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Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*

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Summary

Plants must sense and respond to diverse stimuli to optimize the architecture of their root system for water and nutrient scavenging and anchorage. We have therefore analyzed how information from two of these stimuli, touch and gravity, are integrated to direct root growth. In *Arabidopsis thaliana*, touch stimulation provided by a glass barrier placed across the direction of growth caused the root to form a step-like growth habit with bends forming in the central and later the distal elongation zones. This response led to the main root axis growing parallel to, but not touching the obstacle, whilst the root cap maintained contact with the barrier. Removal of the graviperceptive columella cells of the root cap using laser ablation reduced the bending response of the distal elongation zone. Similarly, although the roots of the gravisensing impaired *pgm1–1* mutant grew along the barrier at the same average angle as wild-type, this angle became more variable with time. These observations imply a constant gravitropic re-setting of the root tip response to touch stimulation from the barrier. In wild-type plants, transient touch stimulation of root cap cells, but not other regions of the root, inhibited both subsequent gravitropic growth and amyloplast sedimentation in the columella. Taken together, these results suggest that the cells of the root cap sense touch stimuli and their subsequent signaling acts on the columella cells to modulate their graviresponse. This interaction of touch and gravity signaling would then direct root growth to avoid obstacles in the soil while generally maintaining downward growth.

Keywords: ***Arabidopsis*, gravitropism, gravity, root, thigmotropism.**

Introduction

Plant roots must perceive and respond to a variety of external stimuli such as gravity, touch, and nutrient and water availability to maintain optimal growth patterns as they explore the soil. Under most natural situations, root growth will be directed down through gravitropism until encountering an obstacle such as a rock, another root, or a hard-pan layer of soil. At this point the root must re-orient its growth to navigate across or around the obstacle. Under these circumstances, touch should become an important factor that influences root growth. In nature, therefore, there are likely few situations following germination, when a root needs to re-orient its growth with respect to gravity in the absence of another influence, such as a touch stimulus. Thus, root gravitropic and thigmotropic sensing and response are likely closely interrelated. Indeed, gravitropism has even been proposed to represent a modification of an ancestral plant mechanical sensing system (Trewavas and Knight, 1994).

The cells of the root cap are the first to encounter obstacles and new environments as the root grows through the soil, making them strong candidates for the site where sensors of the soil environment may be located. Indeed, these cap cells have long been proposed as sites of sensing and specialized function, including gravity and touch sensing (Darwin and Darwin, 1880). In addition, cap cells are in a dynamic developmental flux, changing from basal amyloplast-filled cells produced from the cap initials through a series of differentiated cell types, to form peripheral cells that are eventually sloughed off in the form of border cells (Hawes *et al.*, 2000). This developmental flux of cells led to the proposal that the positioning (and hence differentiated state) of cap cells might lead to their differential sensory activities (Blancaflor *et al.*, 1998; Fasano *et al.*, 2001). Although the tip and other peripheral cells of the cap are specialized for polysaccharide secretion they also exhibit touch sensitivity, for example, as judged by

showing touch-induced Ca^{2+} increases (Legué *et al.*, 1997). Root graviperception, however, has been localized to the more basal, amyloplast-containing cells of the root cap columella (Blancaflor *et al.*, 1998; Juniper *et al.*, 1966; Konings, 1968; Sack, 1997; Younis, 1954). Studies of the graviperception function of the root cap in the *Arabidopsis* primary root have revealed that even within the columella gravity sensing cells, spatial patterning of cell function is evident. Thus, even though all cells of the amyloplast containing columella appear to contribute to gravity sensitivity, the central columella cells (story 1 and 2) have been proposed to generate the major part of the gravity sensing signal (Blancaflor *et al.*, 1998). These central cells also have the fastest amyloplast sedimentation rates (Blancaflor *et al.*, 1998), and generate the largest gravisignaling-related cytoplasmic pH increase in response to gravistimulation (Fasano *et al.*, 2001; Scott and Allen, 1999). Thus, the cells responsible for touch and gravity sensing processes are likely to be either the same or spatially very closely inter-related in the root cap. Mullen *et al.* (2000) have also suggested that mechanical stimulation of a plant may affect both root growth and the kinetics of its graviresponse, further implying that the touch and gravity sensing systems may interact.

Therefore, to better understand the relative roles and interactions of touch and gravity sensing in directing root growth *in vivo*, we have characterized the growth of *Arabidopsis* roots in response to simultaneous gravity and touch stimulation. We report that upon encountering an object (continuous touch stimulation), roots respond with a growth habit representing an apparent adaptive compromise between gravitropic and thigmotropic response. Transient touch stimulation of root cap peripheral cells led to reduced gravitropic sensitivity of the root, suggesting touch stimulation was downregulating gravitropic signaling processes/responses in the root cap. These results suggest that even though specialized sensing activities may be spatially distributed within the cap, the touch sensing pathway interacts with the gravity sensing system of the columella to co-ordinate root growth.

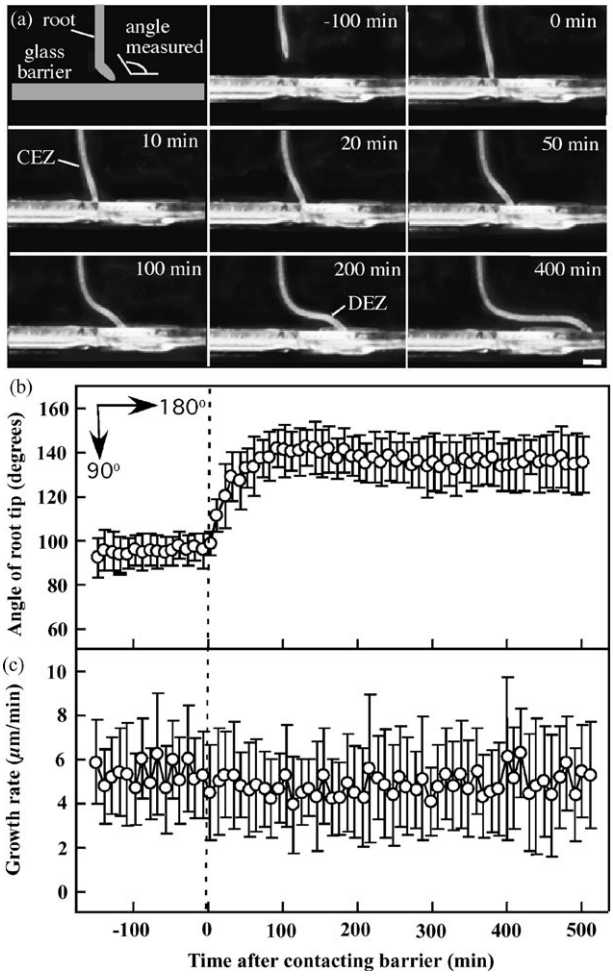

Figure 1. The growth response of a primary root of *Arabidopsis thaliana* as it interacts with a barrier to downward growth.
(a) Roots were grown vertically into a horizontal glass barrier and growth response characterized. Contact with the barrier occurs at 0 min. The CEZ and DEZ indicate the centers of the central and distal elongation zones, respectively. Representative images of $n = 22$ separate roots. Scale bar is 500 μm.
(b) Change in root tip angle upon encountering the barrier to growth.
(c) Growth rate of primary roots of *A. thaliana* encountering a barrier. Results in (b) and (c) represent mean ± SD, $n = 22$.

Results

Growth response of roots encountering a solid barrier

Figure 1(a) shows images of a typical response of a vertically growing *Arabidopsis* root before and during interaction with a glass barrier perpendicular to its direction of root growth. The root grew vertically down to the obstacle (i.e. showed normal gravitropic guidance), but growth direction was changed as the tip encountered the obstacle. There was no bias towards turning left or right in the direction that growth was re-oriented, but interestingly, rather than growing sideways along the barrier, the elongating root proceeded to form a step-like structure (Figure 1a). Formation of the step-like growth habit took 180 min and the root tip progressed from 94.8 ± 7.9° (average for the 5 h prior to encountering the barrier, $n = 22$; vertical angle = 90°) to an angle of 136.0 ± 10.0°(average for 200–500 min after encountering the barrier, $n = 21$; Figure 1b). This angle was maintained as the root tracked along the surface of the obstacle, independent of the length of the barrier.

The step-like structure of the root that occured after contacting the barrier required the formation of two bends. The first bend in the root occurred 685 ± 22 μm from the tip and was detectable 20 min after touching. Thus, the angle of tip to perpendicular was 99.2 ± 5.7° at 0 min, and changed

significantly to $117.4 \pm 9.9^{\circ}$ at 20 min ($P < 0.001$; paired *t*-test; $n = 22$). Under our experimental conditions, the centers of the distal elongation zone (DEZ) and central elongation zone (CEZ) lie at 410 and 700 μm from the tip, respectively (Fasano *et al.*, 2001). Therefore this initial bending occurred in the CEZ. Formation of the step-like growth pattern was completed after a second bend opposite in direction to that in the CEZ became evident at 414 ± 17 μm from the tip (i.e. in the DEZ; Figure 1a). There was no significant difference between growth rate before (4.8 ± 1.5 μm min^{-1}) and after (4.4 ± 1.3 μm min^{-1}) the root encountered the obstacle (Figure 1c; $P > 0.1$; paired *t*-test; $n = 21$). The downward bend in the DEZ implies that cells of the upper flank of the root are elongating faster than cells of the lower flank in this region of the root. Unfortunately, growing the roots under a layer of phytagel prohibited surface marking of the DEZ to track independent growth rates of each flank to confirm this supposition. However, this second bend was maintained at the same distance from the tip (i.e. in the DEZ) for the entire duration of the response, irrespective of how far along the barrier the root had grown. Thus, upon exiting this DEZ bend some compensatory growth control mechanism must equalize the growth rate between the upper and lower flanks of the root such that the CEZ now elongates parallel to the surface of the barrier.

This growth response to the barrier did not involve any obvious buckling or bulging out of the root growth zone, as might be expected of a root lacking any touch sensitivity that was attempting to grow down through an impenetrable obstacle. This observation coupled to the unaltered growth rate upon encountering the barrier suggested to us that the root was actively sensing the obstacle and eliciting an appropriate growth response to the touch stimulation. We, therefore, attempted to define the relative contributions of the gravitropic and touch-related components of this growth response by assessing the barrier response in an agravitropic mutant and in roots where the gravity perceiving cells had been removed by laser ablation.

Growth response of pgm1-1 *to the barrier*

The *pgm1-1* starch-deficient mutant has reduced gravitropic sensitivity and reduced graviresponse (Fasano *et al.*, 2001; Kiss *et al.*, 1989). Figure 2(a) shows that the final tip angle to the barrier of the primary root of *pgm1-1* plants did not differ significantly from the wild-type in the initial timing of angle formation (compare Figures 1b and 2a) or average angle of the tip. Thus the average angle over 200–500 min after contacting the barrier for *pgm1-1* was $143.7 \pm 18.5^{\circ}$ and $136.0 \pm 10.0^{\circ}$ for wild-type ($P > 0.1$; separate-variance *t*-test; $n = 10$). Since *pgm1-1* plants are still able to perceive and respond to gravity, albeit at a reduced rate (Kiss *et al.*, 1989), it appears that this mutant still has sufficient gravisensing ability to control the angle of the root tip as it traverses a barrier. Differences from wild-type roots were seen, however, when the variation in the average angle of the root tip tracking over the object was examined. Figure 2(a) shows that the roots of *pgm1-1* plants developed increasing variability in tip angle after touching the obstacle. For example, at 100 min after hitting the barrier, wild-type maintained a tip angle of $142.4 \pm 11.6^{\circ}$ and *pgm1-1* roots maintained a tip angle of $142.5 \pm 10.5^{\circ}$, whereas by 350 min, although both wild-type and *pgm1-1* had similar average angles (135.1° and 139.3°, respectively), the variation in *pgm1-1* ($\pm 21.5^{\circ}$) was almost twice that of wild-type ($\pm 11.9^{\circ}$). The observed increase in variability in tip angle of *pgm1-1* was seen both within individual roots and between roots (Figure 2b), indicating it was not simply reflecting root-to-root variation in tip angle in the mutant, but rather an impairment in the ability of each individual root to hold a constant tracking angle.

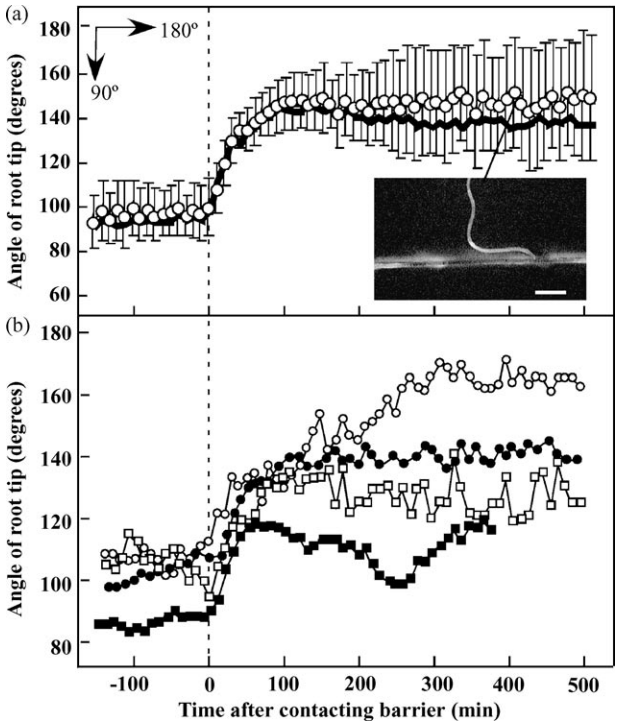

Figure 2. Root tip angle of *pgm1-1* mutants as they interact with a barrier to downward growth.
(a) Roots of the starch-deficient mutant *pgm1-1* were grown vertically into a horizontal glass barrier and growth response characterized. Results represent mean ± SD, $n = 11$. Inset, representative image of *pgm1-1* primary root traversing a barrier. Scale bar is 500 μm. Bold line represents average response of wild-type plants ($n = 22$).
(b) Representative responses of individual root tip angles of *pgm1-1* plants as they grow to and interact with a barrier. Contact with the barrier occurs at 0 min.

Effect of laser ablation of root cap cells on root growth in response to a barrier

The second approach we adopted to perturb gravity sensing in the root was removal of specific columella cells using laser ablation (Blancaflor *et al.*, 1998). Figure 3(a) shows that ablation of the central columella region (Figure 3a, inset), a treatment known to reduce gravisensitivity by three- to fourfold (as measured by changes in presentation time; Blancaflor *et al.*, 1998), produced a tip angle of $165.7 \pm 10.6^{\circ}$ to the barrier, a significantly shallower angle than controls (Figure 3a; average for 200–500 min; $P < 0.001$; separate-variance *t*-test; $n = 11$). Root growth rate was unaffected by the ablation ($4.4 \pm 1.1\ \mu m\ min^{-1}$ for columella ablations versus $3.9 \pm 0.9\ \mu m\ min^{-1}$ for controls; $P > 0.05$; separate-variance *t*-test; $n = 11$). In addition, roots with columella cells ablated maintained this growth rate upon contacting the barrier ($4.5 \pm 1.1\ \mu m\ min^{-1}$ before contact; $4.4 \pm 1.1\ \mu m\ min^{-1}$ after contact; $P > 0.1$; paired *t*-test; $n = 11$).

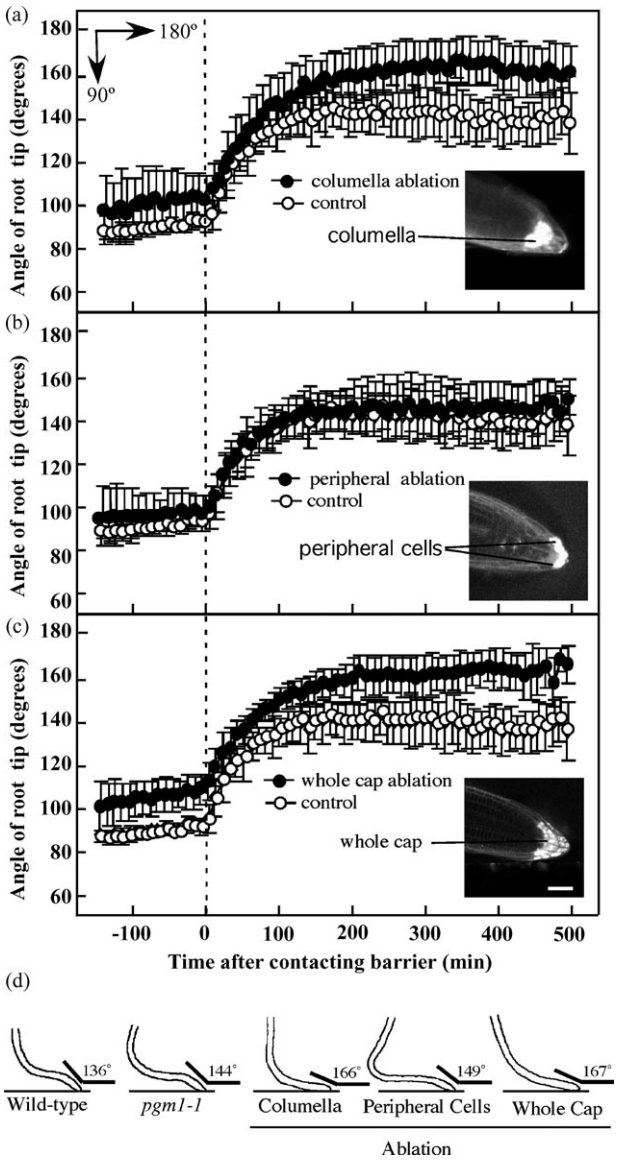

Figure 3. Root tip angle of primary roots of *Arabidopsis thaliana* as they interact with a barrier after ablation of different cap cell types.
Different cells of the root cap of wild-type *Arabidopsis* seedlings were removed by laser ablation and then the roots grown vertically into a horizontal glass barrier. Contact with the barrier occurs at 0 min. Results represent mean ± SD.
(a) Columella cell ablation ($n = 11$).
(b) Peripheral cap ablation ($n = 14$).
(c) Entire cap ablation ($n = 10$).
Inset, ablation patterns as confirmed using propidium iodide staining and confocal microscopy. Note: controls were handled as for laser ablation treatments but not irradiated. Scale bar is 200 μm.
(d) Summary of root tip angles to the glass barrier for wild-type, *pgm1-1* and columella, peripheral cell, and whole cap ablations.

Laser ablation also allowed us to remove the tip and peripheral cap cells (Figure 3b, inset), cells not thought to be involved in gravity sensing (Blancaflor *et al.*, 1998). The response kinetics of roots with this ablation pattern are shown in Figure 3(b). Although the tip angle of these roots was slightly more shallow ($149.1 \pm 12.0^{\circ}$) than the $136.0 \pm 10.0^{\circ}$ of untreated roots, this angle was not significantly different from the angle of the unablated controls ($0.1 > P > 0.05$; separate-variance *t*-test; $n = 14$). Growth rate before ($3.5 \pm 1.4\ \mu m\ min^{-1}$) and after ($3.8 \pm 1.6\ \mu m\ min^{-1}$) the root touched the obstacle was also not significantly different from unablated controls ($P > 0.05$; separate-variance *t*-test; $n = 11$).

Ablation of the entire root cap (Figure 3c) removed all the peripheral and columella cells as well as flanking cells. As noted previously, these roots almost completely lacked a gravitropic response and grew straight in the direction of their previous growth (Blancaflor *et al.*, 1998, data not shown). By placing these cap-ablated roots vertically, they could still be forced to grow into a barrier despite this lack of gravitropic direction. When these whole cap-ablated roots hit the barrier, they adopted a final tip angle of $166.8 \pm 8.6^{\circ}$, i.e. nearly horizontal (Figure 3c) and significantly shallower than either the unablated controls or the peripheral-only ablations ($P < 0.001$; separate-variance *t*-test; $n = 10$). These final tip angles were not significantly different from columella ablations ($P > 0.1$). For comparison purposes, Figure 3(d) shows a summary of the responses of wild-type *pgm1-1* and the ablations (columella, peripheral and whole cap).

Interaction of touch and gravitropic growth

We next reasoned that if touch was indeed interacting with the graviresponse, then this might be evident in altered graviresponse kinetics once the touch stimulation was removed. Figure 4 shows the angle of the root tip as it grew over the edge of a barrier compared to a normal gravitropic response from a root starting at the same tip angle (approximately 136°) but which had not touched a barrier.

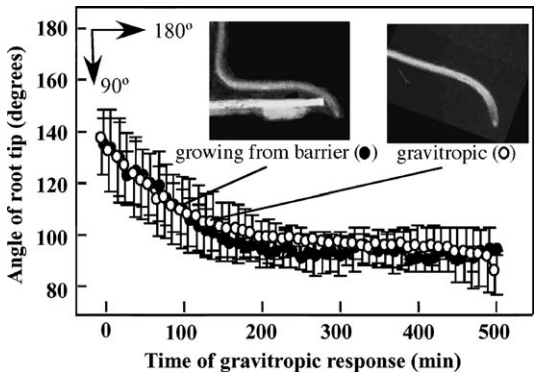

Figure 4. Comparison of root tip angles of primary roots of *Arabidopsis thaliana* re-orienting from 136° (gravitropic, $n = 12$) with those that grew off the edge of a barrier ($n = 10$).
Note: angle of root growing in contact with the barrier is 136° prior to growing off the end of the barrier. Results represent mean ± SD. Representative images of the root growing off a barrier and responding to gravitropic stimulation are shown inset. Scale bar 500 μm.

Table 1 Effect of transient touch stimulation on root growth

Region touch stimulated	Angle of root tip (degrees)
Control	0.7 ± 6.5
Tip cells	1.3 ± 4.7
*Peripheral cap	−1 ± 7.3
*Meristem	2.1 ± 5.0
*DEZ	−2.4 ± 8.6
*CEZ	1.6 ± 9.1

Roots were maintained vertically and the indicated regions of the roots were touch stimulated by contact with a glass micropipette for 10 min. The roots were then immediately placed on a clinostat and the angle of the root tip measured after 4 h subsequent growth. The control was handled as for the touch stimulation experiments but the root apex was not contacted by the micropipette. *The side of the touch stimulation (left or right flank of the root) was recorded and subsequent bending on the clinostat towards this side was scored as a positive angle, away from the touched side as a negative angle. The DEZ and CEZ touch stimulation was centered on cells at 410 and 700 μm from the apex, respectively. Results represent mean ± SD, $n = 14$.

Both sets of roots showed the same rate of gravicurvature (Figure 4; $P > 0.05$; separate-variance *t*-test; $n = 12$ from gravitropic, $n = 10$ from barriers).

These results suggested to us that if the root was integrating information from gravity and touch sensing cells to regulate tropic growth, the touch effect was transient and a full and sustained gravitropic response was rapidly reinstated once the touch stimulus was removed. Thus, several hours of continuous gravity stimulation might mask any short-term effect by a previous touch signal on the gravireponse. Therefore, to isolate these potential transient effects on gravisensing, roots were mounted vertically and a glass micropipette was positioned using a micromanipulator to touch stimulate the root for 10 min. The pipette was then removed and the root rotated 90° to gravistimulate it for 5 or 10 min. The root was then immediately placed on a clinostat to randomize the gravity vector during the subsequent growth period. Tropic growth that developed on the clinostat was then assessed after 4 h. Bending occurring on the clinostat should therefore represent a response to a signal generated during the initial short gravistimulation period and so more likely reveal transient effects of touch stimulation on gravitropic sensing and response. Table 1 indicates that this touch stimulation alone was insufficient to elicit a thigmotropic growth response, irrespective of where on the root surface the stimulus was applied. However, Figure 5 shows that using this clinostat assay we could detect a significant inhibition of gravireponse to an initial touch stimulation ($P < 0.05$ for both 5 and 10 min of touch stimulation of tip cells; *t*-test; $n > 15$). The inhibition of the gravitropic response was observed when the tip or peripheral cap cells were stimulated but not the epidermal cells of the meristematic region ($n = 11$), DEZ ($n = 7$), or CEZ ($n = 8$; Figure 5, data not shown). Figure 5 also indicates that there was no significant difference in the inhibition of gravitropic growth, whether touch stimulation was applied to the future upper or lower flank of the gravistimulated root. These results suggested to us that the interaction of touch and gravity resided in the cells of the root cap, and that the touch sensory system may downregulate gravitropic responsiveness in the *Arabidopsis* primary root.

Movement of the starch-filled amyloplasts of the columella cells is thought to be the initial event of gravity sensing in the root cap, and the mobility (sedimentation) of these organelles correlates with the apparent ability of each columella cell to generate a gravity signal (Blancaflor *et al.*, 1998). We therefore compared amyloplast sedimentation rates in the columella cells of roots touch stimulated using the micropipette to unstimulated controls, to test whether touch might be altering gravisensing via causing changes in amyloplast dynamics. Table 2 shows that touching the peripheral cap cells using a glass micropipette caused a significant reduction in the amyloplast sedimentation rates of story 1 and 2 columella cells, implying that touch stimulation of the peripheral cell surface was capable of altering columella cell function. The largest reduction in rate of sedimentation was seen in the central cells of story 1 and 2, cells also showing the most rapid amyloplast movements upon gravistimulation (Table 2).

Growth responses in other plant species

We also tested the barrier response of the primary roots of other plant species commonly used in gravitropism studies to test for the potential ubiquity of this response system. *Lepidium sativum* (cress) is a dicot in the same family as

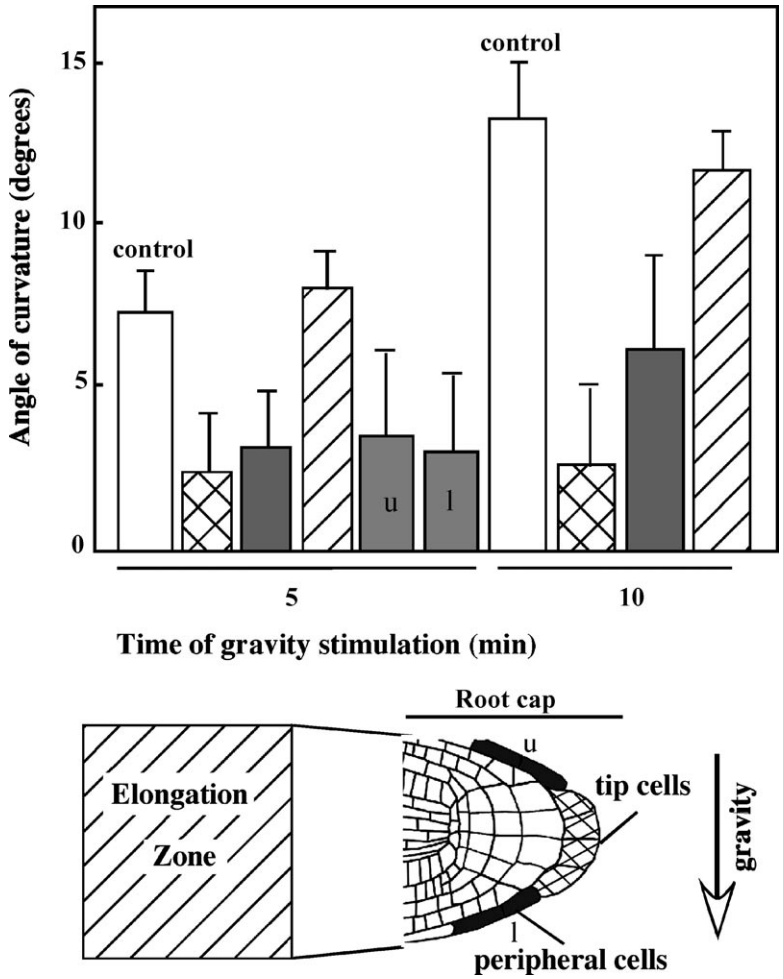

Figure 5. Effect of touch stimulation on gravitropic response.
Roots were grown vertically and the root cap tip and peripheral cells and cells in the elongation zone were touch stimulated by contact with a glass micropipette for 10 min. The pipette was removed and the roots were then gravistimulated for the indicated times. The final angles of the root apex were determined after 4 h of subsequent growth on the clinostat. Any bending occurring during clinostat growth should reflect a response to the initial gravistimulation prior to placement on the clinostat. Controls were handled as for touch treatments but not contacted by the micropipette. Shading of the bars corresponds to the indicated regions of root stimulation shown in the lower diagram. 'u' and 'l' denote touch stimulation applied to cells that will lie on the upper or lower flank of the root upon gravistimulation. Results represent mean ± SD, $n > 15$ separate roots, three separate experiments.

Arabidopsis but with primary roots approximately three times as thick. Figure 6(a) shows that the kinetics of the cress root tip angle, as it interacts with an obstacle, appear very similar to *Arabidopsis* with a final tip angle of $134.5 \pm 8.2^\circ$ which is not significantly different from the angle formed by *Arabidopsis* (time 200–500 min after touching; $P > 0.1$; separate-variance *t*-test; $n = 10$). Analysis using the transient touch stimulation and clinostat as described for *Arabidopsis* in Figure 5 showed that transient touch stimulation of the *L. sativum* root cap also inhibited subsequent gravitropic response by 63% compared to 78% for *Arabidopsis* (compare Figures 5b and 6b; *t*-test; $P < 0.05$; $n = 10$).

The monocot *Phleum pratense* has a root similar in size to *Arabidopsis*. However, as shown in Figure 6(c), the final tip angle of this species as its root traverses the barrier is $151.4 \pm 13.7^\circ$, differing from that of *Arabidopsis* by approximately 15° (time 200–500 min after touching; $0.01 > P > 0.005$; separate variance *t*-test; $n = 8$). In addition,

Table 2 Amyloplast sedimentation rates in columella of control plants and root caps that had been touch stimulated on the tip cells using a glass micropipette

	Sedimentation rate (μm min^{-1})	
Region of Columella	Control	Touch stimulated
S1		
Central	0.9 ± 0.06	0.5 ± 0.08*
Flank	0.5 ± 0.06	0.4 ± 0.07
S2		
Central	1.2 ± 0.06	0.7 ± 0.06*
Flank	0.6 ± 0.06	0.5 ± 0.08
S3		
Central	0.08 ± 0.06	0.1 ± 0.05
Flank	0.07 ± 0.05	0

Roots were then rotated through 135° to induce amyloplast sedimentation. Results represent mean ± SEM, $n \geq 10$. *Mark significantly different sedimentation rates between control and touch stimulated ($P < 0.05$; *t*-test).

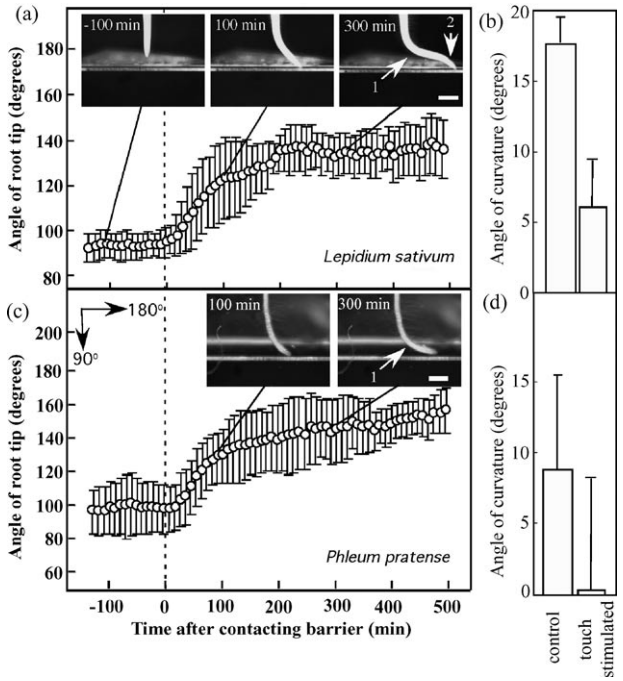

Figure 6. Root tip angle upon traversing a barrier and touch inhibition of gravitropism in primary roots of *Lepidium sativum* and *Phleum pratense*. In (a) and (c), roots were grown vertically into a horizontal glass barrier and the kinetics of growth monitored. Contact with the barrier occurs at 0 min, and insets show representative images of the root before contact, at the point of contact, and after growing along the barrier. Scale bar is 500 μm. Results represent mean ± SD, $n = 10$ (*L. sativum*), $n = 8$ (*P. pratense*). In (b) and (d), roots were touch stimulated by contact with a glass micropipette for 10 min, gravistimulated for 10 min, and the final angles of the root apex were determined after 4 h of subsequent growth on the clinostat. Any bending occurring during clinostat growth should reflect a response to the initial gravistimulation. Note: controls were handled as for touch treatments but not contacted by the micropipette. '1' and '2' mark the bends in the root making up the growth response. Note: *P. pratense* lacks bend 2. Results represent mean ± SD, $n > 30$ separate roots.

the structure formed by the *P. pratense* root as it tracks across the barrier appears morphologically different, without the second bend forming near the root tip (Figure 6c, inset). However, analysis using the transient touch stimulation and clinostat growth showed that, as in *Arabidopsis* and *L. sativum*, touch stimulation of the root cap of *P. pratense* appeared to inhibit subsequent gravitropic response (Figures 6d; *t*-test; $0.1 > P > 0.05$; $n = 30$).

Discussion

Plant roots are known to be sensitive to gravity and touch stimuli (reviewed in Fasano *et al.*, 2002). Indeed, plant graviperception has been proposed to represent a modified touch sensory system as both thigmotropism and gravitropism represent mechanical sensing of some kind (Trewavas and Knight, 1994). However, the inter-relationship between these two mechano-sensory systems remains obscure. Our results suggest that primary roots of *A. thaliana* have the ability to perceive touch stimuli in a manner that modulates gravitropic growth to efficiently circumvent physical obstacles to downward growth. The data for *L. sativum* and *P. pratense* roots taken together with *Arabidopsis* demonstrate that this response is seen in different species with varied root size and root system architecture (tap root versus fibrous root system of *P. pratense*). It is interesting to note that *Phleum* appeared more sensitive to touch inhibition of its gravitropic response (Figure 6) and developed a much shallower angle to the barrier than *Arabidopsis* or *L. sativum*. This apparent variation in sensitivity may reflect differences in tropic responses related to the architecture of their respective root systems (tap versus fibrous) or perhaps to root thickness. This variation in root thickness may also account for apparent differences in tip angle variability before and after touching that were seen in *L. sativum* and *P. pratense*, but not in wild-type *Arabidopsis*. Unfortunately, attempts to repeat this analysis with maize, another of the classical gravitropic model systems, have proven impractical due to difficulties in growing maize roots in the agar medium required for the barrier experiments.

We speculate that the touch stimulus reduces the gravitropic signaling events generated by the cap cells. This modulation of gravisensing would then lead to the tip angle of 136° in *Arabidopsis*, which represents an adaptive compromise between the 90° vertical gravitropic growth and direct sideways growth (180°) to avoid the obstruction. The resumption of downward growth to 90° seen when the root reaches the end of the barrier (Figure 4) implies that graviperception and capacity for full gravitropic response are still present as the root traverses an obstacle, but have been suppressed. It is interesting that root waving and coiling are also believed to involve gravity and touch (Mirza, 1987; Mullen *et al.*, 1998; Okada and Shimura, 1990; Simmons *et al.*, 1995). It remains unclear how circumnutation, gravitropism, and thigmotropism interact to elicit the root waving/coiling phenotype (Mullen *et al.*, 1998; Okada and Shimura, 1990; Simmons *et al.*, 1995), although growth conditions have been shown to modify the wave characteristics (Buer *et al.*, 2000). Root waving is characterized by cell file rotation in the CEZ (Mullen *et al.*, 1998; Okada and Shimura, 1990), and so it is tempting to speculate that the initial bending we report in the CEZ in response to contacting a barrier may be related to the mechanism underlying root waving/coiling. Although, as a note of caution, waving/coiling is also characterized by a handedness (Mirza, 1987; Mullen *et al.*, 1998; Okada and Shimura, 1990; Simmons *et al.*, 1995), and we have no evidence for handedness in the interaction with a barrier in our system.

The involvement of gravity signaling in the growth response to the barrier is suggested from the response of the gravitropically compromised *pgm1-1* mutant. Thus,

Figure 2(a) shows that the average kinetics of the roots of the *pgm1-1* starch-deficient mutant were identical to wild-type, even though previous studies with this mutant (including plants grown under identical conditions to those used in this study; Fasano *et al*., 2001; Kiss *et al*., 1989) indicated that the absence of starch did not completely abolish root gravitropism but rather reduced the gravisensitivity and speed of the response. Therefore, this residual graviresponse of the root appears sufficient to initially elicit the normal barrier contact response in our experiments. However, Figure 2(a) also shows that although the angle of the tip to the barrier was initially unaltered in *pgm1-1* relative to wild-type, these mutants appeared to poorly maintain this set angle as indicated by the increasing variability in tip angle over time. These results suggest that the root tip was continuously using gravitropic signaling to orient itself to the barrier, but the reduced sensitivity of *pgm1-1* led to a more variable response as the root tried to key in on the appropriate 136° angle.

The columella cell ablation results shown in Figure 3 also imply a gravitropic component to maintaining the tip angle upon encountering the barrier. Thus, ablation of the columella led to increased tip angle (166°), i.e. the tip became more horizontal. The columella cells are known to generate most of the gravitropic signal in the *Arabidopsis* root (Blancaflor *et al*., 1998). Thus, the maintained angle of 136° seems to require gravitropic input from these cells. The 166° tip angle was unchanged by the ablation of the entire cap, i.e. columella plus peripheral cap cells, indicating that the columella cells, rather than peripheral cells, were likely responsible for the sensory effects on tip angle of a root contacting the barrier. Ablation of just the peripheral cells confirmed that an intact columella was sufficient to support the wild-type response to contacting the barrier (Figure 3). Therefore, these laser ablation studies indicate that although the tip peripheral cap cells are likely the first to interact with an obstacle as the root grows down, they are not required in the maintenance of the tip angle in response to the touch stimulation. These tip and peripheral cells have been shown not to be important for gravitropic bending (Blancaflor *et al*., 1998), but have been implicated in other secretory and defense functions (Hawes *et al*., 2000) and have been shown to be touch sensitive (Legué *et al*., 1997). Thus, while these cells may be touch sensitive, it appears they are either not responsible for touch signaling in the barrier response or that mechano sensors in the columella or other cells or regions of the root (Wolverton *et al*., 2002) are capable of affecting tropic responses.

The touch component of the response to a barrier is revealed in the transient touch experiments we performed using a micropipette and the clinostat. Touching the root cap elicited a downregulation of subsequent gravitropism. A similar idea has been suggested by Mullen *et al*. (2000) who reported that a lag in the onset of gravitropic growth may be increased when there is a significant mechanical stress caused by the rotation of the root used to elicit the gravitropic stimulation. Within the resolution of our growth measurements (2.5 μm min^{-1}), we could detect no alteration in growth rate upon encountering a barrier. This observation is consistent with rapid sensing and growth reorientation by the root in response to mechanical stimulation.

The interaction of gravitropic and touch responses could occur indirectly through a thigmotropic growth response being superimposed on the gravitropic growth, leading to a growth angle that is intermediate between the two. Alternatively, there may be a more direct interaction through the touch stimulus downregulating the gravitropic signaling. We favor this latter interpretation on several counts. First, controls that were touched with a micropipette but not gravistimulated (i.e. vertically growing roots) and then grown on the clionostat did not elicit bending (Table 1), i.e. this touch protocol did not induce a detectable thigmotropic response, yet the results shown in Figure 5 indicate that it did alter gravitropic response. In addition, this stimulation always had an inhibitory effect on gravitropism irrespective of whether it was applied to the upper or lower side of the cap or symmetrically at the tip (Figure 5, data not shown), implying that the effect on gravitropism could not be the simple addition of a gravitropic and thigmotropic growth response. Similarly, the fact that *pgm1-1* initially adopted a wild-type-like angle might also suggest that the response is not simply a summation of gravitropic and thigmotropic response as the gravitropic response is significantly reduced in this mutant. We speculate that the touch signal may be suppressing gravitropic signal transduction or translocation events to alter the tropic response of the root upon contacting the barrier.

The transient gravitropic inhibition observed in the clinostat experiments might suggest that the initial curve in the CEZ seen upon encountering the barrier should have a significant component of touch-induced reduction of gravitropic sensing. If this idea was true, there may be a significant difference in the kinetics of the CEZ bend in plants where putative touch sensing cells, such as the peripheral cap cells, have been ablated. Conversely, the initial CEZ response might not be altered in roots with inhibited gravisensitivity, i.e. columella cells ablated, as this intial growth response would be dominated by touch-related sensing coupled to the downregulation of the gravitropic sensing system. Consistent with this idea, in roots with the columella cells ablated, the kinetics of the development of the CEZ bend are not significantly different from unablated controls (Figure 3a; 10–60 min; $P > 0.05$; separate-variance *t*-test; $n = 11$), yet the longer-term response kinetics of the DEZ bend are significantly affected, suggesting that this later stage has a significant gravitropic component. However, ablating the peripheral cap cells did not alter the initial

CEZ response (Figure 3a; 10–60 min after contacting the barrier; $P > 0.05$; separate-variance *t*-test; $n = 11$), implying that if there is a significant touch component to the initial CEZ response, touch sensors cannot solely be localized to the peripheral cells.

It is clear that touch sensitivity is not limited to the root cap. For example, touch-induced changes in Ca^{2+} level could be elicited in the elongation zone of the *Arabidopsis* root as well as the cap (Legué *et al.*, 1997). Ishikawa and Evans (1992) also showed that maize roots curved away from agar blocks or sandpaper applied to the tip, even in decapped roots (i.e. roots where the gravisensor was removed). Our results suggest that although many regions of the root show touch sensing, the root cap may be the site where touch and gravity sensing systems interact to coordinately modulate tropic growth. Legué *et al.* (1997) reported that the cap was more sensitive to generating touch-induced Ca^{2+} transients than elsewhere in the root, and we report here that touch stimulation of the cap appears to downregulate gravitropic sensitivity much more effectively than touch stimuli applied elsewhere in the root.

It is likely that the proximity of the touch stimulus to the gravisignaling system of the columella cells is an important factor in the interaction of the touch and gravity signaling systems. The precise cellular mechanism of this interaction remains unknown, but touch does appear to be able to alter the mechanical properties of the columella cell cytoplasm as reflected by reduced amyloplast mobility (Table 2). The actin cytoskeleton has been proposed as a key transduction element in the starch statolith gravisensing system of the root cap (Baluska and Hasenstein, 1997; Collings *et al.*, 2001; Driss-École *et al.*, 2000; for review, see Kiss, 2000; Yoder *et al.*, 2001), although there is also evidence that disrupting actin may not alter graviresponse (e.g. Yamamoto and Kiss, 2002). Thus, touch-induced alterations in the columella cytoskeleton, perhaps mediated via touch-induced Ca^{2+} changes (Staiger, 2000), are strong candidates for one mechanism of integrating touch and gravisignaling. In addition, the proposed roles of auxin and ethylene in thigmomorphogenesis and tropisms (Biro and Jaffe, 1984; Mitchell, 1996; Mitchell and Myers, 1995; and references therein) led us to test several auxin and ethylene-related mutants (*aux-1*, *eir-1*, *rga-1*) known to have defects in gravitropism for their responses to the barrier. However, the relatively random growth direction of the roots of these mutants has made interpretation of their barrier touch response complex, and the results do not provide strong evidence for or against a role of each of these hormones in the gravity/touch interaction (data not shown). Future work will therefore attempt to assess whether the interaction of touch signaling with the root gravity sensing system in the root cap lies at the level of altering signaling events such as pH or Ca^{2+} dynamics in the graviperceptive cells of the root cap columella.

The observation that touch stimulation of the cap peripheral cells was capable of altering amyloplast mobility in the columella (Table 2) further supports the idea that touch is signaling to the columella to alter the function of these cells. The columella is uniquely placed at the root apex to re-distribute auxin to mediate tropic curvature. Thus, it is also tempting to speculate that many stimuli may feed in to the columella, which may effect subsequent tropic responses via changes in how it re-distributes this auxin stream, possibly controlling acropetal and basipetal auxin transport flows independently (Rashotte *et al.*, 2001). One potential mechanism for such an integrating activity between the touch and gravity signaling systems might be through touch-induced Ca^{2+} transients propagating throughout the cap (Legué *et al.*, 1997). Such changes in Ca^{2+} levels in the columella could alter membrane trafficking or targetting, and so affect the dynamics of auxin transporter localization. Indeed, the highly dynamic trafficking system that targets auxin transporters within the columella has been proposed to be a key element of tropic growth control (Friml *et al.*, 2002; Geldner *et al.*, 2001; Steinmann *et al.*, 1999; for review see Morris, 2000; Muday, 2001; Muday and Murphy, 2002). Such touch-regulated redistribution of auxin via auxin transporters could co-ordinately modulate growth in the CEZ and the DEZ to give the tropic response we have observed to contacting the barrier.

Experimental procedures

Plant material and growth analysis

Seeds of wild-type and the *pgm1-1* mutant of *A. thaliana* ecotype Columbia were obtained from the *Arabidopsis* stock center (Columbus, Ohio). Seeds were sterilized according to Legué *et al.* (1997). Seedlings were grown in 0.5% (w/v) Phytagel (Sigma Chemical Co., St. Louis, MO) on #0 cover glasses (Thomas Scientific, Swedesboro, NJ), as previously described (Blancaflor *et al.*, 1998). Seeds of *P. pratense* and *L. sativum* cv. Tioga were obtained from Dr Gabrielle Monshausen (Botanisches Institut, Universitat Karlsruhe, Germany), and sterilized and planted in the same manner. Plants were used after 3 days (*Arabidopsis* and *P. pratense*) or 1 day (*L. sativum*), when the root was approximately 1 cm in length.

To determine the effect of a physical barrier to root growth, sterile pieces of #0 cover glass were inserted into the Phytagel, perpendicular to both the growing root and the surface of the cover glass to form a wall 3–4 mm in front of the growing root. The root was then oriented vertically and a movie of its growth made as described in Blancaflor *et al.* (1998), as it encountered the barrier. The minimum point-to-point resolution of the root growth movies was 25 μm. Frames were captured every 10 min for 10 h. Roots were followed, for at least 3 h prior to contacting the barrier, to ensure they were growing straight and at a growth rate of approximately 200 μm h^{-1}. Frames from the movie were analyzed for root length and angle of the root apex to the barrier using IPLabs Spectrum image analysis software (Signal Analytics, Vienna, VA). For each time point, growth rate was calculated by measuring the difference in root length between consecutive movie frames divided by the growth interval between each frame (10 min). High

resolution measurements were made by imaging the root on a vertical stage Diaphot 300 microscope (Nikon, Melville, NY, USA) using a 40×, 0.75 numerical aperture objective. Point-to-point resolution of the root tip from these movies was approximately 2 μm. Amyloplast sedimentation rate was assayed according to Blancaflor *et al*. (1998).

Laser ablations

Laser ablations were performed according to Blancaflor *et al*. (1998). Ablation patterns were confirmed following each experiment using propidium iodide staining and confocal microscopy (Blancaflor *et al*., 1998). Only the movies of root growth from roots showing clear ablation patterns in this confocal assay were used for kinetic analysis. As ablation experiments required more handling of the plants than the other imaging approaches used, controls for these ablation treatments were handled identically to the ablated roots but were not subjected to laser irradiation.

Transient touch stimulation

In order to transiently apply touch stimulation, roots were mounted vertically on the vertical stage Diaphot 300 microscope and imaged using the 10×, 0.3 numerical aperture objective. A glass micropipette (1.5 mm square borosilicate glass pulled on a PC 580 puller; Wymer *et al*., 1997) was positioned using Narashige micromanipulators to lie either perpendicular or parallel to the direction of growth, i.e. for stimulation of tip cells, the pipette was placed horizontally; for peripheral cell stimulation, the pipette was placed at an angle parallel to the surface of the lateral root cap; for elongation zone stimulation, the pipette was placed vertically parallel to the surface of the elongating cells. The pipette was then moved to contact the root. In each case it was ensured that the pipette tip was deflected by the root by 1–2 μm to confirm contact. To approximately calibrate the force applied by this pipette-based stimulation, identical micropipettes to those used on the root were caused to bend 1–2 μm by contact with the pan of an analytical balance (AB104, Mettler, Toledo, OH, USA). The weight registered by the balance ranged between 5 and 10 μg ($n = 10$). After stimulation, the root was gravistimulated by rotation through 90°, and gravitropic growth assessed either during 8 h of subsequent constant stimulation, or after brief (5 or 10 min) stimulation and subsequent 4 h of growth on a 1 r.p.m. clinostat (Fasano *et al*., 2001). As these touch experiments required more handling of the plants than the other imaging approaches used, controls for these treatments were handled identically to the touch-stimulated roots except that the micropipette was not brought into contact with the root tip. Thus, for the controls with 0 min of gravistimulation, roots were touch stimulated whilst vertical, and then immediately placed on the clinostat. Where indicated, the side of stimulation on the root (left or right side of the root) was recorded and related to what would become the upper and lower flank of the root upon rotation and gravistimulation.

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Supplementary Material

The following material is available from http://www.blackwell-publishing.com/products/journals/suppmat/tpj/tpj1637/tpj1637sm.htm

Fig. S1. Quicktime movie of the growth response of a primary root of *Arabidopsis thaliana* as it interacts with a barrier to downward growth.
A 3-day-old seedling growing in phytagel was placed vertically such that its primary root would grow down into a horizontal glass barrier. Images were taken every 5 min for 12.8 h.

Fig. S2. Quicktime movie of the growth response of a primary root of *Lepidium sativum* as it interacts with a barrier to downward growth.
A 1-day-old seedling growing in phytagel was placed vertically such that its primary root would grow down into a horizontal glass barrier. Images were taken every 10 min for 13.7 h.

Fig. S3. Quicktime movie of the growth response of a primary root of *Phelum pratense* as it interacts with a barrier to downward growth.
A 3-day-old seedling growing in phytagel was placed vertically such that its primary root would grow down into a horizontal glass barrier. Images were taken every 10 min for 8.5 h.

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