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Physiology of Growth in Specific Organs: Roots, Stems, and Leaves

In this chapter we will investigate the physiology of some specific growth characteristics of roots, stems and leaves to get a handle on how plants are able to grow and prosper as stationary organisms in an incredibly dynamic environment. Other organisms have the luxury of being mobile which allows them means of avoiding certain obstacles to their growth and development. Plants on the other hand are stuck where they are and must utilize some pretty fascinating mechanisms to cope. Some of the mechanisms we'll look at are stress avoidance mechanisms while others are simply growth responses to a myriad of environmental signals.

Roots

Geotropism (gravitropism)

Roots grow down because of gravitropism, a tactic plant movement in response to the pull of gravity. Remember that tactic means the response is related to the direction of the stimulus. Roots move towards the gravity stimulus, thus the gravitropic response of roots is considered a positive tactic response. Shoots move away from the gravity stimulus, thus the gravitropic response in stems is considered a negative tactic response. In the context of plant physiology, positive and negative have nothing to do with good or bad, but rather is referring to the direction of the response. In tissues that grow seemingly neutral to the stimulus, such as leaves or sideways growing roots, the response is considered plagiotropic.

If a seedling is placed on its side, the roots will begin to grow downwards and the shoot will begin to grow upwards, both gravitropic responses occurring at the same time in the same plant. The upright growth of the shoot could be at least partially attributed to light, but this response to light is really secondary since shoots will grow upright, away from gravity even in the dark.

The starch-statolith hypothesis

The most widely accepted hypothesis of how plants respond to gravity is called the starch-statolith hypothesis (Fig. 4.1). This hypothesis has been around for over 100 years and continues to gain acceptance today (Blancaflor and Masson, 2003; Perrin *et al.*, 2005).

SIGNAL PERCEPTION Cells which detect the gravitropic signal are called statocytes. The statocytes in roots are specialized cells called columella cells. They are located just behind the root cap itself. If columella cells are carefully examined, small grains of starch called statoliths can be seen in plastids known as amyloplasts. The statoliths, being rather dense and heavy, will settle to the bottom side of the columella cell. Whenever a root's orientation is altered, the statoliths will settle to the side of the cell closest to the gravity stimulus. Thus the mechanism for signal perception is the location of statoliths in columella cells (Chen *et al.*, 1999). But the columella cells do not directly respond to the stimulus (i.e. their growth does not change). It is the cells in the growth zone (also called the zone of elongation) which change. There has to be some signal transmitted from the cells containing the statoliths to the cells in the growth zone that causes the growth of the growth-zone cells to change.

In shoot gravitropism, the statocytes are endodermis or bundle sheath cells. In these cells, statoliths sense a change in the direction of the pull of gravity in much the same way as in root tissue. The position of statoliths in these cells generates a signal that moves laterally to peripheral tissues causing differential growth there which results in shoot bending. Again, the site of signal perception is not the same as the site of the response to the signal, thus some sort of signal transmission must occur.

CELLULAR PERCEPTION The perception of gravity in plant cells relies on statolith position. But how is their position detected? There are filaments

Auxin-mediated mechanism of gravitropism

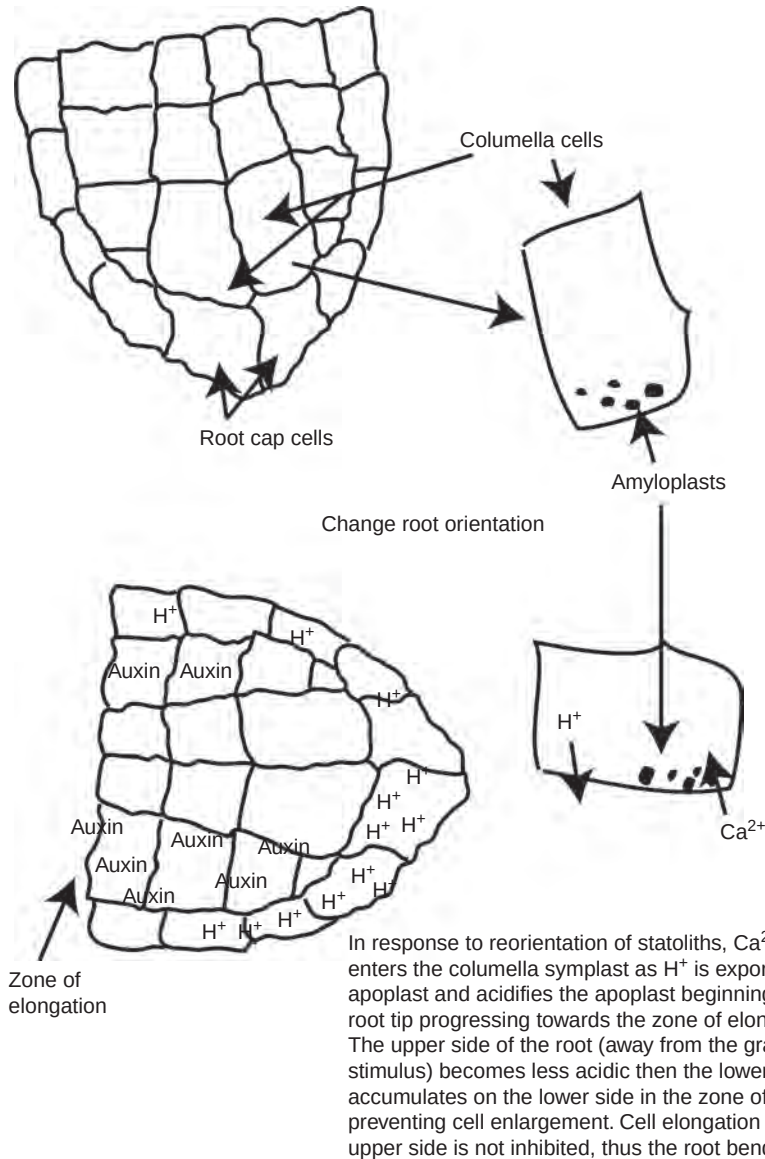


Fig. 4.1. The starch-statolith hypothesis of geotropism.

of proteins throughout the cellular cytoplasm. The thin filaments are composed of the protein actin, while thicker, tubular filaments called microtubules are composed of the protein tubulin. These filaments and microtubules are attached to many cellular components and are important in many cellular processes including cell division, cell signaling, cell expansion, and overall cell structure.

Statoliths travel through this network of filaments as they move in response to gravity. This disruption of the network causes a response at the plasma membrane. The response is due to a mechanical disruption of the filaments by the statoliths which causes a change in the tension exerted by the filaments at their points of attachment to the membrane. The change in tension at the membrane

triggers the movement of calcium ions into the cytoplasm through specific protein channels in the membrane with a concomitant movement of protons out of the cell (Yoder *et al.*, 2001; Boonsirichai *et al.*, 2002).

The protons moving out of the cell acidify the apoplast (the area outside of and between cell membranes). An asymmetric change in root surface pH is first observed at the root cap and then progresses along the root to the elongation zone. The side of the root closest to the gravity signal becomes more acidic than the side opposite the signal. The acidification of the root surface seems to influence the activity and/or distribution of auxin in stimulated root tissues (Scott and Allen, 1999; Fasano *et al.*, 2001).

AUXIN PRODUCTION, TRANSPORT, AND GRAVITROPIC CELL GROWTH Auxins are mainly synthesized in young shoot tissue, and are transported passively through the phloem into the root. In general, auxin inhibits elongation in root cells and promotes elongation in shoot cells. Auxin movement is called polar transport, indicating that movement is due to a gradient from regions of high concentration to regions of low concentration. This movement is accomplished by means of transmembrane transporters, proteins specifically designed to move auxin. One type of transporter moves auxin into a cell and another moves auxin out. The auxin transporters may be distributed asymmetrically in cells which can lead to asymmetric auxin concentrations in cells, depending upon the particular circumstance. This model of auxin transport is called the fountain model (Wolverton *et al.*, 2002).

ARABIDOPSIS Much of our understanding of gravitropism comes from work with *Arabidopsis thaliana* (L.) Heynh. *Arabidopsis*, also known as mouse-ear cress, is a small flowering plant in the family *Brassicaceae* (mustard family) which has little horticultural significance as a crop, but is extremely important in the study of basic molecular mechanisms in flowering plants. Its small genome with only five chromosomes has been completely mapped. It produces an abundance of seed in only 6 weeks from germination, thus the turn-around time for genetic studies is very short. Since a mature plant is only 20–25 mm tall, it takes up very little space when cultivated. Additionally, many mutant lines have been identified and genetic transformation is fairly easy using *Agrobacterium tumefaciens*.

ARABIDOPSIS AND THE AUXIN STORY Auxin produced in young shoot tissue is transported through the phloem to the root tip from cell to cell via auxin transporters. The genes responsible for coding the proteins responsible for transporting auxins have been extensively studied in *Arabidopsis* (Friml, 2003). The transporters responsible for auxin influx are coded for genes collectively called AUX while those responsible for cellular efflux of auxin are coded for by genes collectively called PIN. Auxin must be transported from the vascular tissue into the root tip through protophloem cells. The AUX transporters are located primarily on the basal side of the membrane while the PIN transporters are located on the apical side. Remember in the case of root cells, basal is up and apical is down. Thus the movement of auxin will be in an acropetal direction, away from the vascular tissue to the root cap.

At the root cap, a specific transporter (PIN4) causes auxin to accumulate in the upper layers of columella cells in the root cap. In these cells, a specific transporter called AUX1 ensures uptake by these cells and a specific transporter called PIN3 regulates efflux. Where PIN3 is located on the membrane depends on root orientation. When the root is oriented vertically, PIN3 is symmetric on the plasma membrane of columella cells. When the root is reoriented, PIN3 relocates to a position now sensed to be the new cell bottom. The distribution of PIN3 in the root columella cells could be related to changes in the filament network brought about by statolith settling or by gravity-induced changes in calcium and pH caused by statolith reorientation.

With the PIN3 efflux transporters located at the bottom of the cell, there is an efflux of auxin into the apoplast on the lower side of the root with a concomitant increase in apoplast auxin concentration. The auxin then moves basipetally towards the elongation zone via passive polar transport. The increased auxin concentration in elongation-zone cells reduces their elongation growth (remember, auxin inhibits cell elongation in root tissue). On the opposite side of the root, auxin concentrations have not increased, thus elongation-zone cell elongation is not inhibited. Since cell elongation on the lower side of the root is inhibited while elongation on the upper side of the root is not, the root curves downwards.

When a shoot is tipped on its side, statoliths collect on the bottom side of the stem resulting in an

efflux of auxin from cells, which is probably due to the reorientation of PIN3 transporters. Auxin would then accumulate on the lower side of the stem, promoting cell elongation (remember auxin promotes cell elongation in shoot tissue). The shoot would then curve upwards due to the differential cell elongation caused by auxin.

Cytokinin gradients may also play a role in the root gravitropic response, however, evidence is still limited for this involvement (Wolverton *et al.*, 2002).

BUT HOW CAN AUXIN STIMULATE CELL ELONGATION IN SHOOTS YET INHIBIT IT IN ROOTS? This phenomenon is likely to be due to differences in cell sensitivity to auxin concentration. Root cells are much more sensitive to auxin than stem cells. Low concentrations elicit a response in root cells but have no effect on stem cells. Levels needed for a response in stem cells are inhibitory in root cells.

For example, let's say that the optimum auxin concentration needed for root cell elongation is 10 ppm. Stem cells do not elongate with 10 ppm auxin since they are less sensitive to auxin concentration than root cells. Increasing the auxin level to that needed for stem cell elongation raises it to a level that is inhibitory to root cell elongation. The amount of auxin released by the gravitropic response must be near the optimum level for stem cell elongation, but is at an inhibitory level for root cell elongation.

This type of growth response is quite typical in plants. Think about light levels, water levels, and fertility. Most plants have a level of each that is optimal for growth, above or below these levels, growth is inhibited. It's no different on the cellular level. Why root and stem cells are different in their respective sensitivities to auxin levels is still a mystery.

The gravitational pressure hypothesis

Work with starchless mutants of *Arabidopsis* has led to an alternate hypothesis that the response to gravity is due to the perception of the weight of the entire cell, not starch granules (Wolverton *et al.*, 2002). In general, the gravitational pressure hypothesis for responses to gravity seems only to be applicable to single-cell algae such as *Chara* spp. or unicellular flagellates such as *Euglena gracilis*. The possibility that an alternate gravity sensing mechanism exists in higher plants has been supported in work

with corn (*Zea mays*) roots. Root growth continued to curve as long as the elongation zone was held at any angle away from vertical even when the root cap was vertically aligned. This observation suggests that the cells in the elongation zone could perceive the direction of gravity, even though they themselves do not contain amyloplasts.

Thigmotropic responses in roots

Roots naturally have a propensity to grow downwards into the soil due to gravitropism. When a root encounters an obstacle as it grows, it will turn and grow around the obstacle, apparently overriding the gravitropic response. When small flat pieces of material are placed next to vertically growing roots, the roots will exhibit a thigmotropic response by bending away from the objects point of contact.

In laboratory experiments with *Arabidopsis*, roots will compromise their gravitropic response when they come in contact with a glass plate and exhibit a thigmotropic response instead (Massa and Gilroy, 2003). It is not surprising then that thigmotropic stimuli can delay the onset of a gravitropic response at the cellular level. Touching a root apex can delay one of the first-known gravitropic responses, that is the settling of starch granules in columella cells.

Stems

Phototropism

The physiology of plant stem growth includes a wide variety of stem growth responses to environmental stimuli. Since stem response to light in the form of phototropism is such an extensive subject, it is covered on its own in Chapter 8, this volume.

Thigmotropism

Plant response to any form of touch stimulus is called a thigmo response (Jaffe *et al.*, 2002). Thigmotropic responses are those responses that occur directionally, based on the direction of the stimulus (e.g. the movement of a tendril winding around a trellis). Thigmonastic responses occur independently of the direction of the stimulus, such as the closing of *Mimosa pudica* leaves (Braam, 2005).

Tendrils, vine coiling, and holdfast attachment

As part of their growth and development, many plants have the ability to climb via stem coiling, tendril grasping, or holdfast attachment. Each mechanism is an intriguing physiological activity developed by certain species over millennia of evolution. Part of the climbing response might be considered phototropic as the plant is moving towards a light source while another component may seem gravitropic as the stem is growing away from gravity. The responses are actually more specific thigmotropic responses at the tissue and cellular level.

Tendrils movement

Some species have specialized appendages called tendrils specifically adept at coiling around solid objects which facilitates better exposure to sunlight for photosynthesis. These thin, long, often threadlike organs have a high degree of sensitivity to touch and friction on solid bodies which allows them to wind tightly around stakes, string, or even shoots, giving the appearance that they are physically climbing their support (Jaffe and Galston, 1968).

Plant families with tendrils are relatively few especially when compared with the number of families that have developed other mechanisms for climbing such as stem twining.

TENDRILS FROM STEMS Tendrils can originate from various plant organs. Some tendrils are modified shoots, such as those found in grapes (*Vitis* spp.) (Fig. 4.2), passionflower (*Passiflora* spp.), porcelain berry vine (*Ampelopsis glandulosa*), and evergreen grape vine (*Rhoicissus capensis*). Shoot tendrils originate as a shoot apical meristem and may have minute leaf primordia.

TENDRILS FROM LEAVES Some tendrils originate from a node as a specialized leaf where the blade never really forms and the central axis elongates via apical and intercalary growth. Examples of plants with so called 'leaf tendrils' include garden peas (*Pisum sativum*), sweet peas (*Lathyrus* spp.), many members of the family *Cucurbitaceae* (Fig. 4.3), cup and saucer vine (*Cobaea scandens*), and the Chilean glory flower (*Eccremocarpus scaber*). Leaf tendrils are often further categorized based on their specific origination within the leaf tissue complex.



Fig. 4.2. Grape (*Vitis* spp.) tendril which originates as stem tissue.

TENDRILS FROM LEAF TIPS Some species form tendrils from the tips of developing leaves. *Mutisia* spp., a South American flowering vine, can have leaf-tip tendrils. Asian pitcher plants (*Nepenthes*) form a tendril on the leaf tip. The tip of this tendril then develops into the pitcher.

TENDRILS FROM LEAFLETS A tendril can also form from a leaflet of a compound leaf (*Vicia*, *Lathyrus*, *Pisum*). With this type of tendril, the leaflet tendril, a single leaflet may develop into a tendril (as in many members of the family *Bignoniaceae*) or several of the most distal leaflets may become tendrils (as in *Lathyrus* spp.). Clematis is a temperate zone climber (family *Ranunculaceae*) with leaflet tendrils. *Cobaea*, a vine of the phlox family (family *Polemoniaceae*), forms tendrils from distal leaflets.



Fig. 4.3. Winter squash (*Cucurbita* spp.) tendril which originates as leaf tissue.

TENDRILS FROM STIPULES Stipules are small appendages at the base of a leaf. Greenbrier (also known as catbrier) (*Smilax*) has tendrils formed from stipules.

TENDRILS FROM LEAF STALKS Clematis (*Clematis* spp.) forms tendrils from petioles (leaf stalks).

TENDRILS FROM FLOWERS Pedicel or peduncle tendrils develop from the axis of an inflorescence. Chewstick (*Gouania lupuloides*), a tropical plant, forms tendrils from peduncles. Certain snapdragons (*Antirrhinum* spp.) have tendrils that appear to be derived from pedicels in the inflorescence.

TENDRILS FROM ROOTS Tendrils of the tropical orchids we use as flavoring (*Vanilla* spp.) originate from roots.

Tendril stimulation and direction of movement

Even though tendrils may originate from various plant organs, their movement and growth will be covered in this section on stem thigmo responses since the mechanism for such is the same regardless of origin.

Tendril movement can be either thigmonastic or thigmotropic depending on whether the direction of stimulation has an effect on the direction of coiling. If it does not, the response is thigmonastic, if it does, the response is thigmotropic (Jaffe and Galston, 1968). Some tendrils can bend in any direction when stimulated on any side. Others can

only be stimulated and only bend on the ventral (lower) side, while others can be stimulated on all sides but only bend ventrally. Regardless of the type of bending possible, there are two stages of movement in the bending process.

The first stage is an autonomous movement which does not rely on any particular stimulation. Before a tendril begins to elongate and grow, it is often coiled. It uncoils as it begins to grow and its tip exhibits a circling movement which is caused by increased growth on the upper side of the tendril. By itself, this type of growth would lead to a spiraled tendril. The spiral is prevented by a migration of the growth zone in a screw-like fashion along the longitudinal axis. If a tendril reaches its full length as determined by genetics and has not found any support to coil around, it will wither and die or recoil.

The second stage of movement is an induced movement which begins when the tendril encounters a support. This could be a pole, a string, a stem, or even another tendril. When the support is encountered, a directed growth begins to occur. The tip of the tendril begins to wind itself tightly around the support.

Some of the cells on the epidermis of the tendril are called irritable cells. There are openings called touching pits in the cell wall of these cells which allow for the direct contact between the plasma membrane and the support. Upon contact, a signal is sent such that the turgor of ventral cells (cells closest to the support) decreases while turgor of dorsal cells increases. Dorsal cells therefore elongate more than ventral cells, causing the tendril to coil around the support. The change in turgor is probably due to changes in membrane permeability and flow of ions using metabolic energy.

The distal portion of a tendril is quite selective in its sensitivity. Stimulation as light as a 0.25 mg thread can evoke a response (coiling) in a matter of seconds, yet raindrops touching the tendril elicit no response (Simons, 1992). In addition, the stimulation causing coiling must remain present to maintain coiling, at least until cell wall lignification occurs. Transient stimulation may result in coiling, but the response is often reversed by uncoiling.

In some species basal coiling may occur after the initial tendril tip coiling. This type of basal coiling is called free coiling (Jaffe and Galston, 1968). Its purpose seems to be to bring the stem closer to the support that initiated the coiling at the tip. This suggests that the signal at the tip is

propagated down the tendril to the base where physiological changes induce basal coiling.

Many substances have been investigated as possible chemical messengers for inducing coiling. One such substance, 12-oxo-phytodienoic acid (ODPA), can induce coiling without mechanical stimulation in some species (*Bryonia dioica*, a relative of the cucumber) but not others (*P. sativum*, the garden pea). Application of ODPA leads to the accumulation of indole-acetic acid (IAA), which itself stimulates coiling (Weiler *et al.*, 1993, 1994; Blechert *et al.*, 1999). Even though ethylene increases with the application of either ODPA or IAA, it does not directly induce coiling since if ethylene synthesis is chemically blocked, coiling still occurs with ODPA or IAA (Weiler *et al.*, 1993).

Other mechanisms of climbing

ADHESIVE PADS Some species such as Boston ivy (*Parthenocissus tricuspidata*) and Virginia creeper (*Parthenocissus quinquefolia*) climb via stem tendrils that have touch-sensitive adhesive pads. In these vines, tendril coiling is not a prerequisite for climbing as the adhesive pads will stick to almost any surface.

SHOOT TWINING There are many plants which do not form tendrils, yet still climb to great heights. One of the mechanisms responsible for climbing is shoot twining. Shoot twining consists of the encircling growth of a shoot around an object as the shoot elongates. Some common plants which exhibit shoot twining include morning glory (*Ipomoea* spp.), green beans (*Phaseolus vulgaris*) (Fig. 4.4), clematis (*Clematis* spp.), and honeysuckle (*Lonicera* spp.). There are two different types of twining shoots: (i) twining via stems; and (ii) twining via leaves.

Plants which twine via leaves include clematis, climbing nasturtium (*Tropaeolum polyphyllum*), and *Rhodochiton*. In these plants, young leaves are able to twine around slender objects as the leaves elongate. In doing so the entire shoot is supported.

Many plants have stems which are able to twine around objects. The direction of twining (clockwise or counterclockwise) depends on the species. Some species that exhibit stem twining include green beans (*P. vulgaris*), black-eyed Susan (*Thunbergia*),



Fig. 4.4. An example of stem climbing by twining in green bean (*Phaseolus vulgaris*).

Dutchman's pipe (*Aristolochia*), bittersweet (*Celastrus scandens*), morning glory (*Ipomoea* spp.), moonflower (*Ipomoea* spp.), jasmine (*Jasminum*), honeysuckle (*Lonicera*), wisteria (*Wisteria*), hardy kiwi (*Actinidia arguta*), cup and saucer vine (*Cobaea scandens*), hyacinth bean (*Dolichos lablab*, *Lablab purpureus*), and scarlet runner bean (*Phaseolus coccineus*).

CLINGING STEM ROOTS Plants including climbing hydrangea (*Hydrangea petiolaris*), most ivies such as English ivy (*Hedera helix*) and Irish ivy (*Hedera hibernica*), and also some *Euonymus* spp. climb by utilizing groups of short, stout roots that have the ability to cling to nearly any surface they come into contact with. The roots secrete acids which can damage paint and mortar, thus these vines can be quite troublesome for homeowners.

SO-CALLED CLIMBERS Some plants appear to be climbing as if they were vines but have no mechanism to climb on their own. These plants which include *Bougainvillea* and climbing roses (*Rosa* spp.), simply have very long, flexible stems that are easy to attach to objects giving them the appearance of climbing. Sometimes roses are thorny enough that thorns attach adjacent stems to each other giving a further appearance of climbing.

Leaves

Thigmonastic responses

Physiological responses to touch by leaves is called seismonasty. Probably one of the best known responses is that of leaflet folding. Leaflet folding is observed in a number of species which includes oxalis (*Oxalis* spp.), mimosa (*Mimosa* spp.), Venus fly trap (*Dionaea muscipula*) and many legumes.

When the tip of a mimosa leaf (*M. pudica*), also called the sensitive plant, is touched or heated, the leaflets close in a very orderly fashion down the length of the leaf. The response is rapid, all-or-none and is not limited to the touched leaflet (Simons, 1981; Malone, 1994). The folding is mechanically controlled by specialized organs called pulvini at the base of the leaflets. The folding leaf movement is the result in loss of turgor from extensor cells which are on the top side of the leaflet joint and a stretching of flexor cells, which are located opposite the extensors in the pulvinus. These changes in cell turgor, size and shape are transient and reversible (Braam, 2005). But what causes the turgor of pulvini cells to change? In order to understand the mechanism for turgor changes in pulvinus cells, we must understand the electrical properties of plants.

Electrical potentials in plants

Electrical signals offer a particularly effective means of signal propagation. In studying movement in plants, an understanding of the electrochemistry involved is particularly important, since much of the movement in plants is attributed to changes in cell turgor induced by electrical signals (Volkov and Brown, 2004).

Cell plasma membranes allow electrochemical signals to be passed through the plant over both short and long distances. Changes in the ionic balance inside and outside the plasma membrane

generate an electrical current which can move through the plant via an excitation wave or action potential, both of which we will discuss shortly. The rate at which these signals propagate depend on factors such as: (i) the intensity of the stimulus; (ii) previous similar stimuli; (iii) plant health; and (iv) temperature.

Action potentials, which have been studied extensively in the algae *Chara* and *Nitella*, are electrical signals caused by the depolarization of cell membranes. The depolarization is a direct consequence of some sort of stimulus such as touching or wounding. Once the depolarization occurs the electrical signal in the form of an action potential propagates the stimulus message at rates from 0.05 cm/s to 40 m/s. The response to the stimulus can be local or in a distant plant part. Regardless of the distance from the stimulus, the response is nearly always the activation of an enzymatic system, often membrane-bound, that regulates some aspect of plant growth and development.

Plant action potentials have many of the properties of action potentials associated with animals. This includes: (i) the all-or-nothing response; (ii) some minimum threshold for response; and (iii) a refractory period.

There must be a viable system for long-distance transport of electrical signals in plants. A good candidate for such a pathway is the phloem sieve-tube/companion cell array which consists of a continuous line of connected plasma membranes. It may be the 'wire' in a plant responsible for electrical signaling over long distances.

The major chemicals actively involved in the electric system in a plant are relatively few: potassium, calcium, sodium, hydrogen and chloride ions. In general, as the action potential propagates there are large calcium influxes and concomitant potassium and chloride effluxes from cells. These influxes and effluxes are regulated by membrane-bound enzymes which regulate ion flow in and out of the cell through the membrane.

Mimosa leaflet folding

When a mimosa leaflet is open, potassium ions inside extensor cells of the pulvini maintain a turgor to keep the leaflets open. When a leaflet is touched, there is a rapid movement of potassium ions out of the cell with a concomitant efflux of water (Simons, 1981; Fromm and Eschrich, 1988). The water lost may be up to 25% of the initial cell

volume and it can occur within a second (Fleurat-Lessard *et al.*, 1997). This stimulation of the extensor cell of a pulvinus causes a transient breakdown in the electrical potential due of the membrane and generates an action potential. The action potential of one cell may stimulate neighboring cells if it is high enough to pass a threshold level (thus the all-or-none response), and the signal is passed down the leaf from cell to cell via plasmodesmata, probably through the phloem, at the rate of about 2cm/s. As the signal moves down the leaf, leaflets fold in a regular and orderly fashion. The membrane potentials eventually return to their original state and potassium re-enters the cells. The cells rehydrate, become turgid, and the leaflet opens. While the membranes are returning to their resting potential, they cannot be stimulated to respond again. This is the refractory period. Once they are back to their resting potential and cells are rehydrated and turgid, leaflets may once again be stimulated to close (Braam, 2005).

The actin network may be involved in perception of the initial signal of touching or heating since chemical inhibitors of actin can block leaflet folding (Fleurat-Lessard *et al.*, 1993).

In addition to the short-distance signal transport down a leaf, there may also be long-distance transmission of the signal if the initial stimulus is strong enough. Even though some of the long-distance signal transmission may be electrical, there must be another mechanism since the signal can pass through dead tissue and electricity does not pass through dead tissue very easily. The signal may be changes in hydraulic pressure in the xylem which can be transmitted through dead tissue (Braam, 2005). The long-distance messenger may also be chemical (Schildknecht and Meier-Augenstein, 1990; Ueda *et al.*, 2001).

Regulation of stomata movements

Stomata are part of a plant's epidermal system which regulate gas and vapor exchange between the plant and the atmosphere. They are mostly found on leaves, though there can be stomata on green stems, petioles, sepals, petals, and fruit. The discussion of stomata is often limited to their function in leaves, since in other tissues, their regulatory role in growth and development is often limited. There are usually more stomata on the upper side compared with the lower side of dicotyledonous leaves, while monocots usually have the

same number of stomata on their upper and lower surfaces. In leaves of floating plants, stomata may be limited to the upper surface.

The functional units of stomata consist of specialized cells called guard cells which are loaded with chloroplasts, and subsidiary cells which lack chloroplasts. When guard cells are turgid, stomata are open, when they are flaccid, stomata are closed. This opening is called the stoma, even though the term is also used to refer to the stomatal complex as a whole. It is the guard cell shape and their cell wall anatomy which provide their shape-changing function.

The cell walls of guard cells are unevenly thickened. The wall away from the pore which forms when turgid (the dorsal wall) is thinner than the wall bordering the pore (the ventral wall). In addition, the cellulose microfibrils are arranged radially around the cell. The guard cells are attached to each other at each end. Increased turgor in the guard cell causes the dorsal wall to bulge away from the pore and the ventral wall to become concave. The entire cell appears to bow away from the aperture causing the pore between the two guard cells to enlarge. When turgor is lost, just the opposite occurs.

When environmental conditions are favorable for stomatal opening (high light levels (especially blue light), adequate soil moisture) protons (H^+) are pumped out of the guard cells creating an increasingly negative electrical charge inside the guard cell. When the electrical potential reaches a critical level, enzyme channels open allowing potassium ions to flow into the guard cell. To keep the negatively charged guard cell interior, negative ions (either chloride ions or malate ions) enter the cell accompanying the potassium. This increase in potassium and its attendant negative ion decreases the osmotic potential of the guard cell causing water from surrounding cells (subsidiary and epidermal cells) to enter the guard cell, greatly increasing the guard cell turgor. Because the cellulose microfibrils are radially oriented, which prevents the cell from expanding radially, the guard cell elongates. Since the guard cells are attached at both ends and the dorsal and ventral walls are unevenly thickened, the elongating cells bow outwards creating the stomatal pore.

When light is removed from the equation, the proton pumps shut down and potassium and chloride or malate ions leave the cell. The osmotic potential increases, thus water leaves the cell, it becomes flaccid, and the stomatal pore disappears.

During drought or a water deficit, stress occurs, and abscisic acid (ABA) is produced. The concentration of free Ca^{2+} in the cytosol increases due to influx from outside the cell and the release of Ca^{2+} from internal cellular stores such as the endoplasmic reticulum and vacuole. The increase in free calcium concentration causes the chloride ions and inorganic ions to leave the guard cells and stops any further uptake of potassium. In time potassium begins to leave the cell. This increases the osmotic potential causing water to leave the cell. The cells become flaccid and the stomatal pore closes.

The fact that light stimulates the opening of stomata makes sense since light stimulates photosynthesis, and in order for photosynthesis to proceed, carbon dioxide needs to enter the leaf. It also makes sense that carbon dioxide concentration can also regulate stomatal opening and closing. This mechanism is especially important in species where crassulacean acid metabolism occurs (CAM plants) since their stomata are open at night and close during the day.

Stomata open in response to low sub-stomatal cavity carbon dioxide concentrations and close when sub-stomatal levels of carbon dioxide are high. In C3 or C4 plants ('regular' photosynthesis), sub-stomatal carbon dioxide levels increase in the dark since light is not available for driving the photosynthetic machinery. In CAM plants, sub-stomatal carbon dioxide levels remain low at night since carbon dioxide is being fixed to malate.

Low carbon dioxide levels in guard cells (resulting from low sub-stomatal cavity concentrations) stimulates potassium uptake which reduces the osmotic potential of the guard cell. This causes an uptake of water by the guard cells, they become turgid, and the stomatal aperture opens.

Thigmo responses in carnivorous plants

VENUS FLYTRAP (*D. MUSCIPULA*) The Venus fly trap is probably the most widely recognized carnivorous plant. Specialized bi-lobed leaves with three small trigger hairs on the inside surface of each lobe wait for stimulation by an unsuspecting insect (Fig. 4.5). Upon several stimulations of one hair or concurrent stimulation of two or more hairs at a time, the lobes quickly close, entrapping the victim. Hair stimulation induces an electrical signal which causes uneven enlargement of lobe cells resulting in closure within a second (Curtis, 1834;



Fig. 4.5. Venus fly trap (*Dionaea muscipula*) with specialized bi-lobed leaves with three small trigger hairs on the inside surface of each lobe waiting for stimulation by an unsuspecting insect. (Photo by Noah Elhardt, licensed for use under the Creative Commons Attribution-Share Alike 2.5 Generic license.)

Burdon-Sanderson, 1873; Jacobs, 1954; Jacobson, 1965; Simons, 1981; Fagerberg and Allain, 1991). Attempts to escape further agitate the lobes causing tighter closing and the release of acids and digestive enzymes which results in the victim's demise. This activity supplies the plant with nitrogen in an otherwise nitrogen-poor environment in which they grow.

SUNDEW (*DROSERA* SPP.) There are many different species within the genus *Drosera* that are called sundew plants. All sundews are characterized by having tentacle-covered leaves which exude sticky mucilage. Insects landing on the leaves become entrapped in the gluey mucilage. Their movement to free themselves induces a slow inward curling of neighboring tentacles further entrapping the prey. Tentacle movement is both thigmonastic and thigmotropic (Lloyd, 1942). The curling inwards and towards the insect seems to be at least partially regulated by auxin levels and tissue sensitivity to auxin, since exogenously applied auxin can trigger the curling response, while auxin blockers can inhibit it (Bopp and Weber, 1981). The sensitivity

of the tentacles is incredible in that they can detect prey weighing less than 1 μg , yet do not respond to the force imparted by raindrops falling on them (Darwin, 1880, 1893).

BLADDERWORT (*UTRICULARIA*) The bladderwort is a rootless plant that grows in aquatic and moist terrestrial environments. Attached to its fine leaves are numerous bladders approximately 3 mm in length held up by thin filaments. Each bladder is a hollow sac, two-cells thick with a valve which remains closed until stimulated by an unsuspecting insect. When the trap is closed, the bladder is under significant negative hydrostatic pressure. Upon stimulation of sensory hairs on or near the valve, the valve opens, sucking in water and the prey that triggered the opening. The prey is then digested within the bladder (Lloyd, 1942). In order to catch more prey, the bladder has to reset itself. This is accomplished by active transport of chloride ions along with accompanying sodium ions into the wall of the bladder. An osmotic gradient is established thereby causing water to leave the lumen of the bladder and enter the bladder wall. Hydrostatic pressure builds up within the wall space, expelling the solution into the outside environment. A bladder can be reset in about 30 min.

General growth responses – thigmomorphogenesis

Gradual changes in growth of plants due to stimuli such as touch or wind is known as thigmomorphogenesis (Jaffe, 1973). These growth changes occur slowly over time and are often not noticed. Shoot thigmomorphogenesis is often characterized by decreased elongation growth with a concomitant increase in radial expansion (Biddington, 1986). Other growth responses include: (i) time of flowering; (ii) dormancy induction and release; (iii) senescence; and (iv) stress resistance (Biddington, 1986).

The signal for these growth changes probably involves the plant hormones as well as intracellular calcium levels (Braam, 2005). Intracellular calcium levels have long been suggested as secondary messengers for many of the stimuli affecting plant growth. Rapid increases in intracellular calcium occur in response to touch or wind stimuli (Knight *et al.*, 1991). Reactive oxygen species (ROS) are also thought to play a role in morphogenic signaling (Mori and Schroeder, 2004) and probably interact with calcium in morphogenic regulation (Mori and Schroeder, 2004).

Ethylene has long been implicated in thigmomorphogenic responses. Exogenous application of ethylene can induce thigmomorphogenic growth responses (Goeschl *et al.*, 1966; Brown and Leopold, 1972; Jaffe and Biro, 1979; Erner and Jaffe, 1983; de Jaegher *et al.*, 1987; Telewski, 1995) and ethylene production occurs after mechanical stimulation (Goeschl *et al.*, 1966; Biro and Jaffe, 1984; Takahashi and Jaffe, 1984). However, mutant and inhibitor studies suggest that ethylene is not the primary signaling molecule (Boyer *et al.*, 1983; Biro and Jaffe, 1984; Biddington, 1986; Boyer *et al.*, 1986; Johnson *et al.*, 1998).

A group of genes called touch inducible genes (TCH genes) were discovered in *Arabidopsis* (Braam and Davis, 1990). Since this initial discovery, many more examples of genes induced by touch have been revealed (Ling *et al.*, 1991; Perera and Zielinski, 1992; Gawienowski *et al.*, 1993; Botella and Arteca, 1994; Botella *et al.*, 1996; Mizoguchi *et al.*, 1996; Oh *et al.*, 1996; Royo *et al.*, 1996; Shirsat *et al.*, 1996; Eldick *et al.*, 1997; Mauch *et al.*, 1997; Gilmour *et al.*, 1998; Arteca and Arteca, 1999; Gadea *et al.*, 1999; Hirsinger *et al.*, 1999; Tatsuki and Mori, 1999; Müssig *et al.*, 2000; Oufattole *et al.*, 2000; Lee *et al.*, 2005). Genes that are down-regulated by touch are also known (Lee *et al.*, 2005). Calcium binding proteins and wall modifying enzymes are among the major products of touch up-regulated genes. In addition, genes implicated in disease defense responses are also up-regulated by touch.