Characteristics of Electrical Signals in Poplar and Responses in Photosynthesis[1]

Silke Lautner, Thorsten Erhard Edgar Grams, Rainer Matyssek, and Jörg Fromm*

Fachgebiet Angewandte Holzbiologie, Technische Universität München, 80797 Munich, Germany (S.L., J.F.); and Lehrstuhl Ökophysiologie der Pflanze, Technische Universität München, 85354 Freising, Germany (T.E.E.G., R.M.)

To gain an understanding of the role of electrical signaling in trees, poplar (*Populus trichocarpa*, *Populus tremula* × *P. tremuloides*) shoots were stimulated by chilling as well as flaming. Two kinds of signal propagation were detected by microelectrode measurements (aphid technique) in the phloem of leaf veins: (1) basipetal, short-distance signaling that led to rapid membrane hyperpolarization caused by K^+-efflux within the leaf lamina; and (2) acropetal, long-distance signaling that triggered depolarization of the membrane potential in the leaf phloem. In the latter, the depolarizing signals travel across the stem from the manipulated leaves to adjacent leaves where the net CO_2 uptake rate is temporarily depressed toward compensation. With regard to photosystem II, both heat-induced long-distance and short-distance signaling were investigated using two-dimensional "imaging" analysis of chlorophyll fluorescence. Both types of signaling significantly reduced the quantum yield of electron transport through photosystem II. Imaging analysis revealed that the signal that causes yield reduction spreads through the leaf lamina. Coldblocking of the stem proved that the electrical signal transmission via the phloem becomes disrupted, causing the leaf gas exchange to remain unaffected. Calcium-deficient trees showed a marked contrast inasmuch as the amplitude of the electrical signal was distinctly reduced, concomitant with the absence of a significant response in leaf gas exchange upon flame wounding. In summary, the above results led us to conclude that calcium as well as potassium is involved in the propagation of phloem-transmitted electrical signals that evoke specific responses in the photosynthesis of leaves.

Electrical signaling in plants was first revealed in the 1870s in insectivorous plants by Burdon-Sanderson (1873) and Darwin (1875). In the 20th century, evidence for the existence of action potentials was presented in a broad array of plant species, irrespective of the presence of rapid leaf movements (Bose, 1924; Pickard, 1973). Most of the research on electrical signaling dealt with responses evoked by wounding of aboveground organs, providing insights into various processes of plant physiology. Molecular tools made it possible to detect rapid changes in gene expression (Davies and Schuster, 1981; Stankovic and Davies, 1997) as well as activation of proteinase inhibitor genes within plants (Bowles, 1990; Ryan, 1990; Wildon et al., 1992) upon wounding, even across long distances. Wildon et al. (1992) showed the chemical signals evoked by wounding in the phloem to be significantly slower than the rapid changes in membrane potential. Electrical signals that were generated and transmitted from distant plant parts arrived at responding tissues well before the initiation of transcript accumulation. Vian et al. (1999) induced rapid and systemic accumulation of chloroplast mRNA-binding protein transcripts, in tomato (*Lycopersicon esculentum*), after flame stimulus. In addition to translation and transcription, evidence exists for a role of electrical signals in many processes of plant life, including respiration (Dziubinska et al., 1989; Filek and Koscielniak, 1997), water uptake (Davies et al., 1991), phloem unloading (Fromm, 1991), and phloem translocation (Fromm and Bauer, 1994) as well as fertilization (Fromm et al., 1995). Recently, this account was extended by a study on the inhibition of photosynthesis in *Mimosa pudica* (Koziolek et al., 2004) upon flame wounding, which demonstrated that electrical signals triggered transient changes in chlorophyll fluorescence (PSII electron quantum yield) and leaf gas exchange. Moreover, in this latter case, the transport of chemicals in the phloem was far too slow to account for the induced changes.

In trees, electrically induced action potentials were measured in willow (*Salix viminalis*) shoots, showing that calcium influx as well as potassium and chloride efflux are involved in the propagation of signals within the phloem (Fromm and Spanswick, 1993). Using a vibrating electrode in combination with the standard microelectrode technique, an apparent efflux of anions and cations of 200 to 700 pmol cm^{-2} per action potential was assessed in willow roots (Fromm et al., 1997). These changes in the cellular ion concentrations may be important in intracellular signaling, whereas communication over long distances in trees may be achieved through phloem-transmitted electrical signals. Bridging long distances, these rapid signals possess the capacity for coordinating physiological activities in trees. To ascertain potential functions of long-distance electrical signals in trees that may have a bearing on photosynthesis, noninvasive techniques

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* Corresponding author; e-mail fromm@wzw.tum.de; fax 0049–(0)89–2180–6429.

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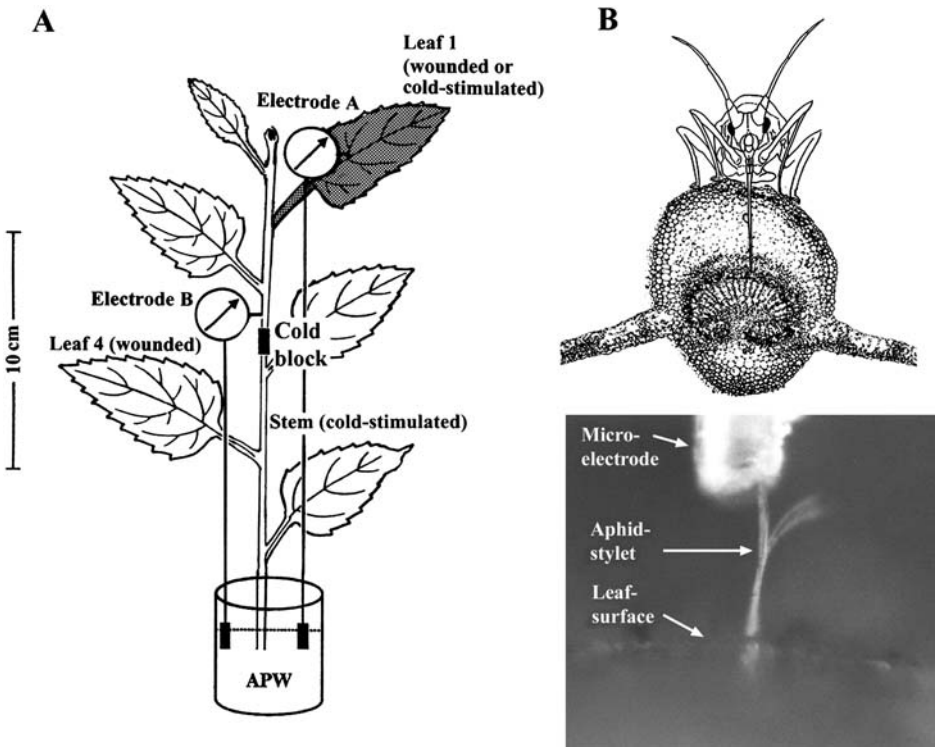

Figure 1. Experimental arrangement of electrical potential recordings. A, The plant was heat stimulated for 3 s by the flame of a lighter either at the tip of leaf 1 or at the base of leaf 4. Cold stimulation (chilling) was applied to the tip of leaf 1 or the lower stem. To disrupt the propagation of electrical signals, a coldblock of 4°C was applied to the stem between the second and third mature leaf underneath the apex. Coldblocking is not equivalent to cold stimulation (chilling) where ice water is rapidly applied for a few seconds, whereas the coldblock at the stem remains in situ permanently in order to block the excitability of the plasma membranes. B, top, An aphid sucking on the underside of a leaf, in the phloem of the primary vein. B, bottom, After the aphid had been severed from its mouthparts by a microscope laser, the stylet stump exuded sieve tube sap to which the tip of a microelectrode was attached by using a micromanipulator.

were employed in this study on poplar (*Populus trichocarpa*, *Populus tremula* × *P. tremuloides*) trees about 80 cm tall. After flame wounding, electrical signals were measured in the phloem by means of the aphid technique (Fromm and Eschrich, 1989). Care was taken to avoid any kind of puncturing stress in the trees, which were grown under nonlimiting nutrient supply or under conditions of calcium deficiency. In parallel, leaf responses were measured in gas exchange (via porometry) and chlorophyll fluorescence (by a two-dimensional imaging approach; Koziolek et al., 2004) at various distances from the wounding site. The hypothesis was tested that (1) heat-induced long-distance electrical signaling affects photosynthesis, and (2) calcium deficiency reduces the capacity for signal transmission. Since intracellular calcium is one of the major elements in the signal transduction pathways of plant cells (Okihara et al., 1991; Trewavas, 2000), calcium-deficient trees were expected to exhibit a difference in behavior.

RESULTS

Long-Distance Electrical Signaling

To detect electrical signals in poplar shoots, the membrane potential was measured either in the leaf mesophyll or in the phloem via severed aphid stylets, at a point in the upper stem (Fig. 1, electrode B) or at the first mature leaf (electrode A). The resting potential of the measured phloem cells ranged between −116 mV to −165 mV in 10 experiments with different plants and was similar to the sieve tube potentials in *M. pudica* (Fromm, 1991) and maize (*Zea mays*; Fromm and Bauer, 1994). In agreement with the common observation that electric activity in plants can be incited through contact with ice water, such stimulation of the tip of leaf 1 resulted, in the sieve tubes, in a basipetally propagating electrical signal with a hyperpolarizing amplitude of 25 mV (recorded at either microelectrode position; Fig. 2, top graph). Transmission velocity was 4 to 8 mm s^{-1}. By contrast, when

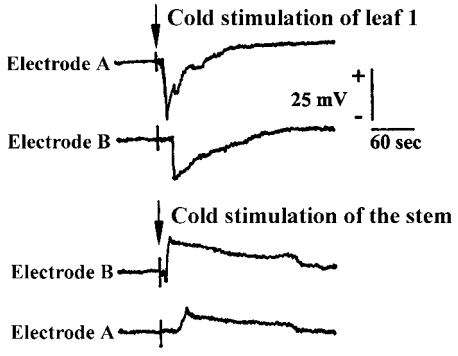

Figure 2. Electrical signals following cold stimulation of detached poplar shoots. As described in Figure 1, the plant was cold stimulated by ice water (4°C) for several seconds at the tip of leaf 1 or at the mid of the stem. Electrical signals were monitored in leaf 1 (electrode A) or in the upper stem (electrode B). The arrows denote the instant of stimulation. Upward and downward deflections signify depolarization and hyperpolarization of the membrane potential respectively. The curves are typical examples from five measurements with different plants.

stimulating the lower part of the stem with ice water at the position of leaf 4, action potentials with depolarizing amplitudes of 12 to 20 mV and velocities similar to those reported above were recorded at the two microelectrode positions (Fig. 2, bottom graph). The original resting potential was reestablished about 3 to 4 min after depolarization, thus indicating typical criteria inherent to action potentials.

Flame stimulation of the tip of leaf 1 evoked a propagating electrical signal with a hyperpolarizing amplitude of approximately 25 mV as measured in the phloem by electrodes A and B as well as in the mesophyll of the flame-wounded leaf, after electrode A had been inserted into the leaf mesophyll (Fig. 3, top graph). Signal transmission velocity in basipetal direction was 1 to 2 mm s^{-1}. Following flame stimulation of the base of leaf 4, either electrode recorded an irregularly shaped propagating electrical signal with an amplitude of over +50 mV in the phloem as well as in the mesophyll of leaf 1 (Fig. 3, central graph). In contrast to the action potentials evoked by stem chilling (Fig. 2, bottom graph), the flame-induced signals reflected failure in reestablishing the original resting potential, indicating that the voltage changes are different from action potentials. The transmission velocity was 1 to 2 mm s^{-1} in acropetal direction and, hence, similar to that in basipetally propagating flame-induced signals. To disrupt the propagation of electrical signals, a coldblock of 4°C was applied to the stem between the second and third mature leaf underneath the apex. In contrast to the unchilled plants, the resting potential of the phloem depolarized only slightly at either electrode (Fig. 3, bottom graph), indicating a strong decrease in signal amplitudes.

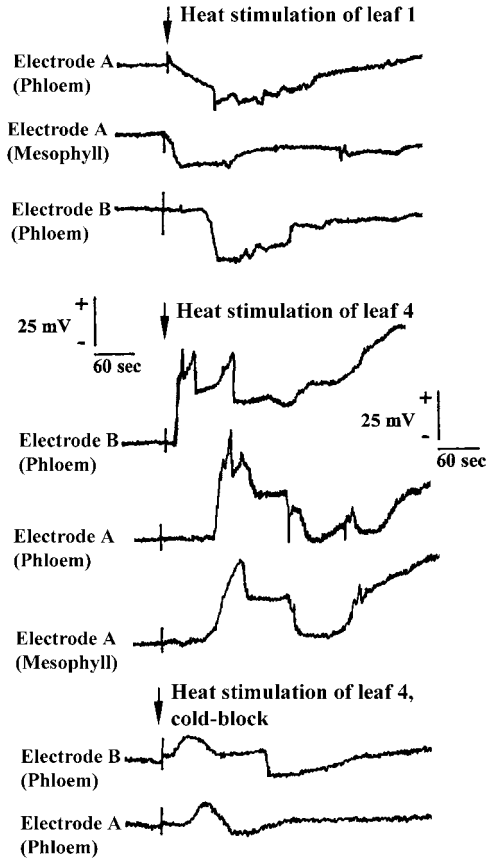

Figure 3. Responses of the membrane potential of the phloem and the mesophyll to flame stimulation of leaf 1 and leaf 4. Stimulation of the tip of leaf 1 evoked the propagation of electrical signals of a hyperpolarized nature (top graph), while stimulation of leaf 4 generated signals of a depolarized nature (central graph). Applying a coldblock to the stem between leaves 2 and 3 significantly reduced the amplitude of the transmitted signal (bottom graph). The curves are typical examples from five measurements with different plants.

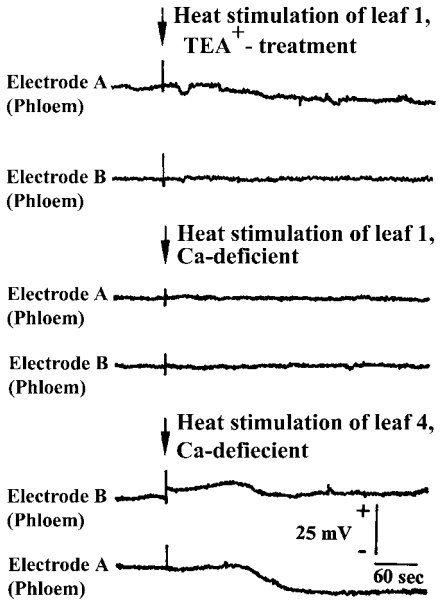

Figure 4. Typical responses of the membrane potential in phloem cells upon flame stimulation in TEA^+-treated as well as calcium-deficient plants. Upon application of TEA^+, a K^+ channel blocker, flame stimulation of leaf 1 did not evoke an electrical signal (top graph). Also in Ca-deficient plants, stimulation of leaf 1 failed to generate any signal (central graph), and wounding of leaf 4 caused weak changes in the membrane potential (bottom graph).

It is remarkable that, independent of the type of stimulation (chilling, heating), signals moving in basipetal direction change toward the negative direction, which contrasts with the opposite response in acropetally traveling signals. The Ca^{2+}-influx/Cl^--efflux/K^+-efflux sequence in signal generation may explain the depolarizing direction, but different fluxes are needed to explain signals of the negative sign, such as K^+-efflux preceding Cl^--efflux. The latter was proved by blocking K^+ channels with 1 mM tetraethylammoniumchloride (TEA^+), acting on both sides of the membranes (Wong and Adler, 1986) and applied to the artificial pond water (APW) medium of the stem (Fig. 1). After 1 d, the inhibitor had been distributed within the plant. When these plants were flame stimulated at the tip of leaf 1, no hyperpolarizing signal was measured in the phloem (Fig. 4, top graph). Obviously, transient hyperpolarization is caused by

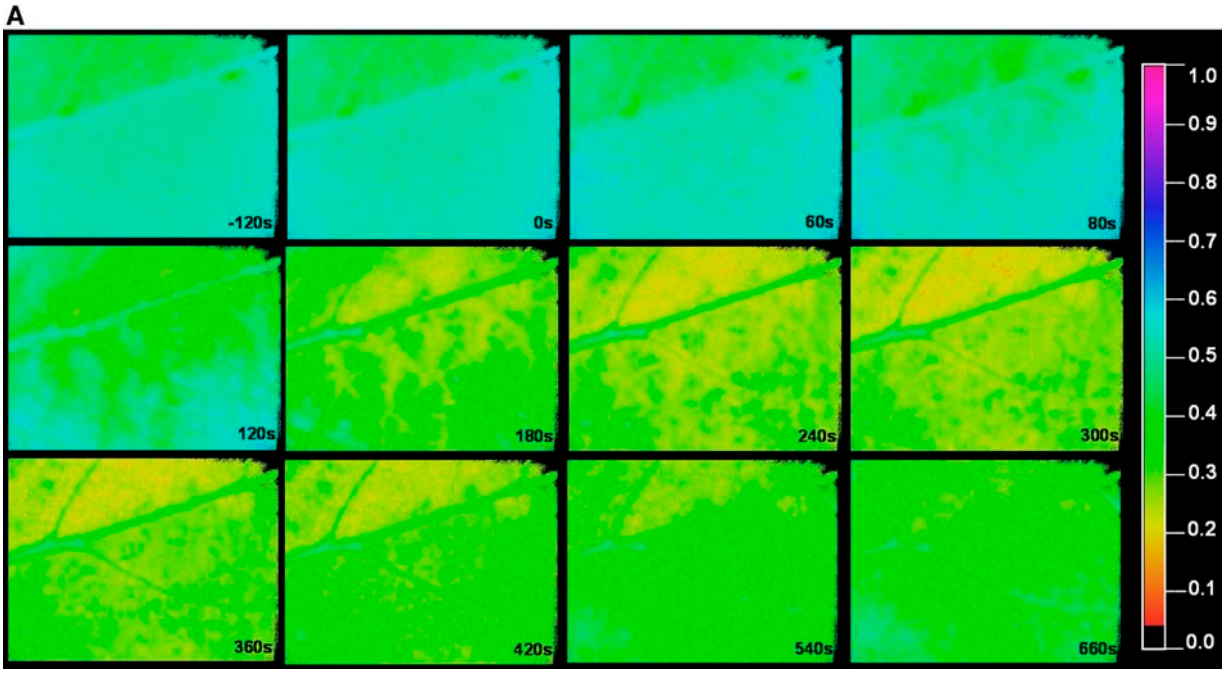

Figure 5. Spatiotemporal changes of PSII electron quantum yield, $\Delta F/F'_m$ assessed by chlorophyll fluorescence imaging. The imaged area (l, 22 mm; w, 17 mm) covers the center of leaf 1. A, The tip of leaf 1 was flame stimulated at a distance of 3 cm. Times are given in relation to the instant of injury (at time 0). Changes in $\Delta F/F'_m$ took 80 s to become apparent. A false-color shift from blue to yellow in the intervein area (equivalent to a lowering of PS electron quantum yield from 0.6 to about 0.2) indicates the decrease in the electron quantum yield of PSII. B, Leaf 4 was wounded at a distance of over 11 cm from the imaged area. Changes in fluorescence were not visible before 240 s had elapsed. After about 420 and 600 s, the left and right side of the leaf, respectively, reached a minimum in the fluorescence response (white and black arrow, respectively). The bar translates the false color code into values of $\Delta F/F'_m$.

the transient opening of K^+ channels in the plasma membrane.

As the ionic mechanism of excitation is based on the movement of potassium, chloride, and calcium (Tazawa et al., 1987; Fromm and Spanswick, 1993), the objective of experiments involving the stimulation of calcium-deficient plants was to characterize the excitation mechanism in poplar. The Ca-deficient plants were distinctly smaller and their leaf size was reduced compared with that in nonlimited plants. Flame wounding of the tip of leaf 1 evoked no detectable electrical signals in the phloem at either electrode position (Fig. 4, central graph). However, signals with strongly reduced amplitudes were measured in acropetal direction after flame stimulation of leaf 4 (Fig. 4, bottom graph), perhaps reflecting the physiological significance of the upwards transmission of electrical signals in plant life.

Chlorophyll Fluorescence Imaging

No photosynthetic response was found upon stimulation of leaves or stem with ice water. However, flaming of the tip of leaf 1 caused a substantial decrease in the electron quantum yield of PSII (Fig. 5A). This effect occurred 80 s after flame stimulation in the intercostal regions of the central part of the lamina, at a distance of 3 cm from the leaf tip. The inhibitory response spread basipetally throughout the leaf, showing a delay in the arrival of the electrical signal at electrode B (approximately 60 s after flame wounding of the leaf tip) in relation to electrode A (after only 2 s; Fig. 3, top graph). In the flame-stimulated leaf, the fluorescence response reached a minimum after about 300 s, prior to incipient recovery.

Flaming of leaf 4 caused a decrease in the electron quantum yield of PSII in leaf 1, evidence of which was found in the leaf veins 240 s of the latter after flame stimulation, and indicating that the signal spreads via the veins into the mesophyll (arrival after 300 s; Fig. 5B). The inhibitory response propagated acropetally throughout the leaf and was delayed in relation to the electrical signal that arrived in leaf 1 only 80 s after flaming of leaf 4 (Fig. 3, central graph). A minimum in the time course of the fluorescence response was reached in the left half of the lamina of leaf 1 after about 420 s (Fig. 5B, white arrow) prior to incipient recovery. However, it took about 600 s in the right half of the leaf for the minimum to occur (black arrow).

The highly resolved time course of the fluorescence response shows the decrease in PSII quantum yield to occur simultaneously in both the veins and the intercostal regions of leaf 1 taking 160 s from the time of flame stimulation of the leaf tip (distance approximately 5 cm; Fig. 6A). By contrast, a distinct two-step

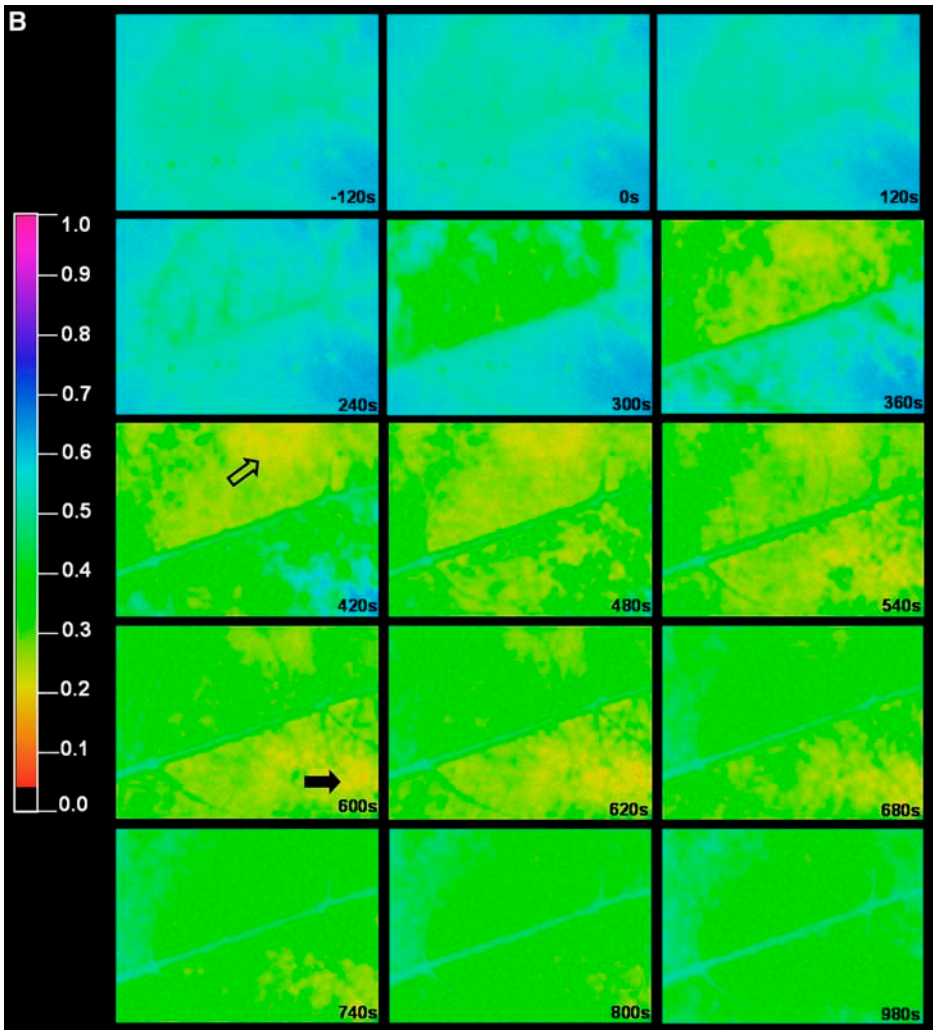

Figure 5. (*Continued.*)

response was observed in leaf 1 during acropetal signaling when leaf 4 was flame stimulated, as the response in veins was faster and stronger than in the intercostal regions (Fig. 6B). In these latter areas, the transient response was delayed by about 60 s compared with the occurrence in the veins. The differences in the response between long-distance signaling along the shoot (Fig. 6B) and short-distance signaling within the same leaf (Fig. 6A) reveal, in the first case, that the signals spread into the mesophyll via the veins, whereas in the latter case, signals travel across veins and mesophyll at similar velocities.

Leaf Gas Exchange

In leaf 1, the net CO_2 uptake rate (J_{CO2}) sharply dropped to about compensation 30 s after flame stimulation of the leaf tip and stayed there for about 90 s before subsequent recovery (Fig. 7A). At the same time, the stomatal conductance (g_{H2O}) remained stable, indicating the absence of stomatal movements. The finding of a photosynthetic reaction not showing any reaction in the g_{H2O} is astonishing. However, the internal CO_2 concentration increased transiently (data not shown), indicating that the relationship between J_{CO2} and g_{H2O} is correct. A similar response occurred in leaf 1, when leaf 4 was flame stimulated at a distance of over 10 cm. At 120 s after stimulation, the J_{CO2} decreased immediately to compensation, staying there for 100 s and then recovering almost completely (Fig. 7B). Chilling the stem slowly down to a temperature of +4°C (coldblock) between leaves 2 and 3 showed that the gas exchange of leaf 1 remained unaltered (Fig. 7C), concomitant with the suppression of the long-distance electrical signal (Fig. 3, bottom graph). This coincidence indicates that the electrical signal has a modifying impact on CO_2 uptake. Experimentation with calcium-deficient plants did not result in any significant response in gas exchange after flame stimulation of leaf 4 (Fig. 7D), which is consistent with the distinctly reduced amplitude of the recorded electrical signal (Fig. 4, bottom graph).

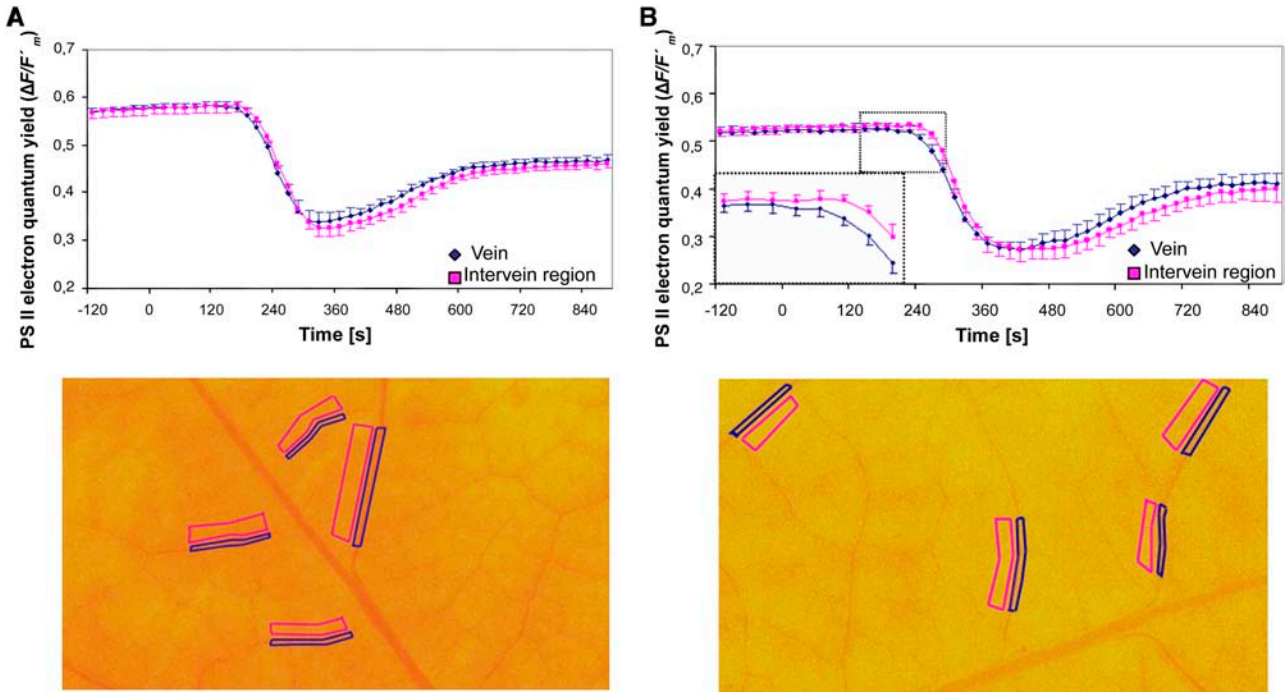

Figure 6. Kinetic changes of PSII electron quantum yield, $\Delta F/F'_m$, measured by fluorescence imaging at representative sites within leaf 1 after flame wounding of A, its leaf tip, and B, the base of leaf 4 at time 0. Blue and red symbols represent vein and intervein field areas, respectively. Data shown are means ± SD, $n = 5$. Parts of imaged areas showing representative vein and intervein field areas used for kinetic analysis are shown below the graphs.

To determine the velocity of chemical signaling, the petioles of excised leaves were fed with ^{14}C-labeled Suc. After a 600-s translocation, macroautoradiography showed that the primary vein became labeled and ^{14}C-Suc extended from the vein across the entire lamina (data not shown). Since the velocity of electrical signal transmission following flame stimulation was 1 to 2 mm s^{-1} in both acropetal and basipetal direction, autoradiography proved chemical signaling to be much too slow to account for the photosynthetic response after flame stimulation.

DISCUSSION

The results presented here show that electrical signals propagated over long distances as well as short distances are capable of modifying photosynthesis in trees. Previous studies had reported on the capacity of many plant species to generate and transmit action potentials as well as variation potentials (Pickard, 1973; Davies, 1987). In ecophysiology, electrical signals play a key role in the communication of environmental stimuli within plants, apparently acting within and across cells, tissues, and organs (Volkov and Mwesigwa, 2001). For instance, the pesticide 2,4-dinitrophenol, considered an environmental problem in agriculture as well as a human health hazard, induced fast action potentials and decreased the variation potential in soybean (*Glycine max*; Mwesigwa et al., 2000). Pentachlorophenol too, a known pollutant and uncoupler of oxidative phosphorylation, induced action potentials in soybean (Volkov et al., 2000). Recent experiments on signal transmission in *M. pudica* demonstrated that a link exists between flame-induced electrical signals and photosynthetic responses, as inferred from two-dimensional imaging analysis of chlorophyll fluorescence in combination with gas exchange assessment (Koziolek et al., 2004).

This study on poplar confirms the latter findings for trees. Moreover, our results demonstrate that different stimulation types and positions incite characteristic electrical signals, each with a specific influence on photosynthesis. We used the aphid technique to measure electrical signals in phloem cells that share fundamental properties with nerve cells in animals, i.e. the existence of excitable membranes by which electrical excitations can be transmitted from cell to cell. In poplar, the nature of the signal depends on the traveling direction. Basipetally propagating signals (induced by chilling as well as flaming) showed negative voltage changes, whereas acropetally moving signals were characterized by transient membrane depolarization (Figs. 2 and 3). The ionic mechanism of action potentials in plants, i.e. depolarizing signals with positive sign, is based on calcium influx as well as initial chloride efflux followed by potassium efflux (Beilby and Coster, 1979; Kikuyama and Tazawa, 1983; Lunevsky et al., 1983; Tsutsui et al., 1986; Kikuyama, 1987; Fromm and Spanswick, 1993). To explain signals

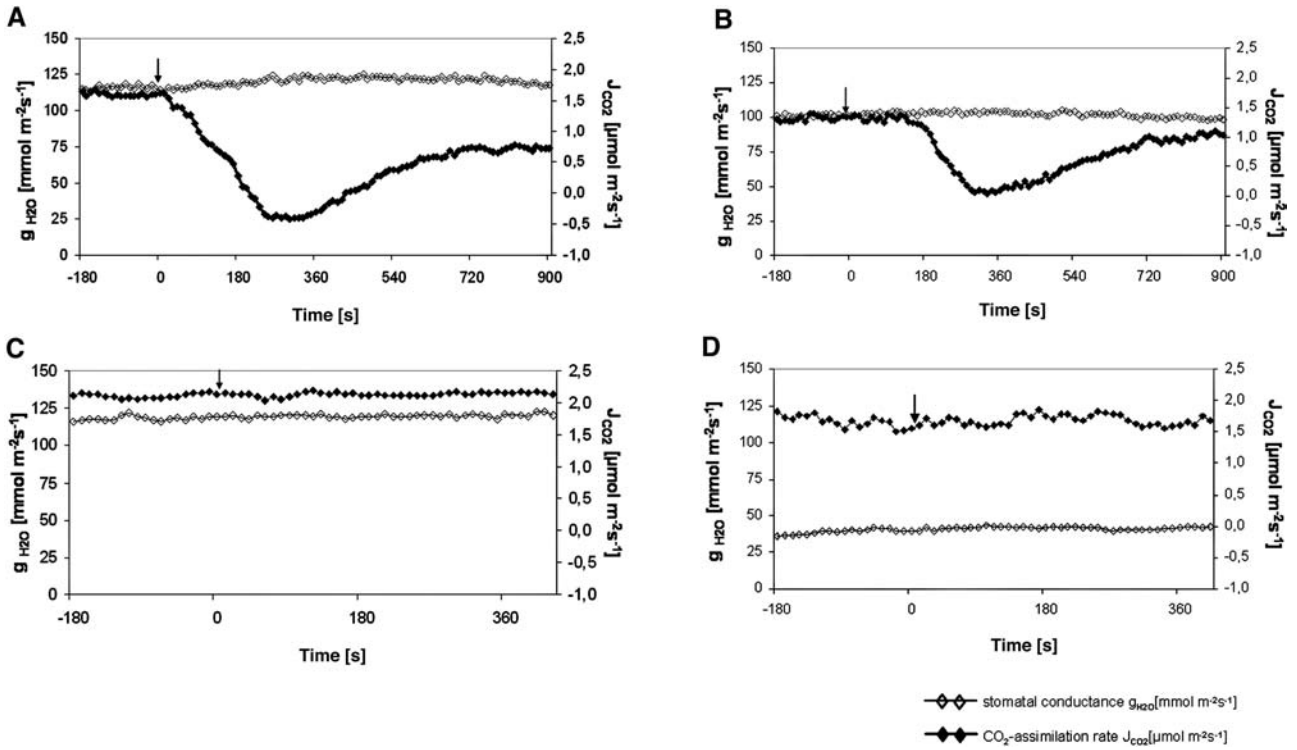

Figure 7. Typical responses of J_{CO2} and g_{H2O} of leaf 1 upon heat stimulation of its tip (A) or of leaf 4 (B). The arrows denote the instant of injury. For heat stimulation of the leaf tip, mean of J_{CO2} before stimulation was 1.35 ± 0.15 and mean of J_{CO2} at transient minimum was −0.2 ± 0.2, while mean of g_{H2O} was 105 ± 15, $n = 5$. For heat stimulation of leaf 4, mean of J_{CO2} before stimulation was 1.38 ± 0.21 and mean of J_{CO2} at transient minimum was 0.48 ± 0.5, while mean of g_{H2O} was 108 ± 17, $n = 5$. C, Chilling the stem with ice water evoked no response in gas exchange. D, No significant response in gas exchange was recorded in calcium-deficient plants following flame stimulation of leaf 4.

of the negative sign, we blocked K^+ channels by TEA^+ and found that no basipetally transmitted signals were induced after flame stimulating the tip of leaf 1 (Fig. 4, top graph), indicating that K^+ efflux causes the hyperpolarization of basipetally transmitted signals. A similar mechanism is reported for the unicellular green alga *Eremosphaera viridis*, where a sudden blockage of photosynthetic electron transport by darkening causes a transient hyperpolarization of the plasma membrane (Schönknecht et al., 1998). In Eremosphaera, the transient hyperpolarization is due to the opening of K^+ channels that is caused by a rapid transient elevation of the cytosolic free calcium concentration. In poplar plants grown under calcium deficiency, phloem cell excitability was completely inhibited after flaming of the leaf tip (compare with Fig. 4). This result indicates first, that calcium channels are involved in the induction of basipetally transmitted electrical signals, confirming the observation that calcium channels and transporters form the basis of a complex calcium signaling network (Trewavas and Malho, 1997; Trewavas, 2000). Second, in optimum nutrient-supplied plants, blockage of the signal by TEA^+ indicates that K^+ efflux is responsible for the occurrence of hyperpolarization, most likely induced by calcium as reported for Eremosphaera (Schönknecht et al., 1998).

In the poplars of this study, electrical signals were induced by chilling as well as by flame wounding. As regards chilling, amplitudes and dynamics of the acropetally transmitted signals were typical of plant action potentials, as previously demonstrated in maize (Fromm and Fei, 1998) and, for trees, in willow (Fromm and Spanswick, 1993). Action potentials are generally self-amplifying signals propagated at constant velocity. It is assumed that they are dependent on voltage-gated ion channels and are capable of propagating through any living cells sharing common membranes, but they are most evident in sieve tubes. In contrast to action potentials, flame stimulation causes the appearance of a slowly moving, irregular, so-called variation potential (Sibaoka, 1966; Sibaoka, 1969). There is widespread agreement in the literature that the variation potential is not a self-propagating signal but, rather, a local electrical response to the underlying passage of chemical substances released from the wound site and transmitted through the xylem by hydraulic dispersal. Malone (1992) reported on hydraulic signals in wheat leaves, traveling at a velocity of at least 100 mm s^{-1} from the site of stimulation. However, using a combination of electrometer and laser-interferometer, Tinz-Füchtmeier and Gradmann (1990) were unable to confirm the hydraulic conductance of excitation in Mimosa.

In poplar, hydraulic signals may play a role in the generation of electrical excitation. Since we managed to measure flame-induced signals in the phloem, the question arises whether pressure or chemical changes in the xylem can activate ion channels in the phloem, making it appear as if a hydraulically induced variation potential were passing through the phloem. However, as the electric signal transmission was disrupted after applying a coldblock to the stem and gas exchange did not respond to flame wounding, it is thought unlikely that hydraulic events play any role in long-distance signaling in poplar. Moreover, variation potentials certainly depend on the prevailing water status of the plant. When shoot water status is saturated, as in the case of the well-watered plants used in this study, xylem tension becomes negligible and variation potentials should not travel at all. We are therefore convinced that the phloem-transmitted flame-induced signals in poplar are self-propagating signals, independent of chemicals traveling through the xylem.

Concerning chemical signaling in the phloem that spreads from the stimulation site through the sieve tubes, the thought cannot be dismissed that photosynthesis is affected by chemical signals. Canny (1975) reported transport velocities in the phloem to range between 50 and 100 cm h^{-1}, i.e. too slow to play a key role in the studied poplar trees. The first response in gas exchange upon wounding of the leaf tip took 30 s to travel a distance of 4 cm, whereas a chemical signal would have moved across a distance of no more than 0.4 to 0.8 cm in 30 s. In consistency with these findings, autoradiography confirmed that chemical signaling is too slow to account for the photosynthetic response (data not shown).

With regard to the photosynthetic response, only flame-evoked signals caused photosynthetic changes, whereas chill-induced signals had no impact on photosynthesis. Interestingly, the noninvasive imaging analysis of chlorophyll fluorescence revealed that short-distance signals within leaves after flame stimulation at the tip cause a simultaneous decrease in electron quantum yield of PSII in both veins and intercostal regions (compare with Fig. 6A). By contrast, long-distance signals, again generated by flame wounding of leaves but arriving after having traveled across the plant, reduce the electron quantum yield of PSII in the veins first and in the intercostal regions afterward (Fig. 6B), hence suggesting that the signal spreads via the veins into the mesophyll. Reduction in photosynthesis upon impact by electrical signals is also known for *M. pudica* (Koziolek et al., 2004) and tomato plants (Herde et al., 1999). Since the PSII quantum yield decreased as well as the J_{CO2}, it can be excluded that the latter is only based on increased respiration. However, the mechanism underlying photosynthetic limitation upon impact by electrical signals requires further clarification. Subcellular alterations in ion fluxes may be involved in the photosynthetic response so that attention should focus on the translocation of ions in mesophyll cells and their chloroplasts. It is reported that enzymes in the cell wall, the plasmalemma, and the cytoplasm can show modified activities during local changes in ion concentrations (Davies, 1987). Bulychev et al. (1986, 1987) reported changes in the chlorophyll fluorescence of intact chloroplasts induced by shifts in membrane potential. The rapid impact on chlorophyll fluorescence and CO_2 uptake in poplar is likely, therefore, to be caused by local changes in ion concentrations. For spinach, evidence is presented for a direct involvement of calcium in O_2 formation of PSII (Vrettos et al., 2001), indicating a possible role for calcium in the photosynthetic response. In summary, this study provides evidence that rapidly evoked and phloem-transmitted electrical signals can affect photosynthesis over long distances in trees. However, the intracellular controlling points in the leaf mesophyll will be the subject of further investigations.

MATERIALS AND METHODS

Plant Material and Growth Conditions

One-year-old plants of *Populus trichocarpa* cv Trichobel were grown in the summer from cuttings under standard greenhouse conditions, at 25°C and 85% relative humidity, in sandy culture medium. In addition, 8-week-old plants of *Populus tremula* × *P. tremuloides* Michx. were grown hydroponically but under otherwise identical greenhouse conditions. The latter plants were provided with macro- and micronutrients in a modified Hoagland solution (Hoagland and Arnon, 1950) containing either low Ca^{2+} (0.1 mM) or full-strength Ca^{2+} (5 mM) concentration. Experiments were performed using fully developed shoots from both groups of plants. Effects of calcium deficiency on electrical signaling were studied using the hydroponically grown plants (*P. tremula* × *P. tremuloides* Michx.); all other experiments were performed on *P. trichocarpa* cv Trichobel.

Electrical Potential Measurements

When about six leaves had developed, plants were cut from their roots and transferred to a Faraday cage for experimentation. They had to be cut from their roots in order to detect the membrane potential by measurements from two sides of the membrane. Microelectrode tips were either inserted into the leaf mesophyll or brought into contact with the exudate droplet on a severed aphid stylet. In the latter case, contact was made at two different positions, either at some point in the upper stem (electrode B) or at the lower side of the first mature leaf underneath the apex (electrode A, Fig. 1). The reference electrode (Ag/AgCl) was immersed into the APW where the cut cross section of the excised stem had also been submerged. The APW was composed of 1.0 mM NaCl, 0.1 mM KCl, 0.1 mM $CaCl_2$, and 1.0 mM MES, adjusted with Tris to a pH value of 6.0.

The electrical potential of the phloem was measured via severed aphid stylets. This involved introducing aphids to a leaf or the stem and allowing them to settle overnight. On the following day, they were severed from their stylets by shots from a laser beam generator (Beck, Neu-Isenburg, Germany), connected to a Zeiss microscope. Electric potential changes were measured through glass microelectrodes with tip diameters of less than 1 μm, back-filled with 3 M KCl. The microelectrode was clamped in an Ag/AgCl pellet holder (WPI) and connected to a microelectrode preamplifier (input impedance $>10^{12}$ ohms) to which a WPI amplifier (model 750, WPI, Sarasota, FL) was attached. The response time of the microelectrodes is about 1 s, which is fast enough to measure electrical responses induced by heat and cold stimulation. The electrodes were inserted into the leaf mesophyll or attached to the stylet stump by micromanipulators: electrode A in leaf 1 at a mean distance of 30 to 40 mm to the stimulated tip; electrode B at some point in the stem (Fig. 1). The resistance for an electric current inside the aphid stylet is relatively low (around 10^9 Ω according to Wright and Fisher, 1981) compared to the high input impedance of the electric equipment used.

Recordings were made between the microelectrodes and a reference electrode (in APW and connected to the cut end of the shoot). Measurement results were logged in parallel by chart recorder and computer.

Chlorophyll Fluorescence Imaging

The two-dimensional imaging approach described by Koziolek et al. (2004; Imaging-PAM Chlorophyll Fluorometer, Heinz Walz GmbH, Effeltrich, Germany) was employed to assess the spatiotemporal variations of the quantum yield of energy conversion in PSII (Siebke and Weis, 1995; Rascher et al., 2001). This method allows noninvasive determination of PSII quantum yield by the saturation pulse method (Schreiber et al., 1986; Genty et al., 1989). Blue light (470 nm) is applied to act as pulse-modulated measuring light, actinic illumination, and saturation pulses. The imaged area of leaf 1 was adapted for at least 10 min to a PPFD of 100 μmol m^{-2} s^{-1} prior to flame wounding of the leaf tip (same leaf) or an adjacent leaf. Heat treatment involved a flame stimulus lasting 3 s. Saturation pulses were given every 10 s to determine the images of fluorescence yield, F, maximal fluorescence yield, F_m', and PSII quantum yield, $\Delta F/F_m' = (F_m' - F)/F_m'$ (for nomenclature, see van Kooten and Snel, 1990).

Leaf Gas Exchange

The gas exchange in the attached leaves was measured using a steady-state CO_2/water diffusion porometer (CQP130, Walz, Effeltrich, Germany) at ambient CO_2 concentration of about 360 μL L^{-1} and a relative humidity of approx. 60%, leaf temperature of about 27°C, and PPFD of about 100 μmol m^{-2} s^{-1}. Leaf 1 was attached to the porometer and its leaf tip or leaf 4 (compare with Fig. 1) were stimulated by flaming. Leaf net-CO_2 exchange was calculated based on single leaf area and expressed as J_{CO2}.

Macroautoradiography

For autoradiographic demonstration of assimilate transport the petiole of a mature leaf was placed into a 5 M ^{14}C-Suc solution. Macroautoradiographs were prepared according to Fromm and Eschrich (1988).

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LITERATURE CITED

Beilby MJ, Coster HGL (1979) The action potential in *Chara corallina*. II. Two activation-inactivation transients in voltage clamps of plasmalemma. Aust J Plant Physiol **6:** 329–335

Bose JC (1924) The Physiology of Photosynthesis. Longman, Green and Co., London

Bowles DJ (1990) Defense-related proteins in higher plants. Annu Rev Biochem **59:** 873–907

Bulychev AA, Niyazova MM, Rubin AB (1987) Fluorescence changes of chloroplasts caused by the shifts of membrane-potential and their dependence on the redox state of the acceptor of photosystem-II. Biol Membr **4:** 262–269

Bulychev AA, Niyazova MM, Turovetsky VB (1986) Electro-induced changes of chlorophyll fluorescence in individual intact chloroplasts. Biochim Biophys Acta **850:** 218–225

Burdon-Sanderson J (1873) Note on the electrical phenomena which accompany irritation of the leaf of *Dionaea muscipula*. Proc Royal Soc (London) **21:** 495–496

Canny MJP (1975) Mass transfer. *In* HM Zimmermann, JA Milburn, eds, Encyclopedia of Plant Physiology. Springer, Berlin, pp 139–153

Darwin C (1875) Insectivorous Plants. J Murray, London

Davies E (1987) Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses. Plant Cell Environ **10:** 623–631

Davies E, Schuster AM (1981) Intercellular communication in plants: evidence for a rapidly generated, bidirectionally transmitted wound signal. Proc Natl Acad Sci USA **78:** 2422–2426

Davies E, Zawadzki T, Witters D (1991) Electrical activity and signal transmission in plants: how do plants know? *In* C Penel, H Greppin, eds, Plant Signalling, Plasma Membrane and Change of State. University of Geneva, Geneva, pp 119–137

Dziubinska H, Trebacz K, Zawadzki T (1989) The effect of excitation on the rate of respiration in the liverwort *Conocephalum conicum*. Physiol Plant **75:** 417–423

Filek M, Koscielniak J (1997) The effect of wounding the roots by high temperature on the respiration rate of the shoot and propagation of electric signal in horse bean seedlings (*Vicia faba* L minor). Plant Sci **123:** 39–46

Fromm J (1991) Control of phloem unloading by action potentials in *Mimosa*. Physiol Plant **83:** 529–533

Fromm J, Bauer T (1994) Action potentials in maize sieve tubes change phloem translocation. J Exp Bot **45:** 463–469

Fromm J, Eschrich W (1988) Transport processes in stimulated and non-stimulated leaves of *Mimosa pudica*. I: The movement of ^{14}C-labelled photoassimilates. Trees (Berl) **2:** 18–24

Fromm J, Eschrich W (1989) Correlation of ionic movements with phloem unloading and loading in barley leaves. Plant Physiol Biochem **27:** 577–585

Fromm J, Fei H (1998) Electrical signalling and gas exchange in maize plants of drying soil. Plant Sci **132:** 203–213

Fromm J, Hajirezaei M, Wilke I (1995) The biochemical response of electric signaling in the reproductive system of Hibiscus plants. Plant Physiol **109:** 375–384

Fromm J, Meyer AJ, Weisenseel MH (1997) Growth, membrane potential and endogenous ion currents of willow (*Salix viminalis*) roots are affected by abscisic acid and spermine. Physiol Plant **99:** 529–537

Fromm J, Spanswick R (1993) Characteristics of action potentials in willow (*Salix viminalis* L.). J Exp Bot **44:** 1119–1125

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990:** 87–92

Herde O, Pena-Cortes H, Fuss H, Willmitzer L, Fisahn J (1999) Effects of mechanical wounding, current application, and heat treatment on chlorophyll fluorescence and pigment position in tomato plants. Physiol Plant **105:** 179–184

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agric Exp Stn Bull **347:** 1–32

Kikuyama M (1987) Ion efflux during a single action potential of *Nitella axilliformis* in medium lacking Ca^{2+}. Plant Cell Physiol **28:** 179–186

Kikuyama M, Tazawa M (1983) Transient increase of intracellular Ca^{2+} during excitation of tonoplast free *Chara* cells. Protoplasma **117:** 62–67

Koziolek C, Grams TEE, Schreiber U, Matyssek R, Fromm J (2004) Transient knockout of photosynthesis mediated by electrical signals. New Phytol **161:** 715–722

Lunevsky VZ, Zherelova OM, Vostrikov IY, Berestovsky GN (1983) Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels. J Membr Biol **72:** 43–58

Malone M (1992) Kinetics of wound-induced hydraulic signals and variation potentials in wheat seedlings. Planta **187:** 505–510

Mwesigwa J, Collins DJ, Volkov AG (2000) Electrochemical signaling in green plants: effects of 2,4-dinitrophenol on variation and action potentials in soybean. Bioelectrochemistry **51:** 201–205

Okihara K, Ohkawa T, Tsutsui I, Kasai M (1991) A Ca^{2+}- and voltage-dependent Cl^--sensitive anion channel in the Chara plasmalemma: a patch-clamp study. Plant Cell Physiol **32:** 593–601

Pickard BG (1973) Action potentials in higher plants. Bot Rev **39:** 172–201

Rascher U, Hütt MT, Siebke K, Osmond B, Beck F, Lüttge U (2001) Spatiotemporal variation of metabolism in a plant circadian rhythm: the biological clock as an assembly of coupled individual oscillators. Proc Natl Acad Sci USA **98:** 11801–11805

Ryan CA (1990) Protease inhibitors in plants: genes for improving defenses against insects and pathogens. Annu Rev Phytopathol **28:** 425–449

Schönknecht G, Bauer CS, Simonis W (1998) Light-dependent signal

transduction and transient changes in cytosolic Ca^{2+} in a unicellular green alga. J Exp Bot **49:** 1–11

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62

Sibaoka T (1966) Action potentials in plant organs. Symposium Society of Exptl Biology **20:** 49–74

Sibaoka T (1969) Physiology of rapid movements in higher plants. Annu Rev Plant Physiol **20:** 165–184

Siebke K, Weis E (1995) Assimilation images of leaves of *Glechoma hederacea*: analysis of nonsynchronous stomata related oscillations. Planta **196:** 155–165

Stankovic B, Davies E (1997) Wounding evokes rapid changes in tissue deformation, electrical potential, transcription, and translation in tomato. Plant Cell Physiol **39:** 268–274

Tazawa M, Shimmen T, Mimura T (1987) Membrane control in the *Characeae*. Annu Rev Plant Physiol **38:** 95–117

Tinz-Füchtmeier A, Gradmann D (1990) Laser-interferometric reexamination of rapid conductance of excitation in *Mimosa pudica*. J Exp Bot **41:** 15–19

Trewavas A (2000) Signal perception and transduction. *In* BB Buchanan, W Gruissem, RL Jones, eds, Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, Maryland, pp 930–987

Trewavas AJ, Malho R (1997) Signal perception and transduction: the origin of the phenotype. Plant Cell **9:** 1181–1195

Tsutsui I, Ohkawa T, Nagai R, Kishimoto U (1986) Inhibition of Cl^- channel activation in *Chara corallina* membrane by lanthanum ion. Plant Cell Physiol **27:** 1197–1200

van Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res **25:** 147–150

Vian A, Henry-Vian C, Davies E (1999) Rapid and systemic accumulation of chloroplast mRNA-binding protein transcripts after flame stimulus in tomato. Plant Physiol **121:** 517–524

Volkov AG, Collins DJ, Mwesigwa J (2000) Plant electrophysiology: pentachlorophenol induces fast action potentials in soybean. Plant Sci **153:** 185–190

Volkov AG, Mwesigwa J (2001) Electrochemistry of soybean: effects of uncouplers, pollutants and pesticides. J Electroanal Chem **496:** 153–157

Vrettos JS, Stone DA, Brudvig GW (2001) Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: evidence for direct involvement of Ca^{2+} in O_2 formation. Biochemistry **40:** 7937–7945

Wildon DC, Thain JF, Minchin PEH, Gubb IR, Reilly AJ, Skipper YD, Doherty HM, Odonnell PJ, Bowles DJ (1992) Electrical signalling and systemic proteinase-inhibitor induction in the wounded plant. Nature **360:** 62–65

Wong BS, Adler M (1986) Tetraethylammonium blockade of calcium-activated potassium channels in clonal anterior pituitary cells. Pflueg Arch Eur J Physiol **407:** 279–284

Wright JP, Fisher DB (1981) Measurement of the sieve tube membrane potential. Plant Physiol **67:** 845–848