Chapter 11 - Fruit growth, ripening and post-harvest physiology

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This Chapter is updated from a previous version written by Rod L. Bieleski, Ian B. Ferguson and Elspeth A. MacRae for *Plants in Action* 1st Edition.

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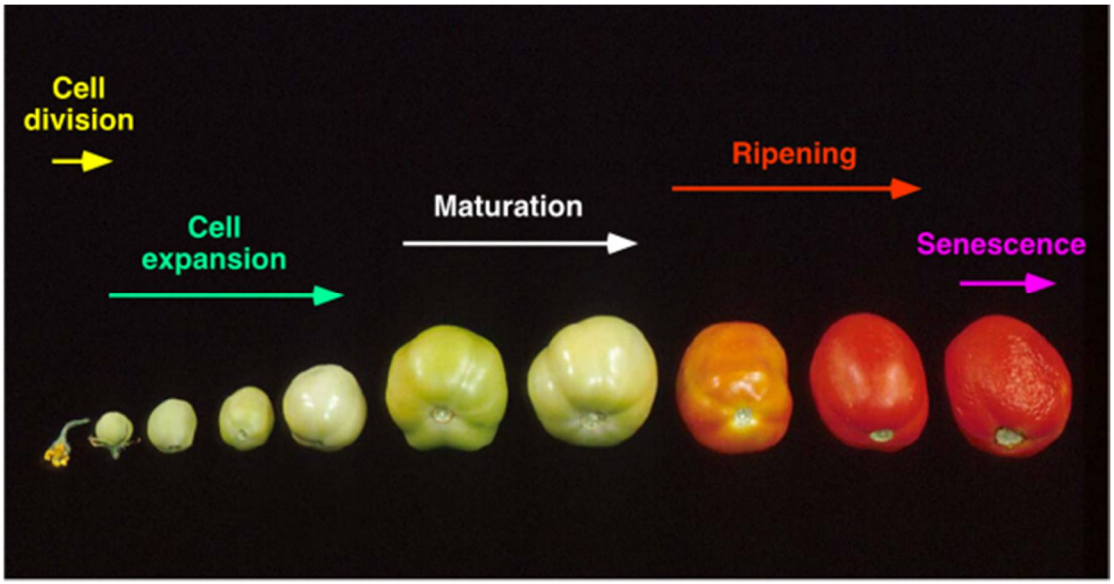

Fruit development can be divided into a series of stages, as shown here for tomato. Early in development fruit are enlarging rapidly and are small, hard, green and accumulating organic acids. The seeds become mature prior to ripening. During ripening fruit become soft textured, and accumulate soluble sugars, pigments and aroma volatiles. Eventually fruit will become over-ripe, cell structures will deteriorate and the fruit will become susceptible to pathogens. (Photograph courtesy D.A. Brummell)

Fruit evolved as vehicles for production and dispersal of seeds. Humans then imposed further selection pressures to develop products for our use. Such development has accelerated over the past century. Our concept of a fruit as a sweet and fleshy object for eating is really quite recent in evolutionary terms.

Despite modifications and exaggerations of fruit structures due to human selection, and with a few seedless exceptions such as banana and sultana grapes, fruit growth is largely dictated by seed development. Processes underlying those influences are considered here, followed by an account of postharvest changes.

Postharvest technology is also a human device to serve human needs, and includes everything that happens to crops between harvest and human utilisation. However, preharvest events affect subsequent postharvest behaviour. Genetic background is particularly important, in part determining crop response to growth and storage environments. Postharvest techniques can be simple and slight, as in grain stores, or highly developed, as in controlled atmosphere storage of fresh produce.

In every instance, some key concepts apply. In particular, *potential quality* of fresh commodities is irretrievably fixed by harvest time, with no further opportunities to manipulate their basic properties. After harvest, storage time may be as short as the few minutes taken for immediate consumption, or extremely prolonged as with years of seed bank storage. Most research discussed here is concerned with storage periods of 2–25 weeks. Handling over this period has a profound effect on final usefulness because a crop is still *alive* throughout this process and vulnerable to adverse conditions. Postharvest physiology is therefore of particular importance to countries such as Australia and New Zealand, which ship a large portion of their crops to distant markets. Accordingly, green kiwifruit (*Actinidia deliciosa*) and yellow kiwifruit (*Actinidia chinensis*) are used here as examples where principles of postharvest research have been applied successfully in establishing a new international crop on a stable and permanent basis.

Wild fruit often contain components that make them unattractive to potential consumers until they are ready to be dispersed. These commonly include calcium oxalate crystals (raphides), bitter flavours and astringent tastes. However, humankind has adapted fruit for personal use by applying intense selection pressure to remove unpleasant components and enhance desired features. These include appealing flavour with well-developed aroma volatiles and sweetness, bright colour, pleasant texture and high food value. As early as the seventeenth century, Gerard recorded that ‘some peares are sweet, divers fat and unctuous, others soure, and most are harsh, especially the wilde peares’ (John Gerard, “Herball or General Historie of Plants”, 1633). Nowadays, all European and Asian cultivars are sweet with greatly reduced acidity and tannin content compared with their various wild parents. Persimmons provide another example where an originally astringent fruit has given rise to a non-astringent form.

During the twentieth century, additional selection pressures have been applied to temperate fruit species in a drive for cultivars that are well suited to postharvest handling and storage. This has not been true for most tropical fruit, which have had less selection for such characteristics and which still present challenging problems for postharvest researchers and breeders.

The history of kiwifruit shows some of the steps that have led to the development of a successful commercial fruit. It was a wild species until 1900, when domestication began. A major commercially valuable green cultivar, ‘Hayward’, was selected around 1930. This event was followed by progressive development of techniques for cultivation, handling and marketing — all required to make a new fruit commercially successful. Further development of the yellow or golden kiwifruit cultivar ‘Hort16A’ in the late 1990s added the unique combination of a highly flavoured fruit with a vibrant yellow flesh. Several features of kiwifruit physiology made it amenable to commercialisation, namely (1) unusual and unique flavours combined with good nutritional value, (2) retention of chlorophyll so that inside tissues remain bright green when ripe (‘Hayward’), (3) a long and manageable ripening period resulting in a long harvest season where fruit can be picked in a

mature but firm condition, and (4), especially important, kiwifruit tolerate lengthy low-temperature storage without subsequent shelf life being compromised.

11.1 - Origin of fruit tissues and fruit set

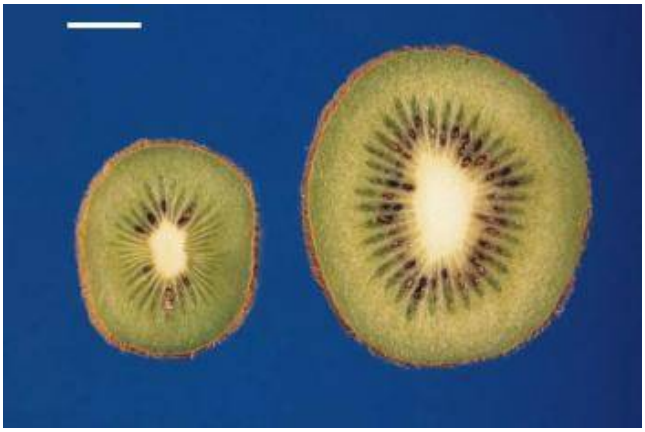

Figure 11.1 Poor pollination (left) compared with normal pollination (right) influences seed number and hence kiwifruit development. A fully pollinated green kiwifruit carries at least 1000 seeds spread more or less evenly lengthwise, and in about 35 locules around its circumference. Faulty pollination causes big disparities in seed number per locule (from around 30 to near zero). There is a corresponding change in relative development of adjacent tissues. Scale bar = 1 cm. (Photograph courtesy M. Heffer and R.L. Bielski)

Pollination, followed by pollen tube growth and fertilisation, instigates fruit growth (Figure 11.1). If pollination does not occur, flowers are shed (only rare exceptions). Nevertheless, a developmental program of gene expression for fruit growth has already been established well ahead of floral biology. Primordia may have been initiated up to 6 months before a particular flower opens, and ovary development continues during flower growth with ovary tissues forming late in this process. As part of that outcome, homology between leaves and sepals is noteworthy and evident in many fruit (Gillaspy *et al*. 1993). Sepals show leaf-like cell layers, stomata and chloroplasts.

The generic term ‘fruit’ covers a wide range of structures, all supporting and protecting seeds, but where the various parts have developed from the original fertilised flower in various distinctive ways. In the simplest form, ovary walls grow along with seeds, and as they develop, the ovary walls dry out to become a pod (legume) or capsule (poppy). In others (particularly fleshy fruit), the main structure can arise by exaggerated development of a particular part of the original floral unit. These include ovary wall or central axis, the receptacle that supports anthers and ovary, or even petals and sepals. In morphological terms, fruit are structures that develop from fertilised or stimulated ovules, plus associated floral parts that originate from the parent plant.

Mechanistically, a fruit is a single dispersal unit that includes seeds and associated tissues, developed as a single body. This broad description includes structures derived from a single ovary (as in simple fruit such as apple, avocado and mango) as well as compound fruit where separate ovaries are joined (an aggregate fruit such as blackberry and cherimoya) or where separate flowers are collected into a single structure (pineapple and breadfruit).

During fruit development, an ovary wall becomes a pericarp: either dry as in a dehiscent pea pod and the indehiscent caryopsis of barley, or fleshy as in berries (grape). Three morphologically distinct

strata are present and developed to varied degrees: exocarp (fruit skin), mesocarp (fruit flesh) and endocarp (inner cell layers).

An exocarp will develop a cuticle and may exhibit a variety of morphological features such as coarse hairs (kiwifruit) or fine hairs (peach). The exocarp plus cuticle restricts gas exchange, and determines the general appearance of ripening fruit. Most cuticles are highly impermeable to gases, so that water vapour, O_2 and CO_2 diffuse mainly via either stomata or lenticels or by mass flow through cavities at the calyx and stem ends of fruit.

Mesocarp tissues usually represent the fleshy part of a fruit, and commonly hold chloroplasts and starch grains. In fleshy fruit such as berries (e.g. tomato, kiwifruit and grape) this tissue typically comprises large parenchyma cells and contains the main vascular network.

Endocarps are less common, but typically develop as a dense hard case around a seed, as in peach, apricot or macadamia.

An ovary must be stimulated in some way for fruit growth to occur; this is normally by pollination and fertilisation. Gibberellins and auxins are involved in the pollination stimulus, and subsequent hormone production by the fertilised ovary is critical to stimulating fruit development (de Jong *et al*. 2009).

By implication, a suitable balance of growth regulators applied to unpollinated fruitlets can result in fruit set, and in practice gibberellins GA_4 and GA_7 are very effective in setting parthenocarpic (seedless) apple fruit. By contrast, parthenocarpy is rare in kiwifruit, although repeated applications of napthaleneacetic acid (NAA) with benzyladenine (BA) and gibberellin have been successful. Such results confirm that growth regulators — alone or in combination — can trigger cell division in ovaries or related tissues that ultimately become fruit.

Seedless fruit have arisen via human selection of genotypes in which ovaries produce an adequate supply of growth regulators without any stimulation from the germinating pollen and developing seed (triploid banana), or where fertilisation is closely followed by seed abortion (stenospermocarpic, as in sultana grape). In the absence of pollination, levels of endogenous hormones such as auxins and gibberellins normally fall markedly (de Jong *et al*. 2009) and flowers abscise or fruitlets stop growing.

11.2 - Dynamics of fruit growth

Fruit can increase in mass or volume by 100-fold or more from fertilisation to maturity, and such changes commonly follow a sigmoid curve (Figure 11.2). Interpretation of such growth curves is complex because a single variable (mass, length, volume) is commonly applied to an object that contains several organs and different tissue types, each developing at their own rate and in accordance with their own programme. Moreover, at a cellular level, comparative levels of division and expansion change with ontogeny, while shifts in airspace percentage also play a part in volume increases. Added to this, changes in storage products (oil, starch and sugar) and structural carbohydrate (endocarp thickening) influence dry matter content. Representative cases are covered later.

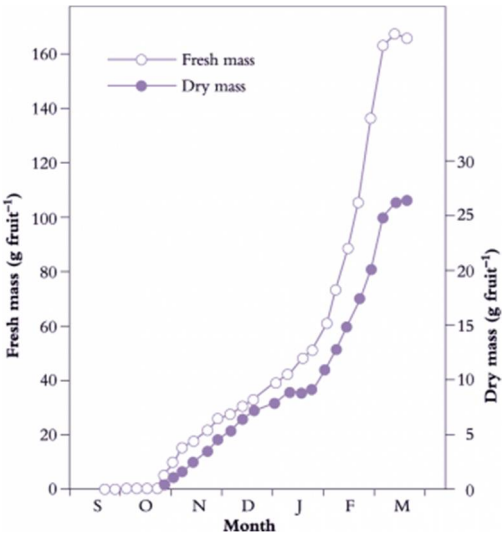

Figure 11.2 Peach growth is biphasic, showing a double sigmoidal pattern in terms of both fresh mass and dry mass. Pericarp cell division is especially active during early stages of phase 1, while enlargement of an existing population of cells is largely responsible for growth during phase 2. (Based on Chalmers and van den Ende (1975) Aust. J. Plant Physiol. 2, 623-634)

11.2.1 - Cell division and enlargement

Despite complexities of fruit growth and development, there are some overall consistencies in patterns of cell division and enlargement, as well as tissue differentiation and fruit enlargement (Figure 11.3). During the first 1–4 weeks, flesh volume increases rapidly and embryo volume remains small. Growth at this time is mainly the result of cell division. In many commercial fruit (e.g. apple, kiwifruit, tomato and peach), cell division may cease a few weeks after anthesis, and fruit growth slows down, reflected as an inflection in the growth curve, and signalling an end to the first sigmoid phase.

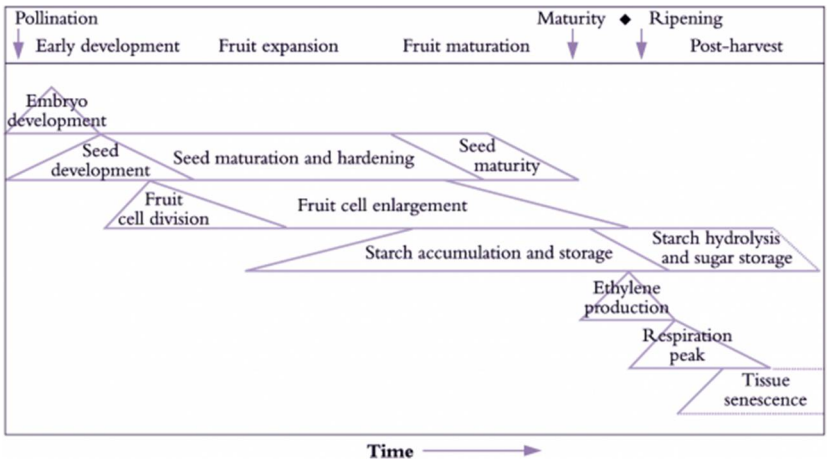

Figure 11.3 A large number of complex processes are integrated in space and time during seed development and fruit growth and are shown here schematically. In broad terms, embryo differentiation and seed development are already well advanced as pericarp enlargement gets underway, and seed maturation usually precedes onset of ripening; consequently fruit ingested prematurely still represent vehicles for seed dispersal. A phase of carbohydrate accumulation during fruit maturation gives way to starch hydrolysis and sugar storage during maturation, accompanied by a peak in ethylene output and respiratory activity as fruit ripen. (Original diagram courtesy I.B. Ferguson)

During early growth, the fertilised embryo and endosperm develop and seeds start to form (Figure 11.3). A second phase begins where the pericarp resumes growth and continues to enlarge until slowing for a second time as fruit mature. This second phase in fruit growth is mainly accomplished by cell expansion in longitudinal, radial and tangential planes. Longitudinal growth, where cells enlarge parallel to the long axis of the fruit, will often be a big factor for development of elongated fruit such as cucumber and marrow. Radial growth increases diameter as in some pumpkins. Increases in cell volume during fruit growth can be considerable. Mature watermelons end up with some of the largest parenchyma cells in the Plant Kingdom, about 0.7 mm in diameter. In contrast to this general pattern where cell division ceases after a few weeks, pericarp cells of avocado fruit continue to divide over the whole growth period so that cells in mature fruit are still relatively small.

Cell enlargement is not a uniform process. Cells in various regions of a fruit often enlarge at different rates and in different planes, so that many mature fruit show strong gradients in cell size from their surface to the centre. In apple fruit, cells closest to the core are smallest, with cell size increasing towards the fruit surface. Conversely in many berries, such as cucumber, kiwifruit and grape, the smallest cells are found in outer regions of the pericarp, with size increasing progressively towards inner regions.

11.2.2 - Cell differentiation

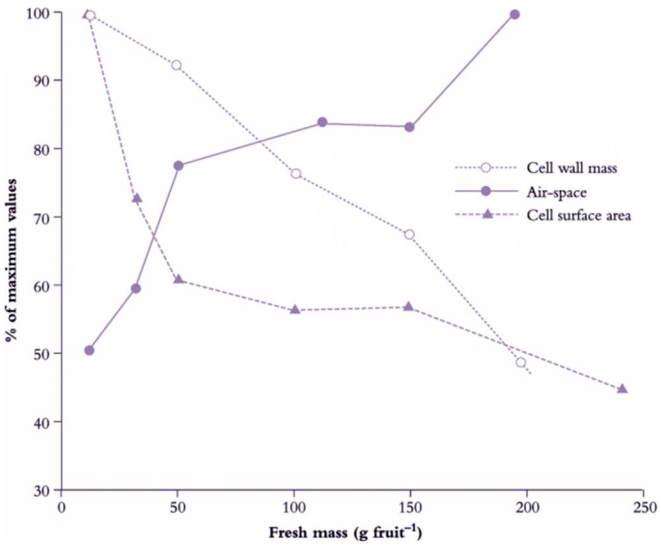

Figure 11.4. Physical characteristics of apple fruit change during growth and development with a notable increase in gas space to a maximum of around 0.1 mL gas mL^{-1} tissue at maturity, and a corresponding decrease in cell wall mass to around 15 mg g^{-1} fresh mass. Cell surface area shows an early rapid decrease from around 340 cm^2 mL^{-1} tissue. (Based on Harker and Ferguson (1988) Physiol. Plant. 74, 695-700)

Patterns of cell growth and differentiation in cell layers can influence the quality of mature fruit. For example, pepino fruit with a compact exocarp composed of tightly packed cells are less likely to bruise during postharvest handling than cultivars having large intercellular airspaces. As cell size increases during development, other accompanying characteristics also change, such as cell wall thickness, differentiation of specific cell types (e.g. sclereids) and the formation of cell inclusions such as oil droplets or calcium oxalate crystals (raphides). In feijoa and pear, development of

sclereids in the mesocarp provides the characteristic gritty texture. As another example, juiciness of orange depends on prior differentiation of juice sacs in the endocarp.

The extent and distribution of airspaces are particularly important, affecting both fruit texture and physiological properties. For instance, in apple, airspace relative to fruit volume can double during development, while cell wall thickness and relative cell surface area both decline (Figure 11.4). Such changes affect gas exchange and diffusion of solutes through pericarp tissues due to increased tortuosity.

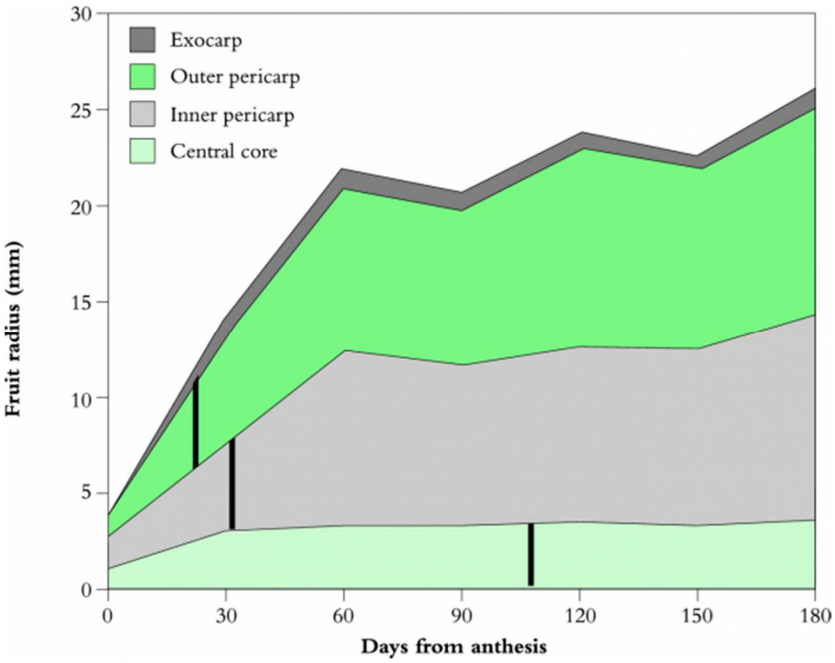

Figure 11.5. Radial growth in kiwifruit is due mainly to enlargement of outer and inner pericarp. Vertical lines indicate cessation of cell division in each tissue. (Unpublished data courtesy K. Gould and I.B. Ferguson)

In kiwifruit all tissues of the mature fruit (exocarp, outer and inner pericarp and central core) are already discernable in the ovary before anthesis and pollination. Each layer grows to a different extent and at different rates, so that the relative contribution of each to the total fruit volume varies with time (Figure 11.5). Cell division ceases first in the exocarp and last in the innermost regions of the central core. The outer pericarp is first seen as a homogeneous population of cells but by c. 14 d after pollination two cell types become visible, namely small isodiametric parenchyma cells full of starch grains, and much larger heavily vacuolate ovoid cells in which the frequency of starch grains per unit volume is low.

Fruit anatomy affects our perception of fruit quality. In kiwifruit, hairs are developed as multicellular projections of the skin, giving a characteristic bristly appearance and rough feel in the case of the green flesh ‘Hayward’ or a silky, smooth appearance in the yellow flesh ‘Hort16A’ cultivar. Tough skin relative to soft flesh is another important character imparted by development of primary cell wall thickenings in the hypodermal collenchyma.

11.2.3 - Seed development and fruit growth

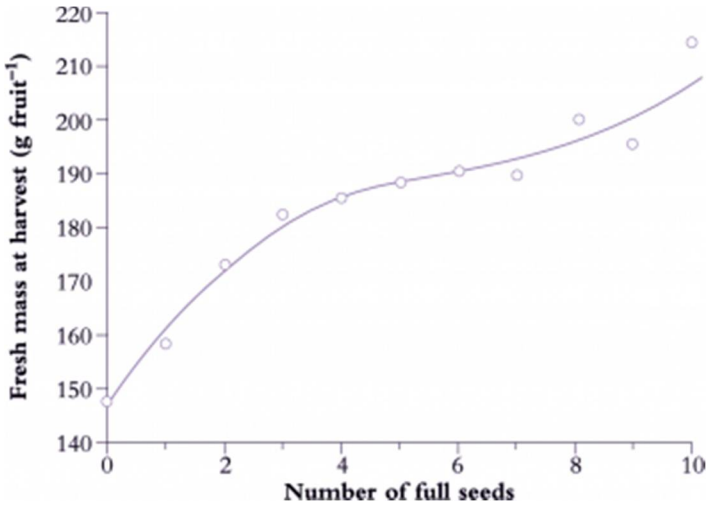

Figure 11.6. Fruit size in Braeburn apple depends closely on the number of viable seeds per fruit (up to a normal maximum of 10 per fruit), emphasising the strong influence that seed development has on fruit growth. (Figure based on data from E.D. Broome)

Fertilisation is generally crucial for fruit set and pericarp development (Figure 11.1). As fertilised ovules develop into seeds, this influence on pericarp growth continues where production of hormones by the endosperm and developing embryo promotes pericarp growth. Indeed, there is usually a positive correlation between the number of seeds in the fruit and final fruit size (de Jong *et al.* 2009). The importance of seeds as sources of hormones for initiation and stimulation of fruit growth is implied by fruit response to exogenous hormones in parthenocarpic systems (development of fruit without seeds).

Applying auxin and gibberellins to unfertilised embryos is one way of achieving parthenocarpy; another is to use auxin transport inhibitors such as chloroflurenol to prevent loss of auxin from embryos so that a threshold level for pericarp response is exceeded. Studies of parthenocarpy in tomato and cucumber indicate that high auxin levels enhance embryo cell division, and this cell division phase seems to be more critical than subsequent cell expansion in determining final fruit size.

Such results imply a cooperative mode of action where gibberellins combine with auxins to initiate cell division. Seed cytokinins and cell division are similarly related because tomato seeds accumulate cytokinins that subsequently influence cell division in surrounding pericarp tissue (Gillaspy *et al.* 1993).

Such interdependence between seed development and fruit growth shows up in final fruit size. Parthenocarpic fruit have reduced auxin content and are generally smaller than wild-type fruit. In apple fruit, seed numbers frequently correlate with fruit growth (Figure 11.6) or with shape and size of fruit. Inadequate pollination of kiwifruit (Figure 11.1) results in distortion, and a curvilinear relationship emerges between seed number and fruit weight. A similar response is obtained when young seeds are surgically removed from immature strawberry fruit, causing a corresponding distortion in flesh development.

Despite ample evidence that natural control of fruit shape is primarily exerted by plant hormones originating from seeds and stimulating growth to varying degrees, this is not true for all fruit. In banana, fertile seeds actually suppress development of the fleshy pulp. In this anomalous case, fertilisation failure *allows* an ovary to grow.

In marrow, tomato and kiwifruit, ovary shape dictates spatial distribution of seeds. They in turn influence pericarp growth, so that fruit size and shape then become a function of initial ovary shape plus subsequent fertilisation and seed development.

11.3 - Resources for fruit growth

As fruit grow, proportions of cell wall, carbohydrate, organic acid, lipid, phospholipid and volatile (aroma) compounds change dramatically; and within each of those groups there are changes in the proportion of individual group members. Of these, by far the most important in practical terms is carbohydrate economy. Two sets of issues are at stake: (1) rate of growth, attainment of maturity and final fruit size, and (2) aroma, flavour and texture in ripe fruit. Both carry commercial implications.

Enlarging fruit require carbohydrate to sustain cell division, enlargement and tissue specialisation. Only in later stages are carbohydrates typically retained as either starch or soluble sugars. Soluble carbohydrate is mainly imported as photoassimilate, with only a minor contribution from local CO_2 fixation, and reassimilation of respiratory CO_2.

During peak fruit expansion, usually early summer, there is an intense flow of photoassimilate from mature leaves (sources) into rapidly enlarging fruit (sinks). Sugars generated by photosynthesis, along with amino acids and phosphate within the plant's vascular network, move via the phloem into enlarging fruit.

11.3.1 - Photoassimilate distribution

Sources of photoassimilate can be identified by providing individual leaves with $^{14}CO_2$ and following the pattern of labelled material into neighbouring organs (Figure 11.7). Leaves typically begin to show a net export of photoassimilate at about 50–60% of full size. In kiwifruit, leaves 49% expanded failed to export the radiolabelled products of $^{14}CO_2$ photosynthesis, whereas those 64% expanded transported labelled photoassimilate into younger leaves.

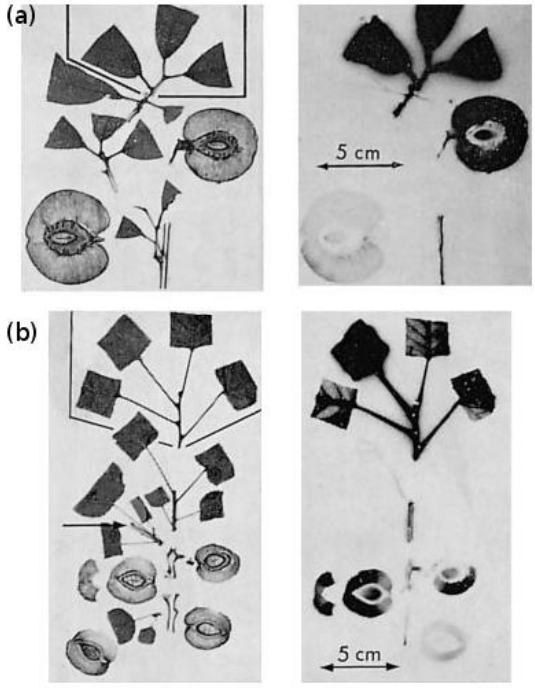

Figure 11.7 Photoassimilate moves from mature leaves of peach (a) and apricot (b) into the pericarp of maturing fruit nearby. $^{14}CO_2$ was administered for about 1 h to source leaves (boxed area top left side in (a) and (b)), and movement of ^{14}C-labelled photoassimilates over the subsequent 24 h was traced by autoradiography of harvested material (right side a and b). Intense labelling of source leaves indicates a high level of residual activity, but strong incorporation of ^{14}C-labelled photoassimilates into the pericarp of adjacent fruit is also evident. Endocarp tissues had hardened and failed to import current photoassimilate, although seeds developing inside the endocarp did become labelled. (Based on Kriedemann (1968) Aust. J. Agric. Res. 19, 775-780)

Distribution patterns of ^{14}C-labelled products relate to developmental morphology of fruiting shoots. Typically, source leaves are nearby on the same lateral branch, both above and below the fruit. In apple, fruiting spurs may develop primary leaves (emerging soon after budburst), then spur leaves (in a rosette at the base of the flower), then bourse leaves (growing on spur bourse shoots). Each in turn provides assimilate for the next phase of leaf growth (primary → spur → bourse); then as leaf expansion ceases, all provide assimilate to the developing fruit. Leaves on adjacent extension shoots can provide some photoassimilate to fruit, but if indeterminate growth continues the furthermost leaves become progressively less important as suppliers, and more significant as competitors. If the normal suppliers are removed, carbohydrate can come from longer distances, sometimes from leaves more than a metre away.

The relative strength of source and sink is a major factor for distribution patterns, but transport options are dictated by vascular connections. During plant growth, development occurs in an orderly and patterned manner, creating separate files of leaves. This pattern (phyllotaxis) is accompanied by a matching pattern of vascular connections. Photoassimilate tends to move along a pathway of least resistance, following these direct vascular connections where they exist, hence distribution patterns generally follow phyllotaxis.

This importance of phyllotaxis in carbohydrate allocation to the fruit is well shown in kiwifruit, where specific leaf–fruit connections exist. Patterns of assimilate distribution from leaf to fruit have been studied by taking a number of matched lateral fruiting branches of kiwifruit vines, then supplying $^{14}CO_2$ to one leaf on each, at various nodal positions along the stem, from node 1 (base) to node 10 (tip). Each lateral also had one fruit each on nodes 1 and 2, while the remaining nodes had

leaves only. Distribution of ^{14}C-labelled photosynthate was allowed to proceed for 6 d, and the total radioactivity in each leaf and fruit on the lateral was then measured. Specifically, node 1 fruit received assimilate from their own subtending leaf (node 1 leaf) and from leaves on nodes 6 and 9. Node 2 fruit was supplied by its subtending leaf and leaves on nodes 7 and 10. Assimilate from remaining leaves was distributed generally within the main body of the plant. However, if the apex of the lateral was removed to stop extension growth, fruit then drew assimilate from all leaves. By implication, a drastic change in source–sink relationships can override restriction on carbon transport imposed by vascular patterns in intact plants.

11.3.2 - Composition of photoassimilates

Table 11.1
Photoassimilate is commonly transported from leaves (sources) to fruit (sinks) as sucrose, and most agricultural plants fit this model. Even genera as diverse taxonomically as Yucca and Vitis (grapevine) transport sucrose almost exclusively, but stonefruit and pipfruit (pomefruit) of the woody Rosaceae (e.g. apple) use sorbito. Ash trees translocate most of their photoassimilate as mannitol. As a further variant, some Cucurbitaceae (e.g. squash) use stachyose and related compounds. In all cases, translocated sugar is an energy-rich source of carbon, but sucrose is not the universally translocated sugar, as indicated below.

Sugar	Yucca	Grapevine	Ash	Apple
Sucrose	97	93	11	22
Glucose	2	4	1	4
Fructose	1	3	1	3
Sorbitol	—	—	—	71
Mannitol	—	—	65	—
Other	—	—	22	—

Radiolabelling of photoassimilates has also been used to identify which compounds are transported into storage organs. Analyses of phloem tissues and phloem sap show that in most plants carbohydrate enters fruits primarily as sucrose. However, other soluble carbohydrates can predominate in some plants of commercial importance (Table 11.1).

In the woody Rosaceae (apple, pear, stonefruit), the sugar alcohol sorbitol is the major photosynthetic product at 60–85% of transported carbon, the remainder being mainly sucrose. Regardless of transport form, photoassimilate arriving in fruits is rapidly converted to the storage products characteristic of the fruit in question (principally starch, glucose, fructose and sucrose). Thus the identity of labelled sugars in fruit often differs markedly from the form transported. For example, sorbitol concentration is high during early development of apple fruit and more or less reflects the composition of photoassimilate in transit. By maturity, sorbitol content will typically decline to below 5% of the total soluble carbohydrate.

If sorbitol reaching fruit is not fully metabolised, apoplastic accumulation results and pericarp tissues become glassy in a disorder called ‘watercore’ (see below; Figure 11.23). This is a common problem

with some apple cultivars such as Fuji. Sugar transport and accumulation can thus have economic importance — both in terms of desired taste characteristics and postharvest fruit quality.

In kiwifruit, the polyol *myo*-inositol may comprise up to 35% of soluble carbohydrate in developing fruit, and up to 20% in leaves. As yet, we do not know whether inositol, like sorbitol, is transported in the phloem, or whether there may be physiological disorders caused by inadequate metabolism of sorbitol within fruit. Such findings challenge our common perception of sucrose as the universal transport carbohydrate in economic crops, and suggest that we still have a lot to learn about the control of carbohydrate metabolism.

11.3.3 - Fruit composition and sensory attributes

Carbon transport and subsequent metabolism in developing fruit cannot be viewed in isolation, particularly when aspects of fruit quality, such as taste and flavour, are directly dependent on such processes. In particular, sugar–acid balance and contents are primary determinants of the taste attributes of fruit, and so are of major significance for consumers. Too much acid and the fruit is tart and unpalatable; too little and the fruit is insipid and bland. In horticultural terms, acid levels are often expressed as titratable acidity (TA), and this is used as one indicator of taste. Another indicator used is the refractive index of the expressed sap (recorded as °Brix). This is a measure of the soluble solids concentration (SSC %) of expressed juice and represents the sum of organic acid, salts and sugar contents. Several organic acids may be present, but certain ones are characteristic of particular species or cultivars. For example, malic acid predominates in pipfruit (pomefruit), citric acid is dominant in citrus, while tartaric acid is dominant in grape. In kiwifruit, malic, citric and quinic acids are the major ones, and in total may exceed 1.5% of the fresh weight.

Acids are not transported into fruit via phloem connections, but are synthesised *in situ*. Part of the acid component comes from metabolism of the sugar imported through the phloem, but part can be synthesised by local fixation. In citrus, dark fixation of CO_2 by mature fruit makes a meagre contribution to acid balance, but inter-conversion of imported carbon is of more consequence. In that case, citrate synthase and subsequent enzymes in the citric acid cycle appear to determine whether imported carbon (as sucrose) is transformed into other sugars or is metabolised further to organic acids.

Starch–sugar balance is a major factor in consumer perceptions of fruit quality. In many fruit, including apple, banana and kiwifruit, starch accumulates throughout development, being laid down as granules in plastids. In kiwifruit, starch may reach 50% of the total dry matter towards the end of fruit growth (at about 15 weeks after pollination). As fruit approach maturity (17–20 weeks after pollination), there is a rapid onset of starch hydrolysis. Starch content at the onset of this conversion is not enough to account for all the sugar present in ripe fruit, and this implies that maturing fruit continue importing sugar up to harvest. Continuing import of ^{14}C-labelled photoassimilate into maturing peach and apricot fruit confirms that pattern (Figure 11.7).

The dynamic between starch breakdown and soluble sugar increase can be a critical index of fruit maturity. ‘Hayward’ kiwifruit, for example, are judged to be mature enough to be harvested and to ripen properly if their soluble solids levels reach a specific target value of 6.2%. Starch pattern tests are used as maturity indices for some apple cultivars. For kiwifruit, the starch content of fruit at harvest may vary according to season, growing system, and cultivar. Starch content is strongly linked

with sugar content of ripe fruit and hence with consumer perceptions of fruit taste. Starch content of fruit at harvest (commonly estimated from fruit dry matter content) is used commercially as a proxy for potential fruit taste.

As an additional factor in their dietary appeal, fruit are rich sources of vitamins, particularly vitamin C (L-ascorbic acid). Moreover, vitamin C can be a major metabolite (greater than 2 g kg^{-1} fresh weight) in fruit such as acerola, rosehip, quandong, kiwifruit, citrus, blackcurrant and guava, and has strong anti-oxidant properties. This may account for a notable absence of browning in kiwifruit and citrus when sliced (in conjunction with relatively low levels of polyphenols and polyphenol oxidase in those tissues). Vitamin C levels increase in the fruit during early growth, and tend to be stable through to maturity.

A number of other important vitamins have fruit or seeds as their major sources in the human diet. The B group vitamins such as B_1, B_2, pantothenic acid and biotin are present in both fruit and seeds, while B_3 and B_6 are particularly abundant in seeds. The vitamin A precursor β-carotene is found in useful quantities in some fruit, for example peach, apricot, melon and cherry.

Phenolics such as anthocyanins and tannins are also important in fruit and are responsible for much of the visual appeal of intact fruit (e.g. tamarillo), exposed flesh (e.g. cherry) or extracted juice (e.g. guava). They also contribute to flavour characteristics, adding a slight and pleasing astringency (as with the dessert apple) or a more aggressive one (as with cider apple and green banana).

Tannins in persimmon fruit are a special feature of that fruit and provide an interesting example of the potential dominance of a single quality characteristic in determining how a given fruit is used. The first cultivars of persimmon originating in China were markedly astringent, having high soluble tannin levels that made the fruit inedible until the tannins became condensed during the softening stages of ripening and early senescence. These original cultivars were therefore not eaten until the fruit flesh had become a glutinous paste. Later selection in Japan produced non-astringent cultivars such as ‘Fuyu’ that lose their astringency during the later stages of maturation, so that they can be eaten in a firm crisp state more typical of a fruit like apple. In persimmon, water-soluble tannins are compartmented in specific tannin cells of the mesocarp tissue. Tannin accumulation ceases with cell growth, and in non-astringent cultivars astringency declines both through soluble tannin dilution and through polymerisation, where soluble tannins are condensed into an insoluble form.

11.3.4 - Mineral nutrients

Just as fruit require an inward flow of carbohydrate and water to provide for seed growth and pericarp expansion, so mineral nutrients are also supplied. As a rule, concentrations of the major mineral nutrients in fruit are lower than in other organs such as leaves, and the patterns of phosphorus, potassium, calcium, magnesium and nitrogen accumulation usually differ.

Mineral nutrients move into the fruit most rapidly during the early stages of development (Figure 11.8) at a time when xylem water flow dominates. As fruit approach maturity, surface to volume ratio declines, the skin becomes less permeable to water loss, and large amounts of photoassimilate are imported via phloem connections. As a result, a significant part of the water reaching fruit now enters through the phloem and is accompanied by photoassimilate. Mobile ions such as K^+ and

HPO_4^{2-} are loaded into the leaf veins along with the photoassimilate, travel in the phloem and so reach fruit over the whole growing season. In contrast, less mobile nutrients such as Ca^{2+} fail to reach fruit during later stages, so that Ca^{2+} concentration remains steady or even declines slightly (Figure 11.8).

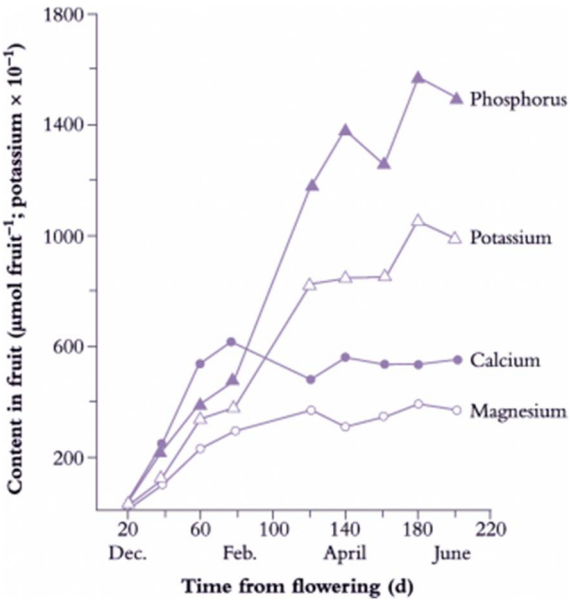

Figure 11.8. In kiwifruit, as in most fruit, accumulation of calcium is confined to early stages of development that coincide with cell division. By contrast, phosphorus and potassium move into fruit over the whole growing season and are able to enter via either the xylem or phloem. Magnesium import is meagre but progressive. Note the expanded scale for potassium. Fruit content of nutrient ions at maturity would be as follows: phosphorus 1600, calcium 600, magnesium 400 and potassium 10 000 μmol per fruit. (Based on Ferguson (1980) N. Z. J. Agric. Res. 23, 349-353)

Nutrient deficiencies in fruit are relatively uncommon, except for those associated with calcium. Calcium deficiencies are expressed in the form of blossom-end rot in tomato, and bitter pit plus lenticel blotch in apple fruit. These apple disorders tend to be expressed during postharvest storage, but symptom expression is somehow related to the previous ripening environment. These disorders show up as a pitting of flesh and skin, reducing fruit value or even rendering those commodities unmarketable. Such commercial penalties have resulted in development of preharvest sprays and postharvest dips of calcium salts that diminish bitter-pit incidence in harvested fruit. Where there is little or no calcium recycling via phloem, calcium needs to be applied directly to fruit to have a beneficial effect.

11.4 - Carbon accumulation

During development, photoassimilate is stored in fruit (and other sink organs such as root vegetables, seeds and flowers) in either a soluble or insoluble form (Figure 11.9). Fruit that store carbon in a soluble form (e.g. berry fruit, peach, persimmon, melon, grape, citrus) need to remain on the plant until nearly ripe if they are to survive postharvest storage and meet customer expectations. In most cases the major and rapid increase in soluble sugar content does not occur until late in development, signalling the beginning of ripening. Because the sugar source is the parent plant, harvesting such

fruit too early reduces their final sugar content to unacceptable levels. In contrast, there are other fruit that store their carbon in insoluble forms, particularly starch. This allows greater efficiency in accumulating carbon, as the storage product is more compact, osmotically inactive and better segregated from metabolic processes. Examples are avocado, which stores carbon as both starch and lipid, and kiwifruit, apple, pear, mango, papaya and banana, all of which store carbon as starch.

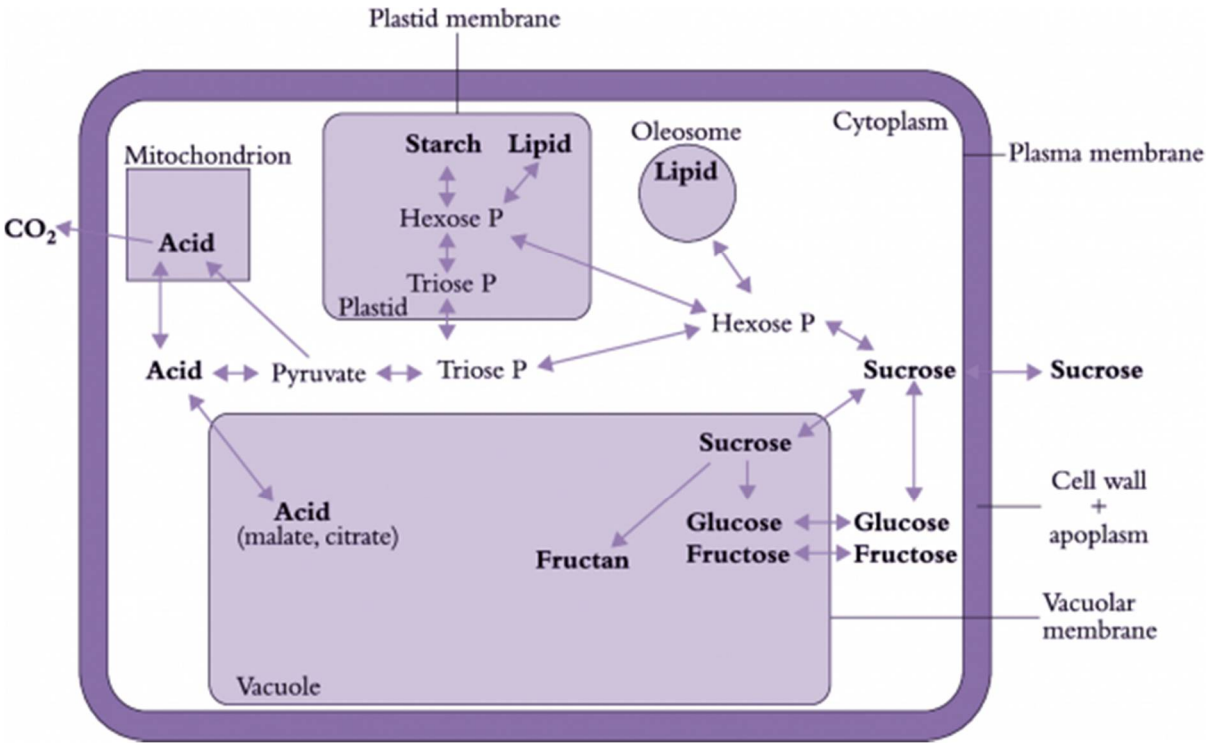

Figure 11.9. Carbohydrate economy in developing fruit is derived by import and recycling of photoassimilates between different metabolic compartments. Sucrose or less commonly other forms of translocated carbon (Table 11.1) arrive via phloem conduits and are loaded into cytoplasmic compartments. As shown schematically, carbohydrate can then be partitioned to vacuolar storage or converted to other sugars and, in the form of hexose phosphate, transferred to plastids where it is used to synthesise starch. Each step is reversible, and as happens during ripening, starch in plastids is transformed back to sugars that subsequently accumulate in vacuoles as indicated. Other specialised organelles (oleosomes) store lipids while mitochondria draw upon imported and locally fixed carbon for ATP generation. (Original diagram courtesy R.L. Bieleski)

In fruit that store carbon as sugars and organic acids during development, colour changes followed by the initiation of softening signal that fruit are becoming mature and ready to harvest. The challenge facing postharvest physiologists is to assess when such fruit are still sufficiently firm to allow easy handling and storage, but have enough sugar for a true ripe flavour to develop. A developing crop is typically monitored with a simple refractometer test (soluble solids concentration based on refractive index of expressed juice). In grape, a rapid rise in sugar content (beginning at ‘veraison’) may need to reach SSC values of around 20% (with, say, 17% sugar and 2% acid as the main soluble components) before a successful harvest can be assured.

There is more to good flavour than high sugar content, with sugar to acid ratio being particularly important, so that a combination of SSC to acid ratio with a minimum SSC level may be required. For example, New Zealand mandarins may not be exported to Japan if the SSC to acid ratio is below

10 (when the proportion of acid is too high). In other crops such as melon, the acid component is unimportant, and sugar content primarily dictates fruit quality.

In fruit that store carbon as starch, time of picking is less dependent on sugar content, since a doubling or more of sugar concentration by starch hydrolysis can still take place after the fruit are picked. Once starch utilisation has started, fruit can be picked without much detriment to final eating quality. SSC measurements alone are then insufficient, and measurements of total solids, dry matter, oil content and starch concentration are used as well. For example, in kiwifruit sequential measurements of SSC are combined with dry matter measurements; in apple and pear, the pattern of starch distribution within the fruit is recorded along with SSC and ethylene measurements; in mango, total solids (and fruit shape, flesh colour and firmness) are recorded; in papaya, colour changes and sequential SSC measurements are recorded; in avocado, dry matter, sometimes in combination with oil content, is used. In banana, shape or ‘fullness’ of fruit is an important criterion rather than starch level because bananas can be harvested over a remarkably wide range of maturities and still ripen satisfactorily.

Thus, when fruit store carbon in insoluble forms, they can often be harvested while still hard, lending greater flexibility to postharvest handling and ensuring a longer storage life.

11.4.1 - Sugar storage

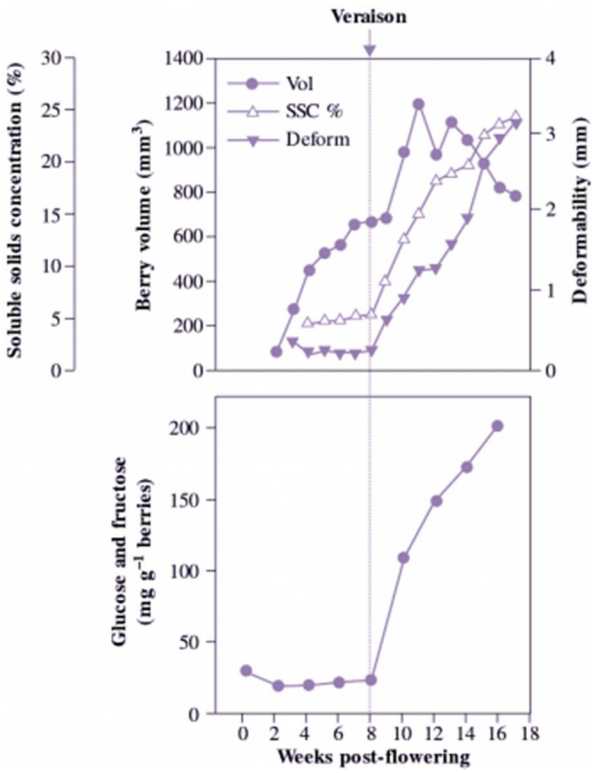

Figure 11.10. Grape undergoes an abrupt change in physiology midway through development. For about 8 weeks after flowering, berry volume increases steadily but fruit are hard (low deformability) and sugar content low. At 'veraison' invertase activity rises abruptly and reducing sugar content increases rapidly, reaching about 20% of fresh weight when ripe. Berries attain full size by 10–12 weeks, and approach an asymptote in sugar content 2–3 weeks later. (Based on Davies and Robinson (1996) Plant Physiol. 111, 275-283)

In sugar-storing fruit a major shift in metabolism generally takes place when fruit expansion is almost complete, heralding a rapid increase in sugar content. Unloading of sugars from the phloem usually occurs by a symplastic route, but in some species is interrupted by an apoplastic step. Control points for sugar entry and accumulation by fruit include:

1. Rate of sugar production by leaves and delivery to transport pathways;
2. Reallocation of sugar from supporting vegetative growth towards fruit growth;
3. Enhanced unloading of sugar from transport streams into fruit;
4. Enhanced transfer of sugar across plasma membranes into cells or through plasmodesmatal connections between cells;
5. Onward metabolism of sugar in the cytoplasm, or transfer to storage in vacuoles;
6. Increased respiratory utilisation of sugar to provide energy for metabolic processes.

As with any biological system, multiple controls operate concurrently to drive a given pattern of maturation. Such events lead us to more robust indicators of ripeness, and improved ways of manipulating maturation to yield higher sugar content and better handling properties.

In tomato, there are different genotypes that accumulate either hexoses or sucrose. Most cultivars are hexose accumulators, in which acid invertase is active during growth and ripening. In transgenic tomatoes in which acid invertase activity was suppressed by expression of an antisense invertase transgene, sucrose accumulation occurred in a normally hexose-accumulating cultivar (Klann *et al*. 1996). Conventional breeding studies using crosses between sucrose-accumulating and hexose-accumulating types of tomato showed that an acid invertase gene is not transcribed during ripening of the sucrose accumulators, and that sucrose accumulators therefore lack acid invertase (Harada *et al*. 1995).

In melon, where sucrose is the main sugar to increase, there is a corresponding decrease in acid invertase and an increase in sucrose phosphate synthase (SPS) activity (this synthesises sucrose from hexose phosphate and adenylated precursors). A complex metabolic transition to allow sucrose accumulation occurs at the end of fruit growth and onset of ripening, of which the loss of soluble acid invertase activity is only one component (Dai *et al*. 2011).

However, in grape berries where hexoses begin to accumulate at veraison and reach very high levels (Figure 11.10), SPS, sucrose synthase and hexokinase activities all increase, but acid invertase mRNA abundance and activity both peak just prior to or at veraison and then decline. This suggests that in grape factors other than invertase activity regulate hexose accumulation. Two sucrose transporters were up-regulated at veraison, and it is possible that these regulate ripening-associated sugar accumulation from the apoplast into the parenchyma cells (Davies *et al*. 1999). Consistent with this suggestion, studies using tracers have shown that at veraison phloem unloading switches from a symplastic to an apoplastic route (Zhang *et al*. 2006).

11.4.2 - Starch storage

Fruit that store starch switch from starch synthesis during development to starch hydrolysis during ripening. Starch–sugar interconversion involves a larger number of enzymes and a greater complexity of control than is required for sugar storage alone. No transgenic plants have yet been

reported in which a starch-storing fruit or lipid-storing fruit has been altered to store only sugars, or vice versa, but results with potato suggest it could be possible. In potato, control of both insoluble carbon storage and sugar to starch conversion has been attained using sense and antisense constructs to alter the expression of specific carbohydrate enzymes (Stitt and Sonnewald 1995).

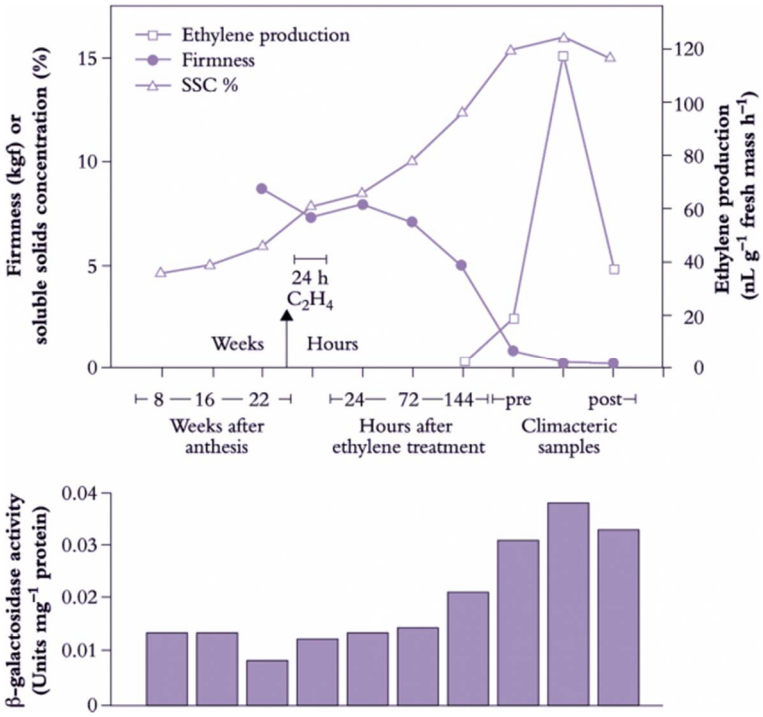

Figure 11.11. Kiwifruit show some dramatic changes in physiological status during development. For 24 weeks after flowering, fruit are hard and sugar content is low. Meanwhile starch content (not shown) rises to about 12% of fresh mass. Ripening in fruit harvested around 24 weeks can be triggered by exposure to an external source of ethylene even though the fruit are not yet producing ethylene by themselves, and are incapable of an autocatalytic response. Within a week of such treatment starch becomes hydrolysed and sugar concentration rises from 7% to 15%. Fruit then soften rapidly and several cell wall-modifying enzymes increase in activity. Fruit do eventually show a peak in ethylene production, but not until ripening is well under way. (Figure based on data from J. Win)

Kiwifruit provide an example of biochemical changes in a starch-storing fruit (Figure 11.11). They are normally harvested with starch contents ranging from 4 to 10% (dry matter concentrations between 14 and 20%; SSC between 6.2 and 12%). At harvest, the main sugars are sucrose, glucose and fructose. As fruit ripen after harvest, sucrose content increases only slightly, while fructose and glucose increase in parallel to become the predominant sugars in ripe fruit. Labelling with radioactive precursors indicates that all three sugars are actively synthesised during ripening, and there are increases in the activities of a number of sucrose-metabolising enzymes, particularly SPS and invertase. The starch-degrading enzyme α-amylase increases two-fold.

The organic acid content of kiwifruit also changes after harvest. At room temperature malate decreases and citrate increases while quinate remains unchanged. However, during cool storage (0–4°C), malate increases. Such changes are enough to alter the flavour balance in the ripe fruit. Where fruit store carbon in an insoluble form, there are several potential control points for sugar metabolism (Figure 11.9) including:

1. Hydrolysis of starch to glucose;
2. Transfer of sugar precursors from starch-containing plastids (amyloplasts);
3. Synthesis or degradation of sucrose;

4. Synthesis of hexoses;
5. Transfer of sugar to vacuoles or export from cells;
6. Carbon flow between sucrose and malate or citrate;
7. Production of CO_2 from sugar or acid precursors;
8. Transfer of malate or citrate across the vacuolar or mitochondrial membranes.

Interference in any of these processes should affect ripening and/or flavour development after harvest. Such interference may be physical (as in storage temperature), chemical (as in atmosphere composition) or genetic (by modifying activities of specific proteins which control flow between particular metabolites).

11.5 - Fruit ripening

Several processes take place as fruit ripen and become edible, and then senesce. These changes may take place while fruit are still attached to the plant or after harvest. Tomato, banana and avocado are examples of fruit that at harvest can be at a mature green but unripe stage and are inedible until subsequent ripening processes have occurred. In contrast, strawberry, orange, boysenberry and grape are examples of fruit that need to stay on the tree or vine until ready to eat in order to have their desired eating characteristics.

Several major changes take place as fruits ripen, and taken collectively they characterise ripening processes. They include:

1. Changes in carbohydrate composition, resulting in sugar accumulation and increased sweetness;
2. Change in colour;
3. Flesh softening and textural change;
4. Formation of aroma volatiles;
5. Accumulation of organic acids with associated development of flavour.

These changes make the ripe fruit attractive to animals, which in eating the fruit will disperse the seeds and enlarge the range and improve the survival chances of the next generation of the plant. Lignified pits and seeds encased in a fibrous core might be discarded after eating the flesh, whereas smaller seeds might pass through the animal's digestive system and be deposited with the animal's excrement.

11.5.1 - Ethylene and the regulation of ripening

Ethylene production is closely associated with fruit ripening in many species, and is the plant hormone that regulates and coordinates the different aspects of the ripening process; colour development, aroma production and texture are all under the control of ethylene (Klee and Giovannoni 2011). Typically, fruit will generate barely detectable amounts of ethylene until ripening when there is a burst of production (Figure 11.12).

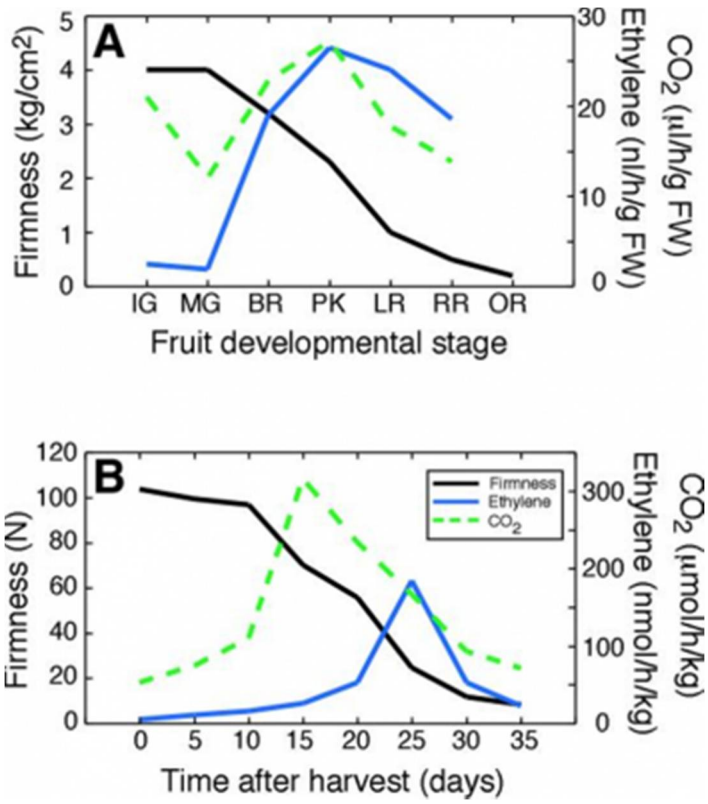

Figure 11.12. Ethylene evolution and respiration (measured as CO_2 production) undergo a rapid increase then decline as fruit ripen and soften. In tomato (A), the peak of ethylene production and respiration occur relatively early in ripening, shortly after the first visible sign of red coloration on the outside of the fruit (known as the breaker stage). Abbreviations of ripening stages: IG, immature green; MG, mature green; BR, breaker; PK, pink; LR, light red; RR, red ripe; OR, over ripe. In kiwifruit (B) that were harvested and stored at 20ºC, the peak of ethylene evolution occurs very late, when substantial softening has already occurred and the fruit are almost at the eating ripe stage. The earlier peak in respiration may have been caused by harvest. The two studies use different units so absolute values cannot be compared between the species. (Data taken from Maclachlan and Brady (1994) Plant Physiol. 105, 965-974; Rothan and Nicolas (1989) HortScience 24, 340-342; Taglienti *et al*. (2009) Food Chem. 114, 1583-1589)

Historically fruit have been categorised into two classes of behaviour with respect to ethylene physiology and respiratory pattern (Table 11.2). In the first type, as fruit progress towards edibility the respiratory rate increases followed by a decline as fruit senesce. This is known as the climacteric rise. Pear, banana and avocado (Figure 11.13) show an especially strong respiratory rise. Ethylene production also increases sharply to a maximum at this time, and then declines before fruit rots intervene and lead to a renewed output. The major rise in ethylene production may take place before, just after or close to the respiratory peak. Such fruit are classed as **'climacteric'**, with apple, avocado, banana, fig, mango, papaya, passionfruit, pear and tomato being classic examples. As with the respiratory rise, the levels of ethylene produced vary widely between species. Climacteric fruit ripen after harvest, and need not remain on the tree or vine. A second category of fruit, exemplified by blueberry, cherry, citrus, cucumber, grape, pineapple and strawberry (Table 11.2) do not show such sharp changes. Respiration rate either remains almost unchanged or shows a steady decline until senescence intervenes, with little or no increase in ethylene production; these are called '**non-climacteric'** fruit, and fruit ripen only if they remain attached to the parent plant.

Table 11.2
Edible fruit are traditionally classified as either climacteric or non-climacteric according to their respiratory behaviour and ripening characteristics. Climacteric fruit ripen off the tree, generate large amounts of ethylene as ripening proceeds, and show a single-phase respiratory response to exogenous ethylene. Non-climacteric fruit ripen on the tree, generate little ethylene as ripening proceeds and can show a multiple-phase response to exogenous ethylene. Listings below reflect a broad distinction that are somewhat arbitrary, and represent two extremes of a continuum in the respiratory physiology of these fleshy fruit.

Climacteric	Non-climacteric
Apple	Cherry (sweet, sour)
Apricot	Cucumber
Avocado	Grape
Banana	Lemon
Blueberry	Pineapple
Cherimoya	Satsuma mandarin
Feijoa	Strawberry
Fig	Sweet orange
Kiwifruit	Tamarillo (tree tomato)
Mango	
Papaya (paw paw)	
Passionfruit	
Pear	
Persimmon	
Rockmelon (cantaloupe)	
Tomato	
Watermelon	

While all fruit were once classified under this either/or nomenclature, more recent work has shown the distinction to be less clear-cut (Paul *et al*. 2012). In some species, notably pepper and melon, different cultivars or genotypes exhibit characteristics typical of climacteric or non-climacteric behaviour. It has been shown that in climacteric fruit some ripening changes occur independently of ethylene, and that some non-climacteric fruit have ethylene-requiring changes during ripening. With the development of more sensitive ethylene measuring devices, many non-climacteric fruit appear to show an increase in ethylene evolution at previously undetectable levels upon ripening. The existence of ethylene-dependent and ethylene-independent pathways in both climacteric and non-climacteric species (Barry and Giovannoni 2007) suggests that regulation by ethylene is ubiquitous, and that climacteric and non-climacteric behaviour are more accurately envisaged as the extremes of a continuum of responses with the acquisition of sensitivity to ethylene playing an important role (Johnston *et al*. 2009). This sensitivity model is supported by the observation that unripe climacteric and non-climacteric fruit both increase their respiration rate when exposed to exogenous ethylene (Paul *et al*. 2012).

With other fruit, such as kiwifruit, a hybrid ripening pattern is seen, with most of the ripening changes occurring in the absence of any detectable rise in ethylene and CO_2 production; a climacteric response occurs only towards the end of ripening. Exposure to exogenous ethylene promotes ripening of kiwifruit, but if exposure to ethylene is insufficient, or fruit are too immature, then removal of ethylene results in non-climacteric behaviour. Ethylene as a ripening trigger is used commercially with banana, avocado and early-season kiwifruit to ensure that fruit are at optimum ripeness when eaten. Conversely, if kiwifruit are to be stored for a long time, then ambient ethylene must be removed (usually by scrubbing this gas from coolstore environments).

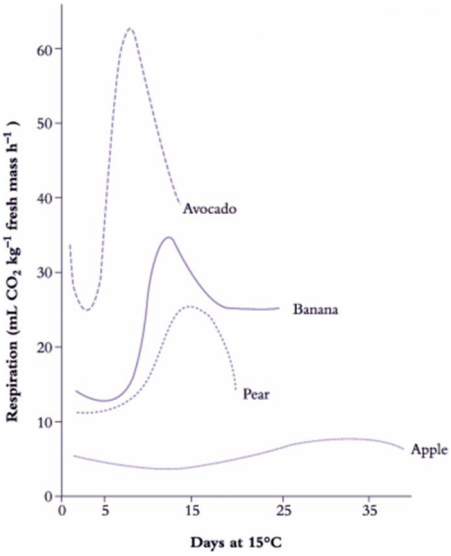

Figure 11.13. Respiratory output of CO_2 can undergo dramatic change as fruits ripen. Early research on apple and pear led to a classic model of a postharvest climacteric rise associated with ripening and linked in time with ethylene production. Studies with tropical fruits such as avocado and banana then revealed characteristic 'waveforms' of even wider amplitude. (Based on Biale (1950) Annu. Rev. Plant Physiol. 1, 183-206)

Ethylene metabolism has been a main focus for biochemical research into fruit ripening (see Feature essay 11.1). The pathway of biosynthesis is as follows (Figure 11.14): methionine (a sulphur-containing amino acid also important in protein synthesis) is converted to SAM (*S*-adenosylmethionine) through the action of SAM synthase; SAM is converted to ACC (1-aminocyclopropane-1-carboxylic acid) through the action of ACC synthase (ACS); ACC is converted to ethylene through the action of ACC oxidase (ACO).

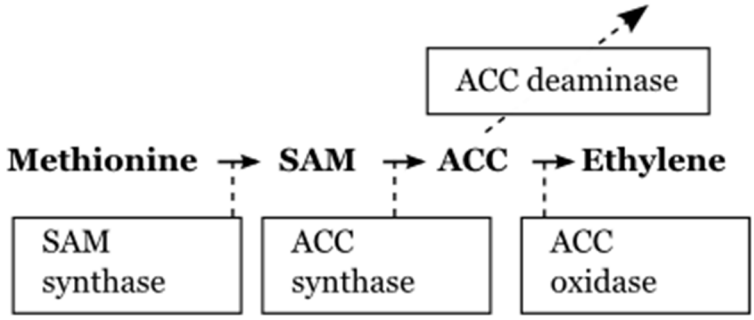

Figure 11.14. The pathway of ethylene biosynthesis in plants. The two genes controlling the committed steps to ethylene biosynthesis, ACC synthase (*ACS*) and ACC oxidase (*ACO*) are highly transcriptionally regulated. In one series of experiments, the biochemical precursor of ethylene, ACC, was depleted by expression of a bacterial ACC deaminase transgene, but this reaction does not normally occur in plants.

In fruit with a climacteric behaviour, ethylene biosynthesis occurs at very low and basal levels during fruit development prior to ripening. This ethylene production is auto-inhibitory, and has been termed System 1 ethylene. At the initiation of ripening there is a change in the regulation of ethylene biosynthesis, which become auto-stimulatory and involves the induction of specific ripening-related *ACO* and *ACS* genes different from those that are responsible for System 1 ethylene (Barry and Giovannoni 2007). Production of ethylene greatly increases due to the autocatalytic regulation, and is known as System 2 ethylene. At the point at which the fruit becomes competent to ripen, there is a

transition from System 1 to System 2 ethylene that may be regulated by developmental genes such as RIN (Barry *et al.* 2000).

Transgenic studies in a number of fruit types have yielded much information about steps in the control of ripening. Tomato fruit with reduced levels of the ripening associated ACC oxidase or ACC synthase, or with depletion of ACC levels using a bacterial ACC deaminase, developed and grew normally but ripening was delayed or almost completely prevented, depending on whether the fruit were attached to the plant or detached (Barry and Giovannoni 2007). Gassing of the ethylene-suppressed fruit with exogenous ethylene caused ripening to resume. Work on melon and apple found that the effects of the suppression of ethylene biosynthesis depended on the species and the extent of suppression achieved. Colour development, fruit softening, the accumulation of sugars and organic acids, and the production of aroma volatiles could in some cases be separated. For example, in antisense *ACO* melon fruit, degreening of the rind, softening and the accumulation of organic acids were sensitive to different levels of ethylene, and flesh pigmentation was ethylene independent. Such experiments show that ethylene does not control all the processes of ripening as once believed, and that additional regulation by other hormones and developmentally controlled factors occurs (see Section 11.5.2). Ripening is a series of parallel processes involving both ethylene-independent and ethylene-dependent pathways, the latter requiring different sensitivities to ethylene to proceed. This leads to a model whereby ethylene acts as a modulator to coordinate ripening in a developmentally choreographed pattern (Johnston *et al.* 2009).

The other important factor in the regulation of fruit ripening is the way in which plants perceive ethylene and the signal transduction pathway that leads to the ethylene response (see Chapter 9). In summary, ethylene is perceived by receptors that are negative regulators of the signalling pathway. In the absence of ethylene the receptors actively supress ethylene responses, but when these receptors bind ethylene they undergo a conformational change, leading to removal of the suppression and this allows de-repression of the signalling pathway. The signal is transduced through a MAP kinase pathway that ultimately leads to the stabilisation of a class of EIN3 (ETHYLENE INSENSITIVE 3) transcription factors. The EIN3 name originated from the ethylene insensitive phenotype observed in Arabidopsis mutants. The stabilisation of EIN3 leads to an increase in the transcription of genes associated with each ripening trait.

Ethylene receptors are multi-gene families (six genes in tomato) encoding two types of closely related proteins, one subfamily with a histidine kinase domain and the other subfamily with a serine/threonine kinase function. In Arabidopsis the receptors appear to act redundantly since removal of any one by mutation does not cause complete insensitivity to ethylene. However, this is not the case in tomato, where suppression of either of two receptor genes caused an early-ripening phenotype (Kevany *et al.* 2007). The importance of receptors in tomato fruit ripening has also been shown by the semi-dominant mutant *Never-ripe* (*Nr*), the fruit of which are unable to ripen. This was found to be due to a mutation in the *ETR3* receptor, making the fruit impaired in its ability to perceive ethylene. Antisense inhibition of this mutant gene restored normal ripening to the *Nr* mutant (Hackett *et al*. 2000).

In tomato the turnover of receptors (degradation of existing receptor proteins and the synthesis of new ones) controls the timing of ripening (Kevany *et al.* 2007). During ripening some ethylene receptors increase in transcription and it appears that receptor expression is used to restore the ability to respond to ethylene, implying that there is a corresponding loss of receptor protein. This suggests a model in which during climacteric fruit ripening there is an increase in receptor turnover, allowing the fruit the ability to rapidly turn off ripening if ethylene is removed from the system. This is observed in tomato, apple and kiwifruit suppressed in *ACO* expression, which require continuous

exposure to exogenous ethylene for ripening. It is also the basis for the temporary inhibition of ripening obtained using 1-methylcyclopropene (1-MCP), a compound that binds irreversibly to the existing ethylene receptors and prevents the physiological action of ethylene (Sisler and Serek 1997) (see section 11.6.4).

11.5.2 - Developmental control of ripening

Ethylene is not the only regulator of fruit ripening. A cold treatment can trigger ripening in detached apple and kiwifruit, acting either independently of ethylene or by increasing sensitivity to existing very low levels of ethylene (Tacken *et al*. 2010; Mworia *et al*. 2012). Other hormones also appear to play important roles; particularly, declining levels of auxin and increasing levels of abscisic acid may control the onset of ripening in non-climacteric species such as grape and strawberry. Abscisic acid may also play a role in controlling the onset of ripening of climacteric species. Uncovering the interaction between auxin, abscisic acid and ethylene in ripening regulation is an emerging area of research (McAtee *et al*. 2013).

While most ripening-associated traits appear to be regulated by hormonal changes, there are also a number of genes that control the developmental switch to ripening (Klee and Giovannoni 2011). Some of these were identified in tomato by the study of ripening mutants that arose spontaneously during commercial tomato production. The *ripening-inhibitor* (*rin*), *Colorless non-ripening* (*Cnr*) and *non-ripening* (*nor*) mutants all have fruit that fail to ripen, though with different characteristics. Although these fruit do not produce elevated levels of ethylene and will not ripen in response to exogenous ethylene, they are not completely insensitive and some ethylene-responsive genes (but not the whole ripening process) can be induced by ethylene treatment. The products of these three genes are transcription factors and are thought to be key developmental genes that control ripening progression, apparently acting upstream of the ethylene production pathway.

RIN encodes a MADS-box gene that clusters in the *SEPALLATA* clade. *CNR* encodes a SQUAMOSA promoter binding protein. Both proteins are necessary for the induction of ripening-related increases in respiration and ethylene biosynthesis, although since they are important in the ripening of both climacteric and non-climacteric fruit they appear to be more global regulators of ripening with some functions that are ethylene independent. Transgenic tomato fruit that had been suppressed in the ethylene signalling pathway and treated with 1-MCP showed an ethylene-independent increase in the expression of ripening-related *ACS* genes and ethylene production (Yokotani *et al*. 2009). This is apparently controlled by developmental factors, and would be sufficient to induce the autocatalytic increase in *ACS* expression and ethylene production typical of tomato ripening.

11.5.3 - Texture and softening

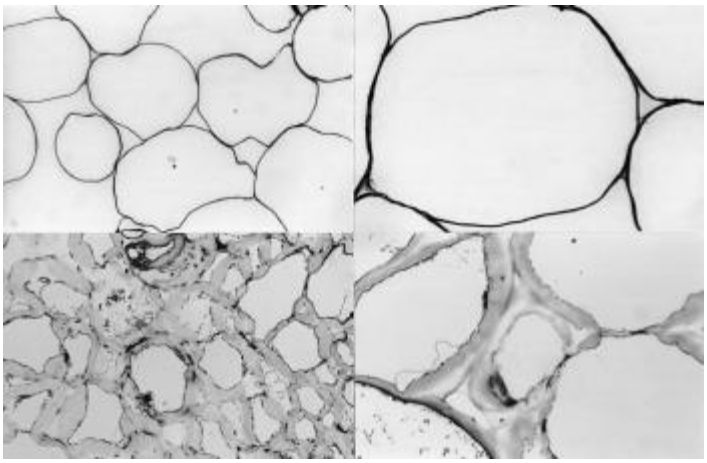

Figure 11.15. Anatomical features such as cell size, wall thickness and the distribution of intercellular gas spaces greatly influence our perception of fruit texture and eating pleasure. Cross-sections of ripe apple flesh (top pair) and ripe kiwifruit flesh (bottom pair) at low (x120) and high (x310) magnification. The apple tissue (top left) shows cells having densely staining thin walls. Tissue from kiwifruit (bottom left) shows cells with thick, swollen and weakly staining walls. The two right-hand figures give views of individual cells, corresponding to the tissue views. (Photomicrographs courtesy I.C. Hallett, E.A. MacRae and T.F. Wegrzyn)

During fruit ripening, softening and textural changes (including the development of juiciness) are components of the suite of modifications that make ripe fruit attractive to animals that might disperse the seeds. The texture of ripe fruit differs drastically between species, with crisp, hard apple and deformably soft avocado representing the extremes. The characteristic textures of different fruit and their manner of softening can be linked both with anatomical features and with changes that occur to the cell wall during ripening (Figure 11.15). Some fruit that are picked while hard, such as kiwifruit and tomato, will subsequently soften markedly as a result of extensive modifications to the cell wall structure that include substantial swelling. Other fruit, such as apple or watermelon, remain crisp and soften only slightly. Their thin cell walls remain relatively unaltered. Both types of softening occur in the pear family: Asian pear (Nashi) shows a crisp apple-like texture, whereas many European pears soften to give ripe fruit with a melting texture. Interspecific crosses between the two types show that texture is heritable (Harker *et al*. 1997).

Many textural characteristics relate to the fate of fruit flesh when it is fractured and crushed in the mouth. Contributing factors include cell size, cell adhesion, turgor and packing, wall thickness, wall composition and the reaction of cells to shearing stress as they are chewed (Harker *et al*. 1997). For example, a ripe apple has large (0.1–0.3 mm diameter), turgid, thin-walled cells that are loosely packed (airspace *c*. 20% of fruit volume). When that flesh is chewed, cells fracture and release their sugary contents as free juice. In contrast, ripe kiwifruit has minimal airspace (*c*. 2% of fruit volume) and cell walls are thick and hydrophilic (Figure 11.15). Such cells tend to pull apart when the flesh is chewed, resulting in a paste moistened by liquid held in cell walls or released by damaged cells. Avocado also has cells with walls that are thick and soft and which tend to pull apart, but also has a high proportion of oil that gives the pulp an oily quality in the mouth.

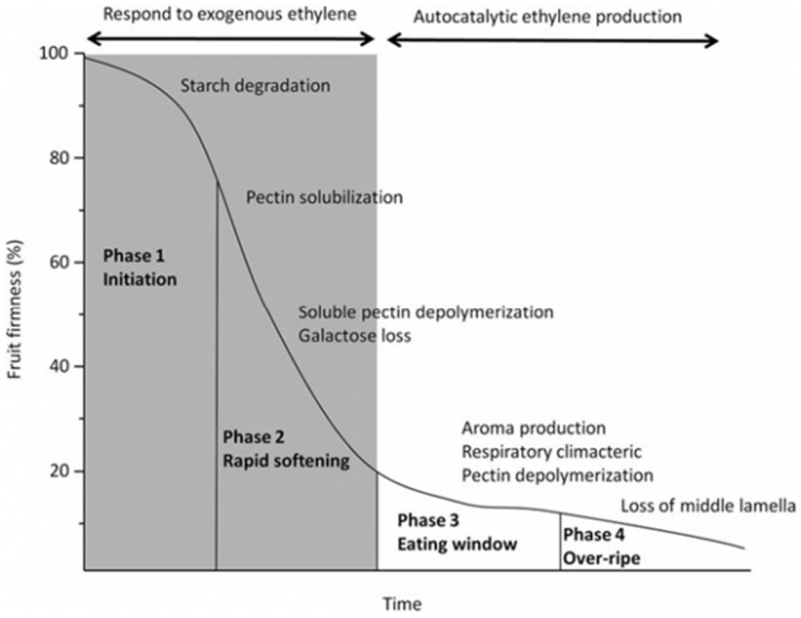

Figure 11.16. Schematic representation of postharvest ripening in kiwifruit, showing the timing of key physiological events. At harvest, fruit do not produce ethylene but are highly sensitive to exogenous ethylene. Softening is initiated (phase 1) and becomes rapid (phase 2). Relatively late in softening, compared with other fruit species, endogenous autocatalytic ethylene production begins, aroma volatiles are produced and fruit become soft enough to eat (phase 3). If fruit progress to the over-ripe stage (phase 4), they become unacceptably soft and exhibit 'off-flavour' notes. (Figure reproduced from Atkinson *et al*. (2011) J. Exp. Bot. 62, 3821-3835, with permission from the Society for Experimental Biology.)

Softening in kiwifruit occurs over a period of weeks, and can be divided into a number of phases (Figure 11.16). Modification of the cell wall plays an important part in determining fruit texture and ripening characteristics. The plant primary cell wall consists of a network of strong, rigid cellulose microfibrils held together by a complex matrix of polysaccharides consisting of two types: the hemicelluloses (composed mainly of neutral sugars) and the pectins (rich in galacturonic acid), together with smaller amounts of structural proteins. The outer part of each cell wall, which abuts and provides attachment to neighbouring cells, is composed mainly of pectins and is called the middle lamella. Fruit softening involves alterations to various pectin and hemicellulose polysaccharide wall components, and changes to the bonding between some polymers. Wall modification has been the subject of much research worldwide, mostly using tomato as a model, but also using other fruit in the search for common themes (Brummell 2006). Chemical analyses of cell wall components in a range of species, notably kiwifruit and tomato, show some consistent changes during the early stages of ripening. In kiwifruit, these include:

1. Solubilisation of pectin (but without further degradation);
2. The cell wall swells and shows an increased affinity for water (become more hydrophilic);
3. Loss of galactose from pectins (especially of a galactan that is tightly associated with the cellulose microfibrils);
4. De-esterification of some pectins.

These changes continue once kiwifruit have begun rapidly softening to ripeness (phase 2 in Figure 11.16). Phase 2 softening is associated with a further increase in pectin solubilisation, loss of galactan and arabinan side chains from pectic polymers, and more cell wall swelling. As softening progresses into phase 3 two more important changes begin, both of which appear to be regulated by ethylene:

5. Depolymerisation (a reduction in size) of the hemicellulosic polysaccharide xyloglucan, which is associated with a reduction in cell wall strength;
6. Depolymerisation of pectin, which is associated with dissolution of the middle lamella and reduced intercellular adhesion.

These six changes have been observed in a wide range of fruit types, although the extent and relative timing varies somewhat between species. Such observations indicate that pectin solubilisation and cell wall swelling are important events in the control of softening in kiwifruit and probably most other species with a melting texture. Cell wall modification is much less extensive in fruit with a crisp, fracturable texture such as apple and capsicum pepper. As fruit become fully ripe, dissolution of the middle lamella means that it eventually virtually disappears as a visible structure under the microscope. Dissolution of the middle lamella results in a great reduction in intercellular adhesion, and cells now have fewer regions of attachment to each other and become more rounded in appearance as they pull away from neighbouring cells. The primary walls are also weakened by the various changes that have occurred, and cells easily rupture when bitten or chewed, releasing the cell contents as juice.

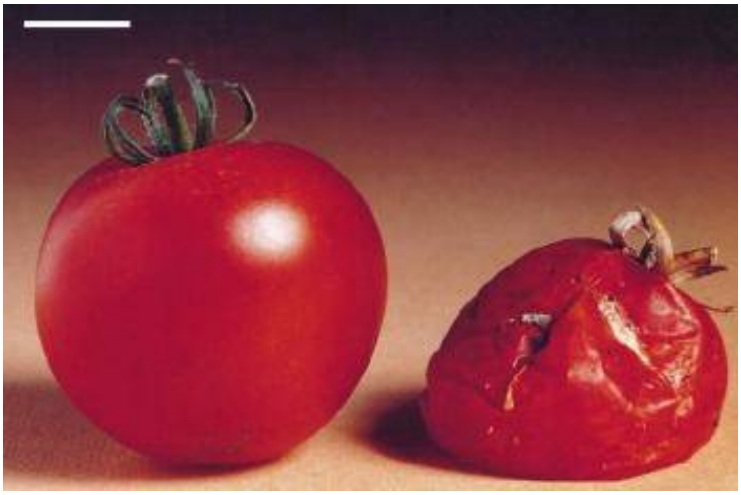

Figure 11.17. Genetic manipulation can have a profound effect on ripening. Normal tomatoes (right) and Flavr Savr™ tomatoes (left) were picked when both were nearly ripe (light red) and held at room temperature for four weeks. By this time normal fruit had softened and rotted but Flavr Savr™ fruit was still firm and edible. This genetically modified fruit was deficient in polygalacturonase and had a much better shelf life, as well as improved flavour and handling qualities. Scale bar = 2 cm. (Photograph courtesy Robert Lamberts)

Many of the cell wall-modifying enzymes produced during ripening are regulated by ethylene. Polygalacturonase (an enzyme that depolymerises pectins) increases *de novo* 10–50-fold in tomato fruit during ripening (Sitrit and Bennett 1998). Understandably, this enzyme was originally accorded a major role in the ripening process. However, studies with transgenic tomato fruit in which polygalacturonase was suppressed found only a small reduction in softening during ripening, although there was a very substantial increase in the storage life of the fruit (Figure 11.17). Because transgenic fruits retained firmness for longer, they were left on vines longer, resulting in more carbohydrate accumulation prior to harvest. Moreover, fruit could be harvested partially coloured rather than mature green, thereby allowing ripening processes to progress more naturally and yielding fruit with better flavour and appearance. To move from experimental results to public availability, the fruit had to go through a series of tests and be de-regulated. Following USDA approval, a transgenic cultivar producing fruit with >99% reduction in polygalacturonase activity was named Flavr Savr™ by Calgene, and was released for marketing in the USA under the brand

identity of McGregor. Tomato paste with increased viscosity derived from similar transgenic fruit was successfully marketed in the UK for several years.

The effects of reduced polygalacturonase activity on firmness and shelf life were probably largely due to decreased degradation of the middle lamellae, and thus the maintenance of intercellular connections and fruit integrity.

Although this work was originally interpreted as suggesting a very minor role for polygalacturonase in fruit softening, the relatively small reduction in firmness observed may have been due to two factors. Firstly, tomato has atypically high levels of polygalacturonase enzyme (far more than in other species), and secondly silencing of the polygalacturonase gene was incomplete, meaning that in this species even 0.5% of wild-type activity was still substantial. Subsequently, silencing of polygalacturonase was found to partially but significantly reduce fruit softening in strawberry and apple, thus confirming that pectin depolymerisation is one part of the softening process (Quesada *et al*. 2009; Atkinson *et al*. 2012). What these studies have also clearly demonstrated is that softening is not controlled by a single cell wall-modifying enzyme. Rather, many different enzymes are involved, with enzymes such as polygalacturonase, pectate lyase, expansin, β-galactosidase and pectin methylesterase making specific contributions to the softening process (Brummell and Harpster 2001; Brummell 2006). It is the action of these many enzymes working together that brings about the wall swelling, reduced wall strength and weakened middle lamellae that result in the final softening and textural characteristics of the ripe fruit. Indeed, the actions of the various enzymes may be interdependent. For example, polygalacturonase requires the prior de-methylesterification of pectin by pectin methylesterase to make the substrate susceptible (Brummell and Harpster 2001), and the action of expansin to increase the accessibility of enzyme to substrate in the cell wall (Brummell *et al*. 1999).

In addition to cell wall disassembly, work in several species has shown that a decrease in cellular turgor accompanies fruit ripening and is an important component of softening (Shackel *et al*. 1991). This is caused partly by internal water movements resulting from the movement of solutes from symplast to apoplast, and partly to the loss of water from the fruit. In tomato, analysis of the non-softening *DFD* mutant attributed its enhanced postharvest firmness to very low water loss from the fruit and therefore to cellular turgor being maintained at higher levels than in wild-type (Saladié *et al*. 2007).

11.5.4 - Colour and flavour

Colour

During ripening many fruit change colour. Their bright colour, which evolved to attract dispersal agents such as birds, browsing animals and primates, has now become a particularly important visible indicator of maturity and ripeness. Bananas, berryfruit and stonefruit provide good examples where colour is a prime indicator of ripeness. Novel colours are also used to market new varieties to consumers, e.g. in kiwifruit, green-fleshed *Actinidia deliciosa* ‘Hayward’ vs. yellow-fleshed *A. chinensis* ‘Hort16A’ (Figure 11.18).

Figure 11.18. Colour diversity in ripe kiwifruit and apple is determined by the presence or absence of chlorophyll, carotenoid and anthocyanin compounds in different fruit tissues. (Photographs courtesy Plant & Food Research)

By analogy with senescence in most green tissues such as leaves, colour change in fruit typically involves chlorophyll loss and an increase in production of yellow, orange, red or purple pigments. In green-fleshed 'Hayward' kiwifruit chlorophyll is retained in the flesh of ripe fruit, whilst in yellow-fleshed 'Hort16A' chlorophyll is degraded during ripening by catabolic enzymes in the chlorophyll degradation pathway. This suggests that in 'Hayward' fruit chloroplasts are not converted to chromoplasts as is typical for ripening fruit.

The gold, orange and red colours of many fruit such as tomato and citrus are formed by enzymes in the carotenoid biosynthetic pathway (Tanaka *et al*. 2008). Carotenoids are divided into two classes: the hydrocarbon carotenes, e.g. lycopene (red) and b-carotene (orange); or the oxygen-containing xanthophylls, e.g. lutein (yellow). Besides providing highly attractive pigmentation, carotenoids protect the plant's photosynthetic apparatus from excessive light energy and are essential requirements for human and animal nutrition.

Other red and purple pigments of the type seen in grapes and boysenberries are anthocyanins, which are products of the phenylpropanoid pathway. Anthocyanin pigments are water-soluble, synthesised in the cytosol and localised in vacuoles. Their basic ring structure can be modified by hydroxylation, methylation or glycosylation and their specific colour is modified by pH, metal ions and co-pigments to produce the subtlety of colours seen in nature. Like carotenoids, anthocyanins have many human health benefits and are widely used as natural food colourants (Tanaka *et al*. 2008).

The accumulation of anthocyanins is regulated by transcription factors of two classes (R2R3 MYB and basic helix loop helix), regulatory proteins that co-ordinate gene expression of the whole phenylpropanoid pathway. In fruit, this regulation system has been well characterised in grape and apple. In white berry grapes, *VvMYBA2* is inactivated by mutations in the coding region and *VvMYBA1* has a retrotransposon in the promoter and is not transcribed (Kobayashi *et al*. 2004; Walker *et al*. 2007). In apple fruit, a mini-satellite repeat structure in the promoter region of the *MYB10* gene up-regulated the expression of this regulatory gene, which increased the level of anthocyanin throughout the plant producing a fruit with striking red colour throughout the flesh (Espley *et al*. 2009).

Flavour and aroma

Flavour is the most important factor determining if consumers will repurchase a particular fruit. Therefore, all varieties are produced and stored to maintain the very best flavour and aroma properties. Two main factors determine a fruit's characteristic flavour – the correct sugar/acid balance and the production of aroma volatile compounds. These volatile compounds can include a mixture of volatile acids, aldehydes, alcohols, esters, terpenoids and aromatics.

Human taste sensations and experiences play an important part in characterising volatile compounds in fruit and wine, so a vocabulary has been developed to describe their sensory nature. The terms used relate a particular flavour sensation to that of a widely available standard, and have led to terms like 'woody, 'grassy', 'floral', 'spicy' and 'citrus' (Figure 11.19).

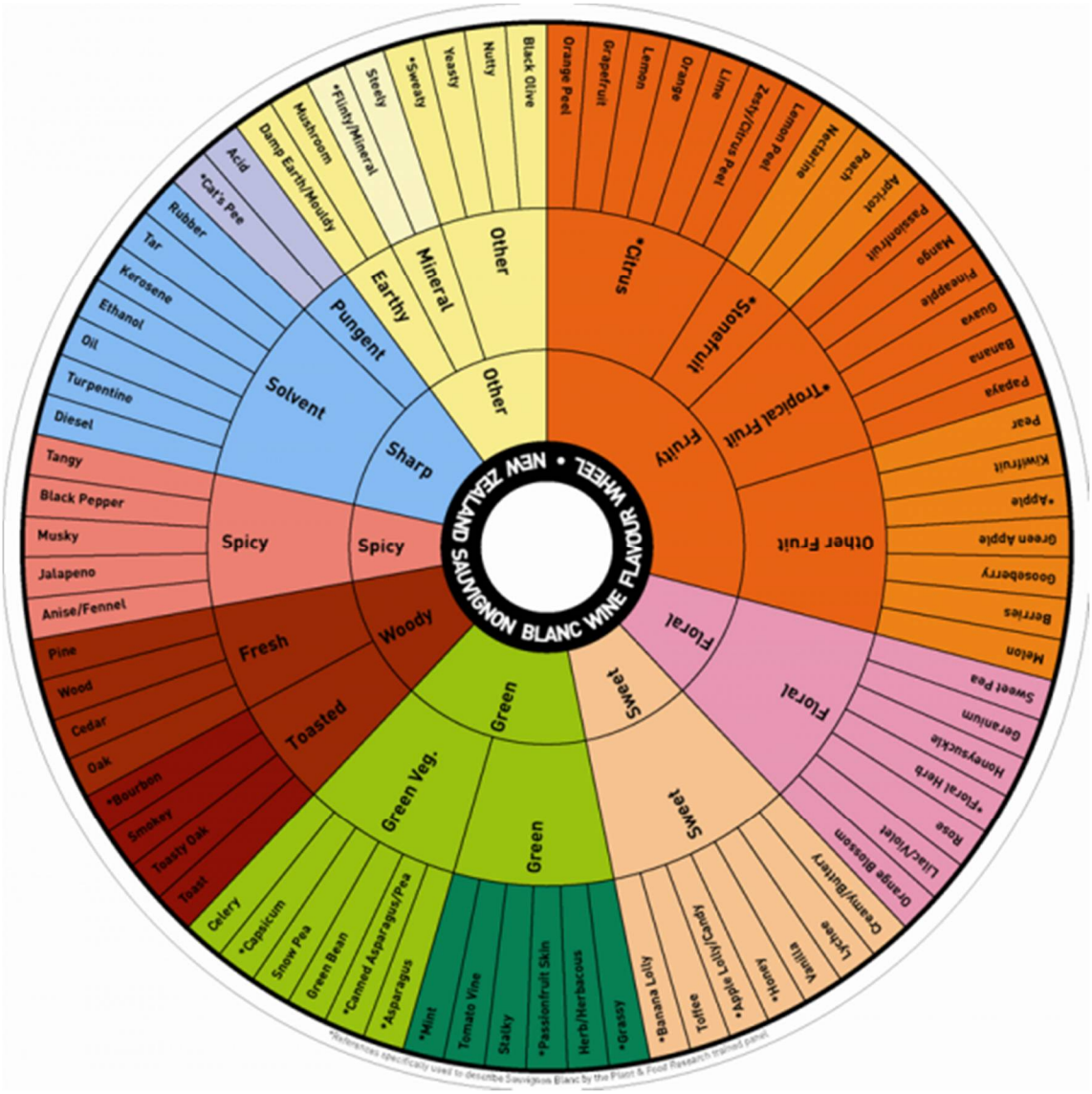

Figure 11.19. A flavour wheel that is used to systematically categorise and define sensory characteristics of wine. (Courtesy F.R. Harker)

With advanced GC-MS techniques many ripe fruit can be shown to contain >100 volatile compounds that contribute to their flavour and aroma. However, the absolute concentration of a volatile

compound itself does not determine how important it is to perceived flavour and aroma. Sometimes compounds found at very low levels, e.g. parts per billion, are required to give a fruit its characteristic aroma. Compounds are given odour activity values (compound concentration divided by the minimum concentration that can be detected by the human nose) to show their importance to aroma.

Sometimes, one or two key volatile compounds can be regarded as characteristic for fruit of a given species or cultivar and are used in synthetic mixtures to represent that commodity. Specific volatiles are especially important in wine grapes where an individual volatile can become the dominant characteristic used in marketing a specific wine type. Examples include the 'grassy' character of methoxypyrazine in Sauvignon Blanc, the 'richness' of b-damascenone in red wine, or the 'foxy' character of methyl anthranilate produced by *Vitis labruscana*. Other examples of important volatiles in fruit include: raspberry – raspberry ketone; 'Hort16A' kiwifruit – ethyl butanoate and 1,8-cineole; 'Hayward' kiwifruit – (E)-2-hexenal and hexanal; apple — 2-methyl butyl acetate and a-farnesene, and strawberry — furaneol.

Our understanding of how flavour compounds are synthesised has rapidly advanced in the last 20 years. Aldehydes, acids and esters are derived from fatty acids and branched chain amino acids. The first committed step in straight-chain ester production is performed by lipoxygenases that produce 13-hydroperoxide linoleic acid from linoleic acid. These compounds are converted by cytochrome p450 lyases to aldehydes such as hex-3-enal and hex-2-enal. Alcohol dehydrogenases (ADHs) can then transform aldehydes to the corresponding alcohols, which contribute 'green' aromas. Alcohol acceptor substrates are then esterified with coenzyme acid donors by alcohol acyl transferases (AATs) to form esters, which generally contribute 'fruity' and 'sweet' characteristics. In apple, the enzyme MpAAT1 can produce a range of esters in ripe fruit and is up-regulated by ethylene during ripening (Souleyre *et al*. 2005). Branched chain esters are produced from isoleucine by branch chain aminotransferases and then pyruvate decarboxylase to produce aldehydes. These aldehydes are then available for ADHs and AATs to form branched chain alcohols and esters, respectively.

Sesquiterpenes and monoterpenes also contribute to fruit flavour and aroma profiles, often by adding 'floral' or 'spicy' top notes. In apple, the most important ripe-fruit terpene is a-farnesene produced by the sesquiterpene synthase AFS1. 'Hort16A' kiwifruit produce 1,8-cineole and *A. arguta* (baby kiwifruit) produce a-terpinolene, which add spicy/minty notes to these fruit. Terpenoid compounds are produced by terpene synthase enzymes, using farnesyl diphosphate (FPP) and geranyl diphosphate (GPP) as substrates. FPP and GPP are produced in plants by the action of short chain prenyltransferases in two compartmentally separated pathways (Lichtenthaler *et al*. 1997). In the plastid, the MEP (2-C-methyl-D-erythritol 4-phosphate) pathway leads to the production of GPP, while in the cytoplasm the mevalonate pathway provides precursors for FPP formation. The primary terpenoid skeletons can subsequently be modified further, e.g. by oxidation, hydroxylation, glycosylation or methylation by a range of other enzymes to increase terpenoid diversity.

Feature Essay - 11.1 A century of ethylene research

Figure 1. Barry McGlasson, University of Western Sydney Hawkesbury, Richmond Campus, using a gas chromatograph (fitted with a flame ionisation detector) to analyse ethylene concentrations in samples of air exiting enclosed containers of harvested fruit.

Plants, fungi and bacteria produce a host of volatile compounds. Some attract or repel animals, some create powerful emotions in humans and some induce morphological and metabolic changes in adjacent plant tissues. Of all these emanations only ethylene is recognised as a natural gaseous plant hormone.

Ethylene has been used unintentionally to manipulate crops such as fig as far back as the third century BC. The sycamore fig originated in eastern central Africa, where it was naturally pollinated by a small wasp that makes its home inside the fruit. When the sycamore fig was taken into the eastern Mediterranean countries, including Egypt, pollinating wasps were left behind. Nevertheless, young fruit which were mechanically injured set parthenocarpically and ripened without seed! A 1633 herbal noted that 'It bringeth forth fruit oftener if it be scraped with an iron knife, or other like instrument'. The fruit is 'like in juice and taste to the wilde fig, but sweeter, and without any grains or seeds within'. We now know that wounding young fruit would have stimulated ethylene production and this gas induced those figs to grow and develop parthenocarpically. David Blanpied summed up this piece of history by recasting Amos 7:14 (OT), 'I was no prophet, neither was I a prophet's son; but I was an herdsman, a gatherer of sycamore fruit', as 'I was an herdsman and *an activator of ACC synthase in sycamore figs*' (Blanpied 1985).

Blanpied's quotation nicely sums up the history of ethylene as a plant hormone because it takes us from simple fruit behaviour to underlying biochemistry. Once the presence of ethylene in plant emanations was proved chemically, a lively debate followed as to whether a gas could really be defined as a hormone. There were two major developments that resolved this issue. First was the invention of gas chromatography which soon enabled measurement of ethylene at concentrations that were physiologically meaningful and in small gas volumes. Second, a non-volatile plant product, 1-aminocyclopropane-1-carboxylic acid, was found to be the immediate precursor of ethylene (Adams and Yang 1979). Any lingering doubts that ethylene was a plant hormone have now been completely erased by application of molecular methods.

This story of ethylene mixes applications of plant physiology with human intuition, and is conveniently related to three eras that represent technical evolution in this area of plant science,

namely, pre-1935 (an age of mystery), 1935–1979 (an age of enlightenment) and post-1979 (an age of opportunity).

An age of mystery

In 1858, Fahnestock in the USA observed that illuminating gas caused plant senescence and leaf abscission, and Girardin (1864) in France subsequently showed that ethylene was a component of illuminating gas. Many suspected that such plant responses were due to ethylene, but it took a Russian student, Neljubov (1879–1926), to establish that ethylene is a biologically active compound. As a young man, he observed that pea seedlings germinated in the dark grew in a horizontal direction when exposed to laboratory air containing burnt gas. He showed that the plants resumed normal growth when the air was first passed over heated CuO to oxidise hydrocarbon gases. This growth response was used as a bioassay for the next 50 years. We now know that these pea seedling responses are induced by as little as 0.06 $\mu L\ L^{-1}$ ethylene.

Many publications from around 1910 indicated that ethylene was produced by ripening fruit such as pears and apples. By 1923, Denny (US Department of Agriculture) had patented ethylene for ripening bananas, tomatoes and pears, removing astringency from persimmons and loosening walnut husks. Finally (1934) Gane in Britain produced conclusive proof that ethylene is a natural product of plants, and to obtain enough ethylene for his tests he collected gases from about 28 kg of apples. He extended this proof to other fruits a year later.

An age of enlightenment

Following Gane's confirmation that ethylene generation was common in fruits, research interests broadened beyond this simple ethylene–fruit connection. By 1940 the postharvest pioneer Jacob Biale (University of California, Los Angeles) showed that green citrus mould (*Penicillium digitatum*) also produced ethylene, thereby extending ethylene physiology to plant–fungus interactions. Hormonal interrelations entered this picture when the synthetic auxin 2,4-D was later shown to stimulate ethylene production by plants. Ethylene was by now acknowledged as instrumental in fruit ripening, but a nagging question remained as to whether ethylene was a true ripening hormone or merely a by-product of ripening events. I entered the field at this stage, and to resolve this issue of hormone status we needed to establish whether ethylene production by fruits increased ahead of ripening. Progress in unravelling cause and effect would hinge on development of a sensitive assay for ethylene.

Strong indications of ethylene involvement in ripening came from experiments using cold mercuric perchlorate solutions to bind specifically ethylene rather than other gases, and thus trap a sufficient amount from ripening tomatoes to measure it manometrically. However, the much greater sensitivity of gas chromatography subsequently allowed demonstration via frequent monitoring that ethylene production actually precedes the onset of ripening in some fruit.

Scientifically, these were exciting times. As a PhD student at the University of California, Davis, I was a member of one of the first teams to use a gas chromatograph fitted with a flame ionisation detector to measure internal ethylene concentrations in a ripening fruit (Lyons *et al*. 1962). We showed conclusively that cantaloupe (rockmelon) was climacteric. Harvested fruit showed an increase in ethylene production with onset of a respiratory climacteric and ripening. Over the next 20 years an explosion of publications documented ethylene involvement in many plant responses. Burg and Burg (1960s) demonstrated that ethylene was essential for ripening as well as other

developmental events in plants. Senescence is a case in point, and a clear ethylene response is shown in Figure 2 for *Cymbidium* flowers.

Figure 2. Ethylene generation influences postharvest behaviour of *Cymbidium* flowers. When the pollen cap is removed from the floral column either by insect pollination or by human mishandling, endogenous ethylene production is triggered in that flower (left side), bringing about anthocyanin synthesis, cupping of petals and swelling of column tissues within 3 d. Intact flowers (right side) remain fresh for three weeks. Scale bar = 1 cm. (Photograph courtesy R.L. Bieleski)

A further practical development from ethylene research dates from 1963 with synthesis of 'Ethephon' (2-chloroethyl-phosphonic acid) (also called 'Ethrel'). This water-soluble compound is readily absorbed by plants, and breaks down to release ethylene above pH 4.6. Ethephon thus provides a convenient way of applying ethylene to plants under field conditions and it has been used to promote uniform maturation of processing tomatoes as an aid to mechanical harvesting.

Three broad research themes in ethylene physiology were now underway: mode of action, inhibition of action and biosynthesis. However, a major problem confounding our best efforts in all three areas was the autocatalytic behaviour of ethylene. This gas stimulates its own production, so how do you distinguish between the external ethylene you have applied experimentally as a stimulus, and the endogenous ethylene which is produced as a response by the plant tissues? Confronted by this dilemma, we devised a neat trick based upon a closely related gas (McMurchie *et al*. 1972). Propylene is a three-carbon analogue of two-carbon ethylene, although about 100 times less active than ethylene, that can stimulate typical ethylene responses! Moreover, propylene is also easily distinguished from ethylene by gas chromatography. We now had an elegant tool for analysis of ethylene physiology.

We applied propylene to citrus fruit (non-climacteric) and to bananas (climacteric) to mimic an exogenous ethylene stimulus, and measured endogenous ethylene production directly. Citrus respiration was stimulated without any increase in ethylene production, whereas in banana both respiration and endogenous ethylene production were stimulated. These outcomes were consistent with our paradigm of ripening in climacteric versus non-climacteric fruit.

Once ethylene was widely acknowledged as a ripening hormone, there was a strong demand by industry for practical control methods in order to extend fruit storage life. Our original approach was to remove ethylene from fruit storage atmospheres by scrubbing with oxidising agents such as permanganate. Commercial absorbents containing permanganate are available but inconvenient to use because the absorbent has to be packaged to prevent contact with stored fruit. The search for other ways of avoiding or inhibiting ethylene action continued. By 1976, Beyer showed that silver

ions are a potent inhibitor of ethylene action, and a new set of management options opened up immediately. Ag^+ is readily bound by plant tissue but not easily translocated and is thus of limited application. However, the silver thiosulphate complex (STS) is negatively charged and can move readily through plant tissues. This observation had little practical value for fruits which are eaten, but has had wide use in slowing the ethylene-driven senescence of cut flowers. In 1979 Sisler introduced volatile unsaturated ring compounds as inhibitors of ethylene action, the most potent being norbornadiene. Sisler has subsequently developed 1-methylcyclopropene (1-MCP), a gaseous compound which is essentially an irreversible inhibitor and safe to use (Sisler and Serek 1997).

While ethylene was gaining wider application in postharvest physiology, research continued with unravelling the biosynthetic pathway. The first clue came when Lieberman and Mapson (1964) supplied the general precursor [^{14}C]-methionine to ripening tissue and found ^{14}C in the ethylene produced. Methionine had been noted as a possible precursor from the discovery that rhizobitoxin inhibits ethylene production. Rhizobitoxin inhibits pyridoxal phosphate-containing enzymes of the kind involved in methionine-utilising pathways. A commercial product (Retain™) containing aminoethoxyvinylglycine (AVG) is now used as a preharvest treatment to delay ripening of apples and peaches. Adams and Yang, working at UC Davis, showed convincingly that S-adenosylmethionine (SAM) rather than methionine was a key precursor in ethylene biosynthesis, then in 1979 they topped this triumph by discovering the immediate precursor of ethylene, namely 1-aminocyclopropane-1-carboxylic acid (ACC). Within another few years, Yang and co-workers had managed to define the biochemical pathways that generate ACC from methionine via SAM.

An age of opportunity

Understanding the role of genes involved in ethylene biology creates opportunities for answering many remaining questions about ethylene-driven behaviour. One that remains unresolved concerns regulation of ethylene production in relation to ontogeny of fruit. I observed that tomato fruit harvested less than 15 d after anthesis failed to undergo normal ripening whereas fruit harvested at 20 d or later ripened normally, although with poor eating quality (McGlasson and Adato 1977). How then are ethylene-driven events coordinated with organ ontogeny? There is now a considerable body of evidence on the mode of action of ethylene that will aid such studies.

The discovery of mutant genes from *Arabidopsis thaliana* plants that are insensitive to ethylene enabled isolation of the *ETR1* gene, which encodes an ethylene receptor and is antagonised by competitors of ethylene binding. Similarly, a ripening-impaired mutant tomato (*Nr*, Never Ripe) has been found to contain a defective homologue of ETR1 that lacks the ability to receive ethylene. The question remains how the different kinds of ethylene receptors might differ in their ethylene response or in their downstream signalling behaviour.

In 1972 we established a distinction between climacteric and non-climacteric fruit in their response to ethylene that led us to propose two systems for regulation of ethylene production. System 1 would be responsible for background ethylene production found in non-climacteric fruit and in pre-climacteric fruit. System 2 would account for the autocatalytic increase in ethylene production associated with ripening in climacteric fruit. Nakatsuka *et al*. (1998) provided elegant proof for this hypothesis in tomato. Their data suggested that System 1 is mediated by constitutively expressed *Le-ACS1A* and *Le-ACS3* and the negatively feedback-regulated *Le-ACS6* (genes encoding ACC synthase enzymes), together with preexisting mRNAs of *Le-ACO1* and *Le-ACO4* (encoding ACC oxidase proteins that convert ACC to ethylene). In contrast, in System 2 there is a large accumulation of two different ACS mRNAs (*Le-ACS2* and *Le-ACS4*), as well as large increases in *Le-ACO1* and *Le-ACO4*. Similar findings have subsequently been reported for other climacteric fruit. Young

developing tomato fruit, while still in System 1, provide excellent experimental material; they behave as a non-climacteric fruit because when treated with propylene, respiration increases temporally without a concomitant increase in endogenous ethylene production. 1-MCP in combination with propylene has turned out to be a very useful tool for distinguishing between events regulated by ethylene and those that are independent (Golding et al. 1998).

The invention of 1-MCP has provided a valuable technology that is widely used to extend the storage life of some fruit, especially several cultivars of apples, and when applied at the right time for each cultivar ensures that the fruit retain good eating quality as well as shelf life. Application of 1-MCP to delay ripening in highly perishable plums can be used to extend shelf life at non-chilling temperatures (<8ºC), leading to a saving in energy costs for refrigeration. The availability of a wide range of new tools that have accompanied the study of ethylene has opened new ways for improving the storage life of fruit that will benefit both domestic and export markets. A current example is the peach, which only has a short cool storage life and responds adversely to treatment with 1-MCP in contrast to the closely related Japanese-type plums that respond beneficially. Furthermore, some late maturing plums have twice the cool storage life of peaches. Imagine the attraction to the consumer if we could transfer these traits from plums to peaches!

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11.6 - Extending storage life

The main method used to prolong the storage life of fruit is through reducing the fruit temperature to slow metabolism. Refrigerated storage slows the rate of ripening and senescence of the fruit, and also slows the development of any rots. The way in which temperature management is implemented after harvest can significantly affect the quality of the fruit at the end of storage, both in the amount of ripening retardation and also the presence or absence of disorders. The basic effect of refrigerated storage on fruit can be supplemented by modification of the atmosphere in the coolstore, by reducing oxygen and increasing carbon dioxide concentrations. More recently, the application of the inhibitor of ethylene action 1-methylcyclopropene (1-MCP) has become common to slow the ripening of a range of fruit, and in particular certain cultivars of apple. The way in which all these technologies impact on the fruit is dependent on the physiological state, or maturity, of the fruit at harvest. What may be described as a 'correct' physiological state at harvest is not fixed, but may differ dependent on the commercial requirements of the fruit, i.e. a short or long storage period. Ultimately, the target for good storage is for the fruit to remain in good condition, to ripen properly, have an acceptable flavour and not have any disorders at the end of storage and when it reaches the consumer.

11.6.1 - Temperature and relative humidity

Temperature

The earliest attempts at temperature management were dependent on fruit being held in cold caves, or using cold night air to prolong the storage life, but experience showed that a 'best' temperature can be sharply defined, and may differ between species or even cultivars (Sevillano *et al*. 2009). To obtain the maximum benefit from cold temperatures, the temperature must be as low as possible without causing damage to the fruit; this is termed the lowest safe temperature. Below the lowest safe temperature, but at non-freezing temperatures, the fruit may develop symptoms of chilling injury (See Section 14.4). At even lower temperatures, generally in the range –0.5°C to –1.5°C, freezing occurs which irreversibly damages a living product. Because of this, –0.5°C is usually the lowest temperature used for storage of fruit, including some apple cultivars, berries or 'Hayward' kiwifruit. Temperatures at which chilling symptoms occur are around 8ºC for subtropical species and may be anything up to 14°C for some tropical fruit: for example unripe banana and mango need to be shipped at 13–14°C. However, it is not only tropical and sub-tropical fruit that are susceptible to chilling injury; even 'Hayward' kiwifruit, which is stored at 0°C or just below, may develop chilling injury.

At 0°C, respiration is reduced to a level that is just enough to maintain cell function. Sugar is slowly consumed during this process so that fruit with a low sugar content at harvest are less durable. Commodities such as kiwifruit, which are picked with large supplies of carbohydrate in the form of starch, have an additional source of sugar to utilise, giving longer storage lives than those entirely reliant on soluble reserves, such as grapes.

Low-temperature storage has played an important part in the development of successful fruit export industries in Australasia, because of the great shipping distances between orchard and consumer. The success of kiwifruit has been largely due to its ability to be stored at 0°C for 6 months or more with no detrimental effect on flavour or texture.

Associated with low-temperature storage is a wide range of techniques to manage temperature changes en route to storage (Kader 2002). There are strong differences between species in their temperature management requirements. Elements of temperature management that need to be considered include the timing of cooling after harvest, the rate of cooling and the final storage temperature. Temperature management may also be viewed as a two-stage process, the removal of the field heat and then temperature maintenance during storage. While it is generally considered that the field heat should be removed from fruit as soon as possible after harvest, there are circumstances where delays may be advantageous for the postharvest performance of the fruit. So whilst highly perishable berryfruit tend to be cooled as soon as possible after harvest, kiwifruit and some stonefruit benefit from a delay at ambient temperature before cooling. Exactly what happens during this delay period is not clear; it may simply be a continued progress of fruit development or the loss of a small amount of water. However, the delay tends to make the fruit more tolerant of storage at low temperatures. In the case of 'Hayward' kiwifruit, the delay period is termed ‘curing’ and is specifically applied to reduce the incidence of stem-end rots caused by *Botrytis*. As a beneficial side effect, the low temperature tolerance of the fruit is also increased. In this sense, curing in kiwifruit is not the same as the curing for wound healing of the skin that is commonly referred to for sweet potatoes.

The rate of cooling is dependent both on what is required commercially and what can be tolerated by the fruit. Simply placing fruit, either in bulk bins or packed, in a coolstore will result in the fruit being cooled, the rate of which will depend on the initial fruit temperature, the cooling capacity of the refrigeration equipment, the airflow in the store and any insulating effects from the packaging, especially if the fruit are packed in boxes with polyliners and held on pallets. The rate of cooling can be increased by forced air cooling, also termed precooling, in which cold air is actively drawn past the fruit. This is a rapid method for removal of field heat, after which temperature management in a coolstore removes the smaller heat load that results from continued respiratory activity during storage. In some cases fast precooling may induce high incidences of chilling damage. This is one reason why 'Hayward' kiwifruit is not always precooled, but may be cooled from about 14–18°C at harvest to about 2°C after about 5 days, with a further 5–7 days to reach the final storage temperature.

Managing the rate of cooling of fruit to avoid chilling injury may be as simple as allowing the fruit to cool slowly, as in the case of 'Hayward' kiwifruit described above, or there may be clearly defined stages of cooling whereby fruit are cooled to an intermediate temperature, held for a period of days before the temperature is reduced to the final storage temperature. In all these instances of slow cooling, there is a trade off between the conditioning effect that increases tolerance to low temperatures and the progression of fruit development that occurs more rapidly at higher temperatures, and reduces storage life of the fruit.

An extreme example of temperature treatment prior to storage is where fruit may be treated at high temperatures (40–50°C) for disinfestation, and in particular to kill fruit fly, after which the fruit ripening may be slower than would occur naturally.

The expression of chilling injury symptoms may be reduced in long-term storage by intermittently warming the fruit. However, whilst there are numerous reports of such treatments in the scientific literature, the practicalities of the procedure and detrimental side effects to fruit quality make it commercially uncommon.

Relative humidity

Once harvested, fruit will continuously lose water to a point where quality will be affected. In some species, a small amount of water loss may accelerate ripening (e.g. avocado), but in all fruit there eventually comes a point at which loss of water, usually first seen as shrivelling, results in the fruit becoming unacceptable. Water loss from the fruit is driven by the vapour pressure gradient between the fruit and the surrounding environment. While the capacity for air to hold water is reduced at low temperatures, there is always a gradient driving water from the fruit into the coolstore atmosphere. The less fruit there is in a coolstore, the greater the water loss from each fruit before an equilibrium relative humidity is reached. Water may be lost from the coolstore atmosphere by condensation on the refrigeration coils that are colder than the room atmosphere, and the greater the temperature differential between the coils and atmosphere the greater the loss of water. When storage is at about 0°C, this can be seen by ice developing on the coils that must be removed by defrosting.

In preventing quality loss of harvested fruit, the relative humidity of the storage environment is one of the first aspects considered, since fruit will lose water more rapidly at lower relative humidity. This is mostly an issue where fruit are held unpacked or in bulk in a coolstore, and water loss is exacerbated where there is only a small volume of fruit in the store, air flow is high and there is a large temperature differential on the refrigeration coil. In other circumstances, such as for kiwifruit that may be stored for months, the fruit is packed into fibreboard packs with a polyethylene liner or bag. In these circumstances, it is the bag that creates a high humidity environment for the fruit and limits the fruit's water loss. A very high relative humidity in the store environment where packed fruit are held may be detrimental to the integrity of the fibreboard packaging, which would soften and lose its strength.

11.6.2 - Controlled and modified atmospheres

The storage life achievable by refrigerated storage can be extended by modifying the store atmosphere by reducing the oxygen and increasing the carbon dioxide concentrations. Elevated CO_2 and reduced O_2, used either separately or together, can delay ripening and slow the onset of senescence (Figure 11.20). When both high CO_2 and low O_2 concentrations are combined then the beneficial effects may be additive. These methods were originally developed on a commercial scale for apple, but have been progressively applied to many other fruit. Container shipping helped their introduction because a sealed container made it easier to maintain the required temperature and atmosphere regimes.

For the bulk storage of fruit in bins, packs of fruit on pallets, coolstores, ships' holds and individual shipping containers, an active process called controlled atmosphere (CA) may be operated. In these, the concentrations of O_2 and CO_2 are monitored and maintained at predetermined levels. Initial low O_2 concentrations may be achieved through the use of nitrogen generators or O_2 scrubbers, or the fruit may be allowed to reduce the O_2 concentration through respiratory activity. To prevent the O_2 concentration from becoming too low, air can be exchanged with the atmosphere. CO_2 accumulates from respiration, but can be prevented from increasing excessively by absorbing it with lime, by removal with an activated carbon scrubber or by purging from the store with nitrogen. In a closed CA system it is also possible to scrub ethylene out of the atmosphere. The removal of ethylene is

particularly important for ethylene sensitive fruit such as kiwifruit, where even low levels (e.g. 30 ppb) in the store atmosphere can reduce the storage life of the fruit.

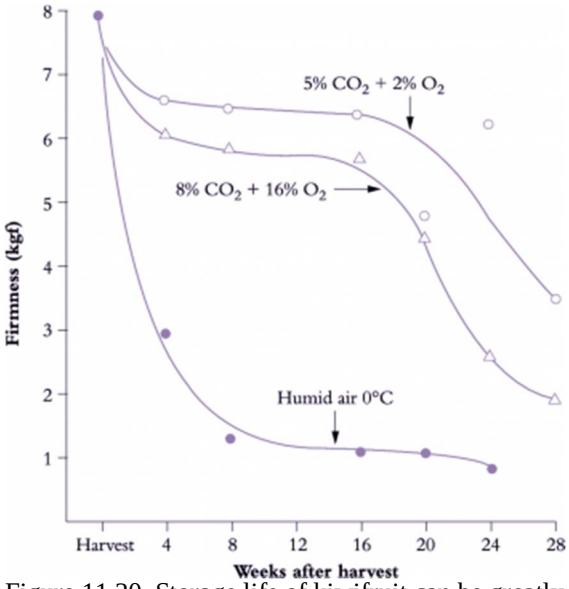

Figure 11.20. Storage life of kiwifruit can be greatly extended by controlled atmospheres. Under standard conditions (humidified air, 0°C) firmness declines exponentially over time, reaching limited acceptability by 8 weeks. Storage life ends once fruit firmness drops below about 0.9 kgf. Fruit are then soft enough to eat. Softening in cold store was slowed and storage life greatly extended by holding fruit in atmospheres containing either 5% CO_2 + 2% O_2 (top curve) or 8% CO_2 + 16% O_2 (middle curve). (Based on McDonald and Harman (1982) Sci. Hort. 17, 113-123)

A more recent approach to CA storage is termed dynamic CA storage, in which the O_2 concentration in the store is determined by the response of the fruit. Dynamic CA optimises the CA process, since using a predetermined atmosphere tends to err on the side of safety by setting the O_2 concentration well above the lowest safe level to allow for the variability in low O_2 tolerance amongst fruit from different orchards or seasons. Although this eliminates the risk of fruit becoming anaerobic, it also reduces the potential benefit. While early attempts at dynamic CA utilised ethanol sensors to detect if fruit metabolism was becoming anaerobic, it was the development of a fluorescence sensor that could give a rapid measurement of the fruit response to low O_2 stress that allowed the commercialisation of dynamic CA. The sensor is placed over a sample of the fruit in the store, the O_2 concentration is decreased until a response is detected from the fruit and then the O_2 concentration is increased slightly above the low O_2 stress point. The procedure can be repeated throughout the storage period so that the O_2 concentration can be continually matched to the capacity of the fruit to withstand low O_2.

An alternative way of utilising the beneficial effects of low O_2 and high CO_2 is termed modified atmosphere (MA) storage. In this system, fruit respiration is used to reduce the concentration of O_2 and increase that of CO_2 inside an enclosed space, usually the export box or retail packs. The fruit is prevented from becoming anaerobic by making such enclosures out of plastic films that are partially permeable to O_2 and CO_2. Both gases come to an equilibrium based on respiration rate, the specific permeability of the film, the surface to volume ratio of the package and the amount of fruit in the package. Hence, this form of storage is highly dependent on being able to control the fruit temperature, since this determines the rate of respiration. The independence of having fruit in smaller packages that can be moved intact throughout handling and retailing suggest that MA may be more

versatile than CA, although in practice any inability to maintain adequate cold-chain conditions can result in fruit spoilage as packages turn anaerobic at higher than desired temperatures.

Coating fruit in waxes or other compounds may act in a similar way to MA, by modifying the gas permeability of the fruit skin, thereby reducing the flow of O_2 in and CO_2 out of the fruit. As with MA, if the restriction of oxygen flow into the fruit is too great, the fruit may turn anaerobic and ferment.

How do altered atmospheres delay ripening and retard senescence? There are several possibilities, mostly involving fruit respiration and ethylene metabolism. One common observation is that fruit respiration is suppressed in response to the changed atmosphere. This could occur via acidification of the cytosol, resulting from an elevated CO_2 concentration redirecting metabolism towards alcohol or lactate/succinate or malate production rather than CO_2 production. Another alternative is a direct effect of ultra-low O_2 concentrations (<2%) on cytochrome *c* oxidase in the mitochondrial electron transfer pathway, preventing that enzyme from functioning properly.

Fruit differ with respect to critical values for tolerance to low O_2 or high CO_2 concentrations, and ideally we might make a model for predicting the tolerance limits for a new cultivar or fruit from specific background information on its physiological behaviour. However, there is a key problem in manipulating atmospheres by static modelling approaches. The critical gas composition exists within the flesh of a fruit, not in the environment around it, while differences in genetic background cause each cultivar to behave differently with respect to metabolism and thus internal gas composition. Species vary in their response to the altered atmospheres of CA, and can even differ according to cultivar and harvest. This variation is seen in both the final concentration of CO_2 and O_2 within stored fruit, and in the time taken to equilibrate. Normally, an internal 0.5% (0.5 kPa) partial pressure is the minimum O_2 level tolerable, and 10% (10 kPa) is the maximum for CO_2.

Conditions during storage are especially critical because optimum levels of CO_2 and O_2 are on the threshold between aerobic respiration (desirable) and anaerobic respiration (undesirable). Fruit differ in their sensitivities to anaerobic respiration, but are normally intolerant of prolonged periods (>3 days), after which disorders and off-flavours appear. Yet the anaerobic metabolites ethanol and acetaldehyde are common volatiles of many ripe fruit, and treatment with these metabolites, or short anaerobic periods before storage, can have beneficial effects on storage life in some fruit, although they are not used commercially. Fruit in which ethanol and acetaldehyde have been induced are able to metabolise these compounds without tissue damage. The effect of anaerobic metabolism is therefore likely to be a question of degree: how much anaerobic metabolism and how sensitive is the tissue?

'Hayward' kiwifruit is a good example of where CA storage can be successful in prolonging storage life. Both low O_2 (2%) and high CO_2 (5%) can independently improve firmness retention during storage, with a synergistic effect when used in combination. However, whilst effective in retarding ripening, there are risks to the fruit. The greatest firmness retention is achieved by a rapid establishment of the CA, although too rapid establishment of 5% CO_2 can result in increased physiological disorders and rots. Also, concentrations of CO_2 at about 10-15% can result in a differential softening of the fruit flesh and core, resulting in a core that is firm relative to the pericarp tissues.

11.6.3 - Blocking ethylene action

With ethylene having a pivotal role in the ripening of many (but not all) fruit, the use of the ethylene action inhibitor 1-MCP has been investigated for prolonging the storage life of a wide range of species through retarding fruit ripening and softening (Watkins 2008). 1-MCP is usually applied after harvest as a gas treatment in a sealed store, container or tent, with the active ingredient released from a powder by dissolving in water. The commercial delivery of 1-MCP is by the SmartFresh(SM) system (www.agrofresh.com).

Successful use of 1-MCP to delay ripening depends on the physiology of the fruit, most likely on the natural rate of replacement of the ethylene receptors that are blocked by 1-MCP. Since binding of 1-MCP to existing ethylene receptors is irreversible, a single period of exposure can delay ripening for several to many days, depending on the rate of synthesis of new receptors. There has been a rapid uptake of 1-MCP use for commercial storage of some apple cultivars, although for other cultivars the treatment has little effect on fruit softening. The rapid uptake for apple is associated with the way in which apple fruit ripen, which involves only limited softening and with firmness retention being a key quality component, i.e., people like crisp apples. This contrasts with the physiology of other species in which ripening involves a softening of the fruit coordinated with changes in flavour and colour. For example, while 1-MCP prolongs storage life in species such as avocado, pear and banana, obtaining uniform ripening afterwards may be difficult (Watkins 2008). This may be because the softening, flavour, and colour aspects of ripening have varying sensitivities to ethylene (Johnston *et al*., 2009) that are affected differently by partial suppression of ethylene perception and the climacteric, resulting in poorer flavour and colour. In stonefruit such as peach, the ripening inhibition is rapidly overcome, and repeated exposure to 1-MCP may be necessary, which can be commercially unfeasible. For all cultivars, careful optimisation of maturity stage, 1-MCP concentration, exposure frequency and duration and storage temperature is required.

11.6.4 Storage disorders

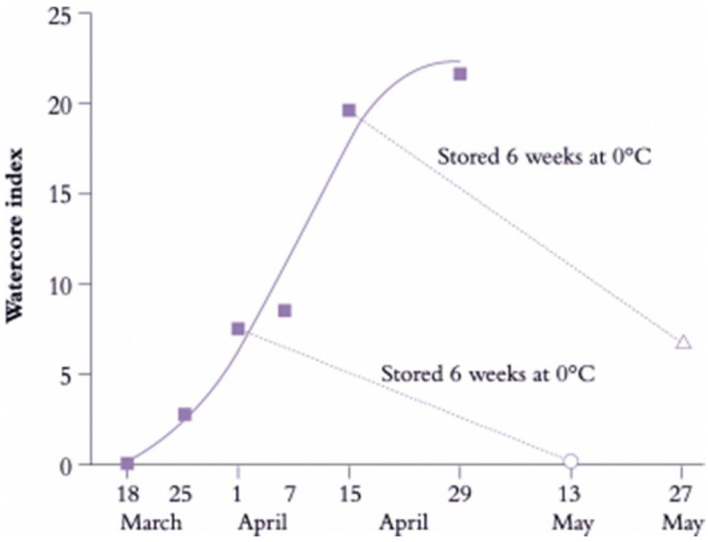

Figure 11.21. Postharvest incidence of the storage disorder watercore in Fuji apple is related to picking date (and thus fruit maturity). Watercore index represents the percentage fruit volume occupied by water-soaked tissue. Fuji is prone to this disorder, especially when fruit are picked mature. Early harvesting thus becomes an important control method. (Original data courtesy F.R. Harker)

When fruit are put into storage, they are on a slow path to senescence and death, and a number of disorders can arise during that time. Several storage disorders have physiological origins, which may be chilling related, and are often highly specific to species, cultivar, season and even growing region. Fruit maturity at picking is one important factor (Figure 11.21), with less mature fruit generally being more susceptible to chilling injury.

Sensitivity to storage disorders depends on many factors, including maturity at harvest, a lack or imbalance of nutrients and adverse growing conditions. Even if fruit are susceptible at harvest, the expression of disorder symptoms is dependent on storage conditions and duration, and symptoms may not always develop. The development of chilling injury is often described as a time by temperature relationship, i.e. it develops sooner at lower temperatures. This is true for damage that is a direct result of exposure to low temperature and which is seen almost immediately after exposure. However, many chilling disorders develop only after long periods in storage and are associated with an inability of fruit to ripen correctly at low temperatures (e.g. kiwifruit, peach, avocado). It seems that at low temperatures the natural highly co-ordinated process of ripening is disrupted by an element that is temperature sensitive. If removed from storage early enough, no symptoms of chilling develop when the fruit ripens at higher temperature.

Thus far, chilling damage has been described as a single disorder, yet there are numerous symptoms that may develop in the fruit flesh or skin that differ among species and cultivars. In addition, there are disorders that develop as fruit start to senesce, irrespective of storage duration or temperature, and that may have similar symptoms to chilling injury in the fruit flesh.

Figure 11.22. Physiological disorders of apple fruit. The top left panel shows bitter pit, a disorder associated with calcium deficiency. It can be partially controlled by preharvest sprays of calcium salts directly onto the fruit. The top right panel shows superficial scald, a low temperature disorder of the skin that can be controlled by 1-MCP treatment prior to cool storage. The bottom left panel shows soft scald, a low temperature disorder with symptoms of brown lesions that extend into the flesh. Incidence can be increased by over-maturity of the fruit at harvest and preharvest climatic conditions. The bottom right panel shows core flush, a browning within the core line, that is a form of senescent breakdown.

Five examples of postharvest physiological disorders in apple are described below (Figures 11.22, 11.23) to illustrate our partial understanding of the problems that occur, and to provide a glimpse of a large and complex area of postharvest physiology.

Bitter pit is a brown, bitter pitting of the skin in some cultivars, particularly 'Cox's Orange Pippin'. It occurs as sunken discoloured pits in the skin with spongy, dry brown flesh beneath. It is primarily a response to inadequate calcium content, and can be greatly reduced by spraying fruit on the tree with calcium-containing solutions during the later stages of fruit development.

Superficial scald is a brown discolouration of the skin surface, particularly in cultivars like 'Granny Smith'. It appears to be connected with the accumulation of the hydrocarbon α–farnesene in susceptible cultivars, the oxidation products of which are brown and may cause cell collapse. Superficial scald can be reduced by a postharvest dip in an antioxidant free-radical scavenger like diphenylamine (DPA). As a postharvest chemical treatment, DPA is being phased out, and in some circumstances the use of 1-MCP before cool storage may mitigate scald expression, since the production of α–farnesene is promoted by ethylene.

Less is known about the factors that affect the occurrence of **soft scald**, which can occur most frequently on cultivars 'McIntosh' and 'Jonathan'. Soft scald or deep scald develops as sharply-defined brown lesions on the skin that usually extend into the flesh. Soft scald is a low temperature disorder, partially avoided by slow (delayed) cooling or by storing at slightly warmer temperatures. Its causes are unclear, but incidence is increased by factors including over-maturity of the fruit at harvest, and by dull, cool, wet summers.

Core flush, most serious in 'McIntosh', is a browning of internal fleshy tissues surrounding the core of a fruit, and may have more than one cause. One factor seems to be the O_2 supply to the core, since conditions potentially causing anaerobiosis (large size, a closed and airtight calyx and a low-O_2 atmosphere) increase incidence. It is most serious in fruit stored for long periods at around 0°C, and may be greatly reduced by storage at 4°C under CA.

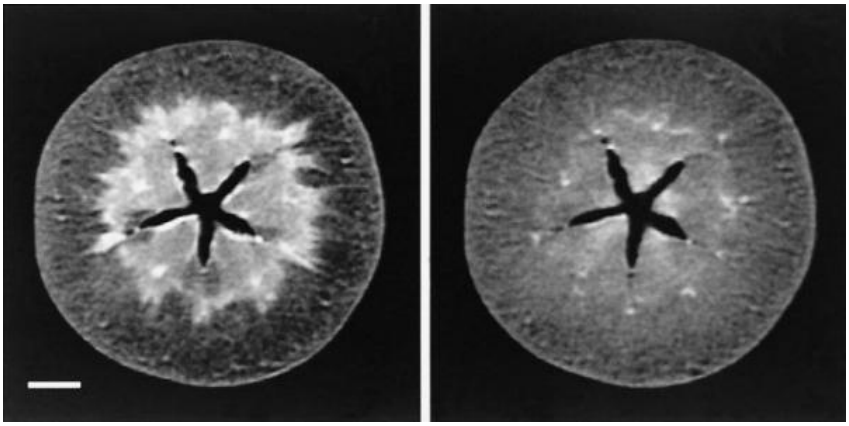

Figure 11.23. NMR images from the equatorial plane of an apple show watercore (waterlogging) as an intense white region. The first scan (left) was taken from a Fuji apple with severe watercore at the time of harvest. The second scan (right) was taken of the same fruit after cool storage for 15 weeks at 0°C, when symptoms had disappeared due to reabsorption of apoplastic water. Scale bar = 1 cm. (Original images courtesy C.A. Clark)

Watercore (Figure 11.23) is a condition where there are glassy, waterlogged sections of tissue towards the centre of the fruit, typically centred around the vascular bundles. Severe watercore leads to anaerobiosis, development of fermentation aromas, and core browning similar to core flush. Fuji is

an especially susceptible cultivar. Watercore is more severe in sweet fully mature fruit (Figure 11.21) and involves a breakdown in transport of sorbitol across cell membranes. As outlined earlier (Section 11.3.2), sorbitol is the main soluble carbohydrate supply for early growth in apple fruit. Unlike other storage disorders, watercore becomes less severe or even disappears during storage (Figure 11.23) presumably because pericarp cells eventually take up intercellular water and sugar and allow airspaces to reform.

11.7 - Future technologies

Classical breeding strategies have successfully driven the production of many desirable cultivars of fruit with improved composition, storage or eating qualities. In postharvest physiology, genetic intervention by conventional breeding has yielded pome- and stone-fruit, citrus, and a range of other subtropical species with improved storage life. Persimmon provides an extreme example of breeding for improved flavour where intense selection of genetic variants has resulted in the non-astringent variant 'Fuyu'. Can fruit growth, maturation and postharvest physiology be modified even further for human convenience, to produce a new generation of 'designer' fruit?

Recent technological advances have driven '-omics'-type research to produce more data, more cheaply, in shorter times. Genome sequences are already available for many fruit species (including grape, apple, strawberry, papaya, tomato, pear, melon, banana, date palm and peach), and it is now feasible to obtain complete genome sequence data for individual cultivars or breeding lines, from which the sequence of important alleles can be determined. Genome sequence, together with data on gene expression (transcriptomics), the accumulation of structural proteins and enzymes (proteomics), and changes in the abundance of metabolites (metabolomics) can be integrated to provide a complete picture of ripening-associated or postharvest changes, or the metabolism that underlies a desired trait. This integrated approach is known as 'systems biology'.

Genes or alleles of genes that have been identified as important in a particular trait can be used as molecular markers to guide targeted conventional breeding efforts. This strategy is known as marker assisted selection and is widely incorporated in breeding of many cereal crops, mainly for disease resistance. In fruit, breeding targets vary between species and between cultivars, and are aimed at improving shelf life, nutritional content, eating quality or disease resistance, or in the alleviation of particular postharvest storage disorders (see Section 11.6.5). Quantitative trait loci (QTLs) for these traits and their underpinning genes are rapidly being identified in many fruit species.

Transgenic strategies have been applied both to investigate gene function and to improve various aspects of fruit quality or production. Genetic engineering was used successfully to suppress ethylene biosynthesis and halt ripening through the silencing of either the *ACS* or *ACO* genes (Barry and Giovannoni 2007). Although biologically successful, this technology did not find a commercial use. Suppression of the cell wall-modifying enzyme polygalacturonase in a line of tomato extended fruit shelf life, and when sold to the public in the USA as 'Flavr Savr' became the first genetically engineered whole food to go on the market. However, consumer resistance led to its withdrawal a few years later. Transgenic modification has been very successful in papaya, a crop that in Hawaii was devastated by papaya ringspot virus. No natural resistance was available, which meant that classical breeding to combat the problem was not a possibility. A transgenic strategy was the only option, and overexpression of a transgene of the virus coat protein successfully interfered with viral

replication and provided resistance (Ferreira *et al*. 2002). Without the development of transgenic papaya cultivars, the papaya industry in Hawaii would have disappeared. Although consumer concerns, either real or perceived, combined with the high costs of de-regulation, have restricted the use of genetic modification in fresh food crops, virus-resistant papaya provides an example of the successful use of the technology, and consumer acceptance of the resulting product.

In cases where the role of a single plant gene (e.g. encoding an important structural or regulatory protein) can be identified to control a key trait, both transgenic and non-transgenic strategies are available to modify gene functionality. One non-transgenic strategy is known as TILLING (Targeting Induced Local Lesions IN Genomes), where a population of seeds or plants is chemically mutated at random, followed by high-throughput screening to identify individuals where the target gene is affected and where a desired trait has been improved. Although large populations are required for screening, individuals with reduced functionality of the encoded protein or even knockout in any non-essential gene can usually be obtained. The technology has been used successfully in melon to identify lines with a mutated *ACO1* gene and improved shelf life (Dahmani-Mardas *et al*. 2010).

11.8 - Concluding remarks

In the USA, the major proportion of maize and soybean production is from transgenic varieties produced for either insect resistance or herbicide resistance. These varieties have been consumed for more than 20 years now without any reported adverse effects. However, in other parts of the world there has been opposition to transgenic fresh food crops. Public concerns may moderate with time, as demands on crop productivity to feed a growing population increase, to cope with changing climatic conditions or pathogen pressures, or as fruit with distinct consumer benefits in flavour, eating quality or nutritional content are developed.

The virus-resistant papaya provides a dramatic example of how molecular techniques can enhance the properties of a crop in ways that can potentially help either productivity or postharvest handling and eating qualities. Political and ethical issues aside, wider use of genetically engineered plants could have a major impact on postharvest handling of many other horticultural products. Consumers will need to be well informed about changes resulting from conventional breeding and those resulting from genetic engineering or from mutations induced by chemical or irradiation treatment. There will also need to be improved physiological and biochemical knowledge about the postharvest responses of each species to be engineered.

Over the past century, fruit production and postharvest technology have been a powerful influence on progress in human societies and personal lifestyles. Very few people in ‘developed societies’ now grow their own fruit or vegetables; mass production has become much more efficient and wastage much lower; food quality has increased and people are better nourished; seasonal fruit are available year round; large amounts of product are distributed worldwide. Even cut flowers have become commodities of global trade instead of specimens from our own gardens, and in all cases postharvest technology has grown from process physiology. This area of plant science still offers exciting prospects for global horticulture, especially in tropical environments where new issues confront physiologists.

11.9 - Further reading and literature cited

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