2 The Plant Hormones

We begin our exploration of plant-based physiology by studying the plant hormones. We will cover 'the big five' then take a look at some of the lesser known, yet equally important ones (Table 2.1; Fig. 2.1). We'll finish up by looking at the substances, both natural and synthetic, we use in horticultural production to modify commodity quality, quantity or both. Though we elucidate very specific roles for each hormone, the net effect on plant growth and development is the sum of all their individual actions and their interactions with each other. It is a very complex and complicated subject, of which we only scratch the surface of understanding.

The Term 'Hormone'

There has always been somewhat of an argument among plant scientists as to whether or not the term 'hormone' is appropriate in our discussions of organic substances which affect plant growth and development. While many think the use of the term 'hormone' is completely acceptable, others consider the term 'plant growth regulator' or a variant thereof more appropriate. The controversy stems from the animal science definition of 'hormone'. In that description, a hormone is an organic substance synthesized in one tissue and transported to another where it elicits an effect on growth and/or development. The main difference in plants is that their 'hormones' can have an effect in the tissue or cell in which it was synthesized; plant hormones don't necessarily have to travel.

In this text, 'hormone' is defined as:

> A naturally occurring organic substance produced by the plant, which at very low concentrations, controls plant growth and development through effects on cell division, elongation and differentiation either in the tissue of synthesis or elsewhere in the organism via long distance transport.

In general there are two classes of plant hormones: (i) inhibitors; and (ii) promoters. Some hormones promote certain plant processes while others inhibit different aspects of plant growth and development. Additionally, some hormones inhibit a process at one stage of development in a plant's life while promoting the same process at another stage. We will look at each specific hormone and answer the following questions about each:

1. Where is it synthesized?
2. What tissue(s) does it affect?
3. What does it do?
4. How does it do it?
5. Why is it important (or how can we use it in horticulture)?

We will not explore each hormone's history or biochemical synthesis to any great extent. The reader is encouraged to review the excellent reference *Plant Hormones: Biosynthesis, Signal Transduction, Action!* by Davies (2004a) for a good discussion of such matters. We will emphasize the basics for each hormone and illustrate horticultural uses of each or related substances. Please note that the description of horticultural uses for any of the hormones discussed does not imply a legal label for use in any country or any endorsement by the author for its use. Please consult local experts for regulations on specific uses in your area.

The Big Five

Auxins

Description

An auxin is any organic substance that promotes cell elongation in tissue segments when applied at low concentrations (Davies, 2004b).

Discovery and nomenclature

The major plant based auxin, indole-3-acetic acid (IAA), was discovered as the substance responsible

Table 2.1. The major plant hormones, their site of biosynthesis, mode of translocation and general function(s) in horticultural physiology.

Hormone	Primary site of biosynthesis	Primary mode of translocation	Primary function(s)
Auxin	Young meristematic tissue	Mass flow in phloem; polar transport	Cell elongation; vascular differentiation; root initiation; apical dominance; stimulates ethylene production
Cytokinin	Root tips; developing seeds	Xylem	Stimulates cell division; overcomes apical dominance; stimulates leaf blade growth; stimulates cell expansion
Gibberellin	Root and shoot apical meristems; young leaves; young fruits; developing seeds	Often synthesized at site of action; phloem; xylem; cell to cell	Stimulates stem elongation; replaces vernalization requirement of some long-day plants; affects floral sex expression; stimulates hydrolases in some germinating seeds; inhibits leaf senescence; inhibits root growth
Ethylene	All living plant tissue	Diffusion	Promotes fruit ripening, senescence and abscission; promotes leaf abscission; promotes (*Ananas*) or delays (*Prunus*) flowering; promotes the production of female flowers; induces epinasty
Abscissic acid	Mature leaves and roots; developing seeds	Mostly phloem	Induces stomatal closure; induces cessation of embryo growth in developing seeds; induces storage of seed proteins and development of desiccation tolerance in seeds
Florigen	Leaf phloem	Phloem	Induces the transition of meristems from vegetative to reproductive
Brassinosteroids	Pollen, seeds and young vegetative tissue	Synthesized at site of action	Promotes organ elongation; inhibits root formation and growth; induces xylem differentiation; stimulates seed germination
Jasmonates	All living plant tissues; leaves; young developing fruit; cotyledons of germinated seeds	Synthesized at site of action; phloem; xylem	Induce tendril coiling; inhibits general stem and root growth, photosynthesis, and seed germination; induces the production of storage proteins in tubers, bulbs and seeds; induces plant defense responses to insect and pathogen attack; increases production of secondary metabolites with a role in plant defenses
Polyamines	All living plant cells especially actively dividing ones	Phloem; xylem	Enhances cell division; prevents mitotic senescence; delays leaf senescence; may help regulate flowering; inhibits ripening and senescence
Salicylic acid	Leaves	Mostly phloem	Signal in thermogenic plants; signaling hormone in plant resistance to pathogens; may be a signaling molecule for flowering

for coleoptile bending towards a light source. The general term 'auxins' is used to refer to the group of substances which have auxin-like properties or elicit auxin-like responses when applied to growing plants.

There are four naturally occurring auxins in plants: (i) IAA; (ii) indole-3-butyric acid (IBA); (iii) phenylacetic acid (PAA); and (iv) 4-chloroindole-3-acetic acid (4-CI-IAA). IAA and IBA are the most widely known of the four. IBA is readily converted into IAA, thus there is some argument that it is a precursor to IAA rather than an individually isolated auxin. The other two auxins, 4-CI-IAA and PAA are recent discoveries, thus have been found in relatively few species. They too may be precursors of IAA (Normanly *et al*., 2004).

The synthetic auxins include IBA, napthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid

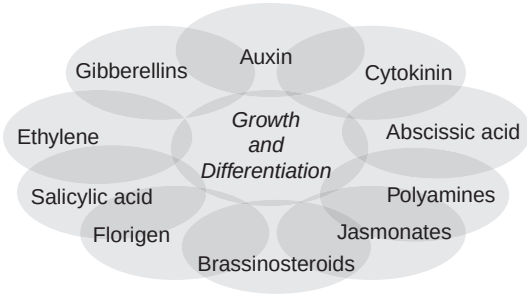

Fig. 2.1. The plant hormones.

(2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCPA).

Production

Auxin synthesis occurs in young meristematic tissues (Normanly *et al*., 2004). This includes the apical meristem of both the root and the shoot, developing embryos, young fruit, and young, rapidly growing leaves.

Transport

While auxin transport is generally described as basipetal, this is not entirely accurate. Basipetal movement occurs from either shoot or root apices towards the juncture of the root and stem. While auxin is synthesized in root apices, little if any moves towards the shoot. For the most part, auxin movement is from the shoot tip towards the root tip.

Auxin transport in cells and tissues is strongly polar, meaning that there is a very discernible gradient in auxin concentration. This gradient gives the 'signal' generated by the auxin direction. In fact, auxin itself may be responsible for developing and maintaining polarity in cells and the plant as a whole.

There are two modes of auxin transport in plants: (i) mass flow; and (ii) polar (Morris *et al*., 2004b). Auxin transport via mass flow occurs through the phloem from photosynthetic sources (leaves) to photosynthetic sinks (fruits, roots, and meristematic regions). Xylem is not involved in auxin transport via mass flow. Young leaves act as photosynthetic sinks early in their development and have a particularly high auxin content due to synthesis of auxin by the young leaf and auxin transported to them from other leaves via mass flow.

The second mechanism of auxin transport, polar transport, is much slower. In this system, auxin is moved from cell to cell from the shoot tip to the root tip. Movement is mediated by membrane-associated protein carriers and requires metabolic energy. Movement is through cambial cells and their derivatives, including developing xylem and phloem cells.

The two transport systems are not independent. Auxin does not enter the mass flow system from the polar system but auxin does enter the polar system from the mass flow system. This transfer from the phloem into the polar system occurs mostly in young tissues in the shoot apex. Movement via mass flow is easy to visualize as auxin going 'along for the ride' with photosynthates from the source to the sink. Polar movement is a little more complex.

Polar transport involves the active efflux of auxin from one cell into the apoplast followed by active uptake (influx) by an adjacent cell. This must occur along a cellular continuum from the shoot tip to the root tip and may occur against a gradient. It does not require cytoplasmic continuity between adjacent cells. In order to understand how these factors fit together, we need to examine polar transport at the cellular level.

Auxin is an acid. Non-dissociated auxin molecules in the apoplast (pH ~5.5) move across the plasma membrane into the cytoplasm (pH 7.0) and quickly dissociate. The auxin dissociates due to pH, thus as more molecules enter the cytoplasm, they dissociate as long as the pH of the cytoplasm remains around 7. The higher pH in the cytoplasm is maintained via an energy-requiring plasma-membrane-bound proton pump that pumps protons out of the cell which in turn acidifies the apoplast. Since auxin molecules continue to dissociate once in the cytoplasm, a concentration gradient for non-dissociated auxin exists across the plasma membrane and auxin can continue to move into the cell via diffusion. Auxin anions cannot freely cross the plasma membrane, thus they remain in the cytoplasm. The only way for them to move out of the cytoplasm is via an efflux carrier molecule in the plasma membrane. Auxin anions may also enter the cytoplasm from the apoplast via influx carriers which transport auxin anions across the plasma membrane into the cytoplasm (Hagen *et al*., 2004). The efflux of auxin molecules from the cytoplasm regulates the degree of the polar gradient.

Effects

CELL AND STEM ELONGATION There are many known effects of auxin on plant growth and development. One of the most widely studied effects is increased stem elongation associated with increased cellular elongation (Cleland, 2004). Auxin induces cell elongation within 10 min of application to stem or coleoptile segments resulting in a five to tenfold increase in length. While auxin stimulates stem elongation, it inhibits the elongation of root cells, particularly in the central zone of elongation. Just as pH influences auxin transport from cell to cell, pH is also important in eliciting the initial cell wall loosening associated with cell elongation due to auxin application. Auxin induces the active transport of protons out of the cytoplasm into the apoplast via an ATP-requiring proton pump. The decreased pH induces the manufacture and activity of wall-loosening enzymes. For prolonged stem elongation via cell elongation induced by auxin, the osmotic potential of the cytoplasm must remain low via import of solutes into the cell. This maintained low osmotic potential ensures the high turgor needed for cell expansion. Additionally, the cell wall must remain susceptible to auxin-induced wall loosening.

TROPIC RESPONSES Auxin is responsible for two very important plant tropic responses: (i) phototropism; and (ii) gravitropism. Both of these responses are discussed in Chapter 4, this volume. Briefly, auxin accumulates on the stem or root side away from the stimulus (the dark side for phototropic responses and the 'up' side for gravitropic responses), greater cell elongation occurs on that side as a direct result of auxin action, and the stem or root bends towards the stimulus.

CELL DIVISION AND VASCULAR DIFFERENTIATION Auxin stimulates cell division in the cambium and also regulates the differentiation of phloem and xylem tissue (Aloni, 2004).

ROOT INITIATION Auxin stimulates root initiation on stem cuttings and is used extensively for propagating hard-to-root species. While auxin stimulates root formation, it inhibits root elongation via stimulation of ethylene production.

LATERAL SHOOT INHIBITION (APICAL DOMINANCE) Auxin inhibits lateral shoot production in many species by inhibiting lateral bud growth. This is known as apical dominance. Growers often remove the apical meristem by pinching to encourage branching, resulting in a green thumb and a bushy plant. Additionally, auxin inhibits adventitious bud growth.

STIMULATION OF ETHYLENE PRODUCTION Auxin promotes flowering in bromeliads by inducing ethylene production. Additionally, auxin-stimulated ethylene stimulates the production of female flowers in cucurbits and other dioecious plants.

FRUIT SET AND GROWTH Auxin promotes fruit set in tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), holly (*Ilex* spp.), okra (*Abelmoschus esculentus*), figs (*Ficus* spp.), and cucurbits. Auxin also promotes fruit growth. In most fruit it is an absolute requirement. Fruit lacking a full complement of seeds are often small and misshapen compared with their fully seeded counterpart. Some species produce parthenocarpic fruit, fruit with no seeds. In these species, auxin is produced by the fruit tissue itself, rather than by the developing embryo in the seed.

FRUIT AND LEAF ABSCISSION Auxin can have completely opposite effects depending on the stage of plant development at the time of application. A great example of this is observed in apple (*Malus domestica*) production. Auxin applied to apple trees in the spring promotes abscission of many, but not all of the flowers. The idea is to remove excessive numbers of flowers to reduce competition among them for photosynthates. This process is called thinning. If not enough flowers or young fruit are removed during the thinning process, fruit quality including size will suffer severely, resulting in many small fruit. Excessive thinning would result in unacceptably low yields. About 3.5 months later, a similar auxin application results in the retention of fruit on the tree, exactly opposite to the earlier effect. Apples have a tendency to abscise prematurely, resulting in reduced yield and bruising of fallen fruit. The timing and rate of application for both uses must be carefully controlled to achieve the desired results.

Auxin helps regulate the formation of leaf abscission zones as well. When the relative level of auxin in a leaf versus its parent stem favors the leaf, an abscission zone does not develop. Once the auxin level in the leaf falls below that of the stem, the abscission zone forms and the leaf is ultimately shed.

Horticultural utilization

The naturally occurring auxin IAA is: (i) very expensive to extract from plant tissue; (ii) subject to degradation by enzymes; and (iii) extremely sensitive to degradation by light. Therefore horticultural uses of auxins involve the synthetic auxins, primarily IBA, NAA and 2,4-D.

One of the most prevalent uses of synthetic auxins is the use of 2,4-D, as a selective broadleaf herbicide. Even though it is considered a selective broadleaf herbicide, grasses may be affected as well. It is normally applied to foliage and is translocated in the phloem to multiple sinks in the plant. It kills plants by inducing uncontrolled growth, resulting in severe stem twisting and leaf malformation and overall plant dysfunction. Death of the affected plant is slow. Many grapes are extremely sensitive (parts per billion) to drift from 2,4-D applications, thus extreme care must be used when using it around vineyards.

Vegetative propagation via stem cuttings is enhanced using auxins. Exposing the basal end of a stem cutting to a powder (usually talcum powder) containing IBA or soaking the basal end in a solution of IBA in water often enhances root initiation of the cutting. The length for soaking and the concentration of IBA needed to enhance rooting varies with species.

Foliar application of NAA at 10 ppm is often used for thinning apples. Later in the season, foliar auxin is again utilized, however, then it is used to prevent pre-harvest drop.

Foliar application of NAA induces ethylene production which subsequently induces floral induction in pineapple.

Fruit set of tomato in the absence of adequate pollination and fertilization can be enhanced with auxin application.

Cytokinins

Description

Cytokinins are substances which promote cytokinesis in tissue culture (in the presence of auxin) (Davies, 2004b).

Discovery and nomenclature

Kinetin, the first known cytokinin, was discovered in autoclaved herring sperm DNA. It has never been isolated from plant tissue. The first plant-based cytokinin was *trans*-zeatin which was isolated from corn (maize) endosperm in the 1960s (Miller *et al*., 1955; Miller, 1961; Letham, 1963). Since then a number of cytokinins have been isolated from plant tissue. The naturally occurring cytokinins are mostly based on zeatin. They are often classified into active, storage or translocated forms. The two major synthetic cytokinins are benzyladenine (BA) and kinetin. Both compounds are stable as they are not subject to degradation caused by zeatin-metabolizing enzymes.

Production

Cytokinins are produced primarily in root tips and developing seeds. However, any tissue with a high rate of cell division may produce cytokinins (Sakakibara, 2004).

Transport

Cytokinins are transported primarily in the xylem. Phloem sap also contains cytokinins, but at much lower levels than xylem sap.

Effects

CELL DIVISION Cytokinins stimulate cell division in tissue culture callus, but growth is limited if auxins are absent. In the presence of auxin and cytokinins, callus growth is robust and undifferentiated. If the balance of auxin to cytokinin in tissue culture is shifted towards cytokinin, shoot production is favored. If it is shifted towards auxin, root production is favored. Thus by altering the levels of these hormones in the growth media, callus tissue can be stimulated to form shoots followed by roots, eventually leading to clonal production of new plantlets.

Stimulation of cell division also occurs in crown gall tumors as a result of infection by the bacteria *Agrobacterium tumefaciens*. The tumors are masses of undifferentiated cells often observed at the base or crown of a plant caused by an overproduction of cytokinins and auxins. This overproduction is caused by a small loop of non-chromosomal DNA called a plasmid carried by the bacteria. The plasmid carries the genetic code for auxin, zeatin and opine production. Opines are nitrogen-containing molecules that serve as food for the bacteria. When the plant is infected with the bacteria, the plasmid DNA is incorporated into the plant's genome and

the overproduction of auxin and cytokinin begins. As a result, a mass of non-differentiated tissue forms on the stem.

Control of cell division by cytokinins is through their control of the movement of cells out of the G2 phase of mitosis, regulated by cyclin-dependent protein kinases (CDKs) and cyclins (Roef and Onckelen, 2004). CDKs are enzymes responsible for adding a phosphate to a protein. Cyclins are proteins which regulate the CDK enzymes. The combination of CDKs and cyclins provide a regulatory switch for controlling the cell cycle.

OVERCOMING APICAL DOMINANCE Cytokinins stimulate the release of lateral buds from apical dominance imposed by auxin from the apical bud. In addition, cytokinin application promotes the development of dormant buds.

STIMULATION OF LEAF BLADE GROWTH Cytokinins stimulate leaf blade growth via cell enlargement. The balance between leaf area and root volume is regulated via this mechanism. A larger leaf area can be supported by a greater number of roots. With more roots, more cytokinins are produced and transported to the leaves, resulting in leaf blade enlargement. If the number of roots is limited, less leaf blade enlargement will occur. In addition, cytokinins promote chloroplast and chlorophyll development and delay leaf senescence. Cytokinins applied to a leaf will induce sink-like activity in the leaf, thereby promoting leaf longevity by directing nutrients to the leaf.

CELL EXPANSION Cytokinins can also stimulate cell expansion by increasing the plasticity of the cell wall. This increased cell wall plasticity is not related to the increased plasticity stimulated via acidification with auxin application.

Horticultural utilization

Use of cytokinins in production horticulture is widespread. Commercial formulations of 6-benzyladenine (BA) are available from a number of manufacturers, and are often a mixture of BA and gibberellins.

Fruit size and weight is enhanced via fruit thinning with an application of BA in apple (*M. domestica*) and pear (*Pyrus* spp.) production. Thinning also promotes annual bearing in normally biennial bearing cultivars. When environmental conditions are not favorable for producing the typical prominent calyx lobes in 'Red Delicious' apple cultivars, application of a mixture of BA and gibberellic acid (GA) promotes such a shape.

Enhanced lateral branching in nursery stock of apple (*M. domestica*), pear (*Pyrus* spp.) and cherry (*Prunus* spp.) can be induced with BA. This lateral branching accelerates the formation of a well-branched tree once transplanted in the orchard. Increased branch angles are also induced with nursery application of BA. Lateral branching of terminal growth in the orchard can be enhanced as well.

Application of a mixture of BA and GA to lily (*Lilium* spp.) bulbs delays senescence of lower leaves and open flowers. If gibberellin-inhibiting growth regulators have caused retarded growth in bedding-plant plug production, treatment with a BA/gibberellin combination helps overcome the growth inhibition.

A similar BA/gibberellin combination spray of poinsettia (*Euphorbia pulcherrima*) plants can promote overall vegetative growth if it is applied before short-day induction of flowering. A late-season spray can be used to promote bract expansion.

Another synthetic cytokinin, forchlorfenuron increases berry size and uniformity in table grape (*Vitus* spp.) and kiwifruit (*Actinidia* spp.) leading to increased yield. Fruit firmness and delayed ambering of green table grapes is also achieved.

Gibberellins

Description

The gibberellins are substances which promote stem, root and fruit growth (Davies, 2004b). There are over 110 different molecular forms of gibberellin and they are identified as gibberellic acid (GA)1, GA2, . . . GA*n*. Although they are similar in structure, they are all very different in their biological activity. Only about 30% of the known gibberellins are physiologically active with the rest likely to be breakdown products or precursors. GA1 is the most biologically active GA and GA3 is the easiest and least expensive GA to extract from fungal cultures for commercial use. All higher plants are assumed to contain GA in one form or another and they occur in free or conjugated forms.

Discovery and nomenclature

A devastating disease of rice causes an excessive elongation of seedling shoots leading to lodging of the

entire plant and ultimate crop losses. This disease is called the 'foolish seedling' disease and is caused by the fungus *Gibberella fujikuro* (Kurosawa, 1926; Brian *et al*., 1954). The substance (GA3) responsible for the pale, spindly weak growth of shoots was isolated from cultures of the fungus and given the name gibberellin. Since its discovery, over 110 forms of GA have been isolated from plant tissues.

Production

Growing meristematic tissue including root and shoot apical cells, young leaves, young fruits, and developing seeds (especially the endosperm) produce GA (Sponsel and Hedden, 2004).

Gibberellins are produced by fungal cultures and purified to obtain GA3 for use in commercial horticulture. GA3 is used because this is the only gibberellin obtainable in commercial quantities. A more expensive mixture of GA4 and GA7 is now available for specific uses.

Transport

GAs are often synthesized in the tissue where they elicit their effect. Transport occurs primarily in the phloem, but may also occur in the xylem and from cell to cell.

There is often a gradient of GA in plants that supports a high level of GA at the shoot apex which decreases basipetally. GA levels are often high in root tips as well. It appears that GAs are transported from the shoots to the roots via the phloem. There they may be transformed into a different form of GA and translocated back to the shoot via the xylem.

Effects

STEM ELONGATION GA induces extensive stem growth in many rosette plants and dwarf mutants. Stem elongation is a combination of enhanced cell elongation followed by increased cell division in the sub-apical meristem. Work with rice has indicated that the GA-induced cell elongation precedes GA-induced cell division. GA first enhances cell elongation via an increase in cell wall elasticity by a mechanism that is unknown and different than that caused by auxin. When cells are large enough, they transition from the G1 to the S phase of the cell cycle (Sun, 2004).

FLOWERING In some long-day plants requiring vernalization (exposure to a cold treatment) where flowering is preceded by stem elongation, the long-day and/or cold treatment can be replaced with GA. Examples of plants with either of these two requirements include celery (*Apium graveolens*), sugarbeet (*Beta vulgaris*), foxglove (*Digitalis purpurea*), and flowering stock (*Matthiola incana*). It seems that in these cases of enhanced flowering, it is the stem elongation prerequisite for flowering that is enhanced by the GA, not flower formation per se. In most other long-day plants whose stems do not elongate before flowering and in all short-day plants, GA treatment does not stimulate flowering.

In some specific long-day plants where stem elongation does not precede flowering (lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) and spinach (*Spinacia oleracea*)) the long-day stimulus can be replaced with GA. Additionally, GA promotes flowering in coneflower (*Asteraceae*), petunia (*Petunia* × *hybrida*) and Douglas fir (*Pseudotsuga* spp.) and enhances flower bud formation in cherries (*Prunus* spp.). GA treatment can also substitute for chilling in artichoke (*Cynara cardunculus*), resulting in earlier flower production.

Besides stimulating flowering in certain species, GA can also affect the floral sex expression. Treatment with GA promotes the production of male flowers in papaya (*Carica papaya*), cucumber (*Cucumis sativus*), and some melons (*Cucumis melo*). The production of female flowers is promoted in begonia (*Begonia* spp.), chinese chestnut (*Castanea mollissima*), and castor bean (*Ricinus communis*).

GAs generally stimulate pollen germination and subsequent pollen tube growth down the style leading to fertilization. In some species such as tomato (*S. lycopersicum*), grape (*Vitis* spp.), stone fruit (*Prunus* spp.), apples (*M. domestica*) and pears (*Pyrus* spp.), GA enhances fruit set above that which normally occurs. Improvement of fruit set in apples and pears is particularly important when adverse weather conditions during pollination results in poor natural fruit set. Parthenocarpy (fruit production without fertilization of the egg) in apples, pumpkin (*Cucurbita* spp.), and eggplant (*S. melongena*) may also be stimulated with GA.

When GAs are applied in the fall to plants which initiated flower buds the previous summer, normal spring flowering is inhibited. Plants in which this response has been observed include apple, grape,

and peach. This phenomenon has been investigated for its potential to avoid frost damage to flowers in the spring by delaying bloom.

SEED GERMINATION Certain seeds have a specific long-day photoperiodic requirement for germination. The long-day requirement (and thus a short night) leads to high levels of phytocrhome far red (P_{fr}) which stimulates germination. GA treatment can substitute for the long-day requirement and therefore the high P_{fr} requirement.

In grains such as barley (*Hordeum vulgare*), GA controls the formation and activity of hydrolases which metabolize starch into maltose, a key sugar in the brewing process. GA also improves the nutrient supply to the embryo during germination. Specifically, GA is produced by the cotyledon (scutellum) of the embryo and stimulates amylase production, which converts starch into simple sugars. These simple sugars are absorbed by the scutellum and translocated to the embryo for growth (Woodger *et al*., 2004).

GROWTH INHIBITION Gibberellins inhibit both leaf senescense and root growth. In addition, GA treatment reduces rind senescence in oranges (*Citrus* spp.) which permits longer 'on-tree' storage. This helps extend the marketing season.

Horticultural utilization

Enhanced stem elongation associated with GA is widely utilized in commercial horticulture. Celery (*A. graveolens*) and rhubarb (*Rheum* spp.) stalks are elongated by GA sprays. In the production of Thompson seedless grapes (*Vitis vinifera*) individual berries elongate in response to GA application. In addition, the entire cluster elongates, resulting in a larger cluster with larger fruit.

Parsley (*Petroselinum hortense*) yield is increased with GA. In roses (*Rosa* spp.), stems are elongated using GA. Tree geraniums (*Pelargonium* spp.) are created by stimulating stem elongation with GA combined with careful pruning.

Sugarcane (*Saccharum* spp.) yield is increased with GA treatment via two mechanisms. Stem elongation is enhanced concomitant with increased sucrose production such that the sugar concentration in the stem is not diluted by the elongation induced by the GA.

There are a number of chemicals which inhibit gibberellin synthesis that are used extensively in ornamental horticuluture including Phosphon D, CCC (cycocel), Amo1618, Ancymidol (A-rest), paclobutrazol and B-Nine (alar). These types of substances will be discussed later in this chapter under 'Synthetic Plant Growth Regulators and Their Uses in Horticulture'.

Ethylene

Description

Ethylene is a single substance which exists in nature as a gas. It is the only hydrocarbon with a pronounced effect on plant growth and development (Adams and Yang, 1981; Davies, 2004b; Pech *et al*., 2004).

Discovery and nomenclature

Ethylene in illuminating gas used to light street lamps in the late 1800s was identified as the substance responsible for premature defoliation and stunted growth observed in plants located around street gas lights, especially leaky ones (Doubt, 1917). Ethylene is a single substance therefore there is no need for a discussion of nomenclature.

Production

Ethylene is produced in all plant tissue, often as a response to stress such as drought, flooding, mechanical pressure, injury, or infection. Ethylene production is often stimulated by auxin. Meristematic regions and senescing tissues, especially fruit, are rich sources of ethylene gas. In general, nodes produce more ethylene than internodes.

Ethylene is produced from methionine, an amino acid (Pech *et al*., 2004). ATP reacts with methionine to form *S*-adenosyl-L-methionine (SAM), a carrier molecule for methionine. SAM is converted to 1-aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase. It is the activity of this enzyme which regulates ethylene production in plant tissues. ACC synthase is coded by a multi-gene family and is found in the cytosol. It is induced by auxins, flower senescence, fruit ripening, wounding, chilling injury, flooding, drought, and ethylene. This stimulation of ACC synthase by ethylene makes ethylene production autocatalytic. This is important. Once ethylene production is induced in ripening fruit,

more and more ethylene is produced, further accelerating ripening and senescence.

There are a number of known ethylene inhibitors. Silver ions, CO_2 and $KMnO_4$ inhibit ethylene at the site of activity by interfering with binding of ethylene to receptors. Aminovinylglycine (AVG) and aminooxyacetic acid (AOA) inhibit ACC synthase, thereby regulating ethylene production rather than reception.

Transport

Ethylene travels through the plant via diffusion from cell to cell. Since it is produced in all tissues, its transport is not normally necessary for an effect to be realized. Ethylene also diffuses out of plant tissue and into the atmosphere, thus elevated levels within plant tissues depends on continued production.

Effects

Ethylene has many known effects on plant growth and development. Much of our knowledge of ethylene activity comes from exposing plant tissues or organs with ethylene in enclosed containers. With the development of ethephon (2-chloroethylphosphonic acid) ethylene application is greatly simplified. Ethephon is applied as an aqueous spray and is absorbed into plant tissues. There it decomposes to release ethylene gas, chloride, and phosphate ions.

FRUIT RIPENING, SENESCENCE, AND ABSCISSION Ethylene promotes fruit ripening, senescence, and abscission.

LEAF ABSCISSION Ethylene promotes leaf abscission. Even though ethylene is generally universally present in plant tissues, auxins produced by the leaf or fruit reduce the sensitivity to ethylene of abscission zone cells in leaves and fruit. As auxin levels decline, abscission zone cells become more sensitive to ethylene and the production of cellulases increases. Cellulases weaken wall connections and the weight of the leaf or fruit is enough to allow the leaf or fruit to fall off the plant.

FLOWERING Ethylene promotes flowering in bromeliads, which includes pineapple (*Ananas comosus*).

On the other end of the spectrum, ethylene can delay flowering in some *Prunus* species. When ethylene applied as ethephon to peach (*Prunus persica*) or cherry (*Prunus avium*) in the fall at approximately 50% leaf fall, flowering is delayed the following spring by as much as 14 days. This fall application of ethylene also increases the cold hardiness of peach flower buds by reducing the size and water content and increasing the sugar content of the pistil. Even though pistil size is reduced with the ethylene application, final fruit size is not affected. In addition, the dehardening response often observed in *Prunus* flower buds upon exposure to warm temperatures after the chilling requirement has been fulfilled is greatly reduced.

Ethylene applied as ethephon promotes femaleness in many crops.

EPINASTY A common malady of houseplants is overwatering. Excessive watering leads to ethylene production by the plant which induces epinasty, a downward bending or drooping of leaves. Caretakers often see this drooping as a sign of water stress and proceed to water the plant even more. This only exacerbates the problem.

THE ETHYLENE TRIPLE RESPONSE A widely used plant indicator of ethylene is the triple response of pea (*Pisum sativum*). Pea seedlings treated with ethylene show a triple response to the gas proportional to the level of exposure. The three responses include: (i) greatly shortened internodes; (ii) increased stem diameter; and (iii) a lack of the normal gravitropic response (stems growing up and roots growing down). Furthermore, leaves fail to expand and the shoot apex remains hooked.

THIGMOMORPHOGENESIS Responses of plants to touch, thigmomorphogenesis, are usually attributable to ethylene action.

ROOT GROWTH Ethylene stimulates auxin biosynthesis and transport towards the root elongation zone where it leads to the inhibition of cell elongation, thus resulting in reduced root growth.

SEED GERMINATION AND BUD SPROUTING The stimulation of germination in cereals and peanuts as well as sprouting in potatoes and bulb crops is also attributed to ethylene action.

FRUIT RIPENING Controlling the level of ethylene in produce storage facilities is crucial in regulating postharvest physiology of horticultural crops.

This aspect of ethylene action will be discussed in Chapter 17, this volume. Ethylene enhances ripening of fruit that are harvested mature but not ripe, for example, banana (*Musa* spp.).

LATEX FLOW Ethephon enhances latex flow in rubber trees (*Hevea brasiliensis*) by delaying the healing of tapping wounds. Tapping wounds are the cuts made to allow latex to flow from the plant.

STEM ELONGATION AND THICKENING Ethylene inhibits stem elongation in terrestrial plants with a concomitant increase in stem thickness. Stem thickening is attributed to reorientation of microtubules and microfibrils from mostly transverse to oblique. In contrast, many semi-aquatic plant stems elongate rapidly upon submergence and its concomitant accumulation of ethylene in the underwater tissue. The stem elongation is a result of increased sensitivity to GA induced by the ethylene. The ethylene also causes a decline in abscisic acid, which is a potent inhibitor of GA. Thus the increased response to GA may actually be due to the reduction in abscisic acid caused by increased ethylene production.

Horticultural utilization

Ethylene is used as a harvest aid to promote fruit abscission in cherry (*Prunus* spp.), apple (*M. domestica*), citrus (*Citrus* spp.), nuts and olives (*Olea europaea*). In addition, ethephon is used to enhance uniform coloration and ripening of tomatoes (*S. lycopersicum*) for mechanical harvesting.

In commercial pineapple (*A. comosus*) fields, natural production of ethylene by the plant is stimulated with applications of auxins or with an application of ethylene via ethephon. Plants are treated when they are 6 months old when they are at the 30 leaf stage, about 3 months prior to their natural time of bloom. Induction of flowering with ethylene promotes uniform flowering, fruiting, and cropping.

Ethylene also promotes female flower production in cucurbits (cucumber, squash, melons (*Cucurbita* spp.)) increasing the number of fruits produced per plant.

Abscisic acid

Description

Abscisic acid (ABA) is often described as an inhibitor. This is unfortunate as ABA promotes several physiological components of plant growth and development.

Discovery

Abscisic acid was initially discovered as an inhibitor of oat (*Avena sativa*) coleoptile growth. Soon after, a 'similar' substance named abscission II was described that stimulated the abscission of cotton (*Gossypium* spp.) bolls. Another substance produced in sycamore (*Plantanus* spp.) leaves that promoted bud dormancy was discovered by another group who named the substance dormin. All three groups were actually working with the same substance and in 1967 the name abscisic acid was given to this new plant hormone. Unfortunately, the name really does not describe the function, since ABA has little involvement in the control of fruit and leaf abscission and relatively little involvement in the control of bud dormancy.

Nomenclature

ABA is a single substance, rather than a group of related substances as in the auxins, GAs and cytokinins. There are a number of forms of the molecule, however, the most prevalent form in plants is (+)-2-*cis*, 4-*trans* abscisic acid, more simply known as abscisic acid or ABA.

Production

ABA is primarily synthesized in mature leaves and roots in response to water stress (Schwartz and Zeevaart, 2004). It may also be synthesized in just about all other plant tissues. Developing seeds are also rich in ABA which is either synthesized *in situ* or imported from leaves or roots.

It is mostly synthesized from carotenoids. Extremely large changes in endogenous levels of ABA can occur rapidly in response to stress and ABA levels are regulated at a number of levels. ABA levels can vary via degradation, compartmentalization, transport to other tissues, conjugation with a sugar, or conversion into phaseic or dihydrophaseic acid.

Transport

Long-distance transport is mostly in the phloem and to a lesser degree, in the xylem. At the cellular level, ABA exists in different forms depending on pH.

At a neutral pH, ABA exists in a dissociated state. At a mildly acidic pH (5.0–6.5) ABA is mostly undissociated. At more acidic pHs (<5.0) ABA is mostly in a protonated form. The undissociated and protonated forms diffuse freely across cell membranes but the anionic form requires active uptake via carriers.

Effects

Although ABA is often classified as a growth inhibitor, this is unfortunate since ABA promotes certain aspects of plant development such as seed maturation, dormancy, and plant survival under certain stresses. As with all hormones, the response to exogenous ABA depends on the tissue and stage of development. ABA applied to hypocotyls, epicotyls, coleoptiles, and leaves generally results in growth inhibition. Application to roots can either inhibit or promote growth. In germinating seeds and excised embryos, ABA inhibits further embryo growth and development. ABA is also known to prevent vivipary, the uninterrupted development of embryos without a dormant period. ABA seems to have very limited involvement in abscission and senescence of leaves and fruits. ABA counteracts the effect of gibberellin on α-amylase synthesis in germinating cereal grains. ABA inhibits cell division in fronds and roots of *Lemna minor* perhaps by promoting the production of a protein which inhibits kinase activity in cyclin–CDK complexes in the cell cycle. There are limited reports of ABA promoting cold hardiness, but much of the work in this area of ABA involvement in plant growth and development has been inconsistent.

PLANT STRESS AND CROSS-PROTECTION ABA increases during times of plant stress, providing protection from the stress via various mechanisms. In this sense, ABA acts as a promotor, promoting plant survival. During drought stress, ABA levels in leaves rise dramatically, causing closure of stomata and the production of proteins known to protect membranes and other cellular structures during dehydration. Proteins also reduce the osmotic potential of the cytoplasm, further preserving what water is left in the cell. Similar proteins are also important in protecting seeds from the severe dehydration which accompanies their maturation. In addition, synthesis of seed storage proteins is enhanced with ABA.

The general response of increased synthesis of specific proteins during many different types of stresses such as stress brought about by salt, heat, and pathogen attack, has led to the description of ABA as the stress hormone in plants. When a plant elicits a protective response to one form of stress, protection from other potential stresses is also incurred. This is known as 'cross-protection'.

WATER STRESS RESPONSES Water stress can be induced by drought, salinity, and freezing temperatures. Independent of the mode of stress induction leading to cellular dehydration, water stress induces a rapid increase in ABA synthesis. The increase in tissue ABA content is transient, since upon removal of the stress, ABA synthesis decreases and levels decline accordingly. Morover, even if the stress is not removed, ABA levels begin to decline. Since ABA levels do not remain high concomitant with the stress, ABA must be a signal for cellular stress-coping mechanisms to occur.

ABA application can mimic drought stress responses by plants, namely reduced bud and shoot growth, stomatal closure, and reduced photosynthesis. Proteins which form in response to the stress can be induced with an application of ABA. ABA-deficient mutants of *Arabidopsis* and tomato (*S. lycopersicum*) cannot tolerate drought stress (their stomata won't close), however, application of ABA to mutant plants under stress elicits responses similar to those of non-mutants under stress.

Dehydration stress induces ABA synthesis which induces the formation of specific stress proteins. But how does a plant sense the dehydration stress to induce ABA synthesis? Several things happen at the cellular level concomitant with dehydration. There is: (i) a decrease in cellular water content along with an increase in solute concentration; (ii) a decrease in osmotic potential of the cytoplasm; and (iii) a reduction in cellular turgor. The component most highly related to ABA synthesis is the loss of cellular turgor and perturbations of the plasma membrane associated with it. How this triggers ABA synthesis is not known.

The initial stimulus of dehydration is probably perceived first by the roots and the signal, in the form of ABA, is sent to the leaves to induce stomatal closure (Fig. 2.2). Stomatal closing has been more closely associated with soil water potential rather than leaf water potential. Lowered soil water potential causes a significant increase in xylem sap ABA concentration.

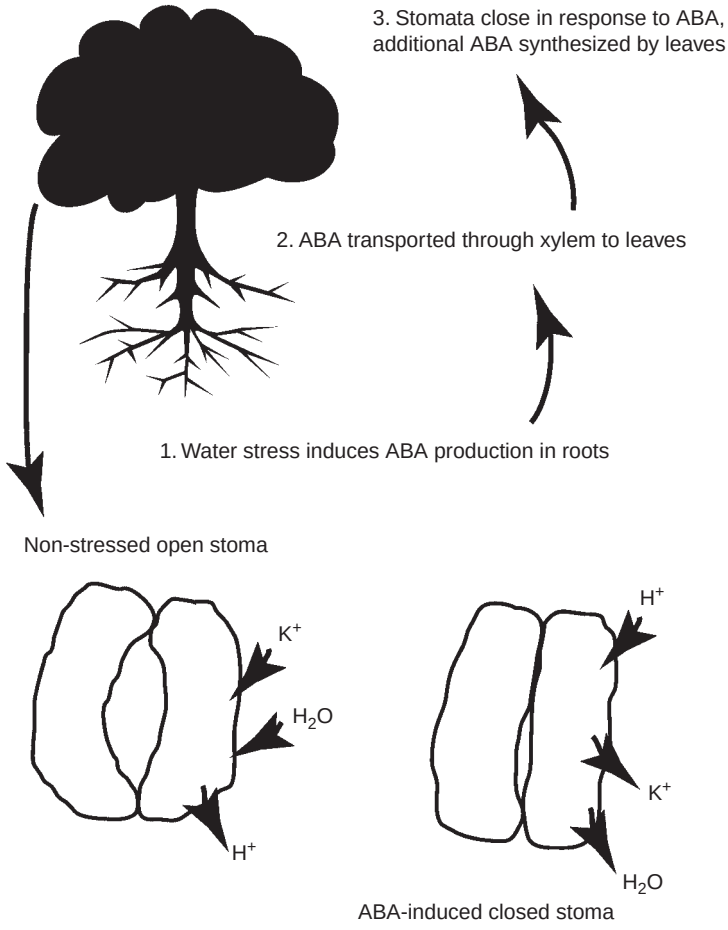

Fig. 2.2. The water-stress-induced response in plants regulated by abscisic acid (ABA).

The concentration of ABA in the xylem is more closely correlated to closing of stomata than either leaf water potential or leaf ABA concentration. While there is convincing evidence for the transfer of the stress signal from the roots to the leaves via ABA in the xylem, one cannot say conclusively that all of the ABA originated in the roots. Some ABA from the shoot may also enter the xylem.

Wherever the ABA originates, it eventually accumulates in the apoplast of leaf epidermal guard cells. Guard cells have no plasmodesmatal connections with any adjacent epidermal or mesophyll cells. Any signal must be transmitted via the apoplast. The ABA receptor at the guard cell remains unidentified. It does, however, seem likely to be a receptor on the plasma membrane. Whether it is located on the apoplastic or symplastic side is still not known. Direct injections of ABA into the cytosol induce responses associated with increased ABA levels, thus at least some of the reception occurs on the symplastic side of the membrane. However, the stomatal response to ABA is more closely related to apoplastic ABA levels, especially under mild to moderate stress conditions.

ABA inhibits stomatal opening and promotes stomatal closure via control of ion-mediated osmotic changes in the guard cells.

Stomatal opening can be triggered by blue and red light, low internal CO_2 concentrations, high atmospheric humidity and the fungal toxin fusicoccin. These stimuli activate ATP-driven proton pumps on the plasma membrane that force H^+ out of the cytosol and into the apoplast. This reduces

the apoplastic pH and increases the transmembrane potential to a more negative value. This in turn stimulates K^+ uptake through voltage-regulated channels in the membrane. Guard cells also take up Cl^- ions, but the mechanism is not known. In addition malate ions act as counterions for the K^+.

During the dehydration stress the apoplastic ABA signal inhibits the ATP-driven proton pumps and induces a decrease in cytosolic pH, an increase in cytosolic Ca^{2+} concentration, inhibits the inward influx channels of K^+ and depolarizes the plasma membrane. The increased Ca^{2+} levels in the cytosol inhibits inward movement of K^+ and at the same time promotes the efflux of K^+ out of the cytosol. The depolarization of the membrane also causes K^+ ions to leave the cytosol. The overall loss of K^+ from the cytosol is concomitant with an efflux of water from the guard cell and the resulting decrease in guard cell turgor allows the stomatal pore to close.

Once the dehydration stress is over, the ATP pumps resume their work and the apoplast pH drops and ABA re-enters the symplast for storage or metabolism. Stomatal opening ensues and apoplastic ABA levels return to pre-stress levels awaiting the next dehydration event, where they will once again increase and the process starts all over again.

The previous description is of the rapid, almost immediate short-term responses induced by ABA under drought stress. Longer-term drought stress incorporates changes in gene expression induced by ABA. In particular, proteins involved in K^+ influx decrease and enzymes involved in carbon metabolism show differential expression after longer-term (2–4 days) drought stress.

SEED MATURATION AND DORMANCY A second major function of ABA is its involvement in seed maturation and dormancy. In general, seed development is often separated into three stages: (i) morphogenesis; (ii) cell enlargement with reserve accumulation; and (iii) developmental arrest and desiccation. The latter two are often combined into a collective stage called maturation. One of the first steps in this process is the cessation of embryo growth due to cell cycle arrest at the G1/S transition which is concomitant with a sharp increase in seed ABA levels. The second part of maturation is the accumulation of storage reserves accompanying cell enlargement and dehydration. It is during this stage that ABA levels in the seed reach their highest. The final stage includes the development of desiccation tolerance, water loss, and a decrease in ABA levels. ABA has a limited role in the induction and maintenance of dormancy in some seeds, but does not seem to play a major role in the regulation of 'true' dormancy that is only broken with low temperature or light.

Horticultural utilization

There are no practical uses of ABA in horticulture. ABA is very expensive to synthesize. Additionally, it degrades rapidly in the light. Analogs of ABA have been developed in the quest to find a chemical which could confer drought resistance. Increases in cold hardiness have been associated with ABA and ABA analog application, however, consistent positive results are lacking.

Some Other Growth Regulating Substances

A number of other substances having growth regulator properties have been identified in plants. Some of the more popular ones are presented in the following section. Whether or not these substances are "officially" classified as plant hormones is academic. They have profound effects on plant growth and development and possess many of the characteristics of the classic hormones, thus they are important.

Florigen

A long popular theory in plant science is that flowering is triggered by the relative levels of the five major plant hormones that are modified by changing environmental conditions. When environmental conditions are favorable for flowering in sensitive species, there is a balance of hormones in the plant which promotes this transition. This idea is rather complicated as simultaneous measurement of the five major hormones is difficult and different environmental conditions can induce flowering in the same species.

Many plant scientists have also sought a single substance, 'florigen', that is responsible for the transition from the vegetative to the sexually reproductive state in plants. The existence for such a substance was controversial, however, molecular-level research with *Arabidopsis* led to the discovery of florigen (Zeevaart, 2008). *Arabidopsis* is a model plant which is induced to flower with long-day exposure. There are two genes responsible for flowering in *Arabidopsis*, the CONSTANS (CO) gene and the FLOWERING LOCUS (FL) gene.

The CO gene encodes a protein that when exposed to long days induces the transcription of the second gene (FT) in the phloem of leaves. Florigen is the FT gene product, a mobile protein of approximately 20 kDa which is transported through the phloem to a receptive meristem causing flowering. While all other hormones are extractable substances which can be applied to plants and induce specific responses, the proteinaceous nature of florigen precludes such extraction and application. However, molecular techniques have allowed transplanting the FT gene to other species. Overexpression of the FT gene has led to flowering under non-inductive conditions in many species. In addition, overexpression of the FT gene greatly reduced the juvenile period in perennial species.

Florigen is required for flowering in all plants and is not species specific. Florigen is produced in leaves of photoperiodically sensitive species under the control of phytochrome. In day-neutral species, the level of florigen, and therefore flowering, is not regulated by daylength.

Brassinosteroids

The term brassinosteroid (BR) refers to the naturally occurring steroids found in plants that elicit growth responses in nanomolar or micromolar doses. A substance first isolated from rape (*Brassica napus*) pollen was given the name brassin and later identified as brassinolide, the first plant steroid with growth regulating activity. Brassinolide caused extreme elongation of pinto bean internodes. Since brassinolide's discovery, more than 50 steroids have been isolated from many different species, thus BRs are likely to be ubiquitous in the plant kingdom. The two most common steroids in plants are brassinolide and castasterone, a brassinolide precursor.

BRs are produced in almost all plant tissues, but especially in seeds, pollen, and young vegetative tissue (Choe, 2004). Their presence in roots has not been confirmed, and exogenous application of BR inhibits root formation and growth. BRs promote stem, petiole, and peduncle elongation in dicots and promotes elongation of colepotiles and mesocotyls in monocots. BRs often promote organ elongation by promoting cell wall loosening and subsequent cell elongation but not cell division. In some species, BRs will stimulate cell division in the presence of auxin and cytokinin. They do so by regulating the production of the cyclin 3 protein which is important in the release of cells from the G1 to the S stage of the cell cycle.

BR-deficient mutants of *Arabidopsis*, tomato and pea all exhibit severe lack of stem elongation. Exogenous BR application fully restores normal stem elongation in such mutants, indicating that BRs are essential for normal stem elongation.

BRs are also involved in plant responses to light. BR-deficient mutants of tomato and *Arabidopsis* grown in the dark often lack an apical hook, have expanded cotyledons, form true leaves, and express genes associated with photosynthesis and anthocyanin production. All of these attributes are normally observed in light-grown seedlings! Thus a lack of BR simulates the presence of light.

BRs also stimulate tracheid formation in differentiating xylem tissue. In BR-deficient mutants, too much phloem and not enough xylem are differentiated. Additionally, deficient mutants are male sterile, and plant senescence is delayed. Pollen tube growth is promoted by BRs. Application of BR enhances ethylene production and subsequently promotes epinasty, senescence, and leaf abscision.

BRs can reverse an ABA-induced seed dormancy while stimulating germination.

Although there are many reports of enhanced yield, vigor and stress tolerance with the application of exogenous BRs, they are extremely inconsistent.

Jasmonates

Jasmonates are a group of oxylipins (oxygenated fatty acid derivatives) which include jasmonic acid and methyl jasmonate (Howe, 2004). Methyl jasmonate is a fragrant volatile component of the essential oils of rosemary (*Rosmarinus officinalis*), jasmine (*Jasminum*), and many other flowers.

In early studies in the 1980s, both jasmonic acid and methyl jasmonate were observed to retard root and coleoptile growth and promote leaf senescence.

Jasmonates are found in many higher plants, green and red algae, and in some fungi. They are produced in all plant tissues, especially upon wounding. Leaves, young developing fruit, and cotyledons of germinated seeds are particularly rich in jasmonates. Even though jasmonic acid is more prevalent in plant tissue than methyl jasmonate, plant responses to exogenous applications are more readily observed when methyl jasmonate rather than jasmonic acid is applied.

Jasmonates can induce tendril coiling, much like that caused by ethylene. Jasmonates inhibit general stem and root growth, photosynthesis, and seed germination. Jasmonates induce the production of storage proteins in tubers, bulbs, and seeds. Jasmonates are important for promoting male reproductive development in *Arabidopsis*. They also stimulate potato (*Solanum tuberosum*) tuberization, fruit ripening, leaf and flower senescence, and abscission.

Jasmonates have been identified as the major compounds responsible for inducing plant defense responses to insect and pathogen attack. When a plant is mechanically wounded or an insect chews on a leaf, an 18 amino acid protein called systemin is produced. Systemin is derived from a larger protein called prosystemin. Upon chewing, the gene for prosystemin production is activated in the vascular tissue and systemin is translocated in the phloem. Proteins associated with increased defense are synthesized in leaves near and far from the attack or injury as a result of the systemin signal.

In addition, oligosacharride fragments may be released from cell walls and the fragments may act as elicitors. Elicitors are compounds (proteins, peptides, lipids, and polysaccharides) of microbial origin which initiate a plant's defense response system. These elicitors may not travel far from the attack site, but can induce the gene for prosystemin which leads to higher systemin levels in the plant. The systemin or oligosacharride fragments may induce jasmonate production which can then induce the defense-related genes. Methyl jasmonate is volatile and may serve as an airborne signal to neighboring plants that an attack is underway.

Jasmonates also increase the production of secondary metabolites that play a role in plant defenses. The plant defense response may be direct or indirect. Direct regulation is accomplished by production of phytochemicals that negatively affect the plant attacker, its feeding, growth, or reproduction. For example, upon feeding by an attacking herbivore, a plant may produce proteinase inhibitors or polyphenoloxidases which reduce the digestibility of the plant tissue. Indirect regulation comes via an interaction of the host, the herbivore, and an enemy of the herbivore, such as a predator or a parasite. When caterpillars feed on certain plants, the plant responds by producing terpenoids in response to fatty acid amide elicitors in the secretions of the feeding caterpillar (Fig. 2.3). The terpenoids released by the plant allow caterpillar parasites, such as parasitic wasps, to locate the caterpillar.

Fig. 2.3. A tobacco hornworm (*Manduca sexta*) infested with a parasitic braconid wasp (*Cotesia congregata*). Upon feeding on the tomato plant (*Solanum lycopersicum*) fatty acid amide elicitors in the saliva of the hornworm induce the production of volatiles by the tomato which are detected by the wasp, allowing the wasp to find the hornworm and parasitize it. Note the elongated wasp larvae on the back of the hornworm.

Polyamines

Polyamines are strongly basic protein-based substances of low molecular weight that exist either free or bound in all plant cells (Ryan and Pearce, 2004). At a physiological pH, all polyamines are positively charged and bind strongly to negatively charged enzymes. When polyamines bind to enzymes, the enzyme's activity is altered. The main polyamines in plants include putrescine, spermadine, and spermine.

Polyamines should be considered hormones for a number of reasons: (i) they are present in all cells; (ii) they exert noticeable regulatory control over growth and development; and (iii) they are effective at micromolar concentrations.

Polyamines enhance cell division which is required for tuber formation in potatoes (*S. tuberosum*). Polyamine levels are very high in actively dividing cells and very low in cells that are not. Polyamines prevent mitotic senescence. In Jerusalem artichoke (*Helianthus tuberosus*) tubers, low polyamine levels are associated with low rates of cell division. Treatment with exogenous polyamine enhances cell division. Treatment with IAA also enhances cell division with an increase in polyamine levels.

Exogenous applications of polyamines delay leaf senescence by preventing chlorophyll loss, membrane peroxidation, and inhibiting RNase and

protease activity. In addition, polyamine levels are higher in green versus senescing leaves.

Polyamines are also implicated in regulating the flowering process. Polyamines accumulate in the shoot apex, buds, and flower parts of many plants. In *Arabidopsis*, polyamine levels are low in the rosette and bolt, but increase dramatically in the flowers. In tobacco, flowering of thin cell-layer explants can be regulated with spermidine. When the explants are programmed to flower, they are very high in spermidine. If spermidine production is inhibited with cyclohexamide, spermadine levels decrease with a concomitant decrease in flowering. If the inhibitor is removed, spermadine levels increase along with flowering. Cultures grown under conditions supporting vegetative growth will flower if treated with spermadine.

Pharbatis nil is a short-day plant that can be induced to flower with one long night. Under long-day conditions, application of putrescine will induce flowering.

In carrot (*Daucus carota*) and *Vigna* tissue culture, callus proliferation occurs if polyamine levels are low. If polyamine levels are increased, embryoid formation occurs. In tobacco (*Nicotiana tabacum*) tissue culture, the overproduction of spermidine leads to anther rather than ovary production.

Polyamines interact with other known plant hormones in a variety of ways. IBA induces root formation in mung bean (*Vigna radiata*) explants which is accompanied by a twofold increase in polyamine levels. If explants are treated with IBA and a polyamine inhibitor, fewer roots are initiated.

Parthenocarpic fruit induced with an application of auxin can be inhibited with the polyamine inhibitor difluoromethylornithine (DFMO). The inhibition of parthenocarpy with DFMO can be reversed with an application of putrescine.

Cytokinin induces cotyledon expansion in cucumbers (*C. sativus*) which is accompanied by increases in polyamine levels. In pea leaves, senescence is accompanied by a decrease in polyamines. Cytokinin-induced retardation of senescence is accompanied by a retardation in the decline of polyamine levels.

GAs are known to increase polyamine levels in plants. GA-induced dwarf pea (*P. sativum*) internode elongation is accompanied by an increase in polyamines. This elongation is primarily due to increased cell division rather than elongation. α-Amylase activity in germinating barley (*H. vulgare*) seeds is enhanced with GA application which is also accompanied by an increase in polyamine levels.

Ethylene and polyamines are antagonistic with respect to their effects on ripening in climacteric fruit and leaf senescence. Ethylene promotes ripening and senescence while polyamines inhibit both processes.

Salicylic acid

Salicylic acid (SA) is a phenolic plant hormone with roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport (Delaney, 2004). It is most widely known for its roles in signaling for plant defense against pathogens, thermogenicity in plants, and flowering in certain species.

Willow (*Salix*) bark was known for its pain-relieving properties by the ancient Greeks and Native Americans. The active ingredient responsible for this pain-relieving attribute was isolated, identified and named salicylic acid in the early 1800s. Commercial production of synthetic SA began in Germany in the late 1800s and Aspirin, a trade name for acetylsalicylic acid, was introduced by the Bayer Company in 1898. Aspirin rapidly became one of world's best-selling drugs.

Salicylic or ortho-hydroxybenzoic acid belongs to a diverse group of plant phenolics and is widely distributed in plants. The highest levels are found in the inflorescences of thermogenic plants and in plants infected with necrosis-inducing pathogens.

SA and flowering

The first indications that SA was involved in flowering were observed in tobacco (*N. tabacum*) tissue culture, but these observations never attracted much attention since many compounds were known to induce flower bud formation in tobacco tissue culture.

The hypothesis that SA was directly involved in flowering came about after the observation that some factor was transmitted by aphids feeding on short-day flowering *Xanthium strumarum* plants to vegetative plants growing under long days. Honeydew extracts from aphids feeding on flowering *Xanthium* could induce flowering in the long-day plant *Lemna gibba* strain G3 under non-inductive conditions. The flower-inducing substance in the honeydew was identified as SA. This flower-inducing effect has been demonstrated in

other short- and long-day species in the family *Lemnaceae*, in the ornamental orchid *Oncidium*, in *Impatiens balsamina, Arabidopsis thaliana*, and in *Pisita stratiotes* L.

Even though SA was identified in the flower-inducing honeydew, SA itself is not the endogenous flowering regulator, but rather a signaling molecule. SA does not induce flowering in *Xanthium*, members of the *Lemnaceae*, and other plants. Additionally, the levels of SA in honeydew from flowering and vegetative plants do not differ.

Thermogenic plants

Heat production (thermogenicity) occurs in some plant species. It occurs during flowering of plants in the genus *Arum* and occurs in the male reproductive structures of cycads and inflorescences of some angiosperm species belonging to the families *Annonaceae, Araceae, Aristolochiaceae, Cyclanthaceae, Nymphaeaceae*, and *Palmae*.

This heat generation, which can be as much as 14°C above ambient temperature, is associated with an increase in cyanide-insensitive, non-phosphorylating electron transport which is unique in plant mitochondria. Oxygen consumption during this heat generation is equal to that of a hummingbird in flight. Besides the activation of the alternative oxidase pathway, the heat generation requires activation of the glycolytic and Krebs' enzymes.

In the voodoo lily (*Sauromatum guttatum* Schott) the heat generation facilitates the release of foul-smelling amines and indoles to attract insect pollinators. In the 1930s it was suggested that some substance was produced in the plant which induced this incredible burst of heat. It was given the name kcalorigen. It wasn't until 1987 that kcalorigen was identified as SA. The production of SA in thermogenesis regulation is controlled by photoperiod.

SA and disease resistance

SA is the signaling hormone in plant resistance to pathogens and plays a key role in regulating systemic acquired resistance (SAR) and the hypersensitive reaction (HR) in disease-resistant plants.

SAR is where a pathogenic attack on one part of a plant induces resistance to pathogens in other parts of the plant. The intra-plant signal for the development of SAR is SA. When SA is converted by the plant to volatile methyl salicylate, the signal can become interplant, where a pathogen attack on one plant can be perceived by another plant where SAR can be induced in the perceiving plant. The protection afforded by SAR may last several weeks and may offer protection against pathogens not related to the inducing organism.

HR is a response to pathogen attack seen in some disease-resistant plants. In plants which have HR, a necrotic lesion develops around the initial point of pathogen attack via death of cells in the necrotic area. This HR may lead to SAR.

As part of the physiological development of both HR and SAR a number of low molecular weight PR (pathogenesis related) proteins are often produced by the plant. Proof that these proteins are directly related to HR and SAR is lacking, however, their presence during acquisition of disease resistance is well known. Some additional evidence that these proteins are involved includes their induction by application of SA even in the absence of pathogens.

Tobacco (*N. tabacum* cultivar 'Xanthi-nc') has an 'N' gene which confers an HR response to tobacco mosaic virus (TMV) which includes production of PR-1 proteins. Leaf treatment with SA induces the same PR-1 proteins and protection from TMV increases with increasing SA concentration. TMV-susceptible *N. tabacum* has the recessive 'n' allele of the 'N' gene and TMV does not trigger PR proteins and the plant exhibits the mosaic pattern in young leaves. Aspirin (acetylsalicylic acid) application induces PR proteins and the mosaic spread is reduced. SA may induce other mechanisms of resistance other than PR proteins. If SA in fact does induce disease resistance, imagine the possibilities for modern agriculture.

Synthetic Plant Growth Regulators and Their Uses in Horticulture

There are many synthetic chemicals acting as plant growth regulators which are used to improve the quality or yield of horticultural crops or to make their management easier. It would be impossible to cover all of the chemicals used worldwide for management of horticultural crops. As an example of the ways in which plant growth regulators might assist production of horticultural crops, plant growth regulator use in apples (*M. domestica*) is closely examined. A general discussion of plant growth regulator uses in ornamental and turf horticulture follows. While

specific chemicals are discussed, recommendations are left to local experts. In addition, mention of a trade name does not imply an endorsement of any product.

Apple crop management

Apples production relies heavily on the use of plant growth regulators, both naturally occurring and synthetics. One important distinction that apples bring to the table compared to the other crops discussed is that apples are consumed by humans. Therefore the health ramifications of ingesting the discussed substances are considered during their development. Products undergo enormous testing before they are labeled for use.

Growth regulators used to improve crop management

Apple nursery stock and non-bearing trees are often treated with Promalin or Perlan (*N*-(phenylmethyl)-1H-purine 6-amine, also known as 6-benzyladenine, a synthetic cytokinin) combined with Gibberellins A4A7 (GA4+7) to increase lateral bud break and shoot growth as well as improving (widening) branch angles. Benzyladenine enhances lateral bud break since it is a cytokinin while the GAs promote the elongation of shoots which grow from the broken buds. Promalin can also be used to improve branching of terminal shoot growth on bearing trees.

Many cultivars and rootstocks of apples are prone to suckering. Suckers emerge from below the ground and can be controlled with NAA, or its ethyl ester, ethyl 1-naphthaleneacetate (Tre-Hold sprout inhibitor). Watersprouts, vigorous upright shoots, often originate around pruning cuts and can be controlled with the same two chemicals.

Apple fruit is normally thinned in the early stages of development to increase fruit size and reduce crop load to encourage annual fruiting. Thinning 'Golden Delicious' fruit can be accomplished by applying the synthetic auxin NAA around 2–3 weeks after full bloom. The auxin application results in abscission of weaker fruit, resulting in effective thinning. If the NAA is applied too early, fruit will not abscise and thinning will not be achieved. This phenomenon highlights the enormous effect the stage of development can have on efficacy of growth regulator application.

In some apple cultivars, NAA is not effective as a thinner. Non-spur 'Red Delicious' and 'Rome' are thinned with the insecticide carbaryl (Sevin). Carbaryl is not used to thin 'Golden Delicious' as it tends to cause russetting in that cultivar. Extreme care must be used with carbaryl as it is extremely toxic to honeybees.

To thin spur strains of 'Red Delicious' a combination of carbaryl (Sevin) + NAA is applied when fruit are small (average fruit diameter of 9–11 mm). Larger fruit (12–15 mm) can be thinned with a combination of carbaryl and ethephon. Application of carbaryl + NAA at this larger fruit size (12–15 mm) to 'Red Delicious' can cause "nubbins" and half-grown fruit which stick to the tree through harvest. Again, this emphasizes the effect developmental stage can have on response to growth regulators.

To remove all the fruit from apple trees if they are not large enough to bear a crop, a combination of NAA + carbaryl (Sevin) + ethephon (Ethrel) can be used. Besides completely thinning the tree, this combination will suppress vegetative growth for a while.

Thinning cultivars such as 'Gala', 'Fuji', and 'Spur Red Delicious' is accomplished using N-(phenylmethyl) -1H-purine-6-amine (Exilis, MaxCel) + carbaryl (Sevin). 'Stayman', 'Rome', 'McIntosh', 'Jonathan', or 'Gala' can be thinned with carbaryl (Sevin), or NAA, or carbaryl (Sevin) + NAA.

Apples often suffer from biennial bearing. Return bloom can be encouraged without a thinning effect if NAA is applied biweekly for 2 months beginning 6 weeks after petal fall.

The growth regulator prohexadione-calcium (Apogee) can be used to reduce vegetative growth and later-season tree canopy volume and density. This improves pesticide penetration and efficiency.

Apple fruit tend to fall off the tree before they are ready for harvest. Pre-harvest drop can be prevented with sprays of NAA. Applying a low rate of NAA before fruit loosening begins (preloading) is much more effective in preventing premature drop than using a higher rate when the fruit begins to loosen. Preloading may also increase return bloom of 'Golden Delicious' and 'Red Delicious' cultivars.

Pre-harvest treatment to improve quality

A number of measures can be taken before harvest to improve the quality of apple fruit; many of these measures include growth regulators. Spraying with

aminoethoxyvinylglycine (AVG) (ReTain) will delay pre-harvest fruit drop and maturity which allows time for an increase in fruit size.

Some apple cultivars such as 'Delicious', 'Gala' and 'Ginger Gold', may not produce fruit with a desirable shape or fruit weight. An improvement in fruit quality can be achieved with an application of Promalin or Perlan, the same chemicals used to induce better lateral branching in the nursery. The combination of cytokinin and GAs in the Promalin or Perlan increases fruit size via increased cell division induced by the cytokinin and increased cell size induced by the GAs. In addition, the GAs reduce fruit russetting.

Some apple cultivars, particularly 'Golden Delicious', are susceptible to russet formation, a corky epidermal growth around lenticels on the fruit skin. To reduce or prevent russetting, GA4+7 can be applied to trees at petal fall, followed by repeat applications 10, 20, and 30 days after petal fall. GAs also help prevent fruit cracking, a particular problem in 'Stayman' apples, if applied every 3 weeks beginning about 3 weeks before anticipated cracking.

Postharvest treatment to improve storage

Scald is a postharvest disorder in apples that appears after about 3 months of storage. It appears as discoloration on the fruit's surface within about 3–7 days after removal from cold storage, greatly reducing their market value. Diphenylamine (DPA) can be applied as a dip or spray to harvested fruit to reduce the occurrence of scald.

Another treatment that reduces scald as well as maintaining fruit firmness and acidity is treatment with 1-methylcyclopropene (MCP) (SmartFresh). Treatment involves introducing MCP into the atmosphere of the storage container or facility for 24 h. Following treatment, fruit can be held in regular cold storage.

Growth control in greenhouse crops

With most greenhouse grown crops, a standard plant size and form is desired for each particular crop. To achieve this 'ideal' plant, many compounds are available for use in greenhouse production. Both floricultural and vegetable crops are greenhouse grown, however, the vast majority of growth regulators are used on floricultural crops. Few plant growth regulators are labeled for greenhouse vegetable production.

The most common growth regulators used in the greenhouse industry include: ancymidol, daminozide, paclobutrazol, chlomequat chloride, uniconazole, benzyladenine, GA3, GA4+7 + benzyladenine, ethephon, and flurprimidol.

Daminozide and chlormequat chloride are usually applied as foliar sprays to provide short-term inhibition of stem elongation. They are often used on plug crops which only need a slight reduction in stem height.

Ancymidol, flurprimidol, paclobutrazol, and uniconazole are applied as foliar sprays, soil drenches, or liner dips to reduce stem elongation. The differences among the products are the strength of their stem elongation reduction and the length of their effectiveness. Ancymidol has the weakest and most short-lived effect while uniconazole has the strongest and longest lasting effect; those in between have moderate effects on stem elongation and medium longevity of action. Uniconazole-P is the first and only plant growth retardant approved for use in greenhouse production of vegetable crop transplants of eggplant (*S. melongena*), pepper (*C. annuum*), groundcherry (*Physalis* spp.), pepino (*Solanum muricatum*), tomatillo (*Physalis philadelphica*) and tomato (*S. lycopersicum*).

GA3 and GA4+7 + benzyladenine are applied as foliar sprays and promote stem elongation and reduce yellowing of older leaves in crops such as lily (*Lilium* spp.) or geranium (*Pelargonium* spp.). They can also be applied to counteract the over application of a stem elongation inhibitor.

Ethephon is applied as a foliar spray and releases ethylene gas which inhibits stem elongation, causes flower bud and flower abortion, and often increases branching. It is sometimes used to maintain crops in a vegetative state as stock plants for propagation.

Benzyladenine applied without GA stimulates lateral branching and sometimes flowering.

Growth control in woody ornamentals

A widely used plant growth regulator in woody ornamental horticulture is auxin. Auxins are available in a number of forms as a rooting stimulant (K-IAA, IBA, K-IBA, K-NAA, IBA + NAA, IBA, naphthaleneacetomide (NAM), IBA + NAA).

Compact plants are produced in the nursery using daminozide, dikegulac sodium and paclobutrazol.

Lateral branching can be stimulated with application of dikegulac sodium and lateral shoot growth of azalea (*Rhododendron* spp.), *Cotoneaster, Juniperus*, and *Taxus* can be promoted with methyl decanoate/octanoate.

Dikegulac sodium suppresses flowering and fruit formation. Ethephon is used to reduce or eliminate undesirable fruit development on many ornamental trees and shrubs. To retard regrowth of most trees, shrubs, and vines, chlorflurenol is often utilized. Maleic hydrazide is often used to retard regrowth of most trees, shrubs, and ivy (*Hedera* spp.).

Turf management

One of the main expenses in turf maintenance is mowing. Three main groups of chemical are used in turfgrass management to retard general plant growth and thus reduce the amount of mowing necessary. These three groups are: (i) herbicides; (ii) Class I inhibitors; and (iii) Class II inhibitors.

Herbicides

Herbicides can be used at low rates to inhibit plant growth. Herbicides commonly used for this purpose include glyphosate, chlorsulfuron, imazameth, imazethapyr + imazapyr, metsulfuron, sethoxydim and sulfometuron. These herbicides act at the cellular level through varying mechanisms.

Class I – cell division inhibitors

The group of chemicals called Class I retardants act by inhibiting cell division. These chemicals include amidochlor, chlorflurenol, maleic hydrazide, and mefluidide. These substances are foliarly applied. Their main effects are retarded leaf growth and inhibited seed head formation. They can be phytotoxic, and as such, are often used on 'lower value' or 'low maintenance' turf.

Class II – GA biosynthesis inhibitors

Class II retardants are GA biosynthesis inhibitors. They are applied foliarly and reduce leaf growth but do not retard seed head formation. One chemical is trinexapac-ethyl which is absorbed through the leaves. Others, absorbed through the roots, include flurprimidol, and paclobutrazol.

Miscellaneous plant growth regulator uses in horticulture

Desiccants for aiding harvest

A number of crops are much more easily harvested if vegetative tissues are desiccated prior to harvest. Some crops benefiting from pre-harvest desiccation include chili peppers (*C. annuum*) for drying, dry beans (*Phaseolus* spp.), potatoes (*S. tuberosum*), sunflower (*Helianthus annuum*) and tomatoes (*S. lycopersicum*) for processing. A number of chemicals are available for use as desiccants including sodium chlorate (Chlorate, Defol), paraquat (Firestorm, Gramoxone Inteon), diquat (Reglone), and glufosinate (Rely).

Pre-harvest treatment to aid harvesting

Tomatoes grown for processing are mechanically harvested all at once, requiring uniform ripening among the fruit. Ethephon (Ethrel) is an ethylene-releasing growth regulator which can be foliarly applied to induce uniform ripening.

Postharvest treatment to prolong quality

In order to prevent sprouting in storage (Fig. 2.4) and the greatly reduced storage life associated with sprouting, onions (*Allium cepa*) and potatoes (*S. tuberosum*) are often treated with a potassium salt of maleic hydrazide. Maleic hydrazide works by non-selectively inhibiting cell division.

Fig. 2.4. Potatoes (*Solanum tuberosum*) sprouting just 3 weeks after harvest. To prevent sprouting, potatoes are often treated with the growth inhibitor maleic hydrazide. Maleic hydrazide prevents sprouting by non-selectively inhibiting cell division.