20 Slow Wave Potentials – a Propagating Electrical Signal Unique to Higher Plants

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Abstract Plants have at least three kinds of propagating electrical signals. In addition to a sustained wound potential (WP) that stops a few millimeters from dying cells, these signals are action potentials (APs) and slow wave potentials (SWPs). All three signals consist of a transient change in the membrane potential of plant cells (depolarization and subsequent repolarization), but only SWPs and APs make use of the vascular bundles to achieve a potentially systemic spread through the entire plant. The principal difference used to differentiate SWPs from APs is that SWPs show longer, delayed repolarizations. Unfortunately, SWP repolarizations also show a large range of variation that makes a distinction difficult. SWPs and APs do differ more clearly, however, in the causal factors stimulating their appearance, the ionic mechanisms of their depolarization and repolarization phases as well as the mechanisms and pathways of propagation. The depolarizations of a SWP arise with an increase in turgor pressure cells experience in the wake of a hydraulic pressure wave that spreads through the xylem conduits after rain, embolism, bending, local wounds, organ excision and local burning. The generation of APs occurs under different environmental and internal influences (e.g. touch, light changes, cold treatment, cell expansion) that – mediated through varying generator potentials – trigger a voltage-dependent depolarization spike in an all-or-nothing manner. While APs and WPs can be triggered in excised organs, SWPs depend on the pressure difference between the atmosphere and an intact plant interior. High humidity and prolonged darkness will also suppress SWP signaling. The ionic mechanism of the SWP is thought to involve a transient shutdown of a P-type H^+-ATPase in the plasma membrane and differs from the mechanism underlying APs. Another defining characteristic of SWPs is the hydraulic mode of propagation that enables them – but not APs – to pass through killed or poisoned areas. Unlike APs they can easily communicate between leaf and stem. SWPs can move in both directions of the plant axis, while their amplitudes show a decrement of about 2.5% cm^{-1} and move with speeds that can be slower than APs in darkness and faster in bright light. The SWPs move with a rapid pressure increase that establishes an axial pressure gradient in the xylem. This gradient translates distance (perhaps via changing kinetics in the rise of turgor pressure) into increasing lag phases for the pressure-induced depolarizations in the epidermis cells. Haberlandt (1890), after studying propagating responses in *Mimosa pudica*, suggested the existence of hydraulically propagated electric potentials at a time when only APs were conceivable. It took a century to realize that such signals do exist and that they coincide with the characteristics of SWPs rather than those of APs. Moreover, we begin to understand that SWPs are not only ubiquitous among higher plants but represent a unique, defining characteristic without parallels in lower plants or animals.

20.1
A New Effort to Decipher the Impact
of Electrical Long-Distance Signals in Plants

For a long time plants were thought to be organisms whose limited ability to move and respond was matched by limited abilities of sensing. Exceptions were plants with rapid, purposeful movements such as *Mimosa pudica*, *Droseras* (sundews)*, Dionea muscipula* (flytraps) and tendrils of climbing plants. These "sensitive plants" attracted the attention of researchers like Pfeffer, Burdon-Sanderson (1873), Darwin, and Haberlandt (1890; 1914). They found that these plants use sensitive mechanoreceptors and action potentials (APs) that implemented these movements. Although hardly appreciated at that time, the discovery that normal plants such as pumpkins had propagating APs just as the sensitive plants (Gunar and Sinykhin 1962, 1963) was an important scientific landmark. First, it corrected the long-held belief that normal plants are less responsive than sensitive plants. Second, it led to a new, eagerly pursued belief that such widely distributed electric signals must carry messages with an importance that could exceed the induction of organ movements in animals and sensitive plants. In different laboratories around the world this anticipation became the driving force for a renewed quest to decipher the meaning of electrical plant signals. Considerable progress was made in linking electrical signals with respiration and photosynthesis (Gunar and Sinykhin 1963; Koziolek et al. 2003), pollination (Sinykhin and Britikov 1967; Spanjers 1981), phloem transport (Opritov 1978; Fromm and Bauer 1994), rapid deployment of plant defenses (Wildon et al. 1992; Malone et al. 1994; Alarcon and Malone 1995; Herde et al. 1995, 1996; Stankovic and Davies 1996, 1998).

However, with only a few scattered laboratories producing results, new data suffered an almost constant lack of confirmation by other laboratories. This slow progress is traceable in the case of the plant-wide or systemic induction of proteinase inhibitors in wounded tomato plants, which was discovered as early as 1972 (Green and Ryan 1972). Although an involvement of wound-induced electrical signals was immediately suspected and tested (Pickard 1973; Van Sambeek and Pickard 1976), it took 20–30 years before the relationship was independently confirmed (Wildon et al. 1992; Malone et al. 1994; Herde et al. 1995, 1996; Stankovic and Davies 1996, 1998).

20.2
Propagating Depolarization Signals in Plants

Three different types of propagating depolarizations in plants have been suggested to reflect three different types of signals: APs, slow wave potentials
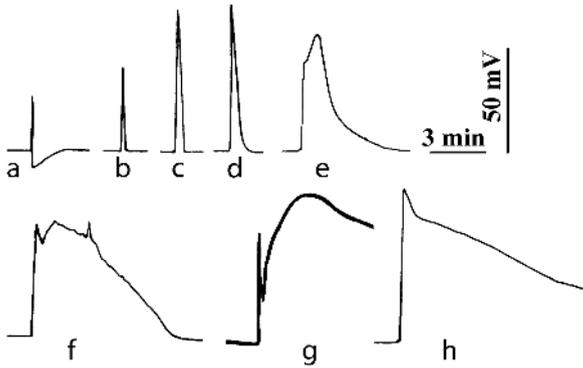
Fig. 20.1. Extracellular and intracellular recordings of the three major types of propagating depolarization signals (action potentials, *APs*, slow wave potentials, *SWPs*, and wound potentials, *WPs*). They show in *a* an AP recorded from the abaxial surface of a *Dionea muscipula* leaf after touching a trigger hair located on the opposite lobe at a distance of 15 mm, in *b* an AP recorded from the epicotyl of a 3-week-old, intact sunflower plant after reduction of bright illumination from 150 to 10 μmol m^{-2} s^{-1} white incandescent light, in *c* a SWP from the epicotyl of an intact sunflower plant after one puncture of the hypocotyl in air 17 cm below the recording site, in *d* a SWP from the epicotyl of an intact sunflower plant after one puncture of the hypocotyl under water and 20 cm below the recording site, in *e* a SWP from the epicotyl of an intact sunflower plant after a 2-mm-deep cut in the submersed surface of the hypocotyl 20 cm below the recording site, in *f* a SWP from the epicotyl of an intact sunflower plant after a 1-s-long torching of the tip of a canopy leaf 15 cm above the recording site, in *g* a combined signal of AP and SWP in the petiole of *Mimosa pudica* after burning of the leaflet tip (redrawn from Houwinck 1935; Sibaoka 1953; Roblin 1985), and in *h* a WP from the epicotyl of an intact sunflower plant after one puncture directly at the recording site. Although APs, SWPs and WPs differ in rates of repolarization, this difference can be too small for reliable identification

(SWPs, also called variation potentials) and wound potentials (WPs). All three signals represent a transient depolarization. However, only SWPs and APs use the vascular bundles to cover longer distances and potentially spread through the entire plant. Examples a and b in Fig. 20.1 show an AP from a flytrap leaf (a) that is of a very short duration and an AP from a sunflower stem (b) that – like most APs in higher plants – lasts for a much longer time of 30−50 s. Within this range APs are characterized by a rapid depolarization and a rapid repolarization. When the depolarization passes a certain threshold, excitable plant cells are able to amplify the signal to a full AP spike by strictly implementing the all-or-nothing rule. Therefore, within the same cell types, the propagation of such APs will proceed in the form of nondecrementing signals with constant amplitude.

SWPs were first discovered by recognition that their kinetic appearance was different from that of APs (Houwinck 1935; Sibaoka 1969, 1991; Umrath

1959). Electrical recordings in *Mimosa* showed an electrical excitation signal in the form of a sequential combination of a short AP spike and a longer wavelike SWP (Fig. 20.1, trace g). Such a sequence was understood to imply that the AP moved faster through the *Mimosa* leaf and therefore appeared earlier at the recording site at the petiole base than the slow wave. The same sequence of signals was found in *Vicia faba* (Roblin 1985), but in sunflowers and cucumbers SWPs can be faster or slower than APs (Stankovic et al. 1997; Stahlberg and Cosgrove 1997c).

Today the "slow" in SWP refers to the slow repolarization and the resulting wavelike appearance rather than to inferior propagation rates (Fig. 20.1, traces e–g). To date a low rate is the most frequently applied criterion to differentiate SWPs from APs (Stankovic et al. 1997; Dziubinska et al. 2001). However, even if measured in the same location (sunflower stem) SWPs can cover an astounding range in repolarization times and their resulting shapes closely approach that of either APs or WPs (Fig. 20.1, traces c–g). Repolarization times are extended after flame induction and root excision (Fig. 20.1, traces e and f), but short when initiated by a needle puncture (Fig. 20.1, traces c and d). SWP signals can be contaminated with action spikes (Fig. 20.1, traces f and g). Such mixed signals have been found among many species, e.g., tomatoes, cucumbers and sunflowers (Roblin 1985; Stahlberg and Cosgrove 1997c; Stankovic et al. 1998b). Moreover, uncontaminated SWPs occur in pea epicotyls (Stahlberg and Cosgrove 1992, 1994, 1995, 1996, 1997c) and perhaps *Tradescantia* shoots (Tsaplev and Zatsepina 1980). In addition to the slower repolarization, uncontaminated SWPs show slower depolarizations than APs and a round rather than pointed signal shape (see Fig. 20.1, traces e and g, plus SWPs from pea epicotyls in Stahlberg and Cosgrove 1992, 1996, 1997a). Unlike APs, SWP induction does not follow an all-or-nothing rule and SWP amplitudes therefore decrement during propagation (see later).

Finally, there are WPs as a direct depolarization response in the vicinity of injured cells (Fig. 20.1, trace h). WPs have very long repolarization times and show a range from less than 1-mm to 40-mm distance (Shimmen 2001; Stahlberg and Cosgrove 1994). The overlap in appearance of the depolarization–repolarization events makes it difficult to distinguish the three signals (Fig. 20.1); therefore, other distinguishing characteristics are needed, e.g., stimuli causing SWPs to appear, ionic mechanisms mediating depolarization and repolarization, in rates, mechanisms and pathways of SWP propagation. What then is a SWP and what are its characteristics?

20.3
SWPs are Hydraulically-Induced Depolarizations

The classic way to induce SWPs is to bring an open flame in contact with a leaf or another part of the plant (Houwinck 1935; Umrath 1959; Roblin 1985; Wildon et al. 1992; Stankovic et al. 1997; Dziubinska et al. 2001). Flaming was considered as a model wound stimulation and an entire set of indirect data suggested that the excitation in *Mimosa* and other plants was mediated by the transpirational transport of wound substances emanating from the burned site (Ricca 1916; Umrath 1959; Schildknecht 1984). However, heat increases gas volume and pressure in the intercellular spaces (reflected as an increase in leaf thickness; Malone 1992, 1996; Boari and Malone 1993) and – more importantly – transiently increases volume and pressure in the narrow xylem conduits of the vascular bundles (Stahlberg and Cosgrove 1997c). Therefore, flaming acts as a strong hydraulic signal that appears as a rapid increase in xylem pressure (Stahlberg and Cosgrove 1997c), turgor pressure (Malone and Stankovic 1991), growth rate (Stahlberg and Cosgrove 1992, 1996), and leaf and stem thickness (Boari and Malone 1993).

The idea that hydraulic signals are accompanied by an electrical depolarization was clearly expressed by two independent studies in the early 1990s (Malone and Stankovic 1991; Stahlberg and Cosgrove 1992). A hydraulically propagated signal had already been suggested to exist in flamed *Mimosa* leaflets (Haberlandt 1890) but experimental evidence for a hydraulic wave paralleling AP propagation did not materialize (Tinz-Fuchtmeyer and Gradmann 1990). It was 100 years later that the exposure of the root of intact pea seedlings to modest pressure steps showed the appearance and propagation of a well-resolved transient depolarization in the pea epicotyl (as in Fig. 20.6). This propagating electrical signal, however, was not an AP but had the typical shape and slow-repolarization characteristics of a SWP (Stahlberg and Cosgrove 1992, 1996, 1997a, 1997c).

The large, propagating depolarizations of a SWP are generated by the application of positive, not negative, steps in xylem pressure (Fig. 20.2b). Rapid axial propagation of the hydraulic signal is manifested by an almost immediate water uptake into the apical growth zone in both pea and sunflower shoots (Stahlberg and Cosgrove 1992, 1995, 1996; Stankovic et al. 1997) and equally rapid changes in turgor and xylem pressure (Malone and Stankovic 1991; Stahlberg and Cosgrove 1995). How does a pressure signal that is almost instantly rising throughout the stem axis relate to an electric signal that takes minutes to climb the stem? An analysis of this question in pea epicotyls found that the induced slow wave depolarizations increased amplitude, rate and range in proportion to the size of the applied pressure steps while their lag phases were declining (Fig. 20.3; Stahlberg and Cosgrove 1997a). Figure 20.3 explains an important point: depolarizations
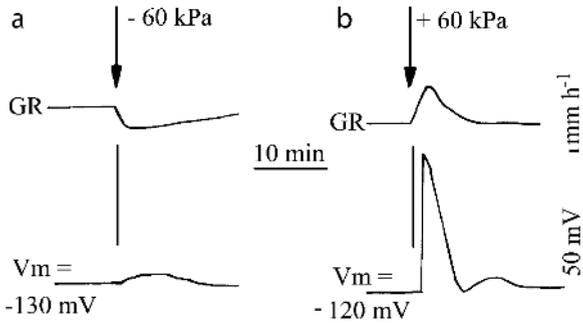
Fig. 20.2. The application of negative (vacuum) and positive pressure steps of equal size (60 kPa) to the submersed basal cut of an etiolated pea epicotyl shows that only positive pressure steps lead to increased growth rate (*GR*) and water uptake as well as the generation of a SWP depolarization, here measured at the epicotyl surface at a distance of 60 mm

will vary in amplitude and lag in appearance only if the xylem pressure in the measured stem section increases between 30 and 80 kPa. For pressure increases larger than 80 kPa, depolarizations will be identical, i.e., they will appear immediately and with similar, i.e., maximal, amplitudes. Thus, a SWP will appear immediately and simultaneously along entire stem or leaf sections as long as their change in xylem pressure exceeds 80 kPa (e.g., in monocot leaves after flame stimulation; Malone and Stankovic 1991; Malone 1992).

Unlike ideal tubes, xylem conduits leak water in a centrifugal direction and they do so preferably upon an increase in xylem pressure (Canny 1995). When a chamber enforced a constant pressure increase of 50 kPa to the vasculature of the submersed basal end of a pea stem segment, it was found this pressure is not transmitted in full amplitude to the apical end of the segment (Fig. 20.4). The transmitted pressure steps decline with increasing length of the measured segments and completely disappear when the epicotyl length exceeds 12 cm. While Fig. 20.4 shows a linear drop of the xylem pressure from the base to the tip of the pea epicotyl, Fig. 20.3 predicts that an axial pressure gradient in the range 30−80 kPa should create a series of depolarizations with increasing lag and decreasing amplitudes. Figures 20.3 and 20.4 therefore provide the basis for understanding SWPs. The apparent, decremented apical "movement" of a SWP is not due to genuine axial propagation but to delayed electrical responses to increasingly smaller hydraulic signals (Stahlberg and Cosgrove 1997c). One cannot avoid being impressed by the accuracy with which plant stems translate pressure steps into electrical signals, distances into increasing lags of the depolarization produced, and by the reliability with which these computations create an always perfect illusion of an electrical signal propagating along the surface.
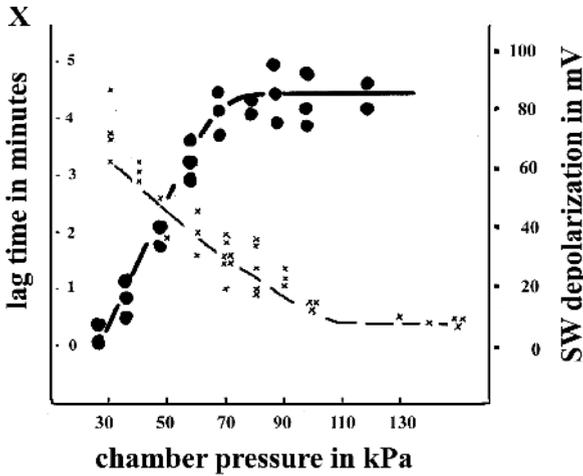
X

Fig. 20.3. A compilation of measurements shows that SWP characteristics such as lag phases (*crosses*) and amplitudes (*circles*) of SWP depolarizations depend on the size of the xylem pressure steps (which are somewhat smaller than the actually shown pressure steps that were applied to the submersed cut end of an etiolated pea seedling at a distance of 60 mm from the recording site, see Fig. 20.4). Note that a propagating SWP appears only at lower pressure steps. At pressures above 100 kPa the induced slow wave (*SW*) depolarizations become indistinguishable in time of appearance (lag phase→0 s) and amplitude. (Compiled from unpublished and published data from Stahlberg and Cosgrove 1997a)
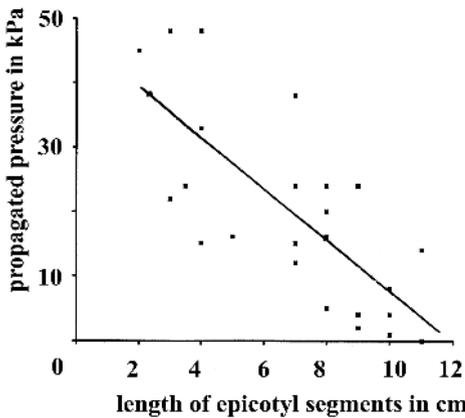

Fig. 20.4. Regression analysis of pressure propagation in epicotyl segments of various lengths shows the linear dissipation of a constant pressure step of 50 kPa from the basal end of application towards the apex. The basal ends of pea epicotyl segments 2–12 cm long were submersed and sealed into a pressure chamber while the apical end was sealed to a pressure probe. A loss of about 4 kPacm^{-1} reflects the radial leakiness of the xylem. (Redrawn from Stahlberg and Cosgrove 1997a)

The SWP phenomenon suggests that the epidermal cells of many species and organs show a depolarization as a consequence of a rapidly rising turgor pressure. The quantitative characterization of this relationship, e.g., with the use of a combination probe measuring turgor pressure and membrane potential, would fill the remaining void about the hydraulic induction of the SWP. Pressure steps can become severely reduced on their centrifugal path from the xylem to the epidermis (Westgate and Steudle 1985). Application of 100 kPa pressure steps to 3 cm-long epicotyl segments was insufficient to cause a measurable increase in epidermal turgor pressure (Stahlberg and Cosgrove 1992). Stankovic and Malone (1991) measured large turgor pressure changes in the epidermal cells of torched wheat leaves but not the increase in xylem pressure. Together with the radial dissipation of xylem pressure steps, a parallel study of the radial propagation of the depolarization from the vascular bundles to the epidermis would be useful to fully understand the conversion of pressure into electrical signals. So far such studies exist only for APs (Rhodes et al. 1996; Herde et al. 1998).

Induction of SWPs by small pressure steps applied without injury to intact plants also presents a powerful argument against participation of chemical wound factors and for a purely hydraulic induction of SWPs (Stahlberg and Cosgrove 1995, 1996). In spite of this, it is still considered that some plant species may use electrogenic substances to induce propagating electrical signals. The idea draws support from the finding that raw extracts from *Mimosa*, *Biophytum* and tomato plants were able to induce propagating depolarizations (Ricca 1916; Umrath 1959; Van Sambeek et al. 1976; Cheeseman and Pickard 1977; Sibaoka 1997). On the other hand, feeding of wound sap to excised pea epicotyls showed clearly that peas do not use wound substances for SWP generation (Stahlberg and Cosgrove 1992). Figure 20.5 shows a test of whether the xylem-mediated transport of strongly depolarizing agents like cyanide and azide is capable of generating a propagating depolarization in excised sunflower shoots. The induced signal moves slowly (less than 1 mm s^{-1}) in comparison with a hydraulically induced SWP (5–10 mm s^{-1}; e.g., Fig. 20.6) with depolarizations being sustained rather than transient. In order to produce SWP-like signals, potential excitation substances must (1) be shown to accumulate in sufficient quantity to cause a large and rapid depolarization, (2) be able easily to access and exit the vascular bundles and (3) cause transient depolarizations. None of the many SWPs recorded so far have been shown to fulfill these criteria.

While experimental methods of induction explore signal character and effects, it is equally important to find natural circumstances under which plants generate SWPs. Such situations include puncture wounds by sap-sucking insects (Alarcon and Malone 1994; Volkov and Haak 1995; Fig. 20.6); embolisms (Stahlberg and Cosgrove 1996), soil hydration during rains and
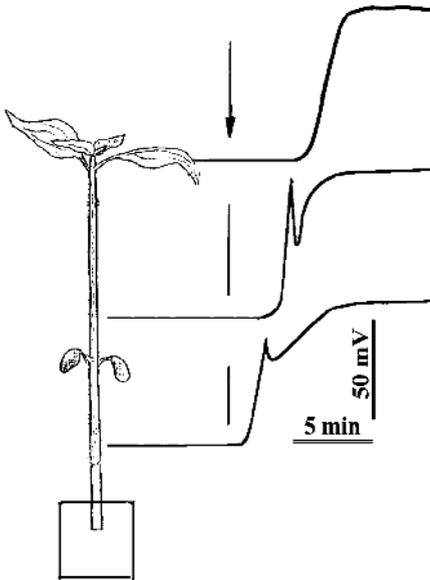
Fig. 20.5. After the submersed excision of the lower hypocotyl of an intact sunflower plant and a waiting period of 60 min that allowed the ascending transpiration stream to return to its normal rate, the basal end of the shoot was subjected to a 5 mM solution of sodium azide (*arrow*), an electrogenic model substance known to cause a large depolarization in plant cells. The transport and release of this substance from the xylem causes a wave of depolarizations that ascends the stem from the hypocotyl (*lower trace*; the distance to the basal cut was adjusted to be 10 cm) to the epicotyl (*center trace*; the distance to the basal cut was 20 cm) to a leaf blade (*upper trace*; the distance to the basal cut was 30 cm). The transport of this depolarizing chemical produces a signal that differs from a hydraulic SWP by a much lower propagation speed and the absence of repolarization and transience

floods (Stahlberg et al. 2005a), and perhaps also the reestablishment of positive xylem pressure during the night in root-pressure-generating plants, and strong bending of plants under wind and other mechanical influences.

For as long as they have been recognized as different entities, SWPs and APs have been believed to originate from different causes (Sibaoka 1953; Umrath 1959). While the cooling of roots and the application of small electric currents in the tissue seem to induce exclusively APs, the induction by heat (leaf flaming) has been reported to induce SWPs as well as APs (Roblin 1985; Wildon et al. 1992; Stankovic et al. 1997, 1998). Repeated flaming of sunflower leaves changed the shape of the resulting stem response from a clear SWP to an AP-like signal (Davies et al. 1991). These data indicate the possibility of an interaction between SWPs and APs that has not been investigated. Both APs and SWPs are propagated within the vascular
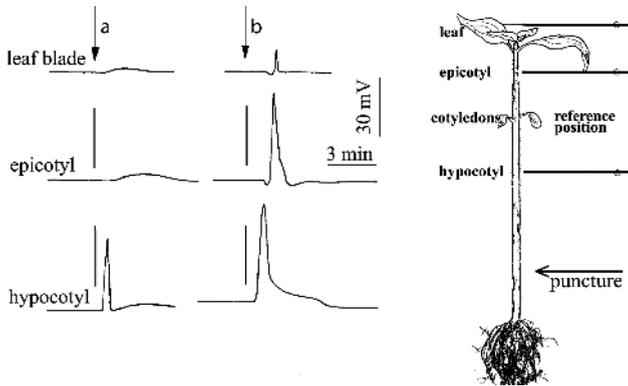
Fig. 20.6. SWPs in the stem of intact sunflower plants arise after a puncturing wound in either a nonsubmersed (**a**) or a submersed (**b**) part of the hypocotyl with a 0.4-mm-wide needle. Caused by the difference between external atmospheric pressure and a transpiration-dependent tension inside the plant, a pressure wave ascends from the wound up the stem in the punctured vascular bundle(s) with high speed and is followed by the appearance of a slower moving wave of depolarizations at the stem surface. In the case of a limited water supply at the puncture wound, the hydraulic signal exhausts itself and the range of the SWP ends between the first and the second electrode position at the stem (**a**). When the puncture is made in the submersed hypocotyl, the SWP becomes systemic, i.e., it increases its range to include all electrode positions along the stem (**b**). The data show that the range of wound-induced SWP depends on the availability of apoplastic water near the wound side

Table 20.1. Characteristics of slow wave potentials in comparison with action potentials and wound potentials. For details and references about the statements see the text

Characteristics of	Slow wave potentials	Action potentials	Wound potentials
Induction	Increase in turgor and xylem pressure	Depolarization beyond certain threshold	Turgor loss of neighboring cells
Amplitude	Graded; amplitude function of stimulus size	Amplitude fixed by all-or-nothing principle	Graded; depends on degree of injury
Induction methods	Heat, wounding, pressure chamber	Heat, cold, touch	Wounding
Ionic mechanism	P-type H^+ pump, $\pm$ ion channels?	Ca^{2+}, Cl^{2-} ion channels	P-type H^+ pump, $\pm$ion channels?
Repolarization	Slow; $> 1-30$ min	Quick; < 1 min	Slow; $3-90$ min
Propagation	As pressure signal in vascular bundles/xylem	As electric signal in vascular bundles/phloem	Unknown

bundles (Table 20.1). Since the depolarization of a SWP lingers longer than that of an AP, SWPs may be more effective than APs in triggering the opening of excitable, i.e., voltage-gated Ca^{2+} and anion channels needed for AP induction and propagation. SWPs in *Vicia*, cucumber and sunflower plants are frequently accompanied by action spikes (Roblin 1985; Roblin and Bonnemain 1985; Stahlberg and Cosgrove 1994, 1997a; Stankovic and Davies 1998; Stankovic et al. 1997). Conversely, the fact that no depolarization has ever been reported to cause a propagating SWP suggests that APs are unable to trigger SWPs.

20.4
The Propagation of SWPs

According to previously introduced considerations (Figs. 20.3 and 20.4), SWPs only undergo an apparent propagation with an appearance and range that is determined by the gradual decline of the inducing pressure signal. Unlike flames and pressure chambers, wounding by puncturing, surface cuts and organ excision generates a hydraulic signal that is based on existing pressure gradients in the plants (Stahlberg and Cosgrove 1997c). This provides useful information of how, e.g., species, age, light and other environmental conditions affect the ability of plants to generate SWPs as well as their size and range (Alarcon and Malone 1994; Stahlberg and Cosgrove 1994, 1995; Stahlberg et al. 2005). An example is given in Fig. 20.6.

Figure 20.6, example B shows the rapid ascent of a SWP from a puncture wound in the hypocotyl to the upper hypocotyl, epicotyl and leaf in an intact, illuminated sunflower plant. When the nonsubmersed part of a hypocotyl was punctured, the limited water supply near the punctured vascular bundle supported only a SWP with a short range limited to the hypocotyl (Fig. 20.6, example A). When punctured under water, the generated SWP had an increased, systemic range. The increased range is a clear indication of the hydraulic nature of SWP propagation. Using a stimulus that is close to the small scale of insect-inflicted wounds, we present here a short SWP that could be more representative than those published before as a consequence of larger injuries. It propagates with the same rate as those induced by root excision and shows the same range extension to wounding under water (Stahlberg et al. 2005b).

Although propagation rates of SWPs and APs overlap (Roblin 1985; Roblin and Bonnemain 1985; Stankovic et al. 1997), SWPs have four distinctive features that clearly separate their movement from that of APs. First, SWPs propagate with a measurable decrement (loss of amplitude) along the plant surface. Excision-induced SWPs in sunflower stems have a decrement of 2.5% cm^{-1} (Stahlberg et al. 2005b). Second, SWPs propagate from the stem

into the leaves and vice versa, whereas APs (at least in sunflowers) cannot do so (Dziubinska et al. 2001; Stahlberg et al. 2005b). A third defining particularity of hydraulic propagation is that it enables SWPs – but not APs – to pass through stem sections where the living cells were killed or poisoned (Stahlberg and Cosgrove 1992). Fourth, SWPs depend on the pressure gradient between the atmosphere and an intact plant interior being under tension. Means to specifically suppress SWP propagation include working with excised sections or intact plants under water or an atmosphere of 100% humidity (Mancuso 1998; Stahlberg et al. 2005b).

20.5
The Ionic Mechanism of SWPs

A highly negative membrane potential in plant cells characterizes an active state that involves the participation of the P-type H^+ pump and generates large potential gradients for ions such H^+, K^+, Ca^{2+} and Cl^- across the plasma membrane. Three major ion fluxes can generate large depolarizations in such cells: (1) an increased inward flow of Ca^{2+} ions from the cell walls into the cytoplasm, (2) an increased outward flow of Cl^- and other anions and (3) a rapid stop in the outward pumping of H^+ ions by the P-type ATPase.

While plant APs have been shown to involve the opening of ion channels (Sibaoka 1969, 1991; Fromm and Bauer 1994); SWPs are thought to reflect a transient shutdown of the P-type proton pump at the plasma membrane (Table 20.1). Evidence for this mechanism is that (1) SWPs depolarize cells by a maximal amount of 90–100 mV, leaving the membrane potential at a negative voltage near the Nernst potential for K^+ ions, (2) amplitudes of the SWP depolarization change continuously with the applied pressure size, (3) SW depolarizations are reduced or suppressed by the use of metabolic inhibitors (Julien et al. 1991; Stahlberg and Cosgrove 1992), (4) no measurable change in the cell-input resistance accompanied the large SWP depolarization of pea epicotyl cells (Stahlberg and Cosgrove 1992, 1996, 1997c), (5) SWP and the pH increase in the apoplast showed matching kinetics when measured with the fluorescent indicator DM-NERF (Stahlberg and Cosgrove 1996), and that the growth rate of apical stems drops with the arrival of the SWP signal (Stahlberg and Cosgrove 1997c; Stankovic et al. 1998). Being the fastest effect on this mechanism known (see Palmgren 1998) the hydraulic or stretch-activated inhibition of the P-type H^+ pump deserves more investigation. One cannot yet exclude that the ionic mechanism of SWPs could be more complex and in some species involve the participation of turgor-controlled or stretch-activated/inactivated ion channels. Unlike pea epicotyls, epidermal cells of cucumber hypocotyls show

transient increases in the cell input resistance during pressure-induced SWPs (Stahlberg and Cosgrove 1997c).

Why is the repolarization of SWPs slower than in APs? Repolarization of plant APs is believed to involve the combined action of voltage-dependent closures of the depolarizing ion channels, the voltage-dependent activation of repolarizing K^+ currents and an increased activity of P-type H^+ pumps. Never going far beyond the Nernst potential for K^+ ions, SWP depolarizations are not likely to be compensated by large outward K^+ currents. Another cause for the delayed repolarization of SWPs is the elimination of the role P-type H^+ pumps play in the repolarization efforts of plant cells. If the second stage of AP repolarization is mediated by a P-type H^+ pumping ATPase (as suggested by Orpitov et al. 2002 for cucurbit cells) a turgor-inhibited pump would explain the slower repolarization of SWPs.

SWPs share one important feature with APs and WPs; a refractory period during which the plant cells are unable to repeat the voltage signal when subjected to the same stimulus (Zawadzki et al. 1991). When a sequence of pressure steps was applied with 10 min intervals in between, all steps caused a transient increase in growth rate but only the first pressure application generated a SWP (Stahlberg and Cosgrove 1996). Systematic studies of refractory periods of SWPs in green plants are completely wanting.

20.6
The Effects of SWPs: Targeted Organs

SWPs trail hydraulic signals and very few studies differentiate whether an effect is caused by either the hydraulic or the electric component. One attempt was the comparison of the growth behavior of cucumber and pea seedlings before, during and after the passage of the electrical SWP signal into the growth zone (Stahlberg and Cosgrove 1997c). Application of small, sustained pressure steps to the stem base rapidly and transiently increased the growth rate due to a hydraulically mediated increase in apoplastic (and turgor) pressure. The delayed appearance of the electrical signal in the apical growth zone coincided with an unexpected, drastic drop in growth rate. The sustained slow wave depolarization in cucumbers paralleled a sustained growth inhibition of their hypocotyls, while a transient slow wave depolarization in peas had a transient effect on the epicotyl growth. Related results from sunflowers show a sustained shrinking of the upper stem after the passage of a flame-stimulated SWP (Stankovic et al. 1998).

Mobility of SWPs in both directions of the plant axis suggests two potential targets: the growing shoots with young canopy leaves and the root. In addition to stem growth, dramatic responses have been reported for leaves known to undergo particularly large, amplified SW depolarizations

(Stahlberg et al. 2005b). Leaf effects range from shutdown of stomata (Wildon et al. 1993; Pena-Cortes et al. 1995), to shutdown of photosynthesis (Koziolek et al. 2003), increased production of jasmonic acid and up-regulated transcription of proteinase inhibitor II and calmodulin in tomato plants (Herde et al. 1996; Stankovic and Davies 1998). Less is known about root responses to SWPs. Sunflower and cucumber plants develop root pressure that is metabolically supported and sensitive to pressure signals that can eliminate it in a rapid and drastic all-or-nothing manner (Stahlberg and Cosgrove 1997b). Leaf flaming generates basipetal electrical and pressure signals that could switch the root pump off and could cause in this way the observed flame-induced shrinking of sunflower stems (Stankovic et al. 1997). This has not been tested yet. Although there is almost no information on SWPs in roots, early work in *Vicia* indicates that the protein metabolism in roots is as sensitive to hydraulic signals as in shoots (Theilet al. 1982).

20.7
WPs and SWPs

Although both APs and SWPs have been called wound signals, there is an electrical signal more deserving of this name. WPs occur not only in higher plants, but also in excised plant organs, nonvascular plants and algae (Shimmen 2001). A tandem pair of *Chara* internodal cells is a simple system to study cellular interactions. When one cell is damaged or killed, the neighboring cell undergoes a WP in the form of a large transient depolarization, sometimes with and sometimes without the occurrence of spikes (Shimmen 2001). Although cellular networks are more complex, cucumber hypocotyls showed identical responses (Stahlberg and Cosgrove 1994). WPs in cucumber hypocotyls extend for a distance of 40 mm (a length corresponding to about 200 epidermal cells), 10 mm in pea epicotyls (Stahlberg and Cosgrove 1994), 5 mm in corn roots (Chastain and Hanson 1982), and 1 mm in barley roots (Mertz and Higinbotham 1976). WPs appear as universal signals with specific extensions for different species and organs. Touch causes a similar response; a slowly repolarizing local depolarization with amplitudes that depend on the strength of the mechanical stimulus (Okamoto 1955; Zerrenthin and Stahlberg 1982).

Evidence exists from *Chara* tandem cells, sugar beet roots and cucumber hypocotyls that the size of the turgor pressure of the victim cell(s) before injury affects the amplitude of the generated WPs (Kinraide and Wyse 1986; Shimmen 2001; Stahlberg and Cosgrove 1997a). WPs in higher plants seem to be caused by a rapid inhibition of P-type H^+ pumps in the effected cells (Chastain and Hanson 1982; Gronewald and Hanson 1980; Kinraide

and Wyse 1986). Accordingly, WPs (1) are accompanied by a strong re-
duction in the growth rate of the cucumber hypocotyl in a 40-mm range
from the wound site and (2) proceed without a change in cell input re-
sistance (Stahlberg and Cosgrove 1994, 1997a). Although WPs and SWPs
seem to share a similar ionic mechanism, WPs lack an important defining
characteristic of SWPs: distant propagation (Table 20.1). SWPs may have
evolved as a type of propagating WP.

Haberlandt (1890) suggested the existence of hydraulically propagated
electric potentials at a time when the only known electrical signals were
APs. It took time to find such signals and to understand that they coincide
with SWPs rather than APs. We slowly begin to realize that SWPs are not
simply ubiquitous but characteristic, defining signals for higher plants that
are missing in lower plants or animals.

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