8

PHYSIOLOGICAL DISORDERS

Physiological disorders refer to the breakdown of tissue that is not caused by either invasion by pathogens (disease-causing organisms) or by mechanical damage. They may develop in response to an adverse preharvest and/or postharvest environment, especially temperature, or to a nutritional deficiency during growth and development.

LOW TEMPERATURE DISORDERS

Storage of produce at low temperature is beneficial because the rates of respiration and of general metabolism are reduced (Chapter 4). Low storage temperatures do not, however, suppress all aspects of metabolism to the same extent. Some reactions are sensitive to low temperature and cease completely below a critical temperature. Several such cold-labile enzyme systems have been isolated from plant tissues. Decreasing temperature does not reduce the activity of other systems to the same extent as it does respiration. For these systems, this differential response leads to an accumulation of reaction products and possibly a shortage of reactants, while the converse occurs with cold-labile systems. The overall effect is that an imbalance in metabolism is created. If the imbalance becomes serious enough so that an essential substrate is not provided or toxic products accumulate, the cells will cease to function properly and will eventually lose their integrity and structure. These collapsed cells manifest themselves as areas of brown tissue in the produce. Metabolic disturbances occurring at subambient temperature are generally divided into two main groups: chilling injury and physiological disorders.

Chilling injury

Chilling injury is a disorder that has long been observed in plant tissues, especially those of tropical or subtropical origin. It results from the exposure of susceptible tissues to temperatures below about 15°C, although the critical temperature at which chilling injury symptoms are produced varies for different commodities. Chilling injury is a separate

phenomenon from freezing injury, which results from the freezing of the tissue and the formation of ice crystals at temperatures below the freezing point. A clear distinction can therefore be made between the causes of chilling and freezing injuries. Susceptibility to chilling injury and its manifestations vary widely among different commodities. In addition, commodities grown in different areas may behave differently, and varieties of the same crop can also behave quite differently in response to similar temperature conditions.

Table 8.1 summarises the physical symptoms of chilling injury and the lowest safe storage temperature for some fruits. A common chilling injury symptom is pitting of the skin, usually due to the collapse of the cells beneath the surface, and the pits are often discoloured. High rates of water loss from damaged areas may occur, which accentuates the extent of pitting. Browning of flesh tissues is also a common feature. Browning often first appears around the vascular (transport) strands in fruit, probably

TABLE 8.1 CHILLING INJURY SYMPTOMS OF SOME FRUITS

Produce	Lowest safe storage temperature (°C)	Symptoms
Avocado	5–12*	Pitting, browning of pulp and vascular strands
Banana	12	Brown streaking on skin
Cucumber	7	Dark coloured, water-soaked areas
Egg plant	7	Surface scald
Lemon	10	Pitting of flavedo, membrane staining, red blotches
Lime	7	Pitting
Mango	5–12	Dull skin, brown areas
Melon	7–10	Pitting, surface rots
Papaya	7–15	Pitting, water-soaked areas
Pineapple	6–15	Brown or black flesh
Tomato	10–12	Pitting, Alternaria rots

* A range of temperature indicates variability between cultivars in their susceptibility to chilling injury.

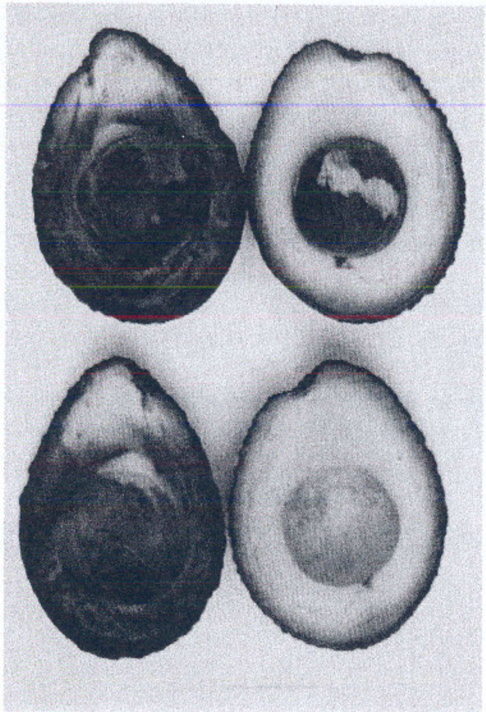

Figure 8.1
Chilling injury in avocado fruit appears as browning of the mesocarp due to the breakdown of cell compartmentalisation and the action of polyphenol oxidases to produce tannins (top). The fruit were stored in air for 21 days at 5°C and then ripened at 20°C for 5 days. The bottom fruit remained free of symptoms after ripening in air at 20°C following storage for 21 days at 5°C in sealed polyethylene bags that generated atmospheres typically 7 per cent carbon dioxide, 3 per cent oxygen and 90 per cent nitrogen.
SOURCE K.J. Scott and G.R. Chaplin (1978) Reduction of chilling injury in avocados stored in sealed polyethylene bags, *Trop. Agric. Trinidad* 55: 87–90. (With permission.)

as a result of the action of the enzyme polyphenol oxidase on phenolic compounds released from the vacuole after chilling, although this has not been proved in all cases. Figure 8.1 shows typical browning symptoms of avocado fruit affected by chilling injury. Fruit that has been picked immature will fail to ripen or will ripen unevenly or slowly after chilling. Degreening of citrus is slowed by even mild chilling. Water-soaking of leafy vegetables and some fruits such as papaya is also often observed. The symptoms of chilling injury normally occur while the produce is at low temperature, but sometimes will only appear when the produce is removed from the chilling temperature to a higher temperature. Deterioration may then be quite rapid, often within a few hours.

Chilling injury causes the release of metabolites, such as amino acids and sugars, and mineral salts from cells that together with the degradation of cell structure provide an excellent substrate for the growth of pathogenic organisms, especially fungi. These pathogens are often present as latent infections or may contaminate the produce either during harvest or postharvest during transport and marketing. For this reason, increased rotting is a common occurrence in tropical produce

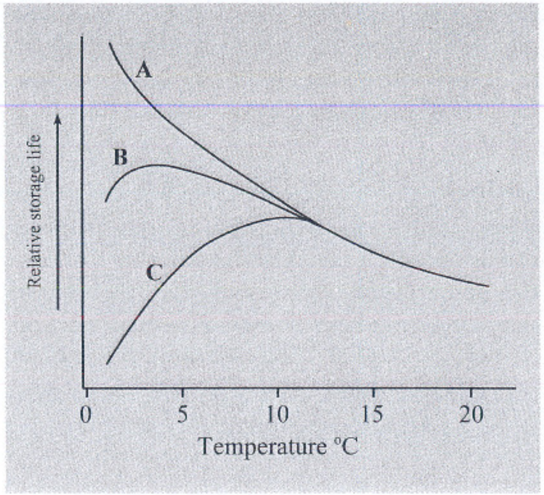

Figure 8.2
Storage life at various temperatures of produce with no (A), slight (B) or high (C) sensitivity to chilling injury.
SOURCE R.G. Tomkins, *The choice of conditions for the storage of fruits and vegetables*, East Malling Research Station, Ditton Laboratory Memoir no. 91. (With permission.)

after low temperature storage. Another consequence of chilling is the development of off-flavours or odours. The complex array of symptoms suggests that several factors are responsible for the development of chilling injury.

The temperatures quoted in Table 8.1 refer to the limiting or critical temperature below which some physical symptom of chilling injury will usually be observed. If the temperature is just below this critical temperature, relatively long exposure to the temperature will be required before injury is observed. Injury will generally appear more quickly and will be more severe the further the temperature is below the critical chilling temperature. Storage of the commodity may be possible for a useful period of time at temperatures slightly below the critical temperature where there is only a slight susceptibility to chilling injury. This relation between storage life at various temperatures and sensitivity to chilling is illustrated in Figure 8.2. Such a relationship also holds for the development of physiological disorders. These disorders are therefore subject to a time/temperature relationship.

The most obvious method for the control of chilling injury is to determine the critical temperature for its development in a particular fruit and then not expose the commodity to temperatures below that critical temperature. But exposure for only a short period to chilling temperatures with subsequent storage at higher temperatures may prevent the development of injury. This conditioning treatment has been found to be effective for preventing black heart in pineapple, woolliness in peach, and flesh browning in plum, but it is not known whether other produce will respond similarly. It has been claimed that modified atmosphere storage can reduce the extent of chilling in some produce. Also, maintenance of high relative humidity, both in storage at low temperature and after storage, may minimise pitting. More research is needed to confirm these claims.

Mechanism of chilling injury

The events leading to chilling injury can be separated into primary events, by which the plant cells sense the lowered temperature, and the long-term responses or secondary events that ultimately lead to the death of the cells. The primary events are more or less instantaneous and are reversible, at least for a period of time. The secondary events are eventually irreversible and are manifest as the various necrotic and other symptoms of chilling injury. This concept is illustrated in Figure 8.3.

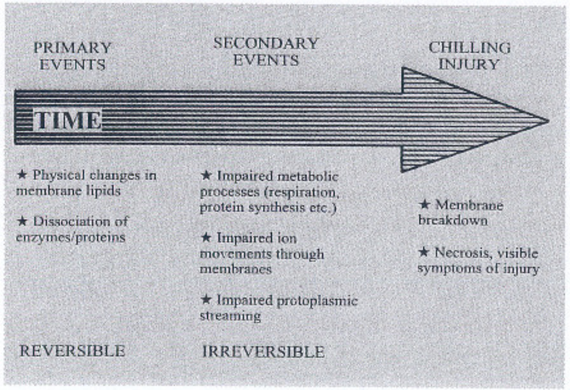

Figure 8.3
Time sequence of events leading to chilling injury
SOURCE G.R. Chaplin, personal communication.

The critical temperature below which chilling injury will occur is characteristic of the species of plant and the commodity from which it is derived. Highly chilling-sensitive plants, such as bananas and pineapples, have a relatively high critical temperature of around 12°C or higher. It has even been suggested that for some pineapple cultivars the critical temperature may be greater than 20°C. Chilling-insensitive plants such as apples and pears have much lower critical temperatures of around 0°C or below. Of course, storage below about -1°C is not possible for fresh produce because of freezing damage.

The two most likely causes of chilling sensitivity are:

- a low temperature-induced change in the physical properties of cell membranes due to changes in the physical state of membrane lipids (the so-called lipid hypothesis of chilling); and

- low temperature-induced dissociation of enzymes and other proteins into their structural subunits, resulting in changes in the kinetics of enzyme activity and changes in structural proteins such as tubulin.

The lipid hypothesis is supported by data obtained using a number of physical techniques, including differential scanning calorimetry, electron spin resonance spectrometry and fluorescence polarisation of molecular probes intercalated into plant cell membranes. Data obtained by these techniques show that there is a change in the physical properties of extracted membrane lipids at characteristic temperatures in the range 7–15°C. The characteristic temperatures were found to coincide with the critical temperatures below which particular plant tissues or fruits showed symptoms of chilling injury. Only a very small proportion (<10 per cent) of the membrane lipids undergo the physical change, which is probably a phase separation. A physical change in the membrane lipids with lowering of the temperature would cause changes in the properties of the membranes. For example, ion and metabolite movements would be affected as would activities of membrane-bound enzymes. These changes could, in turn, cause imbalances in metabolism, with eventual disruption of the various membranes leading to the breakdown of cellular compartmentalisation, death of the cells and the appearance of symptoms of chilling injury.

There is some evidence from studies of both animals and plants that several enzymes of cellular metabolism undergo dissociation at temperatures approaching 0°C. Some multimeric enzymes split into their component subunits, with a consequent loss of enzymic activity and change in some kinetic properties. Some enzymes of both respiratory and photosynthetic metabolism are affected. The consequences of such changes in the relative activities of some enzymes will be imbalances in metabolism that would eventually lead to the death of cells. The toxin hypothesis of chilling injury — in which there is an accumulation of toxic products of metabolism, such as acetaldehyde — could be explained by imbalances in metabolism. Structural proteins of the cell cytoskeleton, such as tubulin, are cold-labile and undergo dissociation at low temperatures. This could account for the effect of low temperature on protoplasmic streaming, which is especially sensitive in chilling-sensitive plants.

Further research is required to fully elucidate the mechanisms of chilling injury. If it should turn out that only a few proteins are involved in the synthesis of the key lipids and cold-labile enzymes of metabolism then it may be possible in future to genetically engineer these proteins to make plants less chilling-sensitive and therefore improve the low temperature storage of subtropical and tropical fruits and vegetables.

TABLE 8.2 SOME PHYSIOLOGICAL DISORDERS OF APPLES

Disorder	Symptoms
Superficial scald	Slightly sunken skin discolouration, may affect whole fruit
Sunburn scald	Brown to black colour on areas damaged by sunlight during growth
Senescent breakdown	Brown, mealy flesh; occurs with overmature, overstored fruit
Low temperature breakdown	Browning in cortex
Soft (or deep) scald	Soft, sunken, brown to black, sharply defined areas on the surface and extending a short distance into the flesh
Jonathan spot	Superficial spotting of lenticels; occurs at higher temperatures
Senescent blotch	Grey superficial blotches on overstored fruit
Core flush (brown core)	Browning within core line
Water core	Translucent areas in flesh; may brown in storage
Brown heart	Sharply defined brown areas in flesh; may develop cavities

Physiological disorders

Physiological disorders mainly affect deciduous tree fruits, such as apples, pears and stone fruits, and most citrus fruits. Most of these disorders affect discrete areas of tissue, whether the produce be fruits, vegetables or ornamentals. (Figure 8.4 in the colour plate section). Some disorders may affect the skin of the produce but leave the underlying flesh intact; others affect only certain areas of the flesh or the core region.

With most disorders, the metabolic events leading to a manifestation of the symptoms are not fully understood and are not understood at all for many disorders. The discovery of most disorders could be considered 'non-scientific'. In the past, cool store operators or shipping agents have held fruit at low temperatures and found that they developed a variety of browning conditions. These conditions were given descriptive names as there was no other way of classifying the disorders. These names are still the only classification used. The apple has been studied more intensely than other commodities and also appears to have the greatest variety of physiological disorders. Table 8.2 lists some of these disorders and their symptoms. These disorders require low temperature storage, usually at less than 5°C, for the development of symptoms. Each disorder is therefore presumed to be derived by a different metabolic route, although this may not prove to be true when their biochemistry is elucidated. Disorders in other fruits are shown in Table 8.3. When more research effort is devoted to commodities other than the apple, the list of physiological disorders will undoubtedly increase. There is no reason to believe that the apple should be more prone to develop disorders than other commodities.

TABLE 8.3 SOME PHYSIOLOGICAL DISORDERS OF FRUITS OTHER THAN APPLES

Produce	Disorder	Symptoms
Pear	Core breakdown	Brown, mushy core in overstored fruit
	Neck breakdown, vascular breakdown	Brown to black discolouration of vascular tissue connecting stem to core
	Superficial scald	Grey to brown skin speckles; occurs early in storage
	Overstorage scald	Brown areas on skin in overstored fruit
	Brown heart	Same as for apple
Grape	Storage scald	Brown skin discolouration of white grape varieties
Citrus	Storage spot	Brown sunken spots on surfaces
	Cold scald	Superficial grey to brown patches
	Flavocellosis	Bleaching of rind; susceptible to fungal attack
	Stem-end browning	Browning of shrivelled areas around stem-end
Peach	Woolliness	Red to brown, dry areas in flesh
Plum	Cold storage	Brown, gelatinous areas on skin and flesh breakdown

Early studies of disorders found that, although a particular variety may be susceptible to a certain disorder, not all fruits develop the disorder.

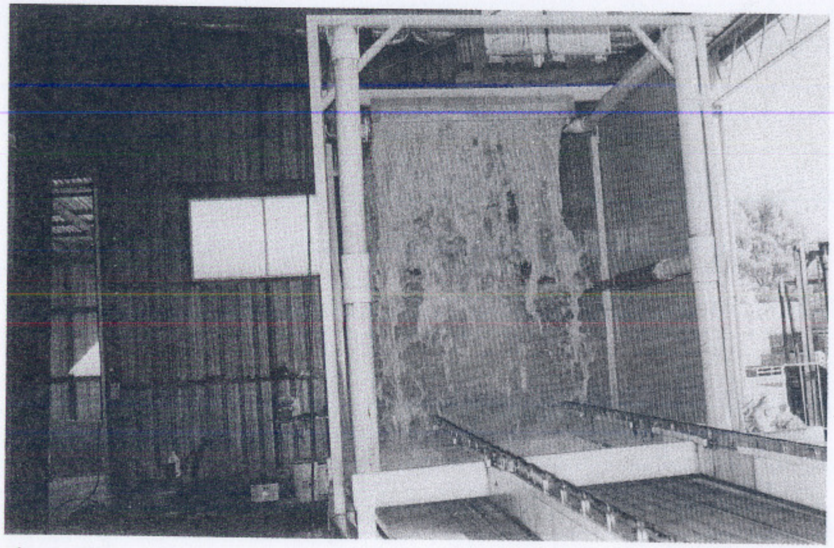

Figure 8.5
Equipment for the flood application of diphenylamine to bulk boxes of apples and pears before cool storage. Boxes stacked two high are conveyed through the applicator. Supplementary jets on the side walls apply solution to the bottom boxes. A fungicide and a low concentration of calcium chloride may be added to the solution.

Susceptibility to disorders was shown to depend on a number of factors, such as maturity at harvest, cultural practices, climate during the growing season, produce size, and harvesting practices. The risk of a fruit developing a particular disorder can therefore be minimised by identifying susceptible fruits and not storing them for long periods. However, the market often has requirements that result in a preference for the type of fruit that is highly susceptible to a disorder. For example, consumers prefer large Jonathan apples with intense red colouration, but such fruits are susceptible to low temperature breakdown. Thus methods had to be developed to enable susceptible produce to be stored to meet consumer demand.

Various systems of temperature modulation have been developed to minimise the development of some disorders. Lowering the temperature in steps from 3°C down to 0°C in the first month of storage effectively minimises the development of low temperature breakdown and soft scald in apples. Low temperature breakdown of apples and stone fruits can also be reduced by raising the temperature to about 20°C for a few days in the middle of the storage period and then returning the fruit to low temperature. Such methods have not been widely adopted in commercial practice because of the logistical problems of having a whole room of produce ready to treat at one time and the difficulty of rapidly changing the temperature of a room full of fruit. A further problem is that any increase in the storage temperature will increase respiration, and thus shorten the storage life of produce held in the same room that is not susceptible to the disorder.

Controlled atmosphere storage can completely prevent Jonathan spot when as little as 2 per cent carbon dioxide is present. The incidence of core flush and various forms of flesh breakdown in apples is also often reduced in controlled atmosphere storage. However, in some instances the level of breakdown has reportedly increased in controlled atmosphere storage. This increase has been attributed to factors associated with controlled atmosphere storage other than the composition of the atmosphere. The enclosed room that is required for controlled atmosphere storage results in high humidity, restricted ventilation rates, and the accumulation of fruit volatiles in the atmosphere. These conditions are conducive to the development of apple breakdown. Superficial scald is another disorder that is enhanced in controlled atmosphere storage by these conditions (see below). Controlled atmosphere storage can also create new disorders if the produce is exposed to very high levels of carbon dioxide or low levels of oxygen for prolonged periods. The critical level of carbon dioxide that induces brown heart in apples and pears varies among different varieties and may be as low as 1 per cent. In addition to browning of the tissue, low oxygen injury is characterised by the development of alcoholic off-flavours produced by anaerobic metabolism.

The ultimate method for the prevention of a disorder is to understand the metabolic sequence that leads to the development of the disorder and then to prevent that metabolism from occurring. Chemical control is an obvious measure to prevent the development of disorders, but it is not necessarily the sole method possible. Storage disorders may also be minimised by physical and cultural treatments and by breeding less susceptible cultivars.

Skin blemishes are generally the more serious problem as even quite small skin marks render the fruit unacceptable in many markets. Internal defects can be tolerated to a greater extent as the consumer buys on visual inspection of the external appearance, and even upon consumption may never be aware of a small amount of internal browning. The skin disorders bitter pit and superficial scald of apple have received considerable attention, and control measures have been developed for both disorders (see Chapter 11).

Much is known about the metabolism relating to superficial scald. Early studies (before 1930) led to the hypothesis that superficial scald was caused by a toxic, volatile organic compound that accumulated in the apple during cool storage. In the 1960s, workers in Australia isolated α-farnesene, a C15 sesquiterpene hydrocarbon from susceptible apple varieties, and suggested that it was the precursor compound involved in superficial scald. Being a fat-soluble compound, it accumulates in the lipid fraction on the skin. Oxidation products of α-farnesene have been claimed to lead to the collapse of the cells and to tissue browning. Control of the disorder is achieved commercially by the application of various synthetic antioxidants, such as diphenylamine and ethoxyquin, which protect α-farnesene against oxidation (Figure 8.5). Chilling injury is usually thought to develop along a different metabolic route to that for superficial scald, but α-farnesene has been shown to accumulate in bananas during the development of chilling symptoms, suggesting that there may be a metabolic similarity in the two disorders. Recent studies in Australia has shown that superficial scald can be controlled by ethanol vapour in the atmosphere surrounding Granny Smith apples. While there may be some resistance to the use of ethanol on religious or liquor-related grounds, it raises the question of whether the use of ethanol vapour is a natural substance as it is metabolised, albeit in small quantities, by apples.

If satisfactory control methods are not available, the ultimate method of avoiding any physiological disorder is to hold susceptible fruits at a temperature high enough to prevent the disorder from being a problem. This temperature is usually 3–5°C, but is sometimes greater that 5°C. This partially negates the idea of using low temperature to minimise respiration, but it is better to market overmature produce than to have a disfiguring disorder present.

MINERAL DEFICIENCY DISORDERS

Fruit and vegetables often show various browning symptoms that have been attributed to deficiencies in some mineral constituents of the produce. These disorders are prevented by the addition of the specified mineral, either during growth or postharvest, even though the actual role of the mineral in preventing the disorder has not been established for most disorders. Plants require a balanced mineral intake for proper development, so a deficiency in any essential mineral will lead to poor development of the plant as a whole. It can be said that the condition is a physiological disorder if the fruiting organ or actual 'vegetable' portion is affected rather than the whole plant.

TABLE 8.4 CALCIUM-RELATED DISORDERS OF FRUIT AND VEGETABLES

Produce	Disorder
Apple	Bitter pit, lenticel blotch, cork spot, lenticel breakdown, cracking, low temperature breakdown, internal breakdown, senescent breakdown, Jonathan spot and water core
Avocado	End spot
Bean	Hypocotyl necrosis
Brussels sprout	Internal browning
Cabbage	Internal tipburn
Chinese cabbage	Internal tipburn
Carrot	Cavity spot, cracking
Celery	Blackheart
Cherry	Cracking
Chicory	Blackheart, tipburn
Escarole	Brownheart, tipburn
Lettuce	Tipburn
Mango	Soft nose
Parsnip	Cavity spot
Pear	Cork spot
Pepper	Blossom-end rot
Potato	Sprout failure, tipburn
Strawberry	Leaf tipburn
Tomato	Blossom-end rot, blackseed, cracking
Watermelon	Blossom-end rot

Calcium

Calcium has been associated with more deficiency disorders than other minerals, and some examples are shown in Table 8.4. Some of these disorders, such as blossom-end rot of tomatoes, can be readily eliminated by the application of calcium salts as a preharvest spray while for others, such as bitter pit of apples, only partial control is obtained. However, this variability in the degree of control is probably related to the amount of calcium taken up by the fruit. For example, the use of postharvest dipping at subatmospheric pressures, which markedly increases the uptake of calcium, usually results in the total elimination of bitter pit.

A substantial amount of the added calcium binds with pectic substances in the middle lamella, and with membranes generally, and may prevent disorders by strengthening structural components of the cell without alleviating the original causes of the disorder. The strengthening of cell components may prevent or delay the loss of cell compartmentalisation and the enzyme reactions that cause browning symptoms.

Calcium has been found to be relocated in apples during storage. This raises the possibility that a local deficiency can be created in one part of the tissue during storage, resulting in a manifestation of a physiological disorder in that region. Calcium has been shown to affect the activity of many enzyme systems and metabolic sequences in plant tissues. The addition of calcium to intact fruit or fruit slices generally suppresses respiration, but the response is concentration-dependent. The activities of isolated pectic enzymes, pectinmethylesterase (PME), exopolygalacturonase (exo PG) and endopolygalacturonase (endo PG), have shown differential responses to calcium concentration. The activity of PME is initially increased by increasing concentrations of calcium but is inhibited at higher concentrations. The large form of endo PG (PG1) extracted from tomato fruit is slightly stimulated by concentrations of calcium that inhibit the smaller endo PG forms of the enzyme. Calcium is needed for the activity of exo PG, kinases and a range of other enzymes. The ability of calcium to regulate these various systems has led to speculation that calcium may have a role in the initiation of the normal fruit ripening process. It is also possible that calcium prevents or delays the appearance of some physiological disorders by maintaining normal metabolism.

Other minerals

Boron deficiency in apples leads to a condition known as internal cork. This condition is marked by pitting of the flesh and is often indistinguishable from bitter pit. The differences between the two disorders are that internal cork is prevented by the application of boron sprays and develops only on the tree, while bitter pit responds to calcium treatment and can develop postharvest.

The major mineral in plants is potassium, and both high and low levels of potassium have been associated with abnormal metabolism. High levels of potassium have been associated with the development of bitter pit in apple so that both high potassium and low calcium levels are correlated with pit development. Low potassium is associated with changes in the ripening tomato and delays the development of a full red colour by inhibiting lycopene biosynthesis.

There may be roles for other minerals in the development of other disorders. Injections of copper, iron and cobalt have induced symptoms similar to low temperature breakdown and superficial scald in apples, but this does not necessarily mean they have a role in the development of the natural disorder. Heavy metals, especially copper, act as catalysts for the enzymic systems that lead to enzymic browning, the browning of cut or damaged tissues that are exposed to air. The levels of these metals are important in processed fruit and vegetables, whether they are derived from the produce or from metal impurities that are included during processing.

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