



## Tansley review

# In touch: plant responses to mechanical stimuli

Author for correspondence:

Janet Braam

Tel: +713 348 5287

Fax: +713 348 5154

Email: braam@bioc.rice.edu

Received: 18 August 2004

Accepted: 13 September 2004

Janet Braam

Biochemistry and Cell Biology, Rice University, 6100 Main St, Houston, TX 77005-1892, USA

## Contents

|  |     |  |     |
|--|-----|--|-----|
| Summary  | 373 | V. Discovery of the <i>Arabidopsis TCH</i> genes       | 384 |
| I. Introduction  | 374 | VI. Microarray identification of touch-inducible genes | 384 |
| II. Fast and fascinating – thigmonasty and thigmotropism | 374 | VII. Regulation of <i>TCH</i> gene expression          | 385 |
| III. Subcellular touch-induced movements                 | 382 | Acknowledgements                                       | 386 |
| IV. Thigmomorphogenesis                                  | 382 | References   | 386 |

## Summary

**Key words:** *Arabidopsis*, mechanical stimuli, mechano-responses, *TCH* genes, touch.

Perception and response to mechanical stimuli are likely essential at the cellular and organismal levels. Elaborate and impressive touch responses of plants capture the imagination as such behaviors are unexpected in otherwise often quiescent creatures. Touch responses can turn plants into aggressors against animals, trapping and devouring them, and enable flowers to be active in ensuring crosspollination and shoots to climb to sunlit heights. Morphogenesis is also influenced by mechanical perturbations, including both dynamic environmental stimuli, such as wind, and constant forces, such as gravity. Even individual cells must sense turgor and wall integrity, and subcellular organelles can translocate in response to mechanical perturbations. Signaling molecules and hormones, including intracellular calcium, reactive oxygen species, octadecanoids and ethylene, have been implicated in touch responses. Remarkably, touch-induced gene expression is widespread; more than 2.5% of *Arabidopsis* genes are rapidly up-regulated in touch-stimulated plants. Many of these genes encode calcium-binding, cell wall modifying, defense, transcription factor and kinase proteins. With these genes as tools, molecular genetic methods may enable elucidation of mechanisms of touch perception, signal transduction and response regulation.

*New Phytologist* (2005) **165**: 373–389

© *New Phytologist* (2004) doi: 10.1111/j.1469-8137.2004.01263.x

## I. Introduction

From the violence of tree strangling and insect trapping to the elegance of roots navigating through barriers in the soil, responses to mechanical perturbation are integral features of plant behavior. Although some plants have very specialized touch-response machinery and rapid and highly noticeable behaviors, touch responses of other plants may occur slowly over time and are often therefore not easily recognized or appreciated. Yet probably all plants sense and respond to mechanical forces. Indeed, cellular responses may be critical for fundamental processes such as turgor regulation, cellular expansion and morphogenesis. The mechanistic bases of touch perception and inter- and intracellular signaling are not well understood. It is also unclear whether the widely diverse responses are related at either the perception or response level or whether the mechanisms and machinery used by single cells to respond to mechanical perturbations such as turgor fluctuations are related to those used at the organ or tissue level to react to externally applied mechanical forces.

For excellent additional information, I refer readers to wonderful reviews written both long ago and more recently (Darwin, 1880, 1893, 1906; Lloyd, 1942; Biddington, 1986; Edwards & Pickard, 1987; Simons, 1992; Trewavas & Knight, 1994; Slack, 2000; Jaffe *et al.*, 2002). Here, I describe briefly some diverse touch responses in higher plants. In addition, I report on aspects of genomic responses to mechanical perturbations and the potential of genetic approaches to this intriguing area of biology.

## II. Fast and fascinating – thigmonasty and thigmotropism

Rapid responses to touch responses are generally thigmotropic or thigmonastic (thigma is the Greek word for touch). Tropic and nastic responses are distinguished by the influence of the stimulus vector on the direction of movement. Tropic responses occur in a direction determined by the placement or direction of the stimulus, such as the climbing behavior exhibited by some mechano-sensitive vines. In contrast, nastic responses are movements, such as the folding up of *Mimosa pudica's* leaflets, which occur in a direction largely independent of the direction of the stimulus.

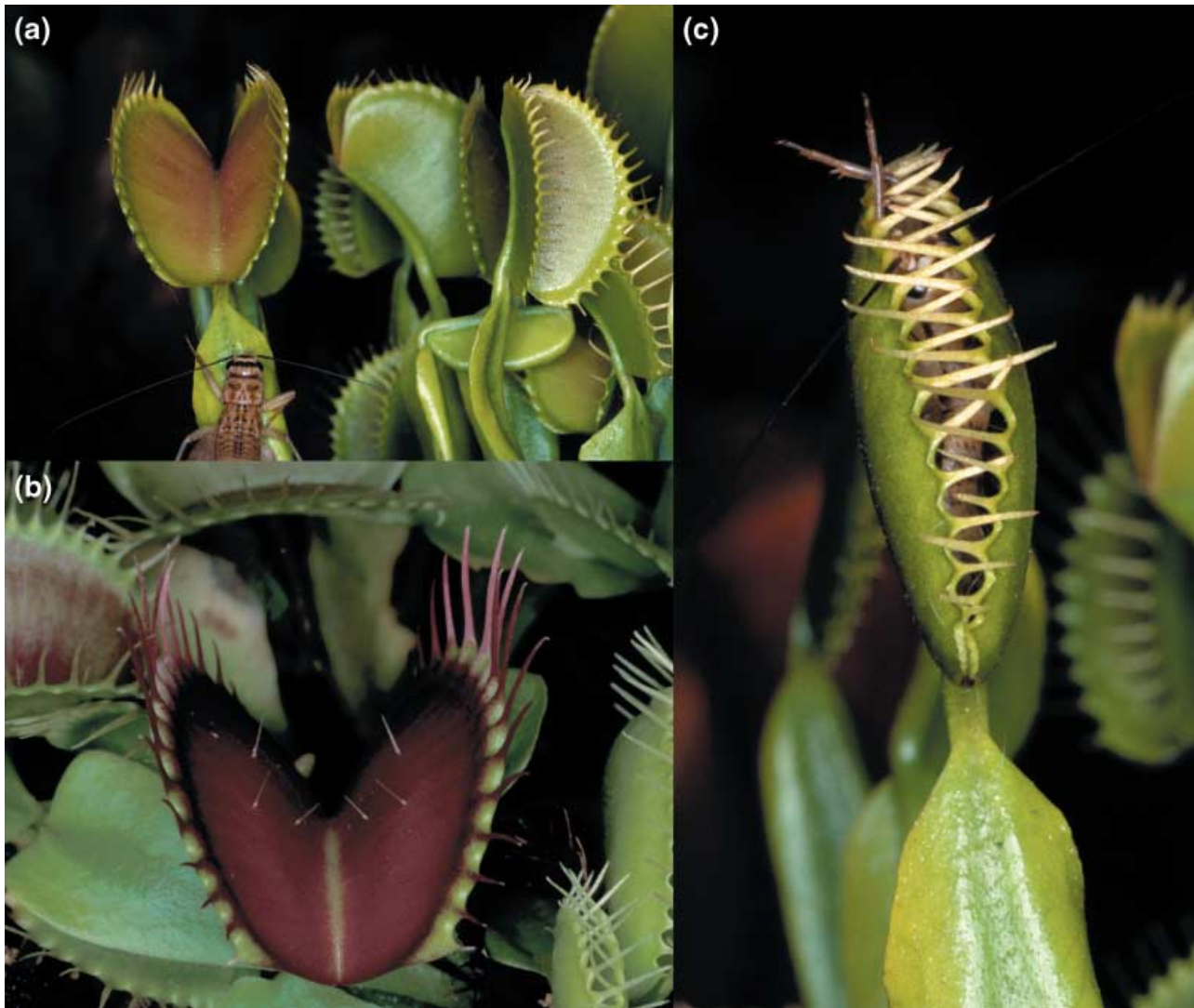
### 1. Carnivorous plants

Venus' Flytrap (*Dionaea muscipula*) accomplishes one of the more spectacular thigmonastic movements resulting in a swap of the role of predator with animals (Simons, 1992). Indeed, Darwin referred to it as 'one of the most wonderful in the world' (Darwin, 1893). The specialized bi-lobed leaves with needle-shaped tines on the leaf margins are generally spread open as invitations to visitors (Fig. 1a). When an unsuspecting insect crawls along the ventral leaf surfaces and bumps into

the three small trigger hairs (Curtis, 1834) (Fig. 1b), inter-cellular electrical signals are generated (Burdon-Sanderson, 1873; Jacobs, 1954; Jacobson, 1965; Simons, 1981) and are thought to trigger differential enlargement of lobe cells (Fagerberg & Allain, 1991), resulting in trap closure in less than a second. Generally, multiple stimulations of one or more of the trigger hairs are required for a response. This first stage of closure interlaces the tines trapping the prey inside the barred cage (Fagerberg & Allain, 1991) (Fig. 1c). Smaller insects, perhaps not worth the energy of further trapping and digestion, may be free to escape through the bars, but larger insects struggle to get free (Darwin, 1893). The insect's further agitation and excretion of nitrogenous compounds, although not always necessary for complete closure (Fagerberg & Allain, 1991), may enhance the subsequent sealing of the lobes around the doomed animal and the secretion of acids and digestive enzymes that kill and digest the nascent meal (Robins & Juniper, 1980). Spatially complex and dynamic changes in cell expansion among distinct tissue layers and sublobe domains effect each stage of the trap closure (Fagerberg & Allain, 1991). This carnivory provides nitrogen supplements to the Venus' Flytrap, enabling them to live in nitrogen-poor environments.

Distinct touch-induced carnivorous behavior is demonstrated by *Drosera rotundifolia*, a species examined in detail by Darwin (Darwin, 1893). The mucilage on the ends of the *Drosera's* tentacles glisten in the sunlight (Fig. 2a,b) and give the plant its common name 'Sundew'. Over 100 tentacles can cover the surfaces of its modified leaves. This showy appearance attracts insects, which upon alighting on the tentacle surfaces become trapped in the gluey substance. The tactile sensitive tentacles detect the presence and further movements of the insect as it struggles to be released. In response, neighboring tentacles bend towards the ones directly stimulated by the prey's presence. Together the tentacles can generate a cup-like indentation that encloses the prey within (Fig. 2c,d). Both thigmonastic and thigmotropic growth-based tentacle movements are involved (Lloyd, 1942). Differential growth may be controlled by changes in auxin levels or sensitivity, as exogenously applied indole-3-acetic acid (IAA) can elicit similar growth behaviors and IAA transport blockers can inhibit touch-induced movements (Bopp & Weber, 1981). The mechano-sensitivity of the tentacles is remarkable, being able to detect a piece of human hair weighing less than a microgram (Darwin, 1880) and yet they fail to respond to the water droplets or even the force of heavy rains (Darwin, 1880; Darwin, 1893). How the plant distinguishes between stimuli unrelated to food, such as rain and wind, from the perturbations of insects is unclear.

The behavior of the bladderwort, *Utricularia*, which may have the most forceful prey capture mechanism among plants, is described in detail by Lloyd (Lloyd, 1942). *Utricularia* have worldwide distribution in aquatic and moist terrestrial environments. Lloyd's writing reveals a particular reverence for this creature and its traps that 'present an astounding

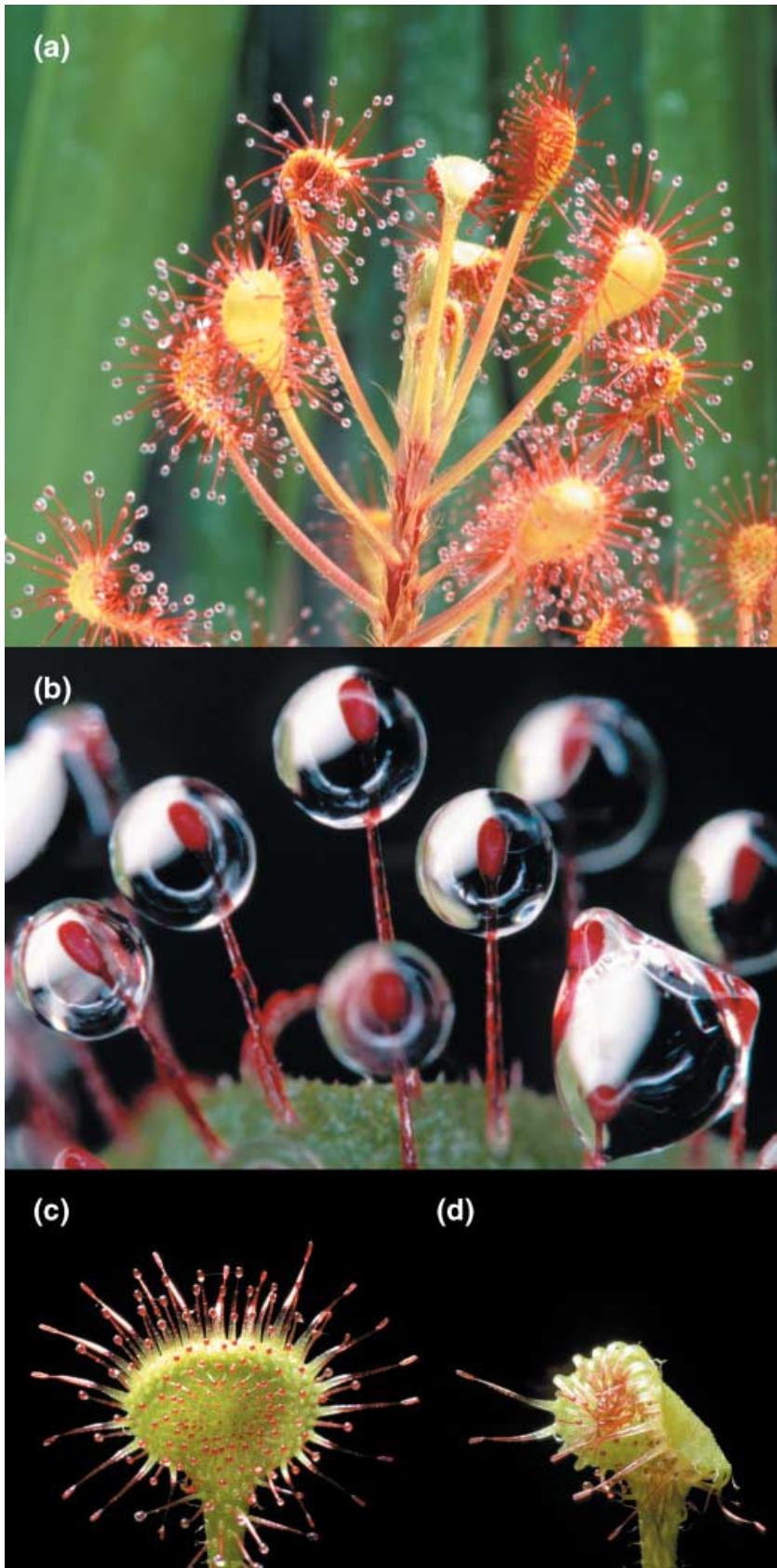


**Fig. 1** *Dionaea muscipula* (Venus' Flytrap). (a) Potential prey nears an open trap. (b) Close-up of trap showing trigger hairs. (c) Interlaced tines trap prey in first phase of trap closure. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.

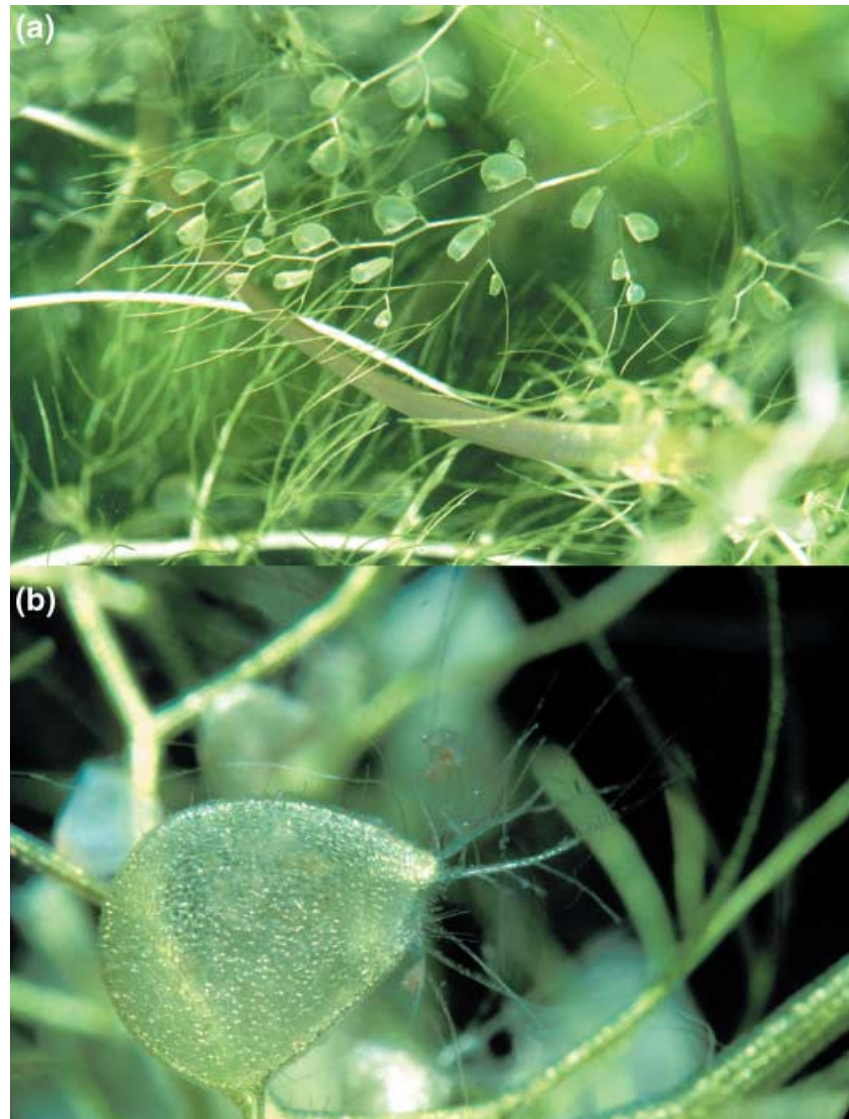
degree of mechanical delicacy depending on a fineness of structure scarcely equaled elsewhere in the plant kingdom'. The *Utricularia* trap, attached via a stalk to the rootless plant body (Fig. 3a), is a thin-walled hollow sac with a watertight trapdoor. When the trap is set, the outer walls are concave and the chamber is under negative hydrostatic pressure. Various appendages, some with mucilage-secreting glands, have roles in luring prey to the trap door entrance and others near the bottom of the door are the touch-sensitive triggers (Fig. 3b). Small creatures, such as water fleas, are examples of those that fall prey to *Utricularia* traps. Within 30 ms of tactile detection, the trap door bursts open, the sac walls move outward to take on a slightly convex shape and, as a result, there is a tremendous suction of water into the bladder. All accompanying creatures are swept in with the current. The trapdoor closes and the meal takes place.

## 2. The sensitive plant

*Mimosa pudica*, known as the sensitive plant, and some of its relatives are other well-known thigmonastic plants. A touch stimulation results in a very rapid folding up of the small leaflets composing the doubly compound leaves (Fig. 4a,b). Remarkably, the touch response is not restricted to the stimulated leaflet, but can propagate to all the neighboring leaflets of the leaf (Simons, 1981; Malone, 1994). A stronger stimulus, resulting in wounding, can elicit folding behavior at distantly located leaf petioles (Simons, 1981; Malone, 1994). The rapid folding of the leaflets may serve to both scare away potential predators and give the appearance of a less voluminous meal. Alternatively, the leaf movements may be a mechanism to expose protective thorns (Eisner, 1981).



**Fig. 2** *Drosera* (Sundew). (a) Open and folded tentacle-laden leaves of *D. madagascariensis*. (b) Close up of mucilage-covered tentacle tips. (c) Open leaf before touch stimulation. (d) Folding leaf following touch stimulation. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.



**Fig. 3** *Utricularia* (Bladderwort).  
(a) Numerous *U. foliosa* bladder traps.  
(b) *U. inflata* side view with tentacles visible  
at right near trap door. Photos by Barry Rice,  
PhD; <http://www.sarracenia.com/galleria/galleria.html>.

The thigmonastic *Mimosa* leaflet closure is mechanically similar to the rhythmic movements of related species, such as *Samanea saman* (Satter *et al.*, 1990). Both of these folding leaf movements result from a loss of turgor from extensor cells of pulvini, specialized motor organs located at the bases of the leaflets and petioles. Opposite in position from the extensors are the flexors. When the extensors lose turgor, the flexor cells stretch. Together these reversible cell volume and shape changes enable elaborate leaflet and petiole movements.

The touch-induced extensor cell volume changes in *Mimosa* may be initiated by a triggered unloading of sucrose from the phloem, resulting in the lowering of apoplastic water potential (Fromm & Eschrich, 1988). Extensor cell turgor and volume loss occur through the passive transport of  $K^+$  and  $Cl^-$  ions and the concomitant water movement driven by osmosis (Simons, 1981; Fromm & Eschrich, 1988). Strong

electrochemical gradients enabling rapid ion movements across plasma membranes are set up by proton pumps (Roblin, 1982), and *Mimosa* motor cells have an abundance of  $H^+$ -ATPase proteins (Fleurat-Lessard *et al.*, 1997), perhaps to accommodate a requirement for high pump activity. The osmotically driven cellular water loss is extensive, with up to a 25% volume change, and rapid, occurring within 1 s (Fleurat-Lessard *et al.*, 1997b). Such a fast and dramatic loss of water from pulvinar cells is likely facilitated through transporters such as aquaporins or solute-water cotransporters (Fleurat-Lessard *et al.*, 1997a,b; Morillon *et al.*, 2001; Moshelion *et al.*, 2002). The actin cytoskeleton may also be involved; inhibitors that affect actin can block movements (Fleurat-Lessard *et al.*, 1993), and movements are correlated with decreased actin tyrosine phosphorylation (Kameyama *et al.*, 2000).



**Fig. 4** *Mimosa pudica* (Sensitive Plant). (a) The doubly compound leaves of *Mimosa pudica* open before stimulation. (b) The folding up of leaflets after touch stimulation. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.

How pulvini located far from the initial site of stimulation receive signals is uncertain. Action potentials, likely traveling through companion cells and phloem parenchyma (Fromm & Eschrich, 1988; Fleurat-Lessard *et al.*, 1997a), may be responsible for touch-stimulated leaflet folding within a single leaf (Houwink, 1935). Electrical signals, called slow wave potentials or variation potentials, are also detected systemically after wounding. However, these systemic signals have properties inconsistent with action potentials in part because they can travel through dead tissue. Indeed, the electrical phenomena detected in wounded *Mimosa* and other plants may not be propagating signals but static responses to hydraulic pressure changes that move through the xylem (Malone, 1994; Stahlberg & Cosgrove, 1997). Xylem pressure alterations can distribute pressure radially to epidermal cell layers and trigger local epidermal cell membrane depolarizations (Stahlberg & Cosgrove, 1997).

Thus, it may be that hydraulic pressure, not the detected electrical changes, serves as the transmission mechanism for distant wound responses. There is also evidence for chemical signals in *Mimosa*. As early as 1916, a role was proposed for a chemical signal, Ricca's factor, that could travel through a water-filled tube connecting severed stem tissues (Ricca, 1916) to signal distant leaf folding in response to wounding. More recently, substances that can induce leaf closing have been isolated and characterized (Schildknecht & Meier-Augenstein, 1990; Ueda *et al.*, 2001), adding evidence for a potential role of chemical signaling in the touch-responsive leaflet folding behavior of *Mimosa*.

### 3. Touch-sensitive tendril coiling and vine climbing

Many plants utilize a sense of touch to achieve vertical height with the goal of rich sun exposure while avoiding the energy

expenditure of developing a supporting trunk. These plants can reach out to grasp sturdy objects and then use them as supports by coiling around them.

Tendrils are often modified leaves or stems (Jaffe & Galston, 1968), appearing as long graceful fingers capable of coiling. The distal portions of tendrils are highly touch-sensitive. Indeed, some tendrils may have greater touch sensitivity than humans. Simons reports that a 0.25 mg thread drawn along a tendril can be enough to evoke a response (Simons, 1992), and Darwin documented tendril responses to stimuli in the range of 1–5 mg (Darwin, 1906). Touch stimulation leads to a rapid onset of tip coiling, often within seconds, that enables a secure association with an object. Some species have thigmonastic tendrils and others are thigmotropic, depending upon whether the direction of contact coiling is determined by the structure of the tendril or the direction or point of touch stimulation (Jaffe & Galston, 1968). The touch sensitivity of tendrils shares a selective sophistication with that of *Drosera*. Application of water droplets causes no coiling response; therefore tendrils avoid nonproductive coiling in rainstorms (Jaffe & Galston, 1968). Furthermore, coiling that occurs in response to transient stimulation are often reversed by uncoiling (Jaffe & Galston, 1968).

Some species undergo a second round of tendril coiling between the tip coiling at the support and the attachment of the tendril to the main body of the plant. This secondary coiling behavior, called free coiling (Jaffe & Galston, 1968), serves to bring the climbing plant closer to its support. Once coiling has occurred, wall changes such as lignification are often induced to stiffen the coils and prevent unwinding (Jaffe & Galston, 1968).

Tendril coiling results from differential growth. The earliest responses include a contraction of ventral cells and an expansion of dorsal cells. Sustained coiling then follows with a differential rate of cell expansion across the ventral and dorsal surfaces (Jaffe & Galston, 1968).

Elmar Weiler and colleagues have found that an increased level of octadecanoids, in particular 12-oxo-phytodienoic acid (OPDA), correlates with touch-induced *Bryonia dioica* tendril coiling (Stelmach *et al.*, 1998; Blechert *et al.*, 1999) and that OPDA, and related analogs that cannot be converted to jasmonic acid (JA), are sufficient to cause tendril coiling with physiologically relevant kinetics in the absence of a mechanical stimulus (Weiler *et al.*, 1993, 1994; Blechert *et al.*, 1999). Octadecanoid pathway signaling may not be universally involved in touch-induced tendril coiling, however, because exogenous OPDA or JA is not sufficient to induce pea tendril coiling (Brosché & Strid, 2000; Engelberth *et al.*, 2001). In *B. dioica*, octadecanoid signaling leads to IAA accumulation (Stelmach *et al.*, 1999), which itself is an active inducer of tendril coiling (Jaffe & Galston, 1968; Jaffe, 1985; Weiler *et al.*, 1993). The central role of IAA in differential growth leading to tropic behaviors is well established (Friml, 2003), so it is not surprising that IAA may have a major role in tendril coiling.

In contrast, ethylene, whose levels increase in octadecanoid- or IAA-treated plants, is not required for the coiling response because coiling still occurs when ethylene synthesis is blocked (Weiler *et al.*, 1993).

Alamethicin (ALA), derived from a parasitic plant fungus and capable of forming voltage-dependent ion channels, can induce tendril coiling in both *B. dioica* and *Pisum* (Engelberth *et al.*, 2001). Because this effect of ALA occurs even when octadecanoid synthesis is inhibited, it may be that ALA-induced membrane depolarization can trigger tendril coiling, perhaps through alterations in IAA accumulation, transport or action (Engelberth *et al.*, 2001).

The *Monstera* vine has an unusual technique for finding a host tree to climb. It travels by growing along the ground in search of the darkness of a tree's shadow; this movement towards darkness is called skototropism (Strong & Ray, 1975). The vine completes its journey when the vine apex senses arrival at the host tree by the resulting touch encounter. A dramatic change in growth direction then occurs. The *Monstera* vine begins an upward climb of its host tree (Strong & Ray, 1975), and leaf and stem development changes in anticipation of increased light and productive photosynthesis.

The strangler fig (*Ficus costaricensis*) has a more sinister interaction with host trees (Putz & Holbrook, 1986; Putz & Holbrook, 1989). Roots of the epiphyte form grow downward from its host tree and, in addition to wrapping around the trunk, roots that touch each other fuse together (Fig. 5). As the roots continue to grow and fuse, they eventually encircle and prevent further growth of the host trunk. Further differentiation of the figs' roots result in such constricting pressure that it damages and eventually kills the host in a manner befitting its name. In this way, the strangler remains alone without competition from its former host.

#### 4. Bold flowers

Many flowers have evolved touch-sensitive organs, with examples of thigmonastic and thigmotropic stamen filaments, petals and pistils among many plant species. These behaviors generally are focused on preventing self-pollination or successfully depositing pollen on insects or hummingbirds. Self-pollination is avoided by a variety of touch-responsive mechanisms. For example, some stigmas bend toward the petals in response to an insect crawling down towards its anthers, apparently to avoid contact with the pollen-laden visitor as it departs (Simons, 1992). Stamens of other species avoid accidental pollination by bending away from the stigma during circadian-regulated flower closure (Jaffe *et al.*, 1977). More commonly, touch-sensitive stamens bend over to dab pollen on visiting insects (Simons, 1992). Some touch-responsive flowers have spring-loaded mechanisms that trigger rather explosive responses (Simons, 1992). One fascinating example is the behavior of the dimorphic flowers of *Catsetum*. Male flowers are poised to respond to visitors that contact antennae



**Fig. 5** *Ficus costaricensis* (Strangler Fig). Strangler fig roots surrounding host tree trunk. Photo by Tim Skyrme; <http://www.artzend.com>.

in the center of the flower by the release of stamen filaments held under tension by petals (Fig. 6a,b). The force by which a sticky disc with pollen sacs hits the chosen pollinator can be strong enough to knock a bee from the flower (Simons, 1992). This experience and the burden of the attached large pollen sac can be so traumatic that the bee will carefully evaluate potential future visiting sites and strongly prefer female flowers rather than the explosive male ones (Romero & Nelson, 1986). In this way, male *Catasetum* flowers may have evolved to compete for exclusive pollination rights of female flowers (Romero & Nelson, 1986).

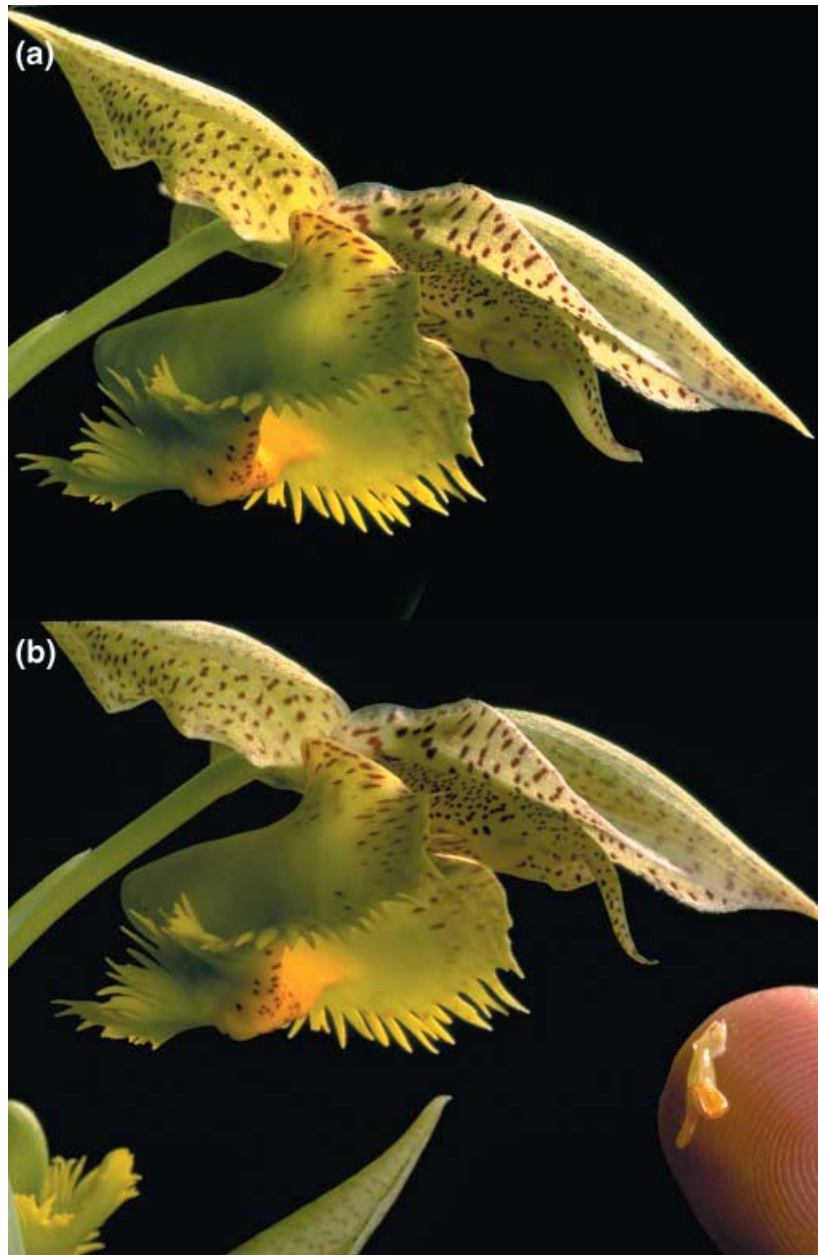
### 5. The struggle of roots

Darwin (Darwin, 1880) contemplated the fate of roots as they grow through the soil encountering many obstacles, such as

stones, in their paths. He observed that when roots are blocked in their downward growth pattern by an obstacle, such as a plate of glass, root tips flatten, take on an oblique shape and turn nearly 90° to take on a new direction of growth over the surface of the glass. Darwin hypothesized that the root apex is touch-sensitive and that contact results in a transmission of a signal to change growth at more proximal root regions. He also subjected young root apices to local mechanical stimulation through the placement of small, flat materials such as sandpaper to the sides of root tips. He observed a loss in gravitropic behavior as thigmotropic growth resulted in movement in a direction away from the point of contact.

More recently, Gilroy and colleagues have reported similar avoidance behavior in *Arabidopsis* roots (Massa & Gilroy, 2003). When *Arabidopsis* roots encounter glass barriers, gravitropism





**Fig. 6** Male *Catasetum* orchid flowers before and after triggered release of pollen sac. (a) The pollen sac is held within the pointed anther cap. (b) When the trigger is touched, the sticky pollen sac is fired at the intruder. Photo by Greg Allikas; <http://www.orchidworks.com>.

appears to be compromised and thigmotropism occurs instead. The touch-stimulated roots neglect their propensity to grow towards gravity vector and instead grow away from the perceived point of mechanical contact. The interaction of touch and gravity may occur at the subcellular level; touch can delay one of the earliest known graviresponses, the falling of starch granules in the root columella cells (Massa & Gilroy, 2003).

This mechanical stimulus avoidance response of roots has been proposed to be an important component of the wave-like turning growth of *Arabidopsis* roots on dense agar plates

held at an incline (Okada & Shimura, 1990). Under these growth conditions, the roots form in the pattern of a sinusoidal wave as a consequence of alternating directions of differential cell expansion in the elongation zone and twisting of tip cells. Mutants defective in this elegant growth patterning have provided evidence of the importance of polar auxin transport and microtubule function for proper waving behavior (Okada & Shimura, 1990; Bennett *et al.*, 1996; Garbers *et al.*, 1996; Deruere *et al.*, 1999; Thitamadee *et al.*, 2002; Nakajima *et al.*, 2004; Sedbrook *et al.*, 2004).



**Fig. 7** Repetitive touch stimulation leads to a delay in flowering and an inhibition of inflorescence elongation in *Arabidopsis*. The plants on the right were touched twice daily; the plants on the left are untreated controls. Photo by Dereth Phillips, Rice University.

### III. Subcellular touch-induced movements

Touch responses also occur at the cellular level; organelles move in directed fashion in mechanically perturbed cells. For example, stimulation with a glass capillary induces chloroplast migration away from the point of contact (Sato *et al.*, 1999). This behavior is dependent upon the activity of gadolinium- and lanthanum-sensitive functions (Gd and La), suggestive of a role for stretch-activated channels, and is dominant to light-induced responses (Sato *et al.*, 2001).

Nuclear migration is also affected by a mechanical perturbation to the cellular surface; nuclei move closer to the site of microneedle contact and induced distortion of the cell wall (Kennard & Cleary, 1997; Gus-Mayer *et al.*, 1998).

### IV. Thigmomorphogenesis

In contrast to the generally rapid thigmonastic and thigmotropic responses of plants or organs specialized to respond to externally applied mechanical stress, gradual morphogenetic alterations in response to stimuli such as touch and wind are common, if not universal, among higher plants. These touch-induced morphogenetic changes occur slowly over time and are therefore often not readily apparent or appreciated; however, these responses can be quite dramatic (Fig. 7). Mark Jaffe, who has carried out systematic analyses of plant growth responses to mechanical perturbations over the past 30 year, coined the term 'thigmomorphogenesis' to describe the touch-induced developmental response of nonspecialized plants (Jaffe, 1973). Biddington (Biddington, 1986) summarizes in detail many of the diverse aspects of thigmomorphogenesis and how responses vary in different species. Briefly, the most

common features of shoot thigmomorphogenesis among many different plant species are a decrease in elongation growth and an increase in radial expansion. These growth changes are likely adaptive, enabling plants to withstand additional mechanical force stresses (Telewski & Jaffe, 1986). Touch-induced morphogenesis changes are correlated with increased production of strengthening tissue and improved resistance to mechanical perturbation induced damage (Jaffe *et al.*, 1984; Biddington, 1986; Telewski & Jaffe, 1986). Some species increase tissue rigidity whereas others have higher flexibility in response to mechanical perturbation (Biddington, 1986; Telewski, 1995).

The earliest detectable physiological touch responses include electrical resistance changes within seconds (Jaffe, 1976) and localized phloem blockages within 1 to 2 min (Jaeger *et al.*, 1988) after stimulation. Younger tissues show greater magnitude responses than older tissues (Biddington, 1986). Long-distance signaling is also likely because growth alterations are not limited to regions that are directly stimulated but are also found at sites not directly stimulated (Erner *et al.*, 1980; Biddington, 1986; Depege *et al.*, 1997; Coutand *et al.*, 2000). In addition to overall growth effects, thigmomorphogenesis can include other changes that are often variable among species. Other processes often affected by mechanical perturbation include flowering time, dormancy, senescence, chlorophyll content, drought resistance, abscisic acid levels, low temperature resistance, pithiness, stomatal aperture and pathogen resistance (Biddington, 1986).

Thigmomorphogenesis may have evolved as a response to wind. Trees allowed to sway in wind tend to have reduced height and greater trunk girth than those tightly staked to prevent movement (Jacobs, 1954). These thigmomorphogenetic-like

growth alterations may be advantageous. Upon freedom from staking, trees suddenly allowed to sway in the wind are more likely to experience wind damage (Jacobs, 1954). Severe wind combined with cold and drought stress can dramatically affect tree form. Krummholz, descriptively referred to as knee forest or twisted wood (Yulsman, 1999), may be an extreme manifestation of thigmomorphogenesis. Krummholz displays an overall stunted phenotype that favors expansion of growth and branching in a leeward direction (Yulsman, 1999).

### 1. The role of strain and feedback mechanisms

Without specialized organs, such as the trigger hairs of Venus' Fly Trap and *Utricularia*, to monitor mechanical perturbations, how do nonspecialized plants perceive such stimuli? Recent experiments indicate that the perception of longitudinal strain is critical, and feedback mechanisms play important roles in determining tissue responses to mechanical perturbation. There is a strong correlation between the degree of longitudinal strain experienced and the extent of the thigmomorphogenetic response (Coutand *et al.*, 2000). Consistent with a central role for strain in inducing thigmomorphogenetic changes, transgenic tobacco plants with xylem of reduced tensile stiffness undergo enhanced xylem development and attain overall stem stiffness comparable to wild type (Hepworth & Vincent, 1999). Enriched production of supporting tissue, such as the xylem cylinder in tobacco stems (Hepworth & Vincent, 1999), may be a mechanism of responding to tissue material properties and their propensity to experience strain. Thus, plants may compensate for defects in wall components by generating a greater abundance of strengthening tissues. This evidence of feedback from wall properties to wall biogenesis is reminiscent of the findings that cellulose-deficient mutants have increased wall pectin content (His *et al.*, 2001), and that inhibition of lignin synthesis results in enhanced cellulose accumulation (Hu *et al.*, 1999). Thus, plants appear to have multiple mechanisms of adapting to wall deficiencies and compensating for potential structural integrity challenges.

Thigmomorphogenesis may not only result in response to externally applied mechanical stress that results in strain. Progressive plant growth itself is sufficient to cause thigmomorphogenetic changes, perhaps by increased weight-triggered tissue strain. For example, increased xylem production occurs in *Arabidopsis* in response to the augmented weight associated with increased height (Ko *et al.*, 2004); enhanced cambium differentiation can also be mimicked by addition of weight to immature inflorescences (Ko *et al.*, 2004). Direct compressive forces applied to callus cells can induce cambium-like development (Lintilhac & Vesecky, 1984; Barnett & Asante, 2000). Just as bone and muscle mass development increase in correlation to the amount of force experienced (Frost, 1992; Goldspink, 1999), tissue strain in plants may induce growth and differentiation to contribute to the reinforcement of

structural tissue integrity. Similar reinforcement must occur at branching points. In this way, plants adapt to morphogenetic and environmental changes, enabling them to maintain continued growth even in windy or otherwise mechanical stress-rich environments.

How the mechanical perturbation caused by strain is sensed at the cellular level is not defined, although two models of mechano-perception at the plasma membrane and/or cell wall have been proposed. As in animal and bacterial cells, plant membranes have stretch-activated channel activity that may be responsible for triggering ion flux changes in response to a mechanical membrane perturbation (Falke *et al.*, 1988; Cosgrove & Hedrich, 1991; Ding & Pickard, 1993; Garrill *et al.*, 1994; Kikuyama & Tazawa, 2001).

An alternative possibility is that proteins that link the extracellular matrix to the plasma membrane and/or cytoskeleton may act as mechano-receptors similarly to how integrins function in animal cells (Wayne *et al.*, 1992; Ingber, 1998; Jaffe *et al.*, 2002; Hayashi & Takagi, 2003). It is also possible that channel activities and tethered transmembrane proteins collaborate to sense cellular mechanical strains and trigger ion fluctuations as intracellular second messengers in plants, as proposed for animal cells (Mano & Driscoll, 1999; Mobasher *et al.*, 2002).

### 2. Involvement of hormones and cellular signaling in thigmomorphogenesis

A number of inter- and intracellular signaling components, including hormones and potential second messengers, have been implicated in touch-induced alterations in plant morphogenesis. At this point, evidence strongly implicates these potential signals as being responses to mechanical stimulation and thus may trigger physiological responses. However, the primary signal controlling the full suite of plant mechano-responses, if such a signal exists, has not yet been identified.

Intracellular calcium ( $\text{Ca}^{2+}$ ) has long been implicated as an important second messenger in mechano-signaling and response in both animal and plant cells. Very rapid cellular  $\text{Ca}^{2+}$  increases are detected in mechanically perturbed cells and tissues (Batiza *et al.*, 1996; Calaghan & White, 1999). Transgenic plants expressing the *Aequoria* jellyfish gene encoding aequorin, a  $\text{Ca}^{2+}$ -dependent luminescent protein, have proven to be valuable tools for monitoring  $\text{Ca}^{2+}$  fluctuations in response to many different kinds of stimuli (Knight, 2000). In particular, aequorin transgenic plants have demonstrated a rapid intracellular  $\text{Ca}^{2+}$  increase in response to touch or wind stimuli (Knight *et al.*, 1991) and have provided evidence that mechano-responsive  $\text{Ca}^{2+}$  increases are derived from internal stores (Knight *et al.*, 1992). The finding that mechanical stimuli, thought to cause organellar membrane stretching, also can evoke organellar  $\text{Ca}^{2+}$  release in *Nitella flexilis* cytoplasmic extracts (Kikuyama & Tazawa, 2001) suggests that mechano-perturbations can be directly sensed by subcellular membrane-bound compartments.

Reactive oxygen species (ROS) are also important signals in plant morphogenesis and responses to stimuli (Mori & Schroeder, 2004). ROS have been detected immediately after a mechanical perturbation (Legendre *et al.*, 1993; Yahraus *et al.*, 1995; Legue *et al.*, 1997; Gus-Mayer *et al.*, 1998). Coincidence of ROS and Ca<sup>2+</sup> changes in many plant behaviors and the evidence for Ca<sup>2+</sup> channel regulation by ROS suggests that these two cellular signals may be interdependently generated and functionally linked. Generation of ROS may be responsible for regulating Ca<sup>2+</sup> channel gating (Mori & Schroeder, 2004).

For many years, ethylene has been proposed to function in aspects of thigmomorphogenesis. Exogenous application of ethylene can result in morphological and physiological changes that resemble aspects of thigmomorphogenesis (Goeschl *et al.*, 1966; Brown & Leopold, 1972; Jaffe & Biro, 1979; Erner & Jaffe, 1983; de Jaeger *et al.*, 1987; Telewski, 1995). Ethylene production occurs after mechanical stimulation of plants (Goeschl *et al.*, 1966; Biro & Jaffe, 1984; Takahashi & Jaffe, 1984). Mutant and some inhibitor studies, however, suggest that although ethylene may have a role in radial expansion aspects of thigmomorphogenesis, ethylene production and/or response are not required for the reduction in elongation growth induced by mechanical perturbation (Boyer *et al.*, 1983; Biro & Jaffe, 1984; Biddington, 1986; Boyer *et al.*, 1986; Johnson *et al.*, 1998). These results and similar studies on tendril coiling (Weiler *et al.*, 1993) are consistent with the idea that ethylene production may be one of the responses to mechanical perturbations, but ethylene is unlikely to be the primary signaling molecule that controls all touch responses.

Octadecanoids have been shown to effect *B. dioica* tendril coiling (Weiler *et al.*, 1993; Weiler *et al.*, 1994) and therefore may have additional touch-response roles. Intriguingly, mechanical stimulation results in an increase in OPDA levels in *P. vulgaris* internodes, and treatment with the OPDA analog, coronatine, can elicit physiological changes reminiscent of thigmomorphogenesis (Stelmach *et al.*, 1998).

## V. Discovery of the *Arabidopsis* TCH genes

Control experiments serendipitously revealed the existence of touch-inducible genes in plants (Braam & Davis, 1990). These genes, originally called the TCH genes and isolated by differential cDNA library screening, are strongly and rapidly up-regulated in expression in response to touch (Braam & Davis, 1990). The TCH genes were identified by their dramatic mRNA accumulation in plants sprayed with gibberellins. However, further northern analyses revealed that TCH expression increased in plants sprayed with a variety of hormones, for example, auxin, cytokinin and abscisic acid, and indeed by a spray of simply water (Braam & Davis, 1990). Finally, a simple mechanical stimulation, administered by touching the rosette leaves and bending them down or

back and forth, was found to be sufficient to elicit a dramatic enhancement of TCH expression (Braam & Davis, 1990). The differential cDNA screening technique tends to identify preferentially genes with high expression levels and indeed the handful of TCH genes proved to be only the tip of the iceberg. Over the past 14 years, many other genes have been discovered, often unintentionally, to have mechano-stimulus-inducible expression (Ling *et al.*, 1991; Perera & Zielinski, 1992; Gawienowski *et al.*, 1993; Botella & 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 & Arteca, 1999; Gadea *et al.*, 1999; Hirsinger *et al.*, 1999; Tatsuki & Mori, 1999; Müssig *et al.*, 2000; Oufattole *et al.*, 2000; Lee *et al.*, 2005).

## VI. Microarray identification of touch-inducible genes

To gain insight into the prevalence of touch-inducible gene expression, a genome-wide search for *Arabidopsis* genes sharing TCH regulatory properties was undertaken (Lee *et al.*, 2005). Hybridization of the 22 810 genes represented on the Affymetrix DNA chip (Affymetrix, Santa Clara, CA, USA) revealed that 589 genes, over 2.5% of the genome, are up-regulated at least twofold in expression within 30 min of a touch stimulation (Lee *et al.*, 2005). One hundred and seventy-one genes have reduced expression (Lee *et al.*, 2005).

TCH1 encodes one of the *Arabidopsis* calmodulins, CAM2, TCH2 and TCH3 encode calmodulin-like (CML) proteins, CML24 and CML12, respectively, and TCH4 encodes a xyloglucan endotransglucosylase/hydrolase (XTH22) (Braam & Davis, 1990; Sistrunk *et al.*, 1994; Xu *et al.*, 1995; Khan *et al.*, 1997; Rose *et al.*, 2002; McCormack & Braam, 2003). Thus, it is not unexpected that Ca<sup>2+</sup>-binding proteins and cell wall modifying enzymes would be among the functions found by microarray analyses to be potentially up-regulated by touch. Indeed, of the 48 CML and 33 XTH genes included on the chip, 19 CMLs and 12 XTHs show greater than twofold up-regulated expression in touched plants (Lee *et al.*, 2005). Genes encoding other putative Ca<sup>2+</sup>-binding proteins, arabinogalactan proteins, pectin esterases, cellulose synthases, expansins and extensins also have touch-inducible expression (Lee *et al.*, 2005). Interestingly, despite the high sequence identity among the seven CAM genes (McCormack & Braam, 2003), only one CAM gene, CAM2/TCH1, was identified by microarray analysis to be touch-inducible (Lee *et al.*, 2005).

Less expected, perhaps, is the finding that genes implicated in disease resistance compose the third most represented functional class of touch-inducible genes. Further investigation into a potential relationship between mechanical perturbation responses and plant disease resistance is necessary. Expression levels of over 10% of the genes encoding kinases and transcription factors are increased in touch-stimulated plants (Lee

*et al.*, 2005), indicating that many kinase transduction pathways and additional transcriptional activities may be impacted by a simple touch perturbation.

## VII. Regulation of *TCH* gene expression

The signaling pathways and transcriptional mechanisms controlling touch-regulated gene expression are not well understood. Intracellular  $\text{Ca}^{2+}$  fluctuations and protein phosphorylation may play roles in touch-regulated gene expression (Braam, 1992; Wright *et al.*, 2002). A functional ethylene response pathway, however, is not required for *TCH* regulation (Johnson *et al.*, 1998).

Intriguingly, expression of the original *TCH* genes is inducible not only by stimuli that appear to share mechanical properties, such as touch and wounding, but also by darkness, temperature extremes and some growth promoting hormones (Braam & Davis, 1990; Braam, 1992; Sistrunk *et al.*, 1994; Antosiewicz *et al.*, 1995; Xu *et al.*, 1995; Polisensky & Braam, 1996). This latter group of stimuli might be predicted to be unrelated in properties, and thus in perception mechanisms, to touch. However, microarray data indicate that the seemingly distinctive stimuli of touch and darkness evoke transcript abundance changes that are highly similar. Indeed, over half of the touch-inducible genes identified by microarray analysis resemble the *TCH* genes in that they are also up-regulated by darkness (Lee *et al.*, 2005). Of the 60 genes found to be up-regulated more than tenfold by touch, 56 are also up-regulated in darkness-treated plants (Lee *et al.*, 2005). These findings are consistent with the hypothesis that the diverse stimuli capable of inducing *TCH* expression may share the common property of being capable of causing mechanical perturbations, perhaps at a cellular level, and thus indirectly inducing *TCH* expression (Braam, 2000).

How might the stimuli known to up-regulate gene expression indirectly result in mechanical perturbations? Darkness-induced stomatal aperture regulation and humidity changes could result in local cellular turgor changes (Braam, 2000). Alternatively, darkness and the growth-promoting hormones, IAA and epi-brassinolide that increase expression of at least a subset of the *TCH* genes (Antosiewicz *et al.*, 1995; Xu *et al.*, 1995) may lead to enhanced growth through cellular expansion. Transient cellular turgor alterations may occur as walls are loosened during cell expansion (Braam, 2000). As individual cells embedded in tissues undergo expansion, forces can be transmitted to neighboring cells, thus propagating cell-to-cell mechanical stimuli (Ding & Pickard, 1993). Sudden temperature shifts may directly affect membrane properties and transmembrane transport activities, and thus trigger cellular turgor alterations. These potential turgor variations may be sensed at the wall, membrane or even intracellular organelles and may lead to activation of intracellular mechano-transduction pathways that regulate *TCH* expression. Consistent with the idea that there is commonality between touch and darkness,

Stankovic and colleagues found that a shift to darkness results in membrane depolarizations similar to that evoked by wounding (Stankovic *et al.*, 1998).

The idea that the diverse stimuli that regulate *TCH* expression may share perception and/or signaling mechanisms is consistent with the finding that a regulatory region consisting of 102 basepairs from 5' untranscribed *TCH4* sequences is sufficient to confer touch, dark, cold, heat and epi-brassinolide regulation (Iliev *et al.*, 2002). A related sequence from the cold- and touch-inducible *CBF2* gene has also been shown to have mechano-responsive regulatory activity (Zarka *et al.*, 2003). These initial studies aimed at defining touch-inducible regulatory elements will be aided by the recent identification of hundreds of additional *Arabidopsis* genes that share this regulatory behavior (Lee *et al.*, 2005). Such elements will be useful tools in employing genetic approaches to investigating plant responses to mechanical perturbation. For example, we are screening for mutants defective in appropriate regulation of a luciferase gene driven by the defined *TCH4* regulatory region in an effort to define functions and identify genes required for perception and response to touch, heat and other environmental stimuli.

The *TCH* genes also show developmental expression patterns. Two possible correlations can be made. *TCH* expression is often seen at sites of potential mechanical strain and/or increased growth. For example, *TCH::reporter* transgenes are expressed and *TCH* proteins accumulate at branching points, the root-shoot junction, elongating hypocotyls and roots, and developing trichomes (Sistrunk *et al.*, 1994; Antosiewicz *et al.*, 1995, 1997; Xu *et al.*, 1995). Furthermore, *TCH2* and *TCH4* expression levels are increased in inflorescence stems when increased weight applied at the apex results in enhanced secondary growth (Ko *et al.*, 2004). Thus, *TCH* regulation occurs not only in response to externally applied stimuli, but also may be induced by mechanical strains that might be generated through morphogenesis (Xu *et al.*, 1995; Campbell & Braam, 1999; Braam, 2000). The developmental expression data are consistent with the idea that transient turgor changes at the cellular level may be sufficient to trigger *TCH* regulation.

Finally, because plant cells maintain turgor and maintenance of turgor is highly regulated, it is likely that plants deal constantly with changes in mechanical stress at the cell surface, both in response to stimuli (like water deficit) and throughout morphogenesis, for example, during the process of cell expansion. Furthermore, it is possible that the machinery and mechanisms used by plants to perceive and respond to external environmental stress may be related to processes used at the cellular level to control fundamental events such as cell expansion. That is, the mechanical force of turgor pressure may be used as a regulatory event to control cellular enlargement and growth. How mechano-perception and response mechanisms of nonspecialized plants may be related to those enabling carnivory, climbing, active pollination and root-avoidance behaviors is unknown. Thus, understanding the mechanisms

of perception and response are likely fundamental to understanding plant biology.

## Acknowledgements

I am very grateful to thank Greg Allikas, Dr Barry Rice, Tim Skyrme and Dr Dereth Phillips for allowing the use of their beautiful photographs. My research in this area is supported by the Department of Energy (grant nos. DE-FG03-99ER20331 and DE-FG02-03ER15394) and the National Science Foundation (grant nos. IBN-9982654, IBN-0321532, and IBN-0313432).

## References

- Antosiewicz DM, Polisensky DH, Braam J. 1995. Cellular localization of the Ca<sup>2+</sup> binding TCH3 protein of Arabidopsis. *Plant Journal* 8: 623–636.
- Antosiewicz DM, Purugganan MM, Polisensky DH, Braam J. 1997. Cellular localization of Arabidopsis xyloglucan endotransglycosylase-related proteins during development and after wind stimulation. *Plant Physiology* 115: 1319–1328.
- Arteca JM, Arteca RN. 1999. A multi-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase (ACS6) in mature Arabidopsis leaves. *Plant Molecular Biology* 39: 209–219.
- Barnett JR, Asante AK. 2000. The formation of cambium from callus in grafts of woody species. *Cell and Molecular Biology of Wood Formation*. R. A. Savidge, J. R. Barnett and R. Napier. Oxford: BIOS Scientific Publishers, 155–167.
- Batiza AF, Schulz T, Masson PH. 1996. Yeast respond to hypotonic shock with a calcium pulse. *Journal of Biological Chemistry* 271: 23357–23362.
- Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Miller PA, Walker AR, Schulz B, Feldmann KA. 1996. Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. *Science* 273: 948–950.
- Biddington NL. 1986. The effects of mechanically-induced stress in plants – a review. *Plant Growth Regulation* 4: 103–123.
- Biro R, Jaffe MJ. 1984. Thigmomorphogenesis: ethylene evolution and its role in the changes observed in mechanically perturbed bean plants. *Physiologia Plantarum* 62: 289–296.
- Blechert S, Bockelmann C, Füllslein MV, Schrader T, Stelmach BA, Niesel U, Weiler EW. 1999. Structure-activity analyses reveal the existence of two separate groups of active octadecanoids in elicitation of the tendril-coiling response of *Bryonia dioica* Jacq. *Planta* 207: 470–479.
- Bopp M, Weber I. 1981. Hormonal regulation of the leaf blade movement of *Drosera capensis*. *Physiologia Plantarum* 53: 491–496.
- Botella JR, Arteca RN. 1994. Differential expression of two calmodulin genes in response to physical and chemical stimuli. *Plant Molecular Biology* 24: 757–766.
- Botella JR, Arteca JM, Somodevilla M, Arteca RN. 1996. Calcium-dependent protein kinase gene expression in response to physical and chemical stimuli in mungbean (*Vigna radiata*). *Plant Molecular Biology* 30: 1129–1137.
- Boyer N, de Jaegher G, Bon M-C, Gaspar T. 1986. Cobalt inhibition of thigmomorphogenesis in *Bryonia dioica*: possible role and mechanism of ethylene production. *Physiologia Plantarum* 67: 552–556.
- Boyer N, Desbiez M-O, Hofinger M, Gaspar T. 1983. Effect of lithium on thigmomorphogenesis in *Bryonia dioica* ethylene production and sensitivity. *Plant Physiology* 72: 522–525.
- Braam J. 1992. Regulated expression of the calmodulin-related TCH genes in cultured Arabidopsis cells: Induction by calcium and heat shock. *Proceedings of the National Academy of Sciences, USA* 89: 3213–3216.
- Braam J. 2000. The Arabidopsis TCH genes: Regulated in expression by mechanotransduction? In: Cherry JH, Locy RD, Rychter A, eds. *Plant Tolerance to Abiotic Stresses in Agriculture: Role of Genetic Engineering*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 83, 29–37.
- Braam J, Davis RW. 1990. Rain-, wind- and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. *Cell* 60: 357–364.
- Brosché M, Strid A. 2000. Ultraviolet-B radiation causes tendril coiling in *Pisum sativum*. *Plant Cell Physiology* 41: 1077–1079.
- Brown KM, Leopold AC. 1972. Ethylene and the regulation of growth in pine. *Canadian Journal of Forest Research* 3: 143–145.
- Burdon-Sanderson J. 1873. Note on the electrical phenomena which accompany stimulation of leaf of *Dionaea muscipula*. *Proceedings of the Philosophical Transactions of the Royal Society of London* 21: 495–496.
- Calaghan SC, White E. 1999. The role of calcium in the response of cardiac muscle to stretch. *Progress in Biophysics Molecular Biology* 71: 59–90.
- Campbell P, Braam J. 1999. Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying functions [Review]. *Trends in Plant Science* 4: 361–366.
- Cosgrove DJ, Hedrich R. 1991. Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba*. *Planta* 186: 143–153.
- Coutand C, Julien JL, Moulia B, Mauget JC, Guitard D. 2000. Biomechanical study of the effect of a controlled bending on tomato stem elongation: global mechanical analysis. *Journal of Experimental Botany* 51: 1813–1824.
- Curtis MA. 1834. Enumeration of plants around Wilmington, NC. *Boston Journal of Nat Histology* 1: 123–237.
- Darwin C. 1880. *The Power of Movement in Plants*. London: William Clowes and Sons, Ltd.
- Darwin C. 1893. *Insectivorous Plants*. London: John Murray.
- Darwin C. 1906. *The Movements and Habits of Climbing Plants*. London: John Murray.
- Depege N, Thonat C, Coutand C, Julien J-L, Boyer N. 1997. Morphological responses and molecular modifications in tomato plants after mechanical stimulation. *Plant Cell Physiology* 38: 1127–1134.
- Deruere J, Jackson K, Garbers C, Soll D, DeLong A. 1999. The RCN1-encoded A subunit of protein phosphatase 2A increases phosphatase activity in vivo. *Plant Journal* 20: 389–399.
- Ding JP, Pickard BG. 1993. Mechanosensory calcium-selective cation channels in epidermal cells. *Plant Journal* 3: 83–110.
- Edwards KL, Pickard BG. 1987. Detection and Transduction of Physical Stimuli in Plants. In: Wagner E, Greppin H, Hiller B, eds. *NATO ASI Series, The cell surface in signal transduction*. Berlin, Germany: Springer-Verlag.
- Eisner T. 1981. Leaf folding in a sensitive plant: a defensive thorn-exposure mechanism? *Proceedings of the National Academy of Sciences, USA* 78: 402–404.
- Eldick G, Jv Ruiter RK, Colla PHWN, Herpen MM, Av Schrauwen JAM, Wullems GJ. 1997. Expression of an isoflavone reductase-like gene enhanced by pollen tube growth in pistils of *Solanum tuberosum*. *Plant Molecular Biology* 33: 923–929.
- Engelberth J, Koch T, Schüler G, Bachmann N, Rechtenback J, Boland W. 2001. Ion channel-forming alamethicin is a potent elicitor of volatile biosynthesis and tendril coiling. Cross talk between jasmonate and salicylate signaling in lima bean. *Plant Physiology* 125: 369–377.
- Erner Y, Biro R, Jaffe MJ. 1980. Thigmomorphogenesis: Evidence for a translocatable thigmomorphogenetic factor induced by mechanical perturbation of beans (*Phaseolus vulgaris*). *Physiologia Plantarum* 50: 21–25.
- Erner Y, Jaffe MJ. 1983. Thigmomorphogenesis: membrane lipid and protein changes in bean plants as affected by mechanical perturbation and ethrel. *Physiologia Plantarum* 58: 197–203.

- Fagerberg WR, Allain D. 1991. A quantitative study of tissue dynamics during closure in the traps of venus's flytrap *Dionaea muscipula* Ellis. *American Journal of Botany* 78: 647–657.
- Falke LC, Edwards KL, Pickard BG, Misler S. 1988. A stretch-activated anion channel in tobacco protoplasts. *FEBS Letters* 237: 141–144.
- Fleurat-Lessard P, Bouché-Pillon S, Leloup C, Bonnemain J-L. 1997a. Distribution and activity of the plasma membrane H<sup>+</sup>-ATPase in *Mimosa pudica* L. in relation to ionic fluxes and leaf movements. *Plant Physiology* 113: 747–754.
- Fleurat-Lessard P, Frangne N, Maeshima M, Ratajczak R, Bonnemain J-L, Martinoia E. 1997b. Increased expression of vacuolar aquaporin and H<sup>+</sup>-ATPase related to motor cell function in *Mimosa pudica* L. *Plant Physiology* 114: 827–834.
- Fleurat-Lessard P, Roblin G, Bonmort J, Besse C. 1993. Effects of colchicine, vinblastine, cytochalasin B and phalloidin on the seismonastic movement of *Mimosa pudica* leaf and on motor cell ultrastructure. *Journal of Experimental Botany* 39: 209–221.
- Friml J. 2003. Auxin transport – shaping the plant. *Current Opinions in Plant Biology* 6: 7–12.
- Fromm J, Eschrich W. 1988. Transport processes in stimulated and nonstimulated leaves of *Mimosa pudica*. II. Energetics and transmission of seismic stimulations. *Trees* 2: 18–24.
- Frost HM. 1992. Perspectives: bone's mechanical usage windows. *Bone Min* 19: 257–271.
- Gadea J, Conejero V, Vera P. 1999. Developmental regulation of a cytosolic ascorbate peroxidase gene from tomato plants. *Molecular General Genetics* 262: 212–219.
- Garbers C, DeLong A, Deruere J, Bernasconi P, Soll D. 1996. A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in Arabidopsis. *EMBO Journal* 15: 2115–2124.
- Garrill A, Tyerman SD, Findlay GP. 1994. Ion channels in the plasma membrane of protoplasts from the halophytic angiosperm *Zostera muelleri*. *Journal of Membr Biology* 142: 381–393.
- Gawienowski MC, Szymanski D, Perera IY, Zielinski RE. 1993. Calmodulin isoforms in *Arabidopsis* encoded by multiple divergent mRNAs. *Plant Molecular Biology* 22: 215–225.
- Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF. 1998. Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. *Plant Journal* 16: 433–442.
- Goeschl JD, Rappaport L, Pratt HK. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. *Plant Physiology* 41: 877–884.
- Goldspink G. 1999. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *Journal of Anatomy* 194: 323–334.
- Gus-Mayer S, Nation B, Hahlbrock K, Schmelzer E. 1998. Local mechanical stimulation induces components of the pathogen defense response in parsley. *Proceedings of the National Academy of Sciences, USA* 95: 8398–8403.
- Hayashi T, Takagi S. 2003. Ca<sup>2+</sup>-dependent cessation of cytoplasmic streaming induced by hypertonic treatment in *Vallisneria* mesophyll cells: possible role of cell wall-plasma membrane adhesion. *Plant Cell Physiology* 44: 1027–1036.
- Hepworth DG, Vincent JFV. 1999. The growth response of the stems of genetically modified tobacco plants (*Nicotiana tabacum* 'Samsun') to flexural stimulation. *Annals of Botany* 83: 39–43.
- Hirsinger C, Salva I, Marbach J, Durr A, Fleck J, Jamet E. 1999. The tobacco extensin gene *Ext 1.4* is expressed in cells submitted to mechanical constraints and in cells proliferating under hormone control. *Journal of Experimental Botany* 50: 343–355.
- His I, Drriouch A, Nicol F, Jauneau A, Höfte H. 2001. Altered pectin composition in primary cell walls of korrigan, a dwarf mutant of Arabidopsis deficient in a membrane-bound endo-1,4-beta glucanase. *Planta* 212: 348–358.
- Houwincq AL. 1935. The conduction of excitation in *Mimosa pudica*. *Recl Trav Botany Néerl* 32: 51–91.
- Hu WJ, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai CJ, Chiang VL. 1999. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nature Biotech* 17: 808–812.
- Iliev E, Xu W, Polisensky DH, Oh M-H, Torisky RS, Clouse SD, Braam J. 2002. Transcriptional and posttranscriptional regulation of Arabidopsis TCH4 expression by diverse stimuli. Roles of cis regions and brassinosteroids. *Plant Physiology* 130: 770–783.
- Ingeber DE. 1998. Cellular basis of mechanotransduction. *Biological Bulletin* 194: 323–327.
- Jacobs MR. 1954. The effect of wind sway on the form and development of *Pinus radiata* D. Don. *Austral Journal of Botany* 2: 35–51.
- Jacobson SL. 1965. Receptor response in the Venus's flytrap. *Journal of General Physiology* 49: 117–129.
- Jaeger CH, Goeschl JD, Magnuson CE, Fares Y, Strain BR. 1988. Short-term responses of phloem transport to mechanical perturbation. *Physiologia Plantarum* 72: 588–594.
- de Jaeger G, Boyer N, Bon M-C, Gaspar T. 1987. Thigmomorphogenesis in *Bryonia dioica*: early events in ethylene biosynthesis pathway. *Biochemistry and Physiological Pflanz* 182: 49–56.
- Jaffe MJ. 1973. Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation. *Planta* 114: 143–157.
- Jaffe MJ. 1976. Thigmomorphogenesis: Electrical resistance and mechanical correlates of the early events of growth retardation due to mechanical stimulation in beans. *Zeitschrift für Pflanzenphysiologie* 78: 24–32.
- Jaffe MJ. 1985. Ethylene and other plant hormones in thigmomorphogenesis and tendrill thigmonasty. *Advances in Agricult Biotechny* 16: 353–367.
- Jaffe MJ, Biro R. 1979. Thigmomorphogenesis: the effect of mechanical perturbation on the growth of plants, with special reference to anatomical changes, the role of ethylene, and interactions with other environmental stresses. In: Mussell H, Staples RC, eds. *Stress Physiology in Crop Plants*. New York, USA: John Wiley and Sons, 25–69.
- Jaffe MJ, Galston AW. 1968. The physiology of tendrils. *Annual Review of Plant Physiology* 19: 417–434.
- Jaffe MJ, Gibson C, Biro R. 1977. Physiological studies of mechanically stimulated motor responses of flower parts. I: Characterization of the thigmotropic stamens of *Portulaca grandiflora* Hook. *Botanical Gazette* 138: 438–447.
- Jaffe MJ, Leopold AC, Staples RC. 2002. Thigmo responses in plants and fungi. *American Journal of Botany* 89: 375–382.
- Jaffe MJ, Telewski FW, Cooke PW. 1984. Thigmomorphogenesis: on the mechanical properties of mechanically perturbed bean plants. *Physiologia Plantarum* 62: 73–78.
- Johnson KA, Sistrunk ML, Polisensky DH, Braam J. 1998. *Arabidopsis thaliana* responses to mechanical stimulation do not require ETR1 or EIN2. *Plant Physiology* 116: 643–649.
- Kameyama K, Kishi Y, Yoshimura M, Kanzawa N, Sameshima M, Tsuchiya T. 2000. Tyrosine phosphorylation in plant bending. *Nature* 407: 37.
- Kennard JL, Cleary AL. 1997. Pre-mitotic nuclear migration in subsidiary mother cells of *Tradescantia* occurs in G1 of the cell cycle and requires F-actin. *Cell Motility Cytoskeleton* 36: 55–67.
- Khan AR, Johnson KA, Braam J, James MNG. 1997. Comparative modeling of the three-dimensional structure of the calmodulin-related TCH2 protein from Arabidopsis. *Proteins* 27: 144–153.
- Kikuyama M, Tazawa M. 2001. Mechanosensitive Ca<sup>2+</sup> release from intracellular stores in *Nitella flexilis*. *Plant Cell Physiology* 42: 358–365.
- Knight H. 2000. Calcium signaling during abiotic stress in plants. *Internal Review of Cytology* 195: 269–324.
- Knight MR, Campbell AK, Smith SM, Trewavas AJ. 1991. Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* 352: 524–526.

- Knight MR, Smith SM, Trewavas AJ. 1992. Wind-induced plant motion immediately increases cytosolic calcium. *Proceedings of the National Academy of Sciences, USA* 89: 4967–4977.
- Ko J-H, Han K-H, Park S, Yang J. 2004. Plant body weight-induced secondary growth in Arabidopsis and its transcription phenotype revealed by whole-transcriptome profiling. *Plant Physiology* 135: 1069–1083.
- Lee D, Polisensky DH, Braam J. 2005. Genome wide identification of touch and darkness-regulated Arabidopsis genes: a focus on calmodulin-like and XTH genes. *New Phytology* (in press; doi:10.1111/j.1469-8137.2004.01238.x).
- Legendre L, Rueter S, Heinstejn PF, Low PS. 1993. Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean (*Glycine max*) *Cells Plant Physiology* 102: 133–140.
- Legue V, Blancaflor E, Wymer C, Perbal G, Fantin D, Gilroy S. 1997. Cytoplasmic free  $Ca^{2+}$  in Arabidopsis roots changes in response to touch but not gravity. *Plant Physiology* 114: 789–800.
- Ling V, Perera IY, Zielinski RE. 1991. Primary structures of Arabidopsis calmodulin isoforms deduced from the sequences of cDNA clones. *Plant Physiology* 96: 1196–1202.
- Lintilhac PM, Vesecky TB. 1984. Stress-induced alignment of division plane in plant tissues grown *in vitro*. *Nature* 307: 363–364.
- Lloyd FE. 1942. The carnivorous plants. Waltham, MA, USA: Chronica Botanica Co.
- McCormack E, Braam J. 2003. Calmodulins and related potential calcium sensors of Arabidopsis. *New Phytologist* 159: 585–598.
- Malone M. 1994. Wound-induced hydraulic signals and stimulus transmission in *Mimosa pudica* L. *New Phytologist* 128: 49–56.
- Mano I, Driscoll M. 1999. DEG/ENaC channels: a touchy superfamily that watches its salt. *BioEssays* 21: 568–578.
- Massa GD, Gilroy S. 2003. Touch modulates gravity sensing to regulate the growth of primary roots of Arabidopsis thaliana. *Plant Journal* 33: 435–445.
- Mauch F, Kmecl A, Schaffrath U, Volrath S, Görlach J, Ward E, Ryals J, Dudler R. 1997. Mechanosensitive expression of a lipoxygenase gene in wheat. *Plant Physiology* 114: 1561–1566.
- Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K. 1996. A gene encoding a mitogen-activated protein kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences, USA* 93: 765–769.
- Mobasher A, Carter SD, Martín-Vasallo P, Shakibaei M. 2002. Integrins and stretch activated ion channels; putative components of functional cell surface mechanoreceptors in articular chondrocytes. *Cell Biological Internation* 26: 1–18.
- Mori IC, Schroeder JI. 2004. Reactive oxygen species activation of plant  $Ca^{2+}$  channels. A signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction. *Plant Physiology* 135: 702–708.
- Morillon R, Liénard D, Chrispeels MJ, Lassalles J-P. 2001. Rapid movements of plants organs require solute-water co-transporters or contractile proteins. *Plant Physiology* 127: 720–722.
- Moshelion M, Becker D, Biela A, Uehlein N, Hedrich R, Otto B, Levi H, Moran N, Kaldenhoff R. 2002. Plasma membrane aquaporins in the motor cells of *Samanea saman*: diurnal and circadian regulation. *Plant Cell* 14: 727–739.
- Müssig C, Biesgen C, Lisso J, Uwer U, Weiler EW, Altmann T. 2000. A novel stress-inducible 12-oxophytodiene reductase from Arabidopsis thaliana provides a potential link between brassinosteroid-action and jasmonic-acid synthesis. *Journal of Plant Physiology* 157: 143–152.
- Nakajima K, Furutani I, Tachimoto H, Matsubara H, Hashimoto T. 2004. SPIRAL1 encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding Arabidopsis cells. *Plant Cell* 16: 1178–1190.
- Oh SA, Kwak JM, Kwun IC, Nam HG. 1996. Rapid and transient induction of calmodulin-encoding gene (s) of Brassica napus by a touch stimulus. *Plant Cell Report* 15: 586–590.
- Okada K, Shimura Y. 1990. Reversible root-tip rotation in Arabidopsis seedlings induced by obstacle-touching stimulus. *Science* 250: 274–276.
- Oufatole M, Arango M, Boutry M. 2000. Identification and expression of three new Nicotiana plumbaginifolia genes which encode isoforms of a plasma-membrane  $H^{+}$ -ATPase, and one of which is induced by mechanical stress. *Planta* 210: 715–722.
- Perera IY, Zielinski RE. 1992. Structure and expression of the Arabidopsis CaM-3 calmodulin gene. *Plant Molecular Biology* 19: 649–664.
- Polisensky DH, Braam J. 1996. Cold-shock regulation of the Arabidopsis TCH genes and the effects of modulating intracellular calcium levels. *Plant Physiology* 111: 1271–1279.
- Putz FE, Holbrook NM. 1986. Notes on the natural history of hemiepiphytes. *Selbyana* 9: 61–69.
- Putz FE, Holbrook NM. 1989. Strangler Fig rooting habits and nutrient relations in the Llanos of Venezuela. *American Journal of Botany* 76: 781–788se.
- Ricca U. 1916. Soluzione d'un problema di fisiologia: la propagazione di stimulo nella Mimosa. *Nuovo G Botany It* 23: 51–170.
- Robins RJ, Juniper BE. 1980. The secretory cycle of Dionaea muscipula Ellis. III. The mechanism of release of digestive secretion. *New Phytologist* 86: 313–327.
- Roblin G. 1982. Movements, bioelectrical events and proton excretion induced in the pulvini of Mimosa pudica L. by a period of darkness. *Zeitschrift für Pflanzenphysiologie* 108: 295–304.
- Romero GA, Nelson CE. 1986. Sexual dimorphism in Catasetum orchids: Forcible pollen emplacement and male flower competition. *Science* 232: 1538–1540.
- Rose JKC, Braam J, Fry SC, Nishitani K. 2002. The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: current perspectives and a new unifying nomenclature. *Plant and Cell Physiology* 43: 1421–1435.
- Royo J, Nass N, Matton DP, Okamoto S, Clarke AE, Newbigin E. 1996. A retrotransposon-like sequence linked to the S-locus of Nicotiana glauca is expressed in styles in response to touch. *Molecular and General Genetics* 250: 180–188.
- Sato Y, Kadota A, Wada M. 1999. Mechanically induced avoidance response of chloroplasts in fern protonemal cells. *Plant Physiology* 121: 37–44.
- Sato Y, Wada M, Kadota A. 2001. External  $Ca^{2+}$  is essential for chloroplast movement induced by mechanical stimulation but not by light stimulation. *Plant Physiology* 127: 497–504.
- Satter RL, Gorton HL, Vogelmann TC, eds. 1990. *The pulvinus: Motor organ for leaf movement*. Current Topics in Plant Physiology. Rockville, MD, USA: American Society of Plant Physiologists.
- Schildknecht H, Meier-Augenstein W. 1990. Role of turgorins in leaf movement. In: Satter RL, Gorton HL, Vogelmann TC, eds. *The pulvinus: motor organ for leaf movement*. Rockville, MD, USA: American Society of Plant Physiologists.
- Sedbrook JC, Ehrhardt DW, Fisher SE, Scheible W-R, Somerville C. 2004. The Arabidopsis SKU6/SPIRAL1 gene encodes a plus end-localized microtubule-interacting protein involved in directional cell expansion. *Plant Cell* 16: 1506–1520.
- Shirsat AH, Bell A, Spence J, Harris JN. 1996. The Brassica napus extA extensin gene is expressed in regions of the plant subject to tensile stresses. *Planta* 199: 618–624.
- Simons P. 1992. *The Action Plant*. Oxford, UK: Blackwell Publishers.
- Simons PJ. 1981. The role of electricity in plant movements. *New Phytologist* 87: 11–37.
- Sistrunk ML, Antosiewicz DM, Purugganan MM, Braam J. 1994. Arabidopsis TCH3 encodes a novel  $Ca^{2+}$  binding protein and shows environmentally induced and tissue-specific regulation. *Plant Cell* 6: 1553–1565.
- Slack A. 2000. *Carnivorous plants*. Yeovil, UK: Marston House.



- Stahlberg R, Cosgrove DJ. 1997. The propagation of slow wave potentials in pea epicotyls. *Plant Physiology* 113: 209–217.
- Stankovic B, Witters DL, Zawadzki T, Davies E. 1998. Action potentials and variation potentials in sunflower: An analysis of their relationships and distinguishing characteristics. *Physiologia Plantarum* 103: 51–58.
- Stelmach BA, Müller A, Hennig P, Laudert D, Andert L, Weiler EW. 1998. Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signalling compound in plant mechanotransduction. *Phytochemistry* 47: 539–546.
- Stelmach BA, Müller A, Weiler EW. 1999. 12-oxo-phytodienoic acid and indole-3-acetic acid in jasmonic acid-treated tendrils of *Bryonia dioica*. *Phytochemistry* 51: 187–192.
- Strong DRJ, Ray TSJ. 1975. Host tree location behavior of a tropical vine (*Monstera gigantea*) By *Skototropism Science* 190: 804–806.
- Takahashi H, Jaffe MJ. 1984. Thigmomorphogenesis: the relationship of mechanical perturbation to elicitor-like activity and ethylene production. *Physiologia Plantarum* 61: 405–411.
- Tatsuki M, Mori H. 1999. Rapid and transient expression of 1-aminocyclopropane-1-carboxylate synthase isogenes by touch and wound stimuli in tomato. *Plant and Cell Physiology* 40: 709–715.
- Telewski FW. 1995. Wind induced physiological and developmental responses in trees. In: Coultts MP, Grace J, eds. *Wind and Trees*. Cambridge, UK: Cambridge University Press.
- Telewski FW, Jaffe MJ. 1986. Thigmomorphogenesis: field and laboratory studies of *Abies fraseri* in response to wind or mechanical perturbation. *Physiologia Plantarum* 66: 211–218.
- Thitamadee S, Tuchihiro K, Hashimoto T. 2002. Microtubule basis for left-handed helical growth in Arabidopsis. *Nature* 417: 193–196.
- Trewavas AJ, Knight M. 1994. Mechanical signalling, calcium and plant form. *Plant Molecular Biology* 26: 1329–1341.
- Ueda M, Takada N, Yamamura S. 2001. Molecular approach to the nyctinastic movement of the plant controlled by a biological clock. *International Journal of Molecular Science* 2: 156–164.
- Wayne R, Staves MP, Leopold AC. 1992. The contribution of the extracellular matrix to gravisensing in characean cells. *Journal of Cell Science* 101: 611–623.
- Weiler EW, Albrecht T, Groth B, Xia Z-Q, Luxem M, Liß H, Andert L, Spengler P. 1993. Evidence for the involvement of jasmonates and their octadecanoid precursors in the tendril coiling response of *Bryonia dioica*. *Phytochemistry* 32: 591–600.
- Weiler EW, Kutchan TM, Gorba T, Brodschelm W, Niesel U, Bublitz F. 1994. The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. *FEBS Letters* 345: 9–13.
- Wright AJ, Knight H, Knight MR. 2002. Mechanically stimulated *TCH3* gene expression in Arabidopsis involves protein phosphorylation and EIN6 downstream of calcium. *Plant Physiology* 128: 1402–1409.
- Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J. 1995. *Arabidopsis TCH4*, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. *Plant Cell* 7: 1555–1567.
- Yahraus T, Chandra S, Legendre L, Low PS. 1995. Evidence for a mechanically induced oxidative burst. *Plant Physiology* 109: 1259–1266.
- Yulsman T. 1999. True nature: twisted trees. *Audubon* 101: 30–35.
- Zarka DG, Vogel JT, Cook D, Thomashow MF. 2003. Cold induction of Arabidopsis *CBF* genes involves multiple ICE (inducer of *CBF* expression) promoter elements and a cold-regulatory circuit that is desensitized by low temperature. *Plant Physiology* 133: 910–918.



### About New Phytologist

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at [www.newphytologist.org](http://www.newphytologist.org).
- Regular papers, Letters, Research reviews, Rapid reports and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2003 average submission to decision time was just 35 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £109 in Europe/\$202 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office ([newphytol@lancaster.ac.uk](mailto:newphytol@lancaster.ac.uk); tel +44 1524 592918) or, for a local contact in North America, the USA Office ([newphytol@ornl.gov](mailto:newphytol@ornl.gov); tel 865 576 5261).