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Rapid alterations in growth rate and electrical potentials upon stem excision in pea seedlings

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Abstract. Excision of the epicotyl base of pea (*Pisum sativum* L.) seedlings in air results in a fast drop in the growth rate and rapid transient membrane depolarization of the surface cells near the cut. Subsequent immersion of the cut end into solution leads to a rapid, transient rise in the epicotyl growth rate and an acropetally propagating depolarization with an amplitude of about 35 mV and a speed of approx. 1 $mm \cdot s^{-1}$. The same result can be achieved directly by excision of the pea epicotyl under water. Shape, amplitude and velocity of the depolarization characterize it as a "slow-wave potential". These results indicate that the propagating depolarization is caused by a surge in water uptake. Neither a second surge in water uptake (measured as a rapid increase in growth rate when the cut end was placed in air and then back into solution) nor another cut can produce the depolarization a second time. Cyanide suppresses the electrical signal at the treated position without inhibiting its transmission through this area and its development in untreated parts of the epicotyl. The large depolarization and repolarization which occur in the epidermal and subepidermal cells are not associated with changes in cell input resistance. Both results indicate that it is a transient shut-down of the plasma-membrane proton pump rather than large ion fluxes which is causing the depolarization. We conclude that the slow wave potential is spread in the stem via a hydraulic surge occurring upon relief of the negative xylem pressure after the hydraulic resistance of the root has been removed by excision.

Key words: Depolarization (growth-induced) – Growth rate after excision – Membrane potential – *Pisum* (growth and electric potential) – Slow wave potential – Surface potential

Abbreviations and symbols: GR = growth rate; P_x = xylem pressure; R_{in} = cell input resistance; SWP = slow wave potential; V_m = membrane potential; V_s = surface potential

Introduction

Studies with intact plants may demonstrate the presence of new mechanisms of growth control in addition to those already found with single plant cells and excised segments. Such a controlling factor could be the plant's xylem pressure (P_x), which has been shown to affect growth rate (GR) in maize, *Vigna*, cucumber and soybean plants (Acevedo et al. 1971; Okamoto et al. 1984, 1989; Cosgrove 1987; Nonami and Boyer 1990). One experimental approach to change the plant's P_x is excision. Excision affects remote plant parts such as the leaves within seconds, with particularly marked effects on leaf water potential and stomatal aperture (Falk 1966; Raschke 1970; Savage et al. 1984). Excision may also rapidly change the growth rate of the stem. Whereas a sharp and rapid decrease in growth rate was reported for cucumber, soybean and zucchini seedlings (Cosgrove 1987), an increase was shown in soybean seedlings (Molz and Boyer 1978), sunflower seedlings (McIntyre and Boyer 1984) and pea seedlings (Cosgrove and Sovonick-Dunford 1989). These apparently contradictory results may find an explanation in the different sign and size of the xylem pressure in these plants.

Likewise, the effects of excision upon electrical potentials seem to differ widely among plants and reports. *First,* there are reports that excision induces a locally confined depolarization of the cells near the cut surface, which may recover over a period of a few hours (Mertz and Higinbotham 1976; Koopowitz et al. 1975; Pierce and Hendrix 1981). These depolarizations can also be measured extracellularly as negative shifts in the surface potential (V_s), which are reported to occur after wounding or mechanical disturbance (Zerrenthin and Stahlberg 1981). *Second,* there are reports that wounding or mechanical disturbance may trigger action potentials, which can be locally confined (Pickard 1971) or rapidly transmitted (Sinyukhin 1964; Sibaoka 1966; Davies and Schuster 1981; Davies 1987; Retivin and Opritov 1987)

Third, wounding has been shown to trigger a propagating depolarization signal, which differs from action potentials by a larger amplitude and slower propagation speed and is called "s-wave" or "slow-wave potential" (Sibaoka 1953; Kawano 1955; Frachisse and Desbiez 1989) and also "variation potential" (Sibaoka 1953; van Sambeek et al. 1976) because of their variability in the repolarization speed and shape.

The mechanism by which these slow-wave potentials (SWP) move is still a matter of controversy. Some authors propose an electrotonic propagation similar to that of action potentials (Tsaplev and Zatsepina 1980). Another possibility for a transmission is that a depolarizing chemical substance is carried by the xylem flow (Umrath 1927; van Sambeek et al. 1976; Schildknecht 1978; Roblin and Bonnemain 1985). Despite many uncertainties about the process of pressure propagation within the xylem (Heydt and Steudle 1991), an hydraulic signal in the form of a pressure wave could also be a rapid transmitter of a stimulus (Haberlandt 1914, pp. 632–646; Sibaoka 1953; Malone and Stankovic 1991).

To identify the mechanism by which excision alters growth and electrical potentials, we carried out excision studies in plants with negative (xylem tension) and positive xylem pressure ("root" pressure). Excision was used as a means to bring positive or negative xylem pressure towards atmospheric pressure and so to induce xylem-pressure changes. This study deals with pea seedlings, representative of plants with negative xylem pressure; a parallel study on cucumber seedlings, representative of plants with positive xylem pressure, will be published separately. Our results indicate that a rapid and transient growth increase and a propagating depolarization result from pressure changes in the xylem after excision.

Material and methods

Plant material. Pea (*Pisum sativum* L. cv. Alaska) seeds were obtained from W. Atlee Burpee Co., Warminster, Penn., USA. All seeds were sown in polyethylene vials of 20 mm diameter and 60 mm high, which were filled with vermiculite and drenched with 0.25-strength simplified Hoagland solution containing only macronutrients (Schmalstig and Cosgrove 1990); they were germinated in the dark at 27° C for 5–6 d. Straight seedlings were selected for use when their stems were about 100–110 mm long, except where noted. Seedlings were handled under dim green light (approx. 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) provided by a 40-W cool-white fluorescent lamp filtered with two amber and one green acetate filters (Roscolene No. 813 and No. 874; Rosco, Port Chester, N.Y., USA).

Surface- and membrane-potential measurements. Seedlings of about 100–110 mm epicotyl length were fitted into the chamber (Fig. 1). With the help of semi-liquid, warmed Vaseline the compartments of the chamber were sealed so that the surface areas of the corresponding shoot segments were electrically insulated from each other. As shown in Fig. 1, three short compartments (having a distance of 25 mm between them) were filled with cool (approx. 30° C), but still liquid 1% agar, containing 10 mM KCl, each making electrical contact to 4 mm of the shoot surface at a distance of approx. 8 mm (6–10 mm, position B), 35 mm (position C) and 60 mm (position A) from the 5-mm-wide cut compartment, which was filled with 1 mM $CaCl_2$ solution. Glass capillaries were filled with the same agar solution and connected by electrode holders to Ag-AgCl half-cells. The holders were filled with 3 M KCl and fitted into holes in the chamber wall making contact with the agar blocks. They were connected to a high-impedance differential electrometer (model FD 223, WPI, New Haven, Conn., USA), whose output was passed to a chart recorder. Normally the most apical electrode compartment (A) was connected to circuit ground. The surface potential difference (V_s) was measured between the basal and central electrodes (B–C) and between central and apical electrodes (C–A) simultaneously. A recording with only two electrodes would not determine whether a V_s change was caused in A or B.

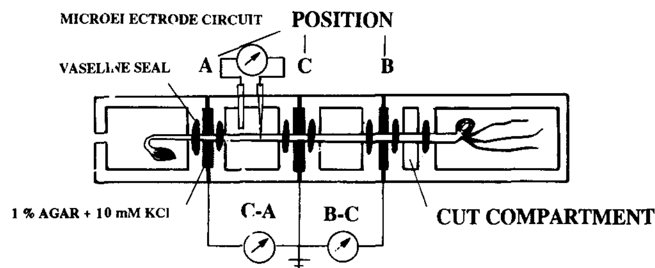

Fig. 1. Diagram of etiolated pea seedling in measuring chamber. *A*, *B* and *C* stand for apical, basal and central electrodes. The electrodes made contact to the surface of the seedling at these three positions via 4-mm-wide agar-filled compartments; these were insulated from the next compartments by Vaseline seals. Micro-electrode measurements of the membrane potential were carried out in the larger compartment between A and C. A similar assembly was used for measuring responses in vertically positioned seedlings. If not indicated otherwise, a cut with a sharp razor blade was carried out under solution in the cut compartment below B

For the measurement of membrane potentials (V_m), the large compartment between A and C was filled with 1 mM $CaCl_2$ solution, which did not shunt the V_s between A and C. A reference electrode of the type already described, as well as a conventional glass microelectrode backfilled with 0.5 M potassium acetate, was inserted into this solution. As described for guard cells (Blatt 1987), acetate proved to be advantageous compared with chloride for long-lasting V_m measurements. Taking into account a slightly increased tip potential (approx. 10 mV), we could register membrane potentials for more than 2 h.

The 1 mm (outer diameter) glass capillary with a tip diameter of < 1 μm was connected to the electrode holders filled with 3 M KCl and the high-impedance electrometer. The tip was inserted into epidermal, subepidermal and cortical cells under microscopic observation with the help of a micromanipulator (Ernst Leitz, Wetzlar, FRG) under green light. For the purpose of comparing V_s and V_m changes the preferred area of insertion was close to position C.

Cell input resistance. For the cell-input resistance (R_{in}) measurements the single-barrelled microelectrode described above was replaced by a triple-barrelled capillary tube system (A–M Systems, Everett, Wash., USA). The tube was twisted and pulled in a vertical pipette puller and backfilled with 1 M KCl. Capillaries with tip diameters > 1 μm were discarded. Triple-barelled capillaries proved to be more stable during twisting, handling and in their tip stability than two-barreled ones; they also provide a choice as only two barrels are needed for this measurement. One barrel was connected by an Ag-AgCl wire to the voltage amplifier, another in the same way to a voltage generator (B&K PRECISION Model 3030; Dynascan Corp., Chicago, Ill., USA), producing alternating 1-Hz positive and negative square-wave voltages (adjustable between 0.17 and 10 V), which were converted by constant resistors (totalling 1 GΩ ± 5%) to current pulses ranging from 0.34 to 20 nA. Normally we used 2 and 4 nA, which alternatingly de- and hyperpolarized the cells by an equal amount of 10–20 mV. The stability of these rectangularly shaped signals was initially checked by magnification with an oscilloscope and then monitored on a chart recorder. The serial resistance of the electrode was lower than 10 MΩ so that its influence on the size of the current impulses was less than 1%.

However, the voltage deflections of the electrode in solution (cross-talk) were measured before and after the impalement of a cell to correct the values for the input resistance.

Growth. Growth rate was measured with the seedlings in vertical position. The differentiated output of a linear displacement transducer (Cosgrove 1982), connected to the apical hook of the experimental plant, was recorded on a chart recorder continously and simultaneously with the corresponding V_s changes.

Expressed cell sap. Fifty pea epicotyl segments, cut from 5 to 50 mm below the hook, were ground in a mortar after adding washed quartz sand and 1 ml of 10 mM $CaCl_2$. Subsequent filtration provided approx. 5 ml of expressed crude cell sap, which was used immediately.

Results

Excision elicits transient changes in V_m and V_s remote from the cut. The V_m of epidermal and subepidermal cells was measured near position C and the V_s between positions C and A was registered simultaneously. Within 30 s after excision of the basal stem, V_m began to depolarize (Fig. 2). Peak depolarization of about 50 mV was attained about 1 min later and was followed by a slower and more variable repolarization toward the original V_m. The surface potential (A–C) closely mimicked the change in the V_m, both in timing and in magnitude (Fig. 2). These results show that epidermal cells 35–45 mm from the cut respond to excision by a large membrane depolarization, which can also be conveniently measured as V_s. The close similarity between V_m and V_s indicates that the depolarization of the surface (epidermal and subepidermal) cells leads to a negative shift in the V_s of that region. It also shows that the measured V_s change (A–C trace) is entirely the result of the depolarization of the cells in C. The depolarization stopped short of position A (60 mm from the cut) the V_s of this position remained unchanged.

This electrical response could be induced only by cutting. High- or low-temperature (60° C and 1° C) treatment of the basal segment and incubation in 20 mM KCN or 50% ethanol failed to produce the effect. It was not necessary, however, to cut through the entire epicotyl. A cut into the surface of the epicotyl, if carried out under solution, was sufficient to elicit the full response. Abraded epicotyl segments lost the ability to produce the signal. A replacement of the standard solution (1 mM CaCl2) in the cut compartment by distilled water, 10 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.7 M mannitol and expressed cell sap from pea epicotyls did not change the characteristics of the signal. It is also worth noting that this electric response was not influenced by the position of the seedling. There was no difference in the response of a pea seedling in a horizontal position to that of one in a vertical position.

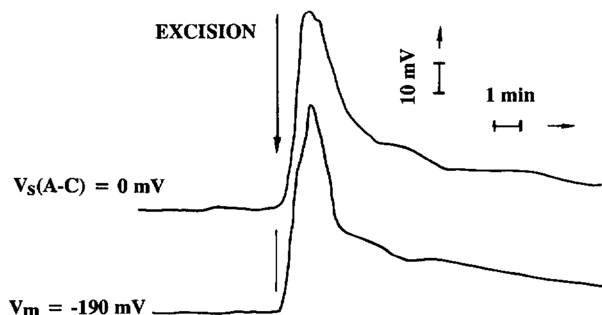

Fig. 2. Comparison of the V_s change in C (A–C trace) and the change in the V_m of an epidermal cell 10 mm from C after excision of the pea epicotyl base. Cells located in the cortex showed smaller V_m changes. Cuts in the basal epicotyl were carried out under solution in the cut compartment. These original trace recordings of an epidermal cell represent a typical example out of 12 similar repetitions with epidermal and subepidermal cells

The electrical response propagates at 1 mm · s^{-1}. With surface electrodes positioned at three sites on the stem, we could record the apical movement of the electrical response (Fig. 3). Upon excision, a negative V_s shift occurred almost immediately near the basal cut site (position B, 8 mm apically from the cut). After about 20 s this negative shift reached C, where it peaked after about 80 s (Figs. 2, 3). The depolarization in C starts before the de- and repolarization in B has come to an end. Therefore the V_s recorded as B–C is a resultant of changes at both B and C (Fig. 3, example 2). This gives a false narrowness to the B–C response. The V_s recorded as A–C, however, does not have this problem because in most cases the depolarization did not reach position A (Fig. 2). In the rare cases where the signal propagated as far as A, the V_s showed the usual positive shift in V_s (A–C) followed by a negative-going shift (Fig. 3, example 3). Since there is no change in the B–C recording at this time we interpret this negative-going shift in the A–C trace as the depolarization reaching position A, which is 60 mm away from the cut and very close to the hook of the seedling (5–15 mm).

Table 1 summarizes the magnitude, timing and velocity of the propagating signal peak. In the rare cases (5 in 40) in which the signal propagated to position A, we could conclude that the signal moved faster from B to C than from C to A. The signal size, however, did not decline while propagating from position C to A. The larger V_s change in B was caused by an additional negative V_s shift, presumably because of mechanical influence and wounding at the cut site (see below).

Whereas Table 1 gives the propagation speed of the signal *peak* (which would be relevant in the case of electrotonic signal transmission), Table 2 shows the average velocity of the *onset* of the signal to be 1 mm · s^{-1} (which would be relevant in the case of a chemical or hydraulic signal transmission). Subsequent cuts repeatedly produced a strong depolarization in B, but induced only minor changes at position C (Table 2, Fig. 3). This shows that only the first cut produces a well-propagating signal; a second one is confined to a non-propagating depolarization, which is a typical wounding response.

Electric or hydraulic mechanism of signal propagation. To determine whether the propagating V_s change involves a hydraulic component, the excision of the pea epicotyls

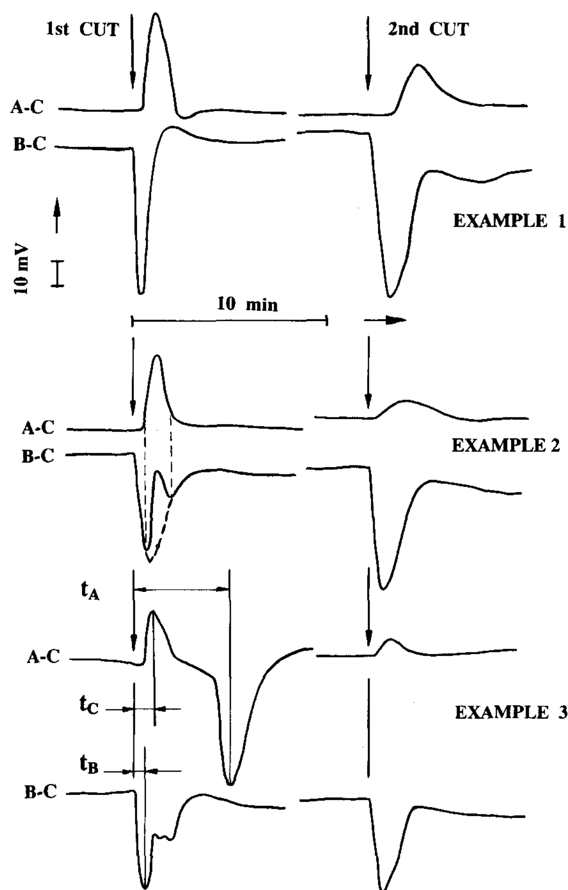

Fig. 3. Effect of a cut in the basal epicotyl of a pea seedling on the electric signals at different epicotyl sites. The cut was carried out in the cut compartment which had been previously filled with 1 mM $CaCl_2$ solution. The cut led subsequently to a rapid, negative V_s shift at position B (as indicated by a negative V_s shift in B–C and no change at A–C circuits) and after about 35 s also at position C (positive shift at A–C and simultaneously at B–C; easy to recognize in *example 2*), but not at position A. In a few cases, however, the depolarization wave reached A (negative V_s shift at A–C, no change at B–C; see *example 3*). Despite the large shift it induced at position B, a second cut given after about 1 h and approx. 2 mm away from the first cut induced only small changes in C. The *arrows* in *example 3* indicate the time for the full development of the signal at B, C and A (= delay as used in Table 1). Here are shown representative examples out of more than 40 repetitions measured with seedlings both in horizontal and vertical positions

was carried out in air. There was either no V_s change at all after the cut (12 in 20 cases), or a negative shift confined to B, probably the result of wounding (Fig. 4). When the cut compartment was filled with $CaCl_2$ solution approx. 5 min after the cut in air, position C depolarized (Fig. 4). This depolarization at C occurred even in cases where B did not respond to the added solution (example 2 in Fig. 4). This depolarization response could not be elicited a second time by eliminating the solution in the cut compartment for approx. 10 min and adding it anew (data not shown). These results, as well as the lack of effect from a second cut (Fig. 2, Table 2), show that a propagating signal is a one-time response or one with a very long refractory period.

The preceding results indicated that the propagating

Table 1. Dependence of signal strength and propagation velocity of the depolarization peak in pea epicotyls upon the distance from the initiating cut. Data represent the average of six rather rare occasions where the electric V_s signal propagated as far as A (as shown in Fig. 3, example 3). These experiments indicate that the signal propagates with a lower speed from C to A than from B to C, but that there is no reduction in signal strength with the distance (decrement). Delays were measured as the time when the depolarization reached its maximal value in B, C and A (t_A, t_B, t_C in Fig. 3, example 3). From the delays at the different positions we can approximate the propagation speed which equals the distance between the electrodes divided by the time differences of the signal peak arriving at B, C and A. Considering that the delay time in B is an underestimate (see Fig. 3), the velocity between B and C might actually be even higher. Standard deviation is provided

Position	Distance (mm)	Signal (mV)	Delay (s)	Speed ($mm \cdot min^{-1}$)
Basal	8	50 ± 14	32 ± 07	–
Central	35	29 ± 15	80 ± 20	34
Apical	60	35 ± 11	202 ± 76	12

Table 2. Comparison of the electric signals in pea epicotyls induced by a first cut with those induced by a second cut, carried out about 1 h after the first one. Despite the fact that a second cut produced a comparable V_s change in B as the first one, there is only a slow propagating minor signal arriving in C (as also shown in Fig. 3). For this table (unlike Table 1) the velocity was calculated for the distance from the cut to C (35 mm) divided by the time taken for the arrival of the *onset* of the V_s shift. Data represent the average of 14 experiments; only five experiments gave enough change in C to measure the onset time for the second cut. Standard deviation is provided. The differences between B and C are reproduced in each experiment and therefore significant. The data provided here characterize the signal as a slow-wave potential

	in position	1st cut	2nd cut
Signal size (mV)	B	57 ± 09	42 ± 20
Onset time (s)	B	~1–2	~1–2
Signal size (mV)	C	37 ± 11	06 ± 07
Onset time (s)	C	34 ± 11	78 ± 46
Maximum (after s)	C	95 ± 22	171 ± 88
Velocity ($mm \cdot min^{-1}$)	Cut to C	62 ± 20	~27

depolarization was associated with an increased water uptake after excision. Such an increase might be expected if the roots presented a substantial hydraulic resistance to water flow. To verify this assumption we followed water uptake by measuring the growth rate of the apical pea epicotyl under the same conditions as the electric response before. When the epicotyl was cut in air, growth rate fell toward zero, but when water was added to the cut, growth quickly resumed, with a rate higher than that preceding the cut (Fig. 5). These results show that the (propagating) depolarizations occur together with a transient surge in water uptake, which is caused by the increase in xylem pressure after excision.

To test the relatedness of these events, both GR and V_s changes in B and C were recorded simultaneously (Fig. 6). Upon excision GR was increased with little delay and peaked after approx. 2 min. The V_s changed immediately at B (not shown) but took approx. 30 s to

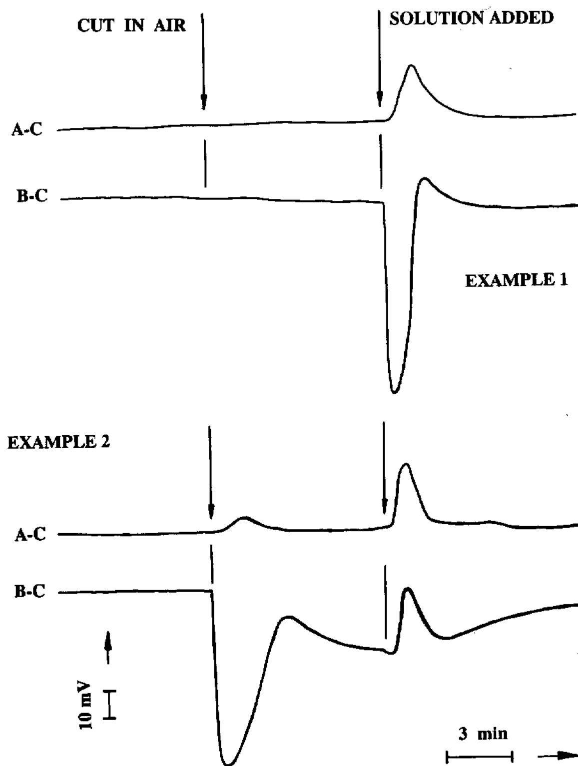

Fig. 4. Cutting through the base of a pea epicotyl *in air* elicited either no V_s change at all *(example 1)* or a local (non-propagating) depolarization at the cut site, i.e. at position B *(example 2)*. Subsequent addition of solution to the cut end, however, induced a depolarization propagating to C. The development of a V_s shift in C without preceding V_s shift in B argues against a passive electric transmission of the depolarization from cell to cell. These examples represent at least eight repetitions for each kind of response. The initial values for B–C and A–C ranged between − 5 and + 10 mV

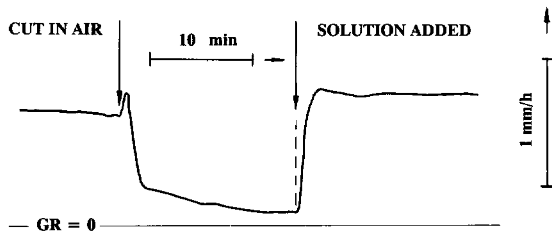

Fig. 5. Excision of the basal epicotyl of a pea seedling in air resulted in a drastic and lasting drop in the GR of the remote apical part of the remaining pea seedling. This was frequently preceded by a short increase in GR during the first seconds after the cut. The subsequent immersion of the cut end into solution then led to a burst in GR overshooting initial values and remaining at these high values for at least 10 min. For these measurements, shorter seedlings (60–80 mm) were fixed with epoxy resin to a heavy, metallic holder, so that the GR of the apical 20 mm could be measured free of disturbance when the basal part was excised 60 mm below the hook

arrive at C, where it also peaked after an additional 2 min. Slowly growing plants exhibited only small changes in GR and small propagating V_s signals (Fig. 6, example 2), despite relatively strong V_s changes in B (not shown). Another, even larger burst in GR and water uptake, which appeared after temporary withdrawal of water

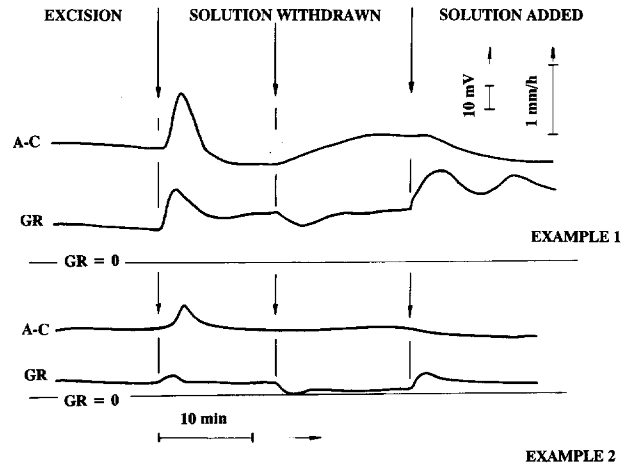

Fig. 6. Simultaneous recordings of V_s and GR were made with pea seedlings in a vertical position and the solution in the cut compartment kept in place by a Vaseline-sealed cover glass. Excision resulted in an immediate rise in GR, followed 30 s later by a negative V_s shift at position C. Note that a second and larger growth acceleration (achieved by withdrawal and resupply of solution to the cut) did not induce another signal, either at B nor C. In a slowly growing seedling, excision-induced changes in GR and V_s were both reduced *(example 2)*. Original trace recordings chosen to represent 14 repetitions. The *thin horizontal lines* indicate zero growth rate

from the cut and subsequent resupply, however, failed to produce a depolarization at B or at C. These results indicate that immediately after excision epidermal cells respond to water uptake with a large depolarization, and this ability or sensitivity was apparently lost subsequently. All attempts to induce a slow wave of depolarization in excised pea epicotyls failed. This indicates a one-time character of the excision-induced depolarization.

Our next step was to study the mechanism by which the hydraulic surge causes the transient depolarization. Cell-input resistance (R_{in}) was measured to address the question of whether the V_m changes were caused by the opening of channels or by the rapid shut-down of the plasmalemma proton efflux pump or a combination of both mechanisms. Proton pumps in higher plants have been characterized as current sources which bring about V_m changes without comparable resistance changes (Felle 1981) while channels have a conductivity per unit which is by orders of magnitude larger than that of pumps (see Bentrup 1989) and therefore change both membrane potential and membrane resistance. We measured R_{in} values ranging from 6 to 9 MΩ, which is in reasonable agreement with other estimates of plant cells in tissues (Higinbotham et al. 1964; Spanswick 1972; Drake et al. 1978). We found no detectable input-resistance change accompanying the large depolarization or the repolarization (Fig. 7). This result indicates that the change in V_m did not involve large ion fluxes coupled to it but rather points to a shut-down of the proton pump. Lack of change in the resistance does not prove beyond any doubt that there is no involvement of channels in the depolarization. A complex response involving opening of anion channels (depolarizing) and closing of other channels might result in a constant membrane resistance.

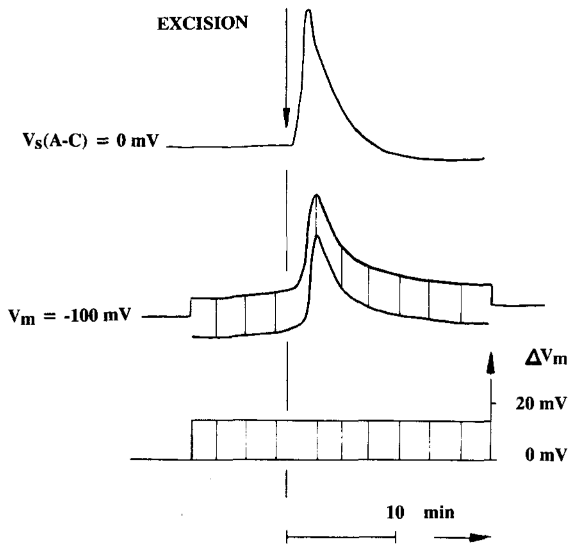

Fig. 7. Simultaneous recording of the excision-induced depolarization and R_{in} in a subepidermal cell near C together with the V_s shift at C (A–C trace). Two lines above and below the V_m trace were drawn from the voltage deflections (ΔV_m) brought about by rectangular current pulses of I = 2 nA, which alternate in depolarizing and hyperpolarizing directions. ΔV_m is proportional ($x2I^{-1}$) to the size of the R_{in}. Original trace recording for a subepidermal cell representing seven similar repetitions in epidermal and subepidermal cells

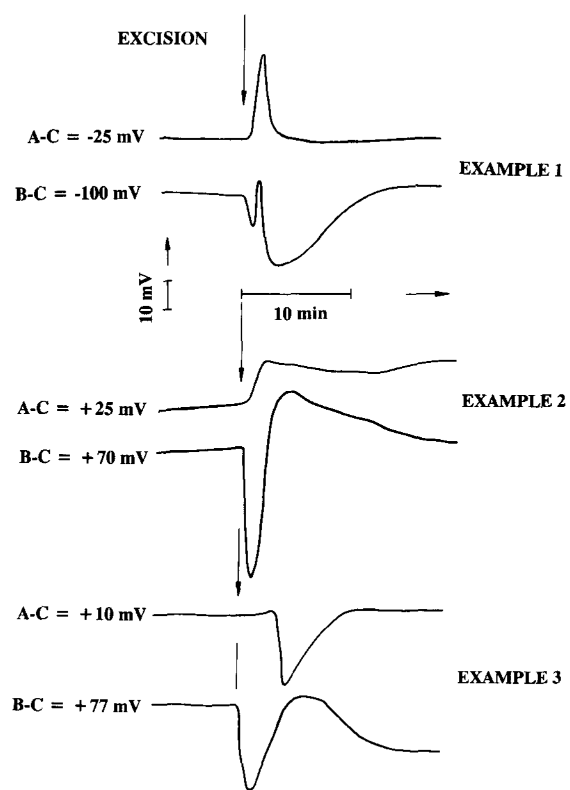

Fig. 8. The effect of cyanide upon the development of the excision-induced electric signal. In these experiments the 10 mM KCl content of the agar making contact between electrode and epicotyl surface was replaced by 10 mM KCN at position B *(example 1)* or at position C *(examples 2, 3)*. Note that CN reduced the depolarization size and that the repolarization process was especially slowed and crippled. *Examples 1* and *2* represent at least six similar repetitions each. *Example 3* is a unique recording where the signal goes from B all the way to A, passing through the poisoned position C without changing the V_s

Likewise, involvement of channels with sigmoidal current-voltage curves (e.g. Bertl and Gradmann 1987) might also fail to show R_{in} changes, under some circumstances. Furthermore, current leakage through plasmodesmata may lead to an underestimation of the membrane resistance by our R_{in} measurement (Spanswick 1972). It should be noted, however, that any current leaking into neighboring cells must still cross a membrane before returning to ground.

Another attempt to find a possible coupling between water and potassium influx was made by using the K-influx inhibitor tetraethylammonium chloride (TEA). Ten-millimolar TEA replaced the KCl in the agar of the surface contact in position C without changing the shape or magnitude of the depolarization (not shown).

To test further the hypothesis of an involvement of the proton pump in the excision-induced depolarization, we inhibited it by replacing KCl in the agar contact of one electrode position (at a time) by KCN. The CN-induced cell depolarization at the treated position was exhibited as a long-lasting negative V_s shift. The KCN treatment reduced the excision-induced depolarization in magnitude and rate in the treated region, i.e. position B (Fig. 8, example 1) and position C (Fig. 8, examples 2 and 3). When compared with control responses, the depolarization was reduced by 50–100%. Particularly affected by KCN was the rate of repolarization. However, KCN did not prevent the passage of the response through the position and its subsequent development in the apical positions (Fig. 8, examples 1 and 3). This is particularly well illustrated in the third example where the signal passed from position B to A, while it was suppressed in the treated position C between them. This result shows that the signal can transverse through poisoned areas and hence supports its hydraulic rather than its electric nature. The inhibition of the excision-induced signal by KCN supports our tentative conclusion that it is caused by a rapid shut-down and resumption of active proton export.

Discussion

It has been suggested that a change in xylem pressure (P_x) may be an important initial factor in the growth reduction which occurs upon sudden exposure of plants to water stress (Acevedo et al. 1971; Nonami and Boyer 1990). Okamoto et al. (1984, 1989) report that even small P_x changes result in considerable and lasting GR changes in perfused *Vigna* stem segments. Our experiments indicate that a P_x increase following excision of the pea epicotyl base increases the GR of the excised segment. This increase in the GR, however, was only a temporary one (Fig. 6), probably because of consequential adjustment of wall properties (Acevedo et al. 1971; Cosgrove 1988). A transient hydraulic surge and increase in GR

come about because the hydraulic resistance of the root is removed and the negative pressure in the xylem of the pea epicotyl segment is relieved. Whereas pea seedlings respond to underwater excision with a *rise* in the epicotyl GR, cucumber seedlings show a steep *drop* in the hypocotyl GR under similar conditions (Cosgrove 1987; Stahlberg and Cosgrove 1990). As cucumber seedlings have positive P_x (as manifested by exudation from their cut surfaces), this difference in the response supports our proposal that the sign of P_x determines the direction in the GR of the plant stem upon excision.

Our results also showed that the transient growth surge was followed closely in time with a transient membrane depolarization (30–50 mV) which propagated from the cut surface at about 1 mm $\cdot$ s^{-1}. The velocity, duration and shape of the signal characterize it as a *slow-wave potential* (SWP) (Tables 1, 2).

Excision itself, when carried out in air, did not give rise to an SWP. It induced a depolarization which was confined to cells near the cut site, did not propagate, and was newly induced after each cut. This kind of electric response seems to be a general *wounding effect*, which has been found in many plants after excision or mechanical disturbance (Koopowitz et al. 1975; Mertz and Higinbotham 1976; Zerrenthin and Stahlberg 1981). Our results confirm the occurrence of local depolarizations that are apparently associated with a wounding effect. The apparent kinetic similarity of the wound response and the propagating signal may be based on similar mechanisms (Chastain and Hanson 1982).

Only excision under solution and a surge in GR caused the depolarization of epidermal cells remote from the cut region. This indicates that this depolarization is dependent on and coupled to the surge in water uptake. Little is known about the mechanism by which the water influx could cause such a depolarization. A large influx of water is likely to be accompanied by growth-induced stretching of the membranes (Coster et al. 1977), which may directly affect active and passive elements of membrane transport. Stretch-activated ion channels have been characterized in *Vicia* guard cells (Cosgrove and Hedrich 1991), tobacco suspension cells (Falke et al. 1988) and many animal cells (Morris 1990). Membrane tension has never directly been shown to activate or inactivate pumps. However, there are several studies supporting the turgor sensitivity of proton pumps (Rubinstein 1977, 1982; Stahlberg and Polevoy 1979; Stahlberg 1980; Kinraide and Wyse 1986). Our R_{in} studies do not demonstrate any large conductance changes and do not indicate the involvement of large ion fluxes in the depolarization, although indirect involvement of stretch-activated channels cannot be excluded (i.e. the opening of a stretch-activated Ca channel might lead to a calcium influx and to pump inactivation without being significant enough to cause a measurable change in R_{in}). The lack of R_{in} changes during the large depolarization and repolarization is more in agreement with the concept of a current change in a pump (Felle 1981), whose unit conductance is much smaller than that of channels (compare Bentrup 1989). The involvement of proton pumps in the generation of the propagating depolarization is further supported by our studies with cyanide, which should reduce ATP levels and thus inhibit the pump. Cyanide did inhibit the depolarization and reduce the rate of repolarization. Thus, our results point towards the presence of a proton pump whose activity is transiently inactivated by membrane stretch to prevent excessively high growth rates. Further studies, however, are necessary to prove its existence.

Whatever the mechanism, the excision-induced depolarization appears to be a *one-time event*, or at least to have a long refractory period. Repeated cuts did not elicit additional propagating depolarizations, nor did the growth surges which occurred when stems were placed in air and then back in water. This one-time response may indicate changes in the characteristics of the membrane, which made it less sensitive to further events of membrane stress. It is worth noting that a similar phenomenon has also been found in the wound-induced depolarization in *Bidens pilosus* (Julien et al. 1991). The role of such a signal in normal plant life has still to be demonstrated. Changes in P_x, however, are known to be a part of plant life on a daily and seasonal basis. They have been shown to be a direct path of root-to-shoot communication, and to induce stomatal responses rapidly (Raschke 1970). Our results and similar findings in leafy wheat plants (Malone and Stankovic 1991) show that another rapid shoot response to P_x changes is the mass depolarization of epidermal cells.

In the *Introduction* we listed three concepts concerning the mechanism of propagation of SWPs. The propagation speed of 1 mm $\cdot$ s^{-1} over a distance of 30 mm in these seedlings is too high to result from the transport of a depolarizing substance in the xylem flow. It is also unlikely that the depolarization is transmitted electrically, i.e. propagating passively from cell to cell. Electrotonic signal propagation over distances larger than 35 mm (C) or even 65 mm (A) without attenuation would require a coupling ratio of approx. 100% among the epidermal cells (coupling ratio is the efficiency of passive electric-signal transmission from one cell to the next). This requirement conflicts with even the highest estimates for coupling ratios in higher plant tissues: 20% in *Elodea canadensis* (Spanswick 1972) and 20–50% in a longitudinal direction for oat coleoptiles (Racusen 1976), or 8% with the same object in a later study (Drake et al. 1978). Only algae of the *Chara* type have been shown to have coupling ratios of approx. 80% between the node cells and even that is not enough to guarantee the transmission of an action potential from one internodal cell to the next one (Sibaoka 1966; Spanswick and Costerton 1967; Sibaoka and Tabata 1981). Electrical coupling, however, may play a role in the coordination of the mass depolarizations successively and acropetally along the plant axis with the xylem pressure change.

Direct evidence for a hydraulic mechanism of SPW transmission comes from the finding that a propagating depolarization only occurs after a surge in water uptake. Cyanide suppresses this response at the application point. The transmission of the depolarization signal and its development behind the poisoned position, however, was not affected by cyanide. This is also in agreement

with a hydraulic rather than an electric signal transmission. A pressure signal can easily pass through a poisoned area and then be translated into the electric signal beyond this region. A hydraulic mechanism was also proposed for the propagation of a wound-induced SWPs in wheat plants, where negative V_s shifts were shown to travel together with turgor increases in the epidermal cells (Malone and Stankovic 1991).

Propagating depolarizations have been found to occur as action potentials and SWPs (see *Introduction*). Our results indicate that the propagating signal in pea seedlings is not an action potential (see Davies 1987), but an SWP. The concept of a propagating signal which is based on fast changes of active pumps (as opposed to altered channel activities in action potentials) was independently put forward for *Acetabularia* ("metabolic" action potentials; Gradmann 1976) and *Bidens pilosus* (Julien et al. 1991). Our results indicate that the SWP type of a propagating depolarization differs from action potentials not only in its electrogenic nature but also in its mechanism of propagation. Whereas action potentials move electrotonically with a high speed in the phloem of higher plants (Haberlandt 1914; Sinyukhin 1964; Retivin and Opritov 1987; Eschrich et al. 1988), SWPs seem to propagate hydraulically and with a lower speed in the xylem.

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