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Water Relations in Pulvini from *Samanea saman*[1]

II. EFFECTS OF EXCISION OF MOTOR TISSUES

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ABSTRACT

Pulvinar motor tissues of *Samanea saman* are often excised for *in vitro* studies of ion transport. Because ion transport may be regulated in part by hydrostatic pressure (P), this study explores how P and water potential (Ψ) change when motor tissues are excised. Water potential (Ψ) of excised extensor and flexor tissues was measured by the Chardakov method and compared with Ψ measurements made on extensor and flexor tissues of intact pulvini (HL Gorton 1987 Plant Physiol 83: 945-950). Ψ values for excised extensor and flexor tissues were always substantially more negative than for the same tissues in intact pulvini. Extensor tissues excised from open pulvini had slightly more negative Ψ than excised flexor tissues, and the opposite was true for closed pulvini. Extensor and flexor tissues elongate immediately when excised from open or closed pulvini, suggesting that in intact pulvini they are constrained from elongating by the nonextensible vascular core. In addition, both tissues in both open and closed pulvini are under compression imposed by oppositely positioned motor tissue. Excision relieves constraint and compression, decreasing P, and thus decreasing Ψ. This finding may explain, at least in part, the difference between Ψ measurements on intact and excised motor tissues. Implications of these data for the planning and interpretation of *in vitro* experiments requiring excised strips of extensor and flexor tissues are discussed.

Pulvini of the nyctinastic, leguminous tree *Samanea saman* (Jacq.) Merrill[3] are often chosen for experimental work because they are large and easily dissected, so it is possible to do *in vitro* work on the antagonistic extensor and flexor sides of the pulvinus separately. Such experiments suggest that ion uptake in swelling pulvinar cells may be driven by an outwardly directed H^+-pump (3, 4) and that the magnitude and direction of H^+ fluxes depends on the Ψ[4] of the bathing medium (6). Ideally, the *in vitro*

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[3] *Samanea saman* (Jacq.) Merrill has been renamed *Pithecolobium saman.* I retain the name *Samanea* for continuity with the earlier literature.

[4] Abbreviations: Ψ = water potential ($\Psi = \pi + P$); π = osmotic potential; P = hydrostatic pressure; Ψ_{dr} = water potential measured on extensor and flexor sides of intact pulvini using the droplet method (from Gorton [2]); Ψ_{Ch} = water potential measured on excised strips of extensor and flexor tissues using the Chardakov procedure; P_{dr} = pressure calculated using Ψ_{dr} and π; P_{Ch} = pressure calculated using Ψ_{Ch} and π; P_{cell} = pressure attributable to the water status and resistance to expansion of individual cells; P_{tissue} = pressure imposed on a given cell by other cells.

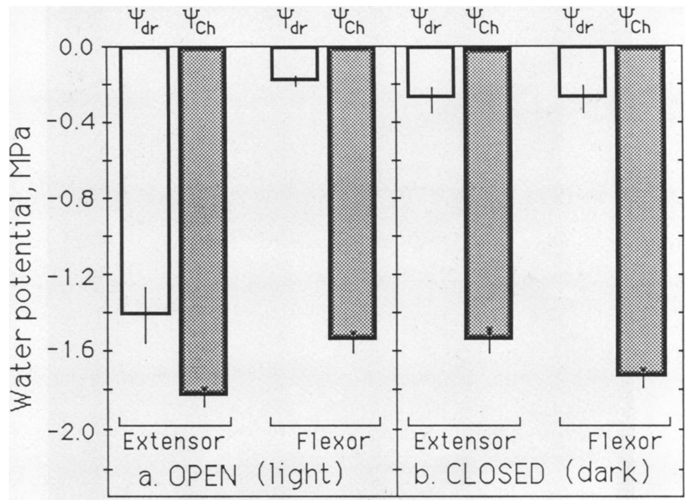

FIG. 1. (a and b) Extensor and flexor Ψ for *Samanea* pulvini that were open in the light (a) and closed in the dark (b). Ψ_{dr} (open bars), data for Ψ determined for extensor and flexor tissues in whole pulvini by the droplet method (from Fig. 5 in Gorton [2]). Ψ_{Ch} (shaded bars), data for Ψ determined for excised extensor and flexor tissues by the Chardakov procedure. Typical data from one experiment are shown. The height of the bar indicates the midpoint of the range of possible Ψ values determined by each method, and the error bars indicate upper and lower bounds.

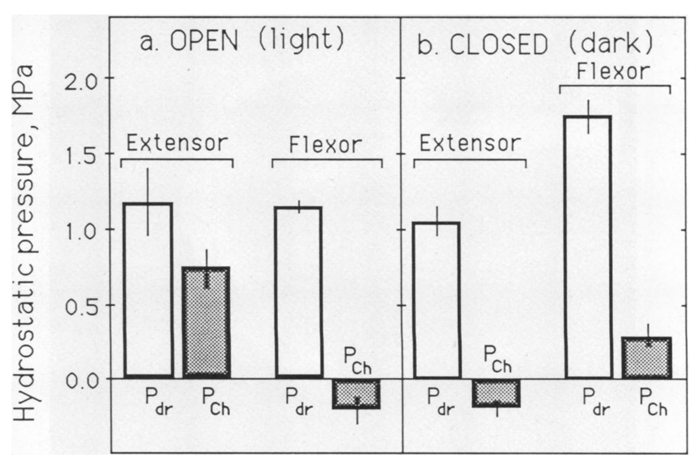

FIG. 2. (a and b) Hydrostatic pressure within *Samanea* pulvini that were open in the light (a) and closed in the dark (b). Pressures were calculated as the difference between π (from Fig. 5, in Gorton [2]) and Ψ (from Fig. 1). Both Ψ_{dr} and Ψ_{Ch} were used, yielding P_{dr} (open bars) and P_{Ch} (shaded bars). Error bars indicate upper and lower bounds.

environment should mimic the *in vivo* environment, with π and P, and hence Ψ, unchanged. This is difficult partly because Ψ of motor tissues in intact pulvini changes during the day (2). A further complication might arise if excision itself changes Ψ of extensor and flexor tissues. Pulvini are organs that accomplish mechanical work, and significant mechanical loads might be released upon excision of motor tissues, thus altering P and Ψ. In this study, I measure Ψ for excised extensor and flexor tissues from pulvini that are open in the light and closed in the dark, compare these values with Ψ values reported in the accompanying paper (2) for of extensor and flexor tissues in intact pulvini, and explore mechanical forces in pulvini that might affect P and Ψ.

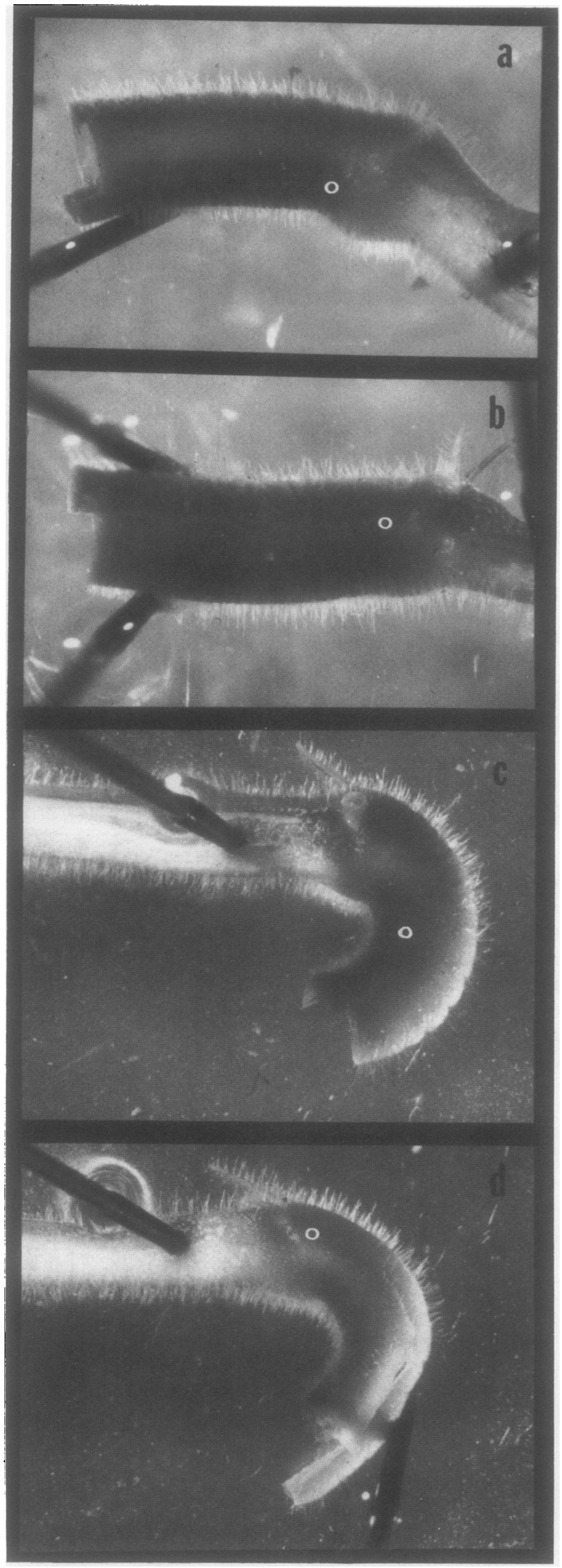

FIG. 3. (a–d), Response of extensor and flexor tissues to partial excision from *Samanea* pulvini. Each pulvinus, kept under fluorocarbon fluid to prevent evaporation, was cut parallel to its longitudinal axis. Cuts were made in the extensor (a, c) and flexor (b, d) tissues of pulvini that were open in the light (a, b) and closed in the dark (c, d). Pins were used to hold the pulvinus during and after the cut. Small white circles mark the bases of the cuts.

MATERIALS AND METHODS

Plant Material. *Samanea saman* (Jacq.) Merrill trees were grown as described in Gorton (2). Pulvinar tissues were excised from terminal secondary pulvini as described in Iglesias and Satter (3).

Water Potential Measurements. At h 8, when pulvini were open, and at h 20, when pulvini were closed, strips of extensor and flexor tissues from 8 to 10 leaves, chosen as in Gorton (2), were excised for Ψ estimation by a modification of the Chardakov procedure (1). Tissue samples were added to tubes containing 10 μl of mannitol solution of a known Ψ (the test solution), incubated for about 1 h, and removed. Incubation took place in the growth chamber, in the light if the tissues were excised during the light period, and in the dark if the tissues were excised during the dark period. The dim, green light used to excise tissue during the dark period is described in Gorton (2). One strip of extensor or flexor tissue was used per tube, and 8 to 10 different concentrations of mannitol were tested in each experiment. After the tissue was removed, a few crystals of methylene blue were added to the 10 μl of test solution. Droplets of the colored solution were introduced with a glass microcapillary into tubes containing 5 ml of control mannitol solution, identical to the test solution, except uncolored and unexposed to tissue. The fall or rise of the colored test droplets in the control solution indicated whether the tissue had taken up or lost water, thereby causing the test solution to decrease or increase in density. Experiments were repeated at least five times with similar results. Data presented here were obtained from the same plant used to obtain data shown in Figures 5 and 6 in Gorton (2). Error due to damaged cells in the tissue sample should be small, since the volume of damaged cells, estimated from the surface area of the cut and a cell diameter of 30 μm, was less than 2% of the volume of the test solution.

Water Content. Extensor and flexor tissues were excised in a humidified chamber and weighed immediately. The tissues were reweighed after drying at 70°C for 24 h, and percent water was calculated as follows:

$$\text{Percent water} = \frac{FW - DW}{FW} \times 100$$

Percent water was determined for extensor and flexor tissues from open pulvini in the middle of the light period and from closed pulvini in the middle of the dark period, with 12 pulvini in each sample. Open and closed pulvini came from the same tree and were matched in age.

In some experiments, pulvini were frozen and thawed to eliminate osmotically driven water movement. Frozen and thawed pulvini, whether originally open or closed, assumed angles of 80 to 120°. One pulvinus of each pair (n = 12 pairs)

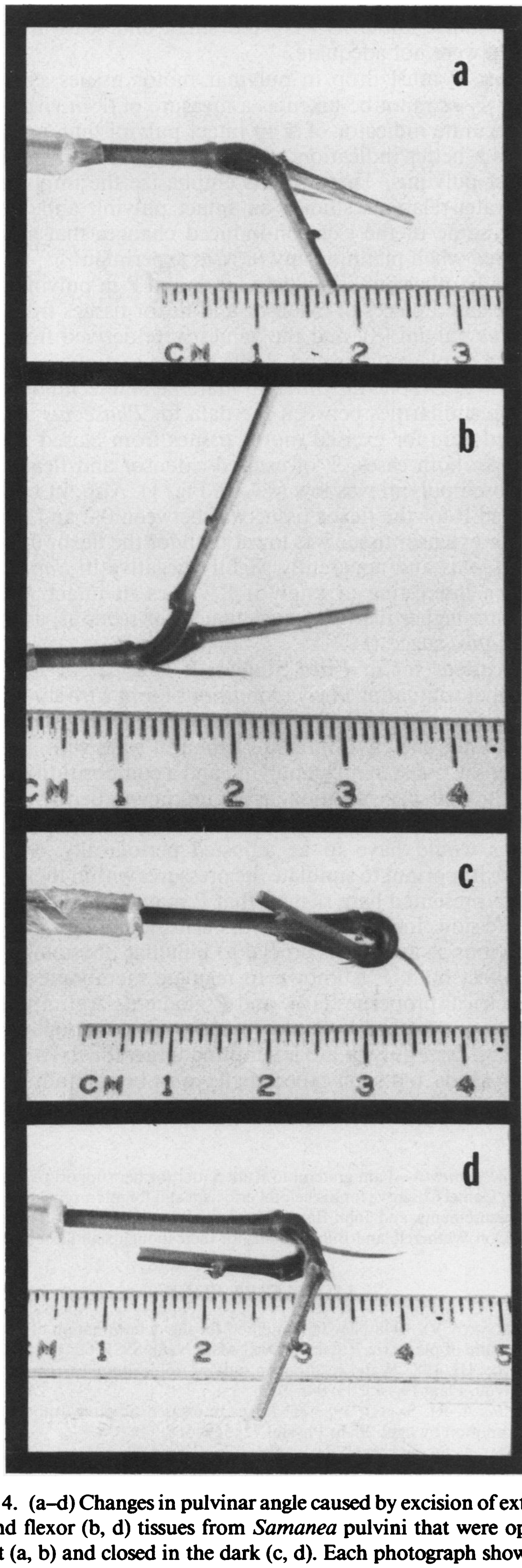

FIG. 4. (a–d) Changes in pulvinar angle caused by excision of extensor (a, c) and flexor (b, d) tissues from *Samanea* pulvini that were open in the light (a, b) and closed in the dark (c, d). Each photograph shows one pair of pulvini, viewed from the side, with the rachis inserted into a tube of water. The two thickened, flexible pulvini are attached to the rachis and a rachilla is attached to each pulvinus. The control pulvinus (in the background in each panel) was not dissected. The pulvinus in the foreground had the extensor or flexor motor tissue excised and the cut surface covered with paraffin oil. If no change in pulvinar angle had occurred, the control pulvinus would be obscured from view and the two rachillas would be superimposed. Changes in pulvinar angle were evident immediately upon excision of extensor or flexor, and were photographed with 30 s.

was held closed (0°) and the other held open (180°) for 15 min, then flexor tissues were excised for percent water determination as described.

RESULTS

Water Potential. Data for Ψ of excised extensor and flexor tissues obtained by the Chardakov procedure (Ψ_{Ch}) are shown (Fig. 1) in comparison with data obtained by the droplet method for extensor and flexor tissues in intact pulvini (Ψ_{dr}, from Fig. 5 [2]). All Ψ_{Ch} values for excised tissues were substantially more negative than the corresponding Ψ_{dr} values for the same tissues in intact pulvini. In open pulvini, Ψ_{Ch} was about 0.3 MPa more negative in the extensor than in the flexor. This gradient was much smaller than the Ψ_{dr} gradient in intact, open pulvini. In closed pulvini, the excised flexor showed slightly more negative Ψ_{Ch} than the excised extensor; no such gradient was evident in intact pulvini.

Hydrostatic Pressure. Ψ data (Fig. 1) were used in conjunction with osmotic potential (π) data (from Fig. 5 [2]) to calculate P. Because Ψ_{dr} and Ψ_{Ch} were different (Fig. 1), both sets of data were used to calculate P, yielding P_{dr} and P_{Ch} (Fig. 2). P_{Ch} values were lower than P_{dr} values for the same tissues, reflecting the more negative Ψ values obtained with the Chardakov method. Extensor had a higher P_{Ch} than flexor in open pulvini, and the reverse was true in closed pulvini. For the flexor in open pulvini and the extensor in closed pulvini, both regions of shrunken cells, slightly negative values of P_{Ch} were calculated.

Mechanical Constraint and Compression. These experiments were initiated to investigate one reason for the difference between Ψ_{dr} and Ψ_{Ch}. The chief difference between the Chardakov and droplet methods for determining pulvinar Ψ was that the Chardakov method required excised tissue. One of the ways excision might alter Ψ is by changing mechanical forces within the tissue. In an intact pulvinus, cells might be prevented from expanding not only by cell wall properties, but also by constraint from nonextensible neighboring cells and/or by compression imposed by other pulvinar tissues. If these interpretations are correct, excising extensor and flexor tissues would relieve both constraint and compression, cells would expand, P would drop, and hence Ψ would drop as observed.

When extensor or flexor tissues were excised from open or closed pulvini, they elongated immediately (Fig. 3), suggesting that they were indeed constrained in intact pulvini. Compression of extensor and flexor tissues by oppositely positioned cells was demonstrated by immediate pulvinar bending after excision. Pulvini always bent toward the side that was excised (Fig. 4). If the extensor was excised from an open pulvinus, the pulvinus immediately started to close (Fig. 4a). If the flexor was excised from an open pulvinus, it immediately opened further, even wider than 180° (Fig. 4b). The same patterns were evident for closed pulvini (Fig. 4, c and d). Because the pulvini bent toward the side from which tissue was excised, I infer that the tissue must have been under compression prior to excision. The force that caused bending after excision caused compression before excision.

Water Content. Small changes in percent water accompanied normal pulvinar movement: open extensor, 78.8 ± 0.9%; closed extensor, 75.1 ± 0.8%; open flexor, 72.8 ± 1.8%; closed flexor, 77.4 ± 0.4% (means ± SE for three experiments with groups of 12 pulvini used for each experiment). To test whether mechanical forces alone can cause water movement across pulvini, pulvini were frozen and thawed to destroy the integrity of the plasma membrane. Pulvini were then manually bent or straightened, and water movement was monitored as a change in percent water. Because only the flexor was accessible for excision in pulvini held in both open and closed positions, percent water was measured only for the flexor in these experiments. Flexor tissues excised from frozen and thawed pulvini that were forced open held 70.0 ± 0.75% water, and flexors from pulvini that were forced closed held 73.3 ± 0.05% water (means ± SE for two experiments with groups of 12 pulvini used for each experiment).

DISCUSSION

Constraint and Compression in Pulvinar Tissues. Cell expansion in both high- and low-osmolality cells is constrained by neighboring tissues, probably the nonextensible vascular core (xylem, phloem, and collenchyma) (Fig. 3). Motor tissues are also compressed by oppositely positioned motor cells (Fig. 4). The magnitude of the changes shown in Figures 3 and 4 was difficult to quantify because excision could not be reproduced exactly. Therefore, these experiments should only be taken as an indication that constraint and compression do exist in pulvini.

Mechanical Forces and Water Movement. That mechanical forces, such as those suggested in Figures 3 and 4, can drive water movement in pulvini was demonstrated by changes in the percent water in the flexors of frozen and thawed pulvini when they were forced into open and closed positions. The percent water in the flexor increased when the pulvinus was forced closed, and the increase was about half that accompanying normal pulvinar closure. Water was not forced out of the pulvinus through the epidermis during the bending, and rapid movement of water into the vasculature may have been prevented by a hydrophobic barrier (10). If water cannot escape the compressed tissue to the outside of the pulvinus or to the vasculature, it must move to the oppositely positioned motor cells.

Effects of Constraint and Compression on Hydrostatic Pressure and Water Potential. The following discussion is based on (a) Figures 3 and 4; (b) Ψ values measured by the droplet method (from Gorton [2]) as compared to the Chardakov method (Fig. 1); (c) measured π values (from Gorton [2]); and (d) differences between the two derived P values, P_{dr} and P_{Ch} (Fig. 2). The largest sources of error are in the Ψ measurements. Many things can contribute to the difference between Ψ_{dr} and Ψ_{Ch}, including leakage and cut surface effects. However, differences between Ψ_{dr} and Ψ_{Ch} were up to 1.4 MPa, much larger than the 0.3 MPa error Knipling and Kramer (5) attributed to leakage and cut surface effects for the Chardakov method.

While leakage and cut surface effects may be partly responsible for the difference between Ψ_{dr} and Ψ_{Ch}, mechanical changes occurring upon excision may be largely responsible for the difference. The effective hydrostatic pressure, P, in extensor or flexor cells apparently has two components, P_{cell} (turgor pressure), which depends only on the water status of that cell and the resistance of its cell wall to expansion (due to cell wall properties and to constraint by neighboring cells), and P_{tissue}, the tissue pressure, which is the pressure acting on a cell from compression by neighboring cells. A similar situation has been described for stomatal guard cells and subsidiary cells (7, 9). When pulvinar tissues are excised, cells are allowed to expand because constraint by the vascular core is removed and because the cells are no longer under compression from oppositely positioned motor tissues. Therefore, P_{cell} drops (because the constraint is removed), P_{tissue} drops (because compression is removed), and Ψ drops. This drop in P must occur, given that constraint and compression do exist in pulvini (Figs. 3 and 4). However, without further experimentation, the relative importance of the leakage and cut surface effects and of the drop in P that occurs upon excision cannot be established with certainty. It would be desirable to obtain direct information on P within intact and excised pulvinar tissues, but brief attempts with pressure-probe methods were unsuccessful with *Samanea*; the cells were too small and seals around the probe tip were not adequate.

Because P must drop in pulvinar motor tissues as they are excised, Ψ_{Ch} cannot be taken as a measure of Ψ *in vivo*. Ψ_{dr} is a more accurate indicator of Ψ in intact pulvini than is Ψ_{Ch}, and P_{dr} gives a better indication of the total hydrostatic pressure in an intact pulvinus. These results emphasize the importance of doing water-relations studies on intact pulvini, and they also indicate some of the excision-induced changes that should be considered when planning any *in vitro* experiment.

The only other published data for Ψ and P in pulvinar tissues are for excised pieces of extensor and flexor tissues from closed *Phaseolus* pulvini (8), and the values were derived from measurements of plasmolysis and elongation of sections in solutions of known Ψ. Despite the different material and techniques, there are some similarities between the data for *Phaseolus* and those presented here for excised motor tissues from closed *Samanea* pulvini. In both cases, Ψ of excised extensor and flexor tissues from closed pulvini was low (Ψ_{Ch} in Fig. 1). Also, in both cases calculated P for the flexor tissue was between 0.2 and 0.3 MPa, and P for extensor tissue was lower than for the flexor (0.02 MPa for *Phaseolus* and apparently slightly negative in *Samanea*). It would be interesting to know if P values in intact *Phaseolus* pulvini are higher than those calculated for sections, as the data for *Samanea* suggest.

Implications for *in Vitro* Studies. It is apparent from these studies that simulating *in vivo* conditions for *in vitro* studies using excised strips of tissue will be difficult indeed. First, the medium should mimic the environment of the cell wall, with a Ψ equal to that of the tissue in intact pulvini, and a composition matching that of the free space solution, as yet unknown. Because Ψ of the tissues changes over the course of the day, Ψ of the bathing solutions would have to be adjusted periodically. Second, it would be important to simulate the pressures within the pulvinus, and data presented here suggest that P may change significantly upon excision. Ion transport across cell membranes and across the pulvinus as a whole is crucial to pulvinar physiology, and P could affect both. P is known to regulate membrane transport and electrical properties (12), and P gradients are important in determining whether bulk flow occurs in the apoplasm (11). Large *Samanea* pulvini are a tempting target for *in vitro* studies of many kinds, but such experiments must be carefully designed to mimic *in vivo* conditions and they must be interpreted with caution.

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