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Effects of Lanthanum and Ethylenediaminetetraacetate on Leaf Movements of *Mimosa*[1]

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ABSTRACT

Lanthanum and ethylenediaminetetraacetate (EDTA) profoundly affect the rapid leaf movements of *Mimosa pudica* L. Lanthanum, which mimics calcium but does not penetrate the plasmalemma, inhibits the closing response but does not affect reopening. A low concentration of EDTA retards the reopening process while a higher EDTA concentration prevents the closing movement. There is evidence that the EDTA effects result from chelation of calcium ions rather than chelation of other cations. These results are discussed with regard to the role of calcium in leaf movements.

When the leaves of *Mimosa* are mechanically stimulated, the paired leaflets fold together (Fig. 1, A and B). This rapid response (seismonasty) requires less than 2 sec. After seismonastic closure the leaflets reopen within 30 min. These movements result from the bending of the pulvinules which join the leaflets to the rachilla. These bendings are caused by changes in the turgor of the motor cells of the pulvinule (1-3, 20). The closing response results from a loss of turgor by the adaxial (upper) cells relative to the turgor of the abaxial (lower) cells. Recovery requires a reversal of these turgor changes. The rapid leaf movements and turgor changes can be correlated with the redistribution of K^+ within the motor tissue and a prevalent hypothesis is that changes in the K^+ content of motor cells alter osmotic potential and therefore affect turgor pressure (3, 15, 16). The accumulation and loss of K^+ by motor cells also seem to account for the ability of the leaves of *Albizzia julibrissin* to close when placed in the dark (nyctinasty) and to reopen in the light (11, 12). The literature supports the concept that leaflet pairs open by an accumulation of K^+ by the adaxial motor cells and stay open by maintaining a high K^+ concentration (11, 15, 16, 18). Leaflet closure is coincident with the loss of K^+ by the adaxial cells which may result from an increase in the permeability of the plasmalemma (3). The response and recovery by the motor cells of pulvinules may be based on changes in the properties of their plasma membranes relative to ion transport. Since Ca^{2+} is known to affect monovalent cation transport (6, 7), we investigated the possibility that Ca^{2+} plays a role in regulating the turgor of *Mimosa's* motor cells by studying the effects on leaf movements of lanthanum, which substitutes for Ca^{2+}, and EDTA, which removes Ca^{2+} by chelation.

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MATERIALS AND METHODS

Mimosa pudica L. plants were grown from cuttings in a University of California, Riverside greenhouse. Prior to use, plants were allowed to adjust to the laboratory environment for 48 hr. This "adaptation" period was indispensable; plants tested within the first several hr in the laboratory recovered from stimuli very slowly and the leaflet pairs did not completely reopen.

Laboratory plants were illuminated with incandescent lamps for 15 hr/day and all experiments were performed within the first 5 hr of this illumination period. The effects of various test solutions were examined in two ways. In some experiments excised leaves had their petioles placed in vials containing distilled H_2O (controls) or appropriate experimental solutions (Fig. 1A). In most of the experiments excised leaflet pairs were floated, adaxial surface up, on solutions in "spot plates" (Fig. 1B). For both types of experiments the samples were illuminated with incandescent lamps (6,500 lux) which were separated from the samples by heat shields. Both the whole leaves and the excised leaflet pairs could be stimulated with a dissecting probe. The angles between paired leaflets were measured before and after stimulation to the closest 10 degrees with a protractor. Each "point" in the graphs (Figs. 2-7) represents an average leaflet angle for four experiments. In each experiment, three leaves or three excised leaflet pairs received each treatment.

Preliminary experiments indicated that distilled H_2O and quarter-strength Hoagland solution produced identical results and distilled H_2O was therefore chosen as the most appropriate control. Lanthanum chloride solutions (lanthanum nitrate yielded the same results) were adjusted to pH 6.5 with NaOH. Solutions of EDTA were adjusted to pH 7 with NaOH. All EDTA "wash-out" solutions were also adjusted to pH 7. The concentrations of the experimental solutions and the length of treatment time are given under "Results."

For a description of the technique for the ultrastructural localization of lanthanum see Thomson *et al.* (14) and Campbell *et al.* (4).

RESULTS

Effects of Lanthanum. Figure 2 compares the extent of closure after stimulation for leaflet pairs floated for various times on distilled H_2O (control), 10 mM $LaCl_3$, and 20 mM $LaCl_3$. Both 10 mM and 20 mM La^{3+} eventually caused total inhibition of seismonastic (rapid) closure of the leaflets but the higher La^{3+} concentration required less treatment time for total inhibition. Complete inhibition of the closing response was also attainable with 1 mM $LaCl_3$ but a long treatment time (8-10 hr) was necessary.

To test the effect of 10 mM La^{3+} on the reopening (recovery) process leaflet pairs were stimulated, floated on test solutions,

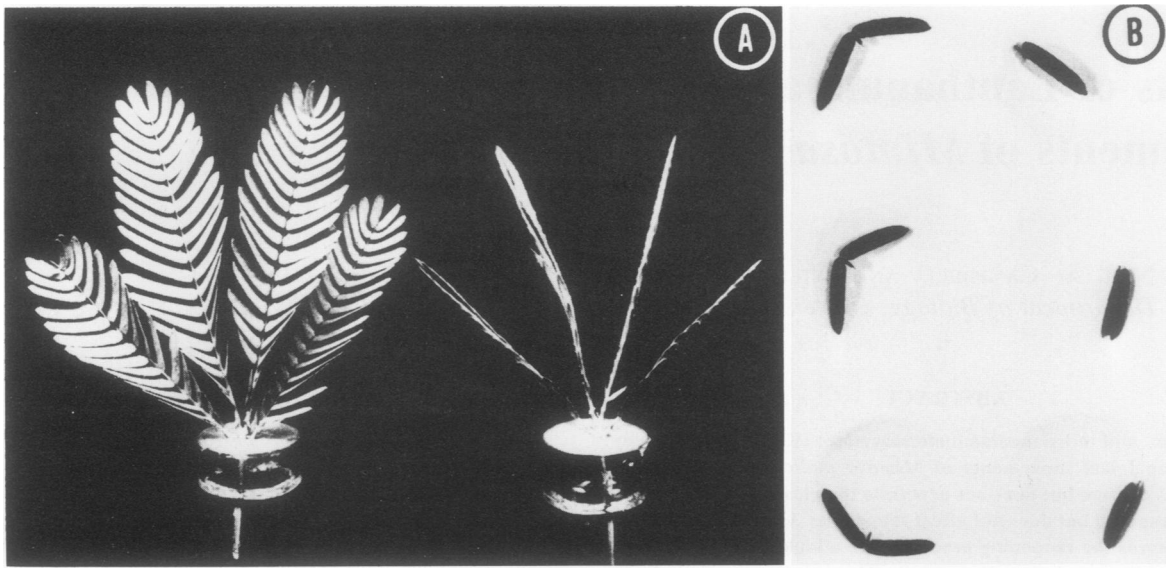

FIG. 1. A: Excised leaves in the open/unstimulated (left) and closed/stimulated (right) states; B: excised leaflet pairs in the open (left) and closed (right) states.

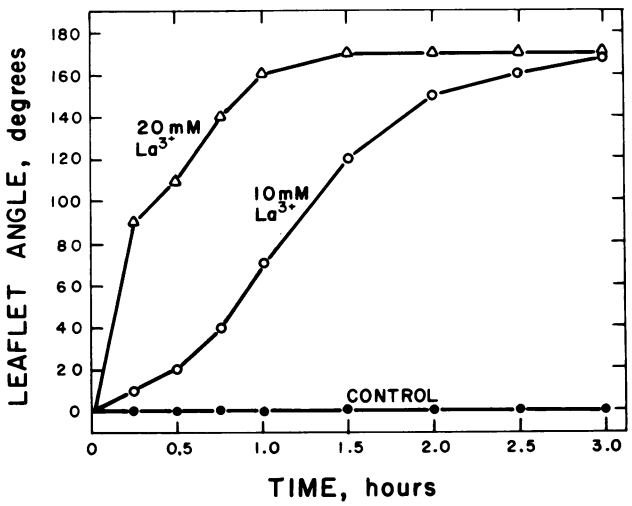

FIG. 2. Angles between paired leaflets measured immediately after mechanical stimulation. Leaflets were floated for various lengths of time on distilled H_2O (control), 10 mM $LaCl_3$, or 20 mM $LaCl_3$.

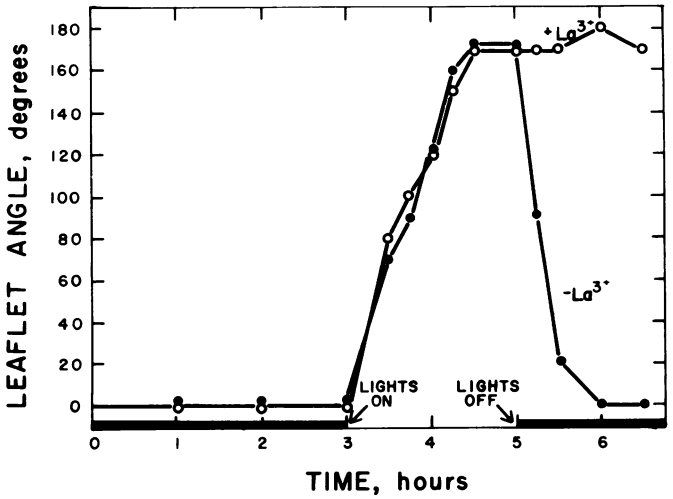

FIG. 3. Comparison of ability of leaflet pairs to reopen when floated on either distilled H_2O ($-La^{3+}$) or 10 mM $LaCl_3$ ($+La^{3+}$) and to close when placed in the dark. The experiment began by the samples being placed in the dark, in the closed state, for 3 hr.

and placed in the dark in the closed state. Neither controls nor La^{3+}-treated experimentals could reopen in the dark. After 3 hr in the dark the samples were transferred to light. Lanthanum-treated samples reopened in the light at the same rate as controls (Fig. 3). However, as soon as the La^{3+}-treated leaflets had reopened, they exhibited the usual La^{3+}-promoted inhibition of seismonastic closure. When the open leaflets were transferred back to the dark, the La^{3+}-treated samples did not close while the controls closed within 30 min (Fig. 3). These observations indicated that La^{3+} inhibited both seismonastic and nyctinastic (dark-promoted) closure but did not affect the reopening process.

Neither $CaCl_2$ nor $MgCl_2$ could overcome the inhibition of closure by lanthanum. Leaflet pairs floated on 10 mM $LaCl_3$ plus either 100 mM $CaCl_2$ or 100 mM $MgCl_2$ were inhibited to the same degree as samples floated on 10 mM $LaCl_3$ alone. Also, after inhibition of closure by lanthanum, the transfer of the samples to distilled H_2O, 100 mM $CaCl_2$, or 100 mM $MgCl_2$ could not restore seismonastic responsiveness.

Effects of EDTA. Treatment of pulvinules with EDTA resulted in two distinct, and apparently opposite, effects. Low concentrations of EDTA (1-10 mM) retarded the reopening process of stimulated (closed) leafet pairs (Fig. 4). This effect was apparent within 15 min of treatment time. The inhibition of the reopening process by EDTA could be rapidly reversed by transferring the EDTA-treated samples to distilled H_2O; the leaflet pairs were able to reopen at a normal rate after 20 min of distilled H_2O treatment. $CaCl_2$ or $MgCl_2$ added to the EDTA wash-out solution did not restore normal reopening any more effectively than did distilled H_2O. However, when samples were floated on 5 mM EDTA and 5 mM $CaCl_2$ simultaneously, no EDTA effect was apparent; these samples reopened as rapidly as controls. Addition of $MgCl_2$ to the EDTA solutions did not prevent EDTA inhibition.

The inhibition of reopening occurred after shorter treatment time with higher (15-25 mM) EDTA concentrations, but after about 1 hr of treatment the leaflet pairs floated on high

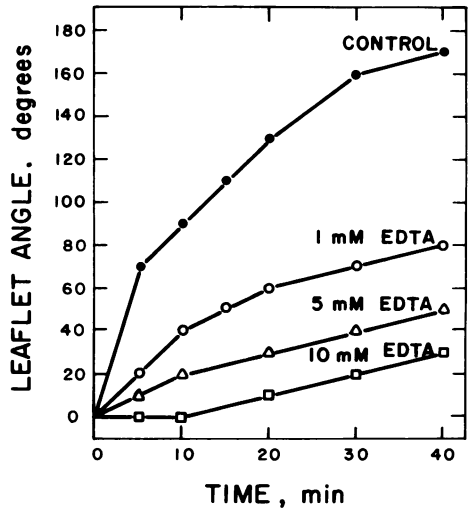

FIG. 4. Rate of reopening of leaflet pairs floated on distilled H_2O (control) or various concentrations of EDTA. Samples were stimulated after 5 min of pretreatment on appropriate solutions.

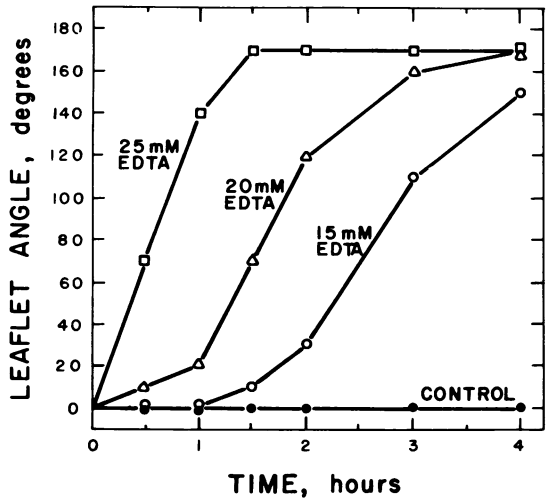

FIG. 5. Ability of leaflet pairs floated on distilled H_2O (control) or EDTA to close after stimulation.

concentrations of EDTA displayed a second effect which was not seen in the samples treated with lower concentrations. For leaflet pairs floated on high concentration of EDTA for more than 1 hr, both seismonastic and nyctinastic closure were inhibited (Fig. 5). These samples remained open after mechanical stimulation and after being placed in the dark. Treatment with a high concentration of EDTA apparently "locked" pulvinules in the open (unstimulated) state.

Not only did 25 mM EDTA prevent leaflets from closing when they were placed in the dark, but high concentrations of EDTA also caused samples to reopen in the dark when placed there in a closed state (Fig. 6).

The inhibition of the closing response by 25 mM EDTA could be completely and rapidly reversed by transferring leaflet pairs from the EDTA solutions to 25 mM $CaCl_2$ (Fig. 7). Transferring EDTA-inhibited samples to distilled H_2O or 25 mM $MgCl_2$ did not restore the closing response.

DISCUSSION

The literature supports the view that the leaf movements of *Mimosa* and other plants result from turgor changes in the pulvini (pulvinule in the case of leaflets) and some authors have proposed that these turgor changes are associated with the observed redistribution of potassium within the motor tissue (3, 11, 12, 15, 16). The "contraction" of the adaxial cells of the pulvinule is concomitant with a loss of potassium and Allen (3) has demonstrated that the motor cell become more permeable to K^+ upon stimulation. Also, Toriyama and Jaffe (18) have reported that seismonastic movement of the primary pulvinus of *Mimosa* is accompanied by a redistribution of Ca^{2+} and suggested that Ca^{2+} has a direct role in the response and recovery of the pulvinus. Further, experiments with lanthanum indicate that it apparently replaces and mimics the role of calcium relative to ion transport by the plasmalemma (7, 8, 13, 21). We suggest that membrane-bound calcium may play a role in the ability of the motor cells of *Mimosa* to maintain solute gradients (probably K^+) during the open leaflet state and with the release of calcium from the plasmalemma these gradients are extinguished and closure occurs. Studies of a wide variety of

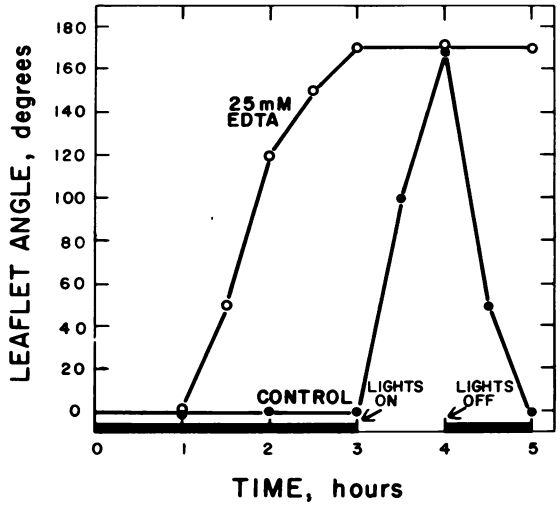

FIG. 6. Comparison of leaflet angles for controls and EDTA-treated samples. Samples were in the dark for 3 hr, transferred to light for 1 hr, and then returned to the dark.

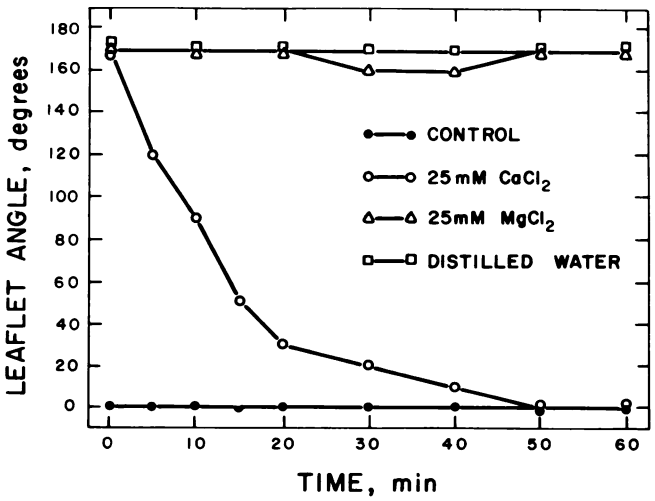

FIG. 7. Extent of leaflet closure attainable after stimulation when 25 mM EDTA-inhibited leaflet pairs are transferred to distilled H_2O, 25 mM $CaCl_2$, or 25 mM $MgCl_2$. Zero time corresponds to time of transfer. Controls were transferred from distilled H_2O to "new" solutions of distilled H_2O.

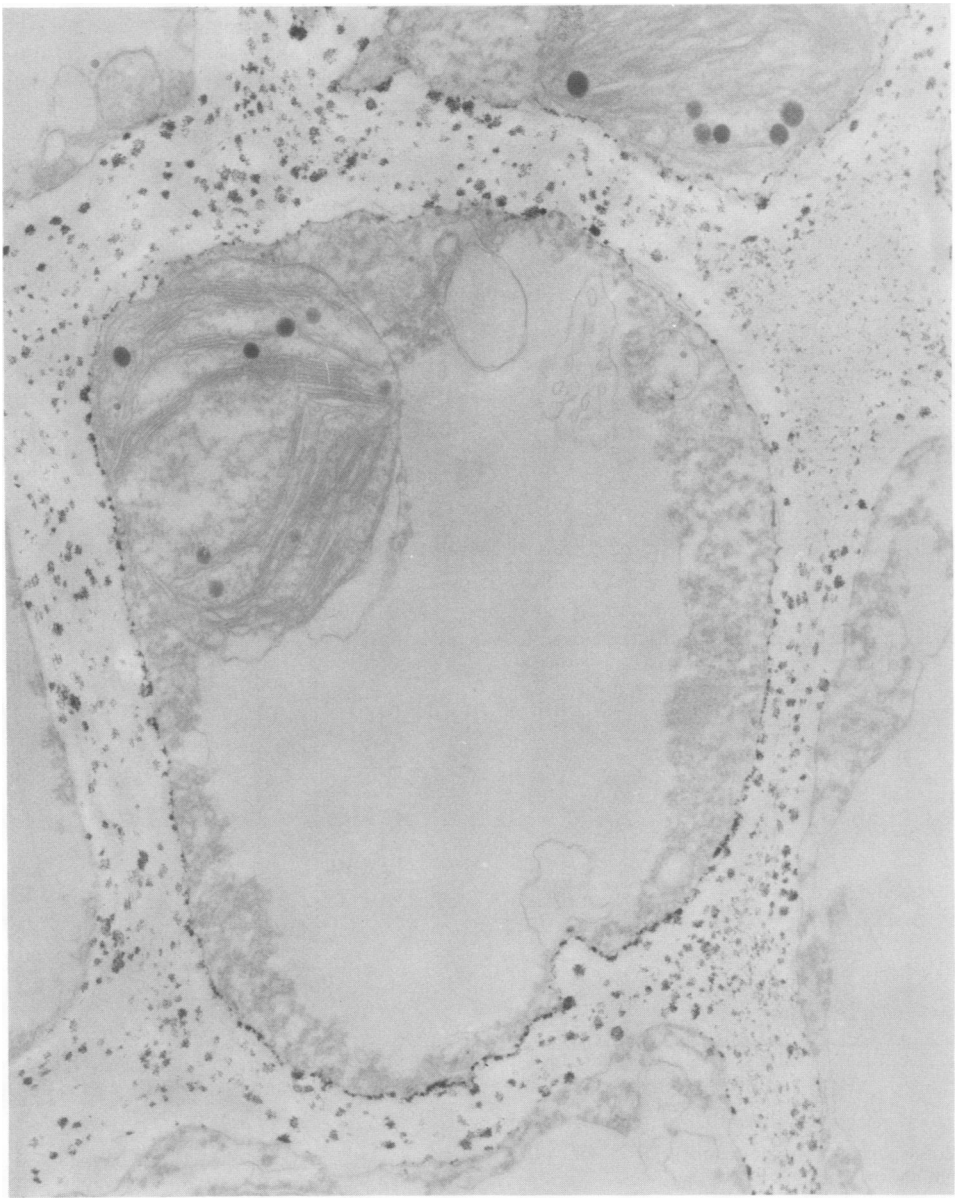

FIG. 8. Electron micrograph of a lanthanum-treated motor cell of a pulvinule. Note electron-dense lanthanum deposits in the wall and on the outer layer of the plasmalemma. Such deposits were not found within the motor cells and were totally absent in control samples untreated with lanthanum (× 28,000).

cells indicate that lanthanum does not pass the plasmalemma (4, 7, 10, 14) and electron micrographs of lanthanum-treated pulvinules of *Mimosa* confirm the inability of La^{3+} to penetrate the plasmalemma of motor cells (Fig. 8). Further, there is evidence that La^{3+} affects monovalent cation transport in both animal and plant cells by occupying Ca^{2+}-binding sites on the plasmalemma (7, 13). Unlike Ca^{2+}, lanthanum appears to bind irreversibly to the outer surface of the plasmalemma (13).

The effects of calcium on ion transport are complex, but one observation is that Ca^{2+} inhibits passive K^+ transport by rendering the membrane less permeable (19). Thus, in certain situations, Ca^{2+} may stabilize K^+ gradients by slowing passive "backflow." Since lanthanum inhibited both seismonastic and nyctinastic closure of the leaflets, but had no effect on the reopening process, we suggest that lanthanum mimics the role of calcium in the accumulation of solutes by the motor cells which is apparently required for the increase in turgor and opening. However, closure may be inhibited because lanthanum is not released from the membrane, as calcium would be, and the decrease in permeability resulting in the loss of solutes and turgor by the adaxial motor cells does not occur. It is also possible that by inhibiting passive ion fluxes, La^{3+} blocks action potentials which may be necessary for transmission of stimuli.

The observation that low concentrations of EDTA retard reopening of leaflets is compatible with our suggestion that Ca^{2+} is necessary for the maintenance of ion gradients. The removal of calcium from the membrane by EDTA may result in the increased permeability or "leakiness" of the membrane and the prevention of a net accumulation of ions by the motor cells. The observation that EDTA does not retard leaflet opening when added with an equivalent concentration of calcium indicates that the EDTA effect is indeed due to chelation of Ca.

The observation that a high concentration of EDTA prevents leaflet closure corroborates similar results reported by Driessche

(5) for *Mimosa* and by McEvoy and Koukari (9) for nyctinastic closure of *Albizzia* leaves. One explanation for the dual effect of EDTA would be that membrane-bound Ca^{2+} is more easily removed than Ca^{2+} in other locations. An excess of EDTA, in addition to extracting Ca^{2+} from the membrane, may also remove significant amounts of calcium from an additional site where the calcium is required for the promotion and/or maintenance of the closed state. Toriyama (17) has suggested that Ca^{2+} could promote seismonastic response by causing changes in the colloidal state of the motor cell cytoplasm. Driessche (5) and Toriyama and Jaffe (18) advanced the idea that Ca^{2+} could activate the response of pulvini by causing the contraction of protein fibers in the motor cells. In addition to the proposed intracellular sites of Ca^{2+}-promoted leaflet closure, it is also possible that cell wall-associated Ca^{2+} is necessary for the response of pulvini. Weintraub (20) and Aimi (1, 2) have shown that differential stresses between the two sides of pulvini due to differences in cell wall thickness are important in seismonastic response. The removal of Ca^{2+} from the cell wall may reduce wall stresses required for the closed leaf state. Whatever the histological location of the EDTA-promoted inhibition of leaflet closure, the rapid restoration of normal closure attainable by transferring the samples to $CaCl_2$ suggests that EDTA is removing Ca^{2+} from a readily accessible site and is causing easily reversible rather than profoundly disruptive effects.

Further experiments may help to rationalize the opposite effects of EDTA but presently we favor the view that Ca^{2+} plays a dual role in leaf movements. The prevention of leaflet closure by lanthanum and the inhibition of reopening by EDTA suggest that Ca^{2+}, probably membrane-bound, is necessary for establishment and maintenance of ion gradients. The locking of leaflets in the open state by a high concentration of EDTA indicates that calcium is also required in motor tissue for the closure of leaflets and for the maintenance of the closed state.

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