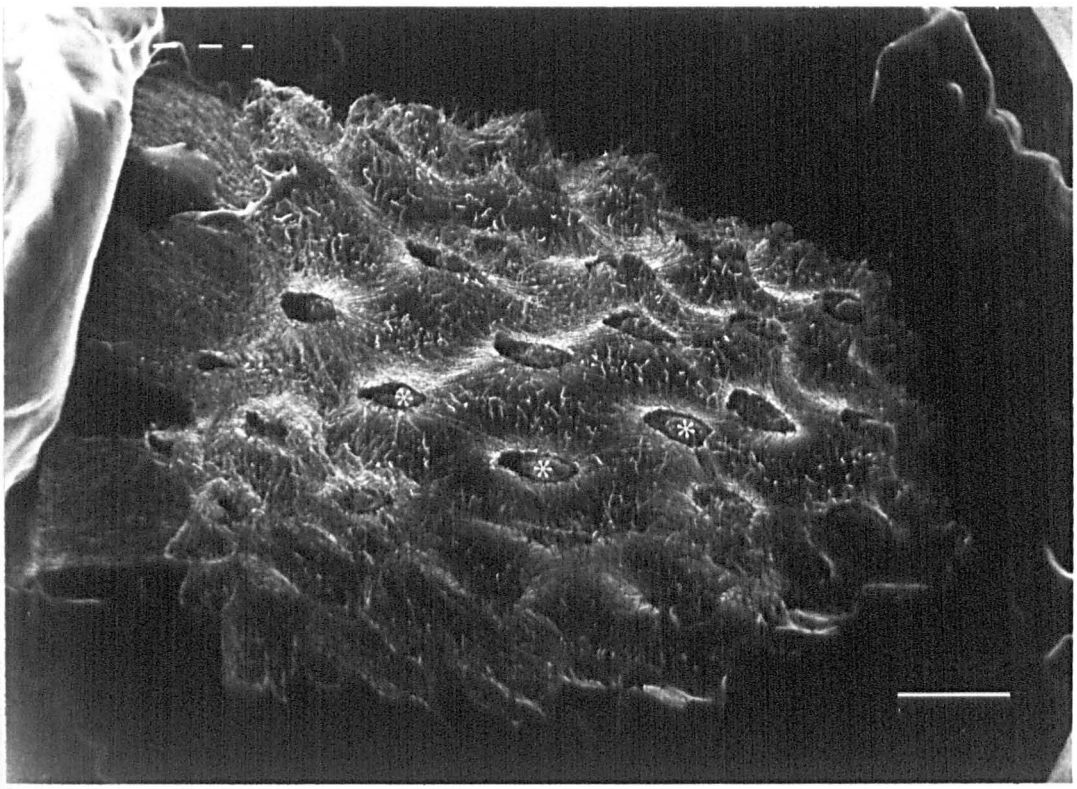


Plate 3.12 SEM of a ripe receptacle of Glen Clova showing abscission scars (*) covering its surface. Bar = 1 mm.

3.1.6 The correlation between ethylene production rates and fruit abscission.

3.1.6 i) Change in ethylene production and fruit retention strength during development using large samples of fruit at each stage of development

The preliminary results obtained by examining batches of fruit (fig. 3.15) showed the EPR of both HG and SG fruit to be very low (<0.2 nl/g/h). These rates increased in a climacteric fashion as fruit developed through M and R reaching values in excess of 10 nl/g/h when PR. If expressed on a temporal basis this increase in EPR would appear more spectacular since the changes between M and PR occur over the final 4 or 5 days of a 30 day developmental period (see fig. 3.2). It also became obvious that the HG fruit had consistently higher EPRs than the larger SG fruit.

The first signs of a reduction in FRS (as a measure of abscission zone integrity), coincided with the first increase in EPR at the M stage of development. This is consistent with the hypothesis that ethylene may be involved in the regulation of fruit abscission, although it does not prove a causal relationship. It is equally possible that ethylene production could be as a result of abscission rather than the cause of it.

3.1.6 ii) Change in ethylene production of individual fruit during development

If ethylene is the inductive agent in abscission it should be possible to establish that an increase in EPR precedes any measureable FRS reduction. Data obtained as averages of large

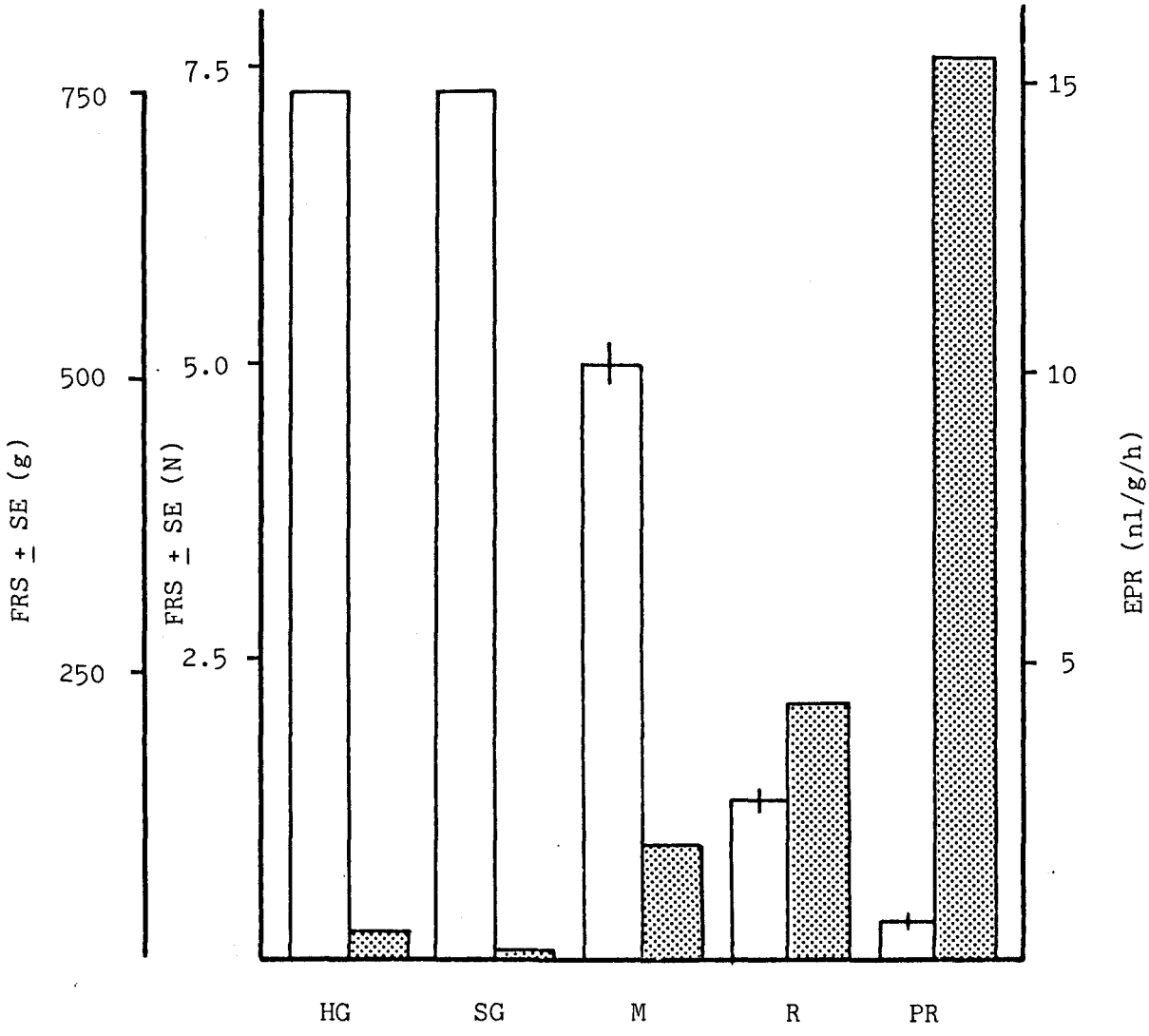


Fig. 3.15 Change in fruit retention strength (□) and ethylene production rates (▩) during fruit development. (30 fruit/sample).

batches of SG and M fruit (as presented in fig. 3.15) could well obscure important variation between individual fruit. For instance, a few SG fruit with relatively high EPRs would be obscured by a large batch of fruit with low EPRs. The identification of these few fruit is important in that they could indicate fruit in the early stages of ethylene induced abscission. Equally a few mottled fruit with low EPRs yet showing reductions in FRS could be obscured in a population with a higher average EPR. Identification of these fruit would cast doubt on ethylene as an inductive agent for abscission if a reduction in FRS had occurred in the absence of increased ethylene production. Since it is feasible to measure the EPR of an individual fruit in the same way as a batch (see Section 2.6) a study of individual fruit was undertaken.

The EPRs of individual fruit over the whole spectrum of developmental stages are shown in fig. 3.16, the fruit in this instance having been classified into a developmental series according to their fresh weights. The data clearly shows EPRs to increase at the higher fruit weights, as might have been expected from the data on bulk samples (fig. 3.15). No account however is taken of small ripe or large unripe fruit and this is to some extent reflected in the scatter of points at the higher weights (above 2.5 g). A point of interest to arise from fig. 3.16 is the presence of fruit <0.5 g showing EPRs higher than the larger HG fruit (up to around 1.0 g). In this instance the use of fresh weight as a method of classification has allowed the detection of these fruit which would otherwise not have been identified from the bulk of the HG category. Classification by weight however fails to distinguish fully between the developmental stages at higher weights, a better

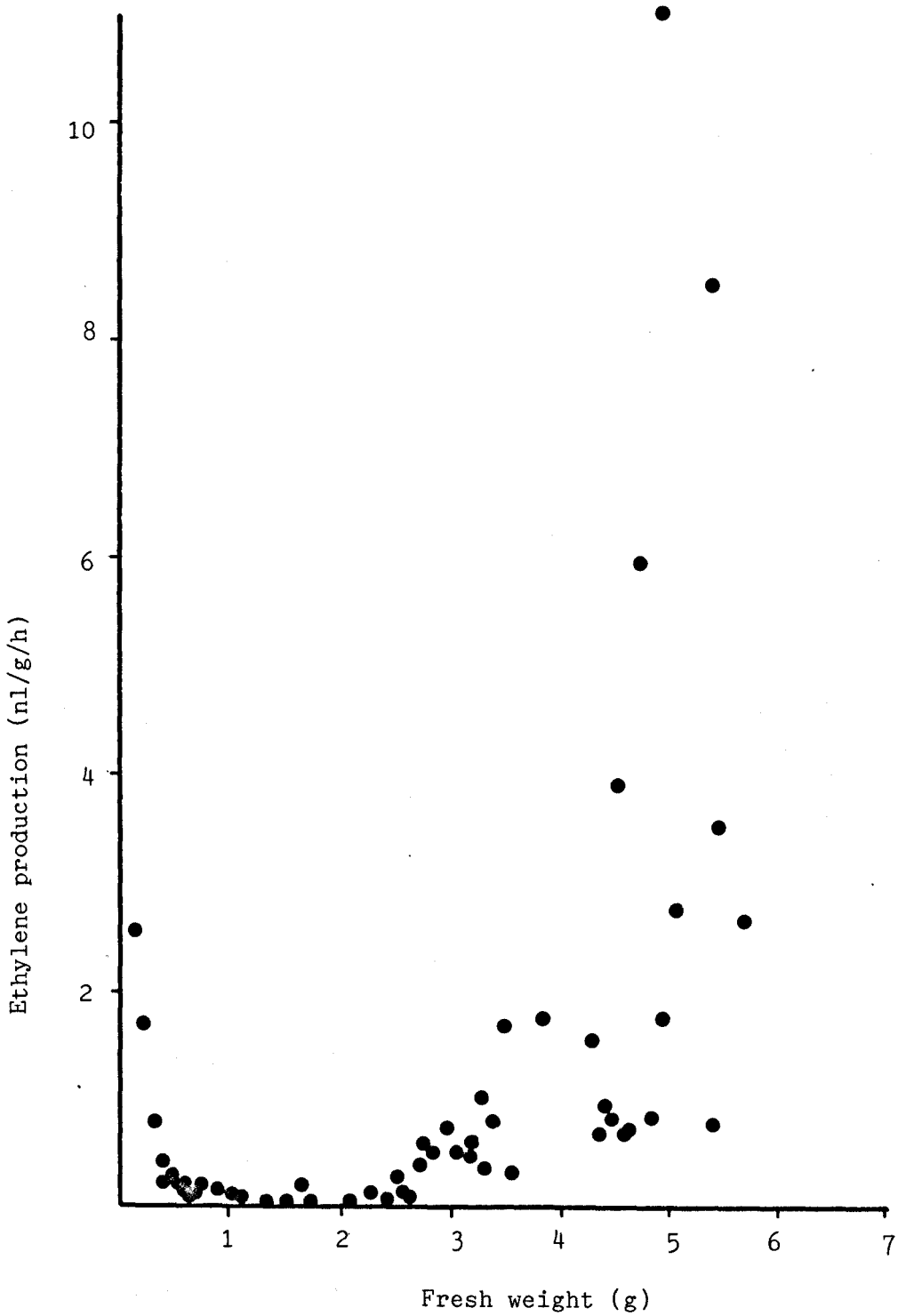


Fig. 3.16 Ethylene production rates of a range of individual fruit classified by fresh weight.

method being to separate fruit according to FRS once weakening has commenced.

Taking these two factors into consideration a further experiment was carried out examining the EPRs of the full range of stages in raspberry fruit development from closed flower buds to fully mature fruit (fig. 3.17). In an attempt to facilitate interpretation of this data the EPRs have been presented against a series of developmental changes. This was particularly important during flowering where the changes between a closed bud and a small berry after flowering are insignificant in terms of weight. As a consequence weight has only been used to separate fruit or flowers within the individual developmental categories. The classification chosen was therefore: i) Closed buds with no sepal opening or petals visible, separated on a weight basis. ii) Buds opening separated into a developmental series by the sequential movements of the sepals and petals. These are described fully in Section 3.2. iii) The numbers of petals lost. iv) The development of the berry as judged by the drying out of the stamens followed by expansion of the drupelets (for fruit weights under 0.3 g). v) Separation by fruit weight once above 0.3 g but not showing any reduction in FRS. vi) The degree of reduction in FRS.

The ethylene peak during flowering coincided with the shedding of petals possibly indicating a role for ethylene in the regulation of petal abscission. This prospect is examined in greater depth in Section 3.2.

The EPRs of fruit declined during the initial stages of development reaching a steady low EPR of less then 0.25 nl/g/h whilst in the weight range 0.4 to 2.6 g. At weights in excess of

Fig. 3.17 Change in ethylene production during raspberry fruit development. The classification series is fully described in the text.

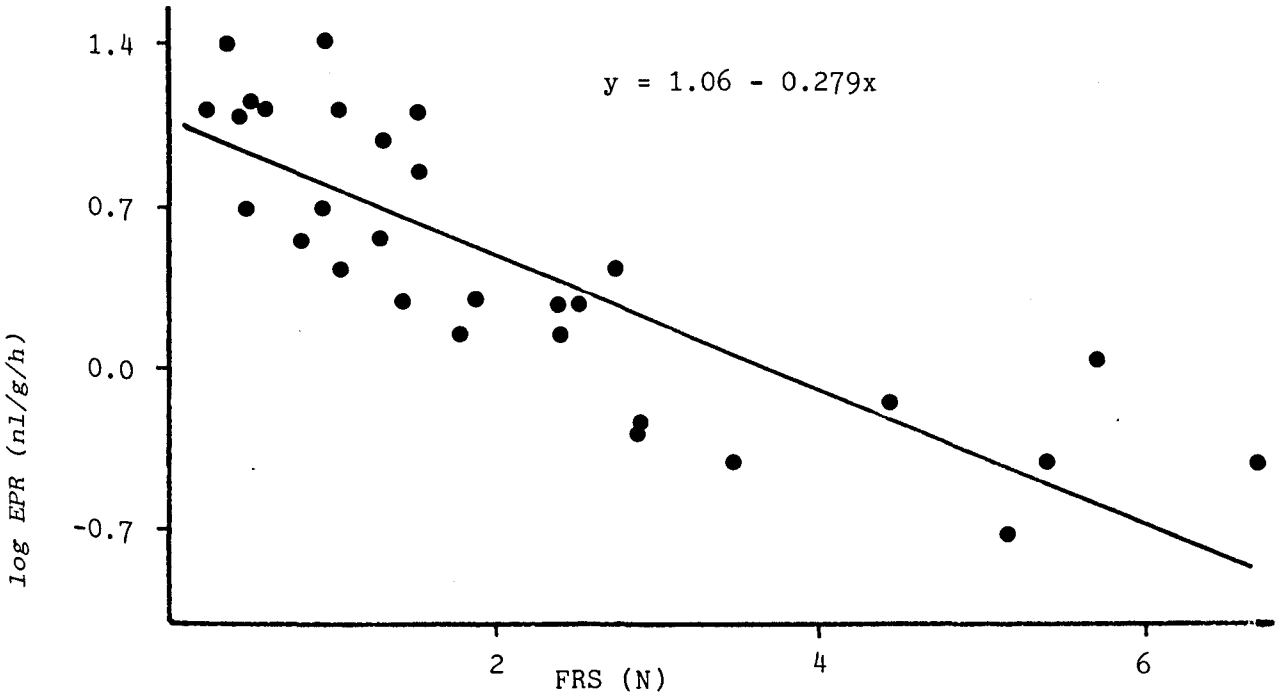


Fig. 3.18 Relationship between FRS and EPR of individual fruit covering the whole spectrum of development.

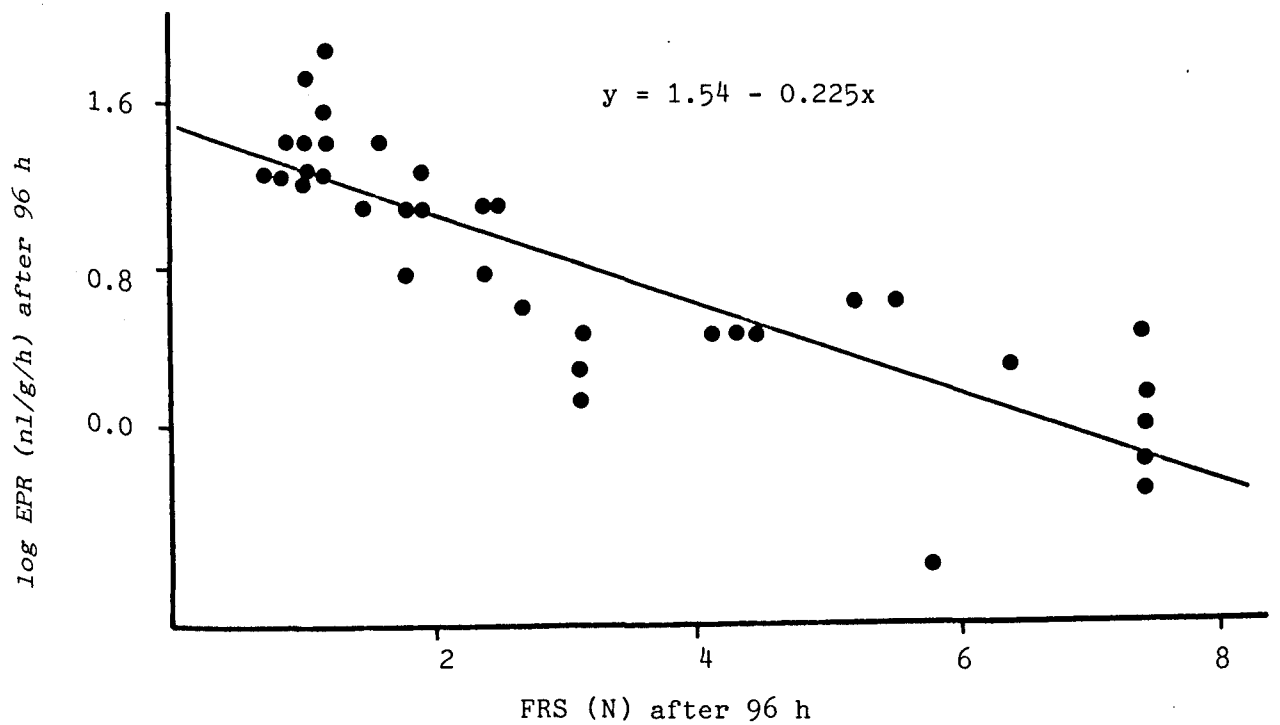


Fig. 3.19 Relationship between FRS and EPR of individual fruit 96 hours after collection at SG.

2.6 g (up to 4.3 g) the EPRs were in the range 0.3 to 1.9 nl/g/h. In these instances there had been no reduction in FRS, thereby suggesting ethylene production to increase to some extent prior to any changes in the abscission zone which would result in weakening. The final division of the developmental continuum was based on a reduction in FRS. In terms of EPR the first few fruit in this category and the last fruit in the previous (fruit weight) category were indistinguishable. Generally those fruit showing FRS between 3 N and 5 N had EPRs slightly above the majority of the fruit in the previous category. Once the FRS had fallen to less then 3 N the EPR increased dramatically in some cases exceeding 20 nl/g/h.

There is therefore some evidence here to support a role for ethylene as an inductive agent for abscission with a clear category of fruit showing increased EPRs prior to any FRS decline. The actual levels of production achieved at this stage however are small in comparison to those occurring during the final stages of fruit separation (FRS < 3 N). There is a clear correlation between FRS and EPR ($F(1,32) = 52.2$; $P < 0.001$) once FRS decline has commenced as illustrated in fig. 3.18.

3.1.6 iii) Ethylene production of soft green fruit as an indicator of their capacity to ripen

The results in the previous section indicated some SG fruit to have higher rates of ethylene production than the bulk of the sample, whilst still showing no decline in FRS. If the hypothesis that ethylene triggers abscission is correct it is possible that these fruit were about to commence abscission zone weakening. Hence it should be possible to predict by their EPR those SG fruit which

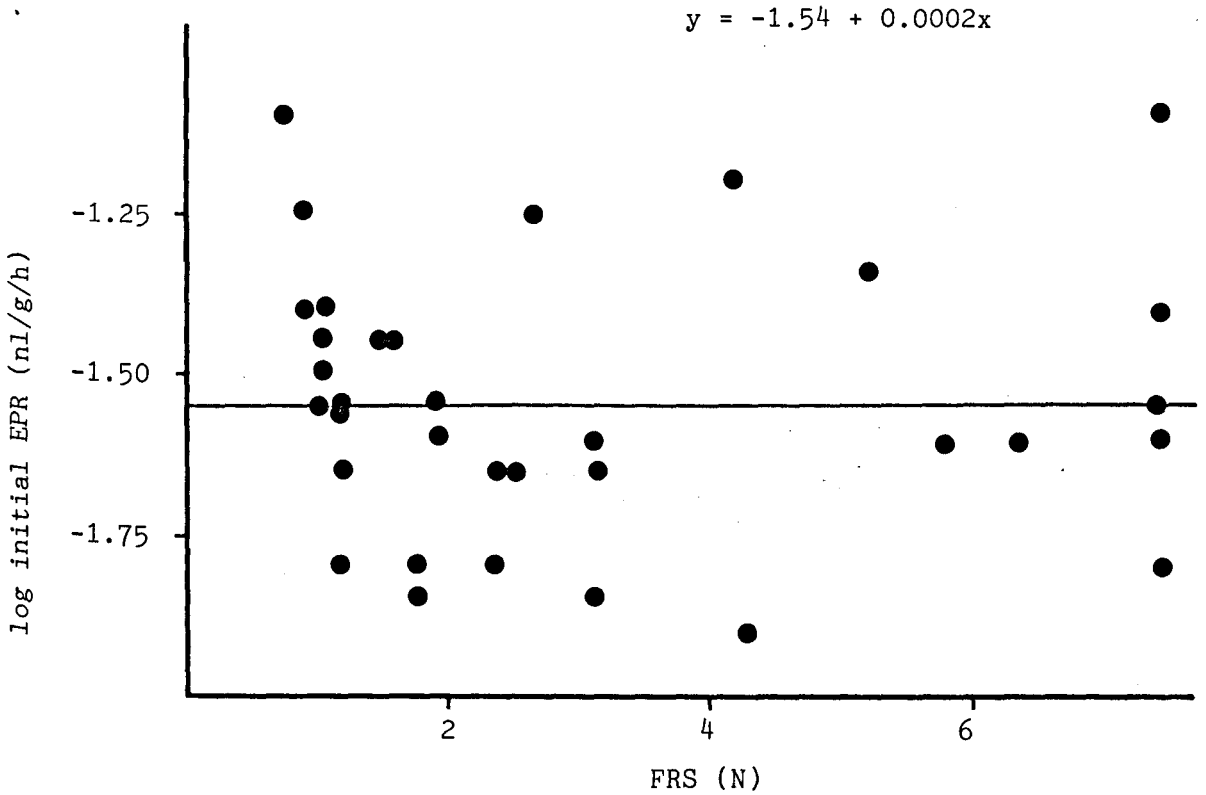


Fig. 3.20 Predictability of final FRS 96 hours after collection and measurement of EPR of individual SG fruit.

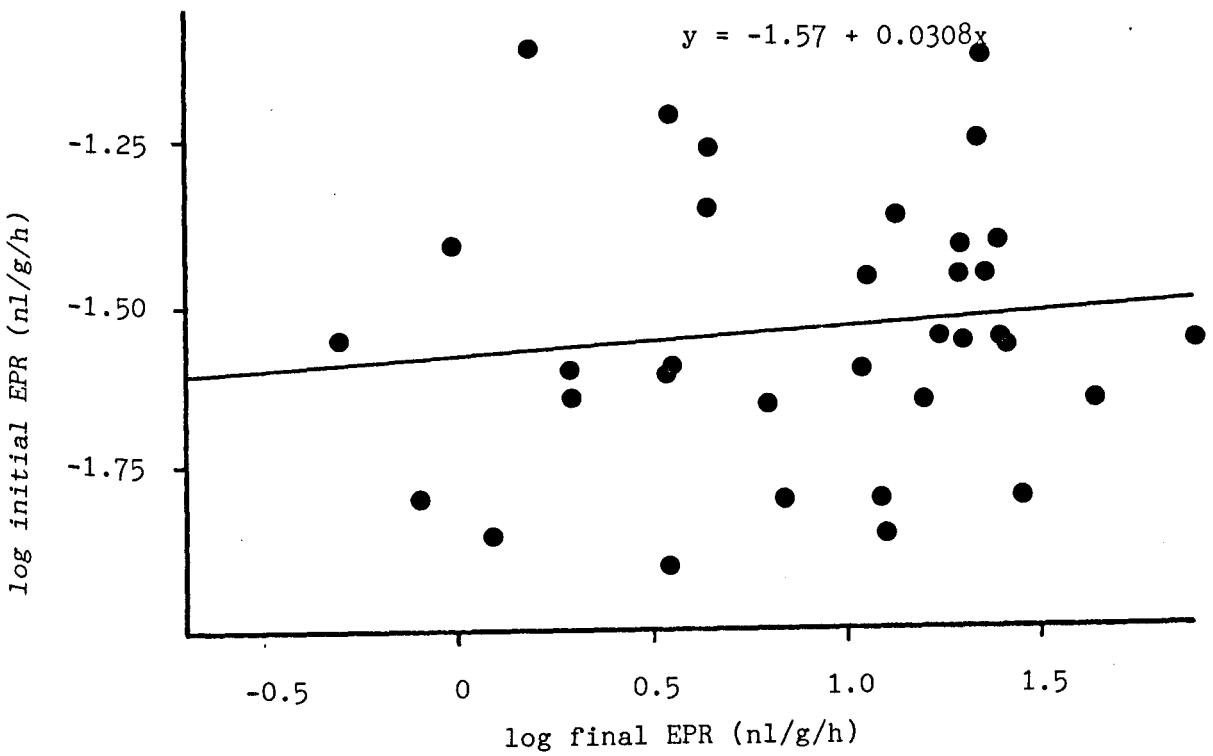


Fig. 3.21 Predictability of final EPR of individual SG fruit 96 hours after initial EPR measurement.

would be first to show a decrease in FRS on detachment from the parent plant. To test this possibility SG fruit were examined for EPRs and then re-examined 96 hours later for both EPR and FRS. During the intervening period the fruit were kept on water in the laboratory at 20°C.

As might have been predicted from earlier work (fig. 3.1) the final EPR and FRS (96 hours after collection) showed a good correlation ($F(1, 32) = 82.23$; $P < 0.001$) with increased EPRs reflecting low FRS values (fig. 3.19). It was not possible to predict the final FRS value of the fruit (fig. 3.20) nor final EPR (fig. 3.21) just from the initial EPR.

3.1.6 iv) Summary

To summarise these results, Glen Clova raspberry fruit produce increasing amounts of ethylene as they develop in a manner indicative of a climacteric fruit. From the bud stage to the fully mature fruit there are two peaks in ethylene production coinciding with flowering and the fruit becoming fully mature. At each point there is an abscission process occurring, petal fall and the shedding of the mature fruit. Abscission and the senescence of the abscinded part are considered to be ethylene mediated processes requiring ethylene involvement for their occurrence and continuation. In raspberry there is therefore a correlation between an increase in ethylene production and abscission. The role of ethylene in flowering is investigated further in Section 3.2 whilst fruit abscission is continued in Section 3.1.7.

3.1.7 The ability to influence abscission through changing ethylene levels

If ethylene is involved in the control of abscission it should be possible to influence the abscission process by adding, removing or interfering with ethylene production or action. The addition of ethylene was achieved through the use of the ethylene releasing chemical chloroethylphosphonic acid (as Ethrel) and by adding ethylene to the atmosphere around fruit. Ethylene was removed from fruit and the atmosphere surrounding them through the use of hypobaric pressures. Finally abscission was examined by the use of chemical inhibitors of ethylene production and action. In those experiments involving hypobaric pressures, or the use of chemical inhibitors of ethylene production, simple control experiments were carried out showing abscission to be reinstated by the supply of saturating levels of exogenous ethylene in the presence of the inhibitor or reduced pressures. This ensured that any measured effect on abscission was as a result of influencing ethylene levels rather than through any other aspects of metabolism.

3.1.7 i) The effect of an ethylene releasing spray (Ethrel) on abscission

The prospect of using ethylene releasing sprays to enhance abscission, and thereby aid mechanical harvesting of raspberries, has been examined in several field trials (Jennings and Carmichael, 1970; Zatyko and Sagi, 1973; Jolliffe, 1975b; Knight, 1976). These trials yielded inconclusive data concerning the capacity of Ethrel to reduce fruit FRS values, the general conclusion being that Ethrel gave slightly higher yields in the following harvest but at the

expense of individual fruit weight. These field trials were carried out with a lack of information on the ethylene physiology of raspberry fruit during development, especially at abscission. Previously only Blanpied (1971, 1972) and Knight (1976) had looked at the natural EPRs of raspberry fruit. Unfortunately none of this work was comprehensive and yielded very little useful information.

It was therefore decided initially to examine the effect of Ethrel on the ethylene levels of fruit at different stages of development. Detached fruit had their EPRs measured prior to being dipped into Ethrel (10 mM), their EPRs were then monitored over the next 24 hours whilst kept on water in the laboratory at 20°C. The results in table 3.3 indicate Ethrel to be very effective at

Table 3.3 Effect of 10 mM Ethrel on EPR of fruit at different stages of maturity (15 fruit/sample).

Maturity	nl/g/h		
	EPR before treatment	EPR 3 h after treatment	EPR 24 h after treatment
R	9.9	208.7	460.6
M	1.7	301.8	697.7
SG	0.4	571.8	1611.7
HG	0.8	867.8	2497.2

increasing ethylene levels of fruit at all stages of development from HG to R. Although expressed as nl/g/h these values are not true EPRs in the sense of a biological production, they are simply a measure of the purely chemical reaction within the cell releasing ethylene from the chloroethylphosphonic acid (Warner and Leopold, 1969).

Having shown Ethrel to be successful at elevating ethylene levels its effect on FRS was examined by individually spraying SG fruit on canes maintained in a greenhouse. Using greenhouse grown canes removed the possibility of climatic interference with either Ethrel's application or effect. Control fruit were sprayed with distilled water. After treatment the fruit were allowed to develop on the plant until being harvested for FRS measurement 120 hours later. For comparative purposes another set of SG fruit were removed from the same canes and sprayed individually with Ethrel or water. These fruit were maintained in the laboratory at 20^{o}C transpiring water until being measured for FRS 120 hours later.

The results in table 3.4 indicate that Ethrel is capable of accelerating the FRS decline in SG fruit kept both on and off the plant. The natural decline in FRS is accelerated by detachment from the plant. This is not altogether surprising in view of the elevated EPRs in untreated fruit maintained off the plant described in Section 3.1.7 ii. It is noticeable however that this effect of accelerated abscission in detached fruit is masked when both sets of fruit are treated with Ethrel. Natural ethylene levels are clearly sub-saturating even in the detached fruit. A similar treatment of HG fruit indicated Ethrel to be capable of enhancing FRS decline in these very immature fruit although requiring a longer duration of

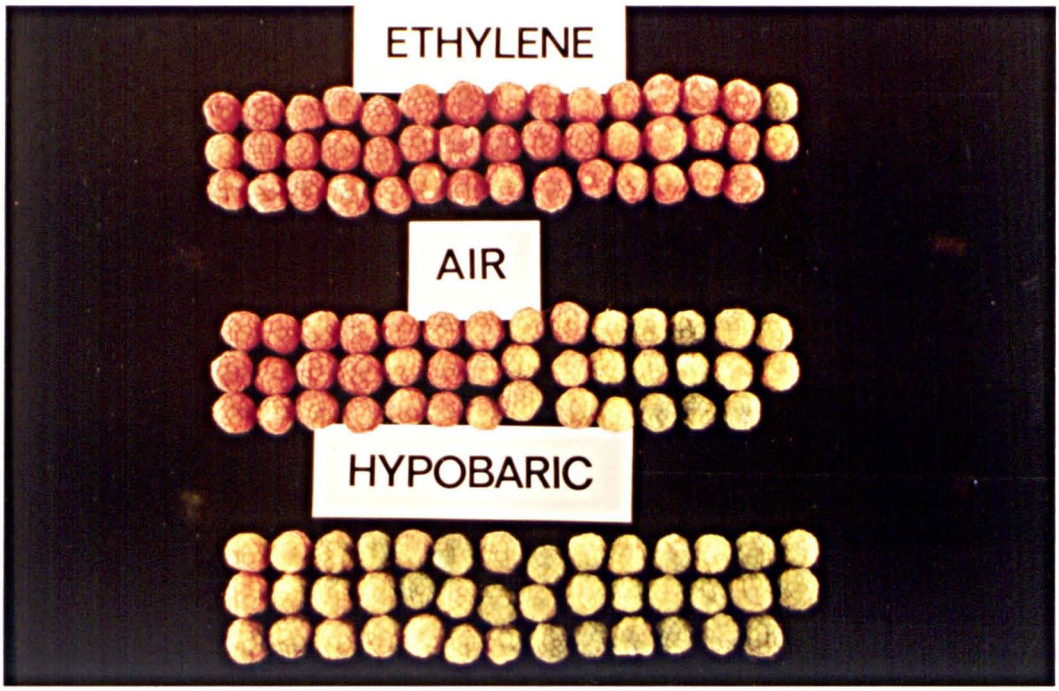
Table 3.4 Effect of 10 mM Ethrel on FRS of SG fruit treated on or off the plant. FRS measured 120 hours after treatment. (15 fruit/sample).

Treatment	FRS + SE (N)	
	Attached	Detached
dH_2O	2.92 ± 0.34	1.18 ± 0.22
Ethrel	0.90 ± 0.11	0.89 ± 0.21

treatment. Control HG fruit on the plant for 240 hours after treatment showed no signs of weakening whereas those treated with Ethrel had FRS values (± SE) of 2.89 N ± 0.55 N.

Accompanying these changes in FRS were very clear changes in drupelet pigmentation. Not only is Ethrel capable of accelerating the reduction in FRS, it is also capable of enhancing the loss of chlorophyll and development of anthocyanin pigmentation as illustrated in plate 3.13.

These findings agree with the tentative conclusions drawn from field trials using Ethrel to facilitating machine harvesting. Treatment with Ethrel causes SG fruits to rapidly become pigmented and show a reduction in FRS. This accounts for the smaller average fruit weight reported by Jolliffe (1975b) where green fruit have been turned red and made to abscise without full drupelet expansion occurring.



A

B

Plate 3.13 Effect of ethylene on pigmentation of SG fruit.
A. Effect of 48 hours exposure to 40 ppm ethylene or hypobaric pressure on detached fruit.
B. Effect of Ethrel and NAA sprays (both 10 mM) on fruit remaining attached to the canes.
Pink tags + Ethrel; yellow tags + NAA; white tags water control. Picture taken 5 days after spraying.

3.1.7 ii) Addition of ethylene

The effect on abscission of increased levels of ethylene in the atmosphere around detached fruit was examined using flow through cabinets. The continuous flow of air prevented any build up of carbon dioxide or endogenously produced ethylene as could occur simply by sealing samples in flasks or chambers. However, it also enhanced the drying out of samples making it essential that fruit were maintained on water as described in Section 2.13. One cabinet was maintained with ethylene free air flowing through it, the other had ethylene bled into it to provide a final concentration of 40 ppm. This concentration ensured that a saturating but non-toxic level of ethylene was present throughout the duration of the experiments. Lower levels of ethylene were tried out but it was found that levels below 25 ppm could not be reliably maintained using the available needle values. At these (< 25 ppm) levels a constant monitoring of the atmosphere would have been required which was often impracticable.

The presence of 40 ppm ethylene in the atmosphere around immature (HG, SG and M) fruit for 24 hours or 48 hours enhanced the reduction in FRS compared to those kept in air (fig. 3.22 and 3.23). This difference after 24 hours was greatest in SG fruit (FRS 5.99 N in air, 3.0 N in ethylene), slight in M fruit (FRS 2.20 N in air, 1.47 N in ethylene) and non-existent in R fruit (FRS 0.65 N in air, 0.69 N in ethylene). The PR fruit at 24 hours and R fruit at 48 hours were in such poor condition that they were discarded. Hence on detachment there was a continuation of the weakening already started in the abscission zones of SG, M and R fruit. In HG, SG and M fruit the presence of 40 ppm ethylene accelerated this

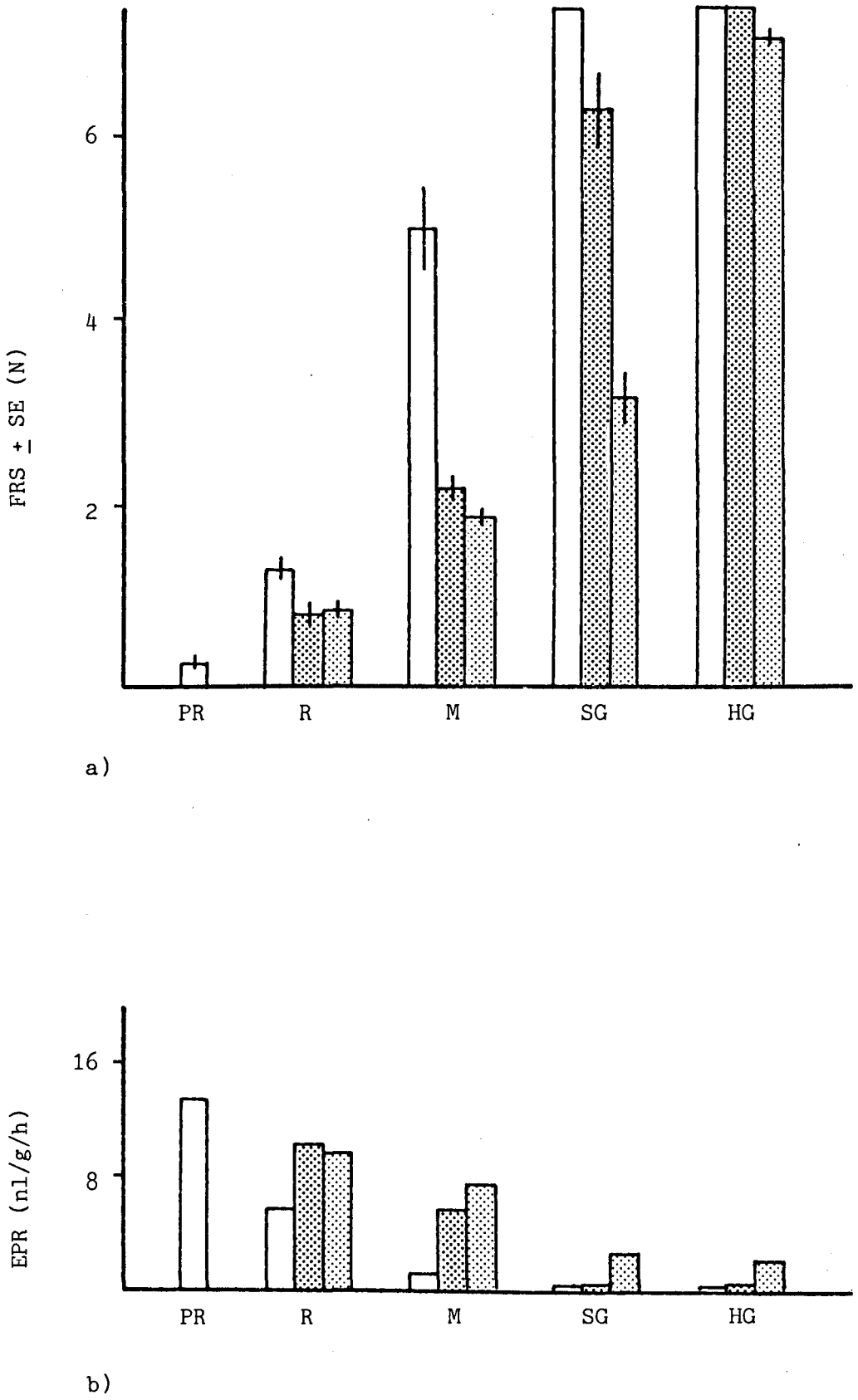
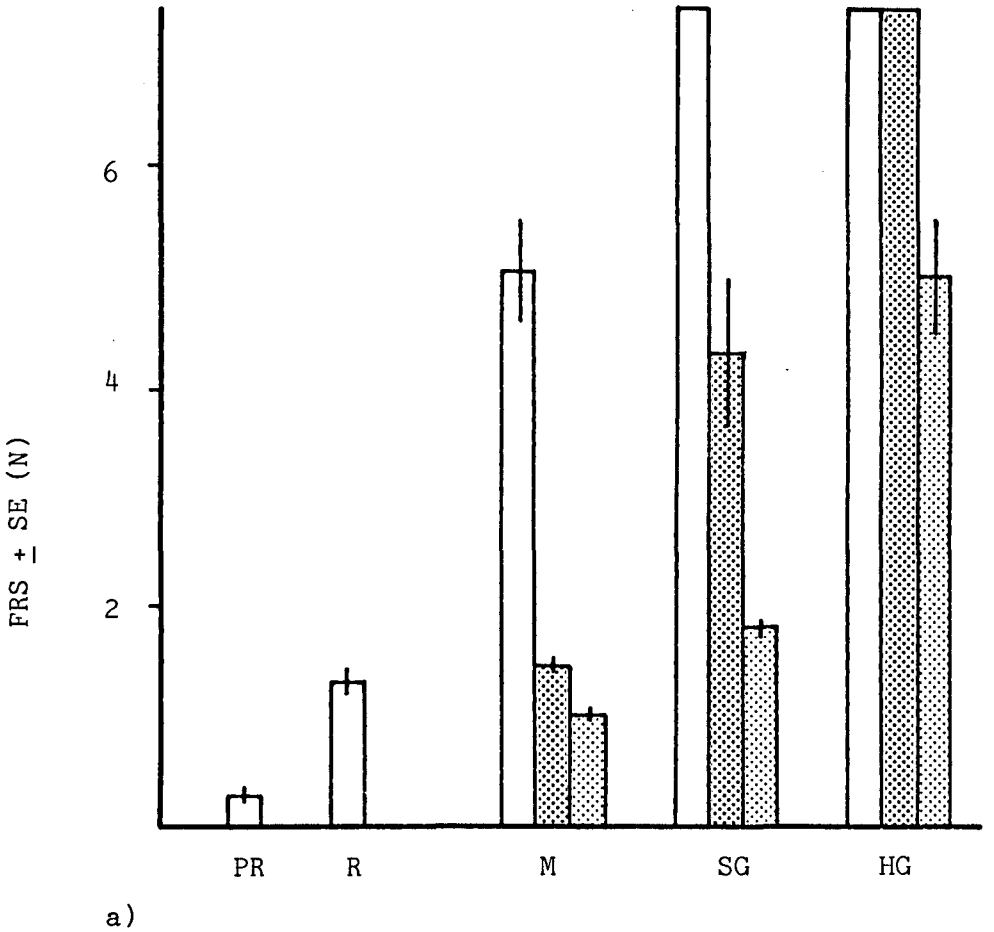


Fig. 3.22 Effect of 24 hours exposure to 40 ppm ethylene on a) FRS and b) EPR of fruit at different stages of development. (Initial values □ , kept in air ▨ , kept in 40 ppm ethylene ▩ ; 20 fruit/sample.)



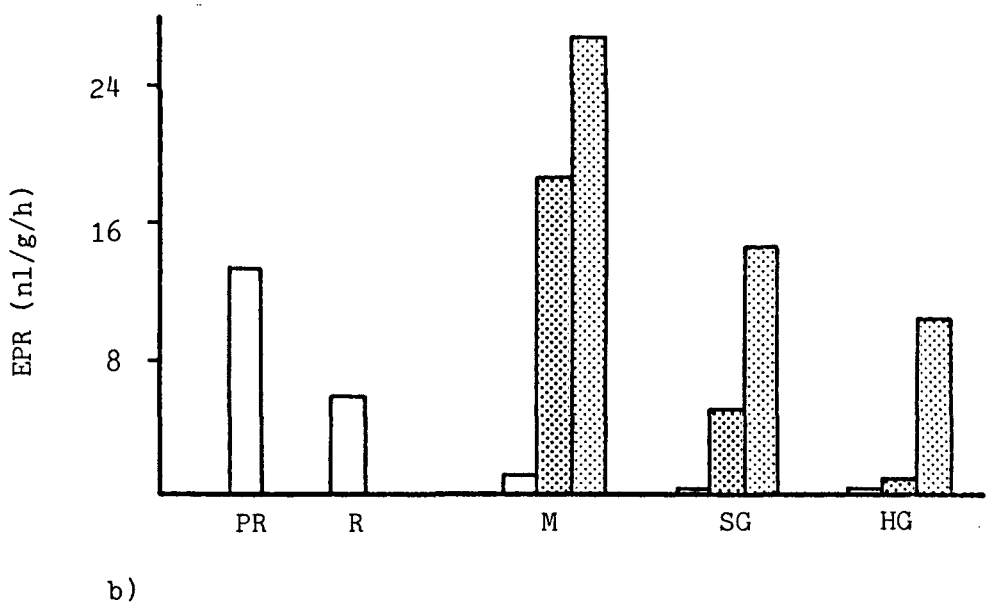


Fig. 3.23 Effect of 48 hours exposure to 40 ppm ethylene on a) FRS and b) EPR of fruit at different stages of development. (Initial values □ , kept in air ▨ , kept in 40 ppm ethylene ▩ ; 20 fruit/sample.)

reduction in the FRS. The HG fruit kept in air showed no decrease in FRS even 48 hours after being detached. After 24 hours and 48 hours in 40 ppm ethylene however their average FRS had declined to 7.08 N and 4.94 N respectively. It therefore appears that as the fruit mature additions of exogenous ethylene become progressively less effective at accelerating the reduction in FRS. This may be because natural ethylene production maintains saturating levels in ripe fruit making additional ethylene ineffective.

Another effect of the presence of 40 ppm ethylene was an increase in the EPRs of fruit at the HG, SG and M stages of development (figs 3.22 and 3.23). This was predictable as ethylene biosynthesis is generally considered to be an autocatalytic process (Yang and Hoffman, 1984). If viewed alongside the change in FRS the increased EPRs correspond to decreased FRS values. Also, as with FRS, the degree of change in EPR caused by the ethylene atmosphere was progressively smaller as the fruit matured, there being no difference between the EPRs of ripe fruit in the presence or absence of exogenous ethylene. One complicating factor is the increase in EPR which occurs naturally even in the detached fruit kept in air. This increase takes the EPRs above those which would normally be associated with fruit at specific stages of development as judged by FRS and pigmentation.

Overall the presence of a 40 ppm ethylene atmosphere increased the EPR and accelerated the FRS decline in HG, SG and M fruit. Once fruit had reached the R stage of development naturally exogenous ethylene was not capable of influencing either factor. The changes are in line with the development of an ethylene climacteric and reduction in FRS which occurs with increased fruit

maturity. The natural development of higher EPRs creating saturating ethylene levels reduced the possibility of influencing ethylene controlled processes through the supply of exogenous ethylene.

3.1.7 iii) Removal of ethylene

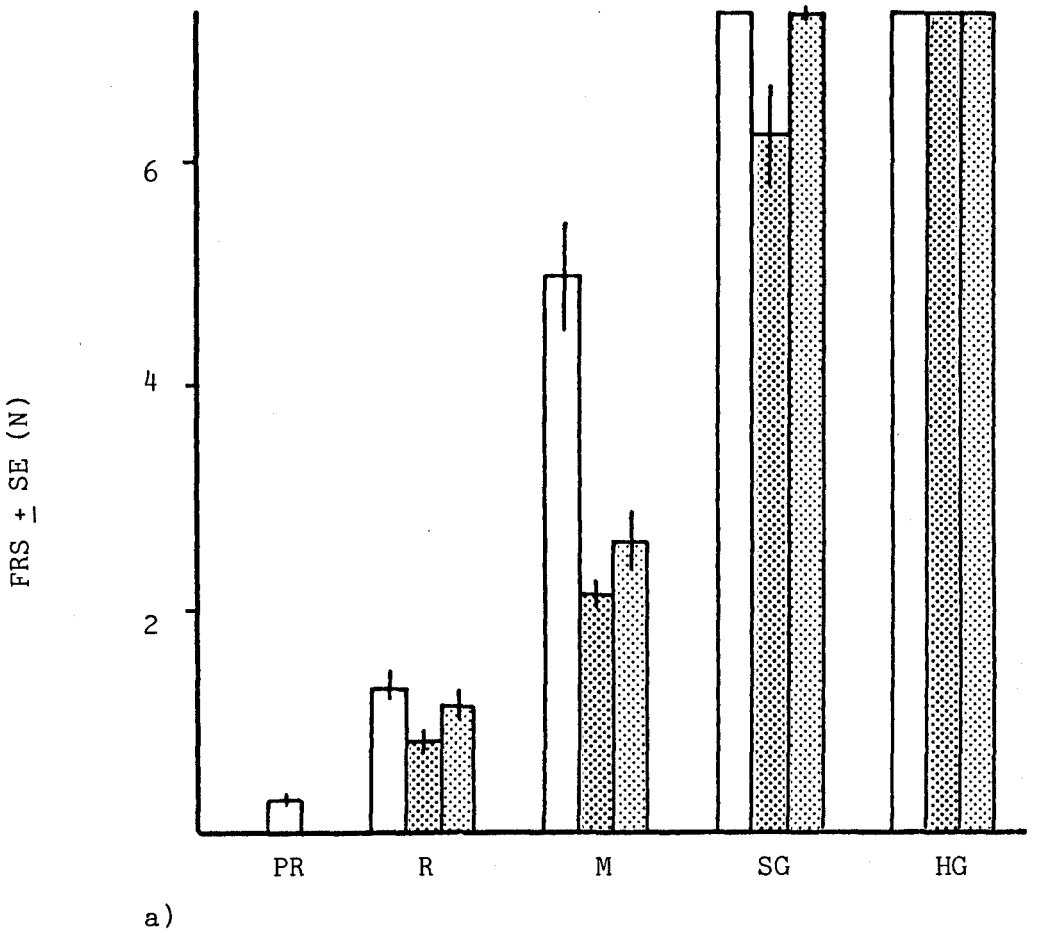
Subatmospheric, or hypobaric, pressures generally in the range 100 to 200 mmHg have been used in the examination of ethylene mediated processes since the mid 1960s (Burg and Burg, 1966). More specifically hypobaric treatment of abscission systems has been used to examine the role of ethylene in the regulation of abscission (see Section 2.14. Hypobaric pressures are considered to alter the internal ethylene levels in plant tissues in several ways, i) an immediate drop in ethylene partial pressure as gases leave the tissues, ii) an increase in diffusive loss from the tissue as the gas phase becomes less dense, iii) a reduced retention as a result of low pressure effects an ethylene solubility, iv) a reduced ethylene biosynthesis and metabolism as a result of limited oxygen availability.

It is difficult to verify that such a general treatment is having an effect on abscission through influencing ethylene levels alone. In reducing the atmospheric pressure around fruit the partial pressure of oxygen is also reduced proportionately. In some cases pure oxygen has been bled into hypobaric atmospheres (0.2 atmospheres) thereby maintaining the normal partial pressure of oxygen to the plant material (see Section 2.14). This attempts to relieve any general effect of a lack of oxygen on abscission. Unfortunately the special oil free pumps required to do this were

not available. The simplest method of checking that effects caused by hypobaric pressures are solely the result of changing ethylene levels is to bleed a saturating level of ethylene back into the hypobaric atmosphere. This treatment should reinstate any response inhibited by hypobaric treatment assuming it to be mediated by ethylene alone. To examine the effects of hypobaric pressure on fruit at different stages of development a flow through system was used with fruit being maintained on water.

The use of 0.2 atmospheres pressure (150 mmHg) was successful at retarding the decline in FRS of SG, M and R fruit over 24 hours (fig. 3.24) and 48 hours (fig. 3.25). There was no decline in FRS in either sample of HG fruit even after 48 hours. The SG fruit clearly showed the natural decline in FRS to be retarded by the hypobaric treatment (FRS at 24 hours, 5.98 N in air, 7.32 N in hypobaric; FRS at 48 hours 4.29 N in air, 6.29 N in hypobaric). In M and R fruit there was less effect with only a slight retardation in FRS decline. The PR fruit at 24 hours and R fruit at 48 hours were in such a poor condition that they were discarded. As with the ethylene addition experiment (previous section) the capacity to influence FRS was reduced in the more mature fruit where abscission was already well advanced.

Comparing EPRs to these FRS values is complicated by the general nature of the treatment making the data difficult to interpret precisely. In SG, M and R fruit the hypobaric treatment reduced the EPRs compared to the control fruit at atmospheric pressure (fig. 3.24 and 3.25). In the HG fruit however both 24 hours and 48 hours of hypobaric treatment resulted in higher EPRs than in the controls, although there was no concomitant reduction in



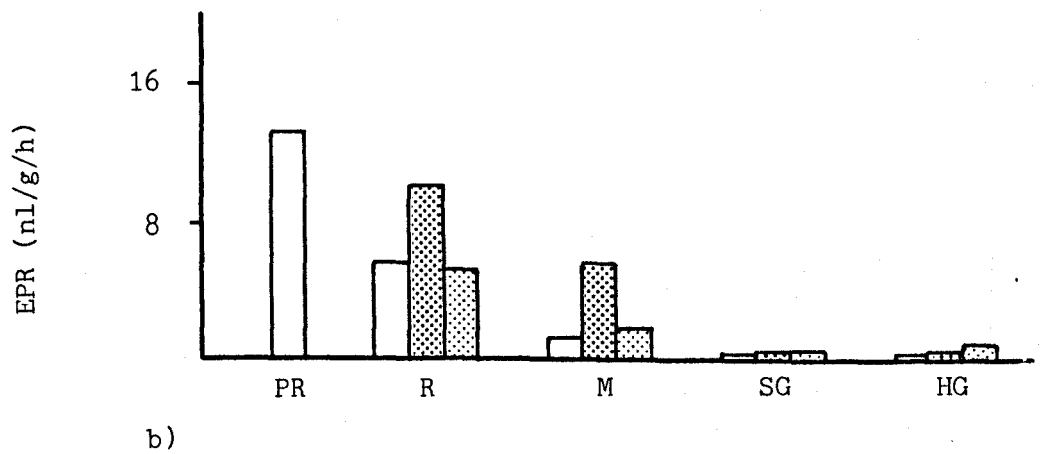
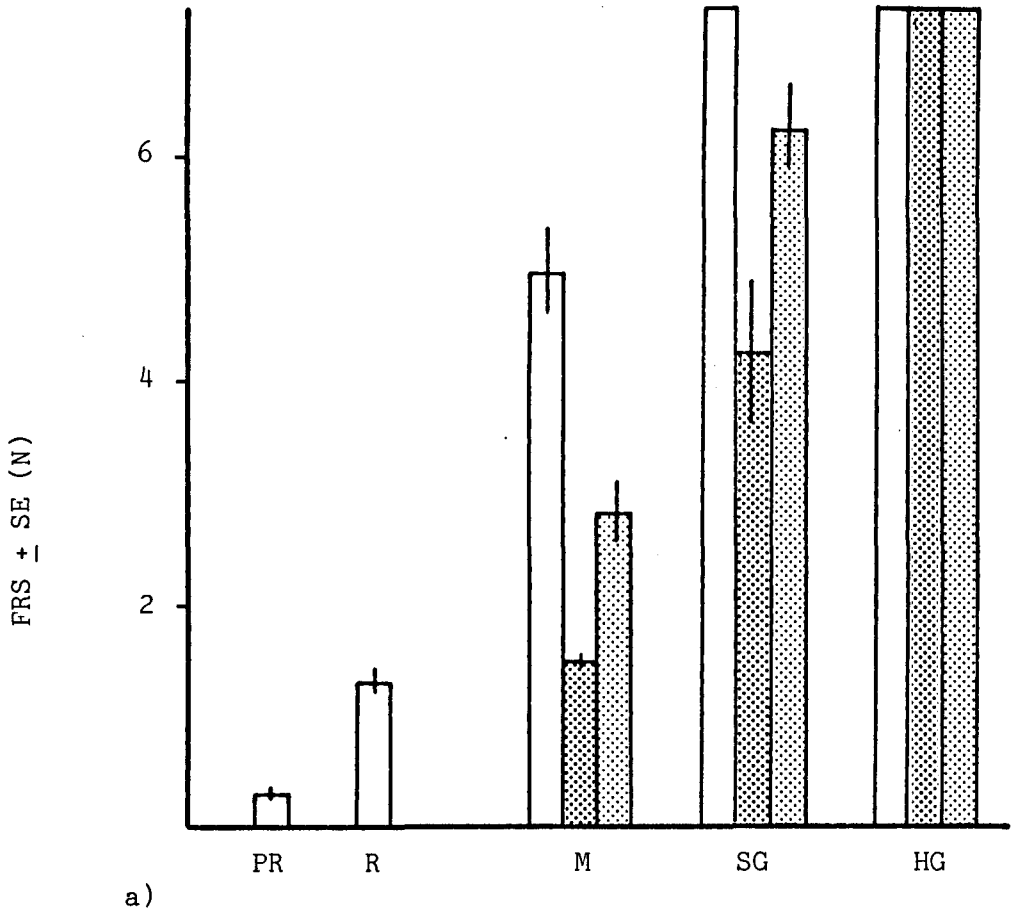


Fig. 3.24 Effect of 0.2 atmospheres pressure for 24 hours on a) FRS and b) EPR of fruit at different stages of development. (Initial values □ , kept in air ▨ , kept at 0.2 atmospheres ▩ ; 20 fruit/sample.)



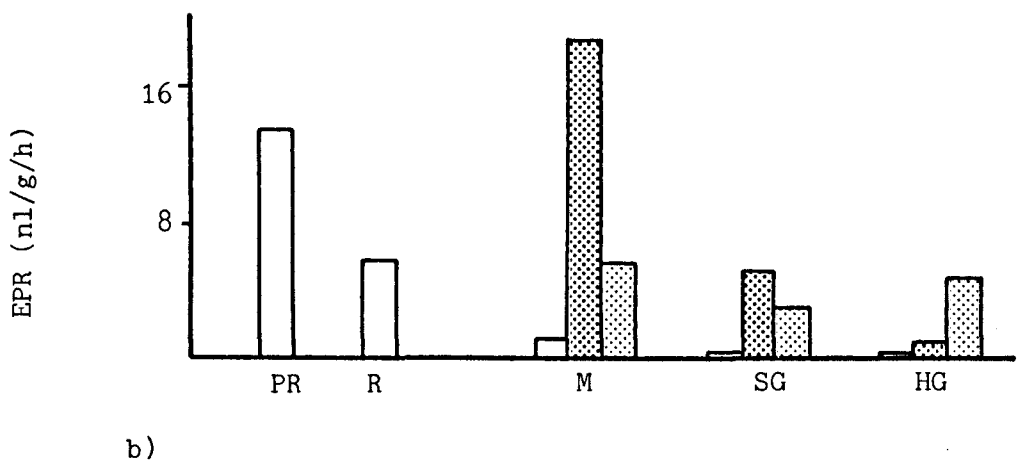


Fig. 3.25 Effect of 0.2 atmospheres pressure for 48 hours on a) FRS and b) EPR of fruit at different stages of development. (Initial values □ , kept in air ▨ , kept at 0.2 atmospheres ▩ ; 20 fruit/sample.)

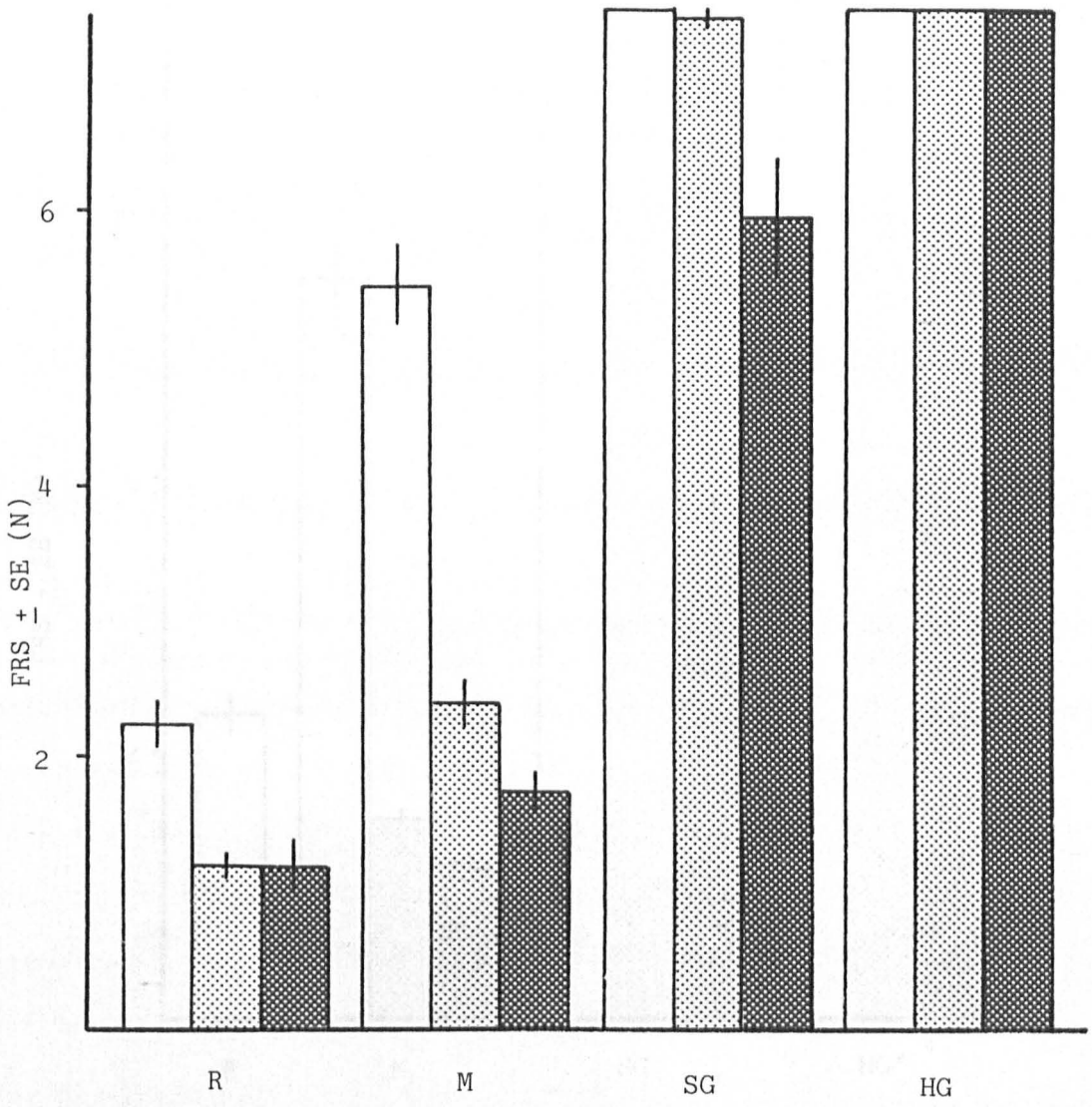


Fig. 3.26 Effect of 0.2 atmospheres pressure for 24 hours ± ethylene on FRS of fruit at different stages of development. (Initial values □ , kept at 0.2 atmospheres ▨ , kept at 0.2 atmospheres + ethylene ■ ; 20 fruit/sample).

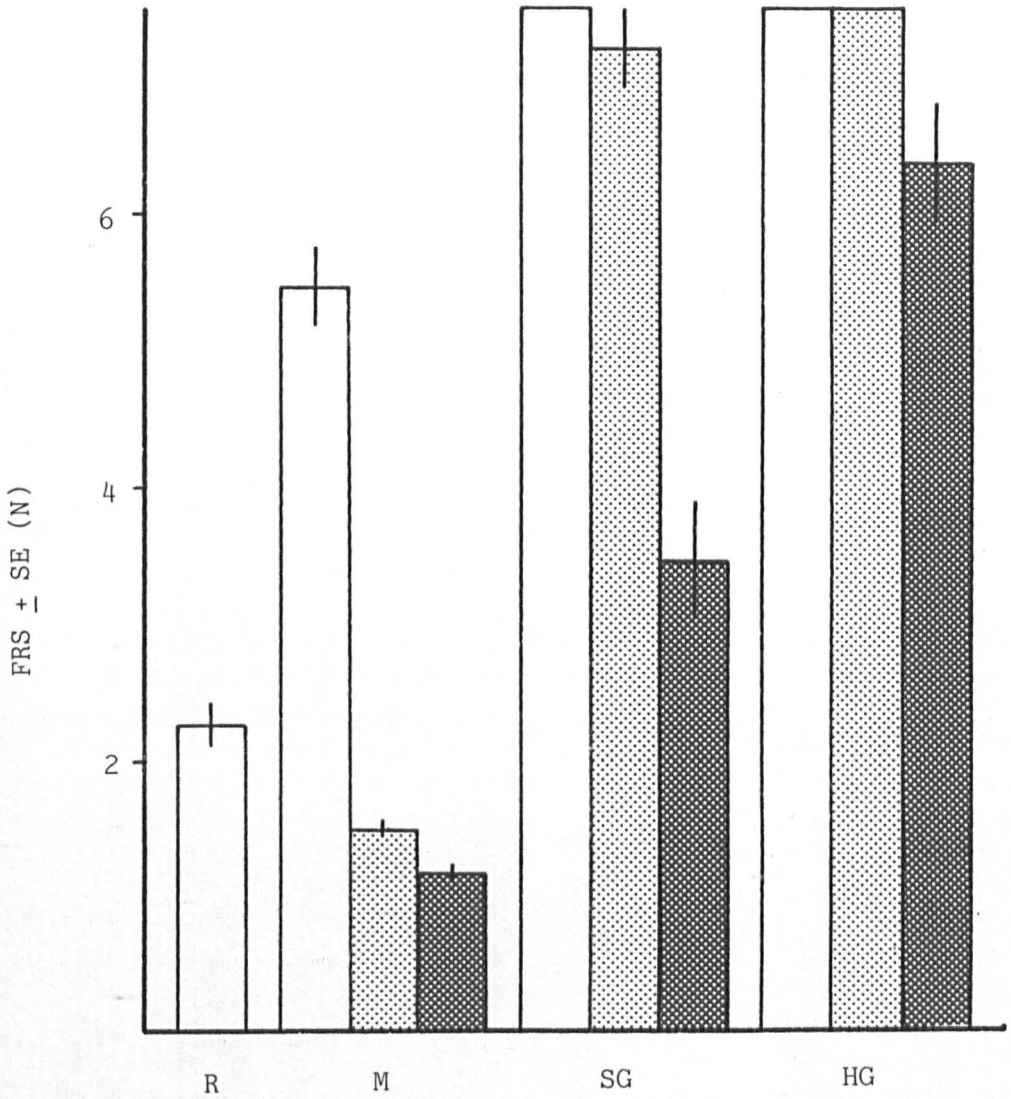


Fig. 3.27 Effect of 0.2 atmospheres pressure for 48 hours ± ethylene on FRS of fruit at different stages of development. (Initial values □ , kept at 0.2 atmospheres ▨ , kept at 0.2 atmospheres + ethylene ■ ; 20 fruit/sample).

FRS.

The changes in EPR were measured on removal from the hypobaric atmospheres and hence may not be a true reflection of those occurring actually under treatment. General metabolic effects of reduced oxygen partial pressures and the specific oxygen requirements for the conversion of ACC to ethylene (Adams and Yang, 1979) could have influenced the EPR. Another factor to consider is whether the EPRs are reduced sufficiently to actually influence abscission, especially in the light of the inexplicable change in HG fruit. It may be that under hypobaric treatment EPRs which would normally be stimulatory to abscission are ineffective.

The practical limitations of the experimental apparatus meant the control experiment of adding ethylene to the hypobaric chambers had to be carried out separately. The results shown in fig. 3.26 and 3.27 indicate that adding ethylene to the system removed the hypobaric effect on FRS. A closer examination of these results in comparison to the basic ethylene addition results at atmospheric pressure (fig. 3.22 and 3.23) shows that the decline in FRS of hypobaric plus ethylene treated fruit was not as great as for just ethylene alone. Hence it is possible to envisage the inhibitory effect of reduced pressures as being the result of 2 components, the direct effect of ethylene and the more general result of reducing the pressure to 0.2 atmospheres.

Overall the results indicate that hypobaric pressures can be used to reduce the rate of FRS decline, but not prevent it totally. This effect can to some extent be overcome by adding ethylene to the system. Again it was found that the capacity to influence FRS was reduced as abscission progressed naturally with fruit development.

3.1.7 iv) Influencing ethylene production or action

a) Aminoethoxyvinylglycine

The rhizobitoxine analog aminoethoxyvinylglycine (AVG) has been shown to be capable of reducing ethylene production through an inhibition of ACC synthase conversion of SAM to ACC (Yu et al., 1979; Boller et al., 1979). AVG was applied to detached fruit either by dipping or through the transpiration stream, as described in Section 2.15. The capacity of AVG to reduce ethylene production in raspberries was readily demonstrated in detached fruit at all

Table 3.5 Effect on EPR (nl/g/h) of dipping fruit of different developmental stages in 10 mM AVG (10 fruit/sample).

Stage of Development		Time (h) after treatment 0	7	20
R	-AVG	12.0	14.2	17.2
	+AVG	8.8	5.1	1.6
M	-	0.80	3.25	3.95
	+	0.85	0.8	0.50
SG	-	0.06	0.85	1.65
	+	0.05	0.10	0.05
HG	-	0.15	0.55	0.25
	+	0.05	0.12	0.03

stages of development (table 3.5). In the HG and SG fruit the AVG treatment maintained low EPRs (<0.12 nl/g/h) compared to the increases occurring in the detached controls. In the M and R fruit the EPRs were actually reduced compared to the initial values whilst the control rates increased steadily. Hence at all stages of

development AVG treatment was capable of reducing or maintaining low EPRs.

Having shown AVG to be capable of holding EPRs down in HG and SG fruit its effect on abscission was examined. SG fruit were allowed to transpire 100 µl of 100 µM AVG in an attempt to ensure the AVG reached the abscission zones. The fruit were then allowed to transpire water for 48 hours before measuring FRS and EPR. To ensure that AVG was influencing abscission through ethylene alone an extra set of AVG treated fruit were kept in a saturating ethylene atmosphere (40 ppm). As with dipping SG fruit (table 3.5) this treatment maintained the EPR at 0.11 nl/g/h after 48 hours compared to 1.10 nl/g/h in control fruit at the same time.

The results in fig. 3.28 show AVG to be capable of slowing the decline in FRS which occurs in SG fruit over 48 hours after detachment (FRS : 3.49 N + AVG; 2.80 N control; significant difference at $P < 0.05$). The treatment was not totally effective in preventing the FRS decline but merely slowed it down. This effect of AVG on FRS could be overcome by adding ethylene (40 ppm) to the atmosphere around the fruit. The ethylene and AVG plus ethylene treatment showed no significant difference in FRS (ethylene, 1.60 N; ethylene + AVG, 1.69 N). Both these values were below the FRS of control fruit (2.80 N). The retardation of FRS decline can therefore be linked to the ability of AVG to reduce ethylene production.

b) Silver Thiosulphate (STS)

STS is considered to block the site of ethylene action through the silver ion (Beyer, 1976; Sisler, 1982) whilst not

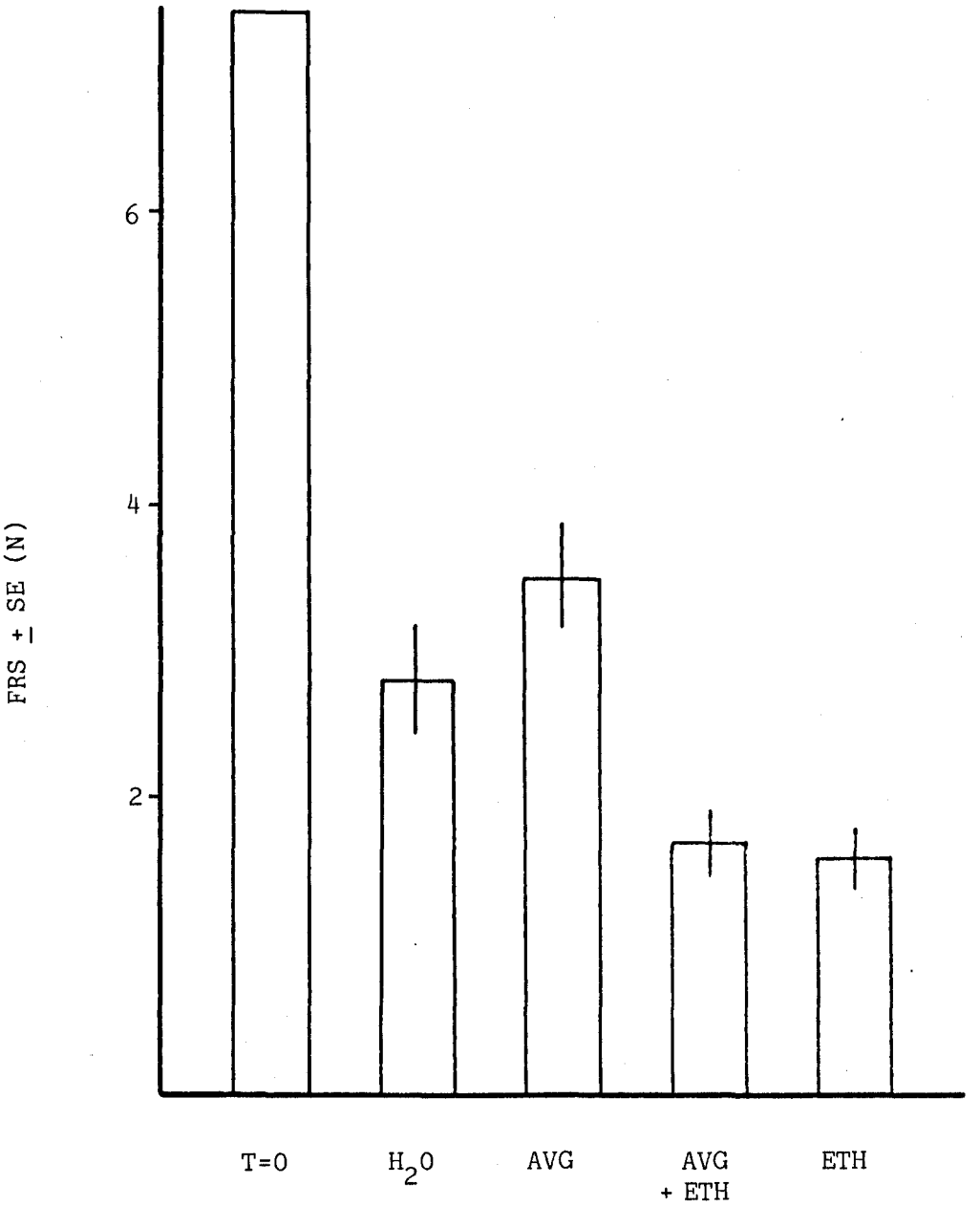


Fig. 3.28 Effect of transpiring 100 µl of 0.1 mM AVG in the presence of 40 ppm ethylene on FRS of SG fruit 48 hours later. (30 fruit/sample).

interfering with the ethylene forming process. The effect of STS on abscission was examined by allowing SG fruit to transpire 100 µl of 0.2 mM STS or water and comparing the FRS values 48 hours later. The intervening period was spent transpiring water. The results in table 3.6 show STS to be capable of reducing the rate of FRS decline significantly ($P < 0.05$), although not preventing it totally.

Table 3.6 Effect of transpiring 100 µl 0.2 mM STS on FRS and EPR of SG fruit 48 hours later (20 fruit/sample).

Treatment	FRS (N) ± SE	EPR nl/g/h
At harvest	7.35	0.08
H_2O	5.36 ± 0.5	4.6
STS	6.68 ± 0.3	1.2

This is what would have been expected from earlier results which showed any reduction in ethylene levels to reduce the rate of FRS decline. It can now be added that preventing ethylene action can also reduce FRS decline. The degree of reduction in FRS in the control fruit is less than might have been expected from previous results, however the effect of STS is still obvious.

The reduced increase in EPR of the STS treated fruit can be ascribed to Ag+ preventing the autocatalytic production of ethylene. In these fruit there is a reduction in FRS decline and a

lesser increase in EPR than in controls. Therefore although STS is considered to function through interfering with ethylene binding it has also reduced the increase in EPR. Hence the effect on FRS could be as a result of Ag+ effecting ethylene's action on abscission directly or indirectly through the lower EPR. This latter prospect however is unlikely since the EPR is similar to that found in mottled fruit which show decreases in FRS.

c) Cobalt

The cobaltous ion (Co^{2+}) administered as the chloride is considered to prevent the conversion of ACC to ethylene by the ethylene forming enzyme (Yu and Yang, 1979). Dipping fruit at

Table 3.7 Effect on EPR of dipping fruit at different stages of development in 1 mM $CoCl_2$. (Measured 48 h after treatment, 15 fruit/sample)

Development stage	EPR (nl/g/h)		
	Initial	Final	Final + Co^{2+}
R	2.15	19.4	9.4
M	0.56	6.6	2.2
SG	0.10	0.72	0.11
HG	0.10	0.45	0.21

different stages of development into 1 mM $CoCl_2$ reduced the EPR of all categories (Table 3.7) when measured after transpiring water for 48 hours. Although Co^{2+} was not capable of maintaining the starting levels of ethylene production in the R and M fruit, it was capable of reducing the increase in EPR. The effect on SG and HG fruit was more impressive with SG fruit showing virtually no increase in EPR whilst the increase in HG fruit was slight.

Those SG fruit which transpired 100 µl of 100 µM Co^{2+} showed a significantly ($P < 0.05$) smaller reduction in FRS than did the control fruit (FRS, 5.75 N:3.94 N respectively, table 3.8). These changes are what would be predicted from the ability of Co^{2+} to prevent an increase in EPR in SG fruit.

Table 3.8 Effect of transpiring 100 µl of 100 µM $CoCl_2$ on FRS and EPR 48 hours later. (15 fruit/sample).

Treatment	FRS (N) ± SE	EPR (nl/g/h)
At harvest	7.35	0.13
H_2O	3.94 ± 0.41	0.73
$CoCl_2$	5.75 ± 0.37	0.12

d) Auxin

Early work has shown auxin to accelerate the onset of fruit ripening although more recently work involving infiltration

techniques has contradicted this view (Frenkel et al., 1975). The role of auxin in abscission is no clearer with its effect being complicated by two factors, i) the practical problem of applying auxin to the abscission zones and ii) the ability of auxin to enhance ethylene production. Hence in abcission auxin has been shown to both accelerate and retard abscission depending on the timing and position of application. This is discussed fully in Section 1.2.

The results in table 3.9 illustrate auxins capacity to increase EPRs of fruit at all stages of development. The effect of auxin on FRS was then examined in SG fruit allowed to transpire

Table 3.9 Effect of dipping fruit at different stages of development in 0.1 mM IAA on EPR 48 hours later. (20 fruit/sample).

Developmental Stage	EPR (nl/g/h)		
	Initial	Final	Final + IAA
R	3.5	7.07	10.70
M	0.72	6.81	12.87
SG	0.06	0.11	0.52
HG	0.21	0.46	1.07

IAA. Having then transpired water for 48 hours the FRS and EPRs were measured (shown in Table 3.10). These results show auxin to enhance the reduction in FRS, as might have been expected from the increased EPRs. The same pattern was obtained from HG fruit with an increased EPR and a reduction in FRS (5.73 N, compared to no weakening in controls after 48 hours).

Table 3.10 Effect on SG fruit of transpiring 100 µl of 0.1 mM IAA on EPR and FRS 48 hours later. (20 fruit/sample).

Treatment	FRS ± SE (N)	EPR (nl/g/h)
Initial	7.35	0.11
dH_2O	3.82 ± 0.64	2.08
IAA	1.78 ± 0.42	12.56

A final auxin experiment involved the treatment of individual SG fruit whilst still attached to the parent plant. Individual fruit were sprayed with a 10 mM NAA solution and the fruit harvested 5 days later and measured for FRS. NAA was used instead of IAA since it is considered to be more resistant to degradation. The results of this experiment showed no difference between the FRS of treated and control fruit (4.92 N and 5.13 N respectively).

In other abscission systems an exogenous supply of auxin

under the correct circumstances can prevent abscission (see Section 1.2). This clearly does not occur in the SG raspberries which would appear to respond to the elevated EPRs by an enhanced FRS decline. However no guarantee can be given that the auxin was reaching the abscission zones in the centre of the fruit, or if it was, in what quantities. The auxin could however stimulate ethylene production in other tissues, thereby influencing abscission.

3.1.7 v) Effects of ethylene other than on FRS and EPR

Ethylene has been shown to increase EPRs and anthocyanin pigmentation whilst decreasing FRS. These are all changes which occur naturally during fruit ripening. Incidental to the main stream of work on abscission a series of experiments were carried out to examine the effect of ethylene on other parameters of fruit maturity. These included changes in drupelet pigmentation, respiration and drupelet sugar and acid levels. Whilst not being directly related to the problem of abscission these effects would provide information of interest to horticulturalists, especially if ethylene releasing sprays are to be considered for the mechanical harvesting of raspberries.

a) Drupelet pigmentation

The most obvious effect of ethylene either supplied directly as the gas or as Ethrel is the change induced in pigmentation. This is clearly shown in plate 3.13 where supplying 40 ppm ethylene for 48 hours enhanced the red coloration of SG fruit. The same photograph also shows the result of ethylene removal, by using hypobaric pressures, reducing the natural change in pigmentation.

Actual anthocyanin values for these treatments are given in table 3.11. Concomitant changes in chlorophyll also occur, the levels decreasing more in ethylene than air. The hypobaric treatment prevented change in either pigment maintaining the levels present when harvested.

All experiments influencing ethylene had predictable effects on pigmentation. An increase in EPR enhanced changes in pigmentation whilst a reduction in EPR or removal of ethylene retarded these changes. This ability to influence pigmentation has been used

Table 3.11 Effect of 40 ppm ethylene or hypobaric pressures on the pigmentation of SG fruit after 48 hours treatment. (20 fruit per sample)

Treatment	Anthocyanins (µg/g)	Chlorophyll (µg/g)
Initial	32	18.9
Air	64	7.8
Ethylene	183	4.1
Hypobaric	35	17.5

commercially to promote colouration of fruits (in cranberries - Craker, 1971). In other fruit the colour changes induced by ethylene occur alongside other ripening changes making the fruit

palatable (tomatoes, bananas, melon, mangoes - Sherman, 1985).

b) Respiration

The respiratory rates of raspberries measured as dark CO_2 efflux per gram (see Section 2.8) decrease during ripening, there being a slight increase in the very ripe fruit (see Section 3.1.3). These changes are not indicative of a climacteric fruit which is what EPR changes suggest raspberry fruit to be.

The presence of an exogenous supply of 40 ppm ethylene for 24 hours increased the dark CO_2 efflux of the HG, SG and M fruit, (fig. 3.29). The degree of increase was reduced as development progressed from HG to R, there being no difference in R fruit. This is as would be expected for a climacteric fruit, the ethylene addition only being effective during the preclimacteric period (Biale and Young, 1981). In a non climacteric fruit the increased CO_2 production would have been expected at all stages of development.

c) Drupelet titratable acidity

During natural development the drupelet titratable acidity decreases in the R and PR fruit (see Section 3.1.3 v). Treatment of fruit at HG, SG and M stages of development with 40 ppm ethylene for 48 hours caused lower levels of titratable acidity (table 3.12).

Bulk samples of drupelets from 20 fruit were homogenised and sub samples used for analysis. The general trend was for a decrease in acidity in both air and ethylene, the latter being greatest. The one anomaly was for the SG fruit in air which showed a slight increase in acidity. This was a recurrent feature of the 3 separate

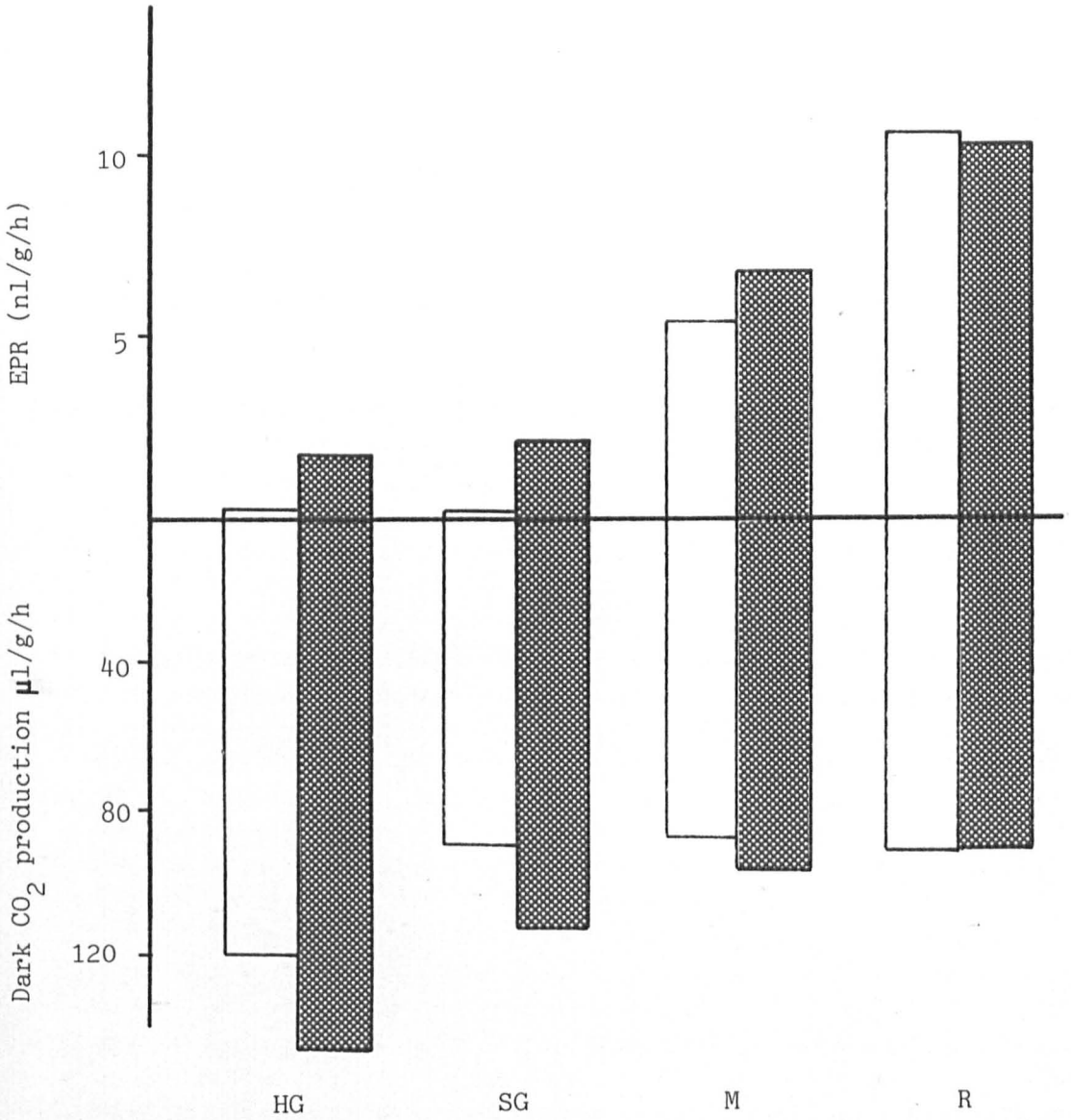


Fig. 3.29 Effect of 24 hours exposure to 40 ppm ethylene on EPR and dark CO_2 production of fruit at different stages of development (10 fruit/sample). Fruit kept in air (□) or ethylene (▩).

Table 3.12 Effect of 48 hours of 40 ppm ethylene on drupelet titratable acidity (20 fruit/sample)

Development Stage	mEq/g fresh weight		
	Initial	Final air	Final + ethylene
M	338.4	303.6	243.8
SG	331.0	352.6	295.1
H	272.8	241.8	208.8

experiments examining the effect of ethylene on acidity. The use of detached fruit complicates the changes occurring but does not detract from the fact that exogenous ethylene caused a decrease in acidity in HG, SG and M fruit beyond that which would be normally expected. This change as with so many ethylene effects (pigments FRS, EPR) is in the direction of increased maturity, thereby accelerating this aspect of ripening.

d) Drupelet reducing sugar levels

During natural ripening reducing sugars increase steadily during development (see Section 3.1.3 vi). Detached SG fruit maintained in the presence of 40 ppm ethylene showed no significant changes in reducing sugar levels in any stages of fruit development. This could be indicative of the dependency of raspberry fruit on the importation of photosynthetic products during

natural ripening. This is the case in cantaloupes where ethylene treatment of immature fruit causes rapid ripening (McGlasson and Pratt, 1964) but no sugar accumulation (Kasmire et al., 1970).

3.1.7 vi) Summary

In all the above instances of EPR manipulation consistent effects on FRS were also recorded. An enhanced EPR resulted in an acceleration of abscission zone weakening reflected as a reduction in FRS. Decreasing the EPR or preventing ethylene accumulation or ethylene action maintained the FRS at a higher value than in control fruit. None of these inhibitory treatments were capable of totally preventing a decrease in FRS, it only proved possible to reduce the rate of decline. The ability to manipulate FRS through the EPR is restricted to HG and SG fruit. The M and R fruit have progressed naturally in terms of abscission and ethylene production to such an extent that it is no longer possible to influence FRS by adding or removing ethylene. This is possibly because the EPR at these stages is high enough to be saturating the FRS response.

As well as affecting FRS ethylene is capable of accelerating fruit development as measured by other parameters such as pigments, EPRs and titratable acidity. Other results (sugars and respiration) are harder to explain, possibly as a result of using detached fruit. Whether the changes induced by ethylene occur as a direct result of the presence of ethylene or indirectly via other ethylene mediated events is not yet clear.

3.1.8 Are the levels of ethylene found in raspberries physiologically significant?

In this section the minimum level of ethylene which must be added to immature fruit to stimulate abscission was determined. This was then compared to the actual levels found in raspberries to determine whether the naturally occuring levels could influence abscission.

3.1.8 i) Ethylene threshold

Fruit of the HG and SG categories when harvested showed no signs of weakening at the abscission zones. These fruit were maintained on a 1 mm thick polystyrene sheet with their pedicels protruding into water whilst being exposed to different levels of ethylene. FRS' were measured after 24 hours exposure. Based on previous work on ethylene levels used to induce abscission in other systems (Sexton et al., 1985) a range of concentrations from 0 to 50 ppm ethylene were examined. These concentrations were prepared in desiccators by diluting pure ethylene. The atmospheres were changed every 12 hours to reduce ethylene or CO_2 accumulation. Atmospheres were checked prior to changing to ensure that no ethylene accumulation had occurred (and none was ever found).

The results presented in fig. 3.30 indicate SG fruit to require a 24 hour exposure to at least 0.5 ppm ethylene to give a significant ($P = 0.016$) decrease in FRS from the control value. The rate of FRS reduction was slightly accelerated in higher levels of ethylene approaching saturation at 5-50 ppm.

In HG fruit a 24 hour exposure to any concentration of ethylene was insufficient to cause a reduction in FRS. By 48 hours

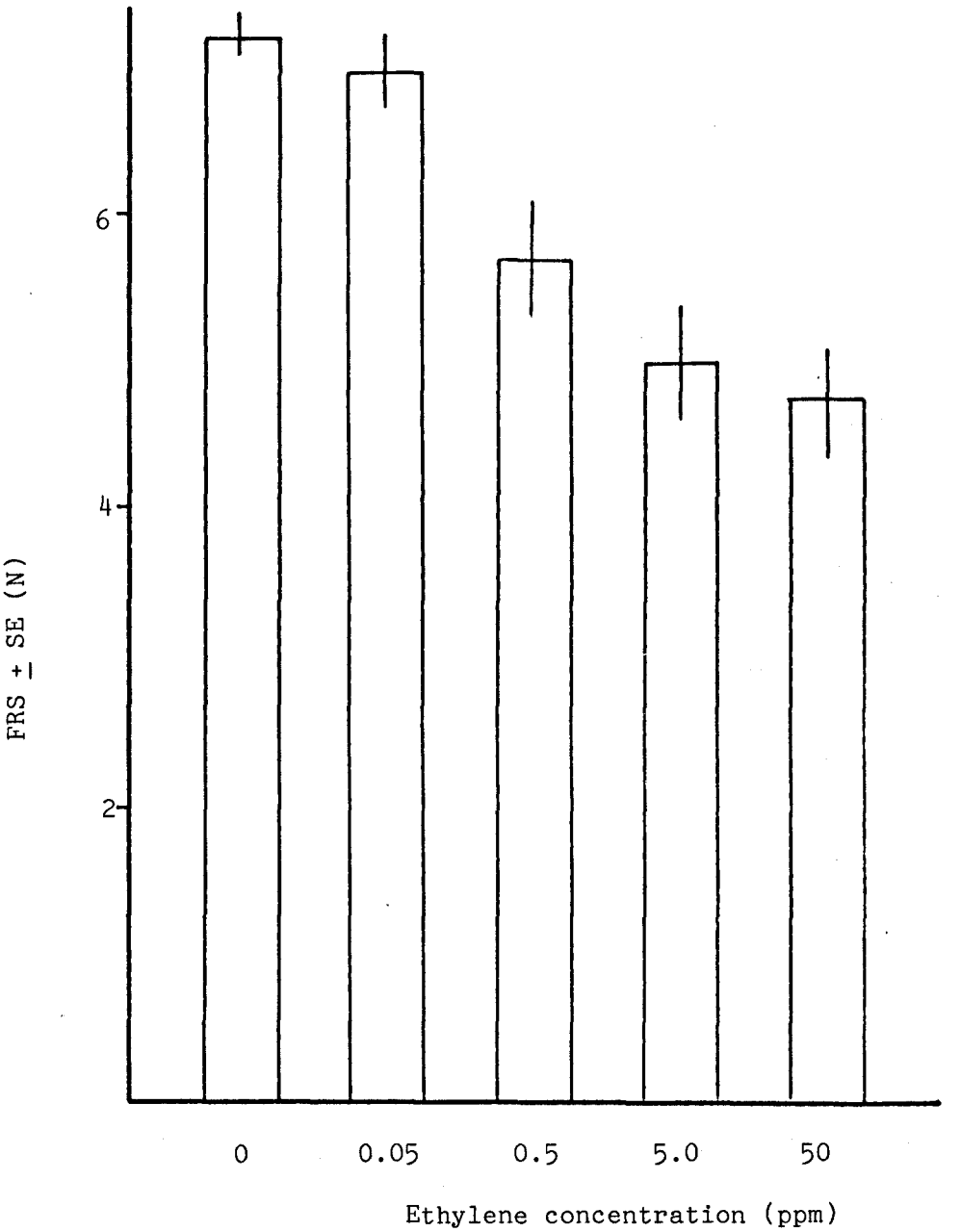


Fig. 3.30 Effect of 24 hours exposure to different concentrations of ethylene on FRS decline of SG fruit (30 fruit/sample).

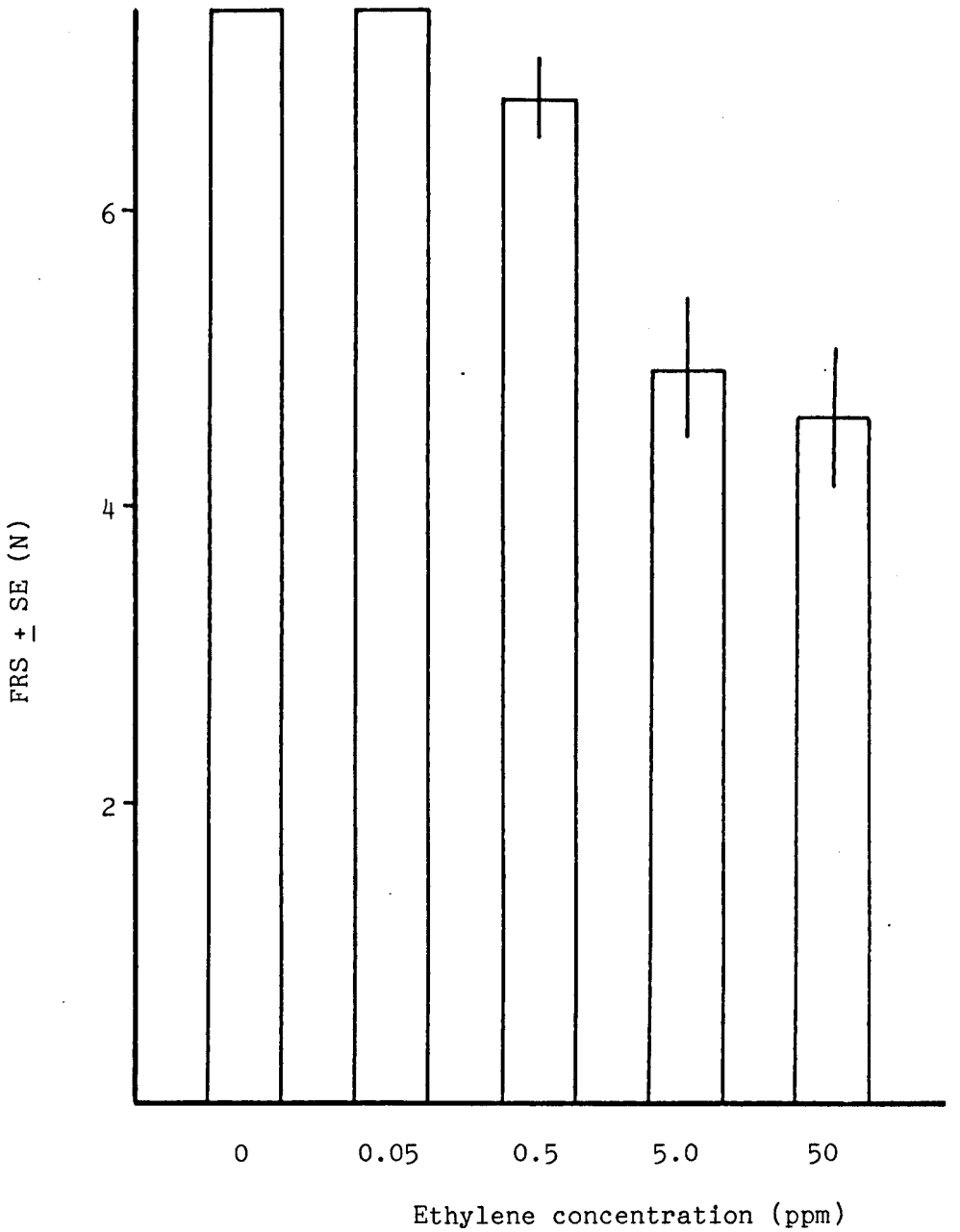


Fig. 3.31 Effect of 48 hours exposure to different concentrations of ethylene on FRS decline of HG fruit (30 fruit/sample).

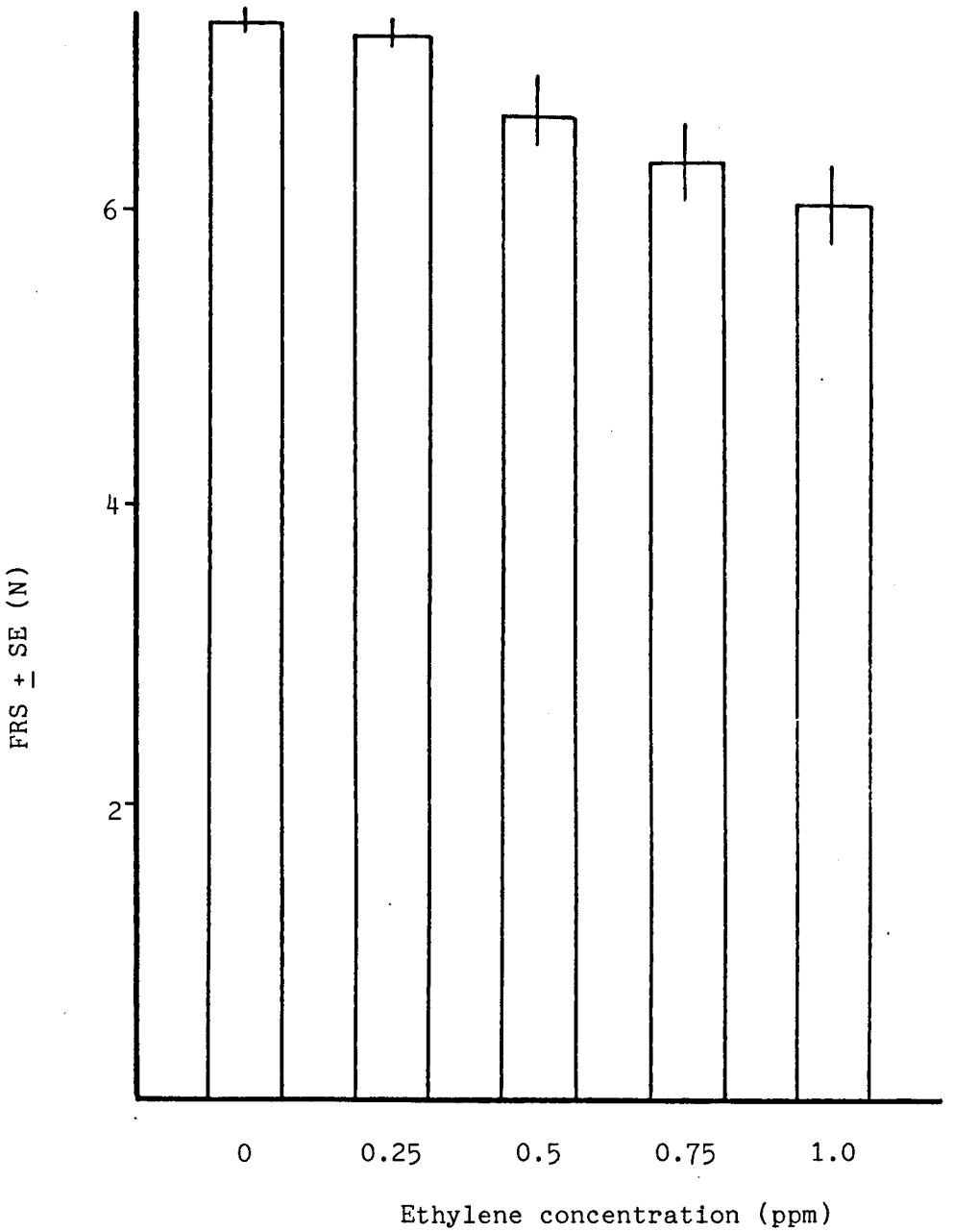


Fig. 3.32 Effect of 24 hours exposure to different concentrations of ethylene on FRS decline of SG fruit (30 fruit/sample).

(fig. 3.31) an exposure to 0.5 ppm ethylene was sufficient to cause a significant ($P = 0.025$) decrease in the FRS of HG fruit. Again by 5-50 ppm the reduction in FRS appeared to be approaching saturation. Hence in both HG and SG fruit the same ethylene concentration was required to cause a reduction in FRS, although the period of exposure necessary was doubled for HG fruit. The first significant FRS decrease in HG fruit was not obtained any sooner even if subjected to higher levels of ethylene. This result may indicate an ageing requirement before the tissues become responsive to the added ethylene. In order to determine a more accurate threshold concentration SG fruit were exposed to a range of ethylene concentrations between 0 and 1.0 ppm for 24 hours. Fig. 3.32 indicates the first significant decline in FRS to occur at 0.5 ppm ethylene, the decrease at 0.25 ppm not being significant. The actual level of FRS reduction in this run was not as great as in previous (figs, 3.30 and 3.31) although the changes still show the threshold for SG fruit to lie between 0.25 and 0.5 ppm ethylene. At these levels the response was not saturated as indicated by the further decline in FRS at 0.75 and 1.0 ppm ethylene. Figures 3.30 and 3.31 indicate levels of 5 to 50 ppm to show no significant difference in FRS reduction and hence be saturating in both HG and SG fruit.

3.1.8 ii) Are the levels of ethylene which must be added to influence abscission in green fruit actually found within the fruit during natural abscission?

The level of ethylene in the internal gases of plant material can be determined by vacuum extracting the gases under a

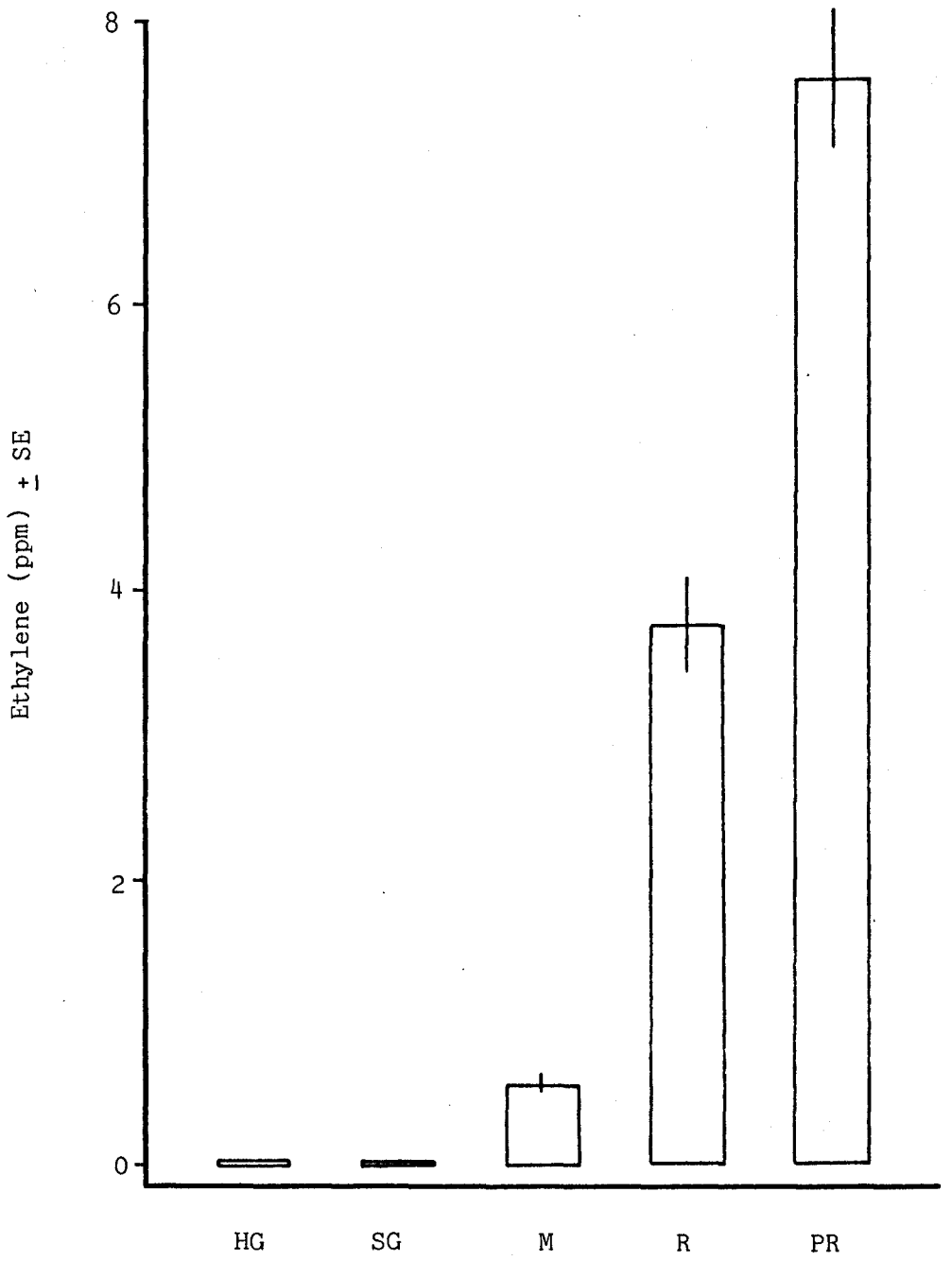


Fig. 3.33 Ethylene level in gases extracted from fruit at different stages of development. Average of 7 samples, each sample of at least 20 fruit.

solution of saturated ammonium sulphate as described by Yeang and Hillman (1981) - see Section 2.16 for precise details. The following series of experiments determined the levels of ethylene in the internal atmospheres of raspberry fruit at different stages of development.

The results in fig. 3.33 show the levels of ethylene in the internal atmosphere of fruit to increase with development. The level was low (< 0.1 ppm) during the HG and SG stages increasing rapidly thereafter through M (0.55 ppm), R (3.8 ppm) and PR (7.6 ppm). The threshold ethylene concentration of between 0.25 and 0.5 ppm required to decrease the FRS of SG fruit is exceeded between the SG and M stages, the very time at which FRS reduction commences naturally. It must however be noted that this value represents the average of all the internal gases from the fruit. Ethylene concentrations may well vary within the different parts of the fruit.

3.1.8 iii) Are ethylene production rates correlated with the internal ethylene levels?

EPRs are generally taken as a meaningful assessment of ethylene levels within the tissue. In some cases the correlation has been shown (Yeang and Hillman, 1981; also listed in Beyer et al., 1984) proving the internal level to be related to the EPR. To establish if such a correlation exists in raspberry fruit EPRs were measured prior to the internal levels of the same fruit. This was done for fruit at all stages of development. The results plotted in fig. 3.34 reveal a linear relationship of :

log internal ethylene (nl/ml) = -0.214 + 0.961 (log EPR (nl/g/h))

Hence in raspberry fruit at all stages of development the internal

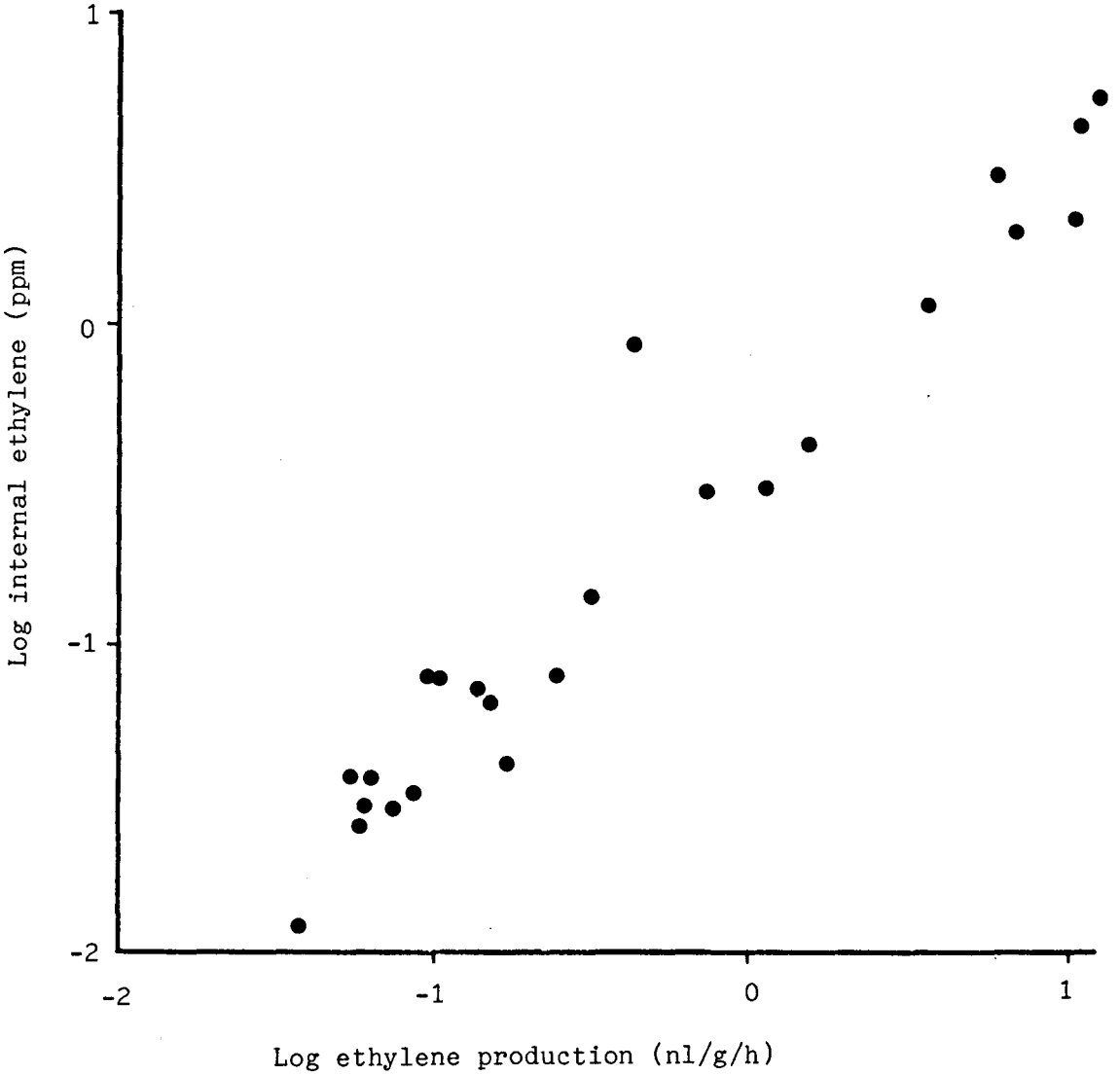


Fig. 3.34 Relationship between ethylene production rate and internal ethylene level. Data for the whole spectrum of fruit from HG to PR from 3 seasons work (1984 to 1986) has been included.

ethylene level can be predicted from the EPR and the above equation. The threshold of 0.25 to 0.5 ppm ethylene correspond to EPRs of 0.4 to 0.8 nl/g/h, these values being found to occur naturally during the M stage of development.

3.1.8 iv) Internal ethylene levels of individual fruit

As with the examination of EPRs the use of bulk samples of fruit for determining internal ethylene levels can hide important differences between individual fruit in the sample. Although examination of the internal gases of individual fruit was not feasible because of the small amounts of gas involved, the internal levels of these fruit can be estimated using the relationship discussed in the previous section.

The data plotted in fig. 3.17 showing the relationship between EPR and FRS has been reworked to convert EPR to internal ethylene levels (fig. 3.35). It is now possible to see if the threshold value of 0.25-0.5 ppm ethylene is exceeded prior to the period of FRS decline. This data reveals a proportion of fruit to have internal ethylene levels in the range 0.25-0.5 ppm whilst not showing any decrease in FRS. This can be considered as providing evidence in favour of the hypothesis that in abscission ethylene acts as an inductive agent. There is however no difference in the levels of ethylene in those fruit showing the initial stages of FRS decline (down to 3N). Fruit having FRS values below 3N clearly showed increased levels of ethylene in their internal atmospheres, the levels increasing with decreasing FRS. It is interesting however to note that in the small (< 0.5 g) fruit, the internal levels are high with no concomitant abscission although the internal

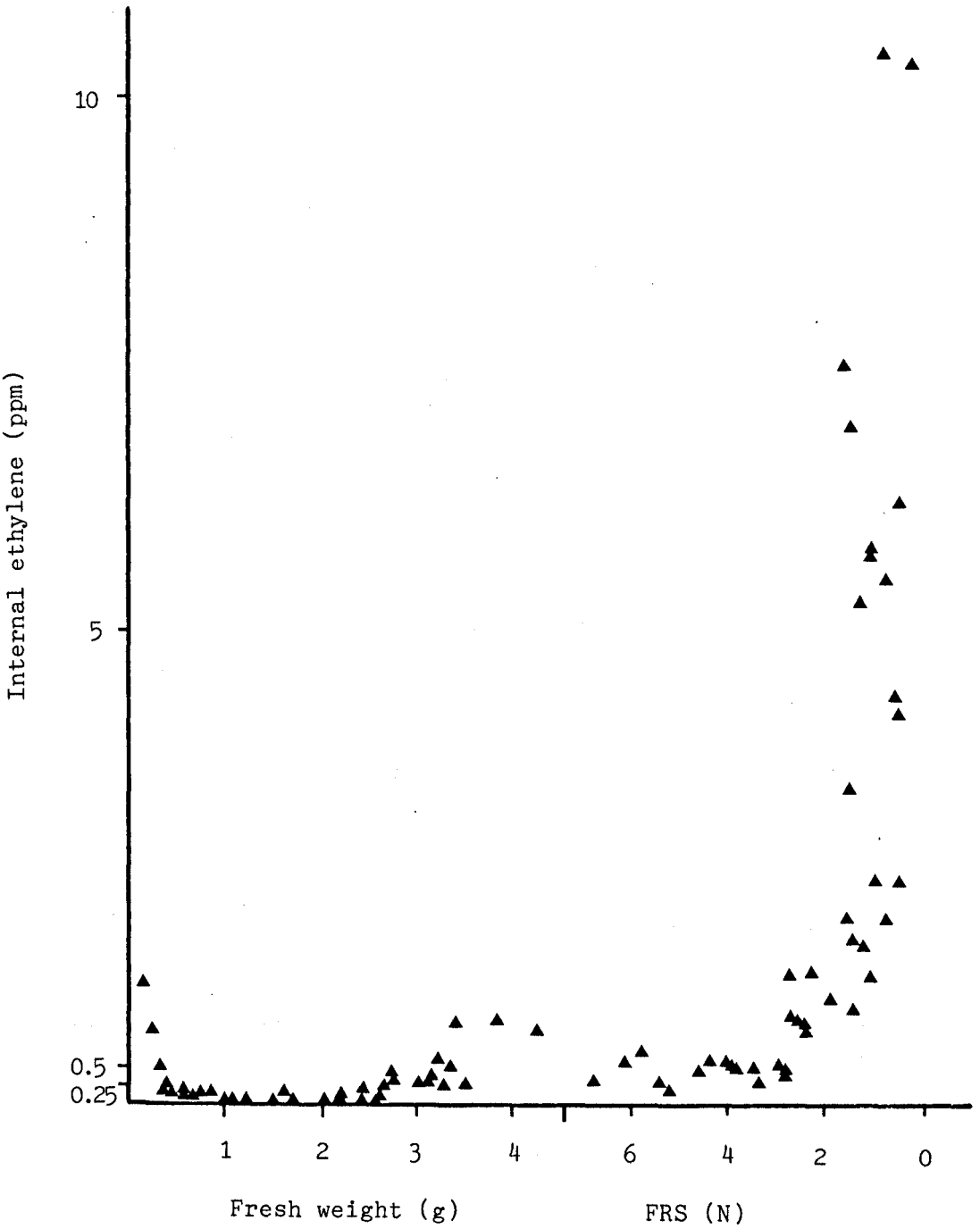


Fig. 3.35 Internal ethylene levels of individual fruit calculated from ethylene production rates. The thresold value of 0.25 to 0.5 ppm is marked.

levels fall rapidly possibly thereby preventing any effect.

3.1.8 v) Summary

In raspberries the mean levels of ethylene accumulating with the berry reach those which if added to SG fruit cause a premature decline in FRS. Measuring the internal levels of ethylene in large samples of fruit shows the threshold level of ethylene to have been reached in mottled fruit, the point at which the FRS declines. There is a constant relationship between the internal ethylene level and the rate at which ethylene is evolved by the berry. Hence the measurement of EPRs by the accumulation of ethylene evolved is a valid technique for assessing the level of ethylene within fruit, once the relationship between the two is known. Calculating internal ethylene levels of individual fruit from their EPRs revealed a category of fruit with internal ethylene levels above the threshold range (0.25-0.5 ppm) yet showing no FRS reduction. This can be taken as evidence in favour of ethylene's inductive role in abscission, the ethylene increasing to significant levels prior to any weakening occurring.

3.1.9 The site of ethylene production

The raspberry fruit consists of two major parts, the drupelets and the receptacle. It has been suggested that it is the production of ethylene by the organ being shed which triggers abscission (Morgan, 1984). The following experiments set out to examine both EPRs and internal ethylene levels of receptacles and drupelets of fruit at different stages of maturity.

Since it is only possible to separate the drupelets and receptacles without major damage from fruit after the M stage, only M, R and PR fruit have been examined. To make measurements of the internal ethylene levels of drupelets and receptacles both were immersed immediately after separation in a 0.1% Triton X-100 solution. This served the dual purpose of preventing the adherence of air bubbles to the outside of the tissues and preventing the loss of internal gases. The gases were then extracted in saturated ammonium sulphate within a 5 minute period as described in Section 2.16. In trial runs where the receptacles were kept in in air, instead of Triton, the ethylene levels were found to decrease the longer they were kept in air prior to extraction under ammonium sulphate. This loss of ethylene is a result of the increased surface area for ethylene to diffuse from, and the greater diffusion gradient from the receptacle on removing the drupelets.

3.1.9 i) Ethylene production rates of separated drupelets and receptacle during development

The results in fig. 3.36 show that both the drupelets and receptacle have increased EPRs as they develop. Also at all stages examined the receptacle produces ethylene at 2-3 times the rate of

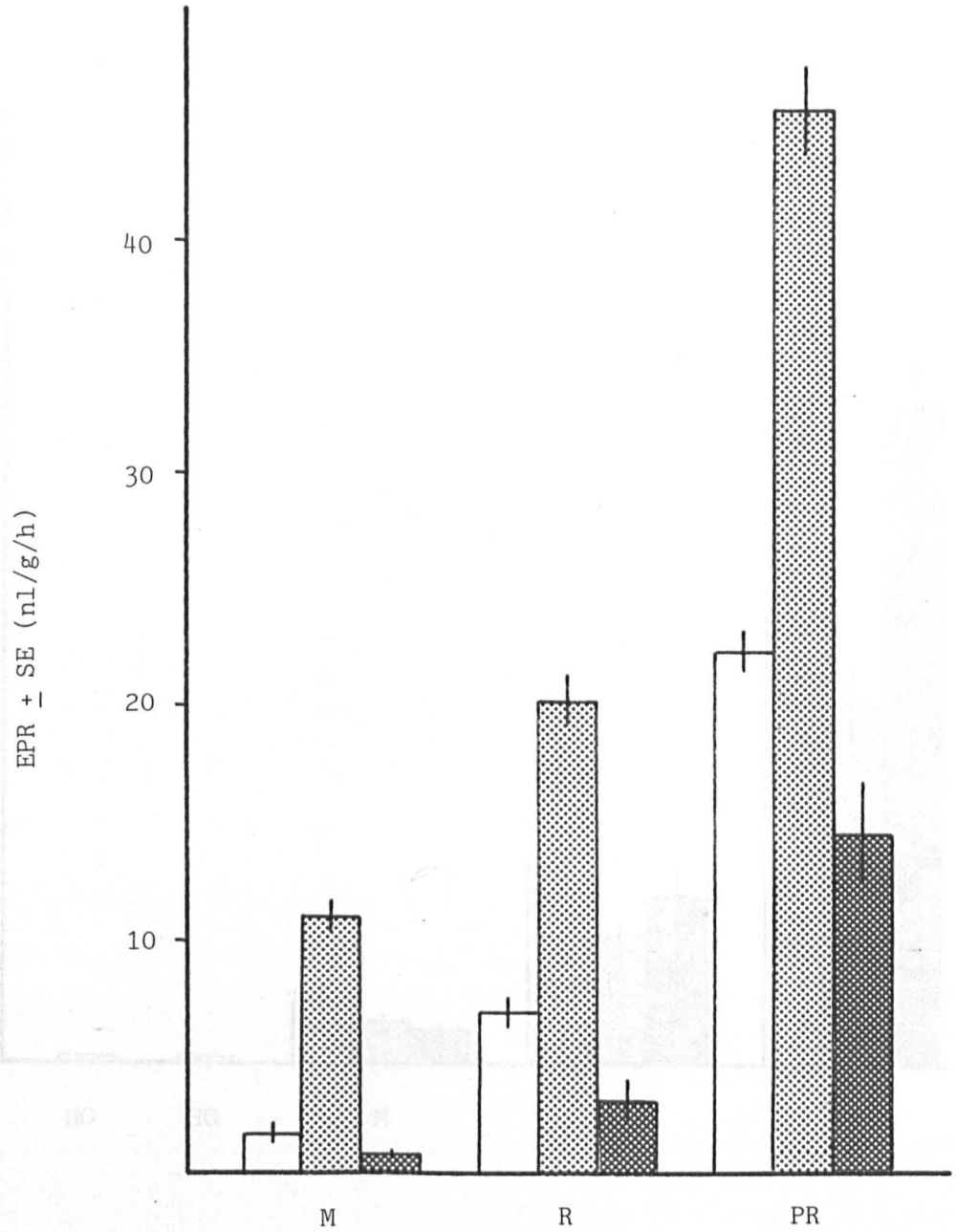


Fig. 3.36 EPRs of receptacles (▨) and drupelets (▩) during fruit development from M to PR. Whole fruit values are also included (□). Average of 5 samples, 20 fruit/sample.

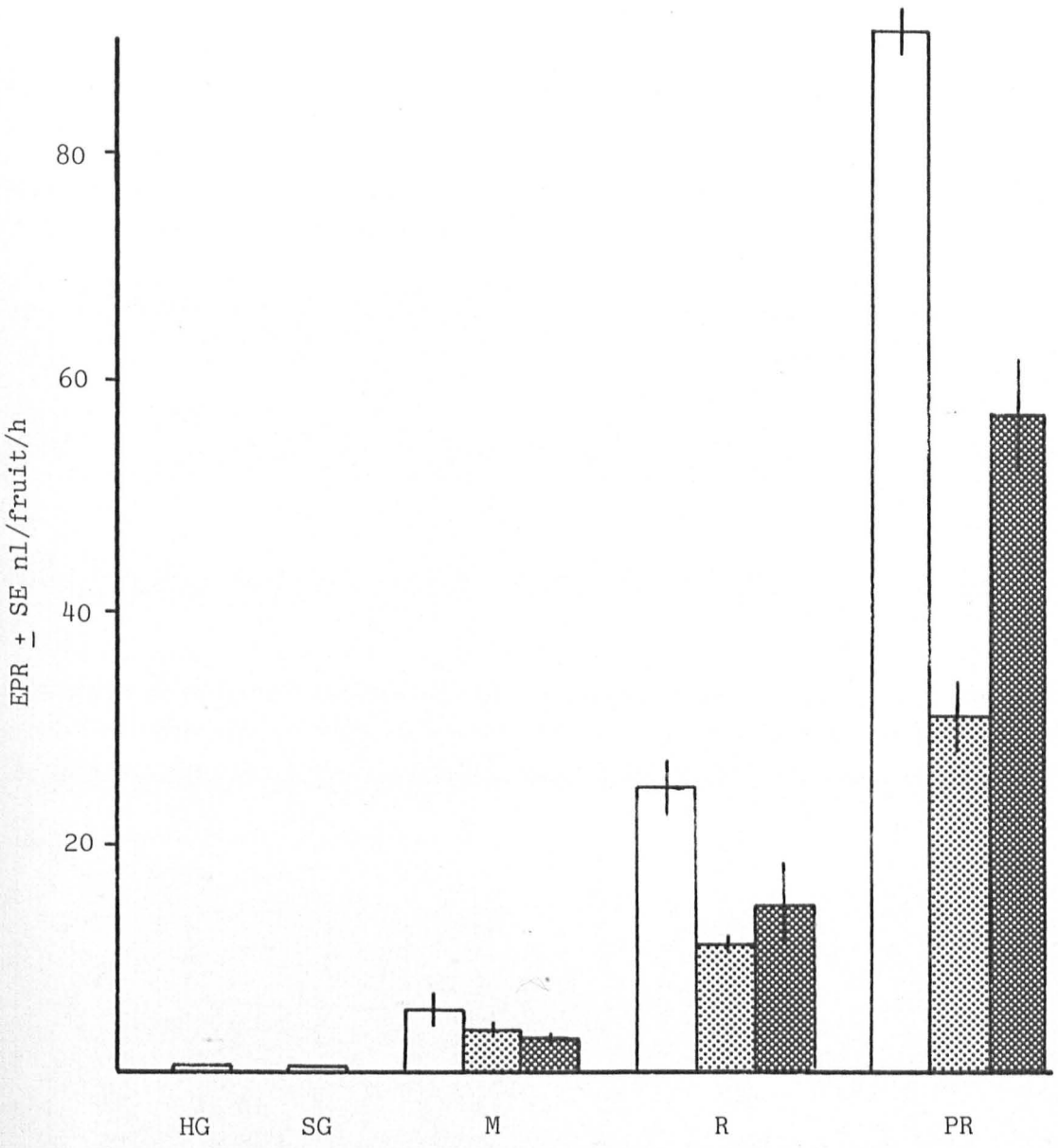


Fig. 3.37 EPRs of receptacles (▨) and drupelets (▩) during fruit development from M to PR. Whole fruit values are also included (□). Average of 5 samples, 20 fruit/sample.

the drupelets. In absolute terms however, at the M stage the receptacle produces 50% of the total ethylene (fig. 3.37), this percentage decreasing when R (44%) and PR (27%). Hence when the first signs of abscission occur the receptacle produces a greater proportion of the total fruit ethylene than in the more mature fruit.

The receptacle can be examined further by dividing it into that area to which the drupelets are attached and where abscission will occur (the apex) and the remainder joining the apex to the pedicel (the calyx). These areas are illustrated in fig. 1.2. The results for ripe receptacles in table 3.13 show the apex region to be the main ethylene producing part of the receptacle.

Table 3.13 EPRs of apex and calyx regions of the receptacles of ripe fruit measured individually (20/sample)

Receptacle part	EPR ± SE nl/g/h	EPR ± SE nl/part/h
Whole receptacle	26.04 ± 4.2	12.9 ± 2.5
Apex region	86.5 ± 10.5	17.7 ± 2.5
Calyx region	16.5 ± 2.3	4.8 ± 0.8

It is clear that the act of cutting the receptacle increased the rate of ethylene production since in absolute terms the sum of the apex and calyx regions exceeds the production of the whole

receptacle. Looking at the situation simplistically it can be assumed that the increase is the same for both halves, each having the same cut surface area. If this is taken as the case the true ethylene production of each part can be assessed. By summing the production of the calyx and apex parts and subtracting the production of the whole receptacle, the amount of ethylene produced by wounding is determined. Assuming the cut surfaces to act equally the amount produced by each is 4.8 nl/hour. In reality however this view may be too simplistic since the cut surface also provides an area of reduced diffusional resistance to ethylene. This may result in an artificially high EPR measurement with an increased rate of ethylene evolution through the cut surface in addition to the natural EPR and any additional wound ethylene. The high EPR of the apex region is significant in that it is to this region that the drupelets are attached and eventually abscise. From this simple calculation the actual EPR of the calyx would appear to be zero. While this is unlikely it does show the production rate to be very small compared to that of the apex (12.9 nl/hour).

3.1.9 ii) Internal ethylene levels of the receptacle and drupelets

Section 3.1.8 ii measured the levels of ethylene in the atmosphere extracted from whole fruit. This section seeks to examine in more detail the sites of ethylene accumulation following on from the sites of production in the above section. As with the EPRs the levels of ethylene accumulation within the receptacle and drupelets increased with development (fig. 3.38). The internal level of ethylene in the receptacle was always 3 to 3.5 times that of the drupelets. Hence in M fruit the level of ethylene in the

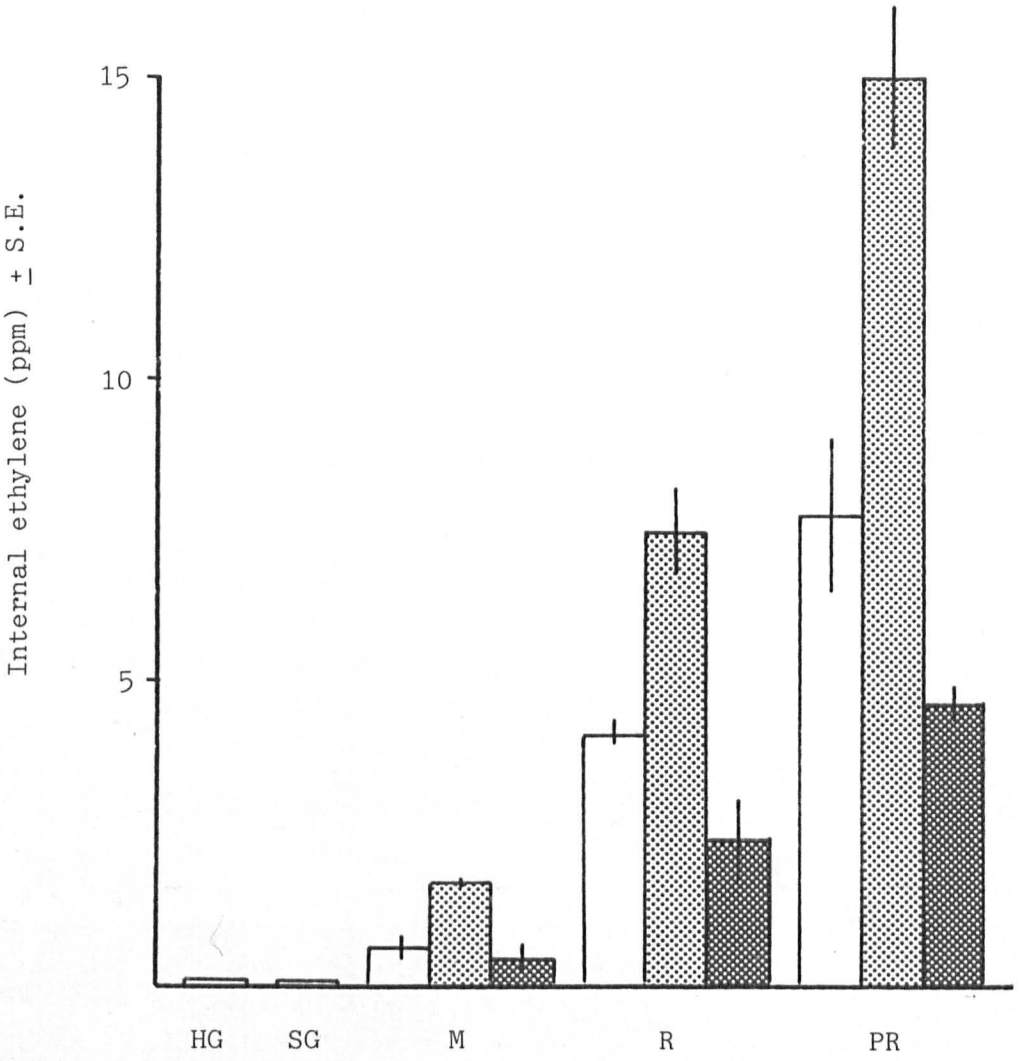


Fig. 3.38 Internal ethylene levels of receptacle (▨) and drupelets (▩) during fruit development. Whole fruit values are also included (□). Average of 5 samples, 20 fruit/sample.

receptacle is 1.7 ppm, well in excess of the sort of level discussed earlier as being significant in terms of causing abscission (0.25-0.5 ppm). The mottled drupelets ethylene content is also within this range (0.48 ppm) indicating the presence in both drupelets and receptacles of ethylene concentrations capable of inducing an abscission response.

3.1.9 iii) Summary

The receptacle produces ethylene at much higher rates than the drupelets in M, R and PR fruit. This is reflected in the internal atmosphere of the receptacle where ethylene accumulates to a greater extent that in the drupelets. During fruit development increased rates of ethylene production may occur first in the receptacle, the autocatalytic nature of ethylene production causing increased ethylene production in the drupelets and receptacle. The levels of ethylene within the receptacle at the M stage of development (1.7 ppm) greatly exceed those determined earlier to be the threshold range (0.25 to 0.5 ppm) required to cause abscission. It is also significant that it is the receptacle apex which contributes the majority of the receptacle ethylene production and can therefore be seen as the factor controlling overall ethylene production and ripening within the fruit, culminating with abscission.

3.1.10 The decline of fruit retention strength and increase in ethylene production rates of other varieties of raspberry

Evaluation of different raspberry varieties for either machine or hand harvesting has led raspberry geneticists to classify varieties as tight or loose. This refers to the FRS of ripe fruit and hence their ease of harvesting. Initial results suggested that those fruit varieties which were difficult to detach when ripe had lower rates of ethylene production when compared to loose varieties. This relationship was examined further using a range of varieties selected by Dr D Jennings of SCRI which included known tight (St Walfried and Landmark) and loose varieties (70/5/32 and 68/14/106 - both SCRI trial varieties). This range is illustrated in plates 3.14 to 3.21 showing the morphological differences between varieties. The berry shapes vary from the very round fruit of Landmark to the more elongate fruit of Phyllis King. Fruit shape is quite variable within a variety but these two examples illustrate the extremes and show least variation, other varieties showing a wider range of shapes. The question as to whether fruit shape is important in terms of FRS is examined later in Section 3.1.11.

3.1.10 i) Fruit retention strength and ethylene producton rates of varieties of raspberry

Stages of fruit development comparable to those already used for Glen Clova were selected for examination. The results in tables 3.14 and 3.15 have been ordered according to FRS when ripe and show the range of FRS and EPR values which occur in the different varieties. The quantitative measurment of the FRS of M and R fruit confirmed the subjective evaluations of the raspberry breeders.

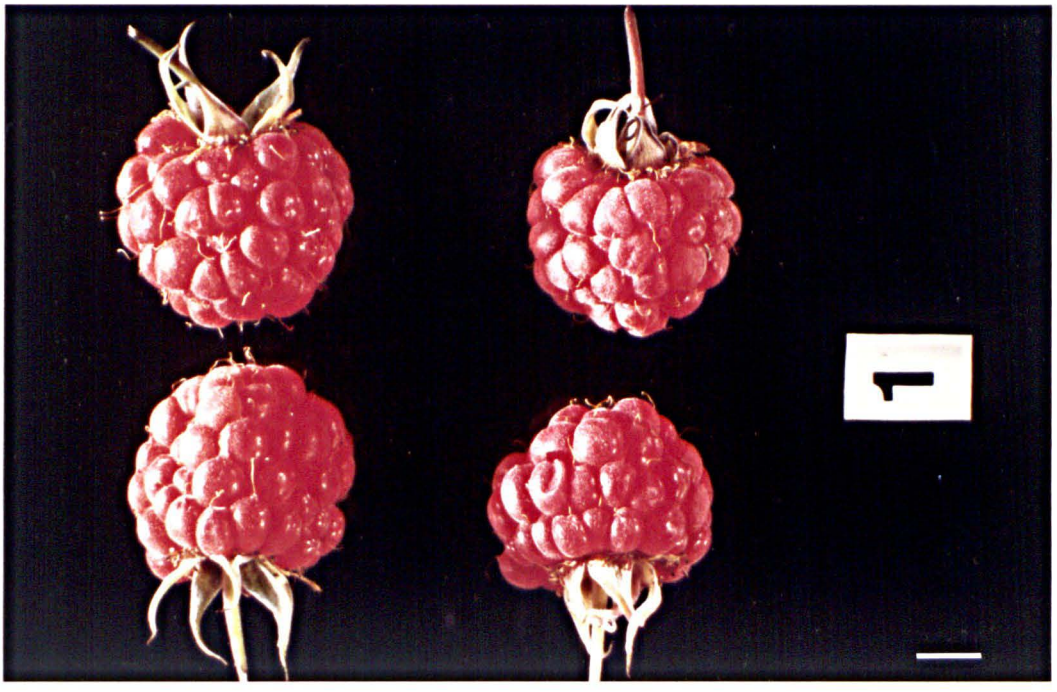


Plate 3.14 Ripe fruit of variety 70/5/32. Bar = 1 cm.

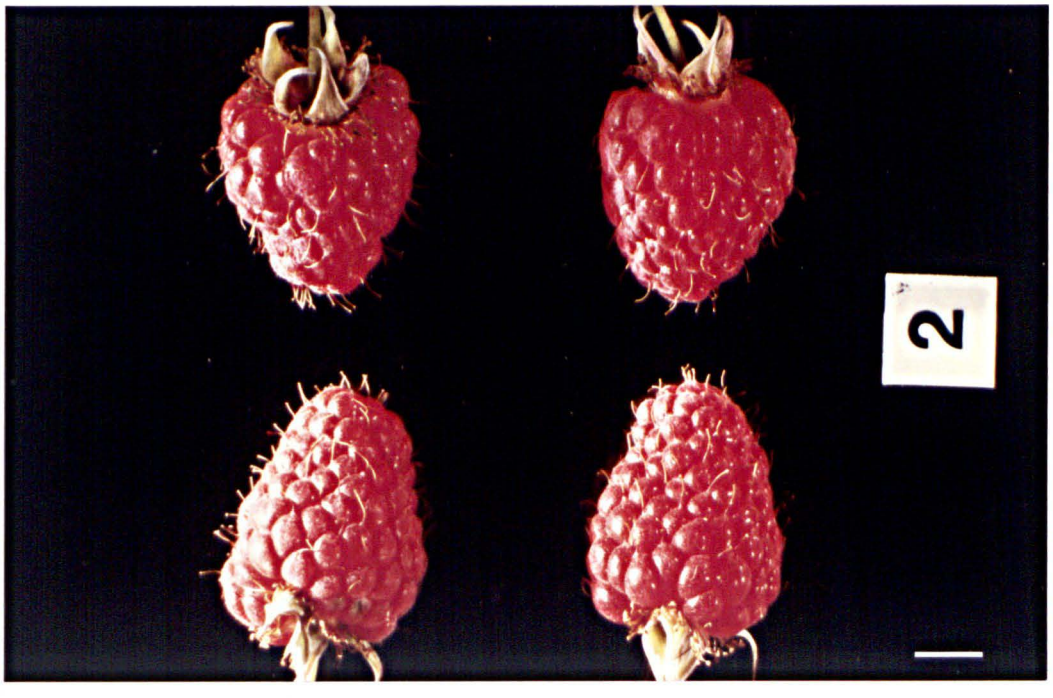


Plate 3.15 Ripe fruit of variety Phyllis King. Bar = 1 cm.

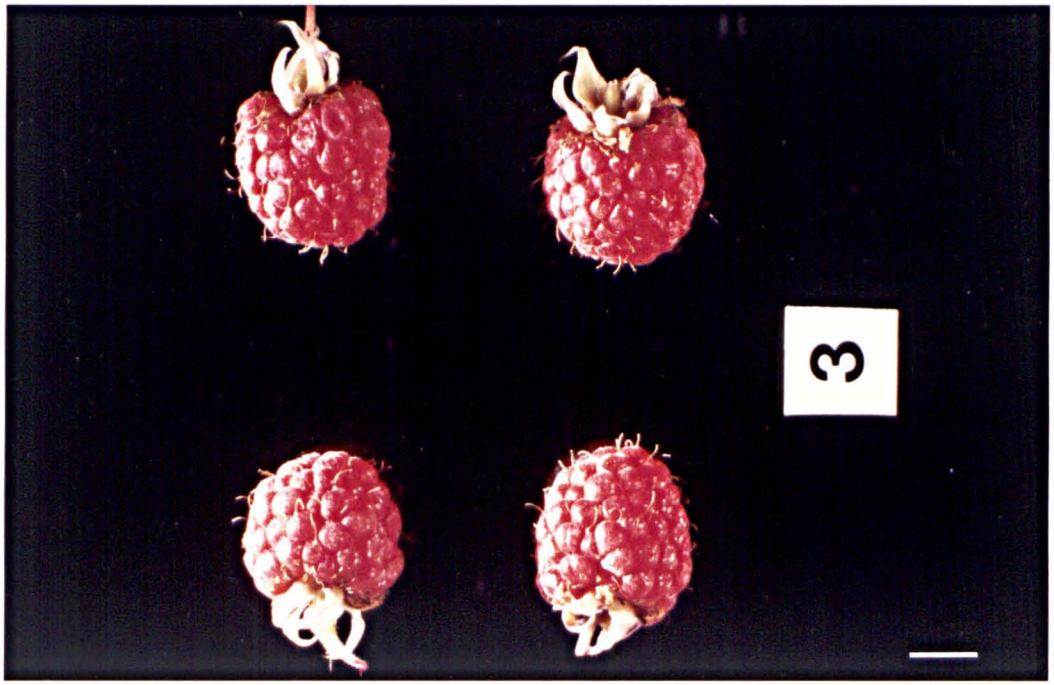


Plate 3.16 Ripe fruit of variety Glen Isla. Bar = 1 cm.

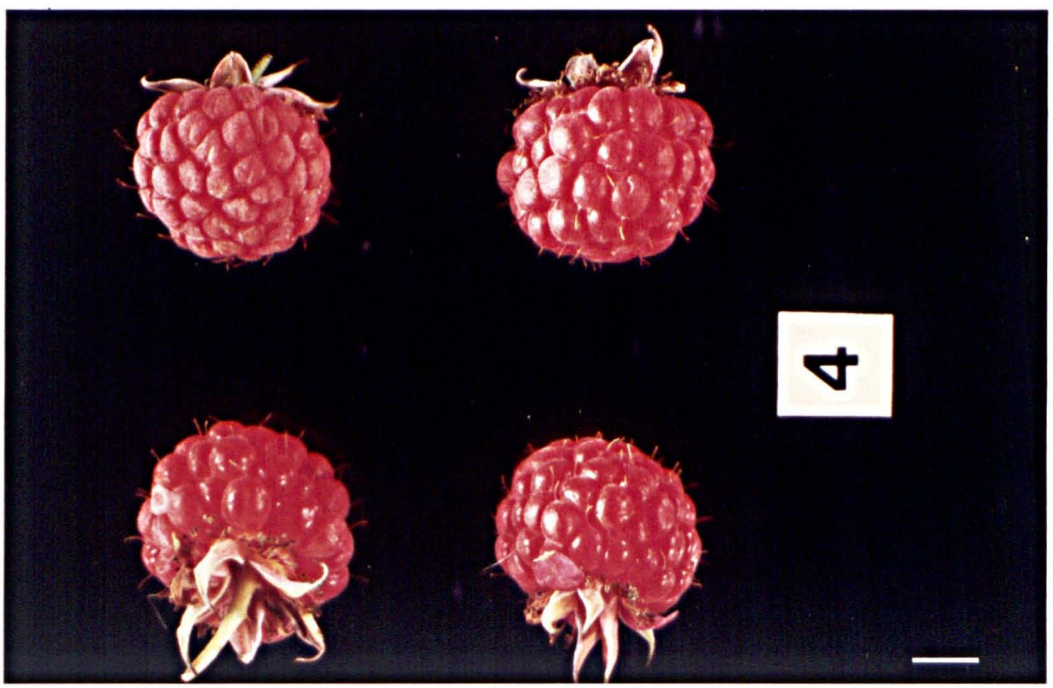


Plate 3.17 Ripe fruit of variety Landmark. Bar = 1 cm.

Plate 3.18 Ripe fruit of variety Malling Jewel. Bar = 1 cm.

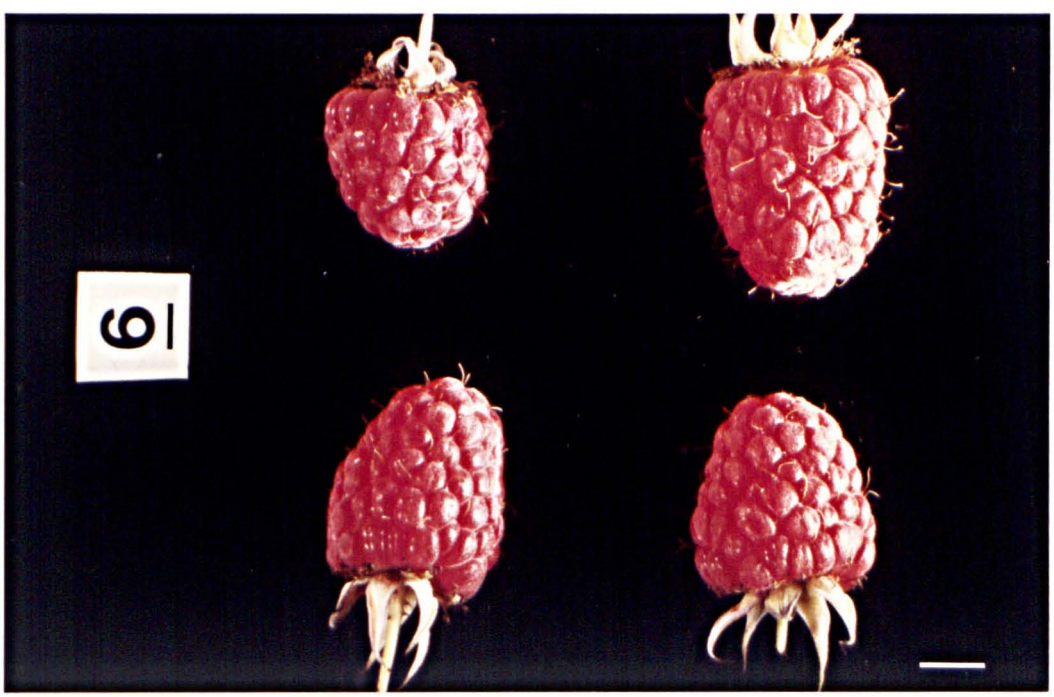


Plate 3.19 Ripe fruit of variety Glen Clova. Bar = 1 cm.

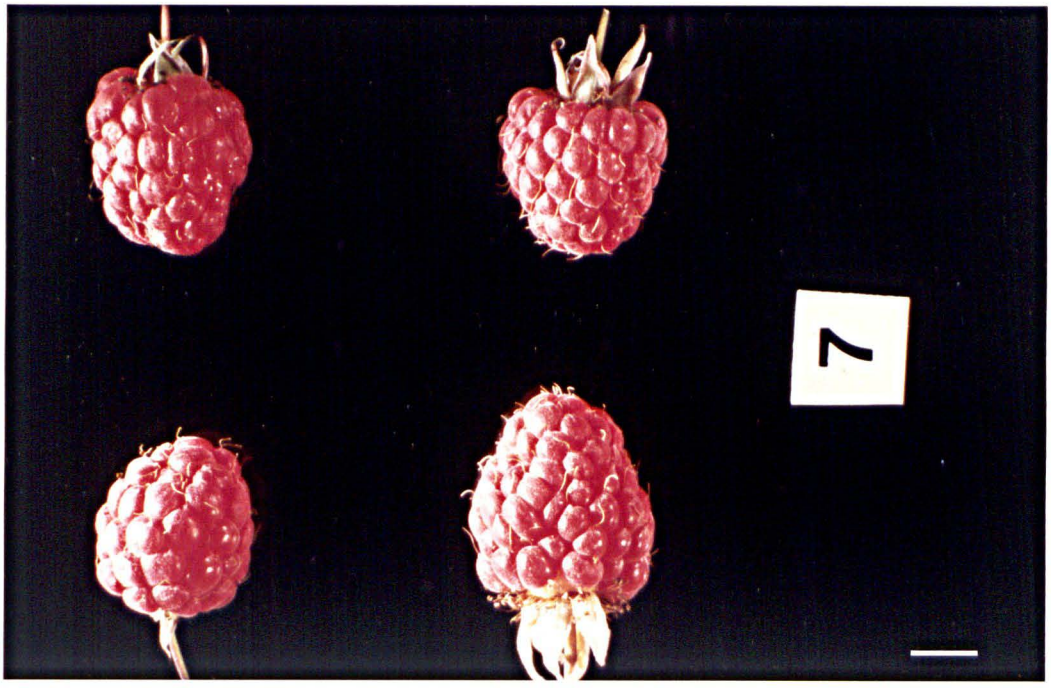


Plate 3.20 Ripe fruit of variety 68/14/106. Bar = 1 cm.

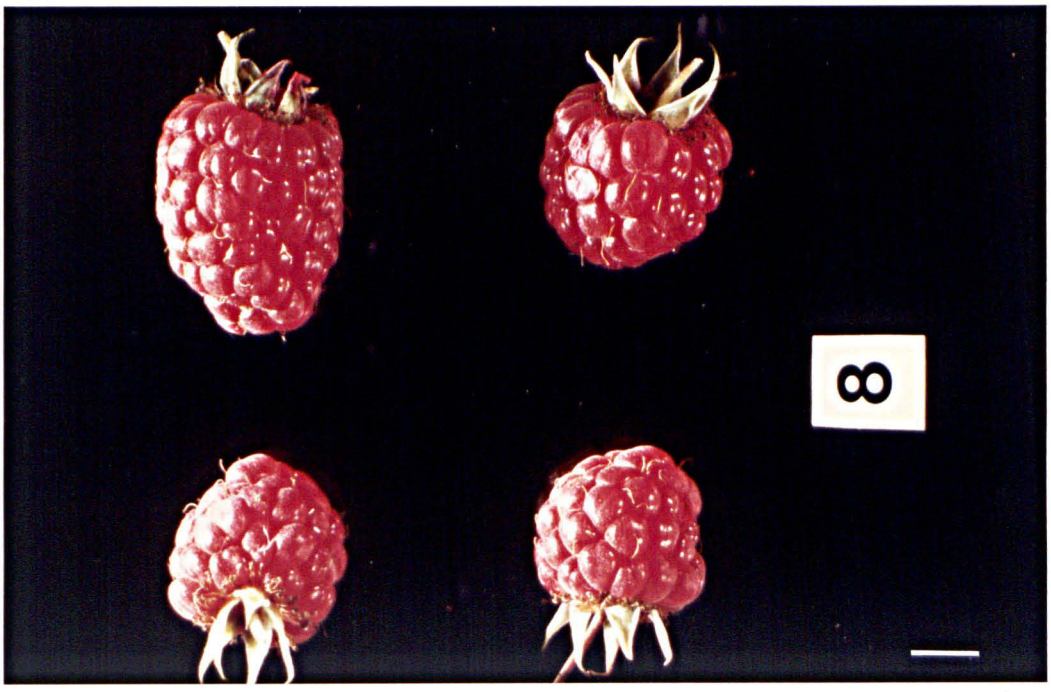


Plate 3.21 Ripe fruit of variety St Walfried. Bar = 1 cm.

Table 3.14 Fruit retention strength of 4 stages of raspberry fruit development in different varieties (15-20 fruit/sample).

Variety	FRS + SE (N)			
	HG	SG	M	R
Phyllis King	7.35	7.35	7.14 ± 0.42	4.20 ± 0.32
St Walfried	7.35	7.35	4.84 ± 0.24	3.42 ± 0.18
Landmark	7.35	7.35	4.51 ± 0.34	2.26 ± 0.07
Glen Clova	7.35	7.35	4.11 ± 0.39	1.61 ± 0.12
Malling Jewel	7.35	7.35	4.93 ± 0.22	1.52 ± 0.12
Glen Isla	7.35	7.35	2.81 ± 0.17	1.31 ± 0.11
70/5/32	7.35	7.35	4.16 ± 0.42	0.88 ± 0.15
68/14/106	7.35	7.35	2.56 ± 0.08	0.64 ± 0.09

Prior to examination it was indicated that Landmark and St Walfried fruit were tight whereas 70/5/32 and 68/14/106 were loose, the FRS measurements quantify and confirm these findings. The differences between varieties were quite remarkable with the ripe fruit of Phyllis King having an FRS of 4.2 N compared to 0.64 N in 68/14/106. These values are for fruit of comparable stages as judged by drupelet coloration and expansion.

The pattern of ethylene production in the different stages of fruit of different varieties showed the same trend as in Glen Clova (Table 3.15). In each variety there is an increase in EPR

from SG to R with the HG fruit having slightly higher EPRs than the SG. The exception to this rule is Phyllis King in which the EPRs increase directly from HG to R. The slightly higher EPR found in the HG fruit of Glen Clova was shown in Section 3.1.6 to be as a result of an earlier peak in ethylene production during flowering.

Comparing tables 3.14 and 3.15 showed that in general terms (excluding Phyllis King) those varieties with the higher EPRs had

Table 3.15 Ethylene production of 4 stages of raspberry fruit development in different varieties (15-20 fruit/sample).

	EPRnl/g/h			
Variety	HG	SG	M	R
Phyllis King	0.117	0.135	0.530	2.232
St Walfried	0.148	0.052	0.056	0.559
Landmark	0.356	0.069	0.084	2.244
Glen Clova	0.163	0.097	0.837	4.198
Malling Jewel	0.352	0.209	0.412	2.647
Glen Isla	0.166	0.067	1.087	5.565
70/5/32	0.430	0.110	0.703	7.060
68/14/106	1.321	0.175	1.087	7.567

lower FRS values at the corresponding developmental stage. Both tables have been ordered for FRS when ripe, from hard to detach at

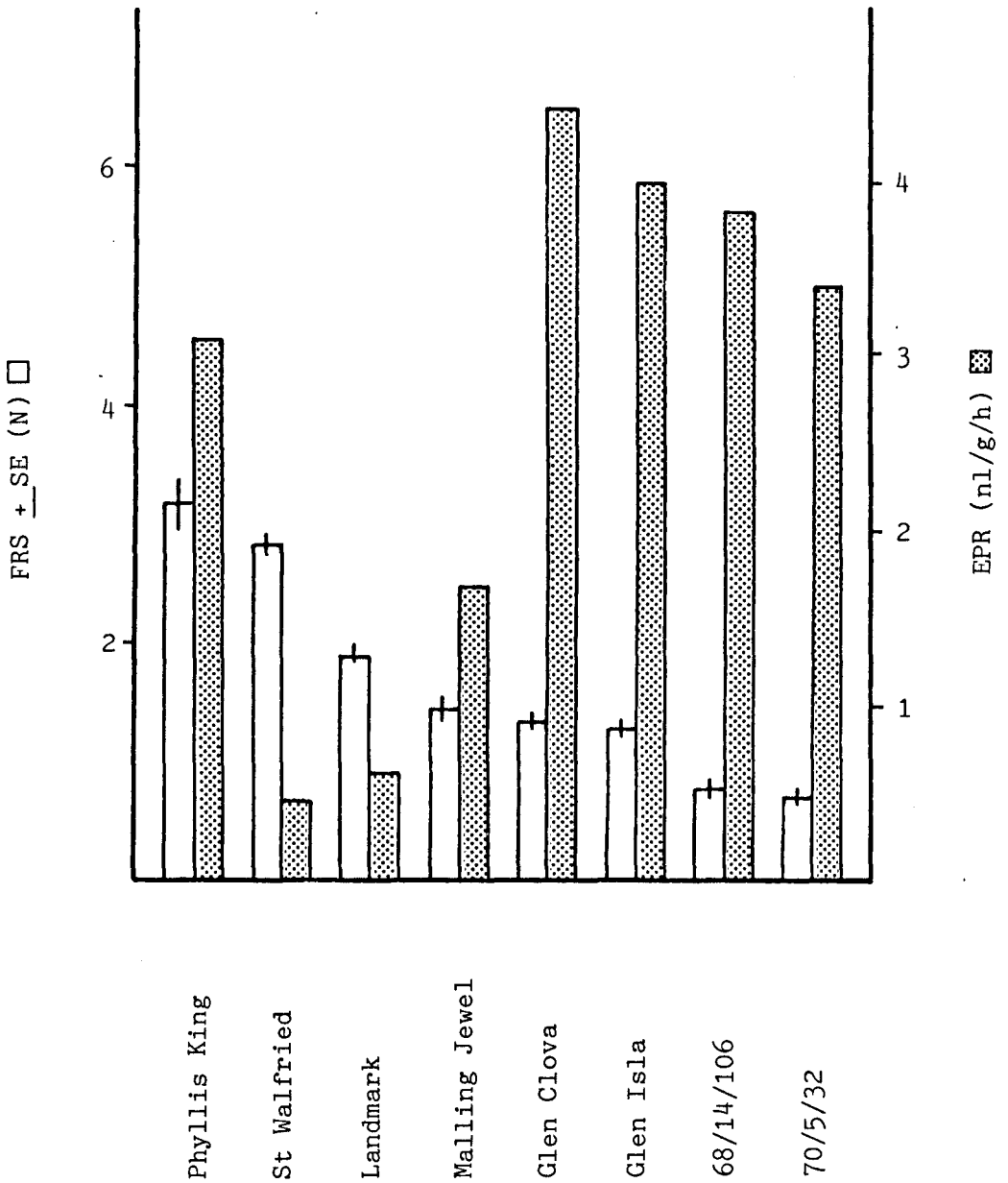


Fig. 3.39 Relationship between FRS and EPR of ripe fruit of 8 cultivars, ordered by FRS. (60 fruit/sample).

the top to easy at the bottom. For example, in M fruit Glen Isla and 68/14/106 were the only varieties with EPRs over 1 nl/g/h and correspondingly the only varieties to have FRS values less than 3 N implying a direct relationship between EPR and FRS. In the mottled fruit both Landmark and St Walfried show a reduction in FRS whilst their EPRs remained below the 0.4 to 0.8 nl/g/h required to give threshold internal ethylene levels in Glen Clova (0.25 to 0.5 ppm). However, as shown earlier, the production rates of the receptacle and drupelets differ greatly and it is possible that the receptacles of Landmark and St Walfried contain significant amounts of ethylene. Once ripe all varieties produced ethylene above those rates expected to create threshold internal levels. The actual EPRs are inversely related to FRS values, the higher the EPR the lower the FRS.

The one exception to this generalisation is the variety Phyllis King. In this case the FRS is high even when ripe (4.2 N) yet there is a relatively high EPR (2.2 nl/g/h). If ethylene alone determined the FRS, fruit of Phyllis King when ripe would be expected to have a much lower FRS, assuming a similar ethylene sensitivity to Glen Clova.

To verify the differences noted above a larger scale experiment repeated the FRS and EPR measurements on ripe fruit of the different varieties. The results (fig. 3.39) confirmed the general view of varieties having high EPRs also having low FRS values, Phyllis King remaining anomalous.

3.1.10 ii) Fruit retention strength and ethylene production of individual fruit of St Walfried and 68/14/106.

The results of the previous section indicate there to be differences between the FRS and EPRs of batches of fruit at apparently the same developmental stages in different varieties. This type of comparison is open to criticism on the grounds that the characteristics used to assess development may vary between varieties. For example the timing of pigment production, drupelet swelling and FRS decline may not be linked in the same way in different varieties. In order to establish a complete range of EPRs and FRS' the complete spectrum of fruit development stages were examined in 2 varieties. St Walfried was taken as an example of a variety exhibiting high FRS and low EPR when ripe, 68/14/106 being at the opposite end of the spectrum, having low FRS and high EPR when ripe. Each individual fruit was classified for development on the categories devised for Glen Clova according to drupelet colour and size (Section 3.1.1) and measured for FRS and EPR. Additional stages were included so as not to omit fruit which would normally be between categories. Pale soft green fruit (PSG) are well advanced SG fruit with little green coloration left, yet don't show any red coloration indicative of M fruit. Pink fruit (P) have just passed the M stage being totally pink but have not developed the red colour of ripe fruit.

The results detailed in table 3.16 show no fruit in the complete St Walfried population ever to reach the extremes of FRS and EPR found in 68/14/106. Even when PR the FRS of St Walfried did not fall below 1.2 N and the EPR at the same stage never exceeded 1.5 nl/g/h. These values can be compared to those of PR 68/14/106

Table 3.16 Fruit retention strength and ethylene produciton rates of individual fruits of St Walfried and 68/14/106 at different stages of development.

68/14/106			St Walfried		
Development	FRS (N)	EPR (nl/g/h)	Development	FRS (N)	EPR (nl/g/h)
PR	0.39	10.404	PR	1.27	1.468
PR	0.54	9.376	PR	1.71	1.058
PR	0.20	4.917	PR	1.96	0.920
R	0.10	6.718	PR	1.47	0.828
R	0.34	4.670	PR	1.32	0.807
R	0.49	3.948	PR	1.47	0.679
R	0.64	2.651	R	2.20	0.359
R	0.73	1.771	R	2.89	0.149
R	0.64	0.755	P	3.33	0.824
P	1.18	0.652	P	7.25	0.156
P	1.76	0.569	PSG	7.35	0.036
P	1.32	0.506	PSG	7.35	0.061
P	1.32	0.488	PSG	7.35	0.084
P	1.42	0.334	PSG	7.35	0.049
PSG	2.20	0.640	SG	7.35	0.040
PSG	3.23	0.152	SG	7.35	0.104
SG	7.35	0.090	SG	7.35	0.055
SG	7.35	0.006	HG	7.35	0.065
HG	7.35	0.055	HG	7.35	0.14
HG	7.35	0.102	HG	7.35	0.467

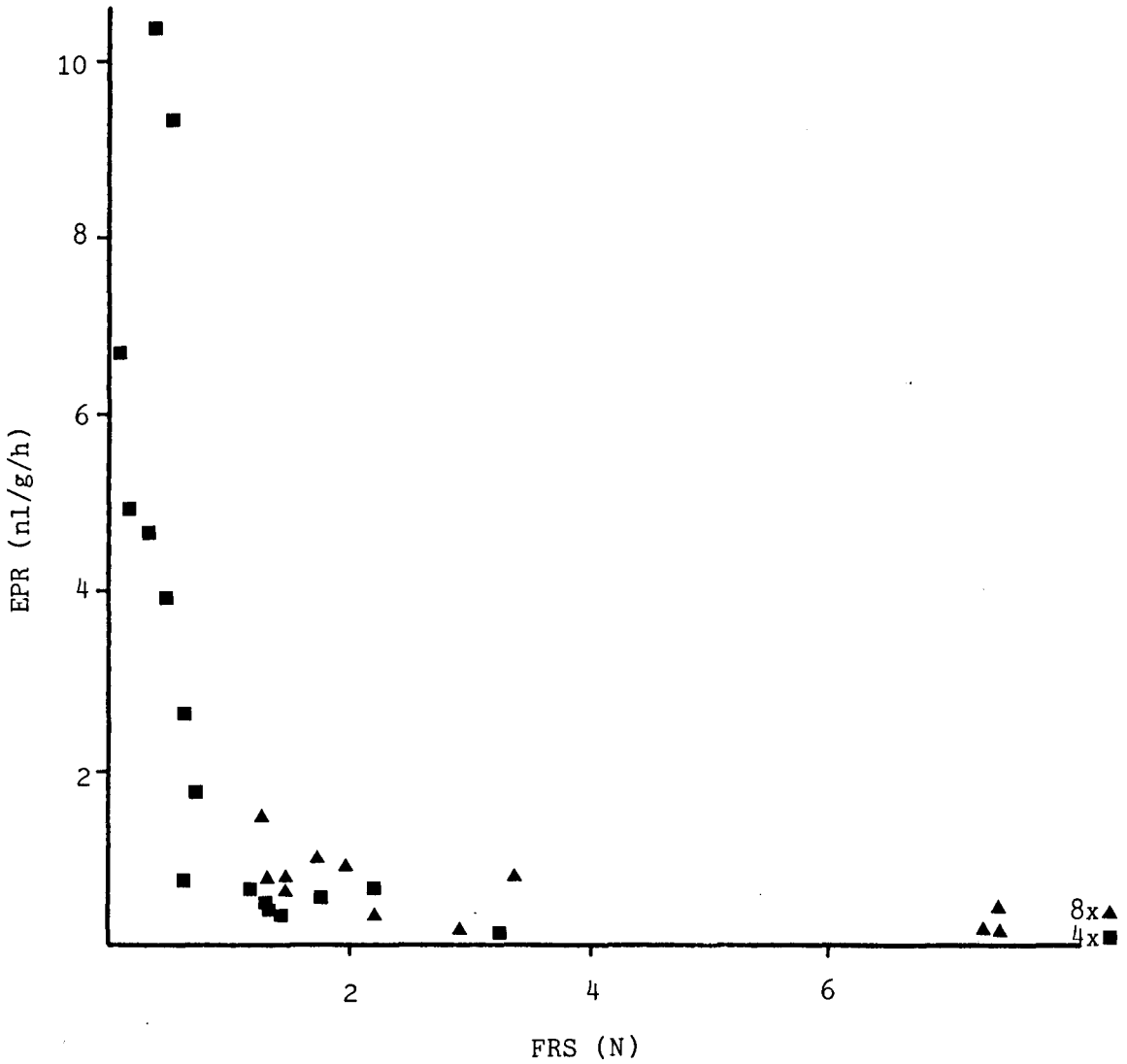


Fig. 3.40 EPR and FRS of individual St Walfried (▲) and 68/14/106 (■) fruit over the whole spectrum of development.

fruits showing FRS values as low 0.1 N and EPRs as high as 10 nl/g/h. This can be seen more clearly by plotting EPR against FRS for the individual fruit of the two varieties (fig. 3.40). Fig. 3.40 however does not distinguish the developmental stage of each fruit. From the data in table 3.16, it can be seen that in 68/14/106 the EPR starts to increase earlier, at the PSG stage rather than the P stage in St Walfried. There is a concomitant earlier decrease in FRS in 68/14/106, occurring at the PSG stage as opposed to the P stage in St Walfried. At both these stages where the FRS starts to decline the EPRs are similar, being in excess of 0.15 nl/g/h.

Hence the difference between the two varieties can be seen as the stage of pigmentation and fruit expansion at which ethylene production increases. In St Walfried the fruit develops as far as the P stage with no FRS decline and low EPRs (< 0.5 nl/g/h). In 68/14/106 the EPR of PSG fruit increases above 0.15 nl/g/h and is sufficient to cause FRS decline. The results indicate that the increase in EPR and concomitant FRS decline are not directly linked to increased pigmentation and drupelet swelling.

3.1.11 Is the shape of the receptacle apex important in determining the fruit retention strength of different varieties of raspberry?

It became apparent whilst examining the FRS of ripe fruit of different varieties (Section 3.1.10) that the receptacle apices showed a wide variation in shape ranging from short and stubby to long and thin. This may provide a possible reason for the differences in FRS not accounted for by changes in EPR, such as arise in Phyllis King. It is therefore possible that fruit having short rounded receptacle apices (such as Landmark) show low FRS values when ripe compared to those varieties (such as St Walfried) having longer apices. The shape of the receptacle apex could clearly be related to FRS in that it is to this part that the drupelets are attached. It develops in a well defined manner throughout the life of the fruit, showing a large increase in volume around the ripe stage of development. These changes have been illustrated in Glen Clova fruit in Section 3.1.1 (plates 3.2 - 3.5). It is possible that the degree of change in volume as well as the general shape of the receptacle could play a mechanical role in the final shedding of the cap of drupelets after the weakening of the abscission zones (Reeve, 1954).

3.1.11 i) The dimensions of the receptacle apices of ripe fruit of different varieties compared to their fruit retention strengths

The possible effects of the differences outlined above have been examined by the measurement of the basal width and length of the receptacle apex of the ripe fruit of the different varieties.

These two dimensions were chosen to examine specific features of raspberry fruit. Measurement of the basal diameter examined the possibility raised by Reeve (1954) that the receptacle expansion pushing the drupelet cap upwards aids separation. Likewise the shedding of a drupelet cap over a short, stubby receptacle can be envisaged as being easier than removing a receptacle penetrating the length of a long thin fruit. The apex weight to length ratio gives a measure of the degree of swelling. This is not ideal since it does not account for variations in shape, some apices being conical, whilst others are more cylindrical. The results in table 3.17 show how these dimensions vary with the average FRS value

Table 3.17 Receptacle apex dimensions and FRS of ripe fruit of different varieties (30-40 fruit/sample)

Variety	Apex Length ± SE (mm)	Basal Width ± SE (mm)	Apex Weight: Length Ratio (g/mm)	FRS (N)
68/14/106	13.05 ± 0.31	7.61 ± 0.17	25.34	0.84
Glen Isla	10.36 ± 0.30	6.88 ± 0.23	16.44	1.27
Glen Clova	13.65 ± 0.47	7.63 ± 0.23	21.98	1.32
Landmark	7.41 ± 0.28	7.44 ± 0.29	33.07	1.57
Phyllis King	14.92 ± 0.44	8.71 ± 0.23	24.55	2.20
St Walfried	16.21 ± 0.22	7.00 ± 0.25	16.38	2.29

of the ripe fruit from which they were taken. Table 3.17 indicates FRS not to be dependent on the basal diameter or the length of the apex, the fruit having the lowest FRS (68/14/106) being intermediate in terms of these dimensions. The apex weight to length ratio also showed no correlation with the final FRS. Overall it therefore appears that these receptacle dimensions are not important in determining the FRS of ripe fruit.

3.1.11 ii) Varietal differences in the receptacle apex development of ripe raspberries

Whilst carrying out the above measurements it became obvious that not only was there variation in the shapes of the receptacle apices of different varieties, there were also differences in the progress of their development. The changes in receptacle development are illustrated for Glen Clova in plates 3.2 - 3.5, the surface topography of the apex can be seen to change between M and R stages. At M the receptacle apex on removal of the drupelet cap has a pimpled appearance, the point of each drupelet attachment being at the tip of a raised area of the receptacle. At this stage the apex is white and changes gradually to a creamy/yellow colour when ripe. Along with this change in colour the receptacle swells between the points of drupelet attachment. The surface appears smoother, the abscission scars now being virtually flush with the receptacle surface. In Glen Clova fruit well into the R category the receptacle apex starts to take on a translucent red appearance, possibly indicating a loss of tissue integrity.

The developmental status of the receptacle apices of ripe fruit of different varieties was assessed by examining both surface

topography and colour. In the examination of surface topography the apices were classified as either being pimpled, smooth or intermediate (table 3.18). An intermediate category was included because of the degree of difference between the extremes. The apex colour differences were examined by classifying the apices on a five point scale; 1 - white, 2 - cream, 3 - less than 30% red coloration, 4 - 30-70% red coloration and 5 - greater than 70% red coloration (fig. 3.41).

Taking the surface topography first it became clear that those varieties having low FRS values when ripe have a majority of smooth surfaced apices whilst those with higher FRS values have more pimpled surfaces (table 3.18). Phyllis King and St Walfried are

Table 3.18 Varietal difference in receptacle apex development as assessed by surface topography compared to fruit retention strength (30-40 fruit/sample).

Variety	% Pimpled	% Intermediate	% Smooth	FRS (N)
68/14/106	13	20	67	0.84
Glen Isla	55	5	40	1.27
Glen Clova	15	70	15	1.32
Landmark	10	45	45	1.57
Phyllis King	100	0	0	2.20
St Walfried	95	0	5	2.29

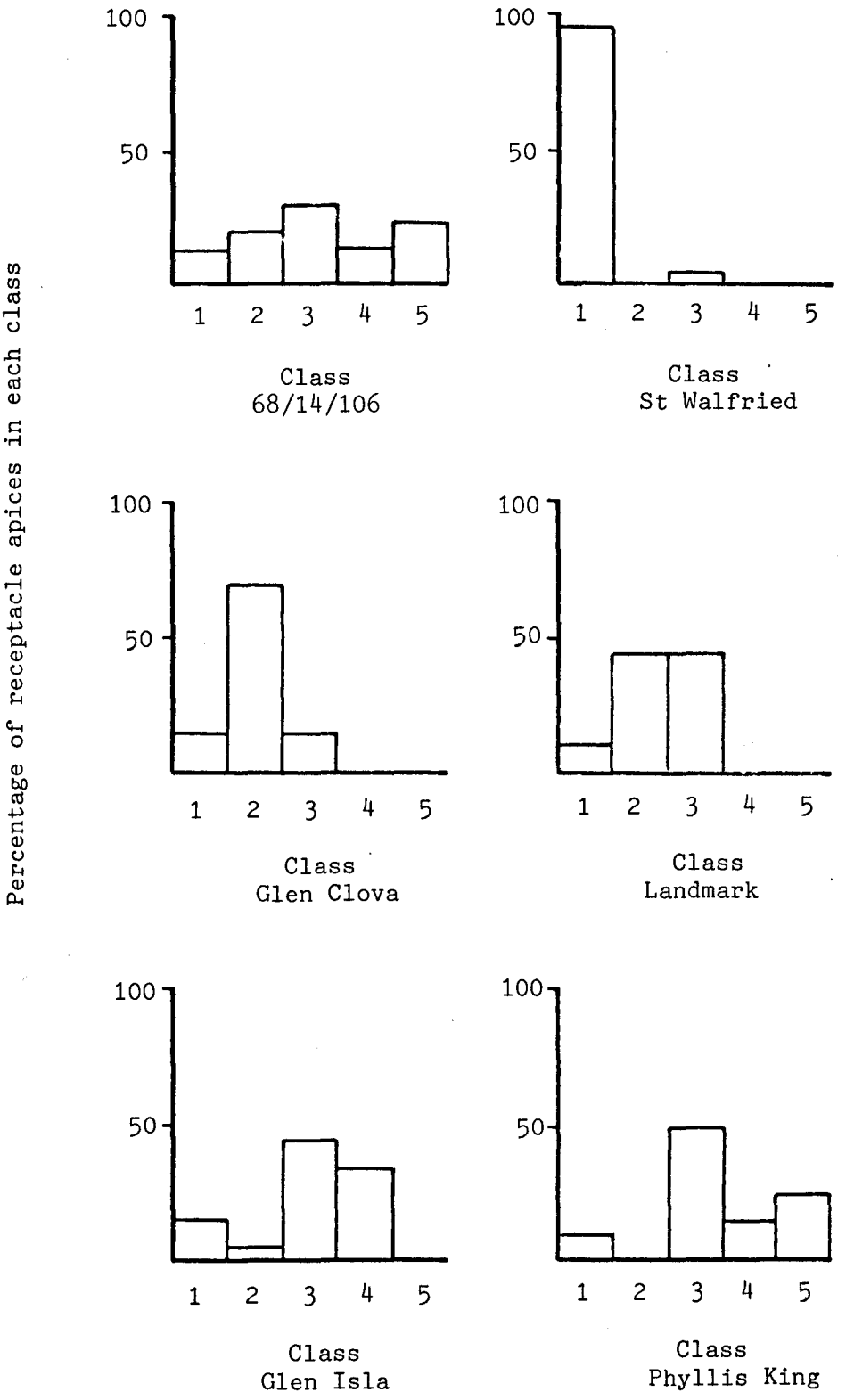


Fig. 3.41 Difference in maturity of ripe receptacle apices from different varieties assessed by colour. Class 1 - white, 2 - cream, 3 - <30% red coloration, 4 - 30-70% red coloration and 5 - 70-100% red coloration. Classes 3-5 refer to the red coloured area of each individual apex.

both good examples of this latter category, 100% and 95% respectively of their populations being pimpled. At the other end of the spectrum the 68/14/106 variety has a low FRS and only 13% pimpled apices with 67% being totally smooth.

Examining apex coloration (fig. 3.41) the variety having least FRS decline (St Walfried) has very few apices above category 1. The percentage of apices in the more mature categories increases through those varieties with intermediate FRS values to 68/14/106 (the lowest FRS) which has over 20% of its apices in category 5 (ie with greater than 70% red coloration). This analysis omits reference to Phyllis King which, as when examining the FRS and EPR of different varieties, proved to be anomalous. The apices of Phyllis King showed increased coloration whilst retaining a relatively high FRS.

Generally apices change from being white and pimpled to white and smooth and hence through the cream/yellow and red coloration gradient. Whilst these transitions in colour and topography are not always totally coordinated in all varieties they do occur in a reasonably predictable sequence. Hence in general a very pimpled receptacle apex would be expected to be white. In Phyllis King all the apices were pimpled yet the majority (90%) were classed on coloration to be in stages 3 to 5 (showing red coloration). This provides an explanation for the apparently contradictory high FRS and high EPR values determined in Section 3.1.10. The FRS value appears to be related to the surface topography and the EPR to the developmental stage of the apex as determined by colour.

The overall conclusion from this piece of work is that the

shape of the apex (ie short, long, fat or thin) has no bearing on the FRS of the ripe fruit, the surface topography of the apex however does have. This change in topography includes a certain amount of swelling of the apex, particularly between the points of drupelet attachment, to produce a smooth surface which has been shown above to be associated with lower FRS values. The change in apex as judged by colour can be related to the ethylene producing capacity of the receptacle, the more mature redder translucent apices being associated with higher EPRs. Taken together these two factors (topography and colour) can account for the difference between the varieties 68/14/106 and Phyllis King. Both have relatively high EPRs as indicated by the red coloration of the apices, however the apices of 68/14/106 are smooth whilst those of Phyllis King are pimpled. This can account for their different FRS values when ripe, 68/14/106 being low whist Phyllis King is relatively high. Clearly the changes in receptacle swelling and surface topography are important in terms of fruit removal.

3.1.11 iii) The dependence of receptacle growth on drupelet development

In the course of the previous examination of receptacle apices of different varieties one feature appeared repeatedly. On removing the drupelets from R and PR fruit the receptacle apex had areas to which poorly developed drupelets were attached. If these undeveloped drupelets were dislodged an unswollen area of receptacle was apparent underneath. The swelling of the receptacle thus seemed linked to the development of the drupelets. Nitsch (1952) showed that the removal of the achenes of strawberries early in development

prevented receptacle swelling (an effect which could be reinstated by auxin). The situation in raspberry was examined by preventing drupelet development. This was achieved by removing half the stigmas from tagged flowers as soon as they became visible and so preventing fertilisation. The way in which the bud opens facilitates this with a tuft of stigmas protruding between the tips of the sepals prior to the sepals opening completely (see Section 3.2 for full details on flowering).

By preventing fertilisation of half of the ovaries only half the drupelets developed normally, the remainder being hard and unswollen (plate 3.22). When these unfertilised ovaries were removed the receptacle underneath was clearly unswollen (plate 3.23). Hence the development (in terms of swelling) of the receptacle is dependent on the development of the drupelet above it.

Plate 3.22 Effect on drupelet development of stopping fertilisation by cutting the styles early in development.

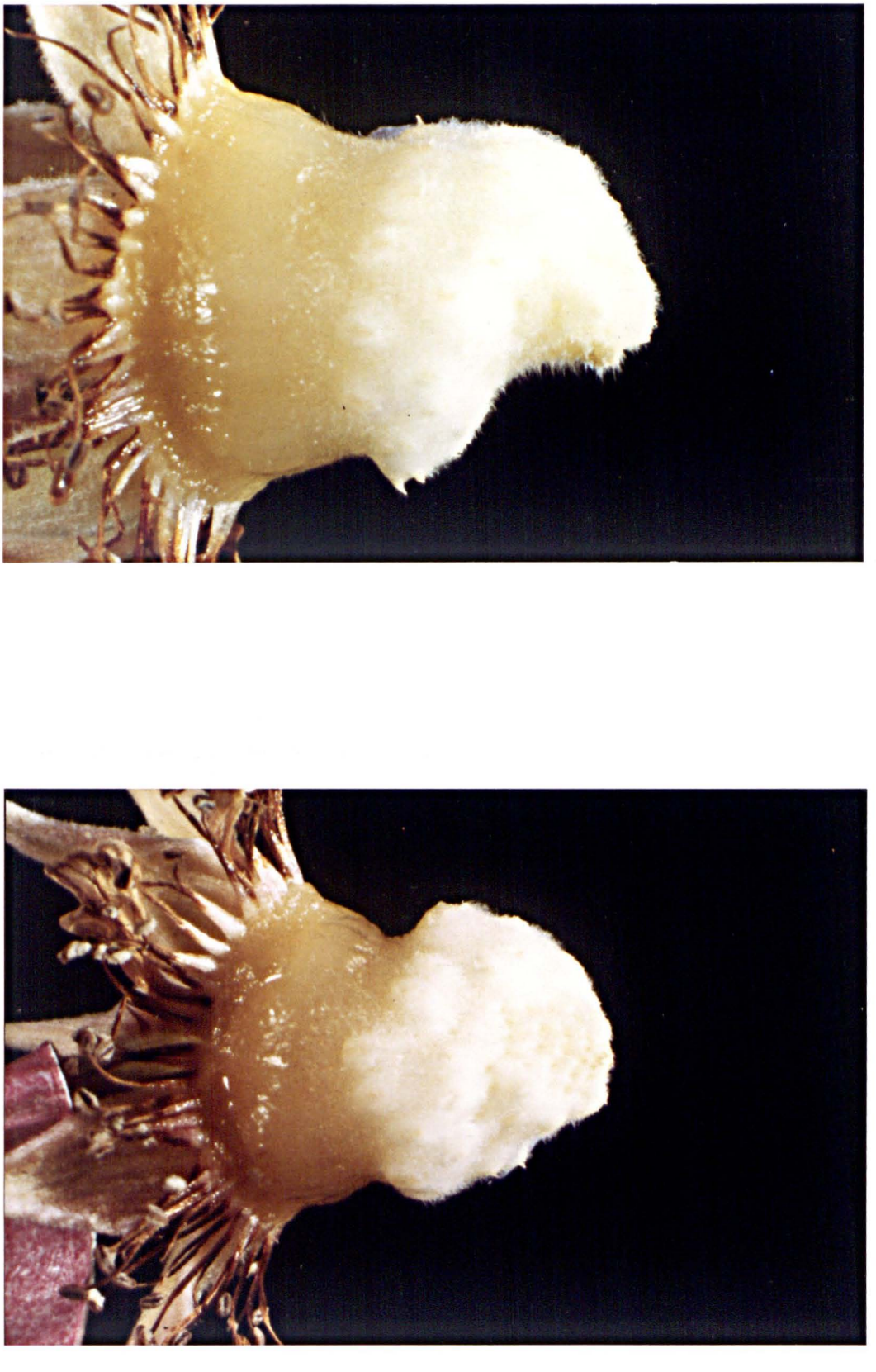


Plate 3.23 Effect on receptacle development of preventing drupelet development by cutting the styles early in development.

3.1.12 The control of ethylene production by the ethylene forming enzyme

The preceding results add weight to the hypothesis that ethylene could be involved in the control of abscission. There was a positive correlation between the onset of ethylene production and the commencement of FRS decline. Also, increasing the EPRs or the level of ethylene around immature fruit accelerated the FRS decline whilst a reduction in EPR or ethylene level around the fruit delayed this decline. However, no matter how far the EPR was reduced the decline in FRS could not be stopped totally.

If ethylene controls or accelerates abscission the next question which arises is what controls ethylene production? In other low EPR systems examined it has been shown that the supply of the ethylene precursor ACC is limiting (Yang et al., 1985). A supply of exogenous ACC is quickly reflected in elevated EPRs showing the ethylene forming enzyme (EFE) is present and non-limiting. Subsequently it has also been shown that the activity of ACC synthase and EFE both increase as natural EPRs increase (Yang et al., 1985). This results in an accumulation of ACC within the tissues coinciding with the increase in EPR. In this section the effect of additions of exogenous ACC will be examined on EPRs and FRS and the endogenous levels of ACC measured.

3.1.12 i) The effect of an exogenous supply of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid on ethylene production rates and fruit retention strengths of fruit during development

The ethylene forming enzyme converts ACC to ethylene and its

activity can therefore be assessed by supplying ACC. If the EFE is present and functional the result of ACC additions will be an increase in EPR, assuming the exogenous substrate reaches the enzyme. As with other chemical additions to raspberry fruit described earlier (Section 3.1.7) there are two methods of applying a test substance. The fruit can be dipped or sprayed thereby mainly treating the drupelets. Alternatively the ACC can be supplied by allowing the detached fruit to transpire the test liquid through the cut pedicel. The latter technique might be expected to deliver the ACC to the entire fruit including the receptacle, assuming vascular connections between the receptacle and drupelets to be intact. Both methods were examined and the results below show them to give similar effects.

Dipping fruit of different developmental stages in 1 mM ACC resulted in transitory increases in EPR for all categories peaking 6 to 8 hours after addition (fig. 3.42). These results clearly show raspberrries at all stages of development to have a greater capacity to produce ethylene from ACC than is expressed naturally. The actual rates achieved cannot be directly compared since the supply of ACC to the different categories of fruit cannot be assumed to be equal, however the extent of the increase is clearly reduced in the more mature fruit. The degree of increase in EPR could be dependent on the actual EPR of the fruit being examined, those having a low EPR being able to respond to exogenous ACC to a greater extent than fruit with an already high EPR. Hence the percentage increases occurring over the control fruit decrease from HG (4000%) through SG (3133%) and M (1037%) to R which only show a 45% increase. It can be seen however that the HG fruit if given sufficient ACC can

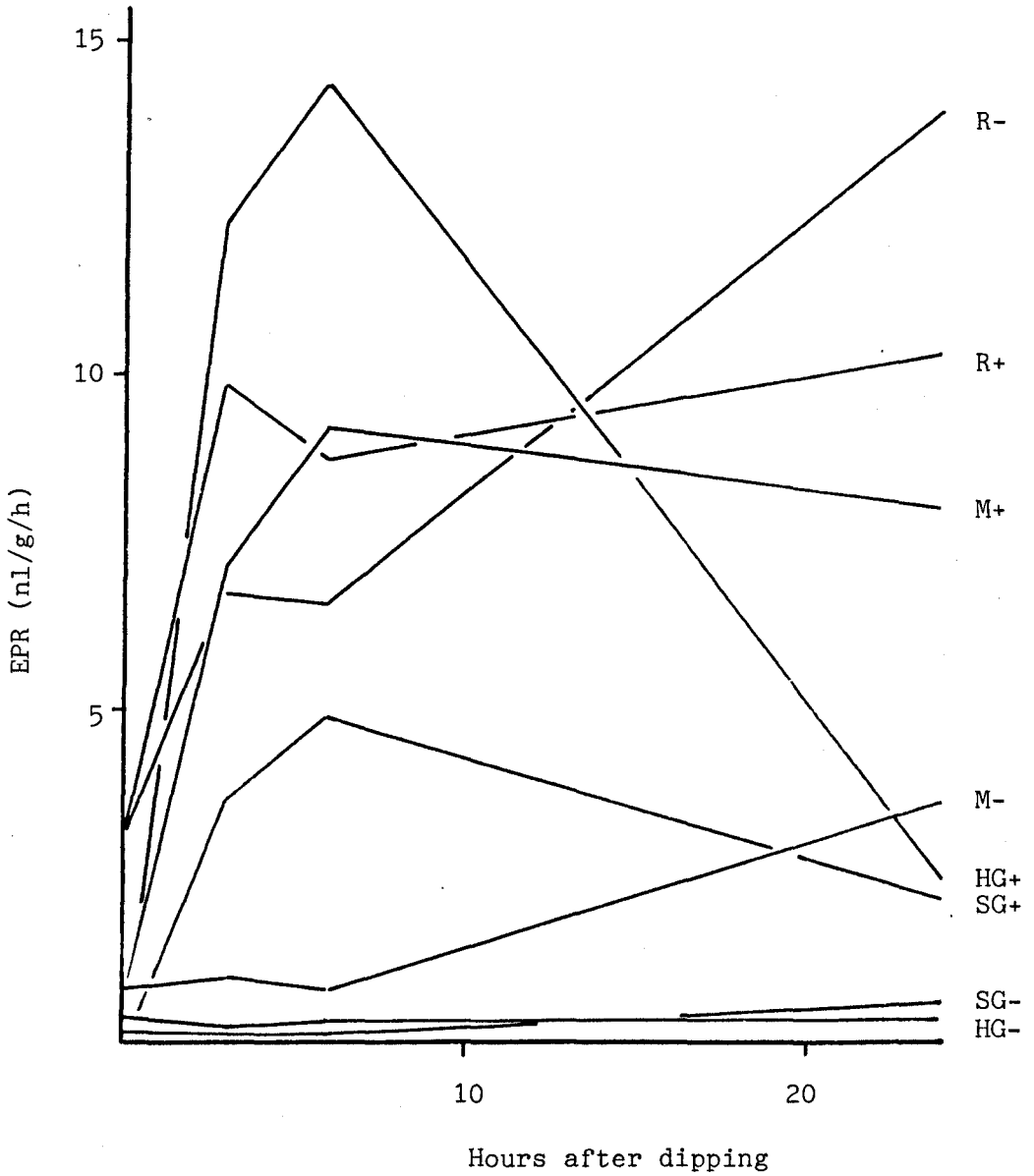


Fig. 3.42 EPR of fruit at different stages of development after dipping in 1 mM ACC (10 fruit/sample).

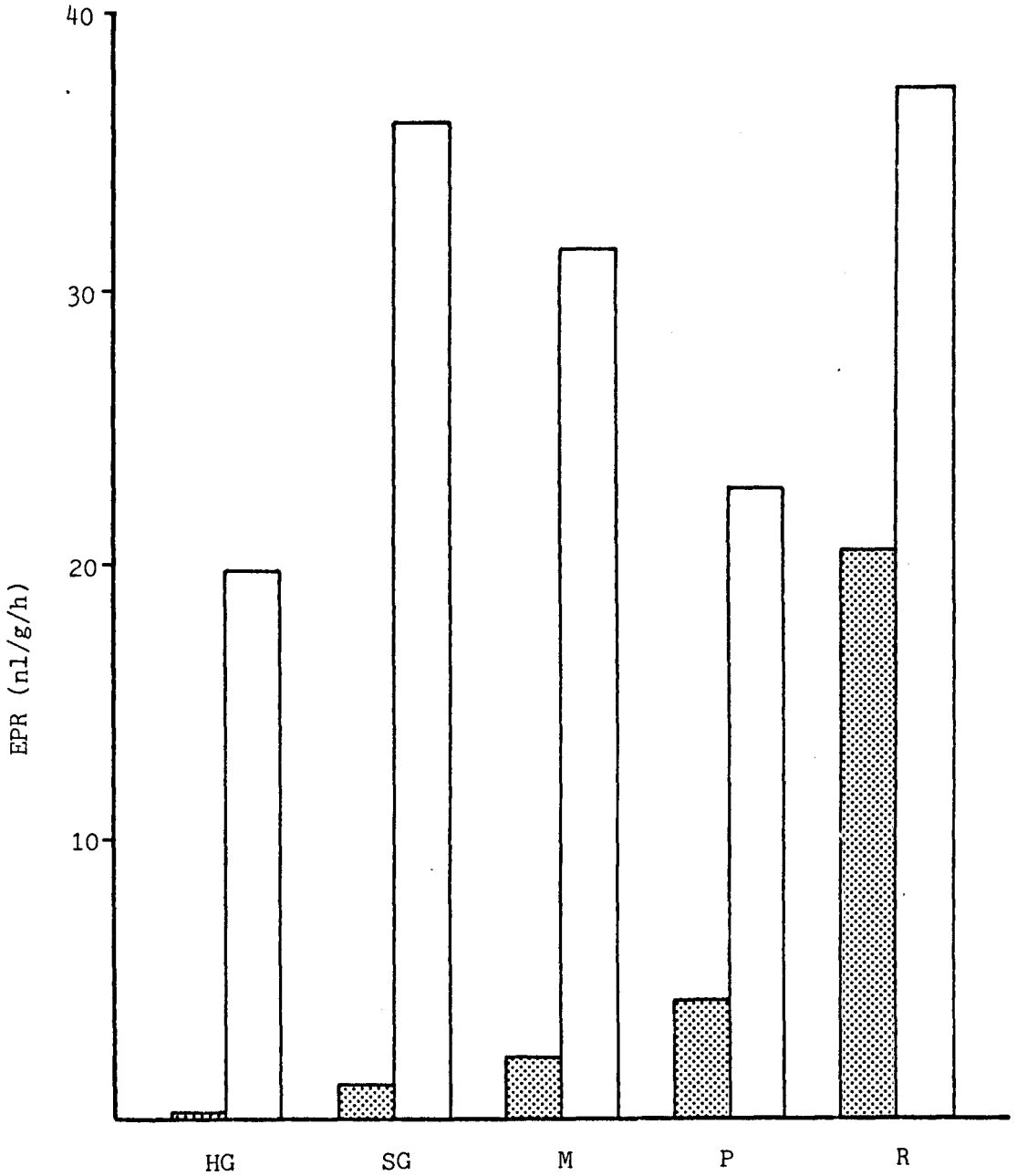


Fig. 3.43 Effect of transpiring 0.5 mM ACC for 24 hours on EPR of fruit at different stages of development (+ ACC □, + water ▨ ; 10 fruit/sample).

produce ethylene at rates equivalent to those found naturally in ripe fruit.

Allowing fruit at different maturities to transpire 0.5 mM ACC continuously also gave the same result of increased EPRs in all categories of fruit (fig. 3.43). The actual EPRs were much higher than those induced by dipping fruit (Fig. 3.42). This could be as a result of dipping providing a limited supply of ACC which on depletion allowed EPRs to return control levels. The disadvantage of using the transpiration stream is that fruit have to be left for a long period to take up the ACC and after 24 hours treatment enhanced EFE activity or even ACC synthase activity (Yang et al., 1985) could have occurred. These possibilities could account for the higher EPRs caused by ACC additions through the transpiration stream.

Table 3.19 Effect of transpiring 0.5 mM ACC on fruit retention strength of M, SG and HG fruit after 36 hours treatment (20 fruit/sample)

Developmental Stage	FRS + SE (N)	
	+ ACC	+ dH_2O
M	1.08 ± 0.07	1.26 ± 0.09
SG	3.08 ± 0.35	5.36 ± 0.50
HG	7.20 ± 0.10	7.35

Since the addition of ACC to immature fruit increased their EPRs it might be anticipated that it would also cause an enhanced FRS decline. Allowing fruit to transpire 0.5 mM ACC for 36 hours to maintain elevated EPRs clearly enhanced FRS decline (table 3.19). The ability to enhance FRS decline by adding ACC was most obvious in SG fruit, being almost totally lost by M stage fruit. This suggests that if it is ethylene which modulates the rate of FRS decline, the levels at M are approaching saturating such that additional ethylene has little effect. This is precisely what was suggested by earlier results (Section 3.1.7).

3.1.12 ii) Levels of 1-aminocyclopropane-1-carboxylic acid in fruit during development

Having demonstrated that a supply of exogenous ACC would stimulate ethylene production and abscission, the natural levels of this ethylene precursor were measured in fruit at different stages of development. Since Lizada and Yang (1979) reported a method of assay for ACC several modifications to the extraction procedure have arisen. The various techniques were assessed for efficiency with regard to recovery and ease of use (details in Section 2.19), extractions finally being carried out in cold 80% ethanol and PVP (giving 62% recovery). The levels of both receptacle apices and drupelets were measured, initally on bulk samples and ultimately on individual fruit.

The results in figs. 3.44 and 3.45 show both drupelets and receptacle apices to contain vast amounts of ACC when PR compared to at other stages of development. In the drupelets the levels increase gradually from SG to R and then massively to PR, the HG

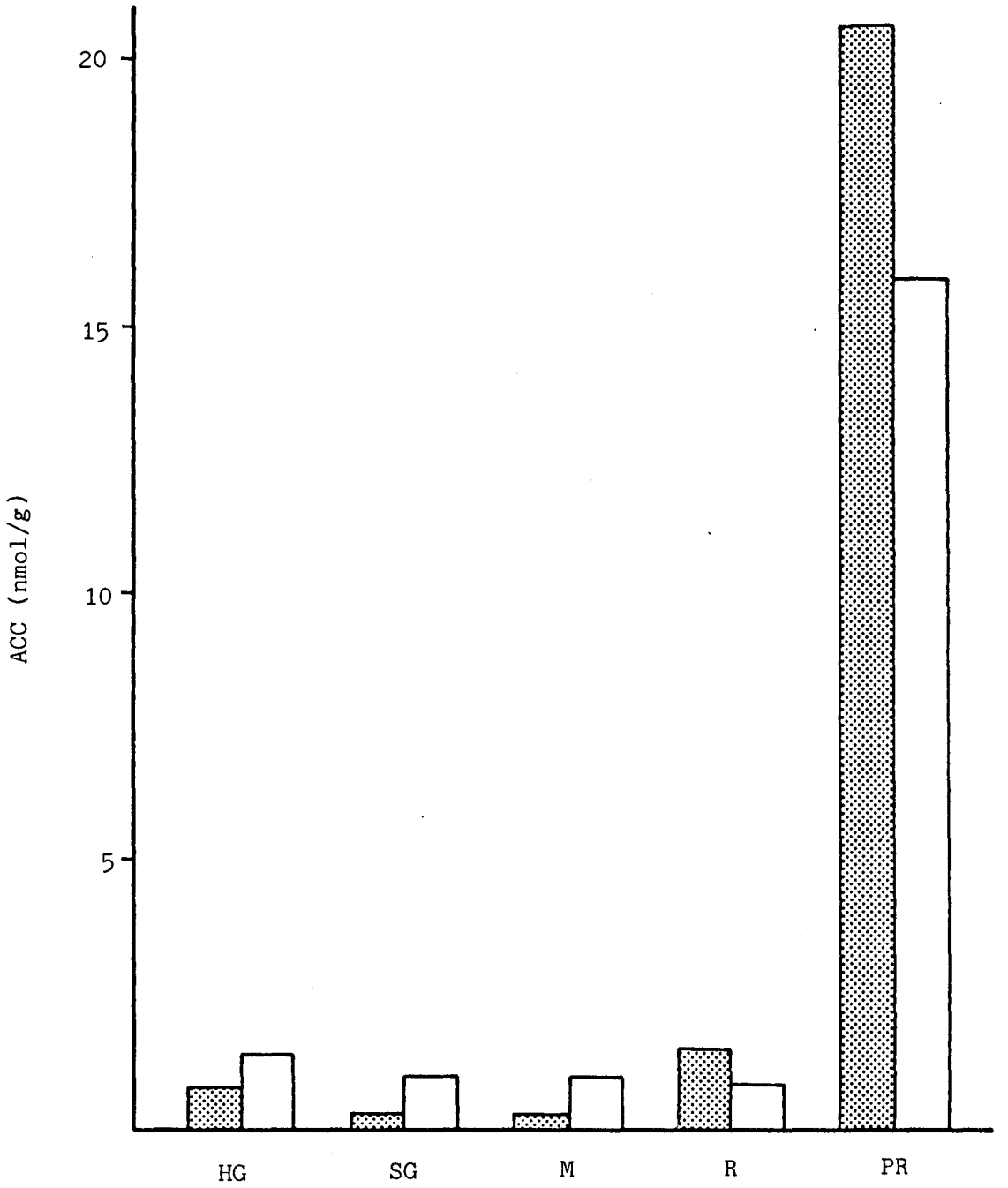


Fig. 3.44 Change in ACC content of drupelets (▩) and receptacle apex (❑) during development (20 fruit/sample).

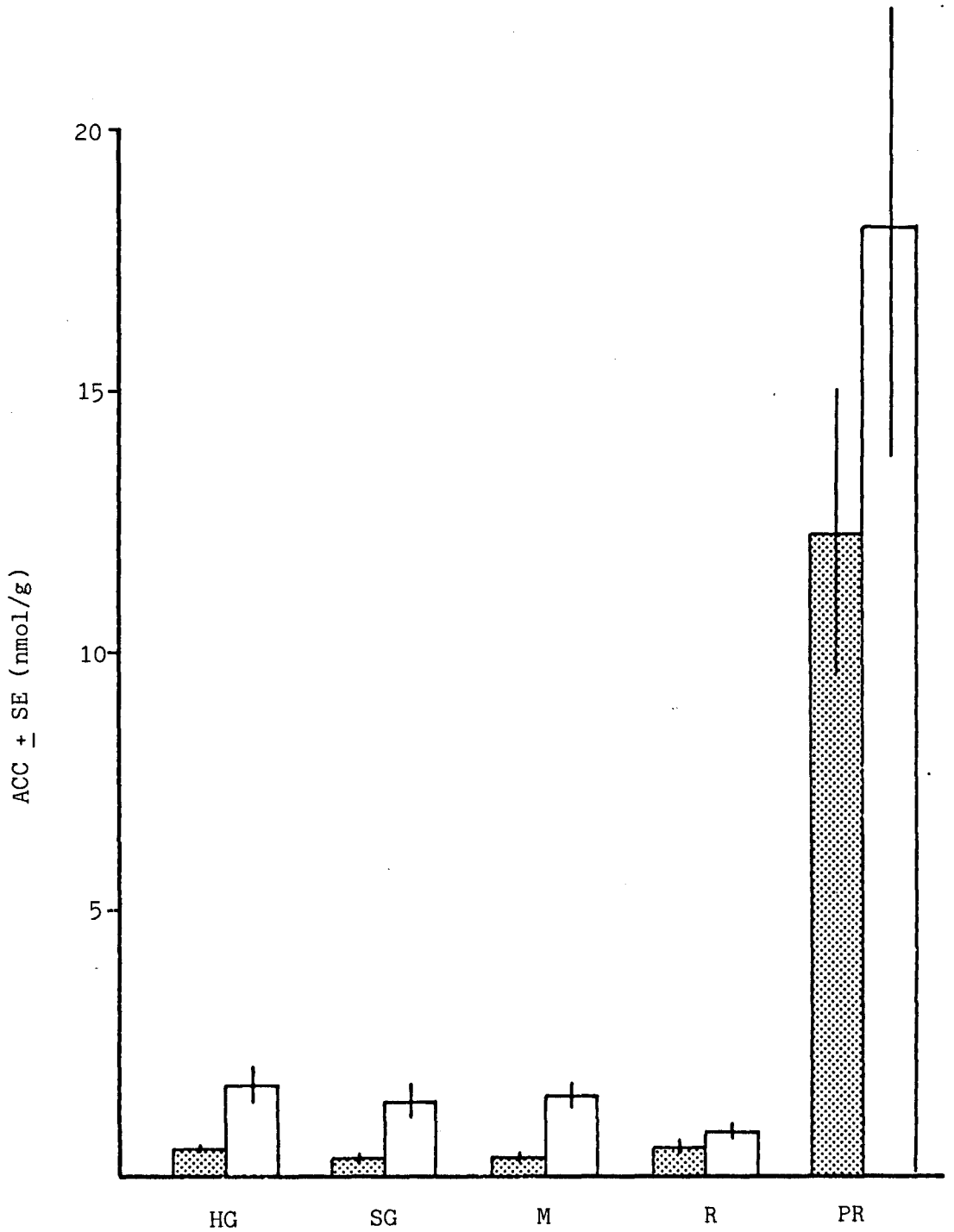


Fig. 3.45 Change in ACC content of drupelets (▨) and receptacle apex (□) of individual fruit during development. (5 fruit/ sample).

fruit having slightly higher levels than the SG fruit. In the receptacle apex the levels decreased slightly from HG to R, then increased dramatically at PR. The levels in the receptacle exceeded those in the drupelets between HG and M, the situation when R being less clear. The bulk sample (fig. 3.44) showed a higher drupelet level than was found examining individual fruit (fig. 3.45). This can be reconciled with the large and rapid changes which occur between R and PR. The presence of a few fruit in the R category which were almost PR could easily have increased the overall average. Hence the examination of individual fruit which is a more reliable method of following changes in ACC levels.

Previously increases in both ACC levels and EPRs have been correlated (Hoffman and Yang, 1980). This is also the case here

Table 3.20 Total level of ACC in fruit at different stages of development (20 fruit/sample)

Developmental Stage	ACC Level (nmol)		
	Whole	Drupelet	Receptacle
PR	53.579	51.979	1.600
R	9.708	9.477	0.231
M	1.038	0.940	0.098
SG	0.470	0.411	0.059
HG	0.681	0.613	0.068

where the drupelet ACC content increases in parallel with the EPR. In the receptacle this relationship was not apparent. This may be a result of an increased ACC usage compared to production, thereby reducing the pool size of ACC being measured whilst giving increased EPRs. The changes in fruit size which occur between stages also complicate the issue. Table 3.20 presents the total amount of ACC in the fruit parts at different stages of development. In this case the drupelets and receptacle apex (and hence whole fruit) show the same trend of changes in ACC level, increasing from SG to PR with a HG level just above that in SG. These are a similar pattern of changes as was found in the EPRs of fruit at these stages of development.

3.1.12 iii) Levels of 1-(malonylamino)-cyclopropane-1-carboxylic acid levels in fruit during development

Intially 1-(malonylamino)-cyclopropane-1-carboxylic acid (MACC) was regarded as a biologically inactive conjugate of ACC, capable of controlling EPR through the ACC pool size (Yang and Hoffman, 1984). More recently however Jiao et al. (1986) have reported MACC at high levels to be capable to some extent of inducing the conversion of MACC to ACC. Hence MACC may prove to play a larger role in ethylene metabolism than was originally considered.

Generally MACC is assayed from the same extract as used in the ACC assay, the extract being hydrolysed to convert MACC to ACC. The ACC levels before and after hydrolysis can be compared to determine the level of MACC (see Section 2.20).

The results shown in fig. 3.46 (including a repeat run)

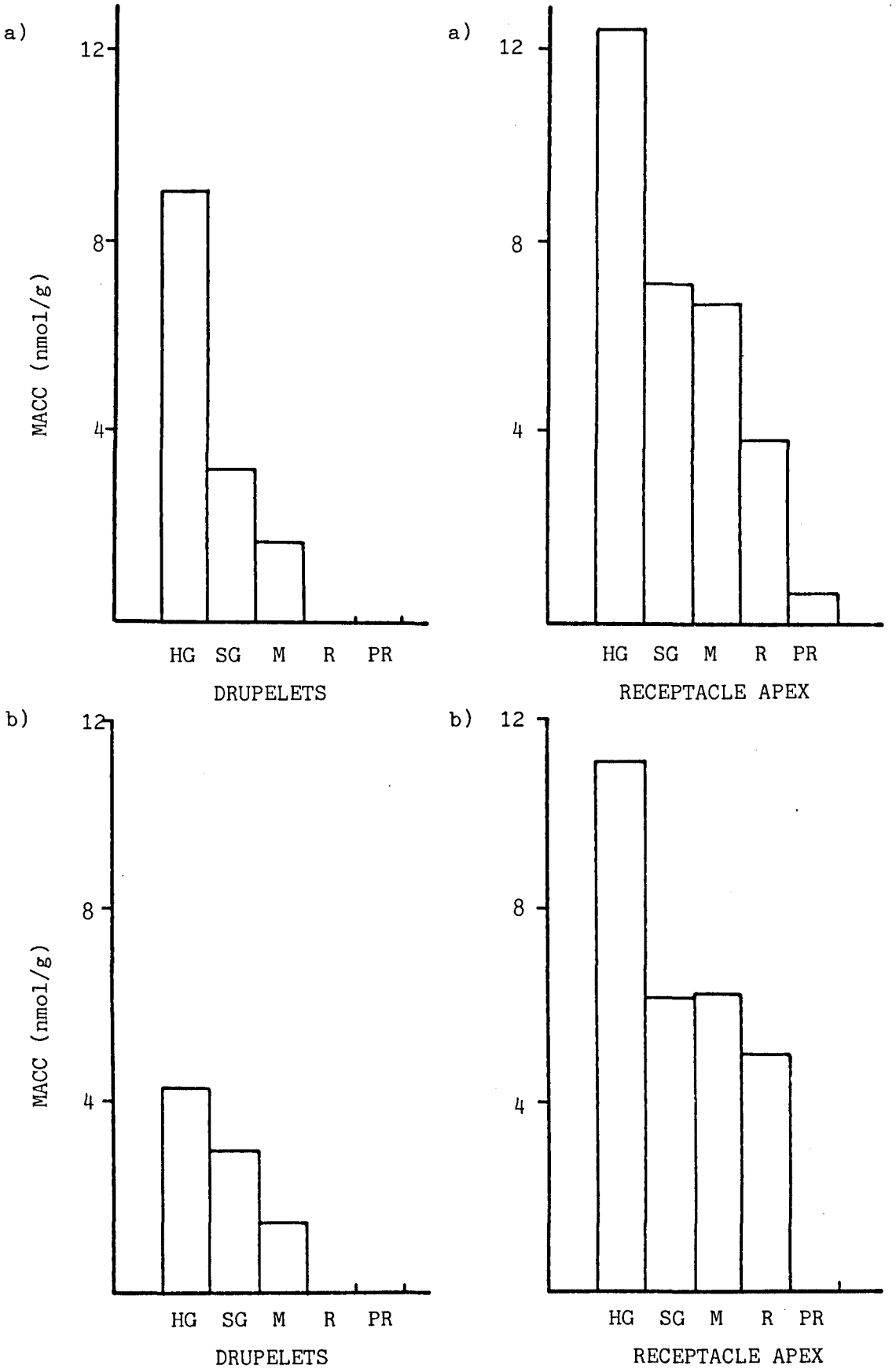


Fig. 3.46 Change in MACC content of drupelets and receptacle apex during development (2 runs; 20 fruit/sample).

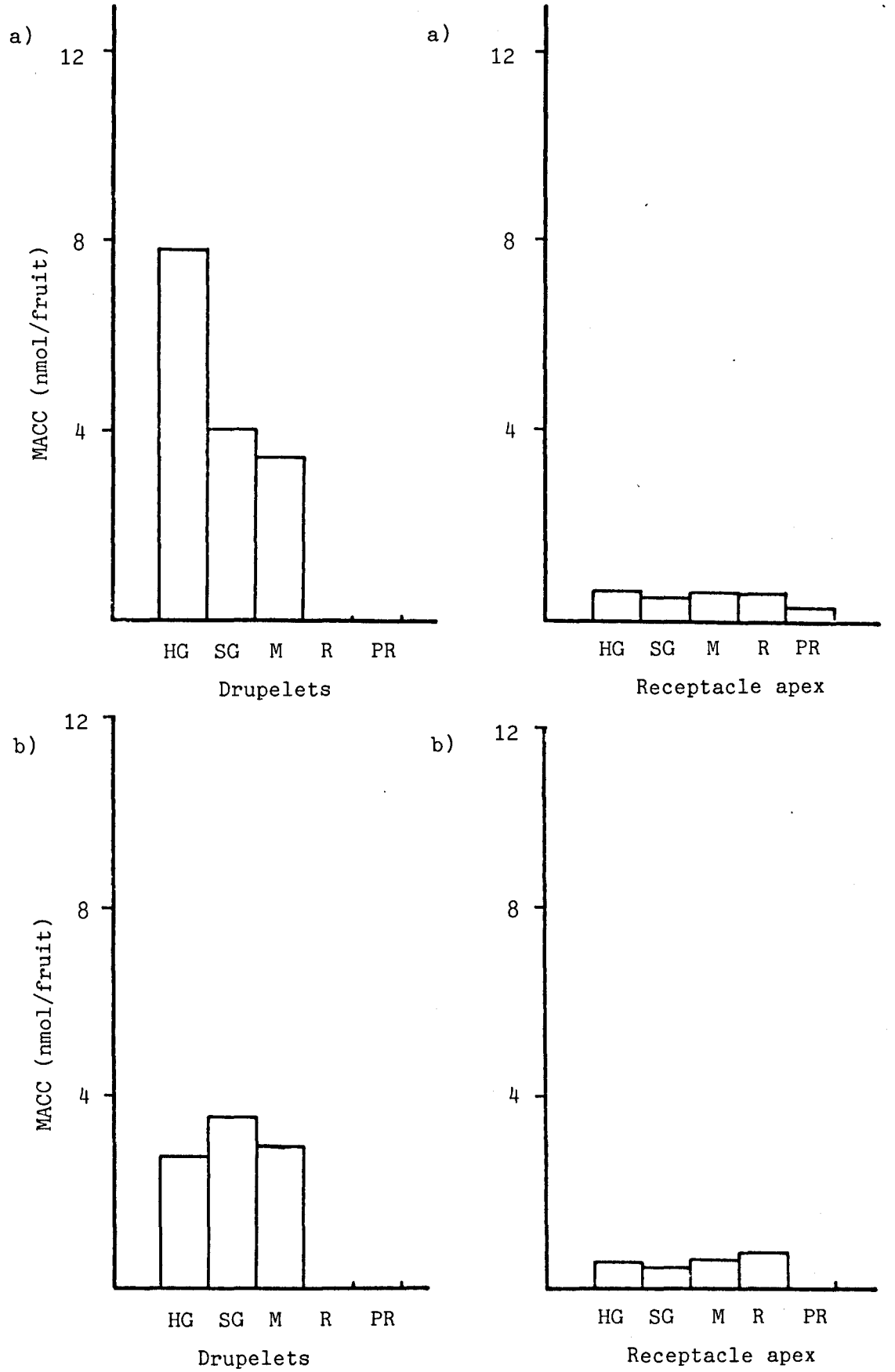


Fig. 3.47 Change in MACC content of drupelets and receptacle apex during development (2 runs; 20 fruit/sample).

indicate that the MACC content of both drupelet and receptacle apex decrease during development. Expressing the data on a per drupelet cap or receptacle apex basis instead of a per gram showed the absolute amount of MACC to remain constant as the apices developed to the mottled stage, there being a slight decrease in the drupelets (fig. 3.47). This however does not account for the situation in R and PR drupelets. The actual values measured were not just zero but negative indicating that the ACC in the sample was being degraded during hydrolysis of the R and PR drupelet extract. This possibility was examined further by the addition of known amounts of ACC to ripe drupelet extracts prior to hydrolysis. Measurement of the ACC post hydrolysis revealed recovery rates sometimes as low as 16%. A lack of reagents in the ACC assay was easily shown not to be the cause. The recovery from similar treatments in immature fruit and receptacles was in the region of 60%. Hence it appears that ACC was being degraded or chemically altered in some way during the hydrolysis procedure in the presence of the R or PR drupelet extract. It is also noticeable however that the PR receptacle apices are also low or lacking in MACC. It is therefore possible that some MACC is converted chemically as development progresses, especially in the light of the recent work of Jiao et al. (1986) referred to earlier. Further examination of MACC metabolism was curtailed as a result of lack of time.

3.1.13 The control of ethylene production by 1-aminocyclopropane-1-carboxylate synthase

Intially attempts were made to extract this enzyme from ripe tomatoes according to the method of Yu et al., (1979). This work was undertaken to ensure that the extraction and assay procedure was being followed correctly. Having successfully extracted ACC synthase from tomato with comparable levels of activity to other workers similar methods were used to extract and quantify ACC synthase in raspberries. Full details have been presented in Section 2.21.

Initially ripe drupelets were examined for ACC synthase activity but none could be detected. It was considered that the drupelet with its accumulation of sugars, acids and pigments could have interfered with enzyme extraction and made the detection of any interfering substances difficult. Hence ripe raspberry receptacle apices, which have high EPRs, were also examined. Having detected ACC synthase activity, albeit at a low level, the work was repeated on the apices of green, mottled and ripe fruit. HG and SG fruit were combined to provide a large enough sample size for extraction (over 100 apices per sample). The results in table 3.21 indicate the overall activity of ACC synthase in the receptacle apex increased with development. On a per gram basis this is not so evident. It would appear that the apex increased its weight faster than its ACC forming capacity.

In an attempt to determine whether the apparently low activities found in the receptacle apices were as a result of some inhibitory factor in the extracts a mixture of ripe raspberry apices and ripe tomato pericarp was extracted and assayed. The activity of

the mixed extract was then compared to the two individually to see whether the raspberry extract would inhibit the tomato enzyme.

Table 3.21 Level of ACC synthase activity in the receptacle apex during development (over 100 apices/sample)

Developmental Stage	ACC synthase activity pmol ACC/h	
	per apex	per gram
Ripe	4.09	18.8
Mottled	2.66	26.6
Green	1.10	14.1

Surprisingly the presence of the raspberry apices during the extraction did not inhibit the tomato enzyme, rather it appeared to increase the total activity by 45%. Full details are given in Section 2.21. It is also noticeable that the ripe raspberry apex activity was higher at 76 pmol ACC/g/h than the result presented in table 3.21 (18.8 pmol ACC/g/h). This discrepancy could not be accounted for.

In another series of extractions no ACC synthase activity could be found in flowers at times of high EPRs (during petal opening and abscission). Unfortunately the raspberry season of 1986 finished before the question of why there was no activity in flowers or ripe drupelets could be resolved. Fruit stored for further work,

both freeze dried and frozen in liquid nitrogen, were subsequently found to retain little activity.

3.1.14 Blackberry and blackberry/raspberry hybrid investigations

A preliminary examination of an unknown blackberry variety found growing wild at Stirling University indicated blackberry to be non-climacteric in terms of developmental changes in EPR. The result was unexpected as raspberry had been shown to produce ethylene climacterically and a recent paper by Walsh et al. (1983) clearly stated blackberry to be climacteric. To clarify the position arrangements were made in 1986 to examine several known blackberry varieties (Ashton Cross, Bedford Giant, Chehalem and Oregon Thornless), courtesy of the Scottish Crop Research Institute at Dundee. Access was also provided to samples of the blackberry/raspberry hydrids Tayberry, Marionberry, Tummelberry and Sunberry, as well as black and purple raspberries. These were all examined for ethylene production rates throughout development.

3.1.14 i) Developmental stages in blackberry

As in raspberry it was necessary to select easily definable stages of physiological development for subsequent examination of FRS and EPR. The stages chosen were small green (SG), large green (LG), brown (BR) and black (BL), as illustrated in plate 3.24. The anthocyanin levels at the different developmental stages of Ashton Cross measured according to Deubert (1978) are shown in table 3.22. In some varieties such as Chehalem the fruit go through a red rather than brown stage prior to turning black.

3.1.14 ii) Abscission in blackberry

Blackberry and raspberry fruit are structurally very similar, having individual drupelets attached to a central

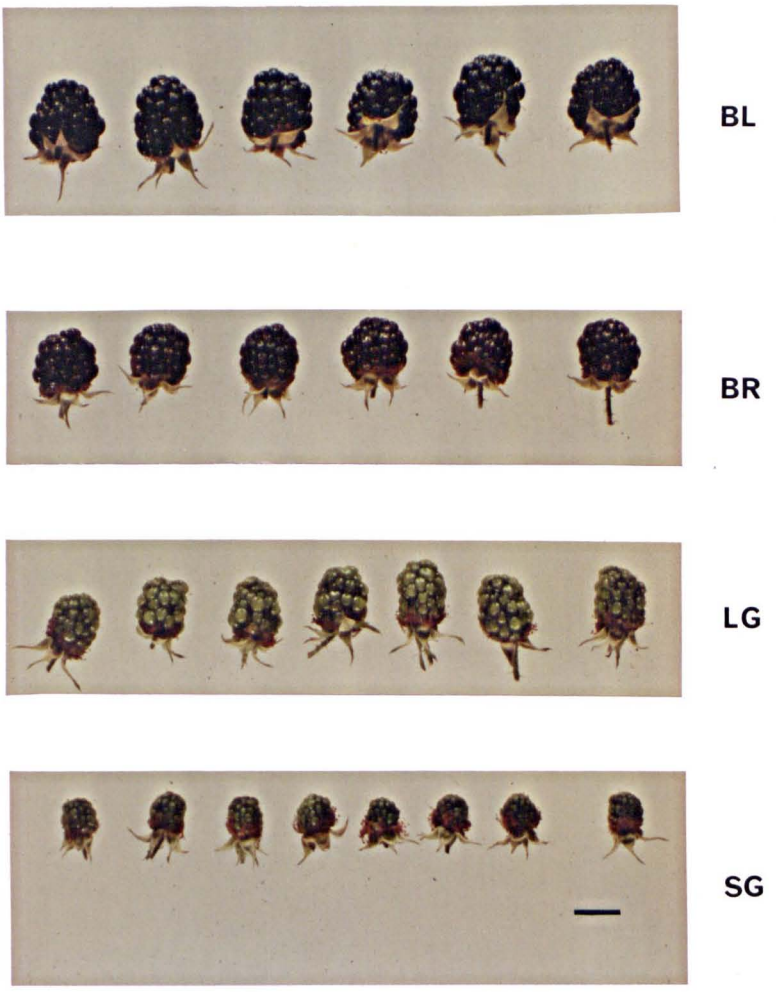


Plate 3.24 Developmental stages of Ashton Cross blackberry fruit selected for future examination. Categories are: SG, small green; LG, large green; BR, brown; BL, black. Bar = 1 cm.

receptacle, the fruit

Table 3.22 Anthocyanin levels of Ashton Cross fruit at different stages of development (15 fruit/sample)

Developmental stage	Anthocyanin level (μg/g)
SG	24
LG	24
BR	187
BL	844

abscission however differs greatly. In raspberry each individual drupelet abscises from the receptacle whilst in blackberry the drupelets remain attached, the whole fruit being shed complete with the central torus. The blackberry abscission zone divides the receptacle into what has been termed the receptacle apex and calyx regions in the earlier raspberry experiments (see fig, 1.2). Hence when measuring the FRS in blackberry it represents a single large abscission zone rather than numerous individual zones at each drupelet as in raspberry. Examination of this large absission zone using a scanning electron microscope revealed that cortical cells at the fracture surface to be whole and rounded whilst the vascular trace had been ruptured (plates 3.25 and 3.26). These features are considered characteristic of true abscission systems (Sexton and

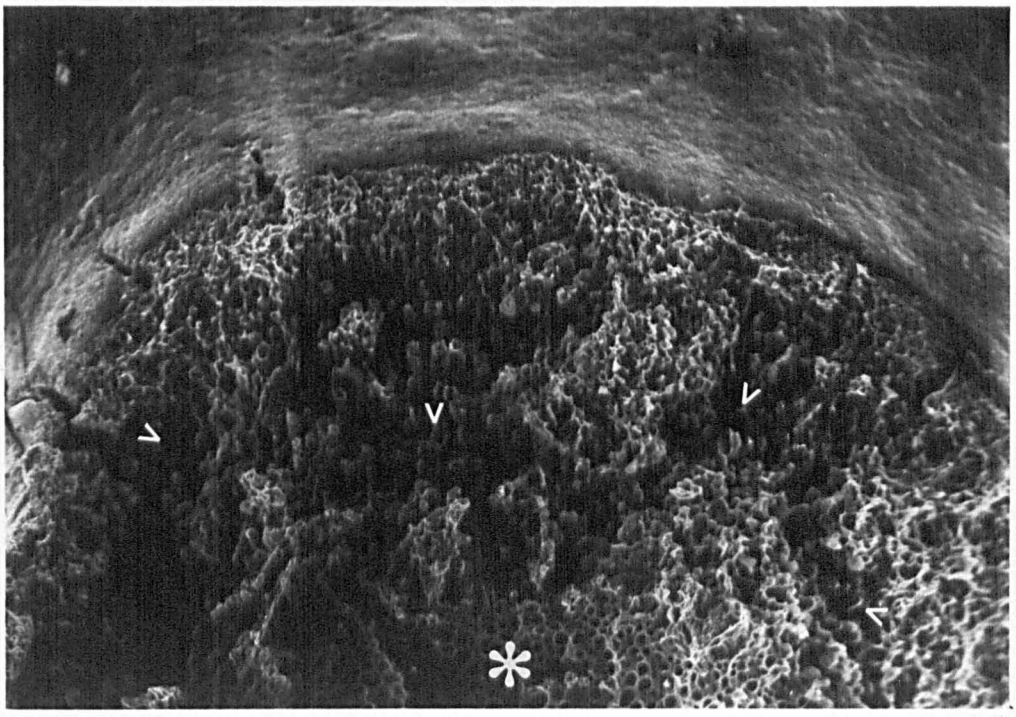


Plate 3.25 SEM of calyx side of a separated Ashton Cross abscission zone from a ripe fruit. Rounded cortical cells (>) and a fractured vascular trace (*) are clearly visible. Bar = 100 μm.

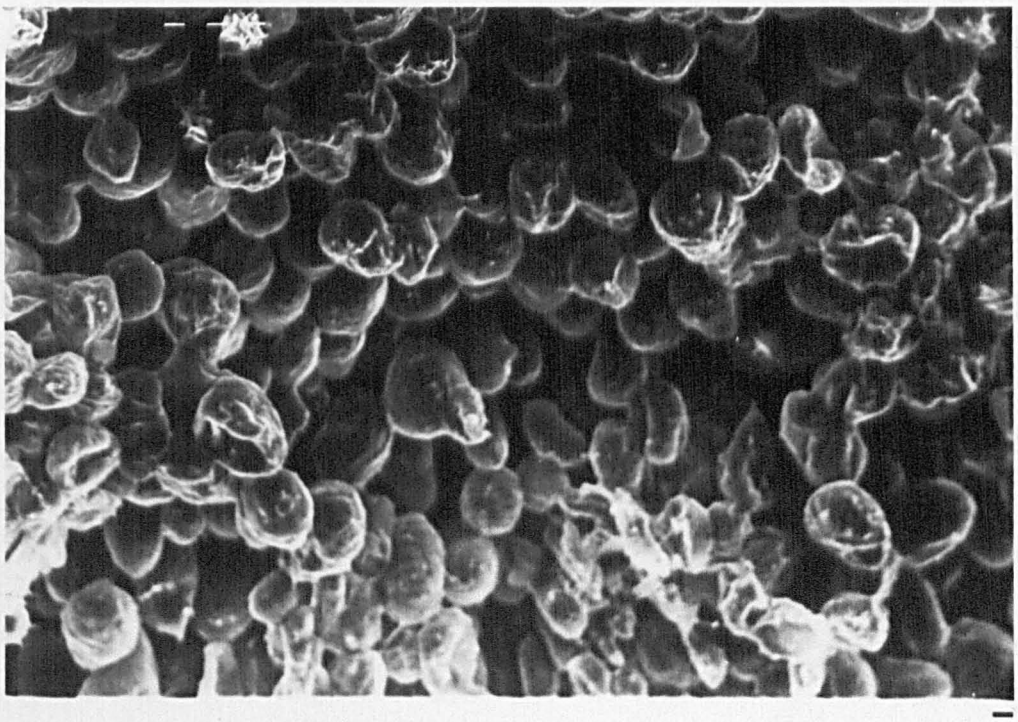


Plate 3.26 Close up SEM of rounded cortical cells on the calyx side of a separated abscission zone of a ripe Ashton Cross blackberry. Bar = 10 μm.

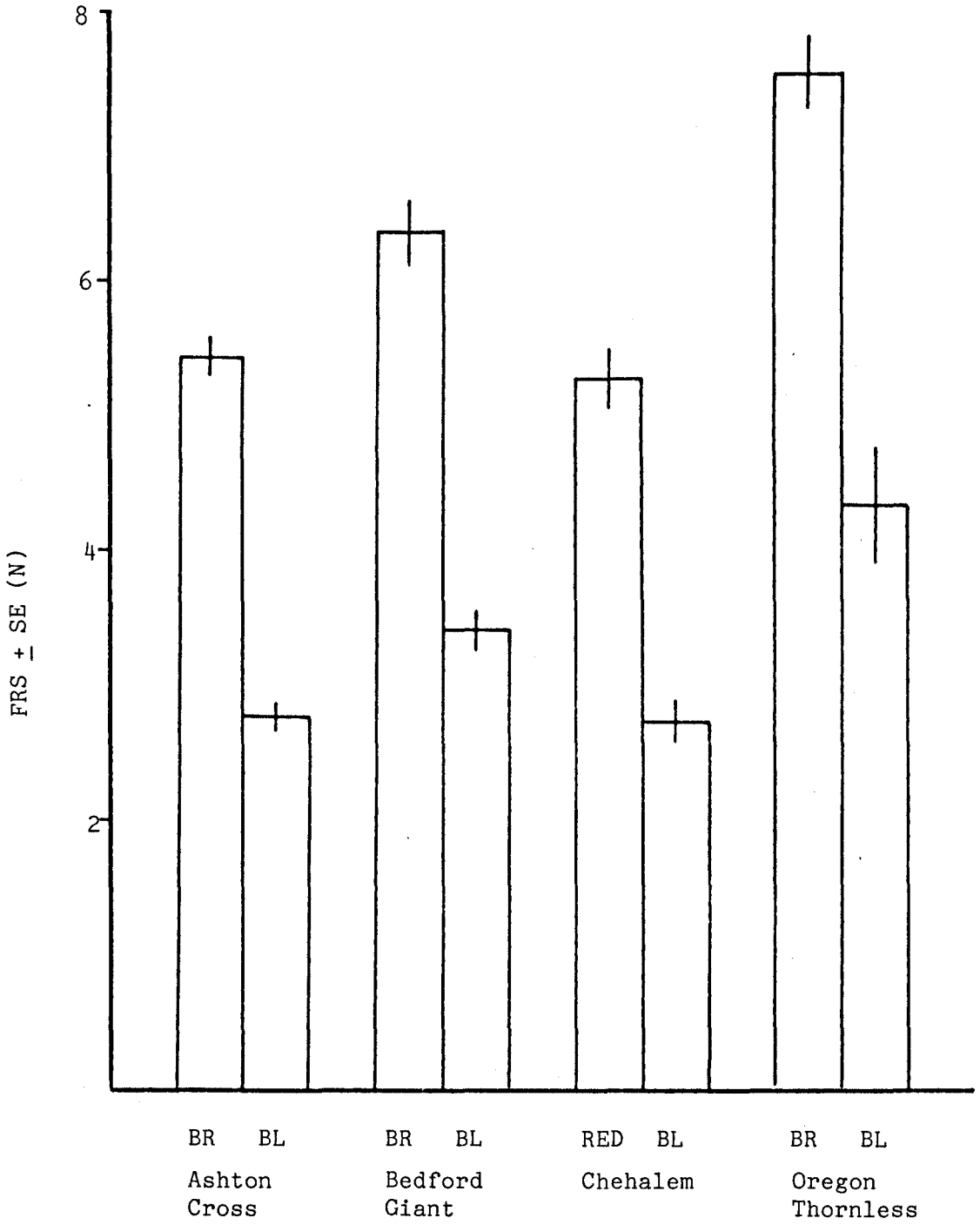


Fig. 3.48 Fruit retention strengths of 4 blackberry varieties at the brown (BR) and black (BL) stages of development. Chehalem goes through a red rather than brown stage. (20-30 fruit/sample).

Roberts, 1982).

The fruit retention strengths of a range of blackberry varieties were determined in the same way as for raspberries (Section 2.5). In all varieties there was no measurable weakening of the abscission zone whilst green, the values for brown and black fruit being shown in fig. 3.48. As would have been expected FRS values declined as development progressed from LG through to BR to BL. These values cannot be directly compared to those of raspberries because of the structural differences between the two systems. The force required to remove ripe black fruit from the pedicel is much greater than that required for ripe raspberry detachment, being in excess of 2.5 N. This contrasts with the situation in some raspberries where the weight of PR fruit is sufficient to cause separation. In Bedford Giant a difference in black ripe fruit was apparent where the drupelets pass through a shiny black stage and then turn a dull black (DBL). At this dull black stage the FRS has declined still further (from 3.43 N when shiny to 2.39 N when dull).

3.1.14 iii) Ethylene production in blackberries

If the blackberry is a climacteric fruit as reported by Walsh et al. (1983), then an EPR profile similar to raspberry would be expected with initially low levels of production in green fruit increasing dramatically during ripening.

The EPRs of the 4 varieties of blackberry were measured throughout development in the same way as for raspberries (Section 2.6). The results for all varieties (fig. 3.49) showed a decrease in EPR from SG to LG, however thereafter the patterns showed

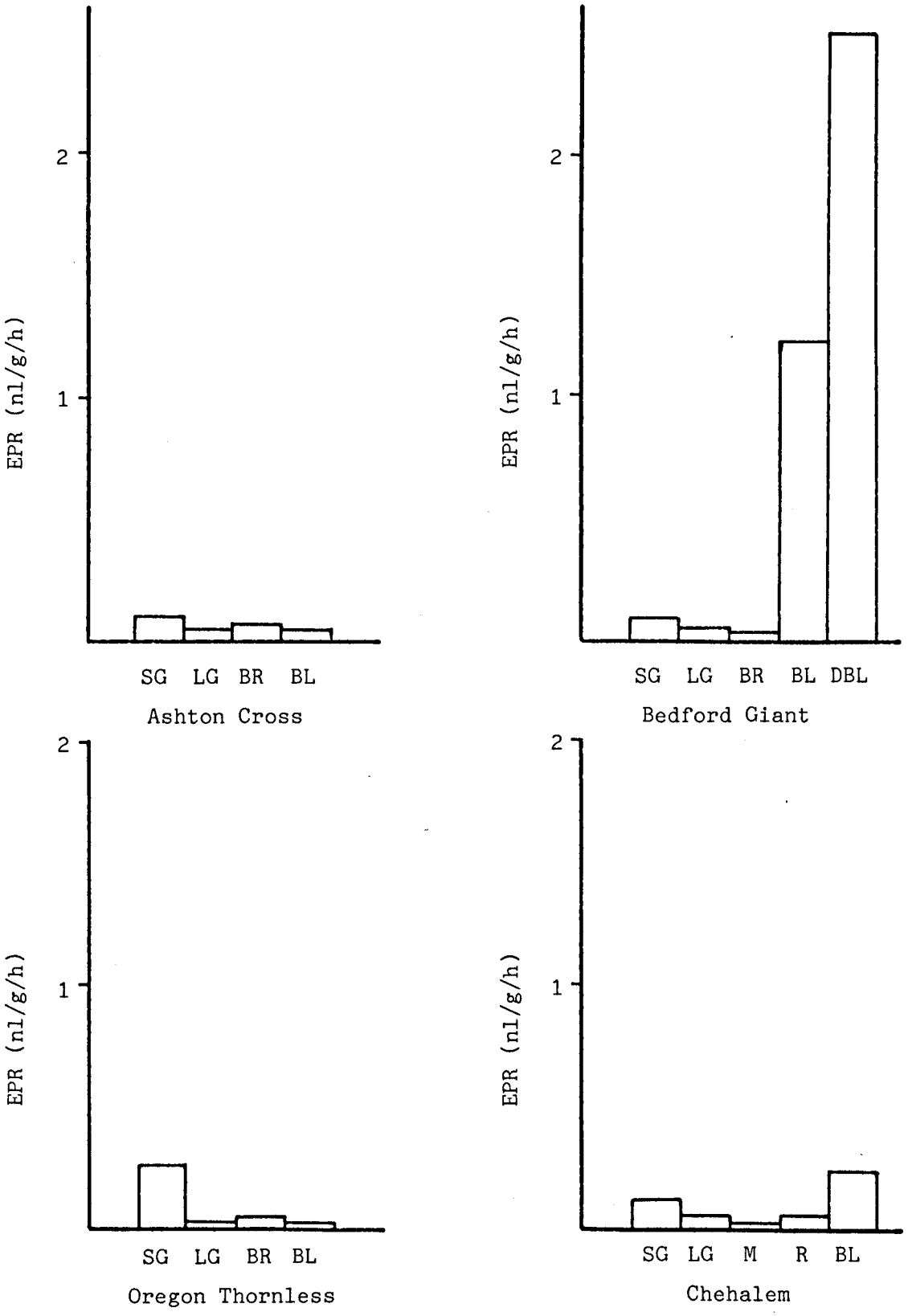


Fig. 3.49 Change in ethylene production rates of 4 blackberry varieties during development. Classification: SG - small green; LG - large green; M - mottled; BR - brown; R - red; BL - black; DBL - dull black. (20 fruit/sample).

differences. In Ashton Cross and Oregon Thornless the low EPR (< 0.1 nl/g/h) in green fruit was maintained throughout the rest of development. In Bedford Giant there was a climacteric type profile with the EPR increasing 30 fold between brown and black with a further doubling of the rate when dull black. A different set of criteria were adopted with Chehalem since it goes though a mottled and red stage similar to raspberry. The decrease in EPR of Chehalem extended from SG through to LG to M, there being a slight increase at R and a 4 fold increase at BL.

Hence in blackberry some appear non-climacteric (Ashton Cross, Oregon Thornless) whereas others show a climacteric rise in ethylene production late in ripening (Bedford Giant, Chehalem). Even when EPR increases do occur in the course of development the decline in FRS preceeds the increased rates thereby making an inductive role in abscission unlikely. Likewise the changes in pigmentation also preceed the rise in EPR. The question of a role for ethylene is even more obscure in Ashton Cross and Oregon Thornless where abscission and pigment changes occur in the total absence of increased EPRs.

3.1.14 iv) Can ethylene influence abscission in blackberry?

Using Ashton Cross as an example of a blackberry showing a non-climacteric EPR profile, the possibility of ethylene influencing abscission in a system which did not naturally produce large amounts was examined. The results shown in fig. 3.50 indicate that supplying 40 ppm ethylene for 48 hours caused an acceleration in the reduction in FRS. The acceleration was obvious at all stages of development, still being quite considerable when BR and BL. This

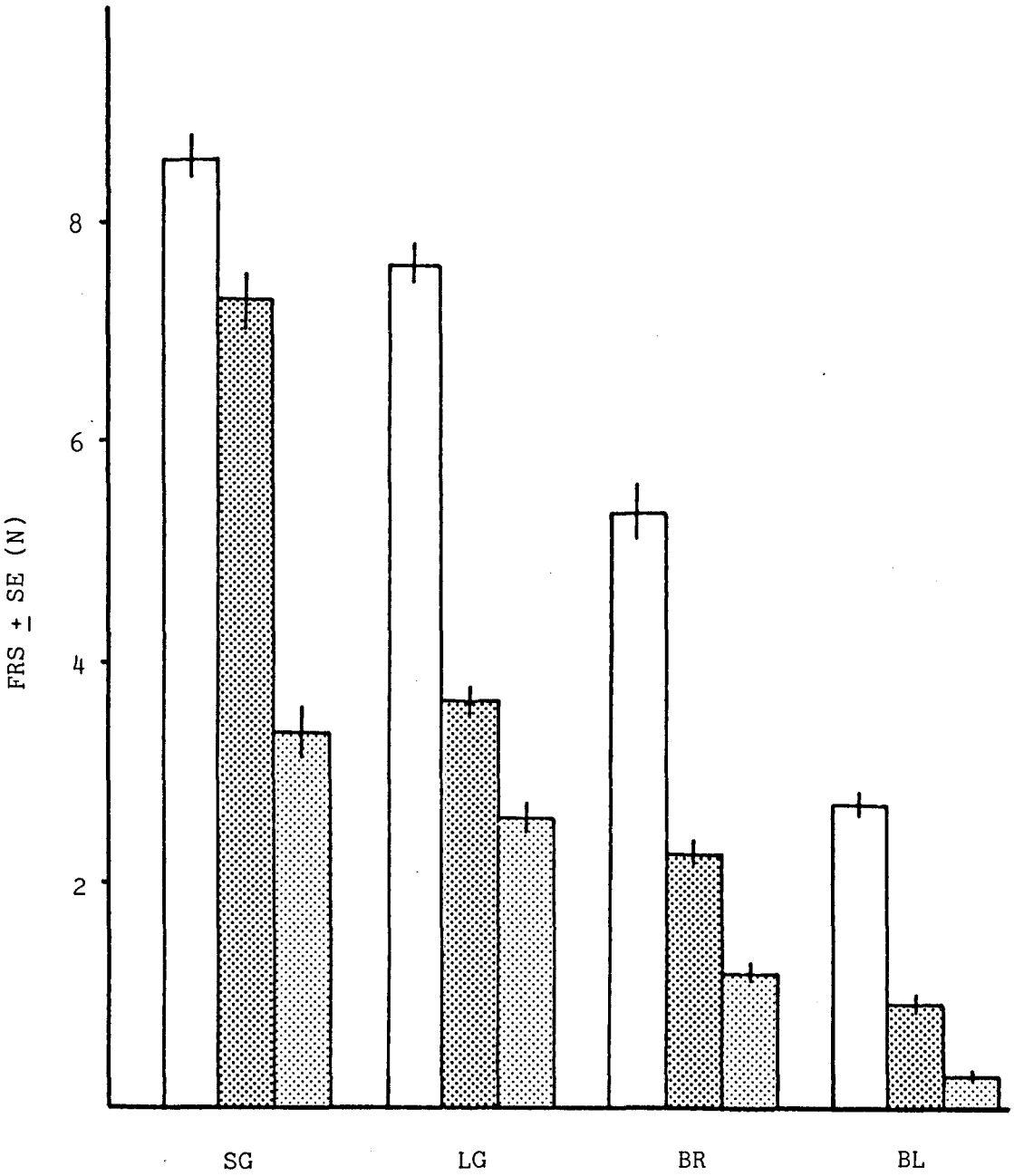


Fig. 3.50 Effect of 48 hours of 40 ppm ethylene on fruit retention strength of Ashton Cross blackberries at different stages of development. Initial FRS at harvest (□), 48 h in air (▩), 48 h in 40 ppm ethylene (▩). 20-30 fruit/sample.

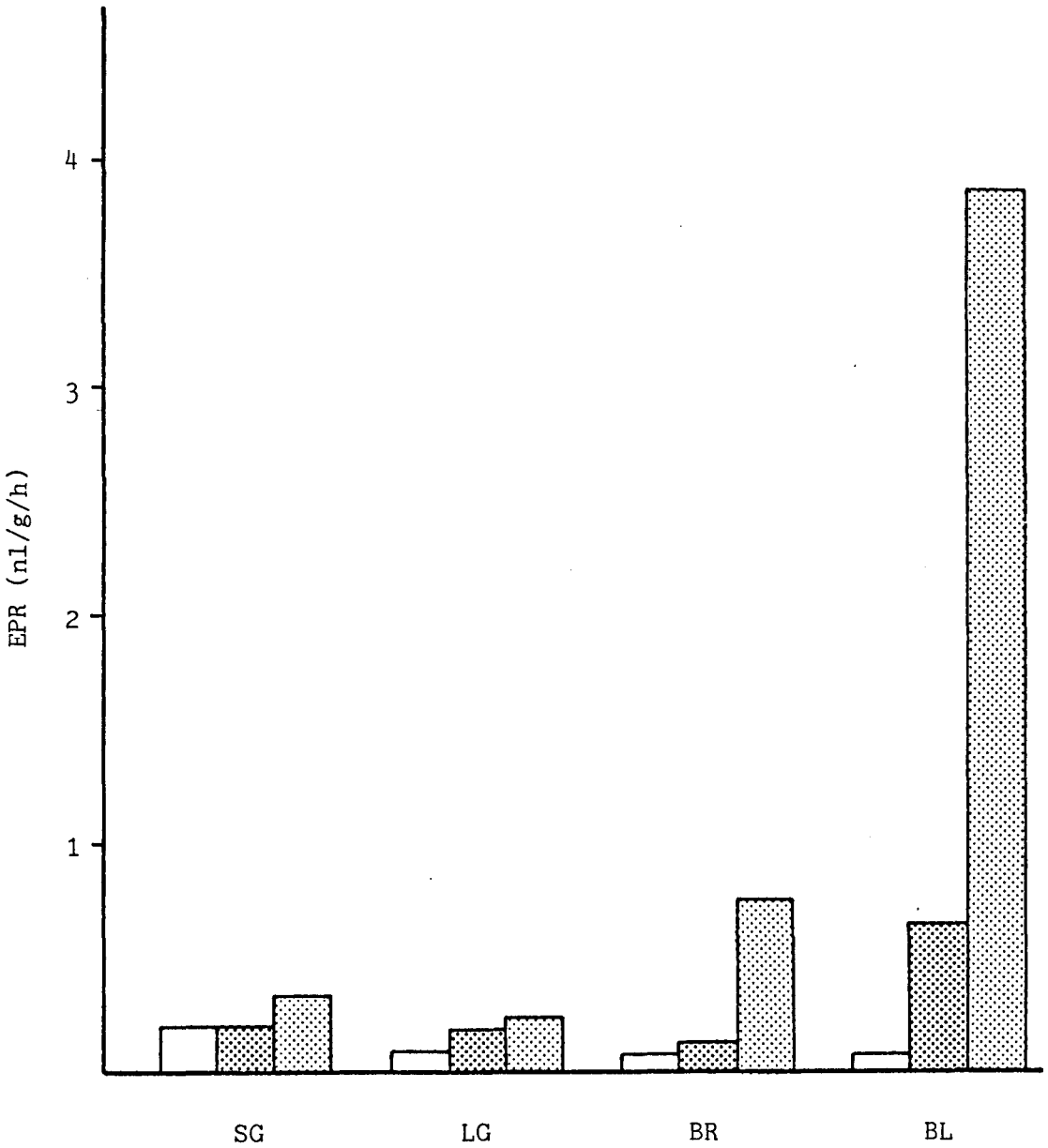


Fig. 3.51 Effect of 48 hours of 40 ppm ethylene on the ethylene production rates of Ashton Cross blackberries at different stages of development. Initial EPR at harvest (□), 48 h in air (▨), 48 h in 40 ppm ethylene (▨). 20-30 fruit/ sample.

capacity to influence the FRS of the more mature fruits differs from the situation in raspberries where the ability to accelerate FRS decline is reduced as development progresses. This is possibly because the endogenously produced ethylene in raspberry is saturating during the latter stages of ripening while in blackberry low levels of endogenous production even in ripe fruit allows the exogenous ethylene to assert its influence.

In addition to accelerating FRS decline the supply of 40 ppm ethylene for 48 hours also enhanced the EPR at all stages of development (fig. 3.51). SG and LG fruit showed increases of 62% and 28% respectively, in the BR and BL fruit however the increases were much greater (445% and 494% respectively). This is at variance with the raspberry results which indicate the EPR of the more mature fruit to be only affected slightly (if at all) by exogenous

Table 3.23 Effect of 40 ppm ethylene for 48 hours on Ashton Cross blackberry anthocyanin levels (20 fruit/sample)

Developmental Stage	Anthocyanin Level ug/g	
	Air	Ethylene
SG	31	184
LG	173	394
BR	1040	1284
BL	1876	2014

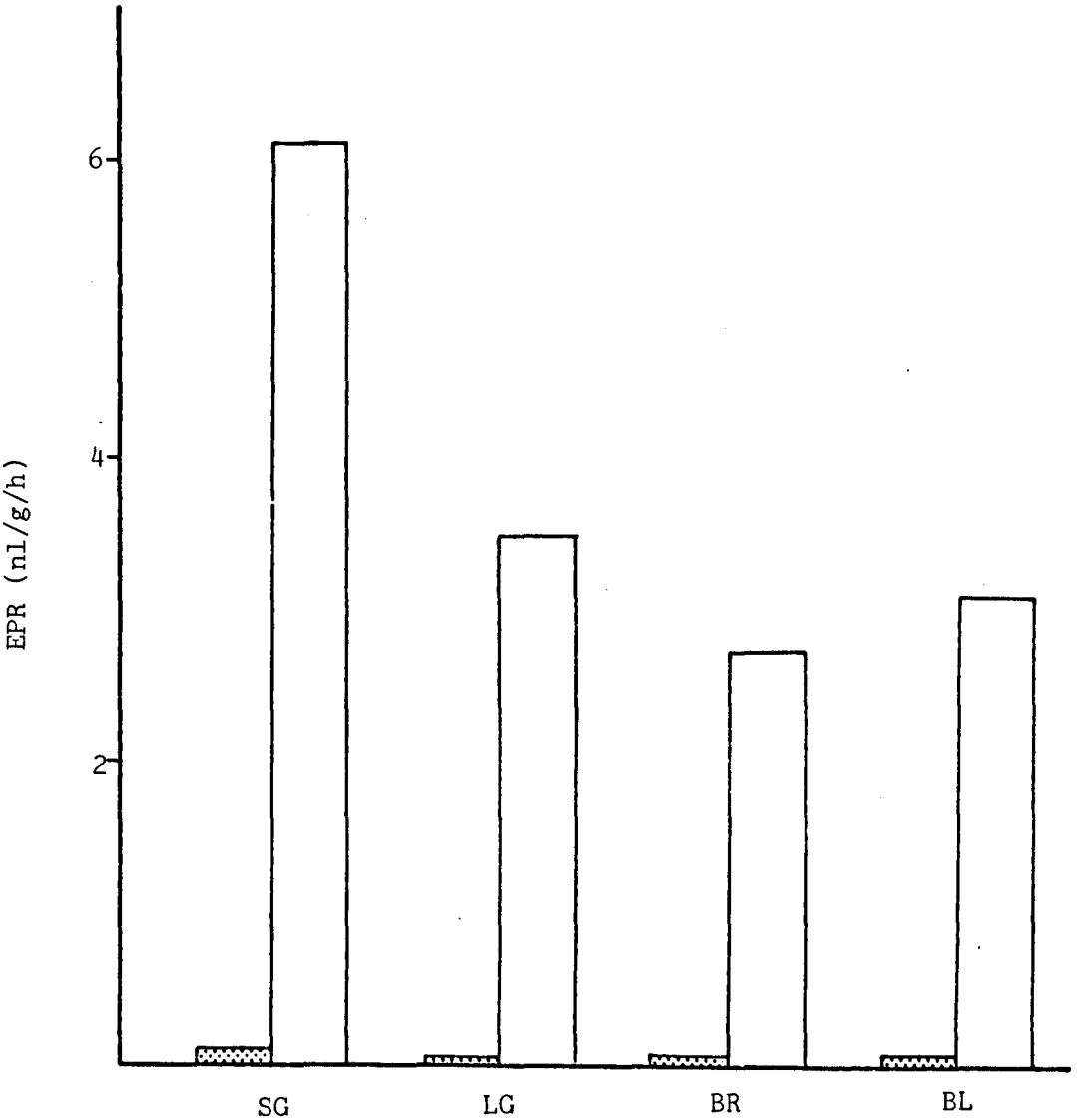


Fig. 3.52 Effect on ethylene production of dipping Ashton Cross fruit at different stages of development in 0.1 mM ACC. EPR measured 20 h later. ACC treated fruit (☐), water treated controls (▩). 15 fruit/sample.

ethylene. Again this may be because natural production is saturating in ripe raspberries.

As with raspberries exogenous ethylene accelerated the pigmentation changes which occur naturally. These stages are clearly visible in plate 3.27 and quantified in table 3.23. Not only did ethylene cause earlier pigmentation changes, but it also increased the level in those fruit which were approaching full pigmentation anyway.

3.1.14 v) The control of ethylene production by the ethylene forming enzyme in blackberry

To examine whether ethylene production was limited by ACC availability or ethylene forming enzyme (EFE) activity, Ashton Cross fruit at different stages of development were dipped in 1 mM ACC as described in Section 2.15. The EPRs of these fruit were then compared to water dipped controls 20 hours later.

The results in fig. 3.52 indicate Ashton Cross blackberries at all stages of development to have a capacity to produce ethylene in excess of that expressed naturally. As this was achieved by supplying ACC it suggests the EFE to be present and functional throughout development and the low EPRs to reflect a limiting supply of ACC, possibly as a result of low ACC synthase activity.

3.1.14 vi) Ethylene production rates of black and purple raspberries and raspberry/blackberry hybrids

The black raspberry (*R. occidentalis* L.) and purple raspberry (a black/red raspberry hybrid) abscise in a fashion identical to the red raspberry already examined. The

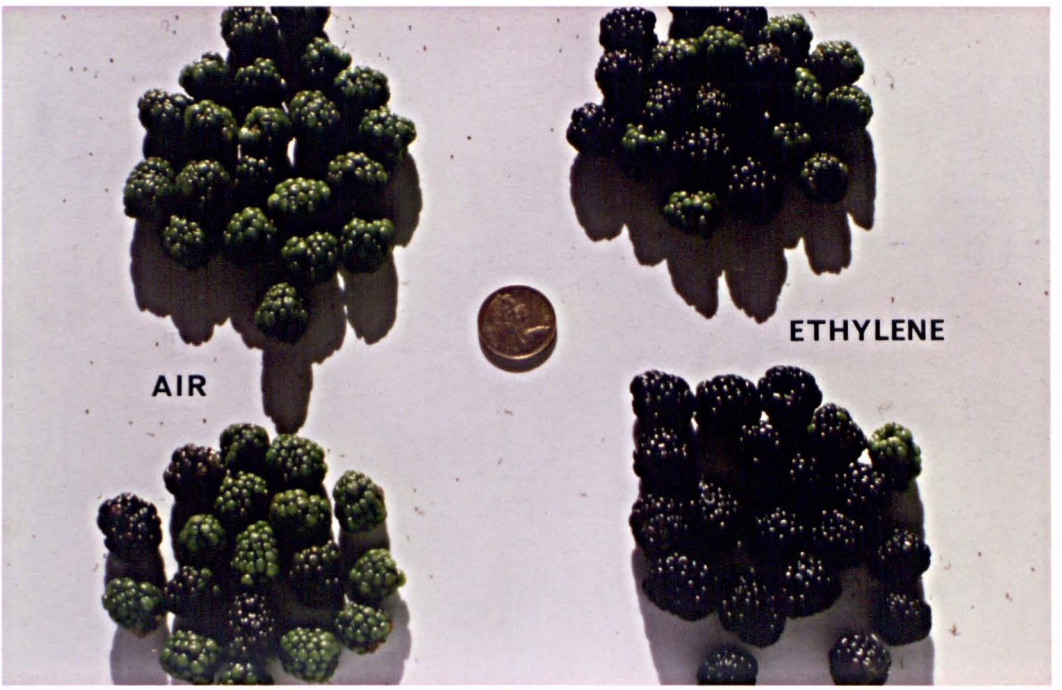


Plate 3.27 Effect of ethylene (40 ppm) for 48 h on the pigmentation of large green Ashton Cross fruit.

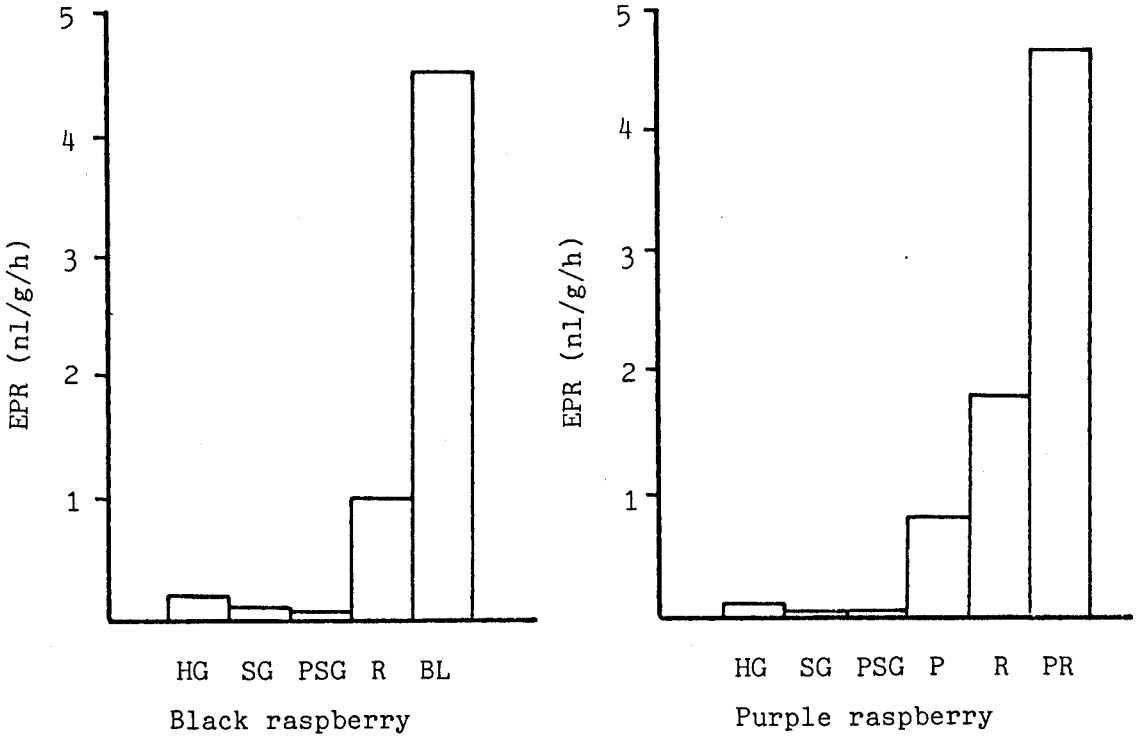


Fig. 3.53 Change in ethylene production rates during development of black and purple raspberries. Classification: HG - hard green; SG - soft green; PSG - pale soft green; P - pink; R - red; BL - black; PR - purple red. (20 fruit/sample).

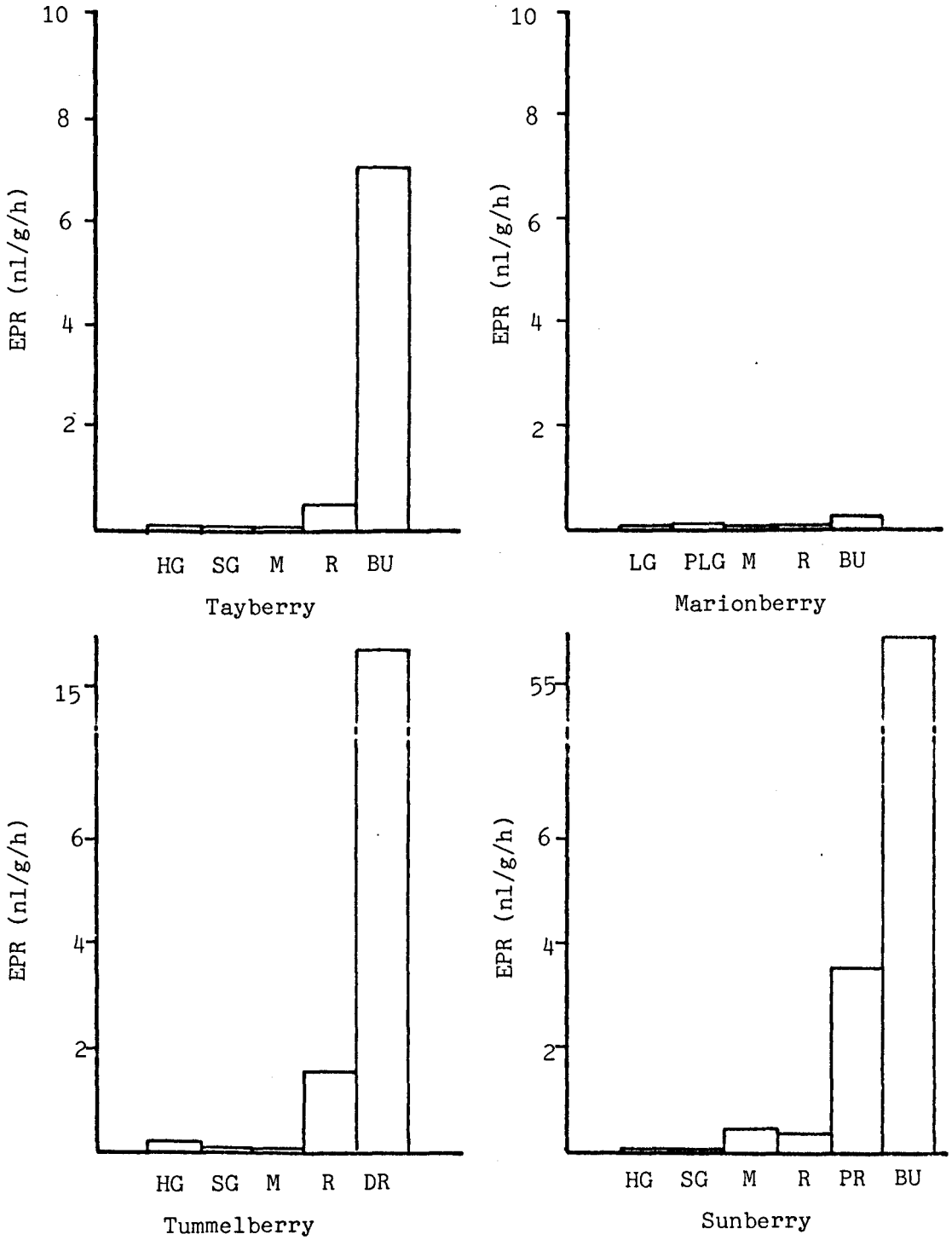


Fig. 3.54 Changes in ethylene production rates during development of 4 blackberry/raspberry hybrids. Classification: HG - hard green; SG - soft green; LG - large green; PLG - pale large green; M - mottled; R - red; DR - deep red; PR - purple red; BU - burgundy. (10-30 fruit/sample).

raspberry/blackberry hybrids (Tayberry, Marionberry, Tummelberry, Sunberry) however separate as in blackberry with the formation of a single abscission zone leaving the receptacle and drupelets whole. These fruit are illustrated in plate 3.28. It was noted however that although abscission in Tayberry normally took place at the base of the receptacle it was possible to separate part of the drupelet from the receptacle apex indicating a degree of weakness in the connection between drupelet and receptacle as in raspberries plate 3.29.

As with abscission, the EPR of both black and purple raspberries (fig. 3.53) are similar to those in red raspberries. Both have higher rates in the HG than SG and increase in a climacteric pattern during the black or purple ripe stages. The absolute rates are similar producing approximately 4.5 nl/g/h at the final stage of maturity.

With the exception of Marionberries the hybrids showed clear climacteric EPRs (fig. 3.54). Because of the differences in pigmentation the categorisation of developmental stages differed and this is outlined in fig. 3.54. The Marionberry shows a slight increase in EPR at the fully mature (burgundy) ripe stage and it may be that those fruit examined for EPR were not fully mature, although they were the most developed fruit available. The changes in EPR of Tayberries, Tummelberries and Sunberries all indicate these fruit to be cimacteric with respect to ethylene production.

3.1.14 vii) Summary

These results indicate the variation found in the EPR profiles of raspberry varieties also exists between blackberry

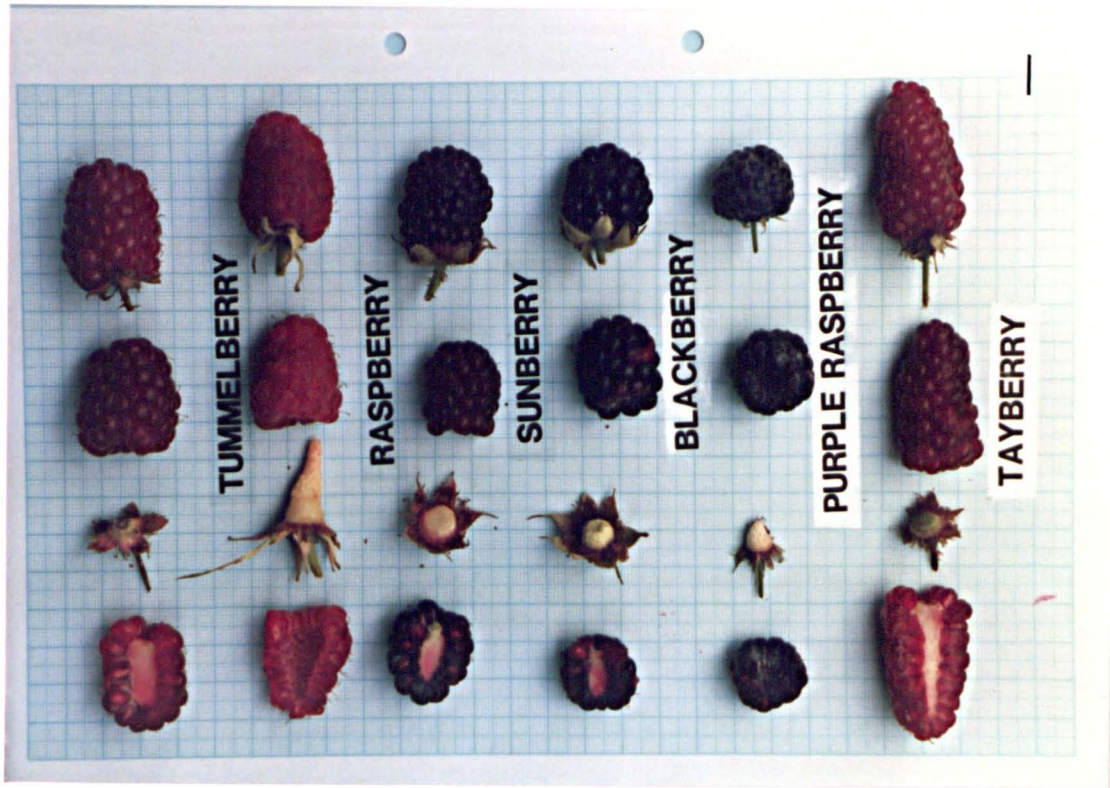


Plate 3.28 Abscission sites in a range of Rubus varieties. Bar = 1

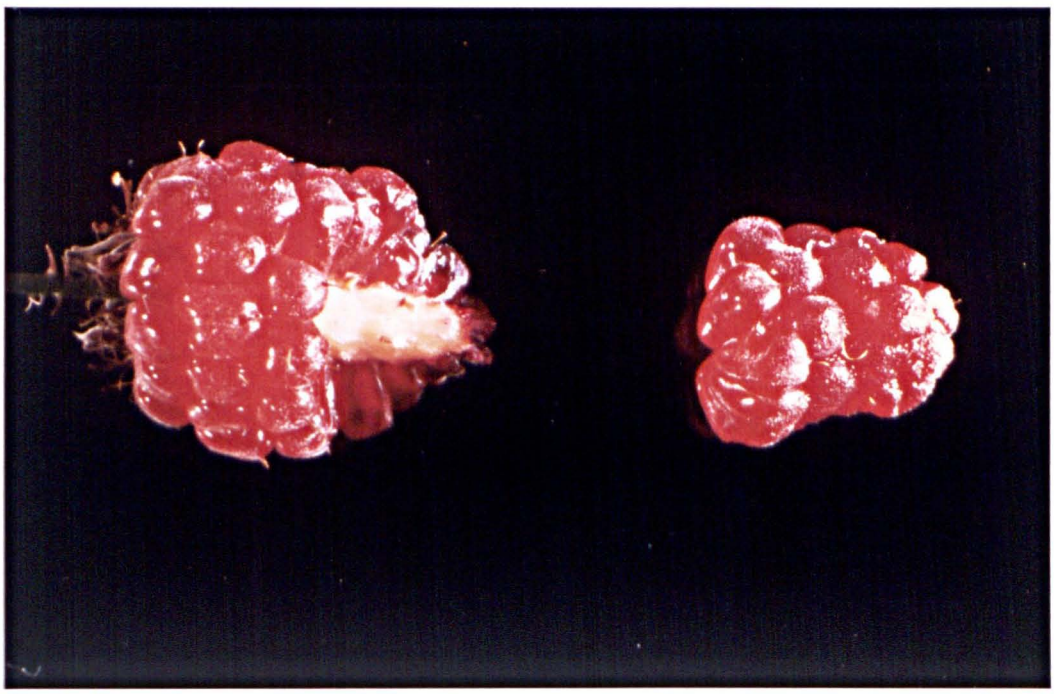


Plate 3.29 The partial separation of a Tayberry at the drupelet/receptacle junction (as in raspberry) as opposed to at the calyx (as found in blackberry). Normal Tayberry abscission occurs at the calyx.

varieties and the hybrids of the two. In the blackberry Ashton Cross the absence of a natural ability to produce ethylene appears to be as a result of a lack of the precursor ACC. An exogenous supply of ACC to these fruit enchanced EPRs at all stages of development showing the EFE to be constitutive. Whilst Ashton Cross fruit do not produce high levels of ethylene naturally this does not prevent them from responding to exogenous ethylene in a fashion similar to raspberry. The lack of ethylene production when mature allows the exogenous ethylene to effect blackberry fruit at all stages of development whereas only the immature stages (when EPRs are low) are influenced in raspberry. The hybrid fruit show the same morphological abscission characteristics as the blackberry but have EPRs more akin to those found in raspberry.

SECTION 3.2 FLOWERS

The initial studies of small fruit (Section 3.1.6 ii) showed ethylene production to be higher in the very small (< 0.5 g) hard green fruit than in the larger fruit of the same category. Extending the range of developmental stages being examined back to the closed buds revealed a peak in ethylene production during flowering (Section 3.1.6 ii). From these results it seemed possible that the high ethylene production rates associated with flowering could induce petal abscission. This hypothesis has been examined below.

3.2.1 Time course of flower development

To be able to study the role of endogenous ethylene in petal abscission it was first necessary to describe in detail the sequence of events occurring during flowering which culminate in petal abscission. This was achieved by following the development of buds kept transpiring water in the laboratory and recording the changes as they occurred. To do this buds were collected from the field with pedicels as long as possible at a stage when the sepals were just parting to reveal the petals underneath. This stage of development is easily recognisable thereby ensuring that a uniform population of buds was available for observation. The pedicels of the buds were trimmed in the laboratory and threaded through 1 cm diameter polystyrene floatation collars. They were then floated in a dish of water with the pedicels protruding into the water. Buds kept in this way opened and abscised their petals in a manner which seemed identical to those in the field.

Progress through the 20 stages of flower development detailed in table 3.24 was determined by following the opening and senescence of 20 flowers kept on water at 20°C. Initially buds were taken from the field in the late afternoon at the stage when they were swollen enough to just reveal the petals between the parting sepals (stage 2). Overnight the buds continued to swell and the petals became more visible until the sepals were only joined at the tips (stage 3) by the next morning. Over the next 6 hours the sepals opened and then curled back towards the pedicel (stage 10). During this period the petals tightly enclosed the stamens and styles. Once the sepals passed the horizontal, the petals started to open taking approximately 10 hours until the first petals were shed. Petal detachment was first evident after the petals reached a position of 45° to the horizontal and once started was complete in a further 3 to 4 hours. A selection of the stages discussed are illustrated in plate 3.30. Whilst these timings are only approximate an important feature of these observations is the synchronous manner by which all the petals are shed by each flower over a short period of time.

Table 3.24 The sequential stages of flower opening and approximate times taken to reach them when kept in the laboratory.

STAGE	DESCRIPTION		TIME FROM STAGE 3 (h)
	SEPALS	PETALS	
1	Joined, bud closed & swollen	Not visible	
2	Just parting	Just visible between sepals	
3	Joined at tips	Visible between sepals	0
4	Separated at tips, not vertical	Tight around stamens and styles	
5	Vertical		2.5
6	60° to horizontal		
7	45° to horizontal		
8	20° to horizontal		4.5
9	Horizontal		
10	Past horizontal, curled		6
11		Loose around stamens and styles	
12		Vertical	10.5
13		80° to horizontal	
14		60° to horizontal	13
15		45° to horizontal	
16		20° to horizontal	
17		One or 2 abscised	17
18		Half abscised	
19		One or 2 remaining	
20		All abscised	20

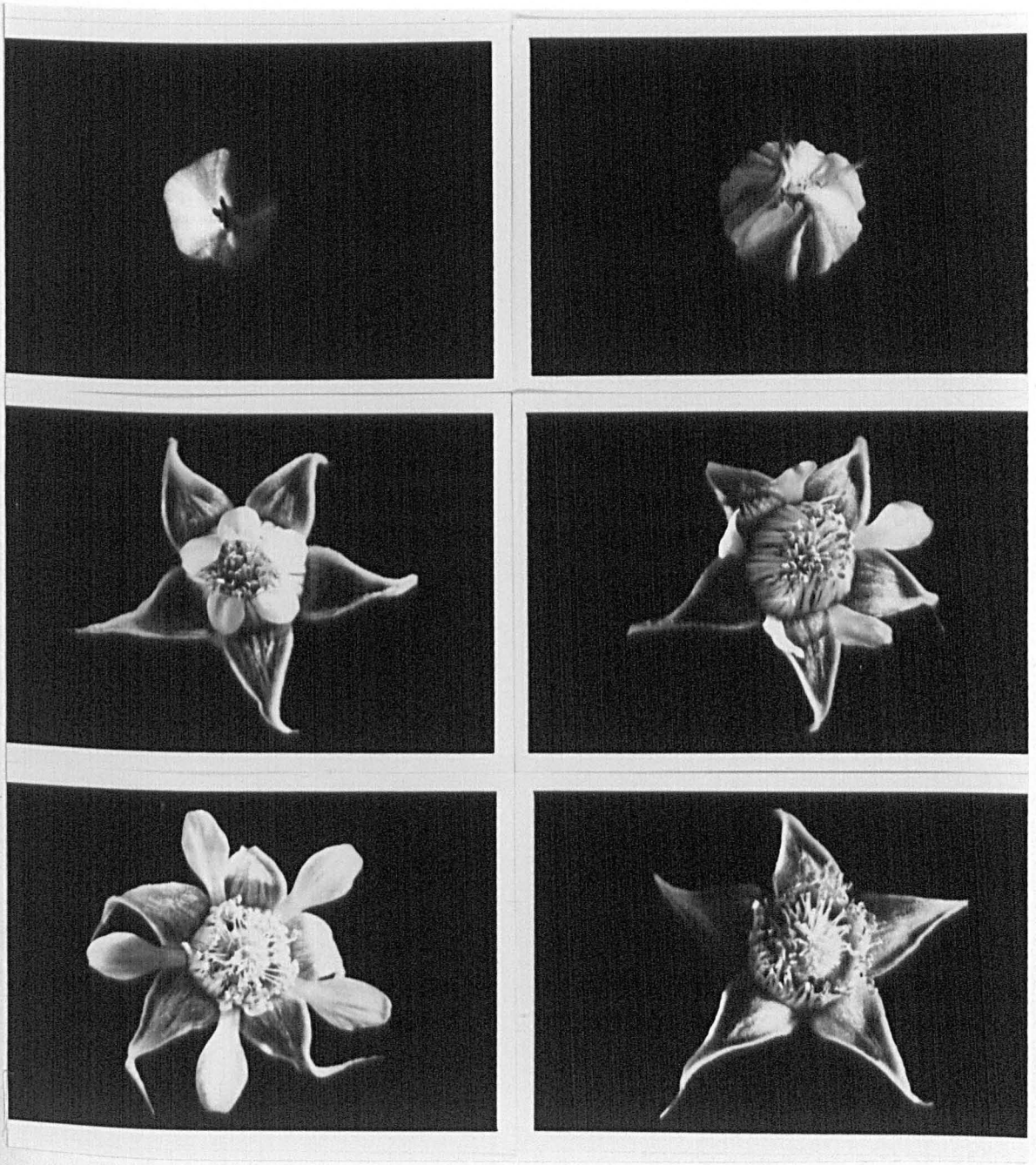


Plate 3.30 Representative stages of flower development in Rubus idaeus L. cv Glen Clova as listed in table 3.23. From top left clockwise, stage 1, stage 3, stage 14, stage 20, stage 16, stage 10.

3.2.2 Ethylene production rates of flowers measured directly after harvest at different stages of development

Ethylene production rates of flowers at different stages of development were measured immediately after harvest. Flowers were split into those with i) sepals opening, ii) petals opening, ii) petals abscising and iv) all petals having been shed. Each batch was then sealed in a glass vial with a suba-seal for up to one hour to allow ethylene to accumulate prior to headspace analysis.

The results in fig. 3.55 are expressed on both a per gram and per flower basis. There is an approximately 10 fold difference between the data expressed on these two bases since the fresh weights increased from 0.08 g to 0.11 g during development. On a per flower basis the EPRs were low (<0.03 nl/flower/h) when the sepals were opening, increasing to ~ 0.5 nl/flower/h during petal opening and peaking at ~ 1.2 nl/flower/h during petal abscission. Once all petals had been shed the rate fell to ~ 0.9 nl/flower/h.

The initial measurements described above were made on batches of flowers at only 4 stages of development. The study was extended to the measurement of individual flowers from the whole range of developmental stages (fig. 3.56). During the stages where the sepals opened and reflexed (stages 1-10) the EPRs were very low (<0.1 nl/flower/h). As the petals opened and abscised (stages 10-19) the EPRs increased to peak at 1.8 nl/flower/h when 1 or 2 petals remained attached (stage 19). Once all petals had been lost the EPRs fell to approximately 0.8 nl/flower/h. This burst of ethylene production which occurred during the latter stages of petal opening coincided with petal abscission and the possibility of ethylene controlling this abscission process is examined in

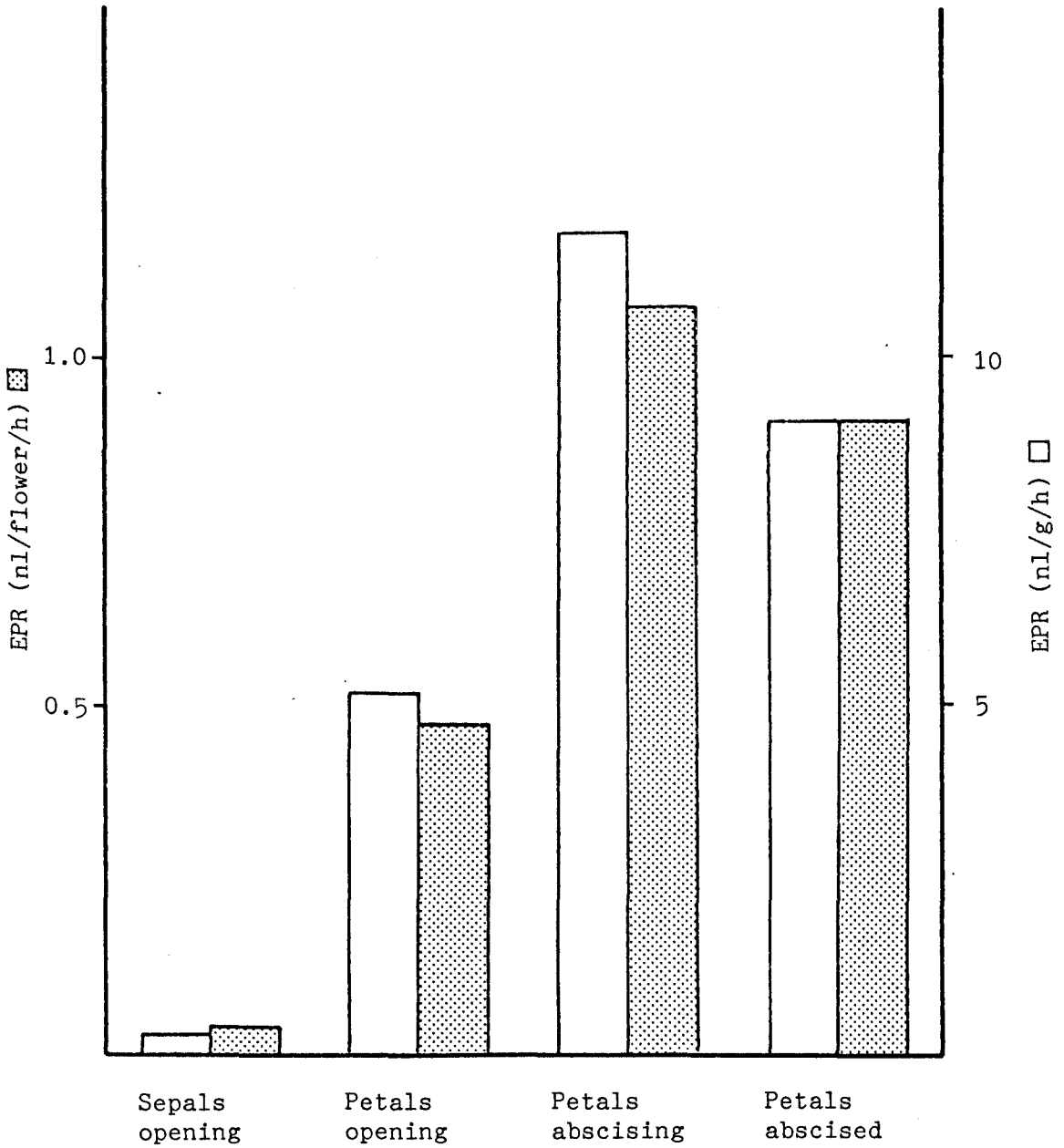


Fig. 3.55 Ethylene production rates at different stages of development measured directly on removal from the plant. (8-11 flowers/ sample).

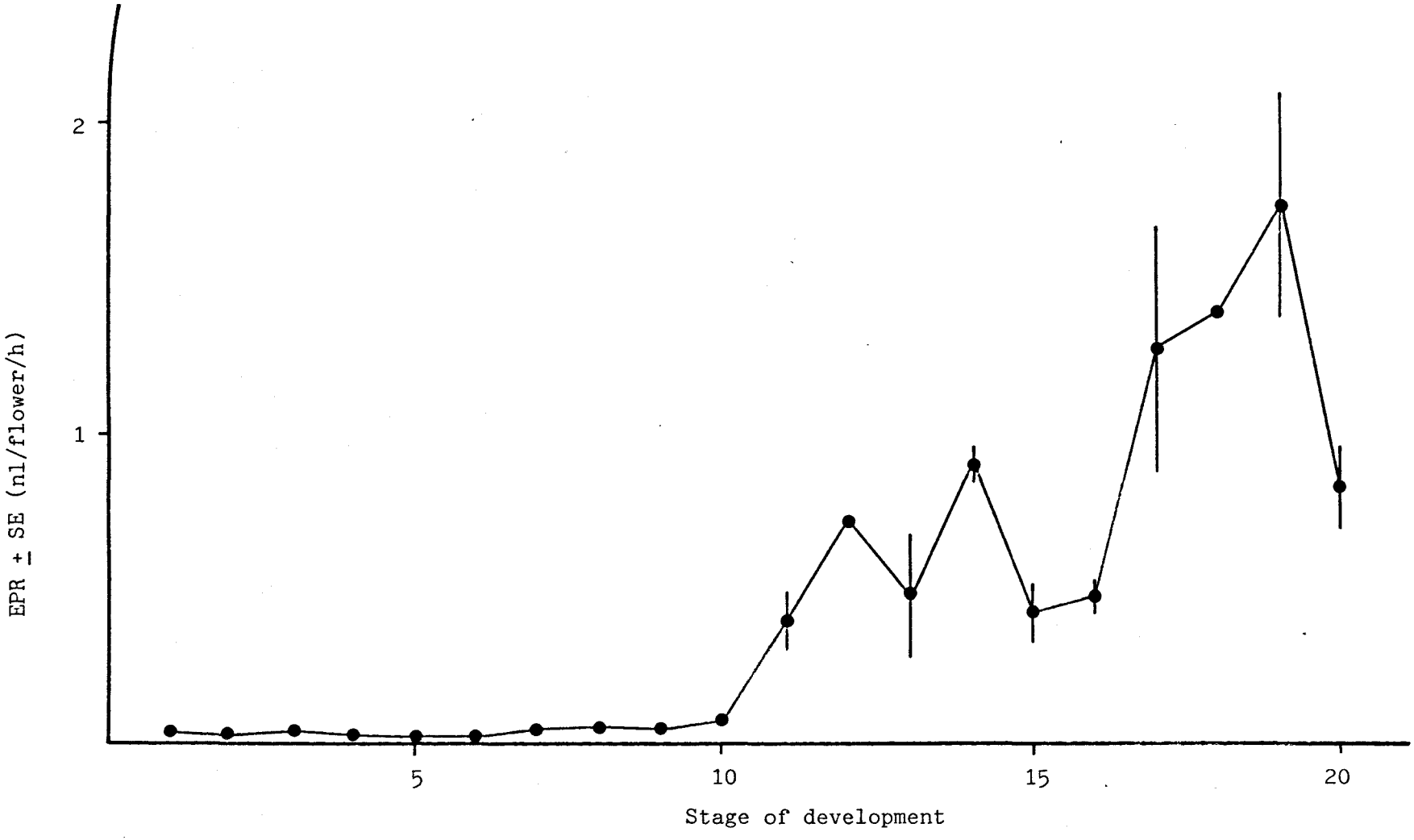


Fig. 3.56 Ethylene production rates of individual flowers measured directly on removal from the plant. (6-10 flowers/sample).

subsequent sections.

A burst of ethylene production of this type has been reported in the following other flowers, snapdragons (Wang et al., 1977) geraniums (Wallner et al., 1979), Digitalis (Stead, 1985), lowbush blueberry and strawberry (Hall and Forsyth, 1967), orchid (Burg and Dijkman, 1967). In the last 3 cases the increased ethylene production is apparently induced by pollination. Experiments were therefore undertaken to examine whether the ethylene production in raspberry flowers is pollination dependent.

3.2.3 Effect of pollination on ethylene production rates

Pollination has long been known to stimulate ethylene production (Burg and Dijkman, 1967; Hall and Forsyth, 1967; Nichols, 1977; Stead and Moore, 1983; Halevy et al., 1984b). The effect of pollination on raspberry flowers ethylene production was examined by opening buds away from pollinating agencies by floating them in isolation on water in the laboratory as described previously (Section 2.23). On reaching stage 6 (sepals 60^{o} to horizontal) pollen was added to the protruding stigmas of half the flowers, the remainder being left untreated. The EPRs of the individual flowers were then measured throughout the subsequent development.

The results in fig. 3.57 show no difference between the EPRs of pollinated or control flowers. However, the results in both sets of flowers show the actual production rates to be higher than in flowers of comparative stages of development measured directly on collection (fig. 3.56). As the sepals opened this increase was slight, the EPR still being low at approximately 0.2 nl/flower/h. As the petals opened and abscised the increase was approximately 10 fold with the peak in production not being so discrete as in fig. 3.56, occurring both during petal opening and abscission. Once all petals had been shed the EPR was still high at just over 2 nl/flower/h compared to 0.8 nl/flower/h in the fresh flowers.

It was possible that the raspberry flowers were self pollinated since fruit developed to some extent on canes grown in greenhouses in February in the absence of pollinating flies. Microscopic examination of untreated flowers revealed the outer whorls of stigmas to be self pollinated during bud opening (plate 3.31). It is therefore not surprising to find there to be no

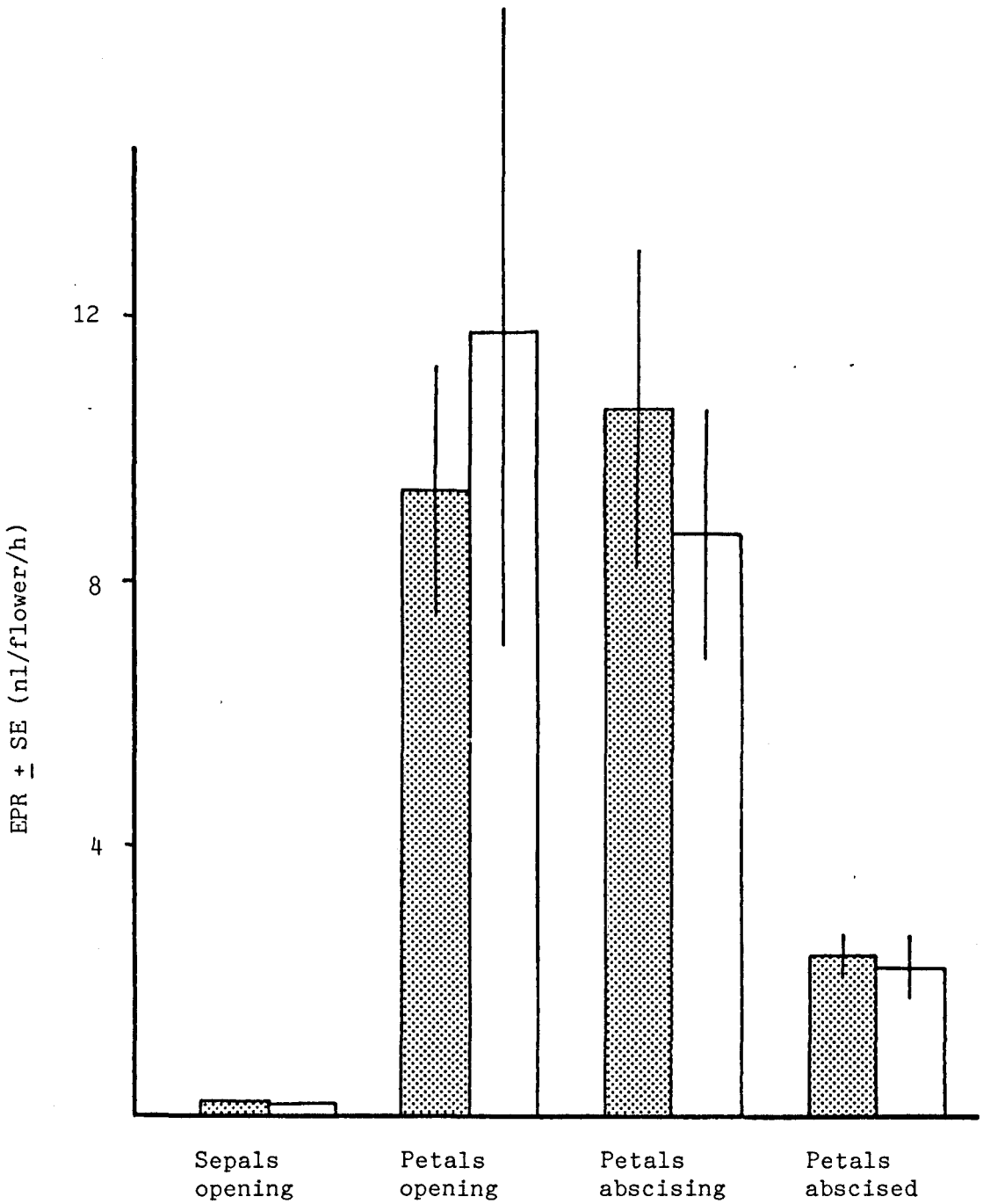


Fig. 3.57 Effect of adding pollen to flowers opened in isolation on ethylene production rates. Pollen treated flowers (⊠), untreated control flowers (□). 6 flowers/sample.

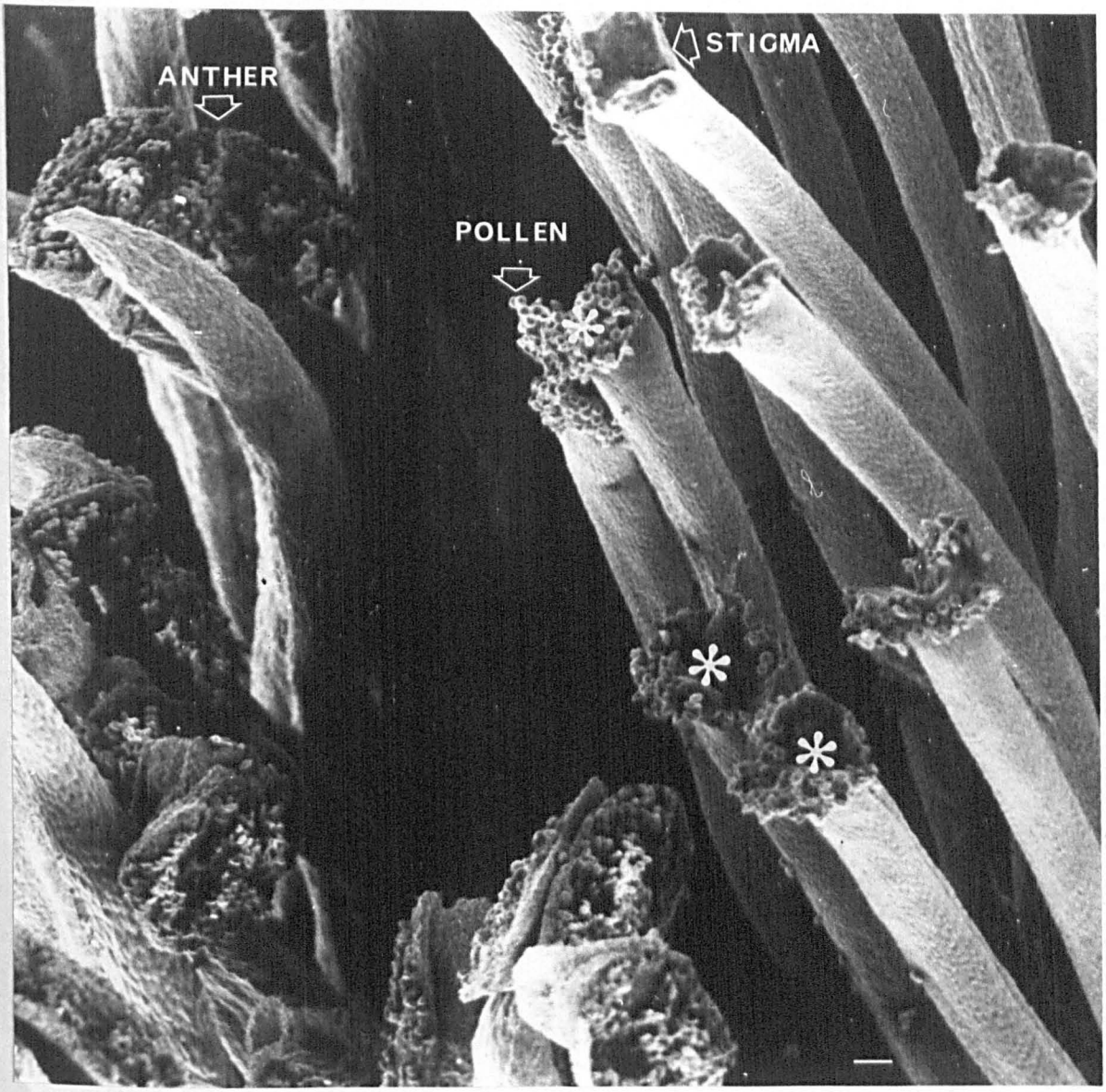


Plate 3.31 Stigmas of a raspberry flower opened in isolation showing self pollination of the outer whorls of stigmas (*). Bar = 100 μm.

difference between the two sets of flowers.

It has been reported in the preceding sections that raspberry flower buds would open and shed petals if kept on water in the laboratory. This appeared to be an ideal system with which to study the involvement of ethylene in petal abscission. Individual flowers could be floated on water in vials and their EPRs continually monitored throughout development. It was therefore possible to examine in detail the correlation between the timing of abscission and the increase in EPR. Also the system lent itself to the administration of inhibitors such as AVG and STS which could be fed into the transpiration stream.

Using this system the following have been examined:

i) The correlation between EPR and the onset of petal abscission.

ii) The effect of the ethylene synthesis inhibitor AVG on EPR, petal abscission and petal senescence.

iii) The effect of the inhibitor of ethylene action STS on petal abscission and petal senescence.

3.2.4 Ethylene production rates during development of flowers opened in the laboratory

To follow the EPRs of laboratory opened flowers individual buds at stage 2 were floated on 5 mls water in small glass vials at 20°C. As each bud reached the stages of maturity listed in table 3, the vials were sealed for EPR measurement. Not all stages of development were monitored on each flower but over 3 different experiments involving 30 buds several replicate determinations of each stage were made (fig. 3.58).

The overall pattern of ethylene production showed very low rates during sepal opening (< 0.2 nl/flower/h), a massive increase during petal opening (approximately 20 nl/flower/h at stage 15) and subsequent decline during petal abscission (approximately 3 nl/flower/h at stage 20). This pattern is similar to those flowers directly harvested at these stages from the field, the increase in EPR occurring from stage 11 onwards. However, the increase was more rapid peaking at stage 15 rather than 19. The actual EPRs occurring at the peaks were in excess of 20 nl/flower/h in the laboratory opened flowers compared to 1.7 nl/flower/h in freshly harvested flowers. In the earlier stages during sepal opening the rates were closer to those found in the freshly harvested flowers (< 0.2 nl/flower/h : < 0.1 nl/flower/h respectively). The increased rates later in development may be due to the higher laboratory temperatures which may raise ethylene production rates (Field, 1985) and synchronise and speed up the development processes. There is also the parallel of increased fruit EPRs caused by detachment reported earlier and increased flower EPRs on detachment reported by Hall and Forsyth 1967.

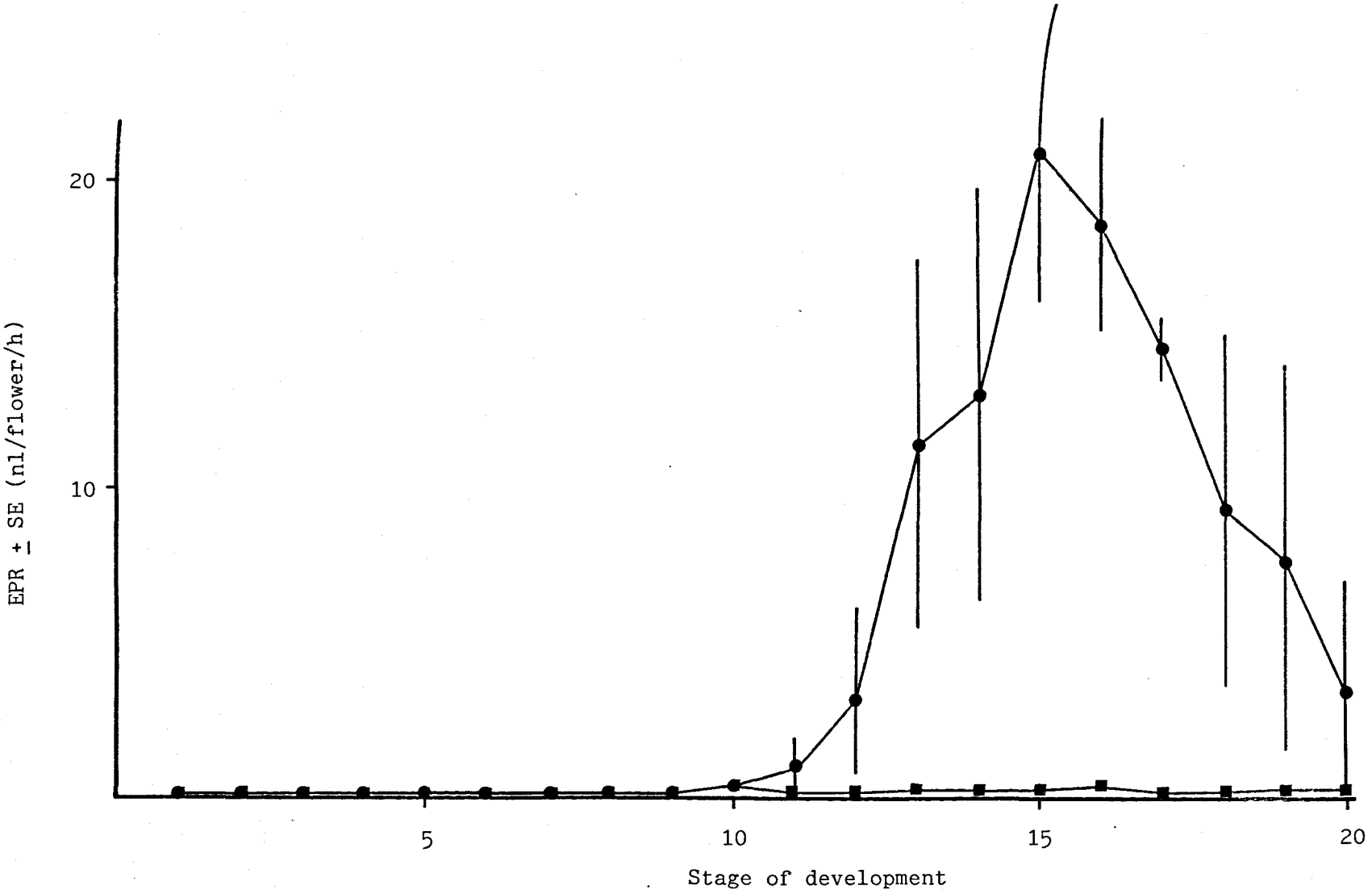


Fig. 3.58 Ethylene production rates of individual flowers opened in the laboratory on water (●) or 0.4 mM AVG (■). 6-10 flowers/sample.

3.2.5 Effect of aminoethoxyvinylglycine on ethylene production rates of flowers throughout development, petal abscission and petal senescence

The effect of reducing EPRs on petal abscission can be examined through the use of the ethylene synthesis inhibitor AVG. Allowing flowers to open whilst transpiring 0.4 mM AVG maintained EPRs below those which would be found in flowers measured directly from the field. The results in fig. 3.58 show that even in the absence of the peak of ethylene production flower development and ultimately abscission occurred. The way in which these results have been expressed however masks an important feature, the time course of development on treatment with AVG was much slower. Hence a further series of experiments were undertaken to quantify these changes in the timecourse.

To examine the effects and specificity of the AVG treatment, flowers were allowed to develop either on water or 0.4 mM AVG in the presence or absence of a saturating level of ethylene (40 ppm). Ethylene additions were used as a control to ensure that AVG was having its effect solely via an inhibition of ethylene production and not through a depressive effect on general metabolism. To examine the delaying effect on AVG of the timecourse of petal abscission the numbers of petals that were shed when touched were recorded 28 and 40 hours after the buds had been collected.

The results in fig. 3.59 clearly indicate AVG to retard petal abscission at both time points. By 28 hours 67% of the petals on the air held control flowers had been shed, exposure to ethylene increased this figure to 88%. The AVG treated flowers had only shed 15% of their petals whilst the addition of ethylene increased this

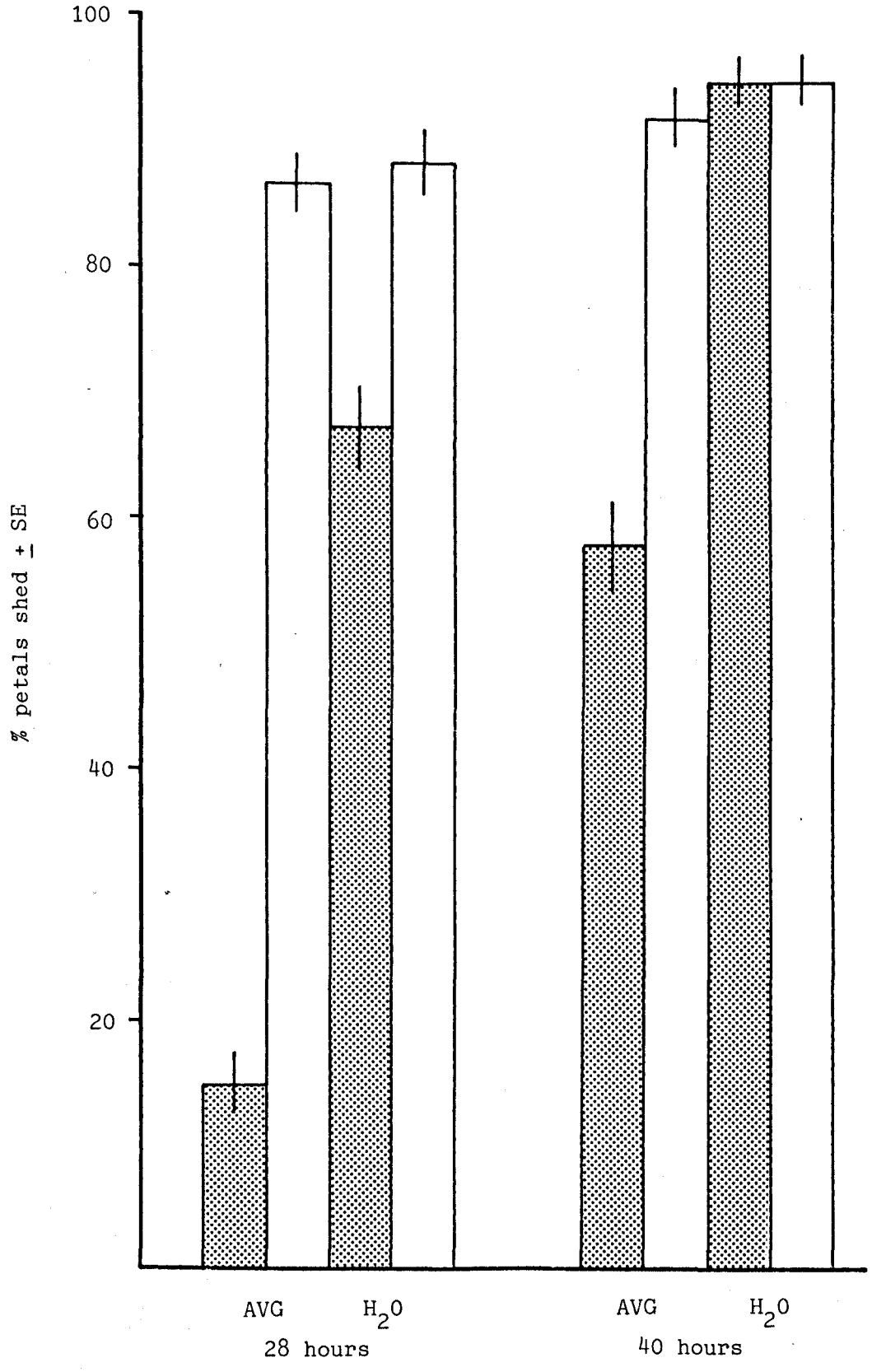


Fig. 3.59 Effect of 0.4 mM AVG on petal abscission in the presence (☐) or absence (▨) of 40 ppm ethylene. 50 flowers/sample.

to 86%. Exogenous ethylene apparently can overcome the retarding effect of AVG on petal abscission. After 40 hours treatment the AVG effect was still apparent with 58% petals shed compared to 94% in the air control flowers. It can also be seen that by 40 hours there was no difference between the air and ethylene treated flowers kept on water. In other words, the acceleration in petal loss caused by ethylene at 28 hours is no longer visible by 40 hours, thereby suggesting that natural ethylene levels are subsaturated during early stages of development. This data clearly shows AVG to retard abscission through its inhibition of ethylene biosynthesis as demonstrated by reinstating abscission with exogenous ethylene.

The effects of AVG on petal senescence were even more obvious (table 3.24). After 40 hours there were virtually no petals

Table 3.24 The effect of 0.4 mM AVG in the presence or absence of 40 ppm ethylene on the numbers of petals showing a brown senescent area at their base after 40 hours treatment (50 flowers/sample)

Treatment	% senescent petals
Floated on water in air	70
Floated on AVG in air	1
Floated on water in ethylene	96
Floated on AVG in ethylene	91

showing brown senescent areas at their bases in the AVG treated flowers kept in air. The other 3 treatments all had at least 70% of the petals showing this symptom of senescence.

During the above experiment it became apparent that the synchrony of petal loss differed between treatments. Normally once the first petal had been lost the remainder on that flower would be shed within a couple of hours. The AVG treated flowers appeared to be losing petals in ones and twos over a long period of time. The results from a further experiment confirmed these observations (table 3.25). After 44 hours treatment with 0.4 mM AVG only 28% of the flowers had

Table 3.25 Effect of transpiring 0.4 mM AVG on the synchrony of petal abscission 44 and 68 hours after collection at stage 2. (50 flowers/sample).

Treatment and duration	% Flowers		% total petals
	Lost all petals	Lost some petals	
44 hours on water	54	80	78
on AVG	14	28	18
68 hours on water	94	100	96
on AVG	14	80	42

lost any petals compared to 80% in the control. Likewise the numbers of flowers having lost all their petals was lower in the AVG treated sample (14% compared to 54%). This 14% of AVG treated flowers having lost all their petals remained constant over a further 24 hours whilst those losing individual petals increased to 80%. This implies that AVG disrupted the normal process by which petal abscission in individual flowers was synchronised. In control flowers after 68 hours treatment 94% had lost all their petals whilst all had lost at least one petal. The AVG treatment results detailed in table 3.25 show the AVG to have been more effective than in the previous experiment (table 3.24). No explanation is offered for the fact that after 40 hours 58% of petals had been shed (table 3.24) whereas by 44 hours only 18% had been shed (table 3.25) in the second experiment. These results indicate that AVG slows the process of petal abscission and disrupts the synchrony of petal shedding in individual flowers.

3.2.6 Effect of silver thiosulphate on petal abscission and senescence

The effect of the inhibitor of ethylene action STS was examined by allowing flower buds to develop whilst transpiring 0.2 mM STS. The flowers opened as normal but the petals were only shed slowly. Table 3.26 shows both the total number of petals lost and the way in which they are lost from individual flowers.

Table 3.26 Effect of transpiring 0.2 mM STS on the synchrony of petal abscission 44 and 68 hours after collection at stage 2. (50 flowers/sample)

Treatment and duration	% Flowers		% total
	Lost all petals	Lost some petals	petals lost
44 hours water	54	80	78
STS	0	18	6
68 hours water	92	100	98
STS	0	26	8

The results indicate STS to be very effective at preventing petal abscission, although the flowers did open as normal. Even by 68 hours treatment no STS treated flowers had lost all their petals and only 26% of the flowers had lost any petals. These abscised

petals were only 8% of the total. This can be compared to all control flowers having lost at least one petal, 92% having lost all and 98% of all petals having been shed.

The transpiration of 0.2 mM STS also retarded petal senescence as measured by the appearance of brown areas at the base of petals. Even 68 hours after collection there were less than 5% senescent petals in the STS treated flowers compared to 92% in the controls.

Hence STS is clearly effective in reducing both petal abscission and senescence. Whether this is simply a result of preventing the action of ethylene is questionable. The concentration of 0.2 mM STS is considered to be the upper limit not to cause toxic effects (Veen, 1983). The duration of the experiment may have resulted in toxic levels accumulating at the abscission zones. This is particularly relevant considering the nature of cellular changes which occur during abscission (middle lamella dissolution and partial cell wall degradation). These are the regions shown to be the sites of Ag^{+} accumulation in STS treated tissues (Hobson et al., 1984). Unfortunately the short duration of the flowering season prevented an extension of these initial observations which were made in 1986.

3.2.7 Effect of adding 1-aminocyclopropane-1-carboxylic acid to flowers at different stages of development and the measurement of its endogenous levels during development

The possibility that the low EPRs of flowers during sepal opening is as a result of a lack of the ethylene forming enzyme or its substrate can be examined by supplying the substrate (ACC). Flowers were taken from the canes at different stages of development and allowed to transpire 1 mM ACC for 5 hours prior to measuring EPRs.

The results (fig. 3.60) show that the supply of ACC caused elevated EPRs at all stages of development giving rise to rates in excess of 100 nl/flower/h at all stages. These rates are well in excess of those measured directly off the canes (<1.0 nl/flower/h) or on opening in the laboratory (<20 nl/flower/h). These increased rates indicate the presence and activity of the ethylene forming enzyme are not rate limiting even during sepal opening when the EPR is normally very low. Hence during sepal opening it would appear that the EPR is limited by the supply of ACC possibly through a lack or inactivity of ACC synthase or an earlier step in the ACC biosynthetic pathway.

The treatment with ACC also enhanced petal abscission. After 44 hours treatment with 1 mM ACC 95% of the treated petals had been shed compared to 78% of the controls. This confirms the earlier finding (fig. 3.59) that the addition of ethylene accelerated petal abscission beyond that found naturally. Hence if ethylene does control abscission, or as seems more likely, coordinates it, the levels produced during petal opening are subsaturating resulting in accelerated abscission when either ACC or

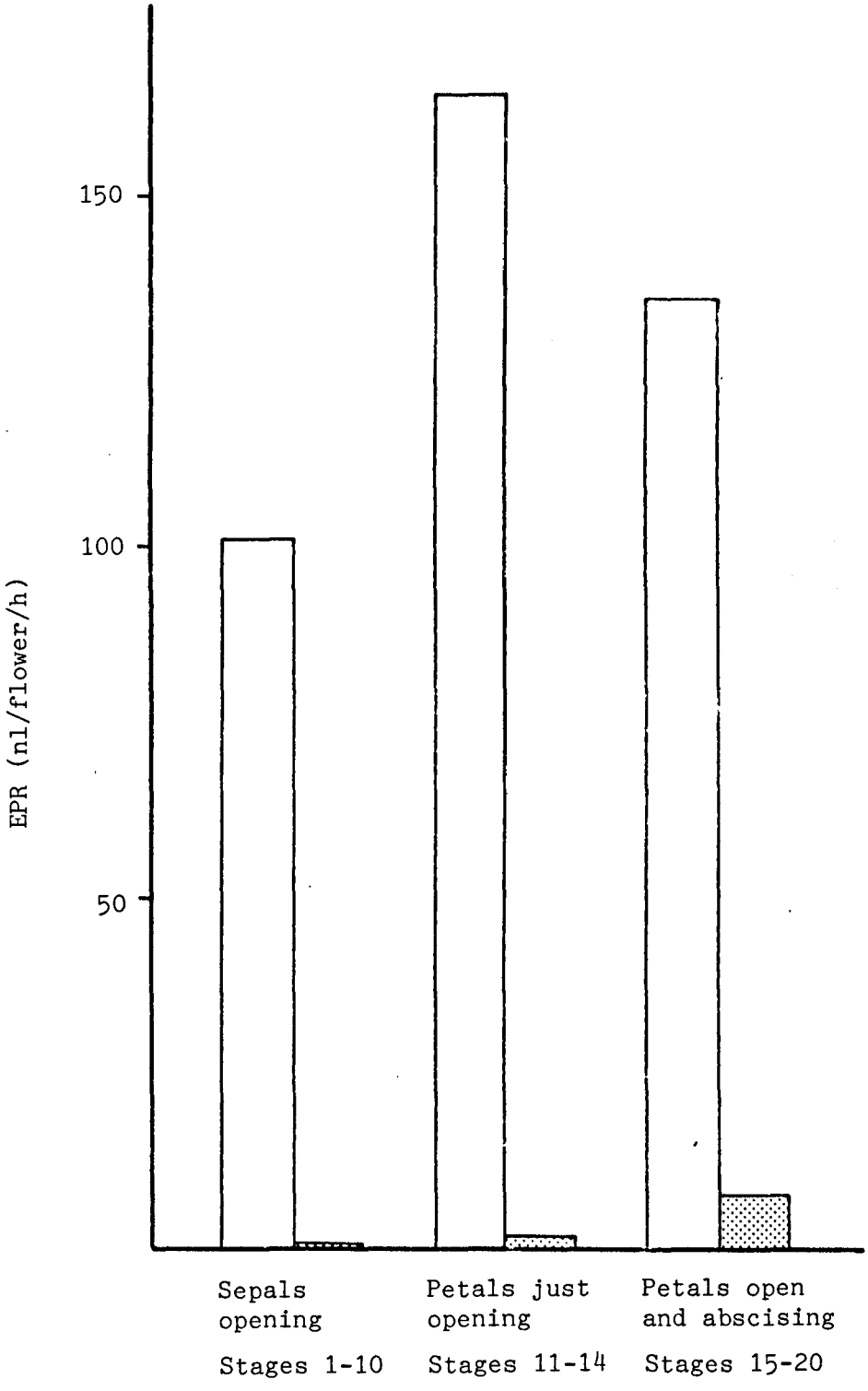


Fig. 3.60 Ability of flowers at different stages of development to convert exogenously supplied ACC (1 mM) to ethylene. Flowers at the 3 stages of development indicated transpired 1 mM ACC (□) or water (▩) for 5 hours prior to measuring ethylene production rates. (6 flowers/sample).

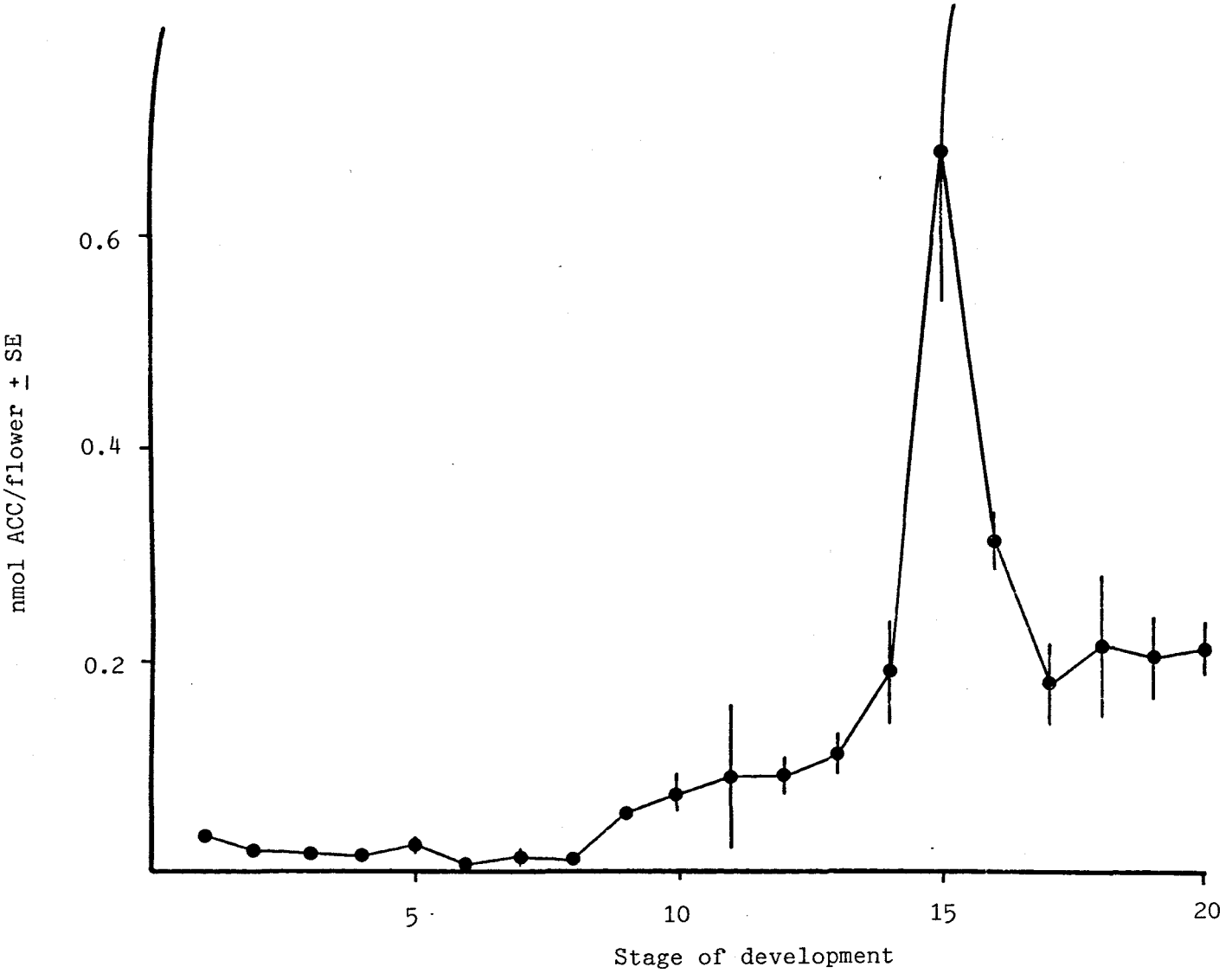


Fig. 3.61 Endogenous levels of ACC in individual flowers at different stages of development. (6-10 flowers/sample).

exogenous ethylene are added.

As mentioned above the EPR early in flowering appears to be limited by the supply of ACC which is the immediate precursor to ethylene, being converted by the ethylene forming enzyme. The ACC content of flowers at different stages of development, measured directly off the canes, are shown in fig. 3.61. This shows a peak in ACC content at stage 15 with accumulation starting at stage 9. There is thus a correlation between the ACC levels and the EPRs of flowers measured directly after harvest which increase from stage 11 and peaking at stage 18. The accumulation of ACC occurs in spite of the EPRs not reaching anything like those found when supplying exogenous ACC. This tends to suggest that the accumulated ACC might represent a pool which is isolated from the site of the EFE. This separation could occur at either the tissue or cellular level.

CHAPTER FOUR

DISCUSSION

For ease of presentation the discussion of the data presented in Chapter 3 has been subdivided as follows:

Section 4.1 The role of ethylene in *Rubus* and other fruit abscission systems.

Section 4.2 The role of ethylene in petal abscission of *Rubus idaeus* L. cv. Glen Clova and other flowers.

Section 4.3 Summary of the main conclusions derived from the experimental work on ethylene in fruit and petal abscission in *Rubus idaeus* L. cv Glen Clova.

Section 4.4 Discussion of the hypothesis that ethylene is a major controlling factor in abscission.

Section 4.5 The role of other growth substances and tissue sensitivity in abscission. This section also includes a critical examination of some assumptions which are often made when interpreting abscission data.

4.1. The role of ethylene in *Rubus* and other fruit abscission systems

It is clear from the results presented in Section 3.1.3 that at least in terms of its ethylene production characteristics Glen Clova raspberry should be classified as a climacteric fruit (Rhodes, 1980; McGlasson, 1985). The EPR was low in green fruit and increased exponentially as the fruit developed from mottled to purple ripe. These data confirm Blanpied's (1972) earlier claim which was based on rather insubstantial evidence.

Normally climacteric fruit show both a rise in EPR and an increase in respiration as they mature (Biale and Young, 1981).

These characteristics have been found in apples, avocado, bananas, mango, pear and tomato (Burg, 1962) and numerous others (reviewed Rhodes, 1980; Biale and Young, 1981). In Glen Clova raspberry however while the EPR increased on a unit weight basis the respiratory rate on the same basis declined. Respiration rates are generally expressed on such a basis although the rate maybe expressed on a per fruit basis. In Glen Clova the respiratory rate clearly increased with development on a fruit basis.

A similar observation of an increased EPR yet no increase in respiration has been reported for Vaccinium fruit by Forsyth and Hall (1969). Conversely Watada and Morris (1967) reported an increased respiratory rate yet no increase in EPR in bean. Even in those cases where both respiration and EPR increase the two processes appear not to be highly linked. The rise in EPR may precede (banana), follow (apple, tomato) or coincide with (avocado, mango, pear) the respiratory peak (Burg, 1962). There is clearly a great deal of variation within the climacteric fruit category and this leaves open to question the usefulness of the classification. Sacher (1973) concluded that ripening was not dependent on the climacteric increase in respiration. This was based on the data of both Frenkel et al. (1968) and McGlasson et al. (1971) who showed CHI to inhibit ripening whilst not affecting the respiratory increase. From a physiological point of view the important grouping is probably all those fruit which show an ethylene climacteric, the significance of the often associated respiratory rise being less clear and it is certainly not directly linked to the EPR.

Whilst there is variation between fruit within the climacteric category the temporal characteristics of the ethylene

climacteric differs between varieties of the same fruit. In raspberries the cultivar St Walfried only showed increased EPRs when ripe whilst other varieties (such as 68/14/106) showed an increased EPR at the earlier mottled or even soft green stages. The situation in blackberry was even more variable. Walsh et al. (1983) classified thornless blackberry as climacteric on the basis of the EPR in very ripe fruit, respiratory data being described as 'inconclusive'. The data in Section 3.1.14 shows a similar EPR profile during development in Bedford Giant blackberry. Other blackberry varieties however showed no rise in EPR during maturation (eg Ashton Cross). Since Ashton Cross appeared to ripen and abscise the result must call into question the hypothesis that ethylene is a major element controlling either process. Thus perhaps ethylene is not essential for ripening but merely accelerates and coordinates the process, a role similar to that which is proposed in abscission (see later).

The difference between climacteric and non-climacteric fruit can be seen in their response to exogenous ethylene (McGlasson, 1985). Climacteric fruit respond with both increased rates of respiration and ethylene production. The non-climacteric fruit also show an increase in respiration although this declines once the exogenous ethylene is removed. The increased EPR of climacteric fruit can only be demonstrated in those fruit with naturally sub-saturating EPRs, as were found in immature Glen Clova raspberries. The ethylene production of non-climacteric fruit is considered to be unaffected by a supply of exogenous ethylene. The apparently non-climacteric blackberry Ashton Cross however showed slightly increased EPRs at all stages of development. In the terminology of McMurchie et al. (1972) the non climacteric fruit

posses the basal ethylene production capacity (System I), yet lack the capacity to autocatalytically produce ethylene (System II).

Positive correlations between elevated EPRs and abscission have been documented in a number of fruit such as cotton (Vaughan and Bate, 1977; Guinn, 1982) peach (Jerie, 1976) mango (Nunez-Elisea and Davenport, 1986) okra (Lipe and Morgan, 1973) avocado (Davenport and Manners, 1982) and apple (Walsh, 1977). This has led to speculation that the increased ethylene production initiates abscission (Morgan, 1984). Consideration of the EPR data from Glen Clova raspberries would lead to a similar hypothesis. All individual fruit showing signs of abscission zone weakening had elevated EPRs and some fruit in the category where weakening was about to occur similarly showed increased rates.

In other cultivars however this relationship breaks down (eg St Walfried) with weakening commencing prior to the increase in ethylene production. On the basis of this evidence it is no longer possible to envisage increased ethylene levels as a major inductive agent. The hypothesis that ethylene controls the rate of weakening however is still viable and perhaps strengthened by these data. The FRS of ripe raspberries differs between varieties yet there is a good correlation between FRS and the EPR of the fruit in all varieties examined except Phyllis King. The timing of the rise in EPR with respect to other ripening characteristics and its magnitude, varied between cultivars. In the fruit of St Walfried which had a high FRS when ripe, the rise in EPR did not occur until the fruit were pink, however in 68/14/106 (low FRS when ripe) the EPR rise occurred in soft green and mottled fruit. As a consequence both varieties had fruit at approximately 1.0 N FRS showing the same

EPRs. In 68/14/106 these fruit were mottled whereas in St Walfried they were ripe. A similar correlation between ethylene production and fruit ripening has been reported in varieties of nectarine (Brecht and Kader, 1984b). It is also clear that in the blackberry variety Ashton Cross abscission will progress to some extent in the abscence of any increased ethylene production. Presumably this is the case in other abscising non-climacteric fruit.

It seems that in raspberries there may well be a slow initial phase of ethylene independent weakening followed by a second more rapid phase of ethylene mediated loss of integrity. In retrospect it would have been productive to investigate whether the weakening mechanisms involved in the two phases were the same.

Lengthy discussions of correlations may prove counterproductive since they seldom provide definitive proof of any hypothesis. In this case Phyllis King was an exception, amongst the raspberry varieties studied, to the general relationship between the increase in EPR and the extent of weakening. It is possible that differences in morphological development of the receptacle may explain this anomally (see later). Alternatively Phyllis King may prove there to be no correlation. Both arguments are easily sustainable and simply point to the problems associated with trying to use correlative data to substantiate the involvement of a plant regulator in the control of a process.

The additions of saturating levels of ethylene to the atmosphere around detached green fruit of Glen Clova accelerated the reduction in FRS. Similarly the addition to ethephon to attached fruit held in the greenhouse confirmed the enhanced ripening and accelerated FRS decline reported by Jolliffe (1975b) and Knight

(1976). This acceleration of fruit abscission by ethylene has been well documented in other fruit including coffee (Browning and Connell, 1970) citrus (Holm and Wilson, 1977) olive, (Blumenfield et al., 1978) and sour cherries (Olien and Bukovac, 1982). There are however two examples where oat (Sargent et al., 1984) and palms (Chan et al., 1972) were not responsive to ethylene.

In Glen Clova raspberries the addition of ethylene only accelerated abscission when added to fruit less developed than the mottled stage. This suggests the endogenous rate of ethylene production to be saturating in the ripe fruit. Ethylene also accelerated the accumulation of anthocyanins in the green fruit whilst at the same time reducing the chlorophyll levels. Ethylene enhanced anthocyanin accumulation has also been reported for cranberries (Craker, 1971) and chlorophyll degradation in citrus (Denny, 1924; Wardowski and Cornack, 1973) and tomato (Goodenough, 1986).

Ethrel treatment of green raspberries caused a rapid change in pigmentation and abscission. The small red fruit produced as a result were unpalatable, not having developed their full size or flavour, although the drupelets did soften. This situation was also found in cantaloupes where ethylene caused the rapid onset of some ripening characteristics (McGlasson and Pratt, 1964) yet these precluded the normal accumulation of sugar (Kasmire et al., 1970).

The identification of ethylene dependent and independent parameters of ripening have recently been examined by Jeffrey et al. (1984) in tomato. Starch, sugar and organic acid metabolism were all shown to be independent of ethylene. The accumulation of lycopene, polygalacturonase and invertase were all dependent on

ethylene. Chlorophyll degradation occurred in the absence of ethylene although exogenous ethylene would accelerate the process.

The actual level of ethylene required in the tissues to stimulate abscission in both hard green and soft green Glen Clova fruit was between 0.25 and 0.5 ppm ethylene. This was determined by maintaining detached fruit in atmospheres containing different ethylene concentrations. These levels agree well with those levels required to stimulate abscission in other fruit (eg cotton, 0.1 to 0.5 ppm, Lipe and Morgan, 1973) but are well below the levels required in avocado (1 to 100 ppm, Davenport and Manners, 1982).

The threshold concentration was similar on both hard and soft green raspberries (both 0.25 to 0.5 ppm), however the period of exposure required to show a response differed. The soft green fruit required 24 hours exposure while the hard green fruit required a 48 hour exposure. This difference could not be overcome by simply increasing the ethylene level supplied to the hard green fruit. Thus in this system the sensitivity in terms of the minimum ethylene requirement does not change. The speed of response to ethylene may just be accounted for in terms of different metabolic rates of the fruit of different maturities. Alternatively it has been shown that in bean leaf abscission zones a period of ageing is required before the tissue becomes ethylene sensitive (Abeles et al., 1967). The time difference between hard and soft green fruit could also be explained in these terms with the ageing period allowing either a decrease in an ethylene action inhibitor or allowing the accumulation of receptors or response machinery.

The internal ethylene levels of batches of Glen Clova fruit were measured using the vaccuum extraction technique described by

Yeang and Hillman (1981). Any air bubbles adhering to the test material were removed by using a wetting agent and those remaining would only make the value determined an underestimation. The main problem is determining where the gas collected was derived from within the tissues, either intercellular or intracellular. Beyer and Morgan (1970) considered a 2 minute extraction at 100 mmHg gave a true value for the intercellular gas phase. Yeang and Hillman (1981) showed that an increased vacuum extracted a greater volume of gas, although the ethylene concentration changed with the amount of gas extracted. It was suggested that using a stronger vacuum extracted intracellular gases in which ethylene was at a lower (Yeang and Hillman, 1981) or higher (Beyer and Morgan, 1970) level. Beyer and Morgan (1970) considered a vacuum in excess of 100 mmHg to be capable of extracting bound or dissolved ethylene. Despite these shortcomings this technique is the best available and the internal gases of raspberry fruit were extracted using a vacuum of 150 mmHg. This allowed a comparison between the natural ethylene levels and those which must be added to enhance abscission. It also allowed the internal ethylene level to be compared to the EPR. In Glen Clova raspberries there was found to be a constant relationship between the EPR and the level of ethylene accumulating within the fruit defined by the equation:

$$\log \text{internal ethylene} = 0.961 \log \text{EPR} - 0.214$$

It is clear from the results that in Glen Clova raspberries the ethylene threshold concentration is exceeded at the mottled stage of development and remains above it thereafter. Thus at the very point when weakening in Glen Clova commences the ethylene threshold is exceeded. Similar claims have been made on the basis

of direct measurements for other fruits (eg Blanpied, 1972; Lipe and Morgan, 1973). However, in most cases the abscission zone is in the fruit pedicel well away from the bulky fruit tissues where the ethylene is extracted. The ethylene levels at the zone may therefore be quite different. This is illustrated by a recent report by Nunez-Elisea and Davenport (1986) where mango fruitlet abscission was accompanied by increased ethylene production by the fruitlet. The increased EPR was not found in the abscission zone. However, they simply took this to mean that the ethylene was diffusing from the fruitlet to the abscission zone thereby causing abscission. This conclusion can be seen as premature in the absence of any hard evidence supporting it.

In raspberry the 50 to 100 abscission zones are actually enclosed within the fruit making the measurement of internal ethylene more significant. In determining an average internal ethylene concentration for the whole fruit it is not possible to exactly define the level in the individual receptacle and drupelets. Analysis of the internal ethylene levels of separate drupelets and receptacles indicated the receptacle to have the highest ethylene levels. This was also suggested by Blanpied (1972). In the mottled fruit both drupelets and receptacle contained ethylene levels in excess of the threshold. The intercellular spaces between the small cortical abscission zone cells are continuous with the extensive intercellular space network of the receptacle (Reeve, 1954) and the drupelet. Unfortunately it is not possible to measure directly the internal ethylene level within the abscission zone, however it may well be intermediate between the levels found in the drupelet and receptacle. It could

even be argued that because the abscission zone is closer to the bulk of the receptacle that the receptacle intercellular ethylene levels are a more accurate reflection of those in the abscission zone. These assumptions are made more plausible by the abscission zones being enclosed within the internal atmosphere of the fruit, thereby reducing diffusive losses from the zones.

Whilst all the evidence so far points to ethylene having a role in controlling the rate of abscission, none of it can be taken as definitive proof. More substantial evidence can be obtained through the inhibition of ethylene production. AVG was used to maintain sub-threshold EPRs in soft green Clen Clova fruit, yet this did not totally prevent the decline in FRS. It did however reduce the rate of weakening, suggesting ethylene (or at least increased levels) not to be an absolute prerequisite for abscission. In other fruit abscission systems where AVG has been used it has been found only to be effective at retarding the rate of weakening, and not to be capable of stopping abscission totally (for example in apple, Bangerth, 1978; Greene, 1980, 1983; Williams, 1980, 1981; avocado, Davenport and Manners, 1982). The same findings have also been recorded for other abscission systems (Sagee et al., 1980; Sipes and Einset, 1982; Kushad and Poovaiah, 1984; Stead, 1985). Greene (1983) however still found AVG to increase fruit set of apples in the year following application in spite of it no longer reducing ethylene production. The specificity of the AVG effect in soft green Glen Clova fruit was demonstrated by reversing the AVG induced retardation of abscission in the prescence of a saturating (40 ppm) ethylene atmosphere. Likewise the cobaltous ion, another inhibitor of ethylene production (Lau and Yang, 1976), whilst maintaining

sub-threshold EPRs could only retard the decline in FRS. Whilst the cobaltous ion has been used to inhibit ethylene production in other ethylene producing systems (mung bean and apple tissues, Lau and Yang, 1976; auxin treated mung bean hypocotyls, Yu and Yang, 1979; submergence promoted petiole growth, Samarakoon, 1985) data on its effect on abscission appears to be lacking from the literature.

The effect of the silver ion (as STS) inhibiting ethylene action has been widely reported in the ethylene mediated process of flower senescence (Halevy and Mayak, 1981; Veen, 1983; Nichols, 1984; Nichols and Frost, 1985). STS has been shown to inhibit abscission in some flower systems (Cameron and Reid, 1981, 1983; Mor et al., 1984a, b) yet is seems to have been largely ignored in examining fruit abscission. Nagao and Sakai (1985) however showed $AgNO_3$ to reduce abscission in young Macadamia fruit.

In soft green Glen Clova fruit STS slowed down the FRS decline, yet was not totally effective at preventing abscission zone weakening. The treatment was also effective at reducing the autocatalytic increase in EPR which occurs in the fruit during abscission. There was however still an increase in EPR and this could possibly account for the inability of STS to prevent the pigmentation changes associated with the ripening of soft green fruit. STS was reported to prevent the ripening associated pigment changes in tomato by Tucker and Brady (1987). Using STS however presents a problem in that there is no satisfactory control experiment to ensure that the silver ion exerts its effect through ethylene action alone. Hence although STS is effective at retarding abscission it is difficult to attribute this to an anti-ethylene action, especially in the light of the potentially phytotoxic nature

of the silver ion (Veen, 1983).

Another technique used to examine the role of ethylene in abscission was the maintenance of fruit at different stages of development under hypobaric pressures. These are considered to reduce ethylene levels within plant tissues (Burg and Burg, 1966) thereby influencing abscission. This technique is discussed fully in Section 1.3. The use of an atmosphere of 150 mmHg retarded the decline in FRS in fruit at all stages of development although the effectiveness was reduced in the more mature fruit. This inhibition was reversed by the presence of exogenous ethylene in the hypobaric atmosphere. The use of this control experiment confirmed the effect of the reduced pressure an abscission to be through a lowering of ethylene levels. The less effective nature of the treatment in the ripe fruit possibly reflects their high EPRs still maintaining significant levels of ethylene in the tissues, even under reduced pressures. Alternatively it can be envisaged that once ripe the machinery for abscission zone weakening is fully functional and the treatment has little influence. Similar reports of hypobaric treatments retarding fruit abscission have been described elsewhere (Cooper and Horanic, 1973; Rasmussen, 1974).

In the past additions of auxin have been shown to cause a range of effects from preventing to accelerating abscission (Addicott, 1970, 1982). Initially the treatment of debladed petioles with IAA showed auxin to retard abscission (La Rue, 1936). Later delayed applications were shown to have a decreasing effect as the delay after excision increased (Barlow, 1950). In some circumstances, such as IAA additions proximal to the abscission zone (Chatterjee and Leopold, 1963; Louie and Addicott, 1970) or delayed

additions (Rubinstein and Leopold, 1964) auxin accelerated separation. The capacity of auxin to enhance ethylene production (Zimmerman and Wilcoxon, 1935; Morgan and Hall, 1964; Abeles, 1966) is now considered to account for the accelerating effect of auxin on abscission.

In raspberry the morphology of the fruit makes it difficult to apply auxin immediately distal to the abscission zone. This is where it must be applied in other systems to have an inhibitory effect (Addicott, 1970). It therefore seems likely that the auxin additions made to raspberry fruit resulted in enhanced ethylene (and its subsequent effects) before any auxin reached the zone to inhibit abscission. More elaborate methods of applying auxin directly to abscission zones would have to be developed to investigate if auxin is capable of inhibiting abscission. The problems of applying auxin to fruit have been experienced in the fruit ripening field where apparently contradictory results have been recorded (Frenkel et al., 1975).

All the above results indicate ethylene to play a role in the control of raspberry fruit abscission. That role appears to be one of modulating the rate of abscission zone weakening, there being no conclusive evidence in favour of ethylene as the inductive agent. None of the experiments reducing internal ethylene levels (hypobaric treatment), reducing the EPR to below the threshold (AVG, Co^{2+}) or preventing ethylene action (STS), were capable of totally preventing the decline in FRS. The possibility of a changing sensitivity to ethylene can be invoked to account for low yet stimulatory ethylene levels. Within the raspberry fruit with its 50 to 100 abscission zones, ethylene can be envisaged as a coordinating

agent both of all the individual zones and of the cells within each individual zone.

In the raspberry there are two major structures, the cap of drupelets and the receptacle. In his cursory examination Blanpied (1972) showed the level of ethylene to be higher in the receptacle than the drupelets of ripe fruit. This was confirmed by the examination of both EPRs and internal ethylene levels of mottled, ripe and purple ripe Glen Clova fruit. At all stages beyond mottled the EPR of the receptacles were far in excess of the drupelets, always exceeding 10 nl/g/h. The drupelets only reached these rates when purple ripe, being less than 1 nl/g/h when mottled. These differences were also reflected in the internal ethylene levels. Differences in EPR of fruit tissues were also reported by Knee (1982) where the rise in the EPR of apples was first noticeable in the core yet ultimately it was the peel which had the highest production rates.

In terms of the threshold (0.25 to 0.5 ppm ethylene) both drupelets and receptacles of mottled fruit showed potentially abscission accelerating levels of ethylene. The presence of high EPRs in the receptacle and the autocatalytic nature of ethylene production make it likely that it is the receptacle which increases its ethylene production first, possibly inducing increased production in the drupelets.

Within the receptacles the apex region was shown to contribute the vast majority of the ethylene production, the calyx region producing very little. The significance of the apex region is that it is to this part that the drupelets are attached and hence from it that they abscise.

Preventing the fertilisation of some ovules (and hence drupelet development) was found to retard the swelling of the receptacle apex. This response is similar to the effect of achene removal preventing the receptacle swelling in strawberries (Nitsch, 1952; Archbold and Dennis, 1986). In these cases auxin was found to reinstate swelling and it may be that a similar mechanism exists in raspberries. Abscission can therefore be envisaged as being controlled by the ethylene in the receptacle, which itself may be dependent on drupelet development.

If ethylene controls the rate of fruit abscission then the question arises as to what causes the increase in EPR. The low EPR of immature soft green fruit was found to be the result of a lack of ACC, the immediate precursor of ethylene (Adams and Yang, 1979). The ethylene forming enzyme was shown to be constitutive by the application of exogenous ACC causing elevated EPRs at all stages of development. The lack of ACC is considered to be a general characteristic of tissues showing low EPRs (Yang and Hoffman, 1984).

The continual supply of ACC was found to accelerate abscission in soft green Glen Clova raspberries although in other cases ACC additions have not accelerated abscission (in olive leaves, Lavee and Martin, 1981). These authors concluded that the ACC was not supplied for long enough and the EPR was elevated for an insufficent period of time to accelerate abscission.

In Glen Clova raspberries ACC accumulated during the later stages of development. Similar observations have been made of ACC increasing in step with EPR in other climacteric fruit (eg avocado, banana and tomato, Hoffman and Yang, 1980). At first sight it was surprising that ACC was found to accumulate even though the EFE was

clearly not saturating as demonstrated by the higher EPRs achieved by the addition of exogenous ACC. The accumulation of ACC might be due to compartmentalisation within the tissues, thereby allowing the ACC to accumulate away from the EFE. Evidence for the existence of pools of ACC with different EFE availability within individual cells has been presented by Guy and Kende (1984).

Another factor making interpretation of ACC levels difficult is the rapid expansion occurring in many fruit tissues, often exclusively due to the uptake of water. This was found to be the case in Glen Clova receptacles where the actual ACC content of the receptacle apex increased with development, yet when expressed on a per gram basis appeared to decrease because of the rapid increase in apex weight.

Unfortunately the complications in the extraction and assay of ACC synthase prevented any further progress on the control of ethylene production in Glen Clova raspberries. The intitial results on ACC synthase levels in the receptacles have provided a start and do show an increase in activity with development. In other work (Boller et al., 1979; Yang, 1981) ACC synthase has been demonstrated to limit ethylene production in fruit tissues although this work has not been done on abscission zones. More recently Bleecker et al. (1986) have used monoclonal antibodies against tomato ACC synthase in its purification and characterisation. This resulted in the identification of a 50 kDa protein representing ACC synthase in extracts of wounded ripe tomato pericarp tissue.

In Glen Clova raspberries the effect of Ethrel applications to aid machine harvesting could have been predicted from the laboratory experiments. The additional ethylene would cause green

fruit to turn red and abscise whilst the ripe fruit containing saturating ethylene levels would not be affected. The rapid nature of the changes in green fruit would preclude full fruit expansion resulting in the smaller average red fruit weight reported from field trials (Jolliffe, 1975b, Knight, 1976). One would also expect the flavour not to develop properly since raspberries depend on a translocated import of sugars (D Mason, pers. comm) rather than a conversion of stored starch as occurs in bananas (Rhodes, 1980). Hence while the use of ethylene releasing sprays does aid fruit removal it is counterproductive because of the undesirable premature ripening and abscission of unpalatable immature fruit and the lack of effect on the naturally ripe fruit. It is possible that in varieties other than Glen Clova, which show low EPRs when ripe, Ethrel could enhance the abscission of the naturally ripe fruit. There would however probably still be the undesirable acceleration in the ripening of green fruit.

One possible way of controlling abscission would be to identify the factor limiting ethylene production in immature fruit and to stimulate ethylene production at a point preceding it. This treatment would not increase the ethylene production of immature fruit but would be effective in the more mature fruit. For example the conversion of SAM to ACC is considered to be limiting in many immature fruit (Hoffman and Yang, 1980), the application of methionine (from which SAM is derived) would be expected to have no effect on ethylene production in immature fruit since ACC synthase is limiting. In ripe fruit with the SAM to ACC pathway functional it would be expected to enhance ethylene production, as was shown in apple tissues by Lieberman et al. (1966). Used in conjunction with

a variety showing a low EPR when ripe (ie sub-saturating) the treatment could selectively influence the more mature fruit. Hence the use of fruit presently disregarded because of their relatively high FRS when ripe could provide a selective method of enhancing the abscission of ripe fruit. For example Jennings and Carmichael (1970) dismissed the variety Norfolk Giant because of its high FRS when ripe, even though Ethrel was shown to enhance abscission.

An alternative strategy could be to use a combination of treatments. These have been shown to prevent premature fruit abscission in olive (Ca^{2+} and ethylene releasing chemicals, Martin et al., 1981) and citrus (cycloheximide and gibberellin, Cooper and Henry, 1974). Using raspberry varieties with low EPRs when ripe it may be possible to keep the less mature fruit insensitive to ethylene whilst accelerating and synchronising the abscission of ripe fruit.

Another consequence of the ethylene producing capacities of ripe fruit of different varieties is the prospect of selecting fruit for fresh marketing with the longest possible post harvest life. At present marketing of fresh Scottish raspberries is made difficult because of their short post harvest life. In many fruit the presence of ethylene in the atmosphere reduces storage life and modified or controlled atmosphere storage is becoming more common (reviewed by Sherman, 1985). The post harvest life of raspberries might be maximised by using low ethylene producing varieties in which the ethylene induced ripening changes do not progress as rapidly as in those varieties with high EPRs when ripe. This was found to be the case in blackberries with the low EPR varieties keeping better than those with higher EPRs (Walsh et al., 1983). It

appears that those raspberry varieties being selected as most suitable for machine harvesting (ie low FRS when ripe) may not have the best shelf life because of the concomitant high EPRs.

Factors other than ethylene production govern the FRS of ripe fruit. This is illustrated in the variety Phyllis King which has a relatively high EPR yet maintains a high FRS when ripe. Analysis of the dimensions of the receptacle apex showed the gross morphological characteristics not to be significant in determining the FRS of ripe fruit. Fejer and Spangelo (1973) also concluded that factors other than receptacle shape determined the FRS of ripe fruit. It was however clear that the development of the apex, in terms of its surface topography, was linked to the FRS. Those fruit having a pimpled apex surface after separation of drupelets and receptacle were associated with the high FRS varieties, smoother apex surfaces being found in those varieties with lower ripe FRS values. The smoothing of the receptacle apex surface is a result of swelling especially between the points of drupelet attachment. In the normal process of development the apices progress from being white and pimpled to being smooth and having a red translucent appearance. Hence in those varieties showing low FRS when ripe (such as 68/14/106) the apex is smooth whilst higher FRS varieties such as St Walfried have pimpled apices when ripe. Likewise the EPR appears correlated with the development of the red translucant appearance, irrespective of surface topography. This accounts for the anomalous situation in Phyllis King having both high EPRs and FRS when ripe. The receptacle apex in ripe Phyllis King fruit was generally found to be pimpled, yet having a red translucant appearance.

4.2 The role of ethylene in petal abscission of Rubus idaeus L. cv Glen Clova and other flowers

The opening and shedding of petals by Glen Clova raspberry flowers is accompanied by increased rates of ethylene production. Whilst the sepals were opening there were very low EPRs, the increased rates being associated with petal opening and abscission. Once all the petals had been shed the EPR decreased.

The occurrence of increased EPRs associated with petal or corolla abscission have been reported elsewhere in cyclamen (Halevy et al., 1984b) and snapdragons (Wang et al., 1977). In Digitalis the pistil EPR was shown to increase prior to pollination induced corolla abscission (Stead, 1985). The maximal rates in whole Digitalis flowers occurred in those flowers in which the corolla had recently been abscised (Stead and Moore, 1977). There has also been a contradictory report that petal abscission in geranium occurs in the absence of increased ethylene production (Armitage et al., 1980).

The ability of ethylene to accelerate petal abscission has long been recognised since the early observations of Fitting (1911) on geraniums, an observation repeated more recently by Wallner et al. (1979), Armitage et al. (1980), Miranda and Carlson (1981) and Sexton et al. (1983). It has also been extended to the abscission of whole flowers and buds in zygocactus (Cameron and Reid, 1981) tomato (Roberts et al., 1984) tobacco (Henry et al., 1974) and Ecballium (Jackson et al., 1972; Wong and Osborne, 1978). The rate of raspberry petal abscission is similarly accelerated by exogenous ethylene. Halevy et al. (1984b) reported ethylene to be incapable of stimulating abscission in cyclamen corollas until they were pollinated, thereby demonstrating a remarkable increase in ethylene

sensitivity.

The effect of pollination enhancing flower abscission has long been recognised since the early reports of Gartner (1844) and Kendall (1918). It is now accepted that pollination can increase the EPR of many flowers (Burg and Dijkman, 1967; Hall and Forsyth, 1967; Nichols, 1977; Stead and Moore, 1983; Halevy et al., 1984b). Stead (1985) has shown that the degree of stimulation of ethylene production in Digitalis is related to the amount of pollen applied. An examination of pollination in raspberries was complicated by the natural self pollination of Glen Clova flowers. It is not practical to measure the EPR of emasculated flowers since the wounding involved would have vastly increased ethylene production in its own right. The method of emasculation usually adopted to leave just the pistils (Hardy, 1932; Eaton et al, 1968) removes the petals, thereby preventing direct observations on the effect of pollination on petal abscission.

The role of ethylene in petal abscission of Glen Clova flowers was examined using inhibitors of ethylene production (AVG) and action (STS). Aminoethyoxyvinylglycine inhibited the natural ethylene production (Boller et al., 1979; Yu et al., 1979) and retarded the rate of petal abscission when supplied continuously through the transpiration stream. Whilst AVG was exceptionally effective at preventing any rise in EPR the flowers still developed and shed their petals. The AVG did however slow down the whole process of petal abscission and reduced the degree of synchrony between the loss of petals from individual flowers. Normally the shedding of one or two petals would result in the rest being shed in the following 2 to 3 hours. The AVG disrupted this coordination

with individual petals being shed over a long period of time. Similar retarding effects of AVG have been reported for corolla abscission in snapdragon (Wang et al., 1977) and foxglove (Stead, 1985). Unfortunately neither of these pieces of work included the control experiment of both ethylene and AVG treatment. In Glen Clova flowers such a treatment showed the AVG effect to be overcome by an exogenous supply of ethylene, thereby indicating AVG to function through an inhibition of ethylene production.

Hence in Glen Clova flowers a rise in ethylene production is not essential for full flower development and abscission which occurs while the petals are white, turgid and having a healthy appearance. The role of ethylene appears simply to coordinate the shedding of all the petals of individual flowers over 3 or 4 hours.

Further confirmation of ethylenes role came from the use of STS, an inhibitor of ethylene action (Veen, 1983). This was found to be more effective than AVG at retarding petal abscission although it was not possible to totally prevent abscission. This retarding effect of STS on petal abscission has also been reported for petals of geranium (Cameron and Reid, 1983), corollas of cyclamen (Halevy et al., 1984a) and Streptocarpus (Agnew et al., 1985) and sweet pea florets (Mor et al., 1984b). Unfortunately there is no satisfactory control procedure available to ensure that STS has its effect simply through an inhibition of ethylene action alone. The effect of the silver ion as an inhibitor of ethylene action has been used extensively to investigate the relationship between ethylene and petal senescence (Nichols, 1984). The aim has been to increase the post harvest lives of cut flowers and pot plants by preventing colour fading, morphological changes and wilting (Cameron and Reid,

1981, 1983; Nichols et al., 1982; Mor et al., 1984a, b; Nowak and Mynett, 1985). These changes may or may not accompany the shedding of the petals. In Glen Clova flowers however (as with the corolla in Digitalis, Stead and Moore, 1983) the petals are shed prior to the appearance of any of these senescence characteristics.

Both AVG and STS retarded the onset of senescence when assessed by the appearance of a brown area at the petal base. This is in agreement with other published work on flower senescence (for example, Veen and Van de Geijn, 1978; Reid et al., 1980; Whitehead et al., 1984; Mor et al., 1984a, b). The AVG mediated inhibition of petal senescence in Glen Clova flowers was reversed by exogenous ethylene, indicating it to be an ethylene mediated phenomenon.

As with Glen Clova fruit abscission there is no evidence to support an inductive role for ethylene in petal abscission. In fact the flowers capacity to develop and abscise petals in the absence of a rise in EPR suggests ethylene not to be an absolute requirement for abscission. Ethylene does clearly play a coordinating (and thus accelerating) role in petal abscission.

The control of ethylene production in raspberry flowers appears to be through the supply of ACC which is limiting in early stages. The conversion of SAM to ACC is usually considered to be the rate limiting step in plant tissues showing low EPRs (Kende et al., 1985). Hence the supply of ACC to various plant organs causes marked increases in ethylene production (Yang et al., 1985) although flowers were cited as exceptions to this case. The results presented here indicate the EFE converting ACC to ethylene to be functional in the early stages of flower development. At early stages of flowering the low EPRs correspond to low levels of ACC,

both of these increase during petal opening and abscission.

This accumulation of ACC occurs despite the EFE not being saturated, as demonstrated by the EPRs being below those possible through the addition of exogenous ACC. This accumulation of ACC with aging has also been shown to occur in carnation flowers by Bufler et al. (1980), Veen and Kwakkenbos (1982/83) and Hsieh and Sacalis (1986). The accumulation of ACC can easily be seen as a result of the existence of pools of ACC having different EFE availability, both at the cellular level (Guy and Kende, 1984) or at the tissue level. The levels of ACC in parts of carnation flowers clearly shows this differential accumulation at the tissue level (Bufler et al., 1980; Veen and Kwakkenbos, 1982/3; Hsieh and Sacalis, 1986).

In raspberry petal abscission, as in corolla abscission in snapdragon (Wang et al., 1977) and foxglove (Stead, 1985), ethylene acts to accelerate abscission. As a result the individual petals of each flower are shed in a coordinated way over a period of approximately 4 hours. Preventing the normal rise in EPR, or ethylene action, removes this coordination. The control of ethylene production depends on the supply of the precursor ACC, which in turn is dependent on ACC synthase or some earlier step in its biosynthesis.

4.3 Summary of the main conclusions derived from the experimental work on ethylene in fruit and petal abscission in Rubus idaeus L. cv Glen Clova

Both fruit and petal abscission were accelerated by exogenous supplies of ethylene and ACC, indicating the natural ethylene levels to be sub-saturating. In the ripe fruit this capacity is lost with the increased EPR providing naturally saturating ethylene levels.

A rise in EPR was found to coincide with both fruit and petal abscission. In the case of petals the EPR increase clearly preceded abscission whereas in fruit this was not so clear cut. There was evidence of an increase prior to abscission in Glen Clova fruit although in some of the other varieties this was clearly not the case and the EPR increased late on in development.

In fruit of Glen Clova the concentration of ethylene accumulating within the fruit easily reached those levels which must be added to accelerate abscission. These ethylene levels were achieved naturally at the mottled stage, the very time at which FRS is starting to decline.

In both fruit and petal abscission the use of inhibitors of ethylene production or action retarded abscission. In none of these inhibitor studies however could abscission be prevented totally.

Hence in both fruit and petal abscission ethylene is involved as a coordinating/accelerating agent. The coordination exists between the 50 to 100 individual drupelets of the fruit and between the individual petals of flowers. On an individual abscission zone basis coordination may also occur between the cells of each zone. The increased coordination results in an accelerated

abscission of both the drupelet cap and the petals.

Although the mechanism of induction of increased ethylene production is unknown the EPR in both fruit and petal abscission is limited by the supply of ACC. This is presumably as a result of low ACC synthase activity. In fruit and flowers the endogenous ACC levels increased in step with the EPR.

In fruit abscission the role of the receptacle is viewed as having great significance. The EPR increased first in the receptacle, possibly inducing autocatalytic ethylene production in the drupelets. The enlargement and swelling of receptacle tissues are important, both in the abscission zone weakening process and in the increased EPR. This swelling of the receptacle appears to be controlled by the development of fertilised drupelets.

The following hypothesis is put forward for the events controlling fruit abscission in raspberries.

Either during flower development or in the early phases after fertilisation an abscission zone is differentiated at the base of each drupelet. The factors that control the abscission zone's positional development are unknown.

The receptive abscission zones remain quiescent during early fruit development, possibly as a result of abscission inhibitors or the absence of an abscission stimulus. The nature of this control is unknown.

Fertilisation ensures the drupelet development which maintains a quiescent abscission zone. Failure to fertilise an ovule results in young drupelet abscission.

The fertilised embryo produces a factor which induces swelling of the receptacle adjacent to the point of drupelet

attachment.

As fruit approach 2.5 g in weight two changes occur. i) The abscission zone separation is induced and weakening commences. ii) Ethylene production increases in the receptacle, presumably through increased ACC synthase activity. The nature of the inductive agents are unknown.

The elevated EPR in the receptacle causes an increase in the EPR of the drupelets (possibly through an increased ACC synthase activity), resulting in threshold levels of ethylene within the fruit. This increases the rate of loss of tissue integrity in individual zones and synchronises the weakening of the whole population of abscission zones within the individual fruit. The rise in drupelet EPR results in accelerated pigmentation changes (chlorophyll degradation and anthocyanin accumulation) and tissue softening.

The level of ethylene in the abscission zone rises to saturating, the FRS declines and the pigmentation changes all occur rapidly.

Unfortunately rather less is known about raspberry petal abscission and at present insufficient hard data is available for even speculative comments to be worthwhile on the mechanism of petal abscission.

4.4 Discussion of the hypothesis that ethylene is a major controlling factor in abscission

There is a large body of evidence in favour of ethylene as a controlling agent in abscission. Of all the diverse abscission systems examined so far only leaves from 4 species (Quercus robur, Fagus silvatica, Pinus silvestris and Hedera helix, Abeles, 1973) and fruit from 2 (oat, Sargent et al., 1984; oil palm, Chan et al., 1972) have been found to be unresponsive to additions of exogenous ethylene. Those systems which have been found to be responsive include amongst others, bud scales (Burdon et al., 1986) flowers (Roberts et al., 1984), petals (Stead and Moore, 1983) styles (Sipes and Einset, 1982) young fruit (Davenport and Manners, 1982) mature fruit (Goren and Huberman, 1976) leaves (Osborne and Sargent, 1976a) and stems (Webster and Leopold, 1972). These responses have been documented in a wide range of plant families. Hence of all plant growth regulator responses ethylene's effect accelerating abscission is perhaps the most widely demonstrated.

The threshold levels of ethylene required to accelerate abscission have also been found to be very similar in widely differing abscission systems. Generally 0.1 ppm ethylene is the minimum threshold required whilst 10 ppm saturates the response (Abeles and Gahagan, 1968; Osborne and Sargent, 1976b; Armitage et al., 1980). The threshold value of 0.25 to 0.5 ppm ethylene determined for immature raspberry fruit is therefore consistent with data already published.

Having shown exogenous ethylene to be capable of enhancing abscission the next question is whether or not it has a role in natural abscission. There is no doubt that in raspberry fruit the

ethylene levels are such as to be significant in the control of abscission. In other systems however it is harder to be certain since it is difficult to get an accurate measure of the ethylene levels at the abscission zone. These levels that have been published are often extrapolated from the EPR of some material, such as an explant, of which the abscission zone is only a small part (Jackson and Osborne, 1970; Nunez-Eilsea and Davenport, 1986). Alternatively the internal ethylene level of a bulky organ such as a fruit is determined and taken to give a level equivalent to that of the abscission zone (for example, cotton fruit, Lipe and Morgan, 1973). This is unsatisfactory as the tissue in which the ethylene level has been determined is clearly not representative of the abscission zone. A clever alternative approach was employed by Jackson et al. (1973) who added CEPA to bean petioles to provide the same rate of ethylene emanation as was found just prior to natural abscission. The treatment was found to accelerate abscission.

Further evidence for the involvement of ethylene in numerous abscission systems comes from the following methods of reducing ethylene levels or action. The ethylene absorbing chemicals mercuric perchlorate and potassium permanganate have been used to maintain ethylene free atmospheres (Jackson and Osborne, 1970; Young and Meredith, 1971). Hypobaric treatments have been used to reduce the levels of ethylene within plant tissues (for example, Cooper and Horanic, 1973; Morgan and Durham, 1980; Kelly et al., 1984). The inhibitors of ethylene production AVG (Sagee et al., 1980; Kushad and Poovaiah, 1984; Stead, 1985), AOA (Nagao and Sakai, 1985) and Co^{2+} and the inhibitors of ethylene action Ag^{+} (Cameron and Reid, 1981, 1983; Mor et al., 1984a, b) and CO_2 (Abeles and

Gahagan, 1968, Jerie, 1976) have all been used to examine ethylene's role in abscission.

Although it is now clear that ethylene plays some role in the control of abscission the question arises as to at what level this control is operating. As discussed in Section 1.2 there are three possible levels of control, separation layer differentiation, induction of cell separation and the rate of abscission zone weakening. Discussions in the abscission literature rarely distinguish at which point in the developmental programme control is being exercised.

4.4.1 Evidence of ethylene involvement in separation layer differentiation

The development of cells in the separation layer to a state capable of responding to an abscission stimulus may well involve control by ethylene, although the prospect has not been examined in depth. The favoured experimental system used to examine separation layer differentiation involves the formation of adventitious (or secondary) abscission zones in positions where abscission does not normally occur.

Webster and Leopold (1972) found ethylene to be capable of inducing adventitious abscission zones in Phaseolus internodes. On the other hand the formation of adventitious zones in apple pedicels (Pierek, 1977, 1980) was not affected by CEPA but was influenced by auxin. A more recent piece of work by Warren-Wilson et al. (1986) also showed the auxin concentration to be critical in the positioning of separation layer differentiation in Impatiens internodes.

In all the above instances the conclusions drawn from auxin or ethylene effects must be considered in the light of possible interactions between the two, ethylene being capable of reducing auxin levels whilst auxin can stimulate ethylene production (see Section 1.2). Once differentiated it is assumed that the separation layer can remain quiescent until activated. Ethylene may have a role as the inductant in both natural and experimental abscission systems. Exogenously added ethylene certainly accelerates abscission (recent examples, Davenport and Manners, 1982; Stead and Moore, 1983; Roberts et al., 1984; Burdon et al., 1986) it is however difficult to be certain that ethylene acts as the inductive agent because of the large number of effects is has on other growth regulators, especially auxin (see Section 1.2).

4.4.2 Evidence of ethylene acting as the inductive agent in abscission

There appear to be two approaches which could provide convincing evidence that ethylene is the inducer of abscission. Firstly it should be possible to block abscission totally by inhibiting ethylene production or action if it is the inductant. From a review of the literature there have been numerous reports of inhibitors retarding abscission (see above and Section 1.3) yet there are no satisfactory reports of abscission having been totally stopped. A common shortcoming in the experimental technique is to terminate the experiment once the controls show total or near total abscission. There is thus no way of knowing whether the inhibitor treated samples would have abscised at a later date if given the chance. This results in the impression being given that the process

of abscission has been totally blocked and that ethylene is the inductive agent. An example of this is the paper by Cooper and Horanic (1973) in which hypobaric treatment of lime fruit was claimed to prevent CHI induced abscission. Whilst counting those fruit which had abscised fully did show this, their measurement of abscission zone integrity showed a 24% reduction in the FRS of the CHI and hypobaric treated fruit compared to controls. Hence the abscission process had only been retarded and it would appear that a larger experimental duration would have allowed full abscission of the CHI and hypobaric fruit.

Some of the most effective inhibition of abscission occurs in flowers treated with Ag^{+}, usually as STS (Cameron and Reid, 1981, 1983; Halevy et al., 1984a, b; Mor et al., 1984a, b; Agnew et al., 1985). The results however are complicated by the methods of assessing efficacy (as above) and the lack of a control experiment to show the silver treatment to have an effect via ethylene action alone. For example Mor et al. (1984b) described STS inhibition of sweet pea flower abscission to be complete 3 days after storage. The vase life of the STS treated flowers however was reported as over 7 days compared to 3 days for the controls. Hence by stopping the experiment at 3 days it is not possible to distinguish between retardation and total inhibition.

The second approach that may eventually show definitively that ethylene acts as the inducer of abscission is to understand the molecular details of the biochemical processes by which it acts. This is a more definitive route since even if it had been possible to totally stop abscission through blocking ethylene production or action, it cannot be said to be the primary inductant since there is

the possibility of it having a secondary role via auxin levels.

This definitive characteristation of an inducer must await the elucidation of the complete molecular mechanism by which ethylene acts. This starts with its interaction with a receptor, a process which is only now in the early stages of examination, and ultimately results in an effect at the gene level. Little progress has been made in this direction in abscission although new mRNA species have been shown to appear in ethylene induced abscission zones (Kelly et al., 1987).

Further progress has been made in ethylene controlled fruit ripening where in tomato an mRNA for polygalacturonase (Grierson et al., 1986) and in avocado an mRNA for cellulase (Tucker et al., 1985) have been shown to appear. Ethylene has also been shown to induce chitinase activity in Phaseolus (Boller et al., 1983; Boller and Vögeli, 1984). There is every prospect that the modern techniques of gene cloning and DNA sequencing may provide answers in the field of abscission in the not too distant future. Only when the regulation of genomic clones can be examined will the full role of ethylene and other growth regulators in the induction of abscission be unravelled.

4.4.3 Evidence of ethylene controlling the rate of abscission zone weakening

There is now a long list of evidence in favour of ethylene modulating the rate of abscission zone weakening including the numerous cases of ethylene accelerated abscission listed earlier in this section. The experiments using AVG (including Kushad and Poovaiah, 1984; Roberts et al., 1984) STS (Cameron and Reid, 1981,

1983; Mor et al., 1984a, b) and Co^{2+} all indicate that an interference with ethylene production or action results in a retardation of the rate of weakening. Likewise the reduction in tissue internal ethylene levels also retarded abscission (Cooper and Horanic, 1973; Rasmussen, 1974; Morgan and Durham, 1980; Sipes and Einset, 1982). Rasberry fruit abscission responded to all these treatments in a fashion indicative of ethylene controlling the rate of abscission zone weakening. In terms of the biochemical basis for this acceleration protein synthesis and mRNA transcription have been shown to be enhanced by ethylene (Abeles and Holm, 1966, 1967; Holm and Abeles, 1967; Abeles, 1968). Ethylene is also capable of stimulating abscission in which transcription is not required, thereby exerting an effect at some translational or post translational level (Henry et al., 1974). Abeles and Leather (1971) showed ethylene to control the secretion of cellulase at the abscission zone. This is as well as increasing the rate of cellulase synthesis and reducing the time lag before it is produced (Abeles, 1969; Ratner et al., 1969; Lewis and Koehler, 1979). Likewise in those systems where an increase in polygalacturonase has been detected ethylene increased its rate of production (Riov, 1974; Greenberg et al., 1975; Huberman and Goren, 1979).

In all the above cases ethylene is shown to be ideally suited to a role accelerating abscission since its influence on diverse processes always acts in a way likely to enhance abscission. In addition, ethylene is produced autocatalytically, it reduces auxin levels and makes the zone more sensitive to itself (Beyer and Morgan, 1971; Morgan and Durham, 1972). Hence ethylene has ideal characteristics as an accelerating and coordinating

agent. This raises the question as to why abscission zones should require coordination by ethylene. The coordination can be envisaged as occurring at two levels. In fruit and flowers such as found in raspberry, the abscission of the numerous drupelets or petals has to be orchestrated. At the tissue level coordination between the cells of an individual abscission zone is required. If only part of an abscission zone was to weaken then the other areas might tear and damage the plant.

The question also arises as to why the rate of abscission should be controlled. In numerous cases natural abscission can be seen to occur slowly as senescence of the distal tissues occurs, but may be accelerated under specific circumstances. In the abscence of pollination there is often a long period of slow senescence in the flower corolla in Digitalis (Stead and Moore, 1983). If pollen is added to the stigma there is rapid corolla abscission which occurs without corolla senescence. This is assumed to be a mechanism to prevent scarce pollinators making return visits to those flowers already pollinated. This accelerating and coordinating effect appears to be the role played by ethylene in raspberry petal abscission . Ethylene accelerated abscission probably also occurs in infected leaves (Wiese and de Vay, 1970; Ketring and Melouk, 1982). Also infection of citrus fruit by Penicillium digitatum results in increased levels of ethylene production (Achilea et al., 1985). Presumably the rapid abscission of an infected organ prevents the spread of infection to the main body of the plant. A similar argument can be made for the shedding of organs damaged by herbivores or those not developing properly (eg in mango fruitlets, Singh, 1961). In these cases abscission must obviously be rapid for

the strategy to succeed. Normally the slow abscission is accompanied by the senescence of distal tissues, a process which allows the recovery of materials from the organ to be shed. When abscission is accelerated complete senescence of the abscised organ does not necessarily take place. There must be a benefit to the plant of this rapid removal which outweighs the loss of nutrients which would normally be recovered. As was found in the Ashton Cross blackberry ethylene was not needed for the natural weakening, yet the fruit were responsive to exogenous ethylene. This presumably allows an ethylene acceleration of abscission should circumstances arise that require it.

In summary there is neither evidence for nor against ethylene controlling abscission through the differentiation of the separation layer. There is much circumstantial evidence in favour of ethylene controlling the activation of the separation layer, although hard proof is lacking. The majority of work on ethylene and abscission points to ethylene simply modulating the rate of weakening, and not being the absolute requirement as was initially thought.

4.5 The role of other growth substances and tissue sensitivity in the control of abscission

In the preceding section the role of ethylene in abscission is discussed. It is clearly the favoured candidate for the roles of both abscission inducer and accelerator. There is however a strong case for the involvement of other potentially regulatory forces.

From the evidence obtained so far the role of auxin must not be disregarded. It has been shown to be a potent inhibitor in explants of leaves (Jackson and Osborne, 1972; Riov, 1974) fruit (Greenberg et al., 1975) pistils (Einset et al., 1981) cotyledons (Morgan, 1976) and bud scales (Burdon et al., 1986). As was found in this study there are however instances where auxin applications accelerate abscission (Rubinstein and Leopold, 1963; Addicott, 1970). This accelerating effect is considered to be the result of auxin enhanced ethylene production (Abeles and Rubinstein, 1964; Morgan and Hall, 1964). Other support for auxin's role comes from the evidence of decreasing IAA levels during natural abscission (Shoji et al., 1951; Roberts and Osborne, 1981).

It is thus possible that IAA is the primary regulator and ethylene has its effect through its capacity to reduce IAA levels. This hypothesis is supported by experiments showing IAA added before, or at the same time as, ethylene to completely negate the latters effects (Jackson and Osborne, 1972; Riov, 1974; Greenberg et al., 1975; Osborne and Sargent, 1976b). In addition ethylene has been shown to inhibit auxin transport (Beyer and Morgan, 1971), increase auxin destruction and conjugation (Riov and Goren, 1979), decrease auxin synthesis (Ernest and Valdovinos, 1971) and reduce auxin sensitivity (Abeles et al., 1971). Through the separate

exposure of leaf blades and their abscission zones to ethylene Beyer (1975) showed that treatment of the source of auxin supply (ie the lamina) alone had no effect, treatment of both the zone and lamina together however resulted in abscission. Beyer concluded from this and other data that the effect of ethylene was two fold. Firstly it increased the zones sensitivity to itself through a reduction in the auxin flow from the lamina. Secondly once the zone was sensitive to ethylene it could induce abscission zone weakening.

There are a number of cases showing positive correlations between the endogenous levels of ABA and abscission (Böttger, 1970, Davis and Addicott, 1972; Bentley et al. 1975; Sargent et al., 1984; Guinn and Brummett, 1987). There are also cases however showing no clear correlation (Perry and Hellmers, 1973; Peterson et al., 1980; Ramina and Masia, 1980; Takeda and Crane, 1980). Doubt is cast on ABA's role in abscission as a result of the widespread failure of ABA additions to stimulate the process (Millborrow, 1974). The capacity of ABA to stimulate ethylene production has also been used as an explanation for its effect in some cases (Jackson and Osborne, 1972; Sagee et al., 1980) although in other systems under conditions preventing ethylene action ABA has been shown still to have an effect (Craker and Abeles, 1969; Cooper and Horanic, 1973; Rasmussen, 1974). None of the above data definitively implicates ABA as having a role in abscission, none the less Addicott (1982) states that ABA's promotive role in abscission is established beyond reasonable doubt. An ethylene independent role for ABA has recently been supported by evidence presented by Sargent et al. (1984). They showed seed abscission in wild oat to be insensitive to ethylene yet to be accelerated by ABA. This does not however prove a role for

ABA in the control of oat seed abscission.

Both gibberellins and cytokinins can influence the rates of abscission either inhibiting or accelerating the process. Gibberellins generally retard young fruit abscission (Rappaport, 1957; Crane, 1969) yet if applied to explants the effect is generally stimulatory (Greenblatt, 1965; Lewis and Bakhshi, 1968a). Low doses of gibberellins applied to cotton explants however have been shown to retard abscission (Carns et al., 1961). Cytokinin is an effective retardant of abscission (Gorter, 1964) although the response depends on how close to the zone it is applied. At sites distant from the abscission zone the application of cytokinin can accelerate abscission (Osborne and Moss, 1963; Carr and Burrows, 1967) by promoting the sink strength of the site of application. Neither gibberellins nor cytokinins are considered to be major controlling factors in controlling abscission. The interrelationships with other regulators, such as gibberellin enhanced IAA synthesis (Muir and Valdovinos, 1970) does show that they play a role in the overall status of the plant which in turn may influence abscission.

A major controlling factor in abscission, as with so many other plant growth processes, is the sensitivity or responsiveness of the tissue (Trewavas, 1982). For instance, the application of auxin to bean leaf abscission zones before or synchronously with ethylene makes the tissue unresponsive to the latter (Jackson and Osborne, 1972). Equally the application of ethylene to abscission zones for 24 hours makes them unresponsive to subsequent IAA additions (Jackson and Osborne, 1972). Jaffe and Goren (1979) reported the ability of auxin to retard abscission to be lost in

parallel with a loss in the ability to stimulate H^+ efflux from the abscission zone. It was therefore speculated that the loss of a common component in the IAA response machinery resulted in an effect on these two different processes. It was shown at the same time that the capacity of microsomal membranes to bind IAA also decreased.

A clear case of the potency of sensitivity changes occurs in cyclamen petals which are unresponsive to ethylene unless the flowers are pollinated (Halevy et al., 1984b). In Lilium the buds go through a 2 week period of being responsive to ethylene, outside this 'developmental window' ethylene has no effect (Durieux et al., 1983). In these cases it is not the levels of plant growth regulators that is the primary controlling factor in abscission, but a change in responsiveness.

Trewavas (1982) has suggested that sensitivity is a reflection of the fact that receptors are important in growth regulator responses. The lack of receptors makes the tissue unresponsive. There is little data on whether this is the case in abscission although Jaffe and Goren's (1979) point described above is in this direction. The binding of plant growth regulators has been thoroughly reviewed recently by Venis (1985) indicating that binding of the five main regulators (auxin, ABA, cytokinin, ethylene and gibberellin) to some cellular fraction does occur. Of these auxin binding systems are best characterised, having been examined since the early 1970's (Hertel et al., 1972). Whilst there is considered to be more than one site, on different cellular membranes, there is general agreement on the site of one in the endoplasmic reticulum. Whilst auxin has been examined extensively

studies on the other growth regulators are less advanced. This resulted in Venis (1985) concluding that at present only one auxin receptor (in maize coleoptile membranes) can be considered to exist with certainty. In cytokinin, gibberellin and ethylene binding sites there is still a lack of evidence to confirm receptor functions. Abscisic acid receptors have been detected (Hornberg and Weiler, 1984) although binding fractions have not yet been isolated. Hence it is not possible to explain changes in abscission responsiveness in terms of changes in the levels of receptors, although progress in this field is being made.

In the literature on abscission there is a wide array of often contradictory information regarding the role of different regulatory factors. As a result no clear concensus has emerged as to how the process is controlled. There is still much debate with some researchers in favour of IAA or ABA (Addicott, 1982) and others favouring ethylene (Morgan, 1984) as the main controlling agent.

The failure to make progress may well be due to two unwritten assumptions which permeate the literature making the interpretation of results difficult. The first assumption is that there is only one control point in the abscission programme and that this is common to all abscission systems. This results in the search for a single control hypothesis capable of reconciling the conflicting data gathered from widely different abscission systems. In different types of abscission zones the progress of abscission may well be suspended at different points in the developmental programme. As has already been discussed (Section 4.4) abscission zone cells are first subjected to control at the differentiation stage. Once competent separation layer cells have developed they

are assumed to be subjected to both repressive and inductive control. Finally once the separation layer has been induced the rate of weakening can be retarded or accelerated. In most experimental systems it is impossible to define at which stage in the sequence the material is at. Thus an experimenter adding or reducing the levels of growth substances does so with a lack of knowledge about the stage of the process being influenced. In some abscission systems (such as stems) it may be necessary to cause the zones to differentiate, to induce them and cause separation, whilst in others (such as petals) the weakening may be well underway and all the growth regulator is influencing is the last stage of the process.

There is evidence that different abscission zones which have been examined experimentally may be controlled at different points. For example petal and flower bud abscission can be very rapid, occurring in 2 to 6 hours after adding ethylene (Henry et al., 1974; Sexton et al., 1983; Halevy et al., 1984b). In these rapid abscission systems the control of the production of abscission specific proteins is at the translational level (Henry et al., 1974; Hänisch ten Cate et al., 1975a, b). In fruit and leaf abscission separation occurs more slowly over 24 to 72 hours (for example in leaves of Melia azedarach L., Morgan and Durham, 1980, Impatiens, Sexton et al., 1984; Ficus, Graves and Gladon, 1985; and fruit of Ecballium, Jackson et al., 1972, mango, Nunez-Elisea and Davenport, 1986). In these systems inhibitor studies have shown the production of abscission specific proteins involved transcriptional control (Abeles, 1968; Abeles et al., 1971). Stem abscission is even slower taking anything from several days to several weeks to occur (Webster

and Leopold, 1972; Warren-Wilson et al., 1986). These large differences in timing have in some cases been illustrated with different abscission zones in the same plant. Warren-Wilson et al. (1986) have shown that explants of Impatiens stems take 25 days to abscise whilst Impatiens leaf explants take only 18 hours (Sexton et al., 1984).

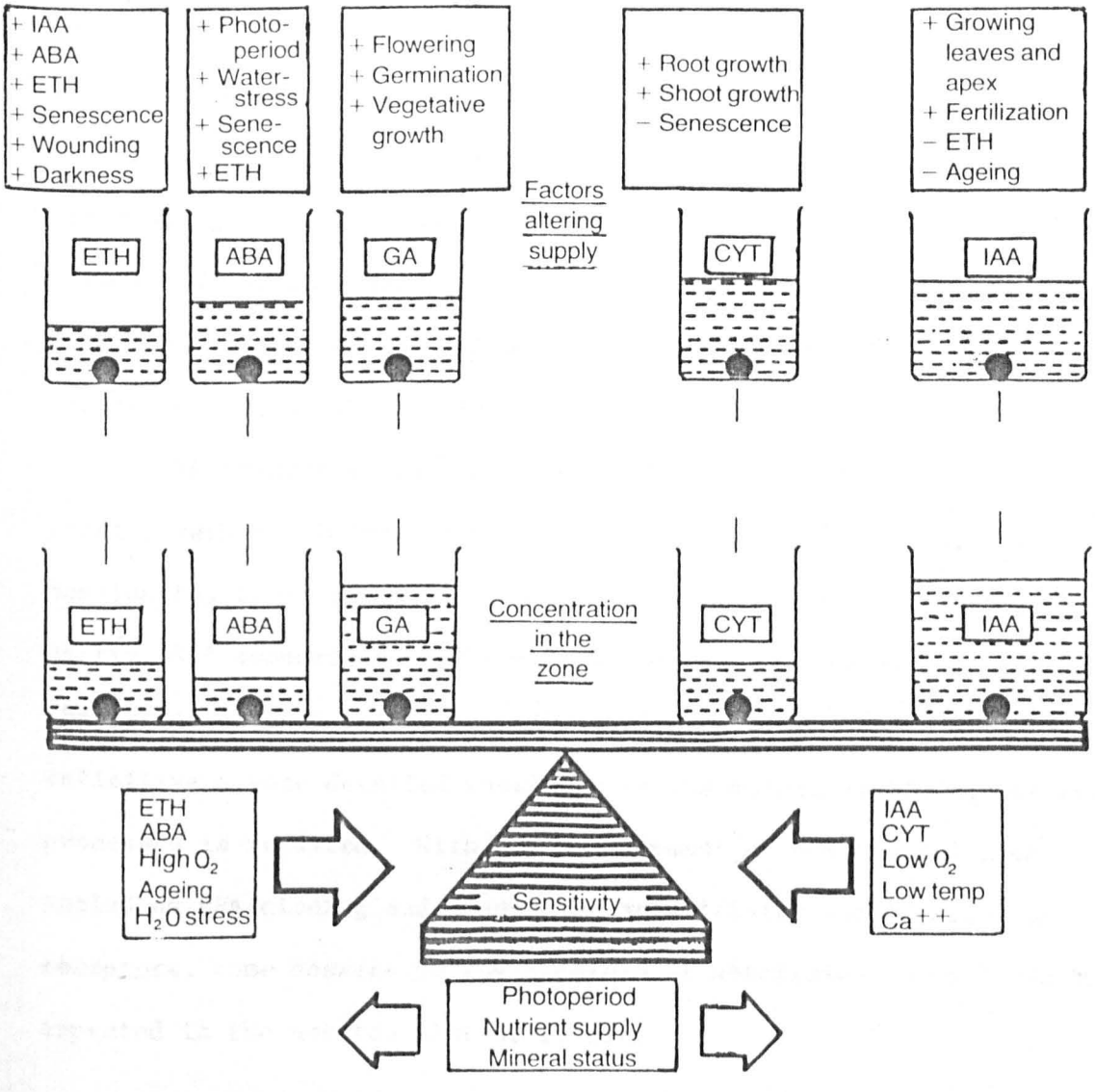
The biochemical evidence which points to control at transcriptional and translational levels in different systems, plus these major variations in the time courses in different abscission zones, argues strongly for different control mechanisms in different types of zone. Thus it may well be that we should not expect a simple concensus model which will reconcile all the data. Instead in each experimental system the point in the developmental process at which abscission is suspended must clearly be defined.

A second assumption in the literature which clouds interpretation is that the process of abscission is simple in biochemical terms. Many authors adopt the simple model that the rate of weakening is solely regulated by wall hydrolase induction. This oversimplification leads to the seemingly plausible view that since only one process is involved only one regulator is required. Abscission is obviously more complex than this. For instance the rate of cell wall weakening is not only dependent on its rate of breakdown but also its synthesis and repair. If ethylene is removed from an already weakening abscission zone the rate of further weakening is retarded (de la Fuente and Leopold, 1969; Abeles and Leather, 1971; Kelly et al., 1984). Another important component in weakening is the production of mechanical stresses across the separation layer which help to break it. By recognising 3 separate

component processes it is possible to envisage 3 different regulatory substances influencing breakdown, synthesis and the differential tissue expansion. Even this is still too simplistic, for example the rate of cell wall hydrolase production can be controlled specifically at the levels of transcription, translation or secretion. Factors which regulate more general features such as overall rates of protein synthesis or respiration may also be involved. The complexity of the abscission process can be compounded further by considering that the different cell types within the separation layer may not all respond biochemically in the same way. In the cortical parenchyma middle lamella breakdown and turgor driven rupture is important (Sexton and Redshaw, 1981). In collenchyma there is breakdown of the primary cell wall as well as the lamella (Sexton, 1976) whilst in the xylem the production of tyloses is implicated in rupture (Sexton, 1976). The overall weakening process can thus be seen to involve numerous elements. If this complexity is recognised it is possible to begin to understand how a myriad of factors could influence the rate of abscission. It also becomes clear that there may well not be a single inducer nor just one way of influencing the rate of the process.

Thus the search for a single regulatory substance may not be realistic. Multifactorial control seems equally likely. Arguments about the precise nature of the abscission inducer seem futile against the background where our detailed understanding of the biochemical changes occurring during abscission remains so rudimentary.

Even if it could be shown that the absence of a single compound could stop abscission (which is far from the case at



A diagrammatic representation of the possible multifactorial control of abscission. Abscission is envisaged as being determined by the relative concentrations of accelerators and inhibitors and the sensitivity of the zone to them. In this diagram the accelerators are shown on the left hand side (LHS) of a beam balance and they are counterbalanced by inhibitors on the right hand side (RHS). If the LHS of the balance goes down, abscission takes place. Ethylene and IAA have been positioned at the ends of the beam since changes in their concentration (weight) are relatively more effective than fluctuations in the regulators positioned nearer the fulcrum. The concentration of each regulator in the zone is determined by the rates of synthesis, transport and loss and this is depicted as a supply dripping into and out of the containers on the beam. Some of the possible factors that increase (+) and decrease (−) the concentrations of each regulator in the zone are shown above the supply tanks. The balance of the beam can also be affected by changing the sensitivity. This is depicted by being able to move the position of the fulcrum along the beam to the right, making the accelerators more effective and to the left decreasing their efficacy. Some of the factors that influence sensitivity are depicted together with arrows indicating how sensitivity is affected. This model oversimplifies the changes in sensitivity since it assumes that responsiveness to all the accelerators will change together; they may well be able to change independently of one another. ETH = ethylene, ABA = abscisic acid, IAA = auxins, CYT = cytokinins, GA = gibberellic acid

Fig. 4.1 Possible multifactorial control of abscission (from Sexton et al., 1985).

present) it would still be difficult with present techniques to prove it to have its effect directly and not through some other growth regulator. The definitive identification of the inductive agents must await the unravelling of the detailed molecular biology surrounding the induction of abscission. It is necessary to understand the interaction of plant growth regulators and their receptors and the way in which effector molecules interact with the genome to control the expression of abscission specific genes.

At present it is not possible to state whether a single plant growth regulator, or the change in sensitivity to one, or a combination of factors, induces abscission. The model illustrated in fig. 4.1 summarises the current knowledge of factors influencing abscission without defining their level of action. To be more definitive a more detailed knowledge of the molecular biology of the processes is required. With the development of new technologies including DNA cloning and sequencing and affinity purification of receptors, some answers to the problems of abscission control can be expected in the not too distant future.

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