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Avocado Fruit Development and Ripening Physiology

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I. INTRODUCTION

The avocado (*Persea americana* Mill.) has a long history as both a subsistence and marketable fruit in the areas of origin in Central and South America. Recently, however, a considerable trade has developed, both locally and internationally, with the fruit becoming well known in the industrialised areas of North America and Europe. The largest producers (Table 7.1) are, nevertheless, not substantial exporters. Marketing remote from the production areas has become important to the avocado industries of the United States (particularly California and Florida), Israel, and South Africa. These three countries are the main exporters and account for 90% of the world's exports, with Israel having by far the largest percentage (Van Zyl and Groenewald 1986). Rapid expansion is also taking place in Spain, with much of the crop destined for Northern European markets.

Trade with distant markets requires the production of high quality fruit and efficient transport so that it may arrive at the market in sound condition. There are, however, many references in the literature to mesocarp discoloration (Van Lelyveld and Bower 1984) and chilling injury (Chaplin et al. 1982) which Couey (1982) attributed to long periods of cold storage during the transport of avocado fruit.

Table 7.1. Annual Avocado Production in the Five Major Producing Areas of the World (Anon. 1986)

Country	Production (million kg)
Mexico	450
Brazil	270
United States of America	190
Dominican Republic	90
Israel	68

There have been a number of comprehensive reviews concerning fruit-ripening physiology in which avocados are invariably mentioned. This fruit is, however, an exception in that it does not follow the patterns of physiological changes associated with ripening in many other fruits (Rhodes 1981). An evaluation of ripening physiology of the avocado is, therefore, necessary in order to examine the possible cause of ripening and storage disorders.

It would be insufficient to examine only postharvest factors affecting final fruit quality, as preharvest conditions pertaining to fruit growth and development also play a role (Bower and Van Lelyveld 1985; Bezuidenhout and Kuschke 1982; 1983). This review will therefore include avocado fruit growth and development, as a background to ripening physiology, transport, and long-term storage, with its associated physiological disorders.

II. AVOCADO FRUIT DEVELOPMENT

While numerous studies on avocado maturity have been made over the last 50 years (Barmore 1977), the physiological development of the avocado fruit has been largely neglected. The anatomical development of the avocado fruit has, however, been thoroughly investigated (Schroeder 1953). No attempt will be made to review flowering as this has been extensively covered by Davenport (1986).

The growth of the avocado fruit follows the single sigmoid curve (Valmayor 1964; Robertson 1971). The early period of fruit growth, regardless of early or late maturing cultivars, is characterized by rapid cell division (Barmore 1977). Variations in fruit size of cultivars maturing at approximately the same time result primarily from differences in the rate of cell division during the first 6 months of development (Valmayor 1964).

The avocado fruit is unusual in that cell division in the mesocarp is not restricted to the initial period of growth but also continues during fruit development and even occurs in the mature fruit attached to the tree (van den Dool and Wolstenholme 1983). In some cases, cell enlargement stops when the fruit reaches about 50% of its size at full maturity, while cell division accounts for the continued growth (Cummings and Schroeder 1942). Variation in size can exist in fruits of the same cultivar due to cultural practices, yield, water relations, and climatic conditions (Barmore 1977; McOnie and Wolstenholme 1982).

The relationship between fruit size and development of maturity can be used to determine maturity only if the factors which affect fruit size are understood. Studies have shown that, in general, larger fruit have higher flavour ratings than small fruit when tested early in the season at the time of minimum market acceptability (Soule and Harding 1955; Hatton and Reeder 1969). However, as the season progresses, differences between large and small fruit become less pronounced (Soule and Harding 1955). In order to understand and possibly manipulate fruit growth, it is necessary to evaluate the influence of environmental, cultural, and endogenous

controlling systems on fruit growth.

A. Environmental Aspects

Avocados are grown as a commercially important agricultural crop in several countries around the world with extremely differing environments. The climatic extremes vary from almost desert conditions in Israel and southern California to highland tropics in Mexico to the cool mist-belt conditions experienced in some areas of South Africa and southern Queensland. Knowledge concerning the role of environment on fruit growth and development is scanty. It has therefore been necessary to use whole tree responses as a guideline.

The avocado is divided into three horticultural races: Mexican, West Indian, and Guatemalan (Bergh 1975; Knight 1980; Davenport 1986; Zentmyer et al. 1987). They have distinctly different temperature tolerances (Malo et. al. 1977) due, primarily, to their areas of origin.

1. Temperature. As a general rule, West Indian avocado trees are the most cold sensitive and are damaged by temperatures below 1.2°C under South African conditions (Joubert and Bredell 1982). While racial ancestry was identified as the most important factor influencing susceptibility to cold, other factors such as tree size, age and vigour, crop load, and cultural practices were also shown to influence cold damage during a freeze in Florida (Malo et al. 1977). West Indian avocados are, however, well adapted to continuous heat (Praloran 1970), and it was probably to this race that Perrin (1975) was referring when he maintained that avocado trees will tolerate any degree of summer heat.

The Mexican race is the most frost tolerant. Mature trees are capable of withstanding temperatures as low as -4°C without damage (Joubert and Bredell 1982). However, this race, having evolved in the cool, subtropical highlands, is heat sensitive and suffers vegetative damage (Praloran 1970), flower and fruitlet abscission, and in some cases even fertilization failure at temperatures above 32°C (Sedgley and Grant 1982). The cold tolerance of the Guatemalan race falls somewhere between the other two but is the most sensitive to high temperatures (Whitmore 1986).

Besides actual frost damage, low night temperatures of less than 12°C can influence flowering and reduce fertilization (Sedgley and Grant 1982) by reducing the number of flowers with a female stage (Sedgley 1977). Cool temperatures will also reduce insect activity resulting in less pollination (Bergh 1967; Peterson 1955). Lahav and Trochoulias (1982) noted that cool temperatures promoted root growth and dry matter accumulation, both of which suffer at higher temperatures. Nirody (1922) cited by Davenport (1986) observed that cooler temperatures facilitated "self-pollination" due to a partial overlapping of the male and female phases of each flower.

The most serious consequences of high temperatures could be a reduction in root growth, the occurrence of abnormal flowering and pollination as well as fruit drop. Lahav and Trochoulias (1982) found that high temperatures altered the root:shoot ratio in favour of the shoot. Sedgley et al. (1985) observed that high temperatures forced the tree into vegetative development to the detriment of fruit set and yield. Temperatures above 30°C are thought to be damaging to roots (Whitmore 1986), and this has led to practices such as mulching and cover cropping to keep roots cool. Lahav and Trochoulias (1982) also found that high temperatures tended to reduce leaf area rather than leaf number, increasing competition between vegetative growth and fruit set. Bower et al. (1978) determined optimum photosynthesis to occur between 19°C and 24°C. Temperatures above 25°C probably decrease dry matter production and yield due to reduced photosynthesis (Bower 1978) or increased photo-respiration (Zelitch 1971) as fruit growth and development make heavy demands on the carbohydrate reserves of the tree (Wolstenholme 1985). It must, however, always be remembered that the trees' response to temperature will

change with age, physiological state, crop size, nutrition, and stress status.

2. Other Climatic Factors Apart from temperature, little information about the other climatic requirements of the avocado, such as rainfall and humidity, is known. However, the climates in the areas of speculated origin give an indication of suitable conditions. Such a study was undertaken by Praloran (1970), and the most important points are highlighted in this review.

Both Mexican and Guatemalan type avocados evolved in a tropical highland environment (altitude 1,300-2,700 m) with an annual rainfall of 660-1,500 mm and a relative humidity of 48-80%. The major difference between the two races is the cooler conditions in the Mexican highlands, giving that race slightly increased cold tolerance.

In contrast, West Indian type avocados evolved in a lowland tropical forest environment (altitude 0-100 m) having tropical temperature (about 29°C) with an annual rainfall of 1,150-1,500 mm and a relative humidity of 75-95%.

Excess water may actually reduce yield and fruit quality (Bower and Cutting 1987) due to a reduction in available root oxygen and the promotion of root rot conditions (Zentmyer 1984). Prolonged wet conditions also reduce general tree vigour (Banks 1980). The rainfall pattern is also important as high precipitation at certain growth periods can hinder fruit set and cause fruit drop (Baxter 1981).

Due to its shallow root system the avocado shows water stress suddenly and without warning at a relatively low soil water tension (Bower et al. 1978) by shedding fruit and leaves or by wilting. Adequate moisture after fruit set is essential to sustain fruit growth because any setback in fruit growth is irreversible (Cutting 1984). Water stress during fruit development is also critical as some cultivars such as 'Edranol' shed fruit if stressed, while others retain their fruit at the expense of size and quality. High humidity during and after fruit set is crucial as a sharp decrease in humidity after fruit set is known to cause fruit drop.

Climatic guidelines for avocado production in South Africa are: temperatures less than 31°C in summer, higher than 4°C in winter, and a relative humidity of more than 32% during the flowering and fruit set period (Human 1986). High humidity conditions during flowering are known to increase the successful fertilization of selfed stage 2 flowers (T. L. Davenport personal communication). The same guidelines can probably be used in most countries, as far as the commercial cultivars are concerned.

B. Endogenous Controlling Systems

Two endogenous systems affecting the physiology of fruit growth are the so called "growth hormones" which control growth and development, and plant nutrients which are the 'building blocks' that enable growth to take place. While the two systems obviously interact, they will be reviewed separately.

1. Hormonal Regulation of Fruit Growth In reviewing the literature on the relationships between endogenous plant growth substances (PGS) and fruit development, remarkably few complete studies are found. This is surprising since interactions of different PGS in the regulation of growth and development are known, and the possibility of such interactions in the regulation of plant development has often been mentioned (Leopold and Kriedemann 1975; Goodwin 1978).

The role of seeds in fruit growth varies with fruit species. Some, such as the strawberry (Nitsch 1950), are dependent upon their "seeds" (achenes) for virtually the entire period of fruit (receptacle) growth, while others, such as the banana, are vegetatively parthenocarpic (Luckwill 1981). Between these examples of the extremes are many fruit types dependent upon their seeds for varying lengths of time (Leopold and Kriedemann 1975).

Goodwin (1978) maintained that there is generally no correlation between endogenous auxin concentration and fruit or seed growth. However, Gazit and Blumenfeld (1972) found avocado seeds to be a rich source of auxin activity. Later work by Cutting et al. (1985) showed that indoleacetic acid (IAA) reached a peak in 'Fuerte' fruits shortly after set. Levels declined rapidly thereafter, and could not be detected 5 months after fruit set. Furthermore, Cutting (1984) showed that first the seed and then the testa had high auxin levels during development. Auxin activity was also detected in the mesocarp of young fruit (Gazit and Blumenfeld 1972). At all stages of fruit development, the mesocarp contained lower levels of IAA than the seed and testa (Cutting et al. 1985). Both groups of workers concluded that auxin increased the sink strength of the fruit and regulated endosperm development.

There is strong evidence that ethylene is involved in the abscission of young avocado fruitlets (Davenport and Manners 1982) even after ovule fertilization (Sedgley 1980). Davenport and Manners (1982) noted that an increase in ethylene did not occur in fruit that failed to abscise. There is thus some regulatory mechanism that determines which fruit abscise and which are retained by the tree. Abscission does not appear to be related to fruitlet size within the fruit cluster (Adato and Gazit 1977). Other studies have shown that the seed often contains the highest concentrations of IAA, e.g., in apple (Luckwill 1953) and avocado (Gazit and Blumenfeld 1972). The high IAA levels in attached flowers and fruitlets could be involved with ethylene promotion, as auxin has been shown to stimulate ethylene synthesis (Dilley 1969; Leopold and Kriedemann 1975; Goodwin 1978), thereby regulating fruit retention on the flower panicle. Cutting et al. (1985) showed that there is a very sharp IAA peak in attached fruit during the fruitlet drop stage. Fruit sampled 2 weeks prior to this drop period had much reduced IAA levels, as was the case with fruit sampled 2 weeks after the main fruit drop period. In addition, ABA levels were low in attached fruit before, during and after this drop period. The prevention of the ethylene peak and associated fruitlet abscission could, therefore, be under the control of other plant growth substances such as the cytokinins, which showed a strong peak during this period (Cutting 1984).

Exogenous gibberellin treatments have been shown to stimulate fruit growth in some species (Weaver and McCune 1959; Luckwill 1959) but not in others (Leopold and Kriedemann 1975). A correlation between fruit growth and extractable gibberellin activity was found in *Phaseolus vulgaris* (Skene and Carr 1961), *Citrus sinensis* (Wiltbank and Krezdorn 1969) and *Prunus* (Martin and Campbell 1976). However, for avocado no such correlation could be established by Blumenfeld and Gazit (1972). Crane (1964) concluded that, in general, gibberellin levels did not correlate well with fruit growth rates. Blumenfeld and Gazit (1972) found high levels of gibberellin activity in the seed and testa of developing avocado fruit. The level in the testa decreased as the fruit developed. No measurable gibberellin-like activity was detected in the meso- and endocarp (flesh) and embryo. They concluded that the testa was the site of gibberellin-like substance production in avocado fruit. No reference to the regulatory function of gibberellins in avocado fruit development could be found in the literature.

The presence of cytokinins in developing fruits is fairly well established, especially during the early cell division period of fruit development (Goldacre and Bottomly 1959; Nitsch and Nitsch 1961). Seeds appeared to be the central source of cytokinins in apple fruit (Letham and Williams 1969). Whether the seed is the site of cytokinin synthesis, or whether the fruit and seed are supplied with cytokinins from the phloem (Kende 1965) or roots (Van Staden and Davey 1979) is unknown. In grape, exogenous cytokinin application brought about increases in fruit growth (Weaver and Van Overbeek 1963).

Blumenfeld and Gazit (1970) found high levels of cytokinins in both the cotyledons and testa of avocado, which decreased with development. At the time of testa shrivel and withdrawal, cytokinin activity was not detected. They concluded that the high levels of cytokinin activity in

the young seed served to increase the sink strength of the fruit for nutrients and other metabolites. In a later study Cutting (1984) found that the cytokinin levels in the cotyledons remained constant after declining from an early peak, while the cytokinin levels in the testa declined to below detection limits 5 months after fruit set. Gazit and Blumenfeld (1970a) found that cytokinin activity in the mesocarp was very low and decreased further with development. A similar trend was observed by Cutting (1984). Whether the reduction in growth was due to increased inhibitor levels or decreased cytokinin levels was not determined. An indication of cytokinin synthesis in young fruit may be obtained from the detection of isopentenyl adenine (2iP) in these extracts. This compound is thought to be a component of the cytokinin synthesis pathway (Letham 1978). There is evidence for the involvement of cytokinins in the regulation of assimilate partitioning (Richards 1980; Goussard 1981). The high cytokinin levels detected in young fruitlets may actively assist in increasing the sink strength of the fruit, and thereby promote fruit growth.

At present the role of abscisic acid (ABA) in fruit development remains unclear, although certain evidence has been presented that it may play a role, particularly in seed development, as was first proposed by Thomson (1961) working on strawberries. ABA levels fluctuate during the development of many types of seeds and fruits (King 1976; McGlasson and Adato 1976; Hsu 1979). Water stress stimulated ABA levels in barley grains (Goldbach and Goldbach 1977). A role for ABA in inhibition of seed germination in developing fruit has been suggested by several investigators (King 1976; Morris 1978).

Gazit and Blumenfeld (1970a) detected an inhibitor in avocado mesocarp which they concluded was not ABA. The level of this inhibitor increased as fruit growth approached maturity. In a further study by Gazit and Blumenfeld (1972), three inhibitors were detected in avocado mesocarp, one of which had chromatographic properties similar to ABA. The level of this compound remained nearly constant throughout development. Later Cutting (1984) and Cutting et al. (1985) found ABA in developing avocado mesocarp, the levels of which rose dramatically with development and reached a maximum at maturity. It is thought that ABA could act by inhibiting or reducing auxin-induced cell expansion (Warner and Leopold 1971) as the ABA levels in developing avocados follow the same trend as the fruit growth curve (Cutting 1984). ABA has also been observed to inhibit cytokinin metabolism (Letham and Palni 1983), and it is thought that ABA has a cytokinin "sparing" action (Letham 1978). This would tend to reduce the rate of cell division (Hill 1980). Therefore, any stress-induced increases in ABA should cause an irreversible loss in fruit growth as was shown by Cutting (1984). The effect would be masked to some extent, as cell division in the avocado fruit continues, although at a reduced rate, until maturity (van den Dool and Wolstenholme 1983). The role of ABA in avocado fruit growth is an avenue that deserves future attention.

2. Nutritional Factors. Avocado nutrition has been extensively researched and documented by Embleton and co-workers (Embleton and Jones 1964; 1966; Embleton et. al. 1955; Embleton et. al. 1959a) and more recently by Lahav and Kadman (1980). Therefore a detailed review of nutrition is not attempted, but rather a highlighting of possible fruit growth and nutritional interactions, summarizing some of the more important findings as they affect fruit yield.

Much of the earlier fertilization work concentrated upon leaf analysis and the rigorous standardization required for such analysis to be of value. Leaf analysis norms have now been established for California (Embleton et al. 1959b), Florida (Iley 1977), Israel (Oppenheimer et al. 1960), and South Africa (T. Koen, personal communication). All of this work concentrated upon yield and postharvest quality.

Yields of avocado on a tree-by-tree basis are extremely variable (Jones et al. 1957). This could be due to the alternate bearing nature of the avocado and its susceptibility to root rot. In

order to measure relatively small differences in yield and to show such differences to be statistically significant, more replication is needed with avocado than with most other tree crops. Fruit trees are difficult to handle experimentally, and nutrient requirement experiments are time-consuming and expensive. Much nutrient culture work with orange trees has been done using seedlings or mature-rooted cuttings (Cary 1970), giving results which are difficult to extrapolate to the orchard situation (Robinson 1986). These results are often complicated by the buffering reserves of certain nutrients masking short term shortages (Taylor and van den Ende 1969). The problems of variability and extrapolation would apply to any study where the nutritional requirements of developing fruits are being researched. Such research could include the nutritional aspects of sink-source relationships, the vegetative reproductive balance in the tree, fruit retention, rootstock effects, and endogenous growth regulation, all areas in which little is known about the avocado.

Of particular interest are those elements which are immobilised in the leaf and lost to the plant during leaf abscission. Such elements could play a critical role in fruit set and growth. Nitrogen, phosphorus, and potassium are largely mobilised from the leaf prior to leaf abscission. In contrast calcium, magnesium, and iron are not mobilised from the leaf and it is estimated that up to 60% of the total tree calcium can be lost each year (Cameron et al. 1952).

Generally, phosphorus and potassium deficiencies have not been documented for field-grown avocados (Embleton and Jones 1966), and the addition of phosphorus and potassium in a 3-year trial did not significantly increase yield (Lynch and Goldweber 1956). Additions of phosphorus and potassium in a normal fertilization program appear to satisfy the plant's requirement.

In contrast, nitrogen and calcium levels markedly affect yield, fruit size, and postharvest quality, and it appears that there is considerable interaction between these two elements in controlling the vegetative:reproductive balance in the avocado tree.

Nitrogen is known to promote vigorous leaf flushing (Embleton and Jones 1966) and reduce yield (Wolfe and Lynch 1940). Nitrogen levels of over 2% of dry mass, when measured by leaf analysis in autumn, promoted vigorous vegetative flushing and reduced yield the following spring. Low levels of less than 1.6% of dry mass were associated with reduced yield due to reduced shoot growth and sparse foliage the following season. Calcium may play a role in yield as calcium accumulated by the leaf or the fruit becomes immobilised and is lost to the tree after abscission or harvest (Cameron et al. 1952). Witney et al. (1986) found that 'Fuerte' and 'Hass' fruits from nonvigorous trees accumulated more calcium during development than the fruits from vigorous trees.

Leaves and shoots are stronger calcium sinks than fruit, especially in vigorously flushing trees, to the detriment of fruit calcium (Witney et al. 1986). Calcium must enter the fruit via the xylem, as it is immobile in the phloem, the usual entry of nutrients into the fruit (Shear 1980). Bangerth (1979) proposed that calcium may be dependent on the opposite transport of auxin. Vigorously growing shoots would be major exporters of auxin, and as such, strong sinks for calcium (and other metabolites). This would be detrimental to fruit calcium and overall fruit competitive ability, thus affecting fruit growth. There is some experimental support for this theory. Cutting et al. (1985) found that fruit auxin levels reached a maximum 5-6 weeks after fruit set, coinciding with maximum calcium accumulation as determined by Witney et al. (1986).

III. AVOCADO FRUIT RIPENING

Wale (1975) described fruit ripening as the processes resulting in changes in colour, taste, and texture, which make the fruit acceptable for consumption. The process involves many catabolic

and anabolic changes, requiring large amounts of energy as well as prolonged integrity of membranes (Bruinsma 1981). Tissue senescence finally occurs, leading to the over-ripe state.

The ripening processes, once started, cannot be reversed. The avocado, however, differs from most other fruits in that ripening does not normally take place on the tree, but only after picking (Schroeder 1953). The reasons for this phenomenon are not well understood, but Tingwa and Young (1975) postulated that some substance, possibly an anion, acts as a ripening regulator and moves either to or from the fruit pedicel once detached from the tree. Notwithstanding this process, normal avocado softening with acceptable taste occurs only when a certain level of maturity, as defined by Spencer (1965) and by Hobson (1979), has been reached. Before this state of maturity is reached, only slight softening may occur due predominantly to shrivelling as a result of water loss (Barmore 1977), and flavour is poor (Ahmed and Barmore 1980). Once horticultural maturity has been reached, the rate of postharvest softening becomes progressively shorter with increasing maturity (Zauberman and Shiffmann-Nadel 1972). With the exception of Florida avocado, maturity is based on lipid metabolism, with rapid oil accumulation beginning at about the time of growth decrease and the onset of maturity (Kikuta and Erickson 1968). In cultivars low in oil content such as the West Indian types grown in Florida, lipid-based maturity standards have been found to be inappropriate, fruit mass being more useful (Ahmed and Barmore 1980).

A. Plant Growth Regulators

1. Ethylene. The avocado is a climacteric fruit. This implies a marked rise in respiration rate at the onset of ripening, followed by a decline. The rate of ripening is rapid compared with that of nonclimacteric fruits. Ethylene is known to play a vital role in this process, leading Rhodes (1981) to define the climacteric as, "a period in the ontogeny of certain fruits during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening." The ethylene peak usually precedes the respiratory climacteric. This means that a key element for normal ripening is the initiation of ethylene formation. Yang (1981) considered ethylene to be essential to the ripening of climacteric fruits.

As previously mentioned, the avocado is different from most other fruit, as ripening does not occur until after picking. If ethylene is the stimulus for ripening in this fruit, exogenous applications should cause ripening even on the tree. Gazit and Blumenfeld (1970b) were unable to demonstrate this, although it is known that exogenous applications of ethylene after harvest do cause an earlier climacteric with consequent ripening (Eaks 1966). Gazit and Blumenfeld (1970b) were unable to induce a ripening response with applied ethylene until 25 hours after harvest, and Zauberman and Fuchs (1973) found the effect to be maturity dependent. Similar findings were made by Eaks (1980), while Biale and Young (1981) found that the climacteric rise could be delayed by ethylene if applied to early-season fruit soon after picking. In addition, Biale and Young (1981) indicated that fairly high levels of ethylene are required to elicit a response. These facts led Lieberman (1979) to conclude that some anti-ethylene factor must be removed, or tissue must acquire increased sensitivity to ethylene before a reaction will occur. McGlasson (1985) postulated such sensitivity changes to be crucial to the ethylene response. However, Beyer (1977) found low oxygen tensions to be inhibiting to the action of ethylene, which is in accordance with the earlier work of Burg and Burg (1967). In a review of ethylene metabolism and action, Sanders et al. (1986) state that there is still no certainty as to the mechanism of ethylene action. Smith and Hall (1985) state that ethylene binding sites appear to be integral membrane proteins. Many factors could thus affect the response. There is further conflicting evidence of the role of ethylene in the normal ripening of avocados. Burg and Burg (1962) found the ethylene peak to precede the respiratory rise in 'Choquette,' while Kosiyachinda and Young (1975) found the opposite in 'Fuerte'. Zauberman and Fuchs (1981)

also found the rapid rise in ethylene formation with accompanying rise in ACC (1-aminocyclopropane-l-carboxylic acid) shown by Hoffman and Yang (1980) to not be the earliest event in avocado ripening. Kosiyachinda and Young (1975) concluded that some other factor is important in the initiation of ripening in the avocado. Rhodes and Reid (1975) reached a similar conclusion for apple fruits. One could therefore conclude that ethylene per se is neither the initiator of ripening, nor directly involved in the ripening of avocados, with its presence a result rather than a cause of normal ripening. There is sufficient evidence, however, that it does indeed play an important role in ripening.

Tucker and Laties (1984) found a clear relationship between ethylene and increased polysome prevalence early in the climacteric of avocado fruit, demonstrating an increase in protein synthesis (Richmond and Biale 1966). Christoffersen et al. (1982) had found an increase in at least three mRNAs with translation products having masses of approximately 16,500; 35,000 and 80,000 Daltons. Later, Tucker and Laties (1984) identified a new gene product, with a molecular mass of 53,000 Daltons, as cellulase, and speculated that ethylene may be directly involved. A number of enzymes may therefore be initiated by ethylene. Although other factors initiate the respiratory rise, alter sensitivity to ethylene, or control its increase, the autocatalytic production of ethylene is of vital importance to normal avocado ripening. Any factors affecting this process could be expected to alter the fruit-ripening pattern.

Commercial manipulation of the ripening process will be outlined later. It is appropriate, however, to discuss some environmental parameters at this stage, as they appear to have a direct effect on ethylene formation.

The temperature before and during the ripening phase is important. Erickson and Takaake (1964) and Zauberman et al. (1977) found temperatures above 30°C had adverse effects on avocado ripening. Eaks (1978) found the ethylene rise to occur earlier as the ripening temperature increased from 20°C to 25°C, while the peak value decreased at temperatures higher than 25°C. Low-temperature storage was also found to affect ripening (Eaks 1976). Unripe fruit held at temperatures at and below 5°C for 2 weeks showed greatly reduced ethylene peaks when ripened at 20°C, while storage for up to a week caused an earlier climacteric (Eaks1983).

Water stress, both before and after harvest, has a profound effect on avocado fruit-ripening physiology. Adato and Gazit (1974) found that avocado fruits that lost water more rapidly after picking, ripened faster. The ethylene peak occurred earlier if fruits were allowed to become dehydrated, whereas the reverse was true if harvested fruits were infiltrated with water. Similar findings were noted by Fukushima et al. (1977) for a number of other crops. Bower and Cutting (1987) found that the rate of fruit water loss during storage affected both ripening rate and fruit quality. Preharvest water relations also affect both ripening rate and ethylene evolution. Bower (1984) found that more negative fruit water potentials at harvest, caused faster ripening after storage. Long-term preharvest stress (and particularly during the first 3 months after fruit set) caused an altered ethylene evolution pattern (Cutting et al. 1986). Earlier initiation of ethylene was in this case evident, but there was a lack of true ethylene increase. Ripening was also uneven, and fruit quality poor. As the fruits in question were not stressed at the time of picking, the results imply that the ethylene-forming system and therefore ripening physiology may be affected by physiological changes occurring a considerable time before harvest, and are irreversible. The condition of membranes as determined by long-term preharvest conditions may well be of importance, affecting the autocatalytic production of ethylene (Kende and Baumgartner 1974), or the ethylene-forming enzyme (Lieberman and Wang 1982) which is membrane bound (Yang 1981). Membrane changes may explain the results obtained by Cutting et al. (1986).

While ethylene does effect important changes related to avocado fruit ripening, it is not the only endogenous substance of importance. The contribution of other plant growth regulators must therefore be considered, bearing in mind the vital role they play in plant growth and development (Dilley 1969; Leopold and Kriedemann 1975). The control of maturation, especially the initiation of ripening, is thought to be a balance between promoting and inhibiting factors. Apart from the role of ethylene as already discussed, it is generally considered that ABA appears to be a ripening promoter, while auxins, cytokinins and gibberellins, are inhibitors of fruit ripening (Rhodes 1981).

2. Auxins. There is some disagreement concerning the role of auxins in fruit ripening. While auxin application can stimulate ethylene synthesis and maturity, as shown by Maxie and Crane (1967) for figs, Frenkel and Dyck (1973) found a delay in ripening of pears after auxin treatment, in spite of increased ethylene synthesis. Rhodes (1981) indicated that products of IAA catabolism due to increased IAA oxidase activity may act as ripening stimulators during the early preclimacteric phase. This work does not, however, seem particularly relevant to avocado, as Adato and Gazit (1976) were unable to elicit initiation of fruit ripening, except with very high doses of IAA. Furthermore, in an extensive study of growth regulators in developing avocado fruits, by Wolstenholme et al. (1985), it was found that IAA decreases to low levels in all parts of the fruit with the onset of maturity. If it is further considered that ripening does not occur until removal of the fruit from the tree, which could be a number of months after attainment of maturity, then a direct role for this growth regulator in the initiation and development of the ripening response is not clear.

3. Cytokinins. Lieberman et al. (1977) found a decrease in ethylene production in avocado fruits with the addition of isopentenyl adenosine. This does not necessarily mean that cytokinins play a pivotal role in normal avocado fruit ripening. The work of Gazit and Blumenfeld (1970a) showed low levels of cytokinin activity in the mesocarp by the time the fruit was mature, using the soybean callus assay. However, it was also found that a nonabscisic acid inhibitor was present, making conclusions as to the role of cytokinins difficult. In later work by Cutting et al. (1986b) using the more exact assay technique of radioimmunoassay, these authors were unable to come to any firm conclusion as to the role of cytokinins in avocado fruit ripening, although there were indications that an interaction with ABA may occur, as suggested by Letham and Palni (1983). Hofman and Wolstenholme (1985) were unable to find any clear trends in cytokinins during avocado ripening and concluded that they probably do not play a significant role.

4. Gibberellins. Bruinsma (1981) noted that gibberellins appear to play little role in the control of the ripening process of fruits in general, although they can inhibit ripening by retarding the overall degradation or senescence phase of fruit ripening. They may even delay the degradation or promote the reassembly of functional chloroplasts, as in degreening of citrus (Goldschmidt 1980), which could be of consequence in terms of the physiological disorders to be discussed later. Lieberman et al. (1977) found exogenous addition of gibberellins had little effect on avocado ripening. Dilley (1969) suggested a link with ABA during fruit ripening, whereby the gibberellins decrease and ABA increases to the point where ethylene production is no longer inhibited and the ripening process begins. Overall, however, there appears to be very little work, particularly for the avocado, on the role of gibberellins in ripening.

5. Abscisic acid. Abscisic acid appears to play a key role in fruit ripening (Goodwin 1978). Rhodes (1981) referred to ABA as a ripening promoter, and it was shown by Hale and Coombe (1974) to advance the onset of ripening in grapes. In their work on tomato, apple, and avocado, Lieberman et al. (1977) showed an increase in ethylene and ripening following application of ABA before the climacteric peak, but depressed evolution if applied after the peak. They concluded that ABA accelerates ageing. Gazit and Blumenfeld (1972) reported little change in

ABA level during fruit development, but once ripening started, a considerable increase occurred (Adato et al. 1976). These workers also found that the increase in ABA closely followed the ethylene curve with peaks at the same time. Furthermore, the ratio of free (active) to bound ABA remained about the same, indicating that the free ABA was the result of synthesis, not activation. This is in agreement with Milborrow and Robinson (1973), who found that ripening avocado fruit could convert labeled mevalonate, which is believed to be a precursor to ABA (Walton 1980), to ABA.

Whether ABA can be considered the primary trigger in avocado fruit ripening or not, is still unknown. In his review, Rhodes (1981) found all fruits contained ABA, and that ABA is somehow involved in the ripening process. The role does not, however, appear to be direct, as Bangerth (1980) found that inhibition of ethylene synthesis inhibited ripening. Bruinsma (1981) considered the likely role of ABA to be the stimulation of ethylene biosynthesis, or alteration of tissue sensitivity to ethylene (Rhodes 1981), once ripening inhibitors from the tree are no longer present. An increase in ABA levels up to a certain threshold could thus be required for ethylene stimulation, which is in accordance with the postulations of Hobson (1979). If this is so, then any factors affecting the synthesis of ABA in the avocado fruit would be important to subsequent normal ripening.

Stress is a major factor affecting ABA levels. It has long been known from work on stomata, that ABA and water stress are linked to stomatal closure. Kriedemann et al. (1972) found ABA to increase to 40 times the normal concentration within 4 hours of leaf wilting. Hiron and Wright (1973) also showed strong links between water stress and ABA synthesis. Similar responses to stress may occur in fruit. Once picked, considerable loss of water from fruit takes place by transpiration (Lurie et al. 1986), which could lead to ABA accumulation. Adato and Gazit (1974) found that the greater the daily water loss from harvested avocado fruits the more rapid the ripening. Infusion of water delayed ripening, and these workers concluded that moisture stress could be an important factor in ripening. It is also known, that as avocado fruit mature, there is an increase in oil content, with a concomitant decrease in water content (Pearson 1975). This could result in more rapid development of moisture stress, with, in turn, more rapid ripening as found with increasing maturity (Zauberman and Shiffman-Nadel 1972). Bower et al. (1986) found that the free ABA content of ripening avocados increased with fruit softening, with the peak at approximately the same degree of softness at which the maximum ethylene peak occurred, and declined thereafter.

Factors other than water stress can also affect both ABA levels and ripening rate. Wang et al. (1972) found that pear fruits subjected to low orchard temperatures ripened faster and also had higher ABA levels. This would be important where fruit are allowed to hang into the winter, and could be an explanation for the high levels of ABA in avocado fruit at the end of the winter when compared with those early in the harvest season (Cutting et al. 1986a).

Thus, while there is still no certainty as to the initial trigger involved in avocado ripening, water stress, ABA, and ethylene all appear to play a role in at least the early stages of ripening.

B. Structural Changes

An important aspect of avocado fruit ripening concerns the cell membrane system and in particular the plasma membrane. It has previously been thought that ripening results in a loss of membrane integrity with increasing permeability (Sacher 1976). Kalra and Brooks (1973) found a change in lipid content of tomato membranes, and Knee et al. (1977) a degradation of strawberry fruit membranes during ripening. In a later study of ripening bananas, Wade and Bishop (1978) showed that properties such as viscosity and permeability do change, but in a controlled manner, and that the membrane remains intact. Thomson and Platt-Aloia (1976) found citrus fruit cell membranes degenerated only after ripening was complete.

In the case of avocados, detailed ultrastructural studies have been made of the cell wall (Platt-Aloia et al. 1980) and of the cell wall contents (Platt-Aloia and Thomson 1981) in ripening fruit. During ripening, it was found that the middle lamella begins to disappear, with pectin removal from the matrix of the cell walls. Later, a loss of organization and density in the walls occurs. Finally, Platt-Aloia and Thomson (1981) found that the walls almost completely disappeared during the postclimacteric phase. Work cited by Huber (1983), indicating that the cell wall may play an active role in controlling ripening, shows this sequence of events could be important. Our unpublished work showed that the cell walls of fruits subjected to water stress undergo changes similar to those encountered in ripening fruits. These changes were visible as early as 3 months after fruit set. The fruits later showed ripening abnormalities (Cutting et al. 1986).

Internally, the fruit cell constituents can be divided into a group of organelles which do not change during ripening and those which do. Platt-Aloia and Thomson (1981) found that chloroplasts, dictysomes, microbodies, the nucleus, vacuoles, and ribosomes did not alter significantly, with the structural integrity remaining. Mitochondria appeared to lengthen, presumably due to increased energy demand during ripening. The most profound changes occurred in the rough endoplasmic reticulum, which showed considerable swelling and vesiculation, and seemed, with ribosomes, to be associated or fused with the plasma membrane. This suggests considerable enzyme synthesis, with the membrane systems remaining intact.

C. Enzymes

A number of enzyme changes are important and various studies on avocados have been reported. Scott et al. (1963) reported cellulose as the major constituent of avocado cell walls, and consequently it is reasonable to expect cellulase to play a major role in avocado softening. Indeed, in his review on cell wall hydrolases, Huber (1983) considers the avocado to be the fruit most representative of those in which cellulases are of primary importance. Pesis et al. (1978) found a rapid increase in this enzyme accompanying softening, which was closely correlated with the respiratory climacteric and ethylene. Application of ethylene in the air surrounding fruit for 48 hours after harvest also caused an increase in cellulase activity, which led Pesis et al. (1972) to conclude that ethylene plays a role in controlling cellulase activity. This is in agreement with the later work of Tucker and Laties (1984), who showed the possibility of ethylene causing gene transcription for cellulase. Hatfield and Nevins (1986) have purified and characterised avocado cellulase. It was found that the enzyme is a (1-α4)-β-D-glucanase, and that the activity is limited to hydrolysing β-D-glucans of four or more glucosyl residues. These authors therefore contend that the solubilization of the cell walls during softening as seen by Platt-Aloia et al. (1980) cannot be due to cellulase alone, and they propose a different role for cellulase. Hatfield and Nevins (1986) consider that xyloglucans or cellulose fibrils may be hydrolysed, which would be consistent with microscopic observations of cellulose xyloglucans, or cellulose fibrils may be hydrolysed, which would be consistent with microscopic observations of cellulose fibrillar changes. Also, hydrogen bonding to other polysaccarides may alter, thus disrupting the cell wall matrix. This could allow greater accessibility to polygalacturans in the wall matrix. Such a process could explain why an increase in cellulase activity precedes that of polygalacturonase in the avocado (Awad and Young 1979). If this is the role of cellulase, then the importance of ethylene initiation and production within the total ripening process becomes apparent. The early stages of softening in the avocado appear to be due to cellulase, controlled at least in part by ethylene, with polygalacturonase responsible for final softening.

The role of pectinmethylesterase is not entirely clear. Huber (1983) quotes considerable conflicting evidence as to the role of this enzyme in general. Gertman and Fuchs (1974) found the enzyme to be associated with lipids, while Awad and Young (1980) concluded that this was not the case in avocados. The same authors felt that phenolics played no role in the control of

pectinmethylesterase in avocado, as the phenols released by cell rupture did not change the enzyme activity. There is nevertheless considerable evidence from other fruits (Huber 1983), that they do. During avocado ripening, a considerable decrease in activity of pectin-methylesterase occurs, although estimates vary (Rouse and Barmore 1974; Awad and Young 1980). Awad and Young (1979) believe that the function of the enzyme is to partially demethylate pectin, such that the latter can be a suitable substrate for polygalacturonase which causes the depolymerization of pectin. If this is so (which from the review of Huber (1983) is not certain), then the likely sequence of events in avocado softening seems to be that of an unknown factor, possibly involving moisture stress and abscisic acid, which stimulates ethylene formation, which in turn, via gene transcription, results in a number of enzymes being produced, including cellulase which allows cell wall degradation. During the preclimacteric phase but after the initiation of ripening, an unknown factor causes pectinmethylesterase to bind to the cell wall, (Jansen and Jang 1960), but at the same time preparing the cell wall substrate for hydrolysis in the presence of polygalacturonase. Intact membrane systems could be vital for the functioning of this chain of events. The role of other enzymes in avocado fruit ripening (Huber 1983) is uncertain as literature pertaining to their presence and activity changes appears to be severely lacking.

D. Calcium

There has recently been much literature concerning the role of calcium in plant physiology, including reviews and symposia covering almost every aspect of known influence including the relationship with fruit physiology (Bangerth 1979; Cheung 1982a; Clarkson and Hanson 1980; Clarkson 1984; Demarty et al. 1984; Dieter 1984; Ferguson 1984; Kirkby and Pilbeam 1984; Macklon 1984; Marme 1983; Millaway and Wiersholm 1979; Moore and Akerman 1984 and Poovaiah 1979;1985). A further review appears elsewhere in this issue (Poovaiah et al. 1988). With such a large volume of literature available, an attempt will be made to summarise those factors known to directly affect fruit ripening physiology. The effects of calcium on the important aspect of fruit quality, will be discussed separately.

Calcium is known to affect fruit ripening to a considerable extent. Infiltration of calcium can delay the overall softening process during ripening (Davenport 1984; Tirmazi and Wills 1981; Wills and Tirmazi 1982). Tingwa and Young (1974) also found that avocados with higher endogenous levels of calcium had slower rates of ripening. Wills and Tirmazi (1982) showed that the infiltration of calcium into avocados greatly reduced the ethylene peak and respiratory rise, with a resultant longer ripening period. Eaks (1985) obtained similar results, with softening, the ethylene and respiratory rises being completely inhibited at high concentrations (0.5M) of calcium infiltration. Van Rensburg and Engelbrecht (1986) found similar results after a long storage period of 30 days, and with various calcium carriers. The effects of calcium are, nevertheless, more complex than appears from fruit infiltration work. Evenson (1984) found calcium to reduce lipid peroxidation, implying a membrane stabilization function. However, the same author also found that calcium in an incubation medium surrounding potato tissue slices, could enhance ethylene formation as a result of enhanced ACC synthesis. The role of calcium is best summed up by Bangerth (1979), as being the effect on enzymes, membranes, cell walls, and interactions with plant growth regulators, all of which can be related to the ripening process.

Ferguson (1984) reviewed the role of calcium in ripening and senescence, and concluded that the important effects of calcium were related to membrane and cell wall structure and function. The overwhelming role of calcium in the cell wall can be gauged by the review of Demarty et al. (1984), who noted that calcium can control the charge density of the wall, and thereby affect ionic selectivity, which could be expected to have a considerable effect not only on cell wall bound components, but also on the metabolism of the cell in general. Calcium also is known to be particularly important in the maintenance of membrane stability and permeability control

(Roux and Slocum 1982; Ferguson 1984). This alone could modify many cellular functions. Although Ferguson (1984) says that a considerable amount still needs to be understood to elucidate calcium metabolism, it would seem important that high concentrations of calcium be maintained outside the cytosol, acting on the cell walls and plasma membrane. The longer this condition can be maintained, the slower the ripening and senescence process. A characteristic of senescence is indeed the breakdown of this clear difference in calcium position due to a change in the calcium regulatory processes (Ferguson 1984).

The role of calmodulin as an enzyme modulator in animal cells has been known for some time (Cheung 1980), but was first identified in plants by Anderson and Cormier (1978). Thus far, calmodulin has been identified and characterised only for a small number of plants, and as far as we know, the avocado is not among them. Nevertheless, a number of authors consider it to be ubiquitous (Muto and Miyachi 1974; Poovaiah 1985). The former authors have developed a radioimmunoassay which in future may help to clarify the cellular occurrence and functions of the complex. Recent reports indicate that calmodulin (Cheung 1982b; Dieter 1984) is critical to both calcium metabolism, as well as the cytosolic calcium pump (Poovaiah 1985). Calcium can bind to a large number of proteins (Kretsinger 1976), but the concentration of calcium in the cytosol is generally too low for the binding affinity of most. Calcium-rich membranes would be a more likely site of action, with, for instance, membrane-bound ATPases (Kylin and Kahr 1973) active in membrane transport. Calmodulin, which is a low-molecular-mass cytoplasmic protein, appears to fulfill the requirements of a cytosolic calcium binder, with the ability to alter cell metabolism. Of note is the finding of Muto (1982), that most of the calmodulin is situated in the cytosol. This finding corresponds with the postulations of Poovaiah (1985) and Moore and Akerman (1984) who propose an ATP-dependent calcium extrusion, resulting in uptake by the endoplasmic reticulum and chloroplasts in particular. This process could be mediated by calmodulin, and may be reversible (Cheung 1982b).

The physiological role of calcium is reported to be that of a second messenger (Poovaiah 1985). Once the level within the cytosol reaches a certain threshold, it combines with calmodulin. This creates an active calcium-calmodulin complex, which in turn combines with a receptor protein creating an active enzyme complex (Roux and Slocum 1982; Poovaiah 1985). To what extent the enzymes involved in fruit ripening are controlled in this way is uncertain. However, Poovaiah (1985) clearly shows the vital role played by the calcium-calmodulin complex in plant growth and development, including the onset and rate of senescence development. Ferguson (1984) examines the possible modification of fruit-softening enzymes, and concludes that polygalacturonase activity could be the key enzyme involved. The mode of action, however, is complex, with a number of alternatives. Considerable work in this regard is still necessary before clarity will be gained. An interaction between calcium and growth regulators also seems to exist. Whether this is direct or not, is still open to question. The effect of calcium on ethylene could, for instance, be explained by its effect on membrane structure (Ferguson 1984). Modification of membranes may affect growth regulator binding sites. Auxin binding, for instance, is known to be calcium dependent (Poovaiah and Leopold 1976). The same maybe true of gibberellins (Pauls et al 1982). Cytokinins appear to play some role in regulating calcium concentrations and movement (Ferguson 1984), but the importance is unclear bearing in mind the lack of evidence for cytokinin involvement in avocado fruit ripening. Recent reviews on calcium metabolism do not discuss a direct link between calcium and ABA which, as previously discussed, could be important in avocado ripening. Indirectly, however, the effect of calcium on membrane permeability and stability may be important.

Overall, therefore, the macro-effects of calcium, such as the effects on ethylene evolution and respiration during avocado fruit ripening, are known. However, mechanisms behind these effects are complex and varied, controlled by many external and internal stimuli (Hepler and

Wayne 1985).

E. Modification of the Ripening Process

The avocado has a high rate of postharvest respiration and limited shelf life (Aharoni 1984). Successful marketing, notably by the largest exporters, Israel, South Africa, and the United States (California), must rely on decreasing the rate of ripening sufficiently to allow for shipping time (which is primarily by sea) and marketing. The South African industry needs to be particularly efficient as the total shipping time from packhouse to sale is between 20 and 30 days, while export from Israel takes only about half this time.

A number of methods or combinations thereof are available for decreasing the rate of ripening, and they are used for avocados; however, the combination of high metabolic activity and their susceptibility to chilling injury (Couey 1982) means that care is necessary in the choice of storage conditions. The ongoing research evident in the literature is an indication that problems have not been solved satisfactorily yet.

1. Temperature. Low temperature storage is the most commonly used method of extending storage life in the avocado. The extent to which the avocado can be chilled depends on the cultivar (Ahmed and Barmore 1980), temperature of storage, and period of storage (Eaks 1976; Zauberman et al. 1977). As international trade consists mainly of the 'Hass' and 'Fuerte' cultivars, and Eaks (1976) found no significant difference in the response of these cultivars to low temperature storage, further discussion will apply to both these cultivars.

Chilling injury in avocados has been described by various authors as a grey or dark brown discoloration of the mesocarp (Chaplin et al. 1982; Couey 1982). While these symptoms are associated with low temperature storage, Vakis (1982) also found chilling-like symptoms in nonchilled fruit, thus leading to the conclusion that there are other causes than chilling. A full discussion of the symptoms and physiology of possible causes will therefore be given in the section on physiological disorders. A further symptom of chilling damage is given by Swarts (1984) who described it as a blackening of the exocarp with no internal disorder. The degree of damage is directly related to the temperature and duration of the damaging temperature.

Eaks (1976) found no evidence of ripening during storage for avocados held at 5°C or lower. No chilling injury occurred if fruit was held at 10°C, but at 5°C injury was found to increase with storage time longer than 2 weeks. Engelbrecht and Koster (1986) also found that storage for longer than 5 days at 5.5°C caused abnormalities in subsequent respiratory patterns during ripening at 18°C. This problem is probably of most consequence to South African exporters, who require the longest storage times with satisfactory subsequent shelf-life. Swarts (1982) found that later in the season (increasing on-tree maturity) storage temperatures lower than 5°C could be used. Smith and Lunt (1984) further found that orchard temperatures interacted with fruit maturity in the response to low temperature storage, implying that an acclimation to low temperatures can occur prior to harvest. It is also known that temperature sensitivity alters during the ripening phase, with Kosiyachinda and Young (1976) finding that during the post-climacteric phase, lower temperatures were tolerated. Kosiyachinda and Young (1977) found that phase changes in lipids of mitochondrial membranes occurred during ripening, which presumably affect membrane transport and membrane-bound enzymes. The phase changes occur at critical temperatures corresponding to chilling injury temperatures. In the post-climacteric period, the phase change occurs between 2°C and 5°C, indicating that the lipids have become unsaturated.

2. Modified Atmosphere Storage. The use of storage conditions containing high concentrations of carbon dioxide and low concentrations of oxygen coupled with low temperatures has proved successful in delaying ripening and senescence in many fruits

(Rhodes 1981). Low levels of oxygen decrease overall respiration rate (Tucker and Laties 1985), and also appear to block the ethylene-forming system (Burg and Burg 1967; Beyer 1977). High carbon dioxide concentrations also decrease ripening, possibly by acting as a competitive inhibitor of ethylene (Rhodes 1981). A general discussion of the physiology of controlled atmosphere storage has been covered by Smock (1979).

Research into controlled atmosphere of avocados has been conducted sporadically for over 20 years (Ahmed and Barmore 1980). In the case of 'Fuerte' and 'Hass' avocados, an oxygen concentration of 2% and carbon dioxide of 10% at 5.5°C was found best for extension of storage life and decreasing postharvest physiological disorders (Eksteen and Truter 1982; 1985; Truter and Eksteen 1983). This is in agreement with Spalding and Reeder (1974) who were able to double storage life under similar conditions. However, they did find an increase in fungal damage during the post-storage ripening period which they attributed to a slower ripening rate. Both groups also found a decrease in temperature sensitivity of the fruit, which could be related to alterations in fatty acid composition (Ahmed and Barmore 1980) which occur under controlled atmospheric conditions. Unfortunately the ratio of oxygen to carbon dioxide is important (Smock 1979). The careful control of the gas concentrations requires a gas-tight container with accurate monitoring equipment for use between production areas and final marketing point. In general, such containers are not readily available at an economical tariff. In order to overcome the logistical difficulties involved with modified atmosphere storage, a second approach termed CO_2 shock has been used (Collin 1984; Eksteen and Truter 1985). The latter authors examined the effect of surrounding fruit with 15-30% CO_2 immediately after harvest or 4 days after harvest. Fruits were left in sealed containers for 3 days. Results indicated that shock immediately after harvest was preferable, but considerable research is still necessary to define correct gas ratios as well as duration of treatment. The treatment nevertheless holds promise, as initial work indicates results similar to controlled atmosphere storage, but with fewer practical problems.

Hypobaric storage has received attention. Ahmed and Barmore (1980) quote work by a number of authors, showing that avocados can be stored successfully for long periods under low pressure. Normal ripening and quality was attained after 70 days storage of 'Hass' at a pressure of 60 mm mercury in combination with low temperature. The success of hyperbaric storage appears to be in the increased diffusion of ethylene from the fruit, thus lowering internal concentration. The lower partial pressure of oxygen may also play a role (Rhodes 1981). Unfortunately, practical problems similar to those encountered with controlled atmosphere storage, have limited hyperbaric storage to a largely academic exercise.

3. Gamma Irradiation. Extension of shelf life by gamma irradiation has been successful for some fruits, but appears to hold little prospect for avocado. Akamine and Goo (1971) found avocado fruits to show surface and internal damage at 5 kRad. At this dosage and lower, the climacteric in respiration occurred earlier than that for controls, thus giving no storage advantage. Smith and Jansen (1983) found 2.5 kRad to be the maximum safe dosage, but again without advantage.

4. Other Storage Enhancement Treatments. Various other treatments can be applied to avocado fruit to enhance storage life. The most commonly used postharvest treatment is that of waxing fruit. Durand et al. (1984) found a considerable reduction in moisture loss due to artificial waxing. Bearing in mind the earlier discussion concerning moisture loss as an initial trigger for ripening, the greater retardation in moisture loss will thus increase the potential for extending storage life. Durand et al. (1984) found a small increase in shelf life after 14 days storage at 5°C. Lunt et al. (1981) found a shelf life advantage of up to 5 days where fruit had been stored for 28 days at 5.5°C before ripening at ambient temperature. In addition to conserving moisture, waxing is also believed to modify the internal atmosphere of the fruit tissue, decreasing the internal oxygen and increasing carbon dioxide concentrations (Durand et al. 1984), which will

affect ripening (Rhodes 1981).

A variation to the technique of modified atmosphere is the use of polythene bags to enclose the fruit and allow a modified atmosphere to develop. Fruits are placed in polythene bags and stored at low temperature (Oudit and Scott 1973; Eksteen and Truter 1985). An extension of shelf life was attained, but gas concentrations within the bags were very variable and this could pose a problem. In addition, Eksteen and Truter (1985) found that bags had to be removed before ripening at ambient temperature (20°C) as oxygen deficiencies promoted the development of physiological disorders. The necessity of such bag removal constitutes a severe commercial disadvantage.

A procedure not yet in commercial use, but which may have a future application, is that of shrink wrap. Durand (1984) evaluated a number of shrink wrap materials, and although the procedure showed potential, severe oxygen deficiency with internal physiological disorder development occurred during the ripening phase at ambient temperature. The same disadvantages found with polythene bags are therefore evident. Future development of more suitable materials could make the technique more attractive.

A further perspective to the modification of ripening is the stimulation of ripening at sales points using ethylene. Pryor (1987) reports considerable increases in sales when fruits are exposed to ethylene for 24-48 hours; ripening then occurring in 3-4 days.

F. Postharvest Physiological Disorders

Physiological disorders are common in many fruit crops, particularly where storage for long periods at low temperatures are required. The major physiological disorders are pulpspot and mesocarp discoloration. The symptoms of these disorders are described by Swarts (1984). Pulpspot is a blackening of a region surrounding cut vascular bundles, and is usually localized in nature. Swarts (1984) reports the incidence to be higher early in the season. The other major disorder is that which is often referred to in the literature as chilling injury (Eaks 1976), although, as mentioned by Vakis (1982), it is not necessarily associated with low temperature. This will further be referred to as mesocarp discoloration. The disorder results in an overall grey to brown flesh discoloration, usually most intense in the distal half of the fruit. The symptom is more predominant towards the end of the season. Both disorders involve browning reactions, and as such implicate in particular the enzyme polyphenol oxidase (PPO) (Kahn 1975), and phenolics (Kahn 1977). Zauberman et al. (1985) were unable to find a role for peroxidase where disorders were associated with low temperatures. Factors affecting PPO therefore appear more important.

1. Polyphenol Oxidase Biochemistry as Related to Avocados. The importance of polyphenol oxidase (PPO) in tissue browning and physiological disorders in avocado is clear, as it is in other fruits such as banana, peach and mango (Van Rensburg and Engelbrecht 1986). Although there have been many studies concerning the enzyme, much of which is incorporated in general reviews by Mayer and Harel (1979) and Butt (1980) as well as more specific aspects such as function (Vaughn and Duke 1984a), there is still a considerable amount of conflicting evidence.

It is known that PPO, otherwise known as catechol oxidase, phenolase or o-diphenol oxygen oxidaseductase (EC 1.14.18.1), catalyses the oxidation of o-diphenols to the corresponding quinone, with the loss of hydrogen. The quinones are irreversibly oxidized to melanin pigments, hence a darkening in color and the appearance of physiological disorders. The presence of oxygen is necessary.

Vaughn and Duke (1984a) discuss the location of the enzyme in their review, and conclude that in healthy green tissue PPO exists in a latent form on the thylakoid membranes of chloroplasts. In addition it is often present in rudimentary thylakoids in leucoplasts, proplastids,

and amyloplasts. Tolbert (1973) was also of the opinion that the major site of the enzyme was in chloroplasts. Sharon and Kahn (1979) added that microbodies associated with chloroplasts are an important site of the enzyme in avocados, although Engelbrecht (1982) agrees with the view of Tolbert (1973). Sharon and Kahn (1979) ruled out mitochondria as being a significant site of the enzyme in avocados, while Sharon-Raber and Kahn (1983) found no correlation between carotenoids and PPO activity in avocados. PPO exists in at least two forms, a bound or latent form (Kahn 1977) where the enzyme is attached to membranes such as the thylakoids or similar structures, and a free or active form which is immediately available for reaction in the presence of substrate and oxygen. PPO is not usually involved in uncontrolled tissue browning, nor is it a phenol oxidase (Vaughn and Duke 1984a), yet as Tolbert (1973) noted, active PPO present in a latent form exists in chloroplasts. Some form of regulation or suppression of PPO must therefore occur. Vaughn and Duke (1984a) found that PPO is nuclear coded, but is inactive until incorporated into a plastid. Vaughn and Duke (1984b) found that PPO is first incorporated into the chloroplast inner membrane. However, at this stage it appears to be in an unprocessed form, and although identified immunologically as PPO, is inactive. From the inner membrane, incorporation into thylakoids with, at this stage the probable addition of copper, is necessary. Further, regulation of PPO within the thylakoids must also occur. Some form of cellular damage (Vaughn and Duke 1984a) appears necessary, with subsequent removal of suppression, release from the membranes and activation, before browning as found in physiological disorders, will occur. Alteration of membrane structure and function is therefore a possible means of activation. There are also a number of other possibilities which require investigation as a background to a discussion of the possible causes of physiological disorders in the avocado.

Conformational changes due to pH alteration are a possibility (Lerner and Mayer 1975). Lerner et al. (1972) found this to occur in grapes. These authors found that prolonged exposure to low pH could cause irreversible aggregational changes. There does not appear to be an indication of whether this is so during the development of avocado disorders, but if anaerobic respiration occurs, then an acidification of cytoplasm is possible. Dizik and Knapp (1970) found avocado PPO to consist of five fractions. Of these, a 28,000 Dalton fraction (a dimer) was the most active. Van Lelyveld et al. (1984) found a change in subunit prevalence with the development of mesocarp discoloration. Altered cellular pH could result in a change in subunit fractions. Flurkey (1986), suggests that post-translational changes take place resulting in glycosylation. The added carbohydrate would change the apparent molecular mass. An enzyme-inhibitor complex could also play a role in activation control (Tolbert 1973). Soderhall et al. (1985) found high calcium concentrations could activate the enzyme in carrots. This has not been confirmed in avocados, but may be of importance and requires further research. Although future investigations should not necessarily be limited to these avenues, it should be noted that for browning to occur, PPO and substrate must be in contact with each other. This implies physical cellular damage. The factors causing cellular damage are therefore important.

2. Factors Inducing Physiological Disorders. Factors affecting or inducing physiological disorders appear to be divided into three groups. Bezuidenhout (1983) ascribed the reasons for physiological disorders to postharvest conditions such as temperature, period of storage, or storage atmosphere as well as preharvest orchard factors. While the two aspects will be examined separately, there is nevertheless an interaction between the two. Bower and Van Lelyveld (1985) found that fruit from trees subjected to water stress during the first 3 months after fruit set, showed higher levels of soluble and total PPO activity as well as visible mesocarp discoloration, than fruit from nonstressed trees, when subjected to restricted ventilation during ripening after storage.

a. Preharvest Factors. The preharvest factors affecting postharvest characteristics must be

particularly important during the early cell division phase when structural characteristics of cell components such as cell walls and membranes are determined (Bangerth 1979). Calcium has been shown important in the expression of physiological disorders of many plants (Bangerth 1979; Millaway and Wiersholme 1979; Ferguson 1984). Poovaiah (1979) attributed the slower rate of senescence in tomatoes having a higher calcium content to prolonged membrane integrity. The known effects of calcium on permeability control and stability under stress (such as low temperature storage) are mentioned by Roux and Slocum (1982). Control of membrane constituent turnover, cell wall precursors, and microtubule assembly all appear to be affected by calcium, as are membrane fluidity alterations, permeability, and enzyme activity changes, as previously discussed under fruit ripening. The latter section discussed calcium physiology in terms of fruit ripening. However, factors leading to abnormal ripening are also likely to contribute to physiological disorders. Thus aspects on calcium previously discussed should be referred to.

If calcium physiology is involved in the manifestation of physiological disorders (Chaplin and Scott 1980 found higher calcium levels to decrease cold-induced disorders in avocados), then the factors affecting the preharvest uptake, movement, and deposition of calcium will be important. This is particularly so during the first 3 months after fruit set, shown by Bower (1985b) to be the period of maximum avocado fruit calcium concentration changes.

Calcium entry into the plant may be influenced by functional root area, soil water content, ion exchange, energy for active uptake, and ion competition, notably $NH4^{+}$ (Fukumoto and Nagai 1983), K^{+}, and Mg^{2+} (Kirkby and Pilbeam 1984). Once in the plant, calcium moves undirectionally in the xylem to areas of young, actively growing tissues (Bangerth 1979). Within the xylem, movement is by a combination of mass flow and ion exchange (Clarkson 1984). Calcium binds weakly to lignins (Shear and Faust 1970). Removal from a binding site allows replacement by further calcium, thus creating a calcium store for further mobilisation. However, of importance is that between binding sites, calcium moves with the transpirational stream, and therefore the faster the transpirational flow the faster the movement of calcium (Hanger 1979). Any factor reducing transpiration, such as water stress due to insufficient soil moisture or excessive environmental demand, or alternatively lack of demand during cool, cloudy days, will reduce calcium movement to both leaves and fruit. In addition, the influence of leaf: shoot ratio must be taken into account. Clarkson (1984) indicates that two distinct sinks exist which alter diurnally. During the day, leaf sinks, particularly old leaves, predominate, due to the direction of maximal transpirational flow. At night, when transpiration ceases, areas of high meristematic activity become more important. Here, apoplastic binding sites unload calcium bound in the xylem walls. In the avocado, early fruit development coincides with a spring growth flush, which is often distal to the fruit. A competition therefore exists between the fruit and developing leaves. Kirkby and Pilbeam (1984) show calcium movement to be strongest towards terminal buds. Witney et al. (1986) found nonvigorous trees to have higher fruit calcium contents than vigorous trees, and ascribed this predominantly to the difference in spring growth flush. The explanation for the difference in shoot and fruit calcium sink strengths could be that calcium transport may be influenced by the movement of auxin (Lee et al. 1984). Vigorously growing shoots would be strong exporters of auxins, outcompeting the fruit calcium sinc strength (F. Bangerth, personal communication). A decrease in spring growth by cultural techniques would be desirable for fruit quality. Of further importance, is overall fruit growth rate. Very rapid cell division and expansion could cause greater dilution of the total calcium pool available in the fruit, resulting in lower concentrations in larger fruit, as were also found by Witney et al. (1986). These authors also consider that the very steep decline in calcium concentration which occurred 6-10 weeks after fruit set, may be caused by a net export of calcium at this time, which Wiersum (1979) found to be possible. A high degree of water stress at this time could be important. Water moves from fruits to surrounding leaves, and transports calcium with it (Bangerth 1979). Bower (1985b) found that trees showing greater water stress, also showed the most rapid decline in fruit

calcium concentrations. It is also noteworthy that such fruits showed greater susceptibility to physiological disorders (Bower and Van Lelyveld 1986). Calcium concentrations within the fruits also vary, with the distal portions having lower concentrations (Bower 1985b). On a subcellular level, however, little is known. It is not known for instance, whether remobilisation of calcium for organic anion neutralisation resulting in subcellular deficiencies and thereby physiological disorders, occurs in avocados as happens in apples, (Perring 1986). Dupont (1981) mentions lipoxygenase activity (to be discussed later) in the presence of calcium. Calcium concentration alone, however, is not necessarily a determinant of fruit quality, due to the dual role of calcium as both physical stabilizer of membranes and a physiological second messenger (Poovaiah 1985). Research into the role of calcium in avocados, particularly physiological disorders, is severely limited, and the role of other elements such as magnesium and potassium even less well known. Using general principals known and specific work discussed as they pertain to other crops, the schematic diagram of the central role of calcium, the factors affecting the mobility, and action within the plant leading to physiological disorders is shown in Fig. 7.1.

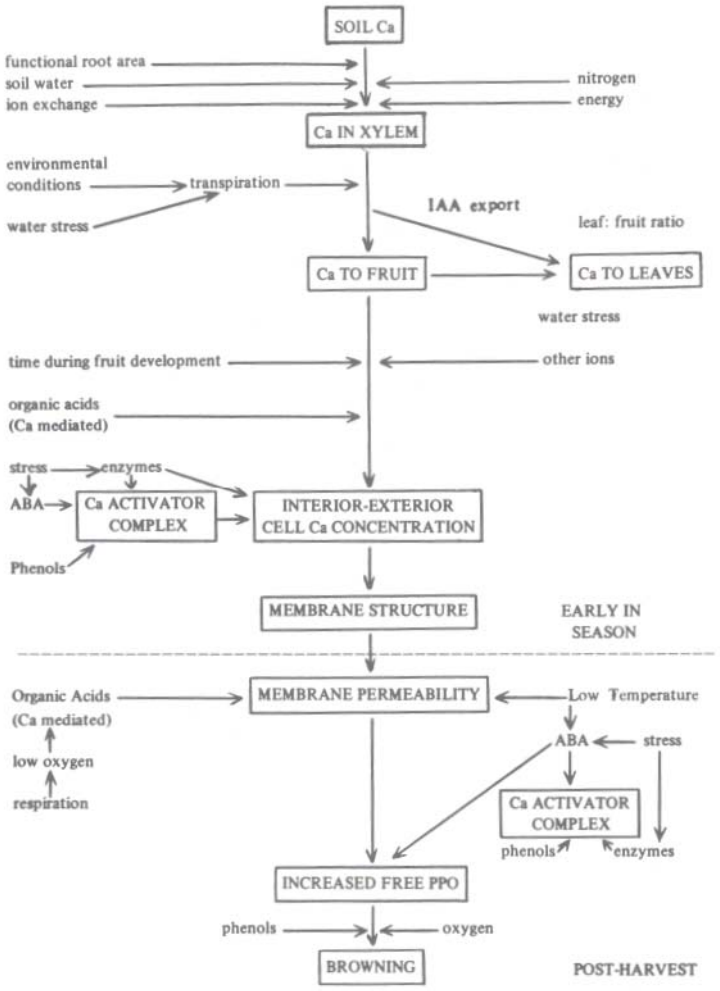

Fig. 7.1. Schematic representation of possible interactions between calcium and other plant and environmental factors in determination of fruit quality.

b. Postharvest Factors.

1. Temperature. Prolonged low temperature storage results in the development of

physiological disorders (Chaplin et al. 1982; Couey 1982). The effect of low temperatures on membrane stability and particularly lipid phase changes has already been discussed in the section on ripening. The recent general review on the response of plants to low temperatures (Graham and Patterson 1982) should also be consulted as a background, although little specifically concerning avocados is mentioned.

Graham and Patterson (1982) mention that in a number of plants, the presence of phenolic compounds increases during periods of low temperature due primarily to an increase in phenylalanine ammonialyase (PAL) activity. Should phenolic substrates for PPO increase, then the intensity of the browning reaction could be greater (Kahn 1977), although other factors must be responsible for cellular damage to allow sufficient access of enzyme to substrate. Van Lelyveld et al. (1984) were unable to show changes in PAL activity with the development of mesocarp discoloration, although higher concentrations of total phenols, hydroxycinnamic acids, and proanthocyanidins were measured. Van Lelyveld et al. (1983) found higher PAL activity in pulpspot-affected fruit, implying greater phenolic concentration (Graham and Patterson 1982).

Respiration changes are associated with storage temperature changes. The avocado is known to contain the alternative salicylic hydroxamic acid (SHAM)-sensitive as well as CN-sensitive pathways (Theologis and Laties 1978; Moreau and Romani 1982). Laties (1982) indicates that as storage temperatures decrease, the alternative pathway becomes more important in total energy production. Nel et al. (1984) found that the activity of the alternative pathway was further enhanced by storage time. They found SHAM-sensitivity only when succinate was used as substrate. Laties (1982) reports that an Arrhenius plot of succinate oxidation by callus of chilling-sensitive *Cornus* showed a sharp break in the presence of SHAM at the chilling damage temperature, indicating the importance of the alternative respiratory pathway at low temperatures. The physiological consequences of this situation in avocado are unknown, but the results of Nel et al. (1984) indicate that lipoxygenase, which may be a key enzyme in the oxidation of structural lipids (Feys et al. 1980) could become important as a source of residual respiration (Solomos 1983) at low temperature, which may result in membrane destruction. The alternative pathway may also result in the formation of superoxides and hydrogen peroxide, also causing membrane damage (Solomos 1977). Although further research is necessary, such a sequence of events may explain the symptom of pulpspot, the browning of the procambial cells surrounding vascular bundles, as opposed to the more general browning reaction of mesocarp discoloration. The avocado is unique, in that the procambial cells remain active, undifferentiated, dividing cells throughout the life of the fruit (Robertson 1971) while the mesocarp cells are more mature. While little is known of the physiology of these cells as opposed to that of the mesocarp cells, their less mature status may imply a greater sensitivity to chilling, particularly early in the normal harvest period, when pulpspot is the more prominent disorder. An ultrastructural study of pulpspot by Engelbrecht (1979; 1982) showed that membrane changes had occurred in the procambial cells before the fruit was cut, which would be consistent with the previous discussion. Later in the harvest season, the procambial cells may, through environmental effects already discussed, be more chilling tolerant, and therefore behave more like mesocarp cells. If sufficient stress to cause membrane damage occurs, damage would be more general, resulting in mesocarp discoloration. Further, Bower (1985a) found a rapid increase in PPO activity towards the end of the normal 'Fuerte' harvest season in South Africa, which would result in a more intense overall browning should sufficient cell damage occur in storage, thereby causing mesocarp discoloration, which is more prevalent at this time.

2. Container Atmosphere. While an alteration in the normal atmosphere surrounding the fruit may have positive effects during storage as discussed in the section on fruit ripening, incorrect ratios of oxygen to carbon dioxide may result in physiological disorders. Van Lelyveld and

Bower (1984) and Bower and Van Lelyveld (1985) found that restricted ventilation leading to oxygen depletion, particularly towards the end of a prolonged (30 days) low-temperature shipping period, caused enhanced PPO activities, both soluble as well as total, implying activation and release of the enzyme. Visible browning also occurred, which is in accordance with the earlier work of Spalding and Marousky (1981). The latter authors found the critical oxygen level for damage to be approximately 1%. At concentrations lower than this anaerobic respiration occurs. Nel et al. (1984) found that lipoxygenase activity then increased, with the resultant lipid (and therefore membrane) degradation. While avocados can also be adversely affected by high carbon dioxide content, it is less critical, with concentrations of up to 25% being nondamaging, provided oxygen is not limiting. In fact, careful manipulation of the surrounding atmosphere as discussed in ripening, can be beneficial in the prevention of physiological disorders. The method of CO_2 shock seems particularly useful. The physiological basis may lie in its effect on phenolics. Prusky et al. (1985) found epicatechin to be an inhibitor of lipoxygenase in avocados. During ripening, epicatechin content decreased, with a concomitant increase in lipoxygenase activity. Under conditions of enriched carbon dioxide atmosphere, epicatechin content did not decrease.

3. Container Humidity. During the continual mechanical cooling of circulating air within containers so as to maintain shipping temperature, water vapor is removed, resulting in a concentration gradient from the fruit to surrounding atmosphere. Even where fruit was waxed to decrease such a loss, Durand et al. (1984) found a 5% loss of mass within 14 days at a container temperature of 5°C. In a simulation of shipping conditions by Bower and Cutting (1987), a significant reduction in fruit water loss over a 30-day storage period was obtained by humidifying the container atmosphere. In addition, a highly significant decrease in postharvest physiological disorders as compared with the control was noted. Post-harvest moisture stress thus seems to play a role in the initiation of physiological disorders. The underlying physiology is not fully understood, but ABA may be implicated. Scriven and Wills (1984) found a positive correlation between the ABA level early after harvest and the degree of core breakdown in apples after storage. Similar results were found by Bower et al. (1986) for avocado, the ABA level at the climacteric correlating well with PPO activity after softening. In further work (Cutting and Bower 1987), varying concentrations of ABA were infused into avocados after harvest. Higher initial concentrations of ABA tended to increase PPO activity, but also increased ABA metabolism such that after softening, residual ABA was negatively correlated with PPO activity. Infusion of water had the reverse effect. The rate of ABA metabolism increased with increasing fruit maturity, as did PPO activity. A decrease in initial ABA synthesis (as with limiting postharvest stress) or prevention of metabolism may enhance fruit quality. The physiological role of ABA metabolism is unknown in relation to the initiation of physiological disorders, but further research may be of considerable value.

Overall, while many aspects of the initiation of avocado physiological disorders are still unknown, and of the known factors the relative influence of each is unclear, sufficient is known to at least severely limit the occurrence of disorders. Membrane structure, function, and stability under stress together with the postharvest conditions of temperature, humidity, oxygen, carbon dioxide, and ethylene to which the fruit is exposed, are all important.

IV. CONCLUSIONS

In reviewing the literature it was notable that certain aspects of avocado fruit growth and ripening physiology have been thoroughly investigated, while others are virtually untouched or are only beginning to receive attention. This may be due to the difficulties experienced when working with the avocado, such as high variability in results and the biochemical and

physiological problems associated with a tissue having high phenol and oil contents.

Wolstenholme (1985) believes that the avocado producer is not as efficient as his deciduous-fruit-growing counterpart. In order to address this problem, we feel that more attention must be given to the question of endogenous growth regulation, particularly the effects of fluctuating yields, fruit retention, growth, and development. A model for the plant growth regulator changes during avocado fruit development is suggested in Fig. 7.2. However, further research is still necessary to understand the endogenous control of fruit development prior to the possibility of effective growth manipulation. Greater tree productivity will become a reality when the concepts of canopy architecture and dwarfing receive long overdue and necessary attention.

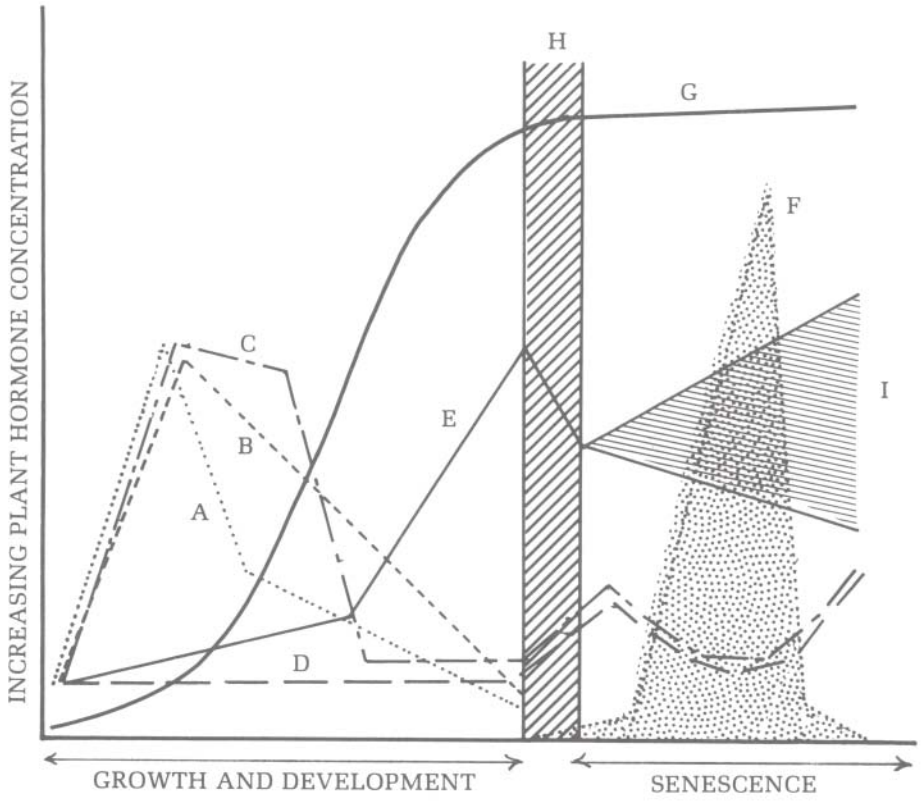

Fig. 7.2. Model for plant hormone movements during avocado fruit development and senescence. Trends for IAA (A), gibberellins (B), free cytokinins (C), bound cytokinins (D), abscisic acid (E), ethylene (F), as well as the growth curve (G) and the `lag' phase between harvest and the onset of ripening (H) are presented. Final abscisic acid levels (I) depend on preharvest conditions.

In this review stress has been shown to be an important contributing factor in the occurrence of physiological disorders. Further, fruit quality research into the biochemical control mechanisms leading to physiological disorders is necessary. In particular, the interactions between plant growth regulators, PPO, phenolics, calcium, and membranes under varying pre- and postharvest conditions require investigation. The majority of countries involved in the international avocado fruit trade (with concomitant long periods of low temperature transport and storage) have climatic conditions differing considerably from the native avocado climate. Preharvest stress in cultivars selected in natural habitats and grown elsewhere can be accepted as inevitable. Therefore more active plant breeding programs with fruit quality as a major objective will be necessary in avocado-exporting countries.

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