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Hormonal Changes During Postharvest

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I. INTRODUCTION

The role of phytohormones in the growth and development of plants is as fundamental as that of much better known biological systems, such as the citric acid or Krebs cycle, for which precise reactions are well known and demonstrated, while those for most plant hormones are still not clear. Most evidence indicates that generally more than one hormone is involved in those physiological responses attributed to hormonal influence, and the effects observed are due more to the hormonal balance than to the activity of any one hormone. On the other hand, sensitivity to growth substances may be the controlling factor rather than the hormone level itself (Trewavas, 1992).

Even less is known about the role of hormones in the postharvest physiology of cultivated vegetable plants, most of which are removed suddenly from the natural environment and sometimes mutilated in the harvest process. Fresh vegetables are living tissues that are subject to continual change after harvest. The potential for change in physiological processes in these edible plant tissues is great, as they lose their normal supply of water, minerals, and organic molecules, including hormones, on removal from the parent plant, with translocation from other parts of the plant being severed. Transpiration is active, although there is little new photosynthesis, and the tissues can transform many of the constituents already present.

While some postharvest changes are desirable, most, from the consumer's standpoint, are not. Because of the well-recognized benefits in human nutrition attributed to adequate consumption of fresh vegetables, there has been increased interest in improving commercial storage systems for vegetables in the fresh state. Postharvest changes cannot be prevented but can be slowed down within certain limits, often by holding in stressful environments—including low temperatures, artificial atmospheres, or both in combina-

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tion— to reduce respiration rates. Respiratory enzyme systems, for example, can be controlled fairly easily by temperature adjustment alone, hence preserving nutritional quality, which accounts for the widespread use of cold storage. It may be that endogenous hormonal activity can be influenced either externally by environmental conditions or internally by breeding technology to improve both storage duration and edible quality.

The few research reports available on stored plant materials have suggested that the usual endogenous plant hormones continue to function and appear to control physiological events. This conclusion is apparent from correlative evidence of hormonal balances and easily observed physiological events in detached plant organs, such as rest, dormancy, and compulsive regrowth. All the commonly identified phytohormones appear to be present—i.e. auxins, gibberellins, cytokinins, abscisic acid, and ethylene, as well as polyamines and jasmonic acid. Their conjugated forms are numerous and act to sequester or inactivate free hormones and to present a reversible system for regulating their levels (Martin et al., 1995). A simple way that hormonal responses are regulated is by controlling the amount of free hormone that is available, release from conjugates in inactive pools activating signal transduction. The consensus seems to be that the balance of endogenous hormones may be the most important criteria (Wareing and Phillips, 1981).

However, the great diversity of plant materials or plant parts that are used as foods is a complication. The kind and extent of physiological activity in detached plant parts can largely determine their storage longevity. The stage of development—whether growth, maturation, or senescence—influences the rate of metabolic activity. Leaves, stems, petioles, etc., are physiologically primed for senescence. Fruit ripening, associated with optimal eating quality, includes the final stages of maturation and can be regarded as a specialized senescence phenomenon. There are numerous plant materials grown as annuals and considered to be vegetables for commercial or legal purposes but which are classified as fruits botanically, such as the fruit vegetables, tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.). However, the question is still raised as to whether tomato is a fruit or a vegetable (Heard, 1996)! The cucurbits are consumed in both the immature and the fully mature or ripe states, such as fresh cucumbers (*Cucumis sativus* L.) in salads and melons (*Cucumis melo* L.) as desserts. Most of the legume fruits are consumed in the immature state or as completely dried seeds, and their hormonal physiology is documented in both forms, especially that of garden peas (*Pisum sativum* L.) and beans (*Phaseolus vulgaris* L.), favorite laboratory research plants. On the other hand, seeds, tubers (e.g., potato, *Solanum tuberosum* L.), bulbs (e.g., onion, *Allium cepa* L.), and fleshy roots (e.g., carrot, *Daucus carota* L.) are morphologically and physiologically adapted to maintain the tissue in a dormant state in which growth is temporarily suspended due to unfavorable conditions—i.e., imposed dormancy, which is the meaning of the term *dormancy* in this chapter. Rest or true, innate dormancy occurs when growth cannot take place even under favorable conditions due to the condition of the plant material itself. Metabolic activity is depressed but not halted. Regrowth is triggered in the spring, probably by a change in the hormone balance. Most vegetables stored over extended periods in the fresh state are biennials that break rest and eventually sprout during storage, terminating their usefulness for commercial purposes. For these reasons more research is now being devoted to the role of hormones in the rest and dormancy phenomena.

The Cruciferae family contains many edible species from which specialized cultivars have been developed and in which the leafy parts, the flower, or the swollen stem or root are used as food. All of these cultivars exhibit some degree of natural storability, some for only a few days to a few weeks at most due to their rapid loss of edible quality, such

as the inflorescence kales (*Brassica oleracea* L., Acephela group), broccoli and cauliflower (both *B. oleracea* L., Botrytis group) and Brussels sprouts (*B. oleracea* L. Gemmifera group), while cabbage (*B. oleracea* L., Capitata group) stores well over many months with some indications of rest even though it is a leafy crop. White cabbage is the most important crop among the Cruciferae that is stored in the fresh state. Like the stored potato, it is an important source of ascorbic acid. However, there is also considerable diversity in inherent keeping quality among cultivars. In seeking cultivars with improved storage quality, the general pattern of hormonal behavior has been investigated under the stress of extended low temperature, either with or without controlled atmosphere (CA).

Botanically, the potato of commerce is a tuber, a modified swollen rhizome or underground stem that accumulates stored reserves, and is capable of producing a new plant asexually. Because it will overwinter in the soil or under proper storage conditions, it shows the principal phases of postharvest behavior for biennials, i.e., innate dormancy or rest, dormancy, and compulsive regrowth. These phases again are regulated by a system of hormonal balances. This is the case with most commercially stored root crops, which, as mentioned above, are biennial plants and have genetic capability for survival under adverse ambient conditions such as prolonged low temperatures. Their general behavior during storage indicates that they also have a hormone-controlled rest period, a dormant period, and a compulsive regrowth period, not yet demonstrated in detail. As with potato, therefore, it can be expected that similar, though temperature-modified, physiological events also occur with stored root crops at dormancy break, with concomitant sprouting or rooting.

Endogenous hormones increase after wounding (Pena-Cortez and Willmitzer, 1995), and exogenous applications can amplify their activities and affect the metabolic activity of cut slices. The wound reactions and aging of cut slices from storage organs, such as potato and sugar beet (*Beta vulgaris* L. ssp. *vulgaris*), have been much studied and include the induction of mRNA synthesis, an increase in nucleolar size and protein synthesis, along with RNase activity. Hormones may work together to stimulate the synthetic activity necessary to "heal a wound."

Exogenous methods have been widely used on plants or their excised parts to study phytohormone action and extrapolate to endogenous hormonal responses. In many cases, there are problems with this approach, often because of difficulties in uptake and distribution into bulky tissues such as fruits or tubers. Such size limitations have often been overcome by the use of cut slices and tissue discs, but this is dealing with wounded tissue with numerous wound effects. Immature flowers grown under standard culture conditions provide a parthenocarpic system to study the effect of phytohormones on fruit ripening (Cohen, 1996).

Genetic approaches are helping to explain how plants perceive and respond to signaling molecules such as phytohormones. Recent advances in molecular biology have opened new windows for the understanding of fruit ripening, for example (see Chapters 10 and 18). Mutations that disrupt a particular phytohormone response have been isolated, along with the introduction of antisense mRNA and its resulting downregulation, which has allowed the construction of mutant genes. A number of phytohormone biosynthetic genes have been cloned in recent years, particularly those involved in the synthesis of ethylene and gibberellins, and also abscisic acid (Chasan, 1995). However, some of these gene product phenotypes are deficient in hormone perception and response rather than in their synthesis, as in the abscisic acid (ABA)–insensitive mutants (*abi*), and, with these genes available, it should be possible to start understanding their expression.

II. ETHYLENE

Ethylene effects are wide and include fruit ripening, flower and leaf senescence, and also leaf abscission, so that ethylene has an obvious result on leafy vegetables. As an example, the commercial storage of cabbage with apples (*Malus* × *domestica* Borkh.) can be disastrous (McKeon et al., 1978). Storage of fruits and vegetables can therefore be prolonged by ethylene removal. This can be done by using ethylene scrubbers—e.g., alkaline potassium permanganate on a silica carrier—flushing with nitrogen gas, or by hypobaric storage. A reactor designed to convert volatile hydrocarbons to carbon dioxide and water by a combination of surface chemistry, using a catalyst bed of zirconia-titania particles, and ultraviolet (UV) radiation was tested under conditions relevant to horticulture and demonstrated ethylene photocatalysis (Cushman et al., 1996). A reactor similar in design has been used for plant growth in space.

Wounding during harvest and transport affects storage, since transient ethylene production is also triggered by stress or injury, and many of the bacterial pathogens have the capacity to synthesize ethylene. On the other hand, ethylene also has a synergistic effect with methyl jasmonate on activating the gene expression of pathogenesis-related proteins, one of the plant defense responses to invasion (Xu et al., 1994; see Chapter 24).

Genetic manipulation of several cloned ethylene synthesis genes has led to understanding of the synthesis of ethylene, but biochemical mechanisms for its perception and response have not yet been so fruitful. Genetic engineering of the signal transducers for ethylene, ETR1 (ethylene-insensitive mutants) and CTR1 (constitutive triple-response or ethylene-overproducing mutants), isolated from *Arabidopsis* but homologous to genes isolated from tomato, may help to clarify the ethylene receptor (Ecker, 1995). Phosphorylation with protein kinases may play a key role in the pathway for ethylene signal transduction (Bleecker and Schaller, 1996). The product of the ETR1 gene acts earliest in the signal cascade, and an interesting unproved possibility is that ETR1 is an ethylene receptor that acts through CTR1 to regulate a mitogen-activated protein kinase cascade (Chasan, 1995). Ethylene-regulatory *cis* regions are identified in several ethylene-inducible genes, and several proteins that specifically react with these *cis* regulatory regions are identified and cloned (Ohme-Takagi and Shinshi, 1995). For an in-depth discussion of ethylene biosynthesis and action, the reader is referred to Chapter 10 of this volume.

A. Vegetable Fruits

In many fruits, ripening is accompanied by an increase in respiration termed the *climacteric*, and there is a pronounced increase in the production of ethylene just before the increase in respiration in climacteric fruit. Ethylene plays a significant role in the rather dramatic integrated sequence of physiological and biochemical changes that occur with the climacteric and ripening, and promotes the loss of chlorophyll. These changes include softening, color change, and the accumulation of sugars (or lipids, as in avocado, *Persea americana* Mill.) and aromatics, along with a decline in organic acids, catalyzed by specific enzymes (Tucker and Grierson, 1987). Ethylene is commonly referred to as the ripening hormone and has a cascade effect in climacteric fruit, leading to the "one rotten apple in the barrel" expression.

Some of the ripening changes, such as softening and carotenoid synthesis, can be separated from the respiration climacteric experimentally (Tucker and Grierson, 1987). However, inhibition of ethylene synthesis or even perception inhibits ripening (Hobson and Grierson, 1993). There is a large variation between species in rates of ethylene produc-

tion during the climacteric and in some cases, such as tomato, ripening can be promoted by simple substances such as galactose or N-glycans (Priem and Gross, 1992).

Nonclimacteric fruits are those that do not normally show a climacteric rise but rather have a simple, gradual decline in respiration, a slow drift down throughout maturation into senescence. Exogenous ethylene application can initiate a transient respiration response even in nonclimacteric fruits such as pepper, but for this continued application is necessary. The main difference between climacteric and nonclimacteric fruits lies in their ability to produce ethylene autocatalytically in response to threshold levels of ethylene. Exogenous ethylene application, in the form of ethephon (2-chloroethylphosphonic acid) or "liquid ethylene," is registered as a harvest aid to promote ripening in tomato, which is one of the few vegetables to which ethylene is applied commercially to influence the rate of ripening. To facilitate shipment for commercial purposes, fruit are frequently harvested at the "mature green" (MG) stage, when they can be expected to ripen completely either off or on the mother plant, and then ripened by ethylene application. There is difficulty in harvesting tomatoes commercially at the precise MG state, since tomato ripening starts in the interior with gel formation in the locule and placenta before the pericarp (Brecht, 1987), so that it is difficult to judge the MG condition in the field. This, together with the fact that immature fruit respond to exogenous ethylene but do not undergo normal ripening, may account for many consumer complaints about the poor edibility of winter-shipped tomatoes. Ripening responses to commercial-type ethylene exposures, made at the MG stage in a comparative study involving several advanced tomato breeding lines and the control cultivar Homestead, suggest that response to ethylene ripening procedures should be one of the criteria for judging cultivar acceptability for the fresh market (Wells et al., 1978).

Parts of the ethylene synthetic pathway have been established using vegetable fruit tissue, from methionine via *S*-adenosyl methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (see Chapter 10):

$$\text{met} \rightarrow \text{SAM} \rightarrow \text{ACC} \rightarrow \text{ethylene}$$

The inside parts of freshly harvested ripening tomato fruit (septa, pulp, and seeds) have higher levels of endogenous ACC and ACC synthase (ACS) than does the outer pericarp. At the preclimacteric, MG stage in tomato fruit, both ACS and ACC oxidase (ACO) activities are low, as well as ACC content, but they increase markedly on ripening following the breaker stage. Addition of exogenous ACC to many vegetative tissues results in greatly increased ethylene production (Cameron et al., 1979) because of low ACS activity, but this is not the case with many preclimacteric fruits, so that here ACO is restricted too. ACO is present in most tissues of higher plants except for unripe fruits. Preclimacteric tomato fruit have low levels of ACO, and exogenous ethylene treatment increases the capability of the fruit to convert ACC to ethylene (Kende, 1993)—i.e., it increases ACO activity before ACS activity. The level of ACO increases markedly during fruit ripening and effectively regulates ethylene production. However, once the fruit becomes overripe or postclimacteric, ACC can accumulate considerably, probably because of ACO inactivation.

An analog of rhizobitoxine, aminoethoxyvinylglycine (AVG), known to block the conversion of SAM to ACC, is also effective in inhibiting ethylene synthesis in slices of green tomatoes but relatively ineffective in pink and red tomato fruit (Baker et al., 1978). However, the isolated enzyme ACS from pink and red fruit is sensitive to low levels of AVG, and the ineffectiveness of AVG with fruit tissue may simply reflect relatively high

endogenous levels of ACC at the pink and red stages of fruit development (Kende, 1993).

Fruit tissue can also conjugate ACC to *N*-malonyl-ACC (MACC). In tomato pericarp, activity of ACC *N*-malonyltransferase mirrors the developmental pattern of ethylene evolution by the whole fruit, increasing dramatically with the onset of ripening, and again declining in the ripe fruit (Martin and Saftner, 1995). Activity in the seed reaches a plateau well after the ethylene climacteric peak has declined in the fruit. Under normal physiological conditions, the malonylation is irreversible, but the reaction is possible, as shown in watercress (*Nasturtium officinale* R. Br.) stems (Kende, 1993). A new conjugate is also described in crude extracts of tomato fruit, 1-(g-L-glutamylamino)-cyclopropane-1-carboxylic acid, or GACC (Martin et al., 1995).

The rate of protein synthesis increases during the early stages of ripening in several climacteric fruits, reflecting an increase in protein turnover and also de novo synthesis of ripening-specific enzymes (Tucker and Grierson, 1987). These include cell wall–degrading enzymes that influence fruit softening. Changes such as softening, color formation, and enzyme activities of cellulase and polygalacturonase (PG) are accelerated by ethephon treatment (Babbitt et al., 1973). PG is absent from green tomato pericarp tissue and increases progressively during ripening, along with acid invertase. PG does not appear until the first appearance of ethylene in tomato fruit, neither does the respiratory climacteric nor the increase in polysomes and cytoplasmic mRNA take place. Ethylene, ACS, and ACO induced by wounding in winter squash (*Cucurbita maxima* Duch.) also induced phenylalanine ammonia lyase (PAL) and peroxidase activity, which resulted in lignification in the first two cell layers as a defense mechanism. This was prevented by norbornadiene, an inhibitor of ethylene reception (Hyodo et al., 1993).

Several nonripening tomato mutants are known, which have aided the investigation of ripening and again have brought into question the role of ethylene as the ripening hormone, for example *Nr*—never ripe; *rin*—ripening inhibitor; and *nor*—nonripening. Neither *rin* nor *nor* fruit display a climacteric rise in CO_2 or ethylene evolution, and they fail to ripen except for seed maturation. Their chlorophyll content remains high and they have reduced lycopene levels, with extremely low PG activity. Exogenous ethylene applications have little effect in inducing proper ripening but will bring about a temporary stimulation of CO_2 evolution, and both exogenous ethephon and light increase some red color development in the mutant fruits, with enhanced beta-carotene and lycopene levels (Buescher and Doherty, 1978). However, the capability to produce ethylene is not lacking, because wounding the fruit causes an increase in both CO_2 and ethylene production (Adato and McGlasson, 1977). Both ACC and ACS were present in *rin*, but at lower levels than in wild-type fruit of comparable age (Theologis, 1992). The *Nr* mutation has been shown to affect ethylene perception rather than synthesis, and *ETR1*-homologous genes isolated from tomato indicate that one is very tightly linked to the *Nr* gene (Wilkinson et al., 1995). Further, *rin* and *nor* may also affect ethylene sensitivity, specifically during fruit ripening (Ecker, 1995). There is also the Alcobaca tomato mutant (with the recessive allele *alc*), which has fruit that ripen partially and have a long shelf life (Mutschler, 1984). Two wild tomato species that ripen on the vine but remain green show ethylene production correlated with fruit softening, while two others have external ripening changes that are not correlated with ethylene production (Grumet et al., 1981). Ripening may thus be determined by changes in sensitivity to ethylene rather than by the amount produced.

Ethylene production is stimulated by chilling temperatures of 0 to 15°C in a number

of chilling-sensitive fruit vegetables. This can occur in preclimacteric fruits, which do not usually produce significant amounts of ethylene. Increased ethylene production in chilled immature cucumber is due to increased capacity to make ACC, but the increase is not seen until subsequent warming. The increase in ACS activity during the warming period is inhibited by cycloheximide treatment but not by cordycepin or α-amanitin. This suggests stimulated production of mRNA coding for ACS during chilling (Wang and Adams, 1982)—i.e., mRNA is transcribed during the chilling stage but translation is not completed until transfer to warmer temperatures. However, in both MG tomato fruit (Brown, 1990) and Honey Dew melon, whether of minimum horticultural maturity or ripening-initiated (Lipton and Wang, 1987), ACC accumulates during the chilling period without waiting for subsequent warming. Thus, not all sensitive fruits seem to respond in the same way to chilling, nor are all fruits likely to show exactly the same ripening control. Prolonged chilling exposure results in a reduction of ACO activity (Abeles et al., 1992). Chilling of fruit vegetables does not usually exceed the few days required for transportation to market in most commercial conditions because they are not typically stored. The ethylene generated upon warming probably is responsible for the chlorophyll loss and pitting found in chilled cucumbers.

B. Underground Storage Organs

Onion bulbs apparently produce some ethylene during storage, especially at the end of dormancy (Abdel-Rahman and Isenberg, 1974). However, applied ethylene using injections of ethephon had little effect on the length of dormancy and very minor effects on rooting and sprouting. Later experiments found that ethephon treatment reduced sprouting in storage in two different cultivars (Thomas and Rankin, 1982). The combination injection of ethephon and abscisic acid partially reduced the latter's effect on length of dormancy but not on any other parameter measured.

A specific effect of ethylene in stored carrots is the development of bitter flavor due to ethylene-induced isocoumarin formation (Sarkar and Phan, 1979). This isocoumarin synthesis and the increased respiration induced by ethylene in carrots were influenced by their physiological state, wounding, and the O_2 level (Lafuente et al., 1996).

Ethylene effects on bulky storage organ slices are important because of possible wound ethylene production after harvest. Treating fresh slices with ethylene results in a sharp rise in respiration, especially in the presence of O_2 rather than air, and the respiration response to cyanide (CN) can be changed. Fresh potato and Jerusalem artichoke (*Helianthus tuberosus* L.) slices are CN-sensitive, but they are CN resistant after ethylene treatment—using the alternative path of respiration. This is also the case with turnip (*Brassica rapa* L., Rapifera group) and rutabaga (*Brassica napus* L., Napobrassica group). Another group yields CN-resistant slices to begin with, as in carrot and parsnip (*Pastinaca sativa* L.). Other changes resulting from ethylene treatment include increased numbers of polysomes and changes in gene expression, reflected in induced mRNA levels. However, the ethylene-stimulated changes in mRNA levels for carrot slices are not necessarily correlated with induced respiration enhancement (Nichols and Laties, 1985). The wound reactions and aging of cut slices from potato tuber include the induction of mRNA synthesis, and an increase in nucleolar size, protein synthesis, and RNase activity. Inactive ribosomes from resting potato tubers can be activated by added mRNA from polysomes of wounded potato.

C. Leafy Crops

Quality loss is accentuated by ethylene, which can induce degreening and leaf abscission to the point of making produce unmarketable. Addition of exogenous ACC to many vegetative tissues results in greatly increased ethylene production. Watercress stems were used to show that the reaction reversing the malonylation or conjugation of ACC to MACC is possible, though not usual under normal physiological conditions (Kende, 1993).

The storage life of broccoli and Brussels sprouts can be shortened by ethylene applications. Postharvest ethylene effects are quite clear-cut—i.e., yellowing in the chlorophyll-containing sepals of the broccoli florets and leaf abscission and leaf yellowing in Brussels sprouts. Transcripts of mRNAs encoded by cDNA clones of two ACO genes from broccoli florets increase markedly in whole florets after harvest, when senescence is initiated (Pogson et al., 1995). Treatment of harvested broccoli with AVG, the inhibitor of ACS, reduces ethylene production and respiration and retards yellowing and senescence at 20°C, resulting in green color retention and compactness and salable condition after 3 days (Wang, 1977). Similarly, treatment with silver nitrate, an inhibitor of ethylene action rather than synthesis, results in maintenance of dark green color in broccoli with tight florets; but decay takes place and such treatment is not a practical solution for vegetables (Ludford and Isenberg, 1987).

In modified atmospheres, it is thought to be the accumulation of respiratory CO_2 and its competitive inhibition of ethylene action, rather than O_2 depletion, that is the main factor in controlling quality in broccoli inflorescences (Aharoni et al., 1985). On the other hand, low O_2 atmosphere (1%) with 0% CO_2 during storage of Chinese cabbage (*Brassica rapa* L., Pekinensis group) suppresses the effect of applied ethylene (100 μL L^{-1}) on leaf abscission at 10°C (Wang, 1985).

Most storage-type cabbage keeps fairly well for several months if it is harvested during low field temperatures and stored with adequate ventilation of cold winter air. This has been the traditional method for common storage, but the product quality declines rapidly as the termination of rest approaches, the cabbage becoming bland in flavor, bleached, and tough. Ethylene is a contributing factor in the rapid decline often experienced with common-stored cabbage. Cabbage can be satisfactorily stored at 2°C under CA conditions of 2.5% O_2 plus 5% CO_2 with ethylene removal over longer periods up to 5 or 6 months. Ethylene begins to accumulate after approximately 80 days at 0°C even in CA storage (Furry et al., 1981). An investigation of the effects of added ethylene on fresh cabbage stored for 5 weeks in air at 0°C showed that high levels (100 μL L^{-1}) of ethylene increased endogenous auxin activity 6-fold and gibberellin activity 12-fold over the air control (Table 1), while inhibitor activity was undetectable (Pendergrass et al., 1976). The external leaves of all heads were bleached, desiccated, and totally separated from the main stalk—a typical response for ethylene-induced abscission. Several instances are documented of total loss of cabbage stored commercially with apples (McKeon et al., 1978). Even 1 μL L^{-1} ethylene has detrimental effects on cabbage stored in air, magnifying or accelerating changes taking place over the long storage period, including degreening and leaf abscission, weight loss, sugar loss, changes in organic acid content, or increased respiration rates (Hicks and Ludford, 1981). However, the increased CO_2 and reduced O_2 of CA conditions seem to counteract the ethylene effects seen in air storage. Thus, 1 μL L^{-1} of ethylene included in an atmosphere of 2.5% O_2 plus 5% CO_2 had little or no effect on appearance or on other measured criteria for storage losses, such as respiration rate or sugar depletion in the cultivar Hidena, though metabolism was somewhat affected by CA

Table 1 Plant Growth Hormone Concentrations Around the Apical Meristem of Cabbage Heads Before and After Storage for 5 Weeks at 1°C in Atmospheres Containing a Range of C_2H_4 Concentrations

	Growth substances μg equiv. 100 gFW^{-1} [a]		
Treatment	IAA	ABA	$GA_{4/7}$
Prestorage	0.460	5.50	0.292
Poststorage in:			
Air (0.04 μL L^{-1} C_2H_4)	0.320	3.00	0.058
Air + 1 μL L^{-1} C_2H_4	0.104	2.80	0.088
Air + 10 μL L^{-1} C_2H_4	0.494	[b]	0.046
Air + 100 μL L^{-1} C_2H_4	21.104	[b]	0.720

[a] Data are means of two heads sampled.
[b] Below sensitivity level of wheat coleoptile assay.
Source: Pendergrass et al., 1976.

in Bartola (Hicks et al., 1982). The effect of CA in modifying ethylene effects in cabbage would again substantiate the fact that CO_2 is a competitive inhibitor of ethylene.

Lettuce (*Lactuca sativa* L.), commercially the most important leafy vegetable, is probably the most difficult crop to grow, harvest, and deliver to the consuming public, resulting in a great deal of handling loss both in quantity and quality. This is especially true in the United States, where transit times and distances between producing areas and consuming centers are great. The severity of russet spotting, a common physiological disorder in lettuce, is determined to a large extent by the ethylene present (Kader, 1985). Senescence begins very shortly after harvest—i.e., wilting, yellowing, and leaf decay. No natural rest period has been shown for lettuce, since it is harvested in the juvenile or actively growing condition.

Ethylene is known to promote loss of chlorophyll and was used to promote the blanching of celery (*Apium graveolens* L.) as far back as 1924 (Thimann, 1980). However, ethylene production stimulated by water deficit is also believed to lead to pithiness (Pressman et al., 1984), as is abscisic acid. Ethylene concentrations of 20 to 100 μL L^{-1} were shown to do so, as measured by density loss in segments (Saltveit and Mangrich, 1996), although lower concentrations that would normally be encountered during processing and marketing had no significant effect. The upper two-thirds of the petiole were less likely to develop pithiness than the bottom region. Ethylene has indeed been implicated in signaling cell death in the formation of aerenchyma or air spaces in the cortex of adventitious roots of maize (*Zea mays* L.), possibly through an increase in intracellular Ca^{2+} (He et al., 1996).

III. AUXINS

Auxins are involved in many plant responses, including cell enlargement and elongation, cell differentiation, and control of apical dominance. The main auxin in most plants is indole-3-acetic acid (IAA). It has been the prevailing theory that IAA is derived from tryptophan, but it now seems that there are additional IAA synthetic pathways in a single plant type (Normanly et al., 1995). The nontryptophan precursors include indole and in-

dole-3-glycerol phosphate, both on the tryptophan pathway but before it, and indole acetonitrile (IAN), found primarily in Brassicas. The regulation is complex and is dependent on developmental stage, other compounds present, etc. For instance, carrot cells cultured in the presence of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) proliferate in an undifferentiated state and synthesize IAA from tryptophan. However, removal of 2,4-D induces somatic embryogenesis and a different pathway produces IAA. This may be one reason for the paucity of auxin biosynthetic mutants, since a block in one pathway could result in compensation by another pathway.

Less is known about auxin signaling mechanisms than for other plant hormones. Most molecular studies show that rapid and specific changes in gene expression are detected when auxin is applied to plants, with multiple auxin response elements and modulation of membrane function (Abel and Theologis, 1996). Auxin appears to affect early auxin-responsive genes, as in the transcription of SAURs (small auxin upregulated RNAs) in both mung bean (*Vigna radiata* L. Wilcz.) and pea, as well as their stability (Hagen, 1995). Some DNA binding proteins are induced that have very short half-lives, and an auxin-insensitive mutant gene *AXR1* has been cloned, both of which suggest that the ubiquitin-activating pathway for protein degradation may play a role (Bowler and Chua, 1994). Therefore, the AXR1 product may degrade a repressor (negatively regulate a negative regulator) or desensitize a signal transduction pathway.

A. Vegetable Fruits

Indoleacetic acid influences ethylene formation through the induction of ACS; therefore its effects are of interest during fruit ripening. The pattern of IAA levels found in stone fruits supports this notion of IAA levels inducing ethylene production during fruit ripening (Miller et al., 1987). However, a different pattern is found in tomato fruit, where endogenous auxin levels are highest after pollination during the early stages of fruit development and lowest during maturation and ripening (Buta and Spaulding, 1994), though this differs from the trend (Fig. 1) presented by McGlasson (1978). The *dgt* mutant of tomato is auxin-insensitive and defective in auxin-induced ethylene production (Abel and Theologis, 1996).

Free, ester, and amide conjugates of IAA and 4-chloroindole-3-acetic acid (4-Cl-IAA) are present in seeds of pea, and exogenous 4-Cl-IAA promotes growth in deseeded pea pericarp. Normal pod or pericarp growth in pea requires the presence of seeds, which regulate the conversion of GA_{19} to GA_{20}, and 4-Cl-IAA can stimulate this conversion in deseeded pea fruit. Two roles for auxin in controlling pea fruit growth are envisaged and both are experimentally supported, one a direct auxin effect on pericarp growth and the other the export of 4-Cl-IAA from the seeds to the pericarp, where it stimulates gibberellin biosynthesis (van Huizen et al., 1996).

Contrasting results obtained by different types of application of exogenous auxin to fruit—e.g., dipping or spraying fruit versus vacuum infiltration—can be explained by limited penetration of auxin into the tissue when applied by dipping. Using dipped, whole tomato fruit and vacuum-infiltrated, cut discs, 2,4-D causes a dual effect, with a delay in ripening but also an increase in ethylene production that promotes ripening (Abeles et al., 1992). The delay prevails but depends on the uniformity of the auxin distribution and its concentration. Wounded tissue, such as cut discs, also has high peroxidase activity, which is known to degrade IAA, and this decarboxylative IAA metabolism noted in cut tissue does not occur in intact fruit (Cohen, 1996).

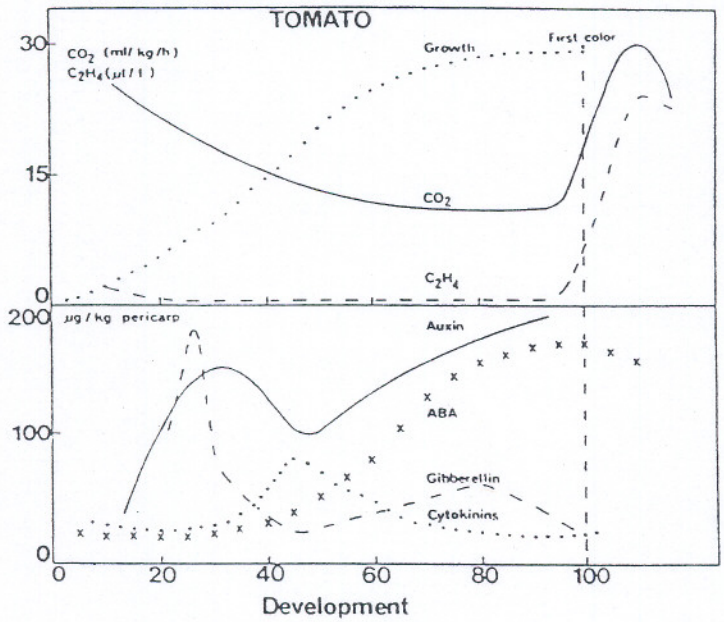

Figure 1 Trends in free hormone levels in tomato pericarp tissue during development and ripening. (From McGlasson, 1978.)

Exogenous auxin treatments can thus provide some delay in ripening and softening, and this was confirmed using a system that got around the previous problems—an in vitro system producing parthenocarpic tomato fruit in culture starting from immature flowers in response to IAA supplied in the medium (Cohen, 1996). Other auxins or conjugates, as well as antiauxins, are not effective. Additional IAA supplied before the breaker stage results in a delay from 7 to 12 days in the period between breaker and red-ripe stages.

IAA is conjugated to both carbohydrates and amino acids to yield IA-glucose, IA-inositol, and IA-aspartic acid, for example. The last of these is an irreversible conjugation (Catala et al., 1992). In green immature tomatoes, IAA is deactivated primarily by conversion to IA-aspartate and further metabolites; while in mature pink fruit, there is more IA-glucose, a potential storage product.

B. Underground Storage Organs

In onion, bulb apices and green leaf tissues show a diminution of auxin in both foliage tops and bulb apices during the change from the lush green foliage stage to the soft-neck stage in the field (Isenberg et al., 1974). Within a week, as the leaf tissues became further desiccated and tops began collapsing at the region of the swollen soft neck, IAA levels remained low but with significant amounts of IAN in the tops. A week later, when all foliage had fallen to the ground, bioassays of the tops showed a significant amount of inhibitor present, but no auxins were detected in the leaf tissues and only small amounts of auxins were present in apices. At this point in onion development it is customary to undercut the crop and to harvest it within a day or so. Growth-promoting hormone activity in the bulb, including that of both auxins and gibberellins, is essentially absent at harvest

in late summer but accumulates gradually throughout the storage period before declining again in spring (Fig. 2).

No important role for auxins has yet been identified in breaking of rest or dormancy or in tuberization in potato. Both exogenous IAA and the synthetic auxin naphthaleneacetic acid (NAA) can promote sprouting of potato eyes at low concentrations (4×10^{-8} M), but they have inhibitory effects at higher (4×10^{-5} M) concentrations (Hemberg, 1947). This suggests that auxins may perform a regulatory function in potato tuber dormancy, but it is likely to be very concentration-dependent. Tuber growth rate and auxin content have been associated (Ewing, 1995).

When carrot roots, cv. Vanity, were treated with growth regulators and then stored in high humidities for 117 days at 0°C, water-treated control carrots were 60% rooted and sprouted (Table 2), while treatment with NAA promoted rooting at the expense of sprouting (Abdel-Rahman and Isenberg, 1974). Only at the lowest concentration of

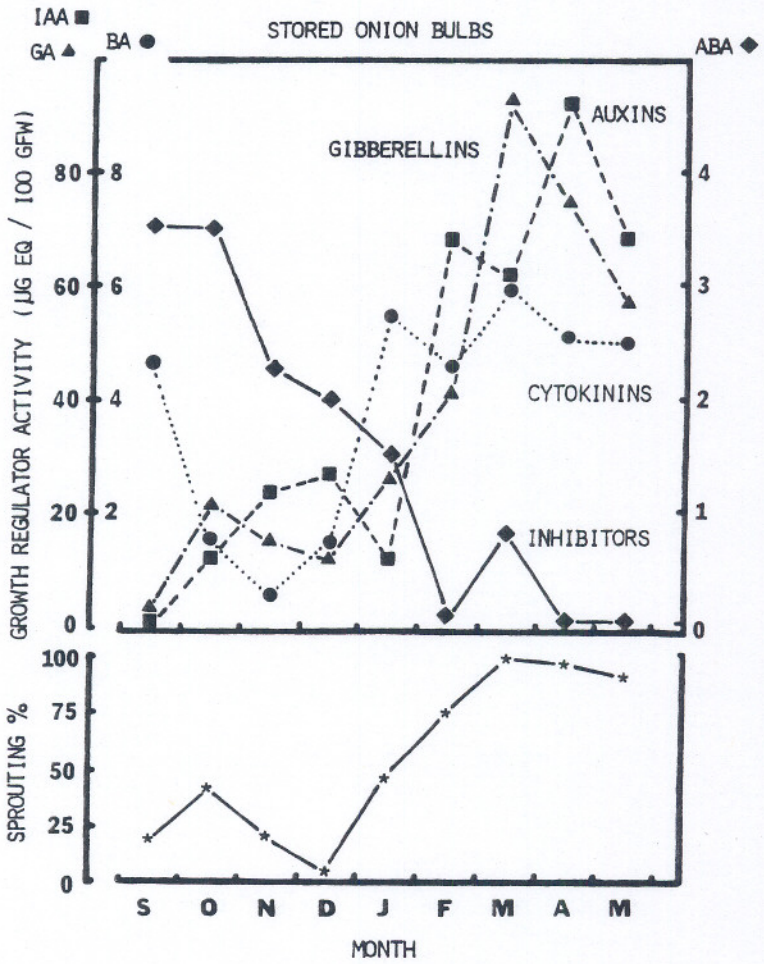

Figure 2 Sprouting and hormonal changes in onion bulbs during storage at 5 to 8°C (3-year average). (From Isenberg et al., 1974.)

Table 2 Growth Regulator Effects on Sprouting, Rooting, and Appearance of 'Vanity' Carrots After 4 Months Storage in Air at 0°C

Treatment[a] and chemical conc. (mg L^{-1})		Sprouted only (%)	Rooted only (%)	Sprouted and rooted (%)	Appearance (3–10 rating)[b]
H_2O		0a[c]	0a	60g	7b
NAA	100	0a	64c	33d	7b
	500	12b	70c	0a	7b
	1000	0a	100d	0a	7b
GA	100	23c	0a	0a	9c
	500	0a	0a	30d	9c
Ethephon	100	10b	0a	10b	9c
	500	25c	0a	24c	7b
Coumarin	100	0a	33b	60g	5a
	500	0a	0a	42e	5a
BA	100	0a	0a	80h	7b
	500	0a	0a	53f	7b

[a] Treatments were applied by dipping for 15 min.

[b] Subjective rating of sound carrots based on appearance, color, degree of sprouting and rooting: 10 = excellent; 9 = very good; 7 = good; 5 = fair; and 3 = poor.

[c] Means within each column not followed by the same letter are significantly different by Duncan's Multiple Range Test at $p = 0.01$.

Source: Abdel-Rahman and Isenberg, 1974.

NAA was there any sprouting, and then in only half of the control. Thus, carrot roots have a physiological system responsive to exogenous growth regulators and do alter growth responses accordingly.

Auxin may also play a role in the wound-induced expression of genes in carrot storage roots. A genomic clone isolated from carrot storage roots encodes a proline-rich cell-wall protein very similar to other proline-rich protein cDNAs (Ebener et al., 1993). This is wound-induced and can also be induced by IAA, 2,4-D, or NAA treatment. The cDNA is not expressed in leaves or in vegetative (nonstorage) roots, whether they are wounded or not, and is expressed beginning at the earliest visible stages of carrot storage root growth—i.e., its expression is also developmentally regulated.

C. Leafy Crops

English gardeners remove the IAA-producing terminal buds of Brussels sprouts after the axillary buds or sprouts have begun to form. This increases the size of sprouts, since decapitation results in increased auxin activity in the top lateral buds, creating new nutrient sinks.

In stored cabbage cv. Green Winter, the level of growth-promoting hormones, including IAA activity, was slightly higher in heads stored in refrigerated air than in CA-stored heads. In harvested iceberg lettuce there appears to be no relation between IAA content and axillary bud development in regard to russet spotting (Ritenour et al., 1996).

Higher concentrations of IAA are found in green spears of asparagus (*Asparagus officinalis* L.) than in white (grown under black plastic or sawdust mulch). This is consistent with the greater elongation of green spears, although white had higher fresh and dry

weights in 5-mm tip samples, and higher levels of inhibitor are also found (Makus and Guinn, 1992). However, IAA levels are also higher in light-grown seedlings of pea than in much taller, etiolated dark-grown seedlings (Behringer et al., 1992), so that this may be more a light/dark sensitivity than an elongation effect.

IV. GIBBERELLINS

Among other effects, gibberellins (GAs) affect cell elongation and bolting in plants. In their synthesis, the conversion of GA_{12}-aldehyde to other GAs can vary from genus to genus, but the four main pathways have a basic sequence of reactions involving the successive oxidation of C-20, leading to its elimination from the molecule as CO_2 and the formation of C19-GAs (Fig. 3). It is the C19-GAs that have biological activity (Sponsel, 1995). In pea seeds, the main two parallel pathways lead from GA_{12} and the 13-hydroxylated GA_{53}, while, in pumpkin (*Cucurbita maxima* Duch.) endosperm, all four pathways are found, from GA_{12}, GA_{53}, 3-hydroxylated GA_{14}, and 3,13-hydroxylated GA_{18}. In most species studied, including pea and spinach (*Spinacia oleracea* L.), the major or only pathway in shoot tissue is the 13-hydroxylated pathway, with GA_1 (3β-hydroxylation from GA_{20}) most likely the active GA controlling internode elongation. In *Cucumis sativus*, GA_4 is more active than GA_1, and is a 3β-hydroxylated C19-GA like GA_1. These are thus active in stem elongation. In reproductive tissue like seeds, GAs are more abundant and more structurally diverse with multiple hydroxylations (Sponsel, 1995).

A. Vegetable Fruits

Pericarp growth in pea normally requires the presence of seeds, which have high GA levels. Exogenous GA can replace this requirement, indicating that transported seed GAs

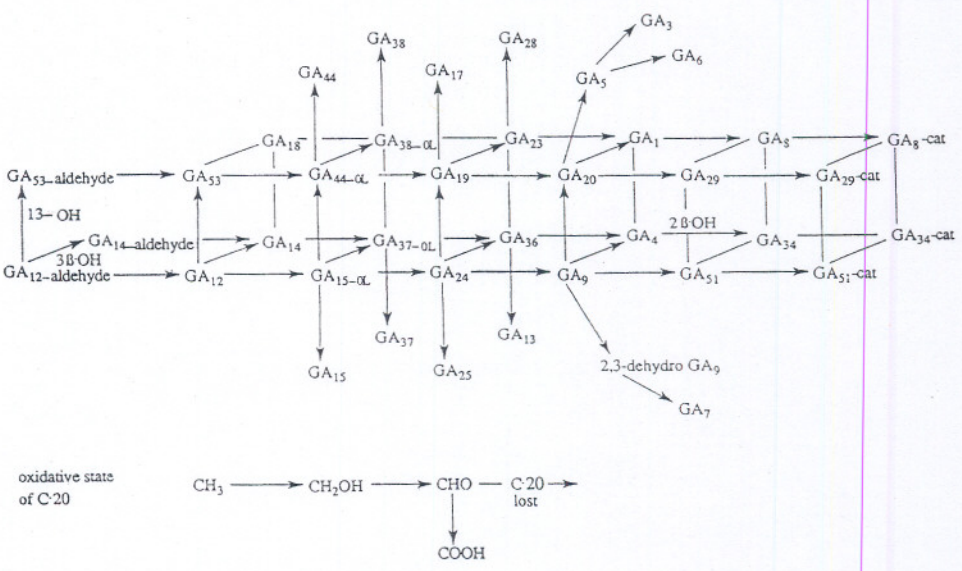

Figure 3 Metabolic grid of GAs in higher plants showing metabolic sequences produced by successive oxidation of C-20 followed by its removal, combined with hydroxylation at C-13, C-3, and/or C-2. Not all reactions operate in all plants. (From Sponsel, 1995.)

regulate pericarp growth, or that seeds promote pericarp growth by maintaining GA synthesis in the pericarp (van Huizen et al., 1996). The gibberellins, GA_1 and/or GA_3, control pod development in pea, while GA_{20} is not active per se (Santes and Garcia-Martinez, 1995), but its activity in the dark must depend on its metabolism, presumably to GA_1 (Sponsel and Reid, 1992). Pea pericarp has the capacity to metabolize GA_{12} to GA_{19} and on to GA_{20} and GA_1, but for the conversion of GA_{19} to GA_{20}, the presence of the seed is needed. The seed factor regulating this conversion is 4-Cl-IAA, which can indeed substitute for the seed in the stimulation of pericarp growth and the GA_{19}-to-GA_{20} conversion (van Huizen et al., 1996). Pericarp tissue responds to seed removal by synthesizing, inhibiting synthesis, or modifying specific proteins. Both 4-Cl-IAA and GA_3 can reverse this process, but their effects are not equivalent.

Endogenous levels of gibberellins are thought to be high in very young fruit (Fig. 1), and they may play a role in retarding senescence. Gibberellins that co-chromatograph with GA_3 and $GA_{4/7}$ increase from anthesis until maturity and decline during ripening in the cherry tomato, cv. Small Fry (Abdel-Rahman et al., 1975).

The respiration response typical of a climacteric fruit is retarded by GA_3 treatment in tomato fruit, which delays the ripening pattern (Babbitt et al., 1973). Fruit softening is delayed by GA, which suppresses PG activity but has less effect on cellulase activity. Similarly, color development in tomato is retarded and modified by GA (Fig. 4). Fruit ripening is associated with the conversion of chlorophyll-containing chloroplasts to carotenoid-containing chromoplasts, and GA_3 delays appearance of plastid-localized lycopene during ripening of tomatoes but does not suppress chlorophyll degradation. Applied GAs do enhance regreening color changes in some fruit, but—despite the effect of applied GAs on delaying fruit coloration in fruit of several species—no close correlation has yet been found between color change in fruit ripening and endogenous GA content (McGlasson, 1978). Color change does not appear to be due to differential transcriptional control during the chloroplast-chromoplast conversion, and there are only moderate changes in the stability of plastid transcripts. The most likely areas of specific gene expression control in plastids are posttranscriptional processing and/or translation (Marano and Carrillo, 1992).

Gibberellins have long been known to inhibit hardening to low temperature. ABA-induced freezing tolerance in bromegrass (*Bromus inermis* Leyss) cells could be inhibited by GA_4, GA_7, and GA_9, separately or together (Gusta et al., 1996). Triazoles are synthetic plant growth regulators that confer tolerance to freezing and chilling by their action as antigibberellins, since they inhibit the gibberellin synthetic pathway. The triazole growth regulators paclobutrazol and uniconizole increase chilling tolerance of green and red bell pepper fruit, and also seem to protect whole plants against various types of stress (Fletcher and Hofstra, 1988).

B. Underground Storage Organs

A rise in endogenous GA activity seems to be involved in the dormancy break of potato tubers along with a decline in inhibitor activity. Increases in both GAs and cytokinins take place during the transition from rest to dormancy, with a movement of GA activity from basal to apical regions of the tuber at the onset of sprouting (Obhlidalova et al., 1979). In confirmation, low-temperature treatment promoted sprouting of tubers in four potato cultivars, with associated increases in endogenous GAs and decreases in inhibitor levels (Thomas and Wurr, 1976).

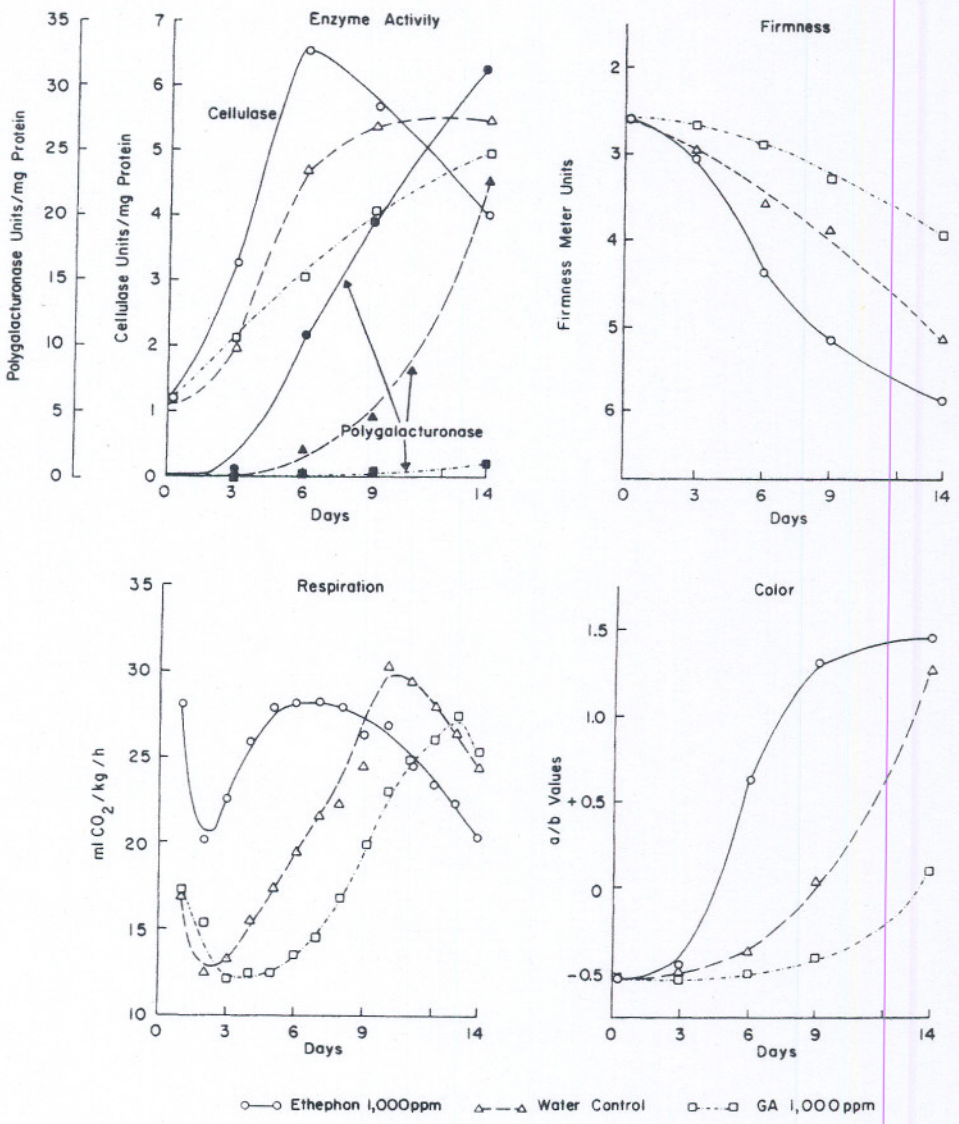

Figure 4 Effect of ethephon and GA on tomato enzyme activity, firmness, respiration, and color in comparison to control. (From Babbitt et al., 1973.)

Endogenous hormone activity increases after wounding, and the addition of GA_3 stimulates protein synthesis, RNA synthesis, nucleolar size and RNA polymerase activity still further in wounded potato tissue, although cells of uninjured tubers are not responsive.

Exogenous GA_3 treatment of cv. Majestic potato tubers during their rest period gave a significant decline in the β-inhibitor complex in the treated tubers over 3 days (Boo, 1961). A number of exogenous GAs over a wide range of concentrations stimulate sprouting in excised potato eye bioassays (Rappaport et al., 1965). GA_3, GA_4, GA_5, and GA_7 are stimulatory, while GA_6, GA_8, and GA_9 are noneffective or slightly inhibitory.

Leaching of eye plugs with distilled water delays sprouting, while GA treatment of leached eyes restores sprouting activity to its original rate. Gibberellins therefore seem to be involved in the dormancy break of potato tubers, and the application of GA_3 to promote sprouting in seed potatoes is an approved commercial practice in some countries.

In contrast to this usual case, where GAs release the tubers from dormancy, exogenous GAs induce a dormant state in bulbils of the genus *Dioscorea* or yams (Tanno et al., 1994). The sprouting of dormant bulbils of *D. opposita* Thunb is promoted by classical inhibitors of GA synthesis, such as 2-chloroethyl trimethyl ammonium chloride choline chloride (CCC) and ammonium (5-hydroxycarvacryl) trimethyl chloride piperidine carboxylate (ACPC or AMO-1618), and newer inhibitors such as uniconazole and prohexadione, which block the pathway later (Sponsel, 1995).

Bioassay results show that onion bulb apical tissue contains very low levels of GAs, auxins, and cytokinins, with a high level of inhibitor activity in late autumn (Isenberg et al., 1974). Inhibitor levels gradually decline to a very low level by midwinter, while growth promoter activity increases (Fig. 2), with GAs peaking in late winter. The interval between late autumn and early spring (November through April in the northeastern United States), the latter being the time of visible sprouting, constitutes the rest and dormancy periods during which hormone-promoted apical development proceeds at a rate responsive to the storage temperatures of 5 to 8°C. Both flower induction and linear growth have been attributed to GA influences, and it was speculated that the GA peak is related to cold induction and sprout extension.

Changes in GA-like substances were examined in several breeding lines of onions considered to have different levels of dormancy, defined as a lack of apical bud growth (Aung and Peterson, 1974). In general, the total GA activity of dormant or resting bulbs is greater than that of nondormant (sprouting) bulbs, and more of it is free, whereas nondormant bulbs have greater quantities of bound GAs, with no striking qualitative differences between the onion lines examined. This could possibly be due to the ability of the nondormant bulbs to mobilize and utilize the GAs during growth in response to external stimuli.

Resting non-temperature-induced onion apices held at 20°C were tested with different exogenous growth substances for breaking rest and were compared with growth responses of a 10°C temperature treatment for 96 h, which is adequate to break rest, as is also 10% sucrose (Mahotiere et al., 1976). Exogenous GA_3 or $GA_{4/7}$ application was not able to overcome the resting state. However, application of ACPC or AMO-1618, the inhibitor of GA biosynthesis, to apices prior to cold induction at 10°C nullified the rest-breaking effect of reduced temperature (Table 3). Since ACPC inhibits GA biosynthesis prior to cold induction, one of the effects of cold-induction treatment might be the stimulation or initiation of endogenous GAs. This is also suggested by work on tulip bulbs (*Tulipa* sp.). Application of GAs to tulip bulbs partly replaced the cold treatment requirement for breaking dormancy (van Bragt and van Ast, 1976), and there was an increase in endogenous GA-like substances during cold treatment (Aung and De Hertogh, 1967). However, there were no significant differences in shoots or basal plates from control or cold-treated bulbs in free GA_4, GA_9, GA_{24}, or GA_{34} (Rebers et al., 1996). This does not have to negate the theory, however, because these GAs are on the GA_{12} pathway, and those from the GA_{53} or 13-hydroxylated pathway were not examined. The latter are the ones that are involved in bolting in spinach. Only GA_1 is lower in cold-treated tulip bulbs, but it is present in very small quantities.

GA_3 treatments were applied to carrot roots, cv. Vanity, which were then stored in high humidities for 117 days at 0°C (Abdel-Rahman and Isenberg, 1974). Sprouting was

Table 3 Effect of Sucrose, Ammonium (5-Hydroxycarvacryl) Trimethyl Chloride Piperidine Carboxylate (ACPC), and Exposure to 10°C on Subsequent Growth of Excised 'Downing Yellow Globe' Onion Shoots at 20°C

Treatment	Growth (% initial length)
Water	20.35a[a]
10% sucrose	38.99b
96 h at 10°C	39.18b
10% sucrose, then 96 h at 10°C	44.55b
100 mg L^{-1} ACPC, then 96 h at 10°C	19.75a
100 mg L^{-1} ACPC + 10% sucrose, then 96 h at 10°C	54.77c

[a] Mean separation by Duncan's Multiple Range Test at $p = 0.05$.
Source: Mahofiere et al., 1976.

stimulated and rooting suppressed at the lowest concentration (100 mg L^{-1}) over water-treated control carrots, which were 60% rooted and sprouted.

When biennial root crops are stored at low temperatures for prolonged periods and permitted to grow to maturity in a second season, they frequently flower, a known cold-induction response. Some species will bolt and flower under noninductive conditions if treated with exogenous GA_3 (Wareing and Phillips, 1981). Hence, one might infer that endogenous GAs play a role in the cold induction that occurs in storage. However, in GA-treated carrot, stem elongation precedes floral differentiation, and there is no flowering without cold induction (Hiller et al., 1979). Thus, while GAs may be involved in stem elongation, bolting precedes floral differentiation and endogenous GAs are not implicated in cold-induced flowering in carrot. Cold induction appears to stimulate most stored roots to early compulsive regrowth.

C. Leafy Crops

As with auxins, the level of most GA activity is slightly higher in heads of cabbage stored in refrigerated air than in CA-stored heads. In air storage, endogenous GAs, especially those co-chromatographing with GA_3, are at very low levels for a month after harvest (Isenberg et al., 1974), but then two quantitative surges result in two distinct peaks (Fig. 5). Under CA influence, there is only one peak, quite out of phase with the air responses. This out-of-phase situation quite possibly disturbs the normal hormonal balances that probably control the cold-induced reproductive apical response. Apices of air-stored cabbage heads show a normal vertical growth pattern, while those in CA remain flat (Fig. 6). Apices that develop in stored cabbage are designated as differentiated (i.e., cold-induced), which grow vertically and reproductively, as compared with undifferentiated, which remain flat and grow vegetatively (Shirokov, 1974). Exposure to low temperature was shown to be necessary for reproductive responses in cabbage. On termination of storage, when stalks are rerooted in compost, most stored in air bolt and flower in 4 weeks, while those under the CA regime do not flower (Ludford and Hillman, 1984).

Lateral bud development of Brussels sprouts is less inhibited than in most other species, and buds attain a large size as the plant matures. Removal of the terminal buds after the axillary buds or sprouts have begun to form increases the size of sprouts, but decapitation at too young a stage results in axillary leader shoots. This is because lateral

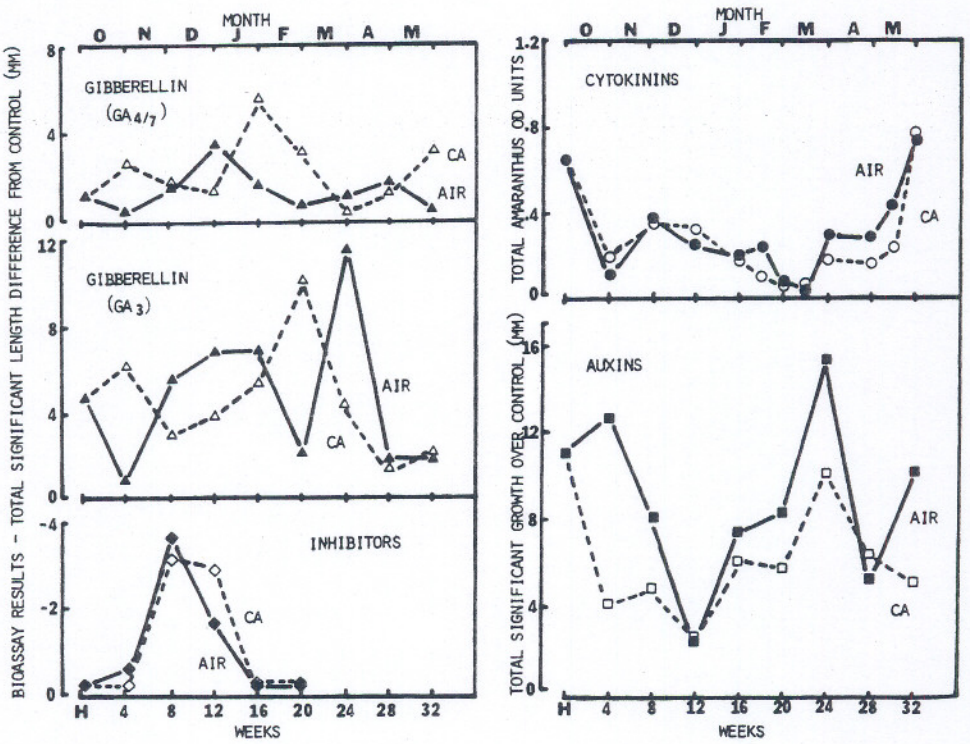

Figure 5 Changes in endogenous growth regulators in cabbage during storage (cv. Green Winter). (From Isenberg et al., 1974.)

buds of the younger plants contain more total GA-like activity than those of older plants, whose buds would not show shoot extension (Thomas, 1972). During accelerated aging of the outer leaves of Brussels sprouts, GA and inhibitor activities increase while endogenous cytokinin activities decrease (Furry et al., 1981). Good-quality sprouts would therefore appear to need low GA content.

The "riciness" disorder of cauliflower is due to elongation of the floret peduncles. It may be GA-controlled and can be retarded somewhat by low-temperature storage (Thomas, 1981). Treatment of cauliflower curds with a number of growth inhibitors, including chlormequat, an inhibitor of GA synthesis, shows some beneficial effects. The principal difficulty encountered is curd-to-curd variation, likely due to the difficulty of harvesting curds at the same state of physiological development. Low GA content would therefore also appear to improve cauliflower quality.

Similarly, in rosette plants such as spinach, low GA levels would be advantageous unless seed stalks were required. Spinach is a long-day (LD) rosette plant in which exposure to LD conditions result in stem elongation and subsequent floral development. The rate of GA synthesis in such LD plants is lower during vegetative growth under short-day conditions than it is under LD conditions, when stem elongation takes place. As found in carrot and noted for Chinese cabbage, both annual and cold-requiring biennial *Brassica* sp., including oilseed rape (*Brassica napus*), bolt in response to GA even when unvernalized, but the flowering that follows is dependent upon photoperiod (Mandel et al., 1992).

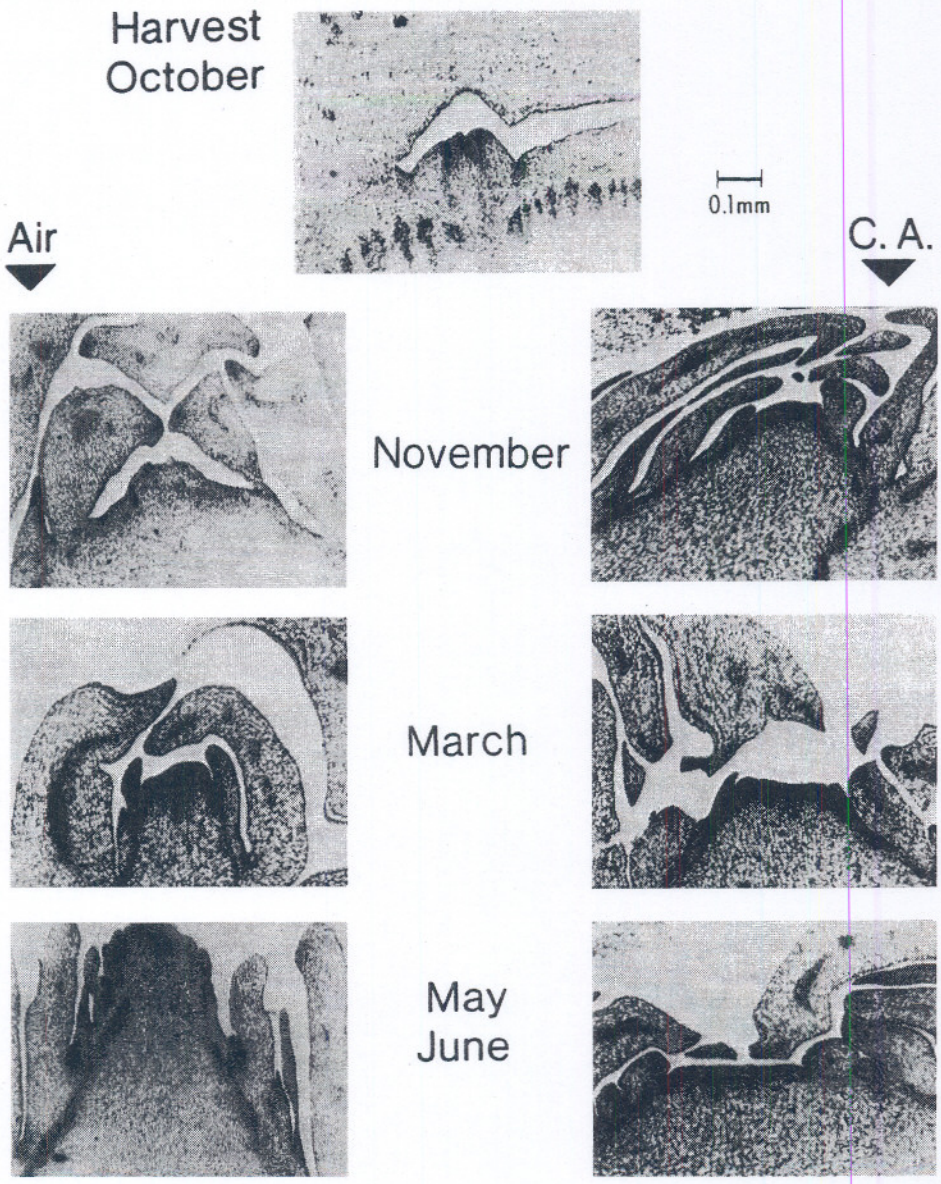

Figure 6 Meristem sections from cv. Green Winter cabbage at harvest and during storage at 1°C in air or under CA (2 to 5% O_2, 5% CO_2). (From Pendergrass AM, Isenberg, FMR, Thomas TH, unpublished data, 1974.)

In spinach, photoperiod has been shown to enhance several steps of the GA biosynthetic pathway, including ent-kaurene accumulation, metabolism of GA_{20}, and the conversion of GA_{53} to GA_{44} and of GA_{19} to GA_{20} (Sponsel, 1995). The level of GA_1 correlates with bolting in spinach. All GAs of the 13-hydroxylated GA pathway start to increase, particularly GA_{20}, but it is finally the rise in GA_1 that is of importance. GA_{20}-oxidase seems to

catalyze the multiple steps of oxidation and elimination of C20 (from GA_{53} to GA_{20}), and the mRNA for this enzyme increases in LD conditions, parallel to the increases in GA_{20} and GA_1 levels (Wu et al., 1996).

Although lettuce seedlings were used for one of the earliest GA bioassays, little is known about the endogenous hormone systems involved in development and postharvest retention. Applied GA_3 is reported to delay chlorophyll loss and decay in harvested lettuce and celery and to prevent decay in fresh Umbelliferae herbs, such as parsley (*Petroselinum crispum* Mill.) and dill (*Anethum graveolens* L.), particularly when the endogenous GA content is depleted (Afek et al., 1995). For celery it is suggested that GA_3 retards decay during storage by slowing down the conversion of (+)marmesin to psoralens, which are linear furanocoumarins, with the higher level of marmesins acting as phytoalexins, thus increasing celery resistance to pathogens (Afek et al., 1995).

V. CYTOKININS

Cytokinins delay senescence and maintain green color and fresh appearance in many leafy vegetables. In leaves, they are relatively immobile, and applying a spot of exogenous cytokinin to a detached leaf can immobilize nutrients at the site of application and demonstrate the ''green island'' effect, as the rest of the leaf senesces while nutrients are transported to the mobilization site. Roots and developing seeds are sites of synthesis for cytokinins, such as zeatin (Z) and isopentanyl adenine (2iP) and their ribosides (ZR and IPA) and ribotides, which are exported in the xylem to other parts of the plant. The major known endogenous hormones have been found in all roots investigated, including carrot root, beet, and sweet potato (*Ipomoea batatas* L.). In carrot root, cambium tissue was found to be the site for cytokinin synthesis (Chen et al., 1985). However, cytokinin conversion is not limited to roots and seeds, since a microsomal enzyme fraction from fresh cauliflower heads can hydroxylate applied 2iP and IPA to Z and ZR (Chen and Leisner, 1984). Since ethylene treatment of this enzyme system reduces the conversion by 28% to 43%, part of the senescence-promoting activity of ethylene could act through preventing cytokinin synthesis.

Further investigations have demonstrated the importance of conjugation in the movement of cytokinins from storage tissue into active meristems. Bud development is apparently dependent on the ability to convert cytokinin glucosides into the active forms, and it seems likely that buds are not themselves the sites of cytokinin synthesis. A relationship between cytokinin activity and Ca^{2+} mobilization is pointed out in various studies (Grabski and Schindler, 1996).

Cytokinin-overproducing mutants have been found, the most dramatic of which are from the moss *Physcomitrella patens*. There is a lateral suppresser mutant of 'Craigella' tomato that has the opposite effect and contains less cytokinin than the wild type. *Solanum tuberosum* tissues transformed with the *ipt* (isopentenyl tranferase) gene from *Agrobacterium* (a gene from *Agrobacterium faciens* that induces synthesis of endogenous cytokinins in transformed plant cells) spontaneously produced shoots, but these could not form roots (Binns, 1994).

Along with other plant hormones, cytokinins may have a role as control agents in pest and disease resistance. Because of the problems of uptake, transport, and metabolism with the use of exogenous cytokinins, the *ipt* gene from *Agrobacterium* is being utilized and introduced into a tomato cultivar susceptible to *Fusarium oxysporum*. This results in higher cytokinin concentrations and enhanced resistance to the fungus (Smigocki, 1995).

A. Vegetable Fruits

High levels of endogenous cytokinin can also delay fruit ripening, and levels may decline as ripening proceeds. Free cytokinin activity was found to be high for 2 weeks after anthesis in cherry tomato, cv. Small Fry, when cell division was most active (Abdel-Rahman et al., 1975), and declined from MG to red ripe stage in standard varieties (McGlasson, 1978). Senescence in leaves can be delayed by cytokinin and, in one aspect, fruit ripening can be regarded as a senescence phenomenon.

Both free and bound cytokinins are found in growing fruits. Cytokinin ribotides have been identified at all stages of growth, particularly during early development, and at ripening, when they may be components of the seeds (Abdel-Rahman et al., 1975). Major cytokinins present during ripening of tomato fruit, cv. Heinz 1439, are probably Z, ZR, 2iP, and IPA (Desai and Chism, 1978). All these compounds declined significantly from the MG to the red ripe condition. A comparison of the normal ripening pattern of cytokinin changes in cv. Rutgers with the pattern in the nonripening mutant *rin* showed that the cytokinin content of both cultivars was quite high at the MG stage and declined quantitatively during growth, but the rate of decline was much greater for cv. Rutgers than for the *rin* mutant (Davey and van Staden, 1978). The latter contained very high levels of zeatin glucoside, which might function as a storage form of cytokinin, to be converted continuously to the active form necessary to prevent or delay normal ripening. Thus, cytokinins appear to be implicated in the delay of fruit ripening.

Endogenous cytokinin levels in seeded tomato fruit can be increased by reducing the ratio of foliage to fruit, which lowers sink competition (Varga and Bruinsma, 1974), and this delays the rate of ripening after the breaker stage. Another way of increasing endogenous cytokinin levels is by transformation of tomato plants. Transformation by a chimeric gene, combining *ipt* from *Agrobacterium* with a promoter from a gene expressed predominantly in tomato ovaries, resulted in plants that had two- to threefold higher levels of cytokinin in ovaries than in the control (Martineau et al., 1995). The fruit also had increased ovary/young fruit sink strength, as shown by higher contents of soluble solids and total solids, although the yield and fruit size were slightly lower, possibly due to increased fruit set.

An exogenous synthetic cytokinin, 1-(2-chloro-4-pyridyl)-3-phenylurea, used to promote fruit set and induce parthenocarpy in watermelon (*Citriullus lanatus* Thunb.), did not adversely affect development and fruit quality (e.g., sugars), in contrast to IAA and NAA, which tended to produce thick rind and deformed fruit (Hayata et al., 1995).

B. Underground Storage Organs

Increases in cytokinin activities are found during the transition from rest to dormancy, with conjugation again being of importance in cytokinin movement to active tissue. There are increases not only in cytokinins but also in GAs during the transition from rest to dormancy in potato tuber (Obhlidalova et al., 1979). Cytokinins increase only up until the initiation of rest termination and then decrease at sprouting. A rest-breaking effect was demonstrated in apical sections of cv. Majestic tubers treated with kinetin and Z (Hemberg, 1970). Bioassays of the peel extracts using the *Avena* coleoptile test to check inhibitor content clearly showed stimulatory effects from the applied cytokinins in rest breaking and reduction in the inhibitor complex.

Tubers stored in the dark for 9 months at 5°C develop a storage disorder called "little potato." Under these constant conditions, the tubers apparently leave the state of

dormancy and enter the state of compulsive regrowth, forming stolons with little tubers. Three endogenous cytokinins—identified as Z, ZR, and zeatin glucoside—are present in the parent tubers and little potatoes (van Staden and Dimalla, 1977). It was suggested that the glucoside was a storage form, and is converted to Z and then to ZR, the latter being present in the highest amount of the three. High amounts of the riboside are found in the stolon tips and the little potatoes, regions of high metabolic activity and cell division. In confirmation, higher levels not only of cytokinins but also of GAs were demonstrated in extracts from little potato tubers than from normal tubers (Wurr et al., 1980).

At the preharvest stage in late summer, when onion bulbs are considered mature, most of the tops have fallen but still retain five to seven partially green leaves. At this stage, well-defined mitotic figures indicating cellular activity are observed in the apical tissues. Such cellular activity is not apparent from autumn through early winter. Two onion cultivars in New York State, Elba Globe and Copper Skin, F1 hybrids of the same parentage, were harvested and cured in accordance with common agricultural practice and then stored at 2°C over 9 months, late summer through spring (Isenberg et al., 1974). Bioassays of onion apical tissue (central plugs containing apices, root plate, and unexpanded leaves) showed cytokinin levels to be quite high initially, declining rapidly to a very low value in late autumn, when there is no visible sprouting and when levels of inhibitor activity are high, but then rising rapidly to a high peak in winter during the beginning of visible sprouting (Fig. 2). The cytokinin rise is probably associated with the resumption of cell division. Histological studies of apices of stored onion bulbs show such a series of correlated cellular level events (Pendergrass, 1969).

When various exogenous growth substances were injected into mature onion bulbs, cv. Elba Globe, the synthetic cytokinin kinetin was the most effective substance in delaying the onset of senescence (Abdel-Rahman and Isenberg, 1974). Regrowth was delayed up to 42 days with an increase in the bulb size, while control bulbs became senescent in 28 days (Table 4). The combination of kinetin with auxin and GA delayed regrowth even longer, doubling both root numbers and growth vigor. At dormancy termination, all growth substances stimulated the number of roots and the vigor of root growth.

Application of exogenous growth substances to excised onion apices also showed that only kinetin could overcome the rest status of non-temperature-induced apices from onions held at 20°C (Table 5), and induced a similar growth response to that of a 10°C temperature treatment for 96 h, which was adequate to break rest (Mahotiere et al., 1976). Only a combination with cytokinin could partially overcome the inhibitory effect of abscisic acid (Table 6), which effectively prolonged the innate dormancy or rest period.

Thus, cytokinins may be part of the breaking of innate dormancy or rest, since only applied cytokinins are able to substitute for the cold induction requirement. This could be through synthesis or metabolism of endogenous cytokinins, or by playing a role in early assimilate availability, since sucrose treatment also overcomes the rest status. The cold induction treatment common under normal storage conditions may have a similar metabolic effect.

C. Leafy Crops

Applied cytokinins are often effective in prolonging the shelf life of leafy vegetables by slowing down senescence. Application of the synthetic cytokinin 6-benzylaminopurine (BA) as a postharvest dip delays senescence and maintains green color and fresh appearance in crucifers as well as many other leafy vegetables, including escarole and endive

Table 4 Effect of Plant Injection with IAA, GA, Kinetin (K) and Their Combinations on Plant Senescence and Size and Dormancy of Onion Bulbs

Treatment[a]	Senescence (days after injection)	Bulb dimensions		Degree of dormancy			
				Rooting		Sprouting	
		Diam. (cm)	Height (cm)	No. roots per bulb	Length (cm)	Bulbs sprouted (%)	Sprout length (cm)
Water	28	4.0	5.0	16	3.5	100	2.1
IAA	31	4.7	5.1	22	3.1	100	2.5
GA	38	4.9	5.7	27	5.4	100	3.2
K	42	5.2	5.5	24	3.5	100	2.6
IAA + GA	38	4.9	5.8	29	5.3	100	3.3
IAA + K	43	4.7	4.9	25	4.9	100	2.8
GA + K	50	4.9	5.8	27	6.3	100	3.6
IAA + GA + K	55	5.6	5.9	34	6.4	100	3.8
LSD ($p = 0.05$)	—	0.23	0.16	3.4	0.48	—	0.42

[a] Plants injected with 1 mL of water or 100 mg L^{-1} solution.
Source: Abdel-Rahman and Isenberg, 1974.

Table 5 Effect of Growth Regulators on Growth of Excised 'Spartan Banner' Onion Shoots at 20°C

Treatment	Growth (% initial length)
Water	18.64a[a]
Kinetin (100 mg L^{-1})	37.13b
IAA (10 mg L^{-1})	17.19a
GA_3 (1000 mg L^{-1})	18.83a
$GA_{4/7}$ (100 mg L^{-1})	18.67a
Ethephon (100 mg L^{-1})	14.83a

[a] Mean separation by Duncan's Multiple Range Test at $p = 0.05$
Source: Mahotiere et al., 1976.

(*Cichorium intybus* L.), spinach, green onions, celery, and asparagus (Zink, 1961). However, application for the use of BA and other synthetic cytokinins on leafy vegetables has not been approved in the United States. The commercial application of synthetic cytokinins would probably be very limited even if approved by the regulatory authorities, since these agents are either relatively immobile or easily degraded in plants, so that only the most exposed leaves would be affected by such treatments. However, due to handling procedures, most external leaves are damaged by the time they reach the point of consumption and require considerable trimming.

Endogenous cytokinin activity during storage of cabbage is high at harvest, declining to a low point during rest and early dormancy, with a rapid increase with the onset of regrowth in the apices (Fig. 5). It is not especially affected by the imposition of CA (Isenberg et al., 1974). Cytokinin profiles showed varietal differences between cv. Excel and three cabbage breeding lines of different storage capabilities (Thomas et al., 1975). The major cytokinin activity cochromatographed with ZR in two lines that exhibit good keeping quality, whereas Excel and one other breeding line with poorer keeping quality had later eluting cytokinin peaks. These latter varieties showed advanced apical regrowth,

Table 6 Effect of ABA, with or Without Kinetin or Sucrose, on Subsequent Growth of Excised 'Spartan Banner' Onion Shoots at 20°C

Treatment	Growth (% initial length)	
	Not exposed to 10°C	Exposed to 10°C for 96 h
None	16.0ab[a]	37.6c
1 mg L^{-1} ABA, then exposed to 10 and/or 20°C	9.0a	7.7a
Exposed to 10°C, then 1 mg L^{-1} ABA	—	11.2ab
100 mg L^{-1} kinetin	40.2c	—
1 mg L^{-1} ABA + 100 mg L^{-1} kinetin, then exposed to 10 and/or 20°C	14.0ab	18.4b
1 mg L^{-1} ABA + 10% sucrose, then exposed to 10 and/or 20°C	7.1a	7.3a

[a] Mean separation by Duncan's Multiple Range Test at $p = 0.05$.
Source: Mahotiere et al., 1976.

leaf color loss, and tissue breakdown. It is possible that the higher regrowth activity could be associated with the later eluting cytokinin activity. The variation in the cytokinin pattern may constitute a technique for selecting long storing cultivars.

Endogenous cytokinin activities decreased during accelerated aging of the outer leaves of Brussels sprouts at 25°C in the dark, while GA and inhibitor activities increased over a 7-day period (Thomas, 1981). Applied synthetic and natural cytokinins extended the storability of Brussels sprouts. When discs from mature green leaves of Brussels sprouts were incubated in the dark for 3 days, those having BA in the external solution lost 51% less chlorophyll than similar control discs, contrasted with 36% greater chlorophyll loss in GA_3. The adverse effects of ethylene on Brussels sprouts leaf senescence can also be overcome by pretreatment with cytokinin-like compounds (Thomas, 1981).

Exogenous cytokinin applications also extended the storability of broccoli (Thomas, 1981). Good quality in broccoli was maintained by BA treatment and low temperatures of 2°C, and this was reflected in sensory evaluations of the cooked broccoli (Ludford and Isenberg, 1987).

Two natural cytokinins isolated from butterhead lettuce have been identified as Z and ZR (Kemp et al., 1979). They are found mostly in the innermost developing leaflets, with no significant amounts occurring in the outer mature leaves. There are numerous reports of chlorophyll retention in lettuce by treatments with exogenous cytokinins, principally BA, but again these are of little practical value, since only outer leaves are affected, and these would be trimmed by the time they reached the consumer because of damage in handling.

Because of the problems associated with using synthetic cytokinins, application of the naturally occurring phytohormones IPA and GA_3 to Romaine lettuce 2 days before harvest was investigated (Aharoni et al., 1975). Since these are endogenous to many species, approval might be more readily obtained if they were shown to be effective in retarding senescence. At harvest, the lettuce heads were trimmed to commercial standards, sprayed, placed in polyethylene bags or lined cartons, and chilled to 0.5 to 1.0°C within 4 to 8 h. After 3 weeks at the low temperature, they were transferred to 18 to 20°C for 3 days. The presence of IPA consistently reduced yellowing and decay, which were expressed as indices whose scale ranged from zero (good condition) to 5. The most effective spray, which reduced both yellowing and storage decay, was IPA at 0.1 mg L^{-1} plus GA at 10 mg L^{-1} (Fig. 7). The reduction in yellowing could have a direct effect on decay, since many storage organisms are bacterial and thrive on tissues that have lost their viability. Lettuce held in lined cartons stored better than that in open bags, for CO_2 levels in the cartons rose to 12% by the end of the experiment, and this modified atmosphere could have suppressed endogenous ethylene effects and further enhanced the results obtained with IPA plus GA treatments. Romaine lettuce, in contrast to the 'Great Lakes' type, is tolerant of elevated CO_2 levels.

It was suggested that opposing effects of applied BA and ABA could be due to a relationship between senescence and stomatal aperture (Thimann, 1980), ABA enhancing closure of stomata in the light, and cytokinins maintaining opening. This may well be the case in intact plants, but harvested leafy vegetables are detached plant parts. Transpiration, with its resulting wilting, is a major factor in initial deterioration. The older view of cytokinins delaying senescence through maintenance of RNA and protein synthesis is more applicable in this case, with a resultant delay in protein and chlorophyll degradation and a retardation of the respiration rate. Some of the benefits claimed for exogenous cytokinin treatments include longer retention of chlorophyll, inhibition of protein degradation, and

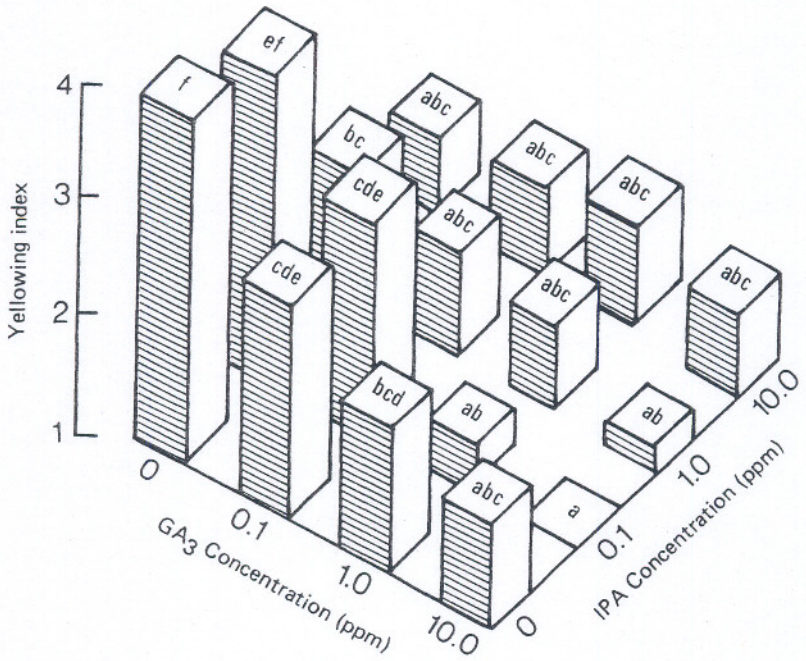

Figure 7 Yellowing of lettuce as affected by GA_3 and IPA. (From Aharoni et al., 1975.)

changes in respiratory rates. Some of these same effects however, can be achieved with prompt refrigeration, which reduces the loss of endogenous cytokinins.

VI. ABSCISIC ACID

Many postharvest phytohormone studies have been concerned with the induction and termination of the rest period, and whether rest is induced and prolonged by an endogenous inhibitor. The nature of the inhibitor has been under study since the presence of a growth inhibitor in a potato peel extract fraction was demonstrated by Hemberg (1947). The inhibitor declined naturally with the termination of rest or when sprouting was artificially accelerated by application of 2-chloroethanol to resting tubers. Hemberg's fraction from potato peels and extracts from other species were shown to have an inhibitory zone equivalent to the "β-inhibitor" of Bennett-Clark and Kefford (1953). It has since been shown with different cultivars that there is a correlation between the level of the inhibitor and the time required to emerge from rest (Wareing and Saunders, 1971) and that the preponderance of the inhibiting activity of the β-inhibitor complex is due not to phenolic compounds as originally thought but to dormin, a single, highly active fraction, subsequently identified with abscissin II or abscisic acid. This is not a very apt name, since the linkage of ABA with abscission is almost limited to young cotton (*Gossypium hirsutum* L.) fruit, where the name originated. It is also not the only controlling factor in true dormancy or rest. ABA is an important hormone, mediating seed development, stomatal closure, and plant stress responses.

Synthesis of ABA occurs in the leaves but also in roots (Davies, 1995), and it is transported from leaves to roots via the phloem and then recycled back to other sinks in the xylem stream. It is preferentially catabolized to phaseic acid and dihydrophaseic acid in the root of sugar beet, and ABA conjugation can take place in both the taproot sink and source leaves (Daie et al., 1981).

ABA has been identified as an agent in the stress-response pathway, inducing the expression of a characteristic set of genes encoding the ABA-responsive Rab (*r*esponse to *AB*A) or dehydrin proteins (Knight et al., 1995). These proteins accumulate in vegetative tissue under stress as well as in later stages of embryogenesis as Lea proteins (*l*ate *e*mbryogenesis *a*bundant). The response to ABA does not always require gene expression, as seen in its control of stomatal closure. But some of its effects are exerted by altering the transcription level of genes (Shen et al., 1996).

Mutants with altered ABA synthesis resulting in reduced levels of ABA, or with reduced sensitivity to ABA are known (Rock and Quatrano, 1995) in potato (*droopy*), pea (*wilty*), tomato (*flacca, sitiens, notabilis*), *Arabidopsis* (*abi, aba*), maize (*viviparous*), *Nicotiana* (*ckr1*), and barley, *Hordeum vulgare* L., (*Az34, cool*). The tomato mutants wilt if subjected to mild water stress, with reduced levels of endogenous ABA. Unfortunately, the potato mutant occurs in *Solanum phureja*, which has a nearly total lack of tuber dormancy, so it is not very helpful to studies of that trait (Suttle and Hultstrand, 1994). The *Arabidopsis aba* mutants are ABA-deficient, while those defective in the normal ABA responses, such as stomatal closure during water stress, are called *abi* mutants (ABA insensitive), and one has been cloned. The gene product also contains a domain highly homologous to a type of serine/threonine protein phosphatase, as are many of the ethylene and GA gene products, and with an N-terminal Ca^{2+} binding site. There is evidence for the involvement of Ca^{2+} in ABA responses (Bowler and Chua, 1994).

A. Vegetable Fruits

Developing sinks serve as sites for accumulation of ABA produced in source leaves, as seen in developing soybean (*Glycine max* L.) seeds. In a number of fruits, which are developing sinks, the level of free ABA is constant during maturation and increases during ripening (Fig. 1), and the rise in ABA is seen in both climacteric and nonclimacteric fruits (Rhodes, 1980). Free ABA accumulates in both attached and detached tomato fruit unless the fruit is detached very early, showing that ABA is also synthesized in the detached fruit and is not dependent only on translocation from the mother plant. Also the ratio of free:bound ABA is about 10:1 throughout ripening in avocado, so that the increase in free ABA in fruit must represent net synthesis rather than release of the bound form (Rhodes, 1980).

ABA-like inhibitors were detected in very young cherry-type tomato fruit, cv. Small Fry, increased in quantity during growth, peaked at the MG stage, and remained high during ripening (Abdel-Rahman et al., 1975). A similar role for both free and bound endogenous ABA was seen in normal cv. Rutgers tomato fruit and in the mutants *Nr, rin*, and *nor* from 9 days after anthesis through maturation and senescence (McGlasson and Adato, 1976). Free ABA concentration was low in Rutgers and *rin* until 19 days after anthesis when it increased to a maximum at 50 days, coinciding with maximum fruit size, reaching a peak at the MG stage and declining prior to the respiration climacteric and ripening, both in the pericarp and in the seed, though the latter peak was reached earlier. Concomitant with completion of growth and maximum ABA concentration, color change

occurred in 'Rutgers' and slight color changes in *rin* several days later. To reach maximum levels of free ABA required 55 days in *Nr* and 70 days in *nor*, which had lower ABA levels (McGlasson, 1978). Changes in bound ABA coincide with changes in free ABA but at approximately one-seventh the concentration, similar to the 10:1 ratio of free:bound ABA throughout avocado ripening.

Exogenous ABA at 3×10^{-5} and 10^{-3} M, infiltrated through the stem scar of detached fruit of 'Rutgers' and the mutant *rin*, reduced Rutgers ripening time by 50% as indicated by the onset of the respiratory climacteric, a rise in ethylene concentration, and the development of color, but did not increase *rin* ethylene production or color change (Mizrahi et al., 1975). It is apparent that some other factor beside ABA is required for ripening in *rin*. In the tomato mutant, *sitiens* (with the sit^w gene), where ABA synthesis is impaired, neither accumulation nor peak is seen in the seed, yet neither dry weight nor storage proteins are affected (Groot and Karssen, 1992).

ABA is a germination inhibitor in many seeds (Rock and Quatrano, 1995). ABA levels in seeds of abscising fruit are higher than those of persisting fruit and a similar tendency is seen in the peel, while shading also enhances the ABA level. Reduced ABA synthesis is associated with most of the viviparous mutants of maize, where precocious seedling development and seed sprouting occur on the ear, but is also found in carotenoid-deficient mutants, suggesting that the mutant genes control biochemical steps that are common to both carotenoid and ABA biosynthesis (McCarty, 1992). Application of the ABA and the carotenoid synthesis inhibitor fluridone to developing seeds results in vivipary. On the other hand, in tomato fruit, the osmotic environment within the tissues was proposed to be more important than endogenous ABA in preventing precocious germination of the developing seeds (Berry and Bewley, 1992). However, ABA-deficient tomato sit^w seeds germinate viviparously in overripe fruit that have been kept on the plant for weeks after fruit maturation. This is contrary to the case in wild-type fruit, and the osmotic potential of the fruit sap was similar in sit^w and the wild type (Groot and Karssen, 1992).

It has been suggested that ABA enhances sucrose uptake (or phloem unloading) by sink tissues and inhibits sucrose uptake by source tissues such as leaves, the latter assumed to represent phloem loading, or possibly reloading in fruit. However, the work on source tissue has conflicting results, and it was concluded that the regulation of sucrose uptake by ABA is complex, depending on the plant species and the type of tissue (Vreugdenhil and Kerckhoffs, 1992). ABA may function in the regulation of assimilate partitioning to developing seeds and to enhance sucrose accumulation, although there are again conflicting results using the ABA-deficient pea *wil* mutant and tomato sit^w mutant (Brenner and Cheikh, 1995).

Both sugar and ABA levels have been linked to chilling injury effects. Sucrose levels increase during chilling in several cultivars of tomato fruit (Crooks, 1985), and reducing sugar levels in peel are highest in 'Marsh' grapefruit (*Citrus* × *paradisi* Macfady) when seasonal resistance of the fruit to chilling injury is highest (Purvis et al., 1979). ABA levels have been linked to chilling injury in many cases, but also in fruit. Chilling MG tomatoes at 2°C for 12 days resulted in a two- to three-fold increase in free ABA in the pericarp (Ludford and Hillman, 1990). Temperature conditioning zucchini squash (*Cucurbita pepo* L.) at 10°C for 2 days increased the ABA content and also reduced the severity of subsequent chilling injury (Wang, 1991). Mefluidide, the acetamide growth regulator, increased chilling resistance in green and red bell pepper fruit (Lurie et al., 1995), possibly as a result of increasing ABA levels, as takes place following treatment in corn (Zhang et al., 1986).

Abscisic acid increases resistance to a range of environmental stresses at the whole plant level (Levitt, 1980), and also to fruit harvested from treated plants. Many genes that are induced by drought are responsive to ABA, and several genes expressed during drought in vegetative tissues are also expressed during desiccation of developing seeds. One such tomato gene, *pLE25*, was expressed only in seeds of MG fruit, shown by hybridization of halved tomato fruit blots, while at the breaker and red stage hybridization was observed in locular tissue (Bray, 1991).

B. Underground Storage Organs

ABA declines naturally with the termination of rest in potato, and several authors have shown a correlation between a low level of ABA and the breaking of bud dormancy in stored potatoes. Tubers on one-leaf cuttings growing under sprout-promoting conditions (35°C plus excised leaf) had a lower level of ABA compared with controls under cool conditions (van den Berg et al., 1991). However, changes in carbohydrate levels preceded this difference in ABA, with a conversion of starch to soluble sugars taking place under warm conditions. Therefore it is not certain whether ABA or sugar levels are a possible growth trigger and, in any case, ABA is not the only inhibitor involved. ABA activity may or may not increase under inducing conditions, and there is little increase in ABA activity of stolons at the earliest stages of tuber initiation (Ewing, 1995). Other studies looking at both endogenous and exogenous ABA and the bleaching herbicide fluridone show that endogenous ABA is essential for the induction and possibly the maintenance of potato microtuber dormancy (Suttle and Hultstrand, 1994). Continual exposure to fluridone, an inhibitor of carotenoid and ABA synthesis, results in the formation of microtubers that are nearly devoid of endogenous ABA and show precocious sprouting, while further treatment with exogenous ABA can both restore endogenous ABA levels and abolish the sprouting. ABA also increased the accumulation of sucrose in sugar beet, where application to root tissue discs caused a threefold increase within 1 hour (Brenner and Cheikh, 1995).

In onion, bioassays of the tops show a significant amount of inhibitor present at the stage when all foliage has fallen to the ground, so hormonal changes that induce the rest period are initiated while the onion is maturing and still in the field. At this point in onion development, it is customary to undercut the crop and, within a day or so, to harvest it. The results of this study (Isenberg et al., 1974) and those of Kato (1966) indicate that inhibitors are initiated in the leaf and presumably translocated to the bulb apex. This belief is strengthened by early sprouting of bulbs whose green tops are prematurely dried by a desiccator spray (Thomas and Isenberg, 1972) or are removed while green (Stow, 1976). Thus, in practice, it is important to avoid premature defoliation in order to allow sufficient inhibitor to move to the bulb apex to establish deep dormancy during storage.

Investigations of endogenous hormone balances in onion bulbs stored at low temperatures for an extended time period analogous to commercial practice suggest a duplication of the natural overwintering physiology of plants in a temperate zone environment. The European onion cultivars Lancastrian and Rijnburger, reputed to have very short rest periods, were stored at 5 to 8°C over a period from autumn through early spring (Thomas and Isenberg, 1972). Bulb tissues contained a high level of endogenous inhibitor activity with very low levels of growth promoters in late autumn. Inhibitor levels gradually declined to a very low level by midwinter, while growth promoter activities increased. A similar study in New York State, using Elba Globe and Copper Skin, which are F1 hybrids of the same parentage, gave similar but more comprehensive results (Isenberg et al., 1974).

The bulbs were cured in accordance with common agricultural practice, and then stored at 2°C over 9 months (late summer through spring). Bioassays of apical tissue (central plugs containing apices, root plate, and unexpanded leaves) again showed a high level of endogenous inhibitors during autumn, followed by a steady decline to about 50% of the original level by early winter (Fig. 2). Even so, the level of growth-inhibiting hormone activities was still quite high in relation to that of the growth promoting hormone activities, which were low from harvest to midwinter. By early spring, when most bulbs have well-developed internal leaves and some sprout even in storage, most hormones decline with the balance in favor of growth promoters. This period characterizes a state of compulsive growth, since no adverse environmental condition short of a lethal one can stop or prevent this growth. This situation terminates the usefulness of the onion bulb for commercial purposes.

When exogenous ABA was injected into onion bulbs, the onset of green leaf senescence could be reduced to 20 days (Table 7), whereas control bulbs required 28 days, and injection of growth promoters showed leaf senescence even later (Abdel-Rahman and Isenberg, 1974). Treatment with ABA also prolonged the dormant period of the resulting bulbs to 74 days as compared with 45 days for control bulbs, reduced bulb dimensions, suppressed rooting, and inhibited bulb sprouting. Of the growth promoters tried, only kinetin in combination with ABA could properly reverse the ABA inhibition by delaying the onset of leaf senescence and reducing the length of the dormant period (Table 7). Application of exogenous growth substances to excised onion apices again shows that only combination with cytokinin could partially overcome the inhibitory effect of ABA (Table 6), which effectively prolongs the innate dormancy or rest period (Mahotiere et al., 1976). Somewhat analogous effects have been reported for ABA applications to buds in other species (Wareing and Phillips, 1981).

The effectiveness of ABA treatments in retaining the bulb apices in a state of rest strongly suggests that ABA is part of the inhibitor complex maintaining this state. Again it seems that transition from the state of rest to dormancy is a complex phenomenon and probably requires the concerted action of several endogenous hormones.

C. Leafy Crops

'Green Winter' cabbage has an inhibitor-controlled rest period for several months after harvest, since the inhibitor rises rapidly for about 8 weeks before peaking, then declines for about the same time interval—i.e., after about 4 months of storage (Fig. 5). An interaction of inhibitors with cytokinins and GAs takes place during its storage life (Isenberg et al., 1974). The imposition of CA (2 to 3%, O_2, 5% CO_2) had no apparent effect on the inhibitors, which are ABA-like hormones.

The rest period can be demonstrated in other ways, reflecting the state of the apical meristems (Fig. 6). The vegetative apex remains flat, without elongating and being transformed into a floral apex. The inhibitor-controlled rest period was seen in the regrowth of axillary buds on decapitated, trimmed cabbage stems, which were rooted in potting mix and grown in the greenhouse after heads were removed from storage (Ludford and Hillman, 1984). The rate and type of regrowth varied with the storage conditions, probably reflecting the hormonal state of the cabbage head (Ludford and Isenberg, 1987). For cabbage straight from the field and after 4 weeks of storage, regrowth was slow and vegetative heads were formed. This could represent the rest period with a high level of ABA. Most plants bolted and flowered after cabbage was stored for 4 months in refrigerated air storage,

Table 7 Effect of ABA and Its Combinations with Other Plant Hormones on Plant Senescence and Size and Dormancy of Onion Bulbs

				Degree of dormancy			
				Rooting		Sprouting	
Treatment[a]	Senescence (days after injection)	Bulb dimensions		No. roots per bulb	Length (cm)	Bulbs sprouted (%)	Sprout length (cm)
		Diam. (cm)	Height (cm)				
Water	28	4.0	5.0	16.0	3.5	100	2.1
ABA	20	3.8	4.3	0.5	0.6	0	0.0
ABA + IAA	21	3.9	4.3	8.0	1.2	25	1.8
ABA + GA	26	3.9	3.5	0.0	0.0	25	2.1
ABA + K	34	3.3	3.2	8.0	1.8	75	2.8
ABA + IAA + GA + K	37	3.3	4.6	7.0	1.8	100	2.8
LSD ($p = 0.05$)	—	0.26	0.18	2.1	0.31	—	0.3

[a] Plants injected with 1 mL of water or 100 mg L^{-1} solution.

Source: Abdel-Rahman and Isenberg, 1974.

reflecting the end of the rest period, whereas those from CA remained vegetative even after 5 months. There was also a large difference in free endogenous ABA:IAA ratios found between air- and CA-stored cabbage, as well as in the subsequent regrowth of the stems. However, these analyses were done on large "apical samples," which included not just apices but stem, leaf bases, and axillary buds as well, the latter containing high levels of free ABA, which could disproportionately slant the hormonal results. The presence of 1 μL L^{-1} ethylene during storage speeded up regrowth and flowering in "Bartola" (Fig. 8A), and flowering took place even in CA-stored cabbage after 6 months with 1 μL L^{-1} and after 10 weeks with 5 μL L^{-1} ethylene present (Fig. 8B), overcoming the influence of the inhibitor (Ludford and Hillman, 1984).

A survey of 20 cabbage cultivars showed that some that held well after 4 months in air storage also tended to have slower axillary bud regrowth. Four were extremely slow, while in others growth was fast and final flowering luxuriant. Only two did not flower after air storage. The most interesting stage was a storage time of 10 weeks in air, since

Figure 8 Regrowth and flowering of stem axillary buds from "Bartola" cabbage heads stored for 10 weeks in air or CA. A. Air alone, air + 1 μl L^{-1} ethylene, air + 5 μl L^{-1} ethylene. B. CA alone, CA + 1 μl L^{-1} ethylene, CA + 5 μl L^{-1} ethylene. (From Ludford and Hillman, 1984.)

some cultivars remained vegetative and formed heads while others bolted and flowered, pointing to a possible borderline endogenous balance between ABA and the growth promoters for this time period (Ludford and Hillman, 1984).

Exogenous ABA application shortens the storage life of broccoli and Brussels sprouts (Thomas, 1981). Endogenous ABA is known to increase with wilting or stress, conditions that are always prevalent in a harvested organ. Thus, although exogenously applied ABA may induce senescence at very high concentrations, these senescence patterns may differ from the natural process.

Pithiness in the edible petiole of celery is a result of the formation of aerenchyma tissue or air spaces that form in the cortex. Root stress stimulates the condition and, while flooding and nutritional deficiency required a prolonged period, water-deprivation stress had a rapid effect (2 to 3 days) and was associated with an increase in endogenous free ABA, a common effect of wilting in leaves (Aloni and Pressman, 1979). However, the ABA level increased before the onset of pithiness. The application of exogenous ABA also stimulated petiole pithiness of detached celery leaves.

VII. OTHER PLANT GROWTH REGULATORS

A. Polyamines

Polyamines are present in all plant cells and are often essential to normal growth and development. However, their endogenous levels are about two orders of magnitude higher than those of traditional plant hormones. Their intercellular transport may also be limited, although there is some evidence for uptake and transport from roots to shoot via the xylem (Bagni and Pistocchi, 1991). There is thus some controversy as to whether they should be classified as hormones, although they do have a regulatory role that is more than a simple nutritional requirement (Galston and Kaur-Sawhney, 1995). The major polyamines in plants are putrescine (a diamine), spermidine (triamine), and spermine (tetramine), with increasing numbers of amino groups. A close physiological link between ethylene and polyamine biosynthesis would not be surprising, since SAM is a precursor common to the synthesis of both ethylene and the polyamines, spermidine and spermine, and these pathways may compete for the common precursor.

Polyamines occur naturally as free bases, which are positively charged at intercellular pH, or bound to phenolic acids such as cinnamic and ferulic (Galston and Kaur-Sawhney, 1995). A previously unknown polyamine conjugate, N^4-hexanoylspermidine, was found to accumulate during ovary and petal senescence in pea (Perez-Amador et al., 1996). The concentrations are high in green tissues and low in nongreen tissues, such as roots and petals at anthesis. Conjugation of spermidine with hexanoic acid is speculated to reduce its positive charge and affect its interactions with anionic groups as in membranes.

Polyamines are associated with rapid cell division, and are found in relatively high concentrations in young, actively growing tissues but their levels often decline with age. Senescence in many plant organs is therefore correlated with a decline in polyamines. A possible working model could involve binding of polyamines to membranes, prevention of lipid peroxidation, and quenching of free radicals (Evans and Malmberg, 1989).

1. Vegetable Fruits

Individual polyamine content varies with species (Casas et al., 1990), with putrescine being the major one in tomato. Free polyamine levels decline during fruit development

in tomato fruit (Saftner and Baldi, 1990). The level of polyamines is high in young tomato fruit during the cell-division phase, mostly in the form of conjugates (Egea-Cortines et al., 1993). During cell expansion and fruit ripening, the levels are comparatively lower, and they are free rather than conjugated.

In tomato, levels of free putrescine are high at the immature green stage, decline at the MG stage, and remain low through ripening. However, things are different in the Alcobaca tomato mutant, with the recessive allele *alc*, which has fruit that ripen partially and have prolonged keeping qualities with a long shelf life. Alcobaca putrescine levels rise again after the MG stage and become three times as high as in normal fruit at the ripe stage (Rastogi et al., 1993). Similar changes are seen in another longer keeping tomato, cv. Liberty (Saftner and Baldi, 1990). The elevated levels of putrescine in *alc* fruit appear to be age-related and take place whether or not they ripen (ripening is light dependent in *alc*). These putrescine increases are not due to changes in conjugation or metabolism, but to an increase in arginine decarboxylase (ADC) activity. However, there is no correlation in ripening *alc* fruit between increasing ADC activity and ADC mRNA levels, which peak at the breaker stage, and this suggests translational and/or posttranslational regulation of ADC expression in tomato fruit.

Both *alc* and 'Liberty' fruit also show a decrease in climacteric ethylene production. Since SAM is a precursor common to both ethylene and the polyamines, spermidine and spermine, if one goes up the other might be expected to go down. However, during ripening of detached tomato fruit, the onset of synthesis and accumulation of ACC, the ethylene precursor, is not a consequence of a decrease in spermidine synthesis (Casas et al., 1990). In addition, putrescine levels are unaffected by norbornadiene (an inhibitor of ethylene binding) in the ethylene-overproducing epinastic tomato mutant (*epi*) compared with normal (Belles et al., 1992).

Exogenous putrescine infiltration of MG fruit of 'Rutgers' or the line 'Alcobaca-red,' which has normal ripening and the *Alc* allele, increase their storage life in darkness. This was achieved by slowing softening, not by slowing down ripening (Law et al., 1991).

Endogenous putrescine accumulation is correlated with chilling injury in a species of *Capsicum*, and increased levels are found in response to other stress conditions. Treatment of cucumber fruit with stress levels of CO_2 (60%) caused increases in both ethylene and polyamine levels, particularly putrescine and spermidine. There were increased levels of respiration, ethylene, ACC, and activities of both ACS and ACO. This was interpreted to show that putrescine accumulation was induced by CO_2 stress, presumably through increased activity of ADC, and that ethylene and polyamine synthetic pathways may not compete actively for SAM, their common substrate, because of the increase in spermidine (Mathooko et al., 1995). When fruit were transferred from a CO_2-enriched atmosphere to air, all CO_2-induced levels declined to control values except for the polyamines, where putrescine decreased but spermidine continued to increase and spermine levels finally increased. If ACS was inhibited, then aminopropyl groups could be transferred from decarboxylated SAM to form spermidine and spermine from the previously high levels of putrescine. This, however, could indicate that there was indeed some competition for SAM earlier. Pretreatment with cycloheximide, an inhibitor of protein synthesis, blocked all the CO_2 stress-induction effects, whereas amino-oxyacetic acid, an inhibitor of ACS, only blocked induction of the ethylene synthesis pathway, including ACC. However, neither SAM levels nor activity of methionine adenosyl transferase were determined, and both of these have been indicated to increase by inhibition of ethylene action (Apelbaum, 1990).

2. Underground Storage Organs

The polyamines putrescine, spermidine, and spermine are found equally distributed in all parts of dormant potato tubers (Kaur-Sawhney et al., 1982), along with their biosynthetic enzymes (ADC, ornithine decarboxylase, and SAM decarboxylase). The breaking of dormancy and initiation of sprouting result in higher levels of these polyamines in the apical buds, but not in the dormant lateral buds or nonbud tissues. Thus the break of dormancy may also involve changes in polyamine levels, although it is uncertain whether these changes are the cause or the result of the breaking of dormancy.

Polyamines inhibit RNase activity in cut potato slices. Exogenous spermidine and spermine inhibit the rise in betacyanin leakage that normally takes place from cut discs of beet root. The presence of more than two amino groups, as in spermine and spermidine, appear necessary for this. They also counteract the detrimental effects of ammonium sulphate or ethylene, applied as ethephon, on cell permeability and pigment leakage in beet root and rose (*Rosa* sp.) petals (Parups, 1984). Free radical scavenging by polyamines is also correlated with the number of amino groups (Evans and Malmberg, 1989). Thus, polyamines may affect wound-induced or senescence-induced destabilization of cell membranes in plant storage organs.

3. Leafy Crops

A study of the involvement of polyamines in GA-induced internode growth in peas suggested that polyamines did not have a role in cell elongation but may be required to support cell proliferation (Smith et al., 1985). When levels were measured in shoot and root apices and expressed on a fresh weight basis, both spermidine and spermine showed a gradient, being very high in the meristematic regions and declining rapidly toward the region of cell elongation (Galston and Flores, 1991). The spermine gradient was still present when expressed on a per unit protein basis. In contrast, putrescine showed a completely opposite gradient, increasing toward the region of elongation. These gradients were found in etiolated pea seedlings, pea and corn roots, and other legume seedlings. Corn coleoptiles, which lack a meristematic region, had no such significant gradients.

Polyamines, particularly spermidine, are generally high in young tissues and decline as organs age and senesce. This is true in both intact and excised leaves, such as those of oats (*Avena sativa* L.), barley, rape, radish (*Raphanus sativus* L.), and pea (Kaur-Sawhney and Galston, 1991). This decline can be prevented by kinetin. However, not all reports support this hypothesis, and the effects of exogenous polyamine application do not always correlate well with trends of endogenous levels. For example, senescence began in the apical buds of a line of peas, where senescence is regulated by photoperiod, before the decline of polyamine levels (Smith and Davies, 1985). On the other hand, defruiting in this same line of peas, which should prevent apical bud senescence, increased polyamine levels and bud size.

Polyamines also provided considerable protection against ozone injury when they were given at low rates in solution to the cut stems of 21-day tomato shoots (Evans and Malmberg, 1989). This study may also indirectly support the possibility of polyamine transport. Polyamines could thus be useful protectants if this positive effect still takes place in intact plants of other species and if they are applied in a more practical way, such as foliar sprays.

B. Jasmonates

Jasmonic acid (JA) and methyl jasmonate are regarded by some as strong candidates for intracellular or intercellular messengers and as members of a signaling pathway leading to gene expression. Jasmonates are widespread in the plant kingdom and have been identified in many plants, including potato leaves and tubers and tomato fruit. However, their location within tissues and cells is not certain, although some data suggests that the chloroplast is the site of synthesis (Harms et al., 1995). They originate from the lipid pathway through linolenic acid via the action of lipoxidase and allene oxide cyclase and move readily in the liquid and vapor phase (Creelman and Mullet, 1995). As a matter of interest, methyl jasmonate is not only a constituent of the essential oil of *Jasminum grandiflorum* L. but also a component of female-attracting pheromones in certain moths, though this may have originated from their feed, green apples (Koda, 1992). Many of the effects of jasmonates are similar to those of ABA. By reprogramming the gene expression of plant cells, jasmonates are also able to deter pathogens and to respond to stress (Reinbothe et al., 1994).

Exogenous JA is even more efficient than ABA in the promotion of leaf senescence, including loss of chlorophyll. However, the role of jasmonates in senescence is not clear because highest endogenous levels are reported in young developing tissues, as in young leaves of soybean and flowers, with lower levels in roots and mature leaves (Creelman and Mullet, 1995).

Externally applied methyl jasmonate stimulates ethylene production, including ACO activity, in all stages of tomato ripening (Sembdner and Parthier, 1993). This is even the case in immature green tomatoes, particularly when chlorophyll in treated fruit has disappeared (Saniewski et al., 1987). Methyl jasmonate stimulates ACO activity in ripe and overripe tomatoes or else inhibits its degradation. However, since it has the opposite effect on ACO levels in other fruit, it is clear that the role of jasmonates through ethylene in fruit ripening needs further study, especially in nonclimacteric fruit.

The severity of chilling injury symptoms in cucumber and zucchini squash was reduced, and their onset delayed by 2 to 4 days, by treatment with methyl jasmonate, possibly through the regulation of ABA and polyamine levels (Wang and Buta, 1994). ABA increased in the exocarps after chilling, and more so in methyl jasmonate-treated fruit. Putrescine also increased after chilling, while spermine and spermidine decreased. Treated fruit maintained higher levels of spermidine and spermine than control fruit through storage at 5°C, but with no effect on putrescine levels.

Much hormone work has been carried out with potato, investigating the process of tuberization under inducing conditions. Jasmonic acid and its methyl ester, tuberonic acid, which is 2-OH-jasmonic acid, and the glucoside of tuberonic acid all have inhibitor activities that counteract the effects of GA and induce tuberization (Ewing, 1995). Tuberonic acid has strong tuber-inducing activities not only in potato, but also in yam (*Dioscorea batatas* Decne.) and Jerusalem artichoke (Koda, 1992). Cucurbic acid (which differs from JA in a hydroxy group instead of oxygen at C-3), its glycoside, and the methylglycoside are also active but not so much so, and were isolated from cucumber seeds (Koda, 1992).

The stimulation of phenylpropanoid metabolism in response to wounding and pathogen attack has been demonstrated in a number of plants. Jasmonates strongly induce the expression of the third and final step in the phenylpropanoid pathway in parsley cell cultures, while pretreatment with a lipoxygenase inhibitor reduces their responsiveness to

the elicitor and to wounding, showing that the elicitor response can be partially mimicked in parsley cells by jasmonate treatment (Ellard-Ivey and Douglas, 1996). Methyl jasmonate is also involved in another plant defense response, the pathogenesis-related proteins. Ethylene and methyl jasmonate have a synergistic effect on activating one of these groups of proteins, the osmotins (PR-5), in *Nicotiana*, and this is not a result of any effect on ethylene production (Xu et al., 1994). Methyl jasmonate is volatile (as is ethylene) and is presumably released to the atmosphere, so that sagebrush (*Artemesia tridentata* Nutt.), for example, which produces constitutively high levels, could induce defense responses in neighboring plants (Reinbothe et al., 1994). In fact, tomato leaves from small tomato plants had elevated levels of proteinase inhibitors I and II when incubated for 2 days in an airtight chamber with 5 g of fresh, leafy branches of sagebrush (Koda, 1992).

C. Camptothecin

Camptothecin is a purified alkaloidal extract from *Camptotheca acuminata* Decne, often called the sour gum tree, and is referred to as a naturally occurring growth regulator, which implies hormone-like characteristics. In the United States and possibly elsewhere, the small round red radishes 'Cherry Belle,' 'Revosa,' etc., are harvested and topped by machine. After washing, they are packaged in plastic bags, usually about 12 radishes to the unit. The distribution and sale of this produce can take from 1 to 3 weeks in the United States, not always under ideal low-temperature conditions. Consequently, 30 to 50% frequently show sprouting, which detracts from their visual sales appeal. A 5-min dip treatment of camptothecin (0.1 mM) suppressed sprouting of topped radishes held for 2 weeks at 10 and 20°C (Wang et al., 1980). Whether this material can be classified as a hormone or as another chemical additive, such as maleic hydrazide, will depend on the future establishment of its particular mode of action, but a use for this material has been shown in an area of need.

VIII. CONCLUSIONS

Endogenous hormones in vegetables do not cease to function after harvest but continue to perform their roles, as do other measurable biological systems, participating in or initiating physiological events during the postharvest regime, such as rest, dormancy, and regrowth of roots or sprouts. They cease to function only with the death of the organism, and they may be precursors to that event.

The effects observed are due more to tissue sensitivity and hormonal balance than to the concentration or activity of any one hormone. Binding and interconversion of various hormones through conjugation may be important prerequisites to the establishment of dormancy and the reactivity of dormant tissues. Interaction and balance between opposing promotive and inhibitory hormonal factors is the idea behind the control of metabolism in postharvest storage, whether this is of fruit vegetables with maturation and ripening or of vegetable storage organs with rest and regrowth. The interesting difference consists in which hormones compose these opposing factors. In leafy vegetative tissue, ethylene causes leaf abscission while cytokinins retard senescence. In fruit, ethylene is one important promoter of ripening and ABA seems to be another, while auxins, GAs, and cytokinins are candidates for the role of ripening inhibitors. In storage organs, ABA is more of an initiator of rest and dormancy and an inhibitor of regrowth, while auxins, GAs, and cytokinins may promote cellular activity and sprouting.

Hormones are thought to be part of a signaling pathway regulating the expression of relevant genes. Responses to developmental and environmental cues occur by stimulus-response coupling, where a signal is generated and transmitted (signal transduction) and a biochemical change is instigated (the response). This requires the recognition of the stimulus by a receptor as well as transmitting second messengers such as Ca^{2+} or effector proteins such as protein kinases to trigger the response, and it also needs negative control and cross-talk between pathways (Bowler and Chua, 1994). However, the same physiological event can sometimes be initiated by a variety of other factors—e.g., minerals, carbohydrate, light, CO_2, temperature, or water—indicating that there is a redundancy in signaling, of which growth substances are a part (Trewavas, 1992). It has been claimed that our known hormones do not have the requisite variety for a signaling system to match the variety of the situation that has to be controlled (Canny, 1985). This is based on the belief that hormone molecules carry complex information, whereas they may be agents with one simple bit—on or off—with concentration dependence for control of magnitude of response (Firn, 1985). There may instead be a multiplicity of target cells, with complexity resulting after the hormonal reaction, and this involves sensitivity. In any case, in addition to the hormone level itself, sensitivity to growth substances must be considered as a possible controlling factor, along with the characterization of receptors (Trewavas, 1992). There is a controversy over the relative importance of the change in concentration of a plant growth substance and the corresponding sensitivity to the rate of plant development.

A new approach to hormonal control looks at some plant hormones as antipodal modulators of elasticity within the actin network of plant cells. Microfilaments (actin) and microtubules (tubulin) form the most dynamic structural elements of the cytoskeleton. Changes in their organization occur as a result of signal-initiated alterations in subunit interactions, such as associated proteins or plasma membrane components. This model depends on control of cytoskeletal tension and organization by the formation and dissipation of temporal and spatial gradients of free Ca^{2+} and pH initiated by signaling hormone molecules. For instance, addition of auxins to soybean root cells results in a decrease in tension within the actin network of transvacuolar strands, which goes along with their effect on acidification of the cell cytoplasm/cell wall (Grabski and Schindler, 1996).

There is no point in continually improving yield if the result is only going to be poured down the drain, which is why postharvest studies are so important. Plant hormones obviously play an important role in the postharvest physiology of vegetables. A more adequate knowledge of the nature of phytohormone activity during postharvest handling and storage could well contribute more to the preservation of quality in harvested fresh vegetables than further improvements in mechanical technology.

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