Alexander G. Volkov *Editor*

Plant Electrophysiology

Signaling and Responses

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Preface

Plant electrophysiology is the study of the electrochemical phenomena associated with biological cells and tissues in plants. It involves measurements of electrical potentials and currents on a wide variety of scales from single ion channels to whole plant tissues. Electrical properties of plant cells mostly derive from the electrochemical properties of their membranes. Electrophysiological study of plants includes measurements of the electrical activity of the phloem, xylem, plasmodesmata, stomata, and particularly the electrical signal's propagation along the plasma membrane. Action potentials are characteristic responses of excitation that can be induced by stimuli such as: applied pressure, chemical substances, thermal stimuli, electrical or magnetic stimuli, and mechanical stimuli.

There are two major divisions of electrophysiology: intracellular recording and extracellular recording.

The electrical phenomena in plants have attracted researchers since the eighteenth century and have been discussed in a variety of books (Baluška et al. 2006; Bertholon 1783; Bose 1907, 1913, 1918, 1926, 1928; Lemström 1902; Ksenzhek and Volkov 1998, 2006; Volta 1816). The identification and characterization of bioelectrochemical mechanisms for electrical signal transduction in plants would mark a significant step forward in understanding this underexplored area of plant physiology. Although plant mechanical and chemical sensing and corresponding responses are well known, membrane electrical potential changes in plant cells and the possible involvement of electrophysiology in transduction mediation of these sense-response patterns represents a new dimension of plant tissue and whole organism integrative communication. Plants continually gather information about their environment. Environmental changes elicit various biological responses. The cells, tissues, and organs of plants possess the ability to become excited under the influence of certain environmental factors. Plants synchronize their normal biological functions with their responses to the environment. The synchronization of internal functions, based on external events, is linked with the phenomenon of excitability in plant cells. The conduction of bioelectrochemical excitation is a fundamental property of living organisms.

Electrical impulses may arise as a result of stimulation. Once initiated, these impulses can propagate to adjacent excitable cells. The change in transmembrane potential can create a wave of depolarization which can affect the adjoining resting membrane. Action potentials in higher plants are the information carriers in intracellular and intercellular communication during environmental changes.

The conduction of bioelectrochemical excitation is a rapid method of long distance signal transmission between plant tissues and organs. Plants promptly respond to changes in luminous intensity, osmotic pressure, temperature, cutting, mechanical stimulation, water availability, wounding, and chemical compounds such as herbicides, plant growth stimulants, salts, and water potential. Once initiated, electrical impulses can propagate to adjacent excitable cells. The bioelectrochemical system in plants not only regulates stress responses, but photosynthetic processes as well. The generation of electrical gradients is a fundamental aspect of signal transduction.

The first volume entitled "Plant Electrophysiology—Methods and Cell Electrophysiology" consists of a historical introduction to plant electrophysiology and two parts. The first part introduces the different methods in plant electrophysiology. The chapters present methods of measuring the membrane potentials, ion fluxes, trans-membrane ion gradients, ion-selective microelectrode measurements, patch-clamp technique, multi-electrode array, electrochemical impedance spectroscopy, data acquisition, and electrostimulation methods. The second part deals with plant cell electrophysiology. It includes chapters on pH banding in Characean cells, effects of membrane excitation and cytoplasmic streaming on photosynthesis in Chara, functional characterization of plant ion channels, and mechanism of passive permeation of ions and molecules through plant membranes.

The second volume entitled "Plant Electrophysiology—Signaling and Responses" presents experimental results and theoretical interpretation of whole plant electrophysiology. The first three chapters describe electrophysiology of the Venus flytrap, including mechanisms of the trap closing and opening, morphing structures, and the effects of electrical signal transduction on photosynthesis and respiration. The Venus flytrap is a marvelous plant that has intrigued scientists since the times of Charles Darwin. This carnivorous plant is capable of very fast movements to catch insects. The mechanism of this movement has been debated for a long time. The Chap. 4 describes the electrophysiology of the Telegraph plant. The role of ion channels in plant nyctinastic movement is discussed in Chap. 5. Electrophysiology of plant–insect interactions can be found in Chap. 6. Plants can sense mechanical, electrical, and electromagnetic stimuli, gravity, temperature, direction of light, insect attack, chemicals and pollutants, pathogens, water balance, etc. Chapter 7 shows how plants sense different environmental stresses and stimuli and how phytoactuators response to them. This field has both theoretical and practical significance because these phytosensors and phytoactuators employ new principles of stimuli reception and signal transduction and play a very important role in the life of plants. Chapters 8 and 9 analyze generation and transmission of electrical signals in plants. Chapter 10 explores bioelectrochemical aspects of the plant-lunisolar gravitational relationship. Authors of Chap. 11

describe the higher plant as a hydraulic-electrochemical signal transducer. Chapter 12 discusses properties of auxin-secreting plant synapses. The coordination of cellular physiology, organ development, life cycle phases and symbiotic interaction, as well as the triggering of a response to changes is the environment in plants depends on the exchange of molecules that function as messengers. Chapter 13 presents an overview of the coupling between ligands binding to a receptor protein and subsequent ion flux changes. Chapter 14 summarizes data on physiological techniques and basic concepts for investigation of Ca^{2+}-permeable cation channels in plant root cells.

All chapters are comprehensively referenced throughout.

Green plants are a unique canvas for studying signal transduction. Plant electrophysiology is the foundation of discovering and improving biosensors for monitoring the environment; detecting effects of pollutants, pesticides, and defoliants; monitoring climate changes; plant-insect interactions; agriculture; and directing and fast controlling of conditions influencing the harvest.

We thank the authors for the time they spent on this project and for teaching us about their work. I would like to thank our Acquisition Editor, Dr. Cristina Eckey, and our Production Editor, Dr. Ursula Gramm, for their friendly and courteous assistance.

Prof. Alexander George Volkov Ph.D.

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Chapter 1
Morphing Structures in the Venus Flytrap

Vladislav S. Markin and Alexander G. Volkov

Abstract Venus flytrap is a marvelous plant that intrigued scientists since times of Charles Darwin. This carnivorous plant is capable of very fast movements to catch insects. Mechanism of this movement was debated for a long time. Here, the most recent Hydroelastic Curvature Model is presented. In this model the upper leaf of the Venus flytrap is visualized as a thin, weakly curved elastic shell with principal natural curvatures that depend on the hydrostatic state of the two surface layers of cell, where different hydrostatic pressures are maintained. Unequal expansion of individual layers A and B results in bending of the leaf, and it was described in terms of bending elasticity. The external triggers, either mechanical or electrical, result in the opening of pores connecting these layers; water then rushes from the upper layer to the lower layer, and the bilayer couple quickly changes its curvature from convex to concave and the trap closes. Equations describing this movement were derived and verified with experimental data. The whole hunting cycle from catching the fly through tightening, through digestion, and through reopening the trap was described.

1.1 Introduction

All biological organisms continuously change their shapes both in the animal kingdom and in plant kingdom. These changes include the internal properties of plants. Among them there are interesting examples that are able to morph extremely

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Fig. 1.1 Venus flytrap in open and closed states

fast. They not only adjust to the changing environment but they also receive signals from the external world, process those signals, and react accordingly. The world "morphing" is defined as efficient, multipoint adaptability and may include macro, micro, structural, and/or fluidic approaches (McGowan et al. 2002).

Some carnivorous plants are able to attack their preys. The most famous of these is the Venus flytrap (*Dionaea muscipula* Ellis). This is a sensitive plant whose leaves have miniature antennae or sensing hairs that are able to receive, process, and transfer information about an insect's stimuli (Fig. 1.1). Touching trigger hairs, protruding from the upper leaf epidermis of the Venus flytrap, activates mechano-sensitive ion channels, and generates receptor potentials (Jacobson 1974; Volkov et al. 2008a), which can induce action potentials (Burdon-Sanderson J. 1873; Volkov et al. 2007; Sibaoka 1969; Hodick and Sievers 1988, 1989; Stuhlman and Darden 1950). It was found that two action potentials are required to trigger the trap closing (Brown 1916).

The history of studying the Venus flytrap spans more than a century. Although the sequence of actions is clearly described in the existing literature, the exact mechanism of the trap closure is still poorly understood. Indeed, quite a bit is known about how the flytrap closes: stimulating the trigger hair twice within 40s unleashes two action potentials triggering curvature changes, which helps the plant rapidly close its upper leaf. When trigger hairs in the open trap receive mechanical stimuli, a receptor potential is generated (Benolken and Jacobson 1970; DiPalma et al. 1966). Two mechanical stimuli are required for closing the trap in vivo (Darvin 1875; Lloid 1942). However, at high temperatures (36–40°C) only one stimulus is required for trap closure (Lloyd 1942). Receptor potentials generate action potentials (Jacobson 1974; Volkov et al. 2008a; Burdon-Sanderson J. 1873; Volkov et al. 2007a; Jacobson 1965), which can propagate in the plasmodesmata of the plant to the midrib (Volkov et al. 2007). Uncouplers and blockers of fast anion and potassium channels can inhibit action potential propagation in the Venus flytrap (Volkov et al. 2008c;

Volkov et al. 2007; Hodick and Sievers 1988; Krol et al. 2006). The trap accumulates the electrical charge delivered by an action potential. Once a threshold value of the charge is accumulated, ATP hydrolysis (Jaffe 1973) and fast proton transport starts (Rea 1983, 1984; Williams and Bennet 1982), and aquaporin opening is initiated (Volkov et al. 2008a, 2011). Fast proton transport induces transport of water and a change in turgor (Hodick and Sievers 1989).

A number of contradictory models were proposed (Bobji 2005; Brown 1916; Darwin 1875; Forterre et al. 2005; Hill and Findley 1981; Hodick and Sievers 1989; Jacobson 1974; Nelson and Cox 2005; Williams and Bennet 1982; Yang et al. 2010), and still there is no agreement between the researchers (Hodick and Sievers 1989). Recently, the focus of interest returned to the original ideas proposed by Darwin in the nineteenth century. In his seminal work, Darwin (1875) demonstrated that the basic catching movement of the Venus flytrap involves the transformation of the leaf curvature from convex to concave resulting in the closing of the trap. Darwin wrote: "We know that the lobes, whilst closing, become slightly incurved throughout their whole breadth. This movement appears to be due to the contraction of the superficial layers of cells over the whole upper surface. In order to observe their contraction, a narrow strip was cut out of one lobe at right angles to the midrib, so that the surface of the opposite lobe could be seen in this part when the leaf was shut. After the leaf had recovered from the operation and had re-expanded, three minute black dots were made on the surface opposite to the slit or window, in a line at right angles to the midrib. The distance between the dots was found to be 40/1000 of an inch, so that the two extreme dots were 80/1000 of an inch apart. One of the filaments was now touched and the leaf closed. On again measuring the distances between the dots, the two next to the midrib were nearer together by 1–2/1000 of an inch, and the two further dots by 3–4/1000 of an inch, than they were before; so that the two extreme dots now stood about 5/1000 of an inch (0.127 mm) nearer together than before. If we suppose the whole upper surface of the lobe, which was 400/1000 of an inch in breadth, to have contracted in the same proportion, the total contraction will have amounted to about 25/1000 or 1/40 of an inch (0.635 mm)."

Darwin established that the upper leaf of the Venus flytrap includes two distinct layers of cells at upper and lower surfaces that behave quite differently in the process of trap closure. The finding of these two independent layers was later confirmed by many authors and their role was related to the turgor pressure (Fagerberg and Allain 1991; Fagerberg and Howe 1996; Mozingo et al. 1970). It is well known that some functions in plants and fungi can only be driven by exploiting hydrodynamic flow, such as stomata guard cell opening and closing, leaf pulvini motor organ, mechanical traps of carnivorous plants, and fungal appressorial penetration (Beilby et al. 2006; Shimmen 2006; Zonia and Munnik 2007).

It is common knowledge that the leaves of the Venus flytrap actively employ turgor pressure and hydrodynamic flow for fast movement and catching insects. In these processes the upper and lower surfaces of the leaf behave quite differently. The loss of turgor by parenchyma, lying beneath the upper epidermis, accompanied by the active expansion of the tissues of the lower layers of parenchyma near the lower epidermis closes the trap (Brown 1916; Brown and Sharp 1910; Darwin 1875;

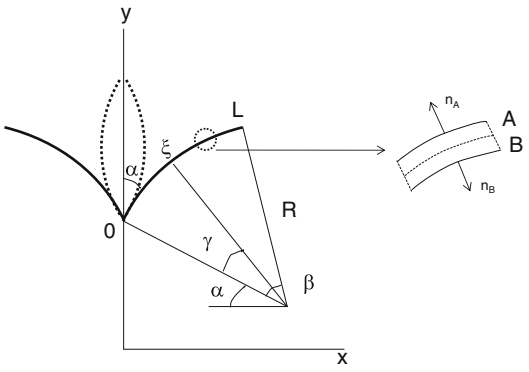

Fig. 1.2 Hydroelastic curvature model

De Candolle 1876; Lloyd 1942; Munk 1876). The cells on the inner face of the trap jettison their cargo of water, shrink, and allow the trap lobe to fold over. The cells of the lower epidermis expand rapidly, folding the trap lobes over (Brown 1916).

Recently, Forterre et al. (2005) reproduced the Darwin (1875) work at the modern technical level with high-speed video imaging and noninvasive microscopy techniques. Forterre et al. (2005) documented in minute details the change of the geometry of the leaf in two dimensions and brought up the idea that elastic energy might play an important role in the closing of the trap.

Recently it was found that the trap of the Venus flytrap can be also closed by electrical stimulation of a midrib (Volkov et al. 2007; Volkov et al. 2008a, b, c, 2009a, b).

1.2 Anatomy and Mechanics of the Trap

It is important to understand the mechanics of the trap closure. One could compare the leaf of this plant to the open book with a fly sitting on the page; the fly can be caught by swift shutting of the book. However, this comparison would be very wrong. In the "book model" there is a pivot at the midrib of the leaf and two flat parts of the book would rotate around this pivot and crush the poor fly. Actual closing of the trap occurs in a different way. The midrib is not a pivot.

The cross-section of the leaf is presented in Fig. 1.2. In the open state (cocked state) the lobes of the leaf have the convex shape (when looking from above). Angle α is the initial angle between the lobe and the vertical line at the midrib. The total angle between two lobes at the midrib is 2α. This angle does not change (at least does not change noticeably) in the process of trap closing (Fagerberg and Allain 1991). The lobes do not rotate around the midrib, but only change their curvature. As a result the distant parts of the leaf move in the space and approach each other—the trap closes. Every point ξ of the lobe moves with different velocity $v(\xi, t)$.

In this text we shall designate the width of the lobe by L. The initial radius of curvature is R, ξ is an arbitrary point along the leaf with corresponding angle γ. We measured a number of typical leafs of Venus flytraps to find the following averaged parameters: $\alpha = 34^{o} = 0.593$ rad, length $L = 2$ cm. These two parameters remain constant. The angle at the center of curvature is β. It changes in the process of closing; its initial value is $\beta_1 = 52^{o}$, initial radius of curvature is $R_1 = 2.2$ cm, or curvature $C_1 = 0.454\ \text{cm}^{-1}$. After closing angle β changes to $\beta_2 = -2\alpha = -1.186$ rad, $C_2 = -0.593\ \text{cm}^{-1}$.

1.3 The Hydroelastic Curvature Model of Venus Flytrap

As we previously mentioned, there is a number of models attempting to explain the fast morphing in this plant. In very detailed work, Forterre et al. (2005) reproduced the Darwin (1875) work with high-speed video imaging and documented, in minute details, the change of the geometry of the leaf in two dimensions and brought up the idea that elastic energy might play an important role in the closing of the trap. They described the kinematics of closing of the Venus flytrap as a relaxation of the leaf to the new equilibrium state after triggering. They wrote: "Upon stimulation, the plant 'actively' changes one of its principal natural curvatures, the microscopic mechanism for which remains poorly understood." So, the mechanism of active change of one of principal curvatures of the leaf remained beyond the scope of their work.

This issue was addressed by Markin et al. (2008) who tried to elucidate what causes the change of spontaneous curvature of the leaf. The accumulated data suggest that elastic energy does play an important role, but driving force behind this event involves another process that determines the transformation from an open to a closed state. Markin et al. (2008) developed the *Hydroelastic Curvature Model* that includes bending elasticity, turgor pressure, and water jets. The closure of the Venus flytrap represents the nonmuscular movement based on hydraulics and mechanical elasticity. The nastic movements in various plants involve a large internal pressure (turgor) actively regulated by plants.

In the *Hydroelastic Curvature Model* (Markin et al. 2008) the leaf of Venus flytrap is visualized as a thin, weakly curved elastic shell with principal natural curvatures that depend on the hydrostatic state of the two surface layers of cell A and B (Fig. 1.2), where different hydrostatic pressures P_A and P_B are maintained.

Two layers of cells, mechanically connected to each other, behave like a very popular in membrane mechanics bilayer couple where the inplane expansion or contraction of any of them causes the change of curvature of the whole leaf. The bilayer couple hypothesis was first introduced by Sheetz and Singer (1974). They noticed that the proteins and the phospholipids of membranes are asymmetrically distributed in the two halves of the bilayer, which is most substantial for the erythrocyte membrane.

The two halves of the closed membrane bilayer may respond differently to various perturbations while remaining coupled to one another. One half of the bilayer may expand in the plane of the membrane relative to the other half of the bilayer, while the two layers remain in contact with one another. This leads to various functional consequences, including shape changes of the intact cell. This concept is called the bilayer couple hypothesis because of the analogy to the response of a bimetallic couple to changes in temperature. It remains very popular and applied to explanation of numerous phenomena, such as red blood cell transformations (see for example Lim et al. (2002) and references within) and the gating of mechanosensitive channels (Qi et al. 2005).

The bilayer couple properties were also extensively studied in connection with bilayer fusion, fission, endo and exocytosis (Markin and Albanesi 2002; Volkov et al. 1998). This technique was applied for the design and analysis of the hydroelastic curvature model of the Venus flytrap. The model is based on the assumption that the driving force of closing is the elastic curvature energy stored and locked in the leaves due to pressure differential between the outer and inner layers of the leaf (Fig. 1.2).

Unequal expansion of individual layers A and B results in bending of the leaf, and it was described in terms of bending elasticity. Unequal expansion means that the torque M appears in the leaf. The energy of the bent layer A is described by the equation.

$$E_A = \frac{1}{2}\kappa_0(C_{AM} - C_{A0})^2 + \kappa_G C_{AG} \tag{1.1}$$

Here C_{AM} is the total curvature of the layer A, C_{AG} is the Gaussian curvature, C_{A0} is the spontaneous or intrinsic curvature of the layer, and κ designates the elasticity. Usually, spontaneous curvature of layers is considered a constant b_A, depending on the composition of the layer, and describes the intrinsic tendency of the layer to bend. There is an additional source of bending—different pressure in two adjacent layers. One can easily visualize the number of mechanical models in which spontaneous curvature is proportional to the pressure in which curvature is $C_{A0} = a_A P_A + b_A$. The same equations are valid for layer B.

The geometrical mean and Gaussian curvatures are defined as $C_{AM} = 1/R_1 + 1/R_2$ and $C_{AG} = 1/(R_1 R_2)$, where R_1 and R_2 are the main radii of curvature of the layer. The shape of the leaf was approximated by a spherical surface; then $C_{AM} = 2/R$ and $C_{AG} = C_{AM}^2/4$. The leaf is thin and hence the two layers have the mean curvatures that are equal in absolute value but have opposite signs: $C_{AM} = -C_{BM} = C_M$. The sign of curvature was defined with respect to the normal directed outside of the layer (Fig. 1.2). Total elastic energy of the lobe was presented as

$$E_L = \frac{1}{2}\kappa_0\left[(C_M - a\,P_A - b_A)^2 + (-C_M - a\,P_B - b_B)^2\right] + \frac{1}{2}\kappa_G C_M^2 \tag{1.2}$$

Here, the coefficients a_A and a_B are assumed to be equal to a.

At the given pressures P_A and P_B, the equilibrium value of the mean curvature can be found from the minimum value of elastic energy (1.2):

$$C_M = \frac{1}{2 + \kappa_0/\kappa_G}\left[a(P_A - P_B) + b_L\right] \tag{1.3}$$

Here b_L designates the difference between two intrinsic curvatures, $b_L = b_A - b_B$. This equilibrium shape is maintained if the pressure difference does not change.

In the open state, the pressure in the upper layer is higher than in the lower layer, maintaining the convex shape of the leaf. The fact, that the hydrostatic pressure in different parts of the plant can vary, is very well known. It is also known (Tamiya et al., 1988) that stimulation of a *Mimosa* plant causes very fast redistribution of water. Tamiya et al. (1988) found that after stimulation, water in the lower half of the main pulvinus is transferred to the upper half of the main pulvinus. Movement of the water in conjunction with *Mimosa* movement was visualized by a noninvasive NMR imaging procedure (Detmers et al. 2006). This fast water redistribution is obviously driven by the pressure difference between different parts of the plant, and exchange occurs through open pores. Unfortunately, the anatomy and the nature of these pores are not currently known. So, for the mechanical analysis their existence was simply accepted.

At the resting state water pores between the two hydraulic layers are closed. The external trigger, either mechanical or electrical, results in the opening of these connecting pores; water rushes from the upper to the lower layer, the bilayer couple quickly changes its curvature from convex to concave and the trap closes.

If the trigger reaches threshold value at the moment t_s and the characteristic time of the opening kinetics is τ_a then the open probability of the pores (after $t \geq t_s$) will be given by $n_{op}(t) = 1 - Exp\left[-(t - t_s)/\tau_a\right]$. The rate of fluid transfer can be presented as $J = n_{op}L_H(P_A - P_B)$, where L_H is the hydraulic coefficient of pore permeability. If the pressure in the layer is proportional to the amount of fluid confined in it, the pressure will change with a rate proportional to the fluid transfer between the layers: $dP_A/dt = -k_rJ = -dP_B/dt$. This means that the sum of the two pressures remains constant: $P_A + P_B = \text{const} = P_{\text{total}}$. Then the variation of pressure can be described by the equation

$$\frac{dP_A}{dt} = -k_r n_{op} L(P_A - P_B) = -\frac{n_{op}}{\tau_r}\left(P_A - \frac{1}{2}P_{\text{total}}\right) \tag{1.4}$$

Here the characteristic time of fluid transfer, $\tau_r = 1/(2k_rL_H)$, is introduced. A similar equation for the mean curvature can be easily obtained from Eqs. 1.3 and 1.4:

$$\frac{dC_M}{dt} = -\frac{n_{op}}{\tau_r}\left(C_M - \frac{b_L}{2 + \kappa_G/\kappa_0}\right) \tag{1.5}$$

Initial curvature C_1 in the open state can be introduced arbitrarily, while the final curvature C_2 in the closed state is found from Eq. 1.5 automatically: $C_2 = \frac{b_L}{2+\kappa_G/\kappa_0}$.

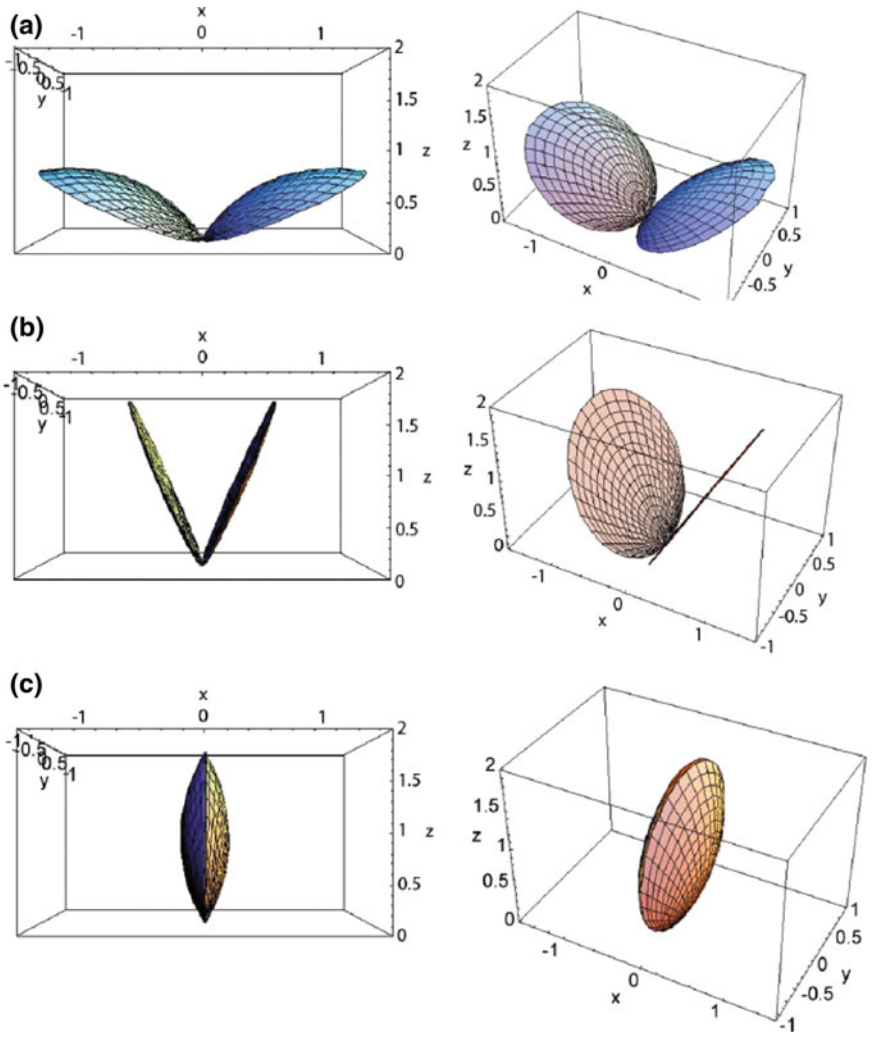

Fig. 1.3 Computer modeling of venus flytrap closing

When solving this equation, one has to have in mind that the open probability n_{op} is the function of time found above. If initial mean curvature at the moment $t = t_s$ is $C_M = C_1$, the solution of Eq. 1.5 at $t \geq t_s$ is

$$C_M(t) = (C_1 - C_2)\ \exp\left\{\frac{\tau_a}{\tau_r}\left[1 - \exp\left(-\frac{t - t_s}{\tau_a}\right)\right] - \frac{(t - t_s)}{\tau_r}\right\} + C_2 \qquad (1.6)$$

The Gaussian curvature was calculated in a similar way. Based on these equations the computer modeling gave the sequence of shapes in the process of trap closing. This sequence A, B, C is presented in Fig. 1.3. The left column gives

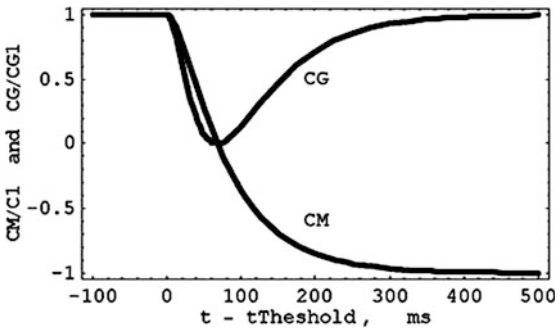

Fig. 1.4 Variation of curvature during Venus flytrap closing

the front view, and the right column the right and above. In panel A the trap is open, the lobes have convex shape. In the process of closing the curvature is changing and panel B shows intermediate state with flat lobes. The final closed state is presented in panel C with lobes having concave shape. Notice that the angle between the lobes at the midrib does not change.

Variation of mean and Gaussian curvature of the lobes is illustrated in Fig. 1.4 with parameters $\tau_a = 20$ ms, $\tau_r = 70$ ms, and $C_2 = -C_1$. As was shown before these curvatures in the given approximation are $C_{AM} = 2/R$ and $C_{AG} = C_{AM}^2/4$. In Fig. 1.4 they are normalized by their initial curvatures, so that initially both of them are equal to 1. It means that the lobes are convex. When closing begins ($t = t_{\text{thershold}}$) both curvatures start to decrease and at some point reach zero. This corresponds to flat lobes in panel B of Fig. 1.3. After that the mean curvature continues to decrease and goes into negative range until it reaches value of -1. This signifies that the lobes became concave. In contrast to that the Gaussian curvature does not become negative; it is the product of two principal curvatures both of which change sign at flat position so that the Gaussian curvature remains positive and returns to value of +1.

When experimentally observing the closing of the Venus flytrap, one can register the change of leaf curvature, but it is easier to measure the change of the distance, X, between the edges of the leaves of the Venus flytrap. Let us designate the initial distance as X_1, and the final distance as X_2. We shall use the normalized distance defined as $x = X/X_1$. It was shown that both distance and mean curvature of the leaf are described by the same function of time.

When the trigger signal opens the pores between the hydraulic layers at the moment $t = 0$, the fluid rushes from one layer to another. The leaf relaxes to its equilibrium state corresponding to the closed configuration. The distance between the edges of the trap was found to vary with time as

$$x(t) = (1 - x_2)\ \exp\left\{\frac{\tau_a}{\tau_r}\left[1 - \exp\left(-\frac{t - t_s}{\tau_a}\right)\right] - \frac{(t - t_s)}{\tau_r}\right\} + x_2 \qquad (1.7)$$

This function was experimentally verified by studying the closure of the Venus flytrap.

Fig. 1.5 Kinetics of the trap closing stimulated by a cotton thread

1.4 Comparison with Experiment

The Venus flytrap can be closed by mechanical stimulation of trigger hairs using a cotton thread or wooden stick to gently touch one or two of the six trigger hairs inside the upper leaf of the Venus flytrap. The cotton thread was removed before the leaves closed. Consecutive photos of the trap are presented in Fig. 1.5. It could also be closed by small piece of gelatin. Plants were fed a $6 \times 6 \times 2$ mm cube of 4% (w/v) gelatin. This induces closing by stimulating 2 of the 6 trigger hairs of the Venus flytrap. The photos are presented in Fig. 1.6.

The Venus flytrap could also be closed by an electrical pulse between the midrib and a lobe of the upper leaf without mechanical stimulation. The closing was achieved by electrical stimulation with a positive electrode connected to the midrib and a negative electrode located in one of the lobes. It is interesting that inverted polarity pulse was not able to close the plant, and the closed trap would not open by electrical stimulus lasting up to 100 s.

A single electrical pulse exceeding a threshold (mean 13.63 μC, median 14.00 μC, std. dev. 1.51 μC, $n = 41$) causes closure of a trap and induces an electrical signal propagating between the lobes and the midrib. When charges were

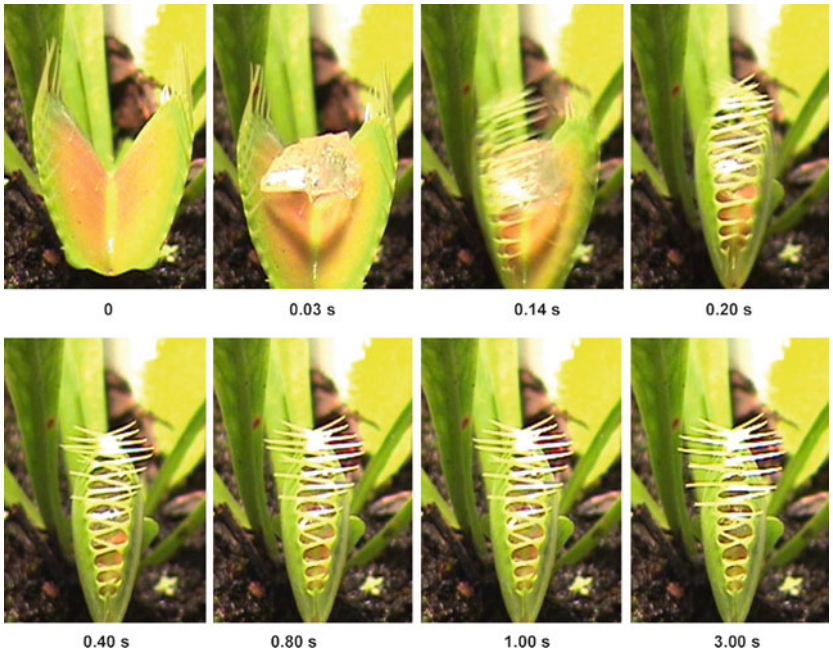

Fig. 1.6 Kinetics of the trap closing stimulated by a piece of gelatin

smaller, the trap did not close. Repeated application of small charges demonstrates a summation of stimuli. Two or more injections of electrical charges within a period of less then 50 s closed the trap as soon as a total of 14 μC charge is applied. Traps closing by electrical stimulus obeys the all-or-none law: there is no reaction for stimulus under the threshold and the speed of closing does not depend on stimulus strength above threshold.

Experimental points in Fig. 1.7 shows the kinetics of closing the upper leaf induced by mechanical or electrical stimuli. Closing consists of three distinctive phases (Fig. 1.7). Immediately after stimulation, there is a mechanically silent period with no observable movement of the plant. This is followed by a period when the lobes begin to accelerate. The third period of fast movement is actual trapping when the leaves quickly relax to the new equilibrium state.

The processes of closing by mechanical or electrical stimuli qualitatively are very similar though parameters of these processes are somewhat different. These parameters were found from curve fitting. They are presented in Table 1.1 (rows A and B). The closing develops at just a fraction of a second. The first mechanically silent phase lasts between 68 and 110 ms and the opening of water channels takes between 10 and 20 ms. These two stages are about two times faster with mechanical stimulation than with electrical one. However, this is not the case for relaxation stage: it is two times slower with mechanical stimulation. Therefore, the fastest stage both with mechanical and electrical stimulation is the opening of

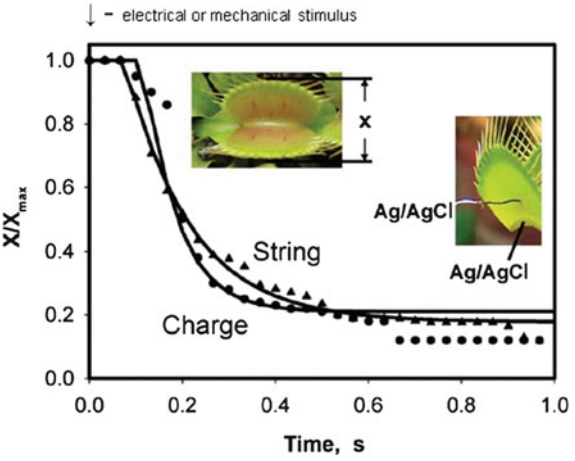

Fig. 1.7 Kinetics of a trap closing stimulated by a cotton thread (*triangle*) or by 14 μC charge (*circle*); x is the distance between the trap margins. Solid lines are plotted according to Eq. 1.7 with parameters from Table 1.1. All these results were reproduced at least ten times. Reproducibility of the initial mechanically silent period with no observable movement of the trap is ±33 ms

water channels. The table shows that the limiting stage of the process is the fluid transfer in the leaf, though it is also very quick due to the small distance between the layers. In both experiment the characteristic time τ_a is always less than τ_r. This means that pore opening is relatively fast and it is not a limiting stage. Final relaxation of the trap to the closed state is much slower.

1.5 Interrogating Consecutive Stages of Trap Closing

The hydroelastic curvature model described three consecutive stages of trap closing: mechanically silent stage of impulse transduction with characteristic time τ_s, opening of water channels with characteristic time τ_a, and relaxation of the elastic shell to the equilibrium closed state with characteristic time τ_r. To verify basic assumptions of this model Volkov et al. (2008c) interrogated these stages using specific inhibitors of different mechanical and biochemical processes involved in the closing process. They presented detailed experiments for comparative study of the effects of inhibitors of ion channels, aquaporins, and uncouplers on kinetics of the trap closing, stimulated by mechanical or electrical triggering of the trap. This gave the opportunity to justify the basic assumption of the hydroelastic curvature model and to determine the variation of kinetic parameters of the Venus flytrap closure.

The first mechanically silent stage of the trap closing involves transduction of electrical signals and hence it is related to ion channel gating. Therefore it should be sensitive to agents interfering with ion channels. To check this hypothesis, the ion channel blockers Ba^{2+}, Zn^{2+}, and tetraethylammonium chloride (TEACl) were used. The altered kinetics of trap closing is presented in Fig. 1.8, panel b. For convenience of comparison, panel a presents control experiment before modification of the plant. Both Ba^{2+} and Zn^{2+} had similar effects on the Venus flytrap:

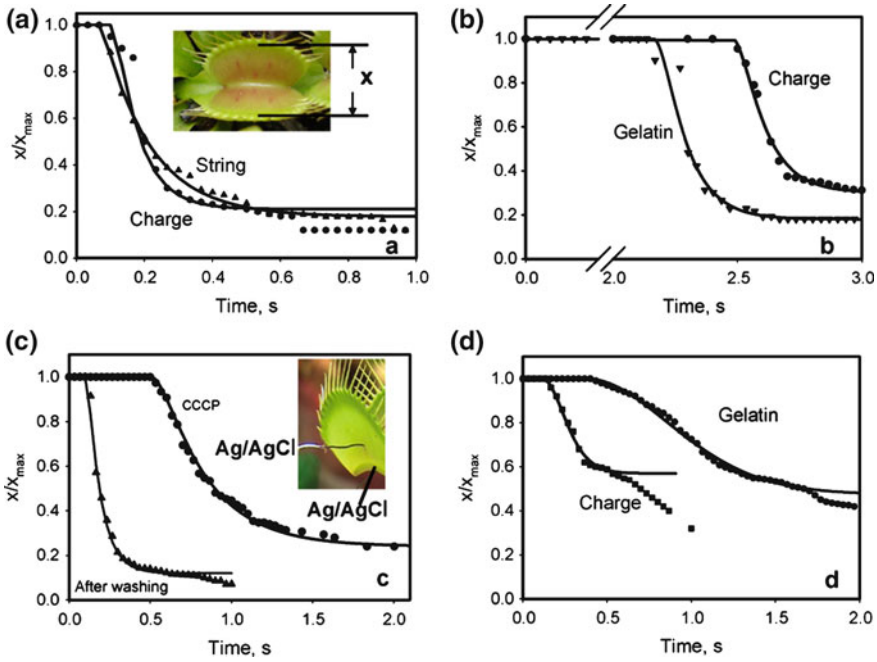

Fig. 1.8 **a** Kinetics of a trap closing stimulated by a cotton thread (triangle) or by 14 μC charge, *x* is the distance between the trap margins; (circle). **b** Kinetics of a trap closing stimulated by a gelatin (*triangle*) or by 28 μC charge (*circle*). 50 mL of 5 mM $BaCl_2$ was added to soil 55 h before experiments. **c** Kinetics of a trap closing after 70 μC electrical stimulation. 50 mL of 10 μM CCCP was added to soil 4.5 h before experiments (*circle*). Soil around Venus flytrap was washed during 2 days with 200 mL distilled water per day to decrease CCCP concentration (*triangle*). **d** Kinetics of a trap closing after stimulation of trigger hairs by a small piece of gelatin (*circle*) or by 28 μC electrical stimulation (*square*). 50 mL of 10 mM TEACl was added to the soil 55 h before experiments. *Solid* lines are theoretical dependencies estimated from Eq. 1.7 with parameters from Table 1.1. Reproducibility of the initial mechanically silent period with no observable movement of the trap is ±33 ms

they significantly extended mechanically silent stage—processing of electrical signals (Fig. 1.8b and Table 1.1). They changed the duration of then first stage from tens of milliseconds to a few seconds—more than an order of magnitude. The effect was more pronounced for electrical stimulation than for mechanical stimulation that intuitively seems quite expected. These blockers of ion channels did not interfere with other two stages: the speed of closing when it started remained similar to nontreated plants both for electrical and mechanical stimulation.

Another agent TEACl is known as a blocker of potassium channels in plants (Volkov 2006a, b). It was found that 10 mM aqueous solution of TEACl decreased the speed of the trap closure induced both by mechanical and electrical stimuli (Fig. 1.8d and Table 1.1). The effect is more pronounced for mechanical stimulation.

The next group of active substances studied in this work included uncouplers of oxidative phosphorylation. They are soluble in both water and lipid phases,

Table 1.1 Estimated kinetic parameters with and without inhibitors

Experiment		t_s (ms)	τ_a (ms)	τ_r (ms)	$\tau_a + \tau_r$ (ms)	x_2
A	Mechanical stimulation	68	10	140	150	0.178
B	Electrical stimulation	110	20	70	90	0.21
C	Electrical stimulation with $BaCl_2$ added to soil	2,480	50	100	150	0.30
D	Mechanical stimulation with $BaCl_2$ added to soil	2,150	50	100	150	0.18
E	Electrical stimulation with CCCP added to soil	480	80	320	400	0.24
F	Electrical stimulation after CCCP washed out	90	20	80	100	0.12
G	Electrical stimulation with TEACl added to soil	120	220	60	280	0.57
H	Mechanical stimulation with TEACl added to soil	370	1900	120	2020	0.475

permeate the lipid phase of a membrane by diffusion and transfer protons across the membrane, thus eliminating the proton electrochemical gradient and/or a membrane potential (Volkov et al. 1998). Hodick and Sievers (1988) reported an excitability inhibition of *Dionaea* leaf mesophyll cells using uncoupler 2,4-dinitrophenol. In our experiments uncoupler CCCP caused the delay of the trap closing (Fig. 1.8c and Table 1.1, electrical stimulation) in addition to significant decrease of the speed of closing as a result of membrane depolarization or dissipation of a proton gradient during ATP hydrolysis. This effect is reversible when concentration of CCCP was decreased by soil washing with distilled water, if an uncoupler was added to soil less than 5 h before. After soil washing with distilled water, the closing time of Venus flytrap treated by CCCP returned back to 0.3 s, but a higher electrical charge is needed for trap closure (Fig. 1.8c). After 48 h incubation of CCCP in the soil, the inhibitory effect of CCCP on the trap closure became irreversible and could not be washed out by distilled water. We found similar effects of significant increase of time closing of the trap in the presence of uncouplers FCCP, pentachlorophenol, and 2,4-dinitrophenol.

Millimolar solutions of $BaCl_2$ and TEACl may affect physiology of the plant since the Venus flytrap is notoriously sensitive to some ions (Hodick and Sievers 1988; Lloyd 1942). Control plants were exposed to similar concentrations of KCl (Fig. 1.9a) and $CaCl_2$ (Fig. 1.9b) added to soil and no inhibitory effect of these salts on the trap closure was found. Usually, concentration of salts in water from lakes and ponds is much higher and varies from 100 to 400 mg/L (Drever 1997). Water from lakes, ponds, and rivers is the traditional source of water for the Venus flytrap in natural habitat and in vitro.

The rate of cellular movement is determined by the water flux induced by a very rapid change in osmotic pressure, monitoring by a fast and transient opening of aquaporins. $HgCl_2$, TEACI, and Zn^{2+} inhibit water channel activity (Detmers et al. 2006; Maurel 1997; Savage and Stroud 2007). According to literature, 1 mM $HgCl_2$ is an efficient blocker of aquaporins (Maurel and Chrispeels 2001; Tyerman et al. 2002). Figure 1.10 shows that the inhibitor of aquaporins $HgCl_2$ hinders the trap closing after mechanical stimulation independently on the type of extraction.

Figure 1.11 shows kinetics of a trap closing after extraction of TEACl from 2 drops of the TEACl solution placed on the midrib. Mechanical stimulation of three

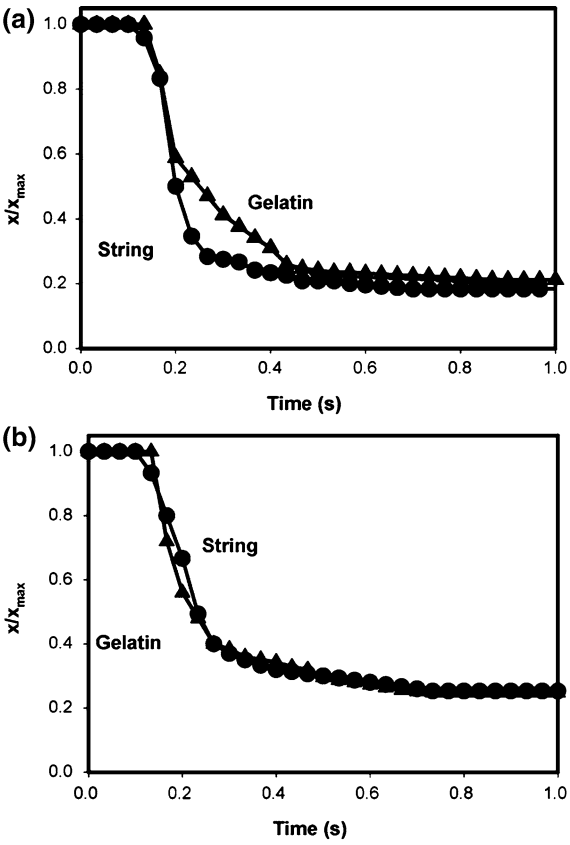

Fig. 1.9 Kinetics of a trap closing stimulated by a cotton thread (*circle*) or by gelatin (*triangle*), x is the distance between the trap margins. **a** 50 mL of 10 mM KCl was added to soil 7 h before experiments. **b** 50 mL of 5 mM $CaCl_2$ was added to soil 7 h before experiments. Reproducibility of the initial mechanically silent period with no observable movement of the trap is ±33 ms

mechanosensitive hairs did not induce the closing of the trap (Fig. 1.11a, line 1). If these mechanosensors stimulated again after short period of time (10–30 s), the trap slowly closes (Fig. 1.11a, line 2). Figure 1.11b shows similar dependencies after electrical stimulation by 14 μC (line 1) and 28 μC (line 2). To close the trap after TEACl treatment, a double electrical charge is required. TEACl is known as a blocker of aquaporins (Demeters et al. 2006) and K^+-channels (Volkov 2006b) in plants.

Figure 1.12 shows kinetics of the trap closing stimulated by a cotton thread after phytoextraction of $BaCl_2$ (Fig. 1.12a) or $ZnCl_2$ (Fig. 1.12b) from aqueous solution placed on the midrib. $BaCl_2$ induces 3 s delay before the trap closing and $ZnCl_2$ decreases dramatically the speed of trap closing.

Figure 1.13 shows inhibitory effects induced by two drops of uncoupler CCCP at the midrib**.** Other uncouplers FCCP, 2, 4-dinitrophenol, and pentachlorophenol decrease speed and increase time of the trap closing similar to inhibitory effects of CCCP.

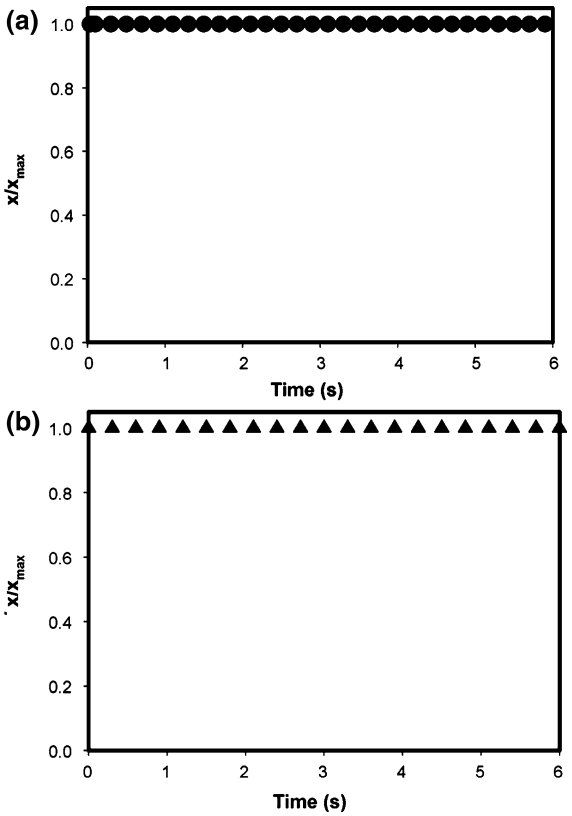

Fig. 1.10 Kinetics of a trap closing stimulated by a cotton thread: **a** 50 mL of 1 mM $HgCl_2$ was added to soil 7 h before experiments. Concentration of $HgCl_2$ in soil was 1 mM. **b** Two 10 μL drops of 1 mM $HgCl_2$ were placed on the midrib 24 h before a mechanical stimulation by a cotton thread of three mechanosensitive hairs. These results were reproduced 16 times on different Venus flytrap plants

So it was found that extraction of $ZnCl_2$, $BaCl_2$, $HgCl_2$, TEACl, and CCCP by the Venus flytrap from soil and from drops of inhibitor solutions placed on the midrib gives the similar results. All these results are summarized in Table 1.1.

The closing of the Venus flytrap develops very quickly; it takes just a fraction of a second. The curve fitting shows that the limiting stage of the process is the fluid transfer in the leaf, though it is also very quick due to the small distance between the layers. In six experiments (A-F, Table 1.1) characteristic time τ_a is always less than τ_r. This means that water channel opening is relatively fast and it is not a limiting stage. Looking for a way to interrogate this stage, we turned to TEACl, which is known as a blocker of K^+ channels and aquaporins in plants. Table 1.1 (rows G and H) shows that in the presence of the aquaporin blocker, τ_r is less than τ_a, and the limiting step of the whole process is the water transport between two layers in the presence of TEACl.

When trigger hairs in the open trap receive mechanical stimuli, a receptor potential is generated (Benolken and Jacobson 1970; DiPalma et al. 1966). Two mechanical stimuli are required for closing the trap in vivo (Darwin 1875; Lloid 1942). Receptor potentials generate action potentials (Burdon-Sanderson 1873; Jacobson 1965, 1974; Volkov et al. 2008a), which can propagate in the

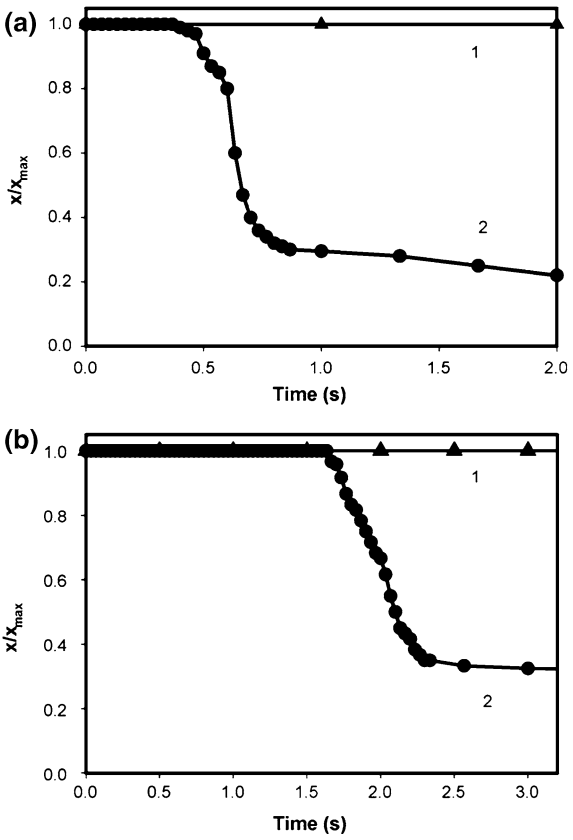

Fig. 1.11 Kinetics of a trap closing stimulated by a cotton thread (**a**) or by electrical charge (**b**). Two 10 μL drops of 10 mM TEACl were placed on the midrib 24 h before a mechanical or electrical stimuli applications. **a** Mechanical stimulation by a cotton thread of three mechanosensitive hairs (1) and repeating of this stimulation in 16 s (2). **b** electrical stimulation by 14 μC (1) or 28 μC (2) between a midrib (+) and a lobe (−). These results were reproduced 25 times on different Venus flytrap plants

plasmodesmata of the plant to the midrib (Volkov et al. 2007, 2008a). Uncouplers and blockers of fast anion and potassium channels can inhibit action potential propagation in the Venus flytrap (Hodick and Sievers 1988; Krol et al. 2006; Volkov et al. 2008c). Once a threshold value of the charge is accumulated, ATP hydrolysis (Jaffe 1973) and fast proton transport start (Rea 1983), and aquaporin opening is initiated. In the presence of aquaporin blocker $HgCl_2$ the trap does not close during sufficiently long period of time. Fast proton transport induces transport of water and a change in turgor (Markin et al. 2008; Volkov et al. 2007, 2008c).

1.6 Electrical Memory in Venus Flytrap

The Venus flytrap has a short-term electrical memory (Volkov et al. 2008a, b, c). It was shown by new charge injection method. As mentioned before, the application of an electrical stimulus between the midrib (positive potential) and the lobe

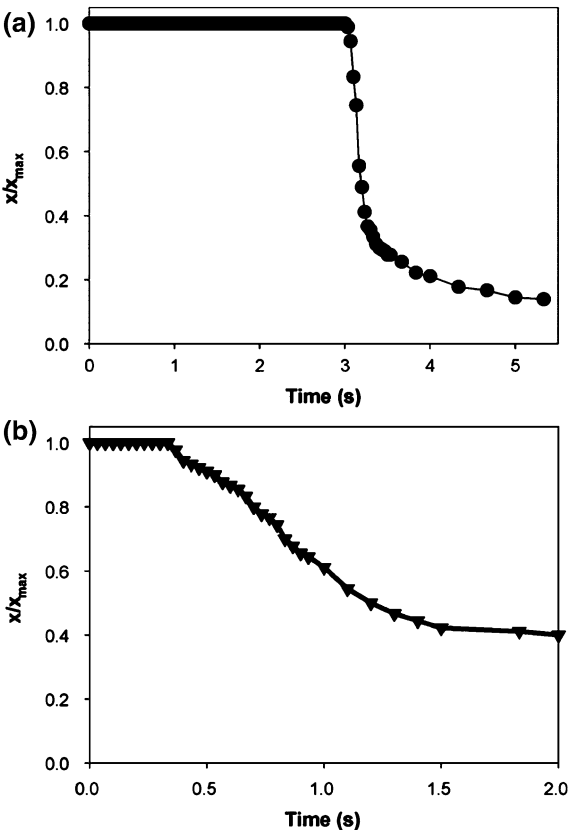

Fig. 1.12 Kinetics of a trap closing stimulated by a cotton thread: Two 10 μL drops of 5 mM $BaCl_2$ **a** or $ZnCl_2$ **b** were placed on the midrib 24 h before a mechanical stimulation by a cotton thread of three mechanosensitive hairs. These results were reproduced 30 times on different venus flytrap plants

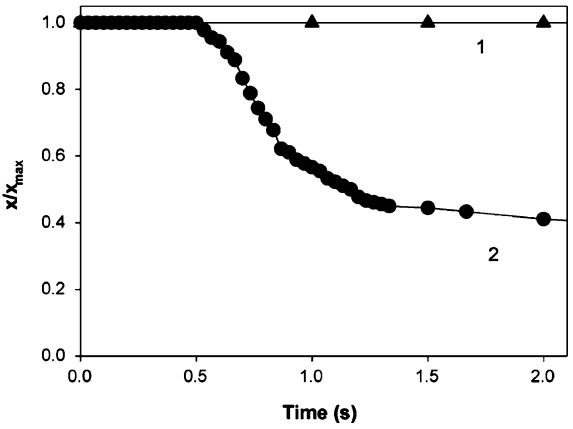

Fig. 1.13 Kinetics of a trap closing stimulated by a cotton thread: Two 10 μL drops of 10 μM CCCP were placed on the midrib 24 h before a mechanical stimulation by a cotton thread of three mechanosensitive hairs (1) and repeating of this stimulation in 16 s (2). These results were reproduced 19 times on different venus flytrap plants