Plant Physiol. (1976) 58, 190-192

Potassium-linked Chloride Fluxes during Rhythmic Leaf Movement of *Albizzia julibrissin*[1]

Received for publication October 14, 1975 and in revised form April 13, 1976

MARTIN SCHREMPF, RUTH L. SATTER, AND ARTHUR W. GALSTON
Department of Biology, Yale University, New Haven, Connecticut 06520

ABSTRACT

Transverse sections of *Albizzia* pulvinules were examined with an electron microprobe to determine ion fluxes associated with turgor-controlled leaflet movements. K^+ and Cl^- concentrations are high in the flexor and low in the extensor region of closed pulvini. Both ions migrate out of the flexor and into the extensor during opening as previously described for K^+. The distribution of these elements is significantly correlated in each phase of the rhythmic cycle examined, but only 50 to 60% of the ionic charge of potassium is balanced by chloride. This value increases to 65 to 85% if one considers only the mobile fraction of the potassium.

The increase in concentration of both ions in the extensor region precedes the decrease in the flexor, thus indicating that there must be a storage reservoir for K^+ and Cl^-. The inner cortex is suggested as such a reservoir, and plasmodesmata are discussed as a probable pathway for ion movement.

Potassium fluxes seem to be a common feature of turgor-mediated movements in higher plants. This is true for stomatal movements (11, 20) leaf movements of certain legumes such as *Mimosa pudica* (2, 22), *Albizzia julibrissin* (18), and *Samanea saman* (17), and petal movements of *Kalanchoë blossfeldiana* (21). How this movement of positively charged potassium ions is balanced electrically is still an open question.

Since it has been shown recently that Cl^- fluxes are involved in some stomatal movements (12), we investigated the possible participation of Cl^- in our system, the movement of *Albizzia julibrissin* leaflets (18).

MATERIALS AND METHODS

Albizzia plants were grown from seed in the greenhouse and were transferred to growth chambers with a 16-hr light/8-hr dark cycle at least 1 week before experiments. Growing conditions, experimental procedures, and light sources have been described elsewhere (18).

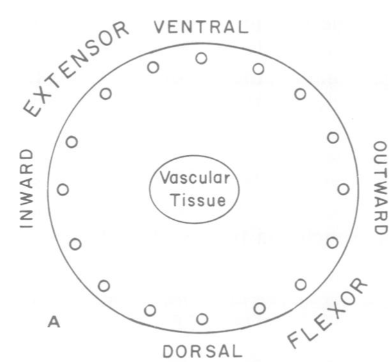
Pinnae were excised 4 hr after the beginning of the dark period (DD = 4), the cut ends were immersed in water, and they were kept in the dark until the end of the experiment. All pinnule (leaflet) pairs for a given experiment were taken from the middle region of the same leaf (third to sixth) (6). At designated intervals, the angles between paired pinnules were measured, pulvini were excised, frozen in Tissue Tek on dry ice, and sectioned (24 μm thick) in a cryostat in preparation for elemental analysis with an Acton electron microprobe (18).

[1] Supported in part by a National Science Foundation grant to A. W. G. and R. L. S. and in part by a fellowship of the Deutsche Forschungsgemeinschaft to M.S.

K^+, Cl^-, and Ca^{2+} were analyzed simultaneously. Since Cl^-, Ca^{2+}, and K^+ have similar atomic numbers, the x-ray emissions representing these elements come from approximately the same tissue volume (3). The spectrometers were tuned with potassium feldspar (K^+) and scapolite (Ca^{2+}, K^+, Cl^-) standards. The instrument was operated with an accelerating voltage of 12.5 kv, a specimen current of 50 nA on brass, a spot diameter of 12 μm, and a counting time of 15 sec (18). The average specimen to background ratios were 20:1 (Ca^{2+}), 70:1 (K^+), and 10:1 (Cl^-).

RESULTS AND DISCUSSION

In contrast with earlier work (15, 18), the present investigation involves transverse rather than longitudinal pulvinal sections. The analyzed regions are indicated in Figure 1A by circles, approximately 50 μm from the epidermis. The dorsal side of the tissue was readily identified by the Ca^{2+} salt crystals (probably oxalate) which occur only in the dorsal region of the inner cortex (Fig. 1B). The "outward" region was marked with India ink to



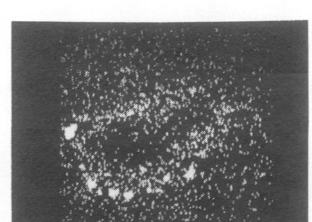


FIG. 1A. Cross-section of a tertiary pulvinus of *Albizzia*. Inward indicates the region where paired pulvini oppose each other; hence, this is the left pulvinus of a pair. Extensor describes the region where cells expand during opening and contract during closure. Flexor cells change in the opposite directions although not in the same magnitude (17). The circles indicate the analyzed regions of the motor tissue 50 μm from the periphery. B. Ca^{2+} scintillations in a cross-section of a closed pulvinus. Approximately 75% of the section is shown. The larger bright spots represent Ca^{2+} salt crystals (probably oxalate) serving as a convenient marker around the dorsal side of the vascular tissue. Inward and outward are left and right, respectively. Microprobe voltage and current are similar to those in Figure 2; total counts = 2000 in 232 sec..

differentiate it from "inward"; such marked pulvini showed no difference in behavior from unmarked controls.

K^+ and Cl^- scintillations are shown as a function of the circumferential distance from a reference point at the middle of the ventral region (Fig. 2). Both ions are high in the flexor region and low in the extensor region at DD = 4.5, when the leaflets are closed, and both ions migrate out of the flexor and into the extensor as the leaflets open to 120°. The distributions of Cl^- and K^+ are strongly correlated, being significant at the 1% level (Fig. 3), both when leaflets are closed and during opening.

It is necessary to apply a correction factor before comparing the absolute magnitude of K^+ and Cl^- values, since these elements are counted with different efficiencies in the microprobe. To determine this correction factor, we measured K^+ and Cl^- scintillations of two standards with known K:Cl ratios, *i.e.* scapolite and KCl-impregnated filter paper (Whatman No. 1). K^+ was measured 5.8 times as efficiently as Cl^- with the filter paper, and 4.5 as efficiently as Cl^- with the scapolite. Obviously, the number of scintillations depends not only on the microprobe operating conditions and the concentration of an element, but also on its chemical environment (5). Since the cellulose matrix of the filter paper is more similar to the cellular matrix than is scapolite, we used a correction factor of 5.8. This correction factor is still considered approximate and the possibility that it might be 2 units higher or lower (*i.e.* 5.8 ± 2) is not excluded. These limits refer to the highest and lowest values obtained with

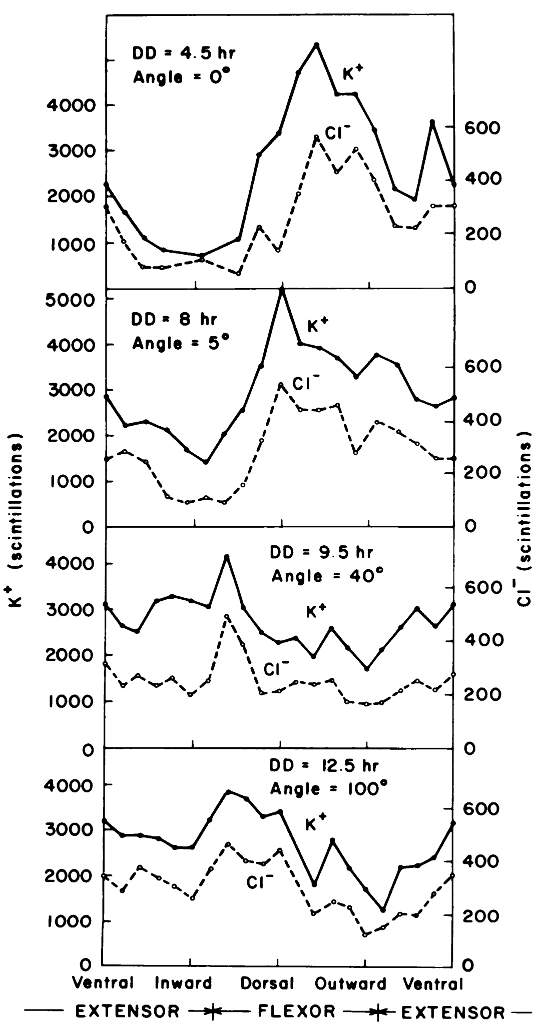


FIG. 2. Electron microprobe analysis of K^+ and Cl^- in the motor region as described in Figure 1A (accelerating voltage: 12.5 kv; specimen current: 50 nA on brass; spot diameter: 12 μm; counting time: 15 sec). The abscissa indicates the distance from a reference point in the middle of the ventral region. The scale of the Cl^- scintillations is enlarged by a factor of 5.8 relative to the scale representing the K^+ scintillations for reasons explained in the text.

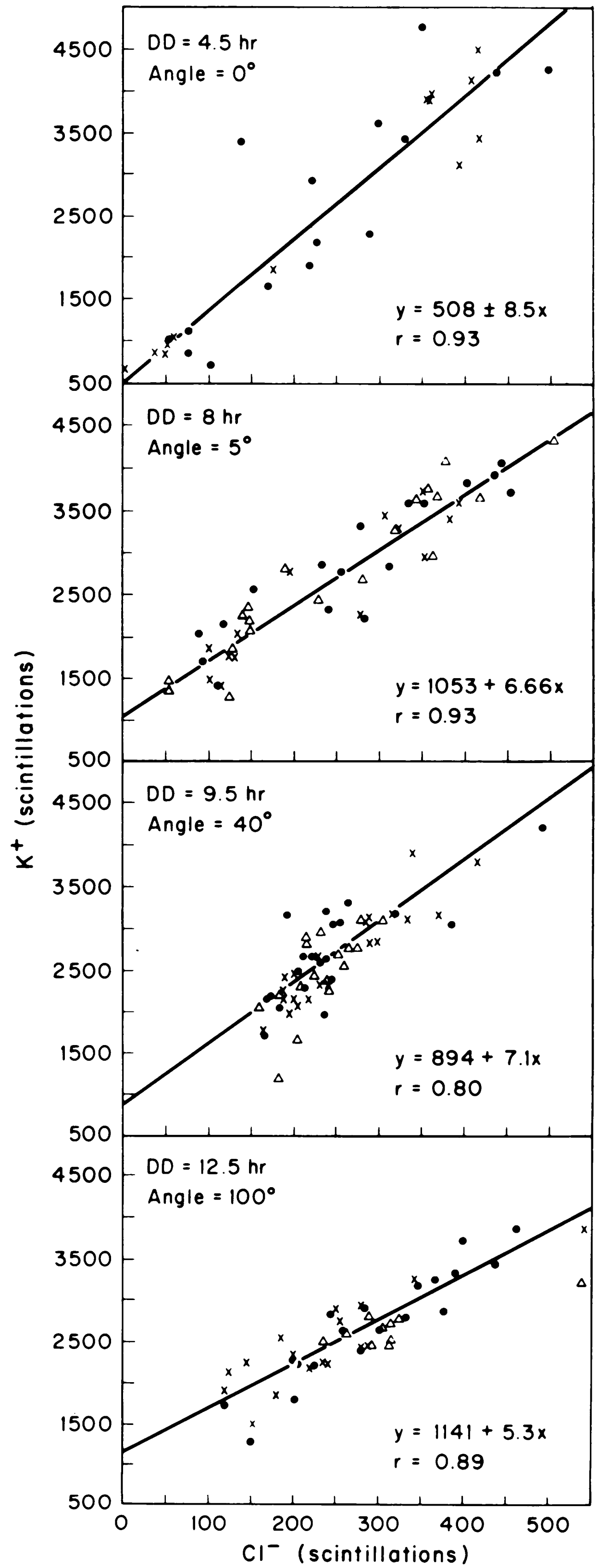


FIG. 3. Regression lines for the K/Cl correlation and the *r* values. The data are from Figure 2 and additional replicate experiments. The different symbols represent different experiments.

different standards, different preparation of the standards, and different tune. The technical data with which the value of 5.8 is obtained are noted in Figure 2.

A comparison of K^+ data with corrected Cl^- data indicates that not all of the K^+ is balanced by this anion. This is supported by regression analysis (Figs. 2 and 3). Corrected Cl^- values are only 50 to 60% as high as K^+ values, although Cl^- balances 65 to 85% of the mobile fraction of K^+, the latter being the difference in K^+ values in a given region of closed and opened pulvini. Since our methods did not permit us to distinguish between intra- and extracellular ions, we could not ascertain whether the large, apparently immobile K^+ fraction is inside the cells or in the free space (cell walls and intercellular space). At least part of it might represent fixed K^+ charges in the cell wall (4).

Both K^+ and Cl^- seem to migrate around the outer edge of the motor tissue, since the peak of the ion concentration shifts slightly from a region near outward (flexor) toward inward (Fig. 2). Since the leaflets fold not only toward and away from each other but also move toward and away from the rachis, thus performing half a circumnutation, the strong correlation between ion content of the motor tissue and leaflet movement (18) is reinforced.

K^+ and Cl^- oscillations in the extensor and the flexor are not 180° out of phase with each other (Fig. 2); increase in the concentration of both ions in the extensor precedes their decrease in the flexor. This indicates that there is not a 1:1 shuttle of ions between extensor and flexor, and there must be a storage region for these ions. The present data, together with those obtained with *Samanea* (17) support the view that the inner cortex acts as a reservoir for Cl^- as well as K^+.

Cl^- fluxes, although in highly varying amounts, have also been detected during stomatal movements in *Vicia faba* (5) and *Zea mays* (12). Whereas Cl^- balances only 5% of the K^+ in *Vicia faba* (5), it balances about 50% of the K^+ in *Zea mays* (12). Another similarity is that the K:Cl ratio varies from cell to cell in *Zea*; although we did not measure single cells, the K:Cl ratio in the examined spots ranged from 1.4 to 2.5 in our experiments. To account for the remainder of the ionic balance, changes in malate (1) and H^+ (13) have been noted, and changes in other organic anions such as citrate (5) and aspartate (24) have been suggested. We intend to study whether these ions are involved in *Albizzia* as well.

The involvement of Cl^- fluxes adds another element to the mosaic of the leaflet movement puzzle in *Albizzia*. We now know that Cl^- and K^+ fluxes are correlated with and probably control the size of pulvinal cells in *Albizzia*. Alteration in cell size and shape in flexor and extensor (about 180° out of phase with each other) are the basis for the oscillations in leaflet angle (15-18). These oscillations are controlled by light (15), temperature (14), externally applied electrolytes (14), metabolic inhibitors (16), and plant hormones (19) in such a way as to suggest rhythmic oscillation between a dominant active phase, requiring metabolic energy (during opening), and an inactive phase (during closure). It is logical to equate the active phase with ion pumping and the inactive phase with ion leakage through channels (16), thus implying apoplastic transport. The participation of transmembrane flux was supported by rhythmic changes in membrane potential that were strongly correlated with leaflet oscillations in *Samanea* (10).

The demonstrated participation of Cl^- in this system causes us to reevaluate our previous model, since the membranes of some plants are known to be efficient barriers for Cl^- (8) and this ion has been detected in the plasmodesmata of several plants (23, 25). There are numerous plasmodesmata in the stomatal complexes of *Vicia* and *Nicotiana* (9) as well as in the pulvini of *Samanea* and *Albizzia* (in preparation). Thus, Cl^- involvement suggests possible migration of ions through the symplast rather than the apoplast; this has the advantage of permitting more rapid fluxes (7), but raises perplexing questions about the nature of the active mechanisms. Resolution of this problem will require intracellular localization of ion flux by radioautography or finer resolution with the microprobe.

LITERATURE CITED

1. Allaway, W. G. 1973. Accumulation of malate in guard cells of *Vicia faba* during stomatal opening. Planta 110: 63-70.
2. Allen, R. D. 1969. Mechanism of the seismonastic reaction in *Mimosa pudica*. Plant Physiol. 44: 1101-1107.
3. Anderson, C. A. 1967. An introduction to the electron probe microanalyzer and its application to biochemistry. Methods Biochem. Anal. 15: 147-270.
4. Dainty, J., A. B. Hope, and C. Denby. 1960. Ionic relations of cells of *Chara australis*. II. The indiffusible anions of the cell wall. Aust. J. Biol. Sci. 13: 267-276.
5. Humble, G. D. and K. Raschke. 1971. Stomatal opening quantitatively related to potassium transport. Evidence from electron microprobe analysis. Plant Physiol. 48: 447-453.
6. Jaffe, M. J. and A. W. Galston. 1967. Phytochrome control of rapid nyctinastic movements and membrane permeability in *Albizzia julibrissin*. Planta 77: 135-141.
7. Lüttge, U. 1969. Aktiver Transport (Kurzstreckentransport bei Pflanzen). Protoplasmatologia VIII/7b.
8. McRobbie, E. A. C. 1962. Ionic relations of *Nitella translucens*. J. Gen. Physiol. 45: 861-878.
9. Pallas, J. E. and H. H. Mollenhauer. 1972. Electron microscopic evidence for plasmodesmata in dicotyledonous guard cells. Science 175: 1275-1276.
10. Racusen, R. H. and R. L. Satter. 1975. Rhythmic and phytochrome-regulated changes in transmembrane potential in *Samanea* pulvini. Nature 255: 408-410.
11. Raschke, K. 1975. Stomatal action. Annu. Rev. Plant Physiol. 26: 309-340.
12. Raschke, K. and M. P. Fellows. 1971. Stomatal movement in *Zea mays*: Shuttle of potassium and chloride between guard cells and subsidiary cells. Planta 101: 296-316.
13. Raschke, K. and G. D. Humble. 1973. No uptake of anions required by opening stomata of *Vicia faba*: guard cells release hydrogen ions. Planta 115: 47-57.
14. Satter, R. L., P. B. Applewhite, D. J. Kreis, Jr., and A. W. Galston. 1973. Rhythmic leaflet movement in *Albizzia julibrissin*. Effect of electrolytes and temperature alteration. Plant Physiol. 52: 202-207.
15. Satter, R. L. and A. W. Galston. 1971. Potassium flux: a common feature of *Albizzia* leaflet movement controlled by phytochrome or endogenous rhythm. Science 174: 518-519.
16. Satter, R. L. and A. W. Galston. 1973. Leaf movements: Rosetta stone of plant behavior? Bioscience 23: 407-416.
17. Satter, R. L., G. T. Geballe, P. B. Applewhite, and A. W. Galston. 1974. Potassium flux and leaf movement in *Samanea saman*. I. Rhythmic movement. J. Gen. Physiol. 64: 413-430.
18. Satter, R. L., P. Marinoff, and A. W. Galston. 1970. Phytochrome-controlled nyctinasty in *Albizzia julibrissin*. II. Potassium flux as a basis for leaflet movement. Am J. Bot. 57: 916-926.
19. Satter, R. L., P. Marinoff, and A. W. Galston. 1972. Phytochrome-controlled nyctinasty in *Albizzia julibrissin*. IV. Auxin effects on leaflet movement and K^+ flux. Plant Physiol. 50: 235-241.
20. Sawhney, B. L. and I. Zelitch. 1969. Direct determination of potassium ion accumulation in guard cells in relation to stomatal opening in light. Plant Physiol. 44: 1350-1354.
21. Schrempf, M. A. 1975. Eigenschaften und Lokalisation des Photorezeptors für phasenverschiebendes Störlicht bei der Blütenblattbewegung von *Kalanchoë blossfeldiana*. Ph.D. thesis. University of Tübingen, Germany.
22. Toriyama, H. 1955. Observational and experimental studies of sensitive plants. VI. The migration of potassium in the primary pulvinus. Cytologia 20: 367-377.
23. Van Steveninck, R. F. M., A. R. F. Chenoweth, and M. E. Van Steveninck. 1972. Ultrastructural localization of ions. *In:* W. D. Anderson, ed., Ion Transport in Plants. Academic Press, New York. pp. 25-37.
24. Willmer, C. M. and P. Dittrich. 1974. Carbon dioxide fixation by epidermal and mesophyll tissues of *Tulipa* and *Commelina*. Planta 117: 123-132.
25. Ziegler, H. and U. Lüttge. 1966. Die Salzdrüsen von *Limonium vulgare*. I. Die Feinstruktur. Planta 70: 193-206.