Voltage Changes Along Geranium Petioles after Leaf Blade Excision

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ABSTRACT

Voltage changes were measured along petioles of geranium (*Pelargonium hortorum*) plants after leaf blade excision. These voltages were detected using a non-invasive, non-polarizable, Ag/AgCl measuring electrode, which was located between 20 and 50 mm from the excision site.

The initial onset of these voltage responses was so rapid that it occurred while the excision was still in progress. After a few seconds, voltage changes typically attained maximum values ranging from 1 to 20 mV, and then returned slowly toward zero. In most cases the predominant voltage change was negative, but in some cases the change was predominantly positive.

In addition to the voltage response to the first cut, a voltage change also occurred in the same petiole after a second cut. This second cut response was usually much smaller than the first.

Experiments using the Scholander pressure bomb indicated that geranium xylem vessel fluid is under a tensile stress ranging from 100 to 300 kPa.

Thus, whenever a leaf was excised, the forces acting on this xylem fluid were immediately unbalanced. This imbalance would have caused the fluid to be accelerated away from the cut until a new balance was achieved.

The resulting fluid motion appears to be responsible for the petiole voltage change which occurred immediately after each excision.

INTRODUCTION

Many authors have reported on the electrical voltage changes which occur in plants as a result of wounding (e.g. Houwink, 1935; Pickard, 1973, 1974; Sibaoka, 1969; Umrath, 1962; Van Sambeek, and Pickard 1976*a,b*).

Previous work has suggested that, in some cases, the onset of the voltage response to wounding is abrupt (Pickard, 1973). However, it is not clear just how sharp this onset really is, or exactly how long a time elapses from the beginning of the wound to the onset of the response.

Our work establishes a well-defined initial time by rapidly excising a leaf blade. We then focus on the initial onset, and the early changes, in the voltage along a geranium petiole in response to this excision.

We shall examine the implications of these data with the objective of clarifying the mechanism responsible for the observed voltage changes.

MATERIALS AND METHODS

All measurements were made on 1-year-old geranium plants, grown in potting soil, and fertilized twice-weekly with household plant fertilizer. For each experiment, one plant was placed in an electrically shielded cage, and one of its petioles was firmly taped to a solid, vibration-damped, wooden base. This base also supported the cutting arm, in which a razor blade could be rigidly mounted in either of two locations (Fig. 1). This design made it possible to make a second cut on the same petiole, 3 mm toward the stem from the first cut. The cutting arm, and the platform supporting the petiole, were designed so that the blade cut all the way through the petiole and continued on until all contact between the two was broken. The cutting arm was moved by hand, and the cutting times ranged between 10 and 50 ms.

The electrical voltage changes along the petiole were transmitted to an electrode through a small drop of conducting gel on the surface of the petiole, located between 20 and 50 mm from the site of the cut. This gel was prepared using 0·1 M KCl, gelled with 1% agar.

The electrode, in contact with the drop of gel, consisted of a glass micropipette which was half-filled with the same gel. One end of a Ag/AgCl electrode wire was inserted through the wide end of the micropipette into the gel, while the other end of this wire was soldered to a shielded cable which was connected to the input terminal of a teledyne Philbrick amplifier (Model 4253). The output of the amplifier was connected to one channel of both a Tektronix oscilloscope (Model 466), and a modified Astro-Med dual-channel chart recorder (Model Dash 2). The ground electrode was located either in the potting soil, or at the base of the petiole being cut. The frequency response of the entire system was such that a step function voltage, sensed by the electrode, appeared to change instantaneously at the oscilloscope sweep speeds used in the experiment.

The oscilloscope was triggered when the cutting arm hit a toggle switch just before the blade touched the petiole. To mark the instant at which the cutting blade first touched the petiole, a 100 pF capacitor, in series with a 6 V battery, was connected between the cutting blade and the ground.
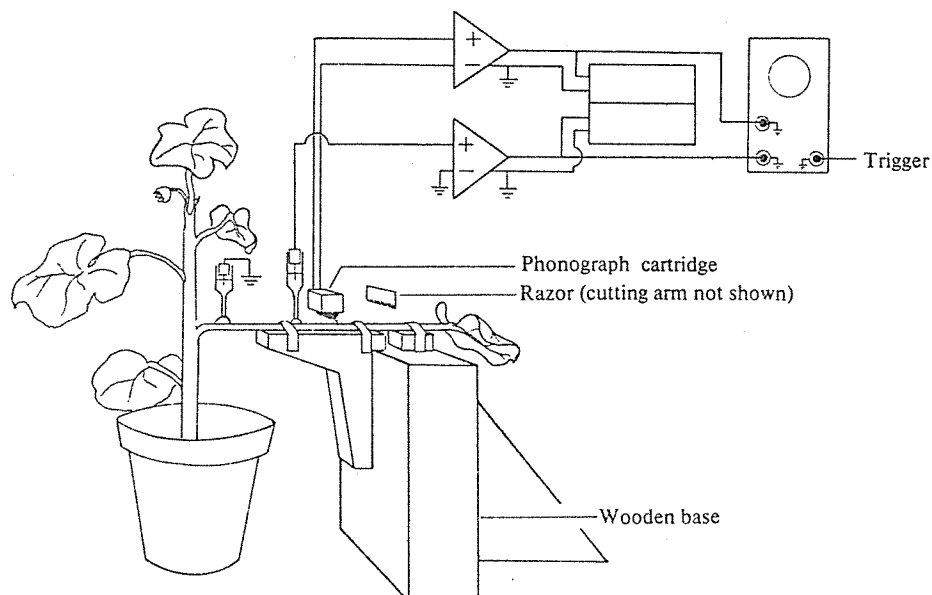

FIG. 1. A schematic diagram of the experiment.

When contact was made, this provided a short pulse of current through the petiole, which appeared as a voltage pulse across the petiole resistance between the ground and the sensing electrode.

Mechanical vibrations along the petiole, which can produce spurious voltages, were monitored with a phonograph pick-up positioned as close as possible to the sensing electrode. These vibrational signals were amplified and displayed in the second channel of both the oscilloscope and the chart recorder (Fig. 1).

In the experiment, the electrode was checked for electrical stability, and then each channel was electronically zeroed. A test run was made, without a blade in the cutting arm, to check the vibrational stability of the electrode. When the electrode was stable, we made first and second cut runs. In many instances these were followed by a final check on the vibrational stability of the electrode.

In addition to the electrical measurements, we made tension measurements on the petiole xylem fluid using a Scholander pressure bomb (Scholander, Hammel, Bradstreet, and Hemmingsen, 1965).

RESULTS

We made simultaneous measurements of the mechanical vibration amplitude and the change in the electrical voltage following both first and second petiole cuts on 20 different geranium petioles. Data from a representative run, including first and second cuts, are shown in Figs 2 and 3. On six runs the voltage was displayed on an oscilloscope, as well as on the chart recorder. The remaining 14 were only displayed on a chart recorder. Although there was considerable variation in the shape, magnitude, and sign of the voltage response from one plant to the next, there was often: (1) a sharp rapid rise in the voltage, starting as soon as the blade began to cut the petiole, and reaching a maximum of ~5 mV about the time the cut was completed; (2) a smooth rapid drop in voltage, starting about the time the cut was completed, and reaching a minimum of ~20 mV within 2 s; and then (3) a slow recovery to a voltage usually near the pre-wound value.

Figure 2A shows the results of the initial vibrational test run. The vibrations are shown in the top channel (ch 1) and the electrode voltage vs time in the lower channel (ch 2). No voltage change was observed in response to the test vibrations.

Figure 2B shows the first cut voltage response in channel 2. The sweep speed is 50 ms cm^{-1}, the voltage sensitivity is 20 mV/large division, and the amplifier gain is 25. The initial negative marker pulse, at ~10 ms, indicated the instant at which the cutting blade contacted the petiole. The second marker pulse, at ~80 ms, was caused by charge flowing back along the petiole to ground after the blade cut all the way through the petiole, and all contact had been broken. The increase in the voltage in channel 2, while the petiole was being cut, is clearly observable. It is also clear that the amplitude of the vibrations during and after the first cut were smaller than the vibration amplitudes created during the test run. The chart recording of these same events is shown in Fig. 2C. The voltage sensitivity is 20 mV mm^{-1}, the chart speed is 0·5 cm s^{-1}, and the amplifier gain is 25.

A similar sequence of events, associated with a second cut, is shown in Fig. 3A, B, and C. A small marker pulse is observable ~10 ms after the sweep has begun. The voltage dropped sharply immediately after this pulse. The chart recording (Fig. 2C) shows this same response over a longer time interval. The scales are identical with those in Fig. 2.

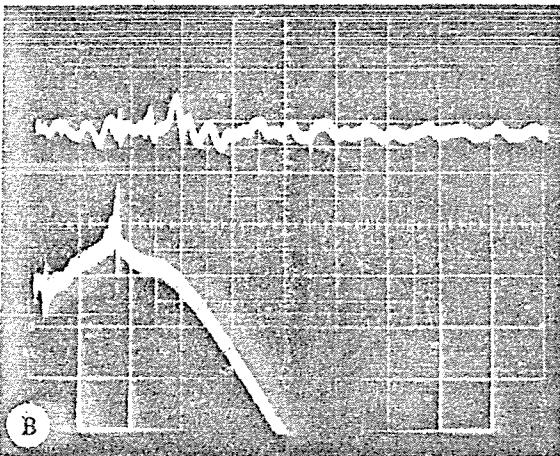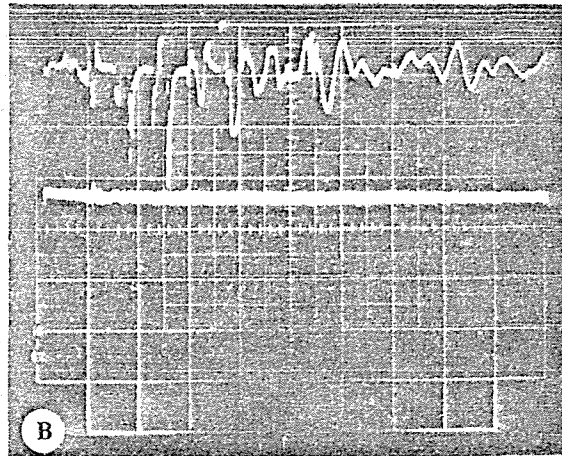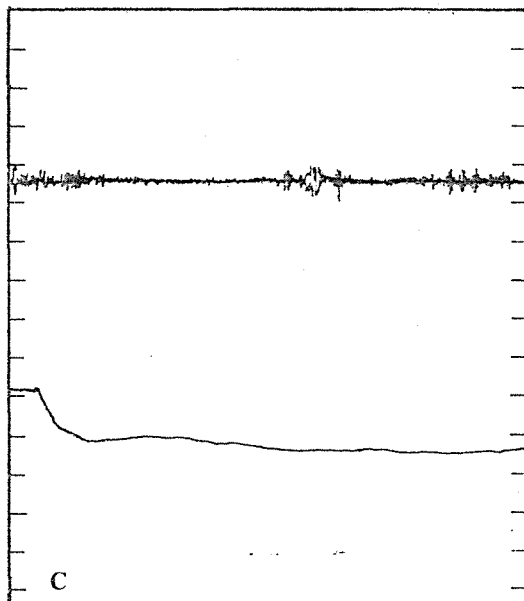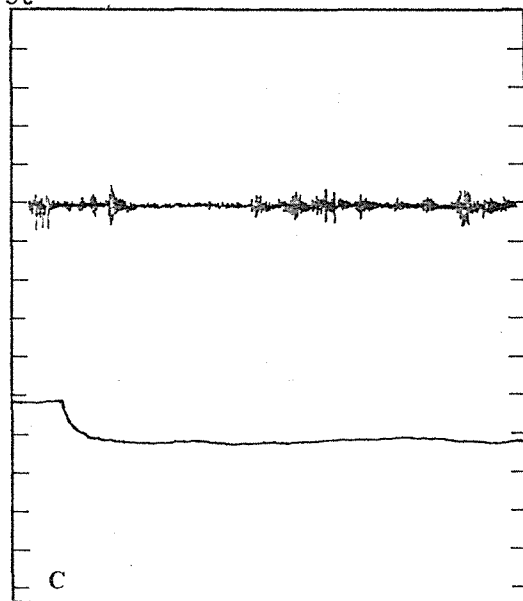Checks were made to establish that this capacitor discharge, in the absence of a
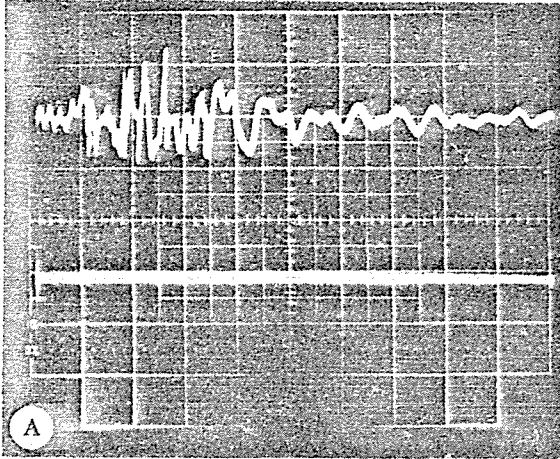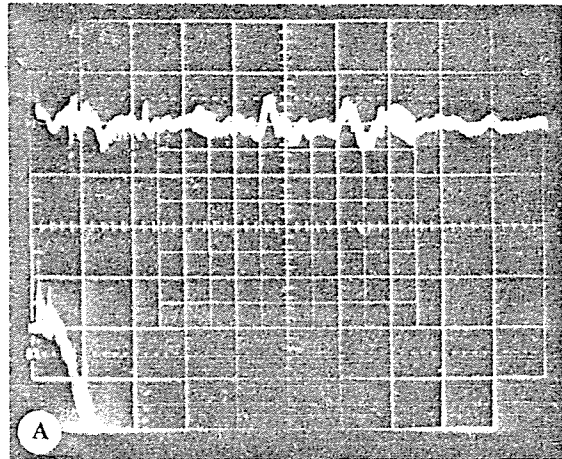
cut, produced no change in the petiole voltage, other than to provide a narrow marker pulse.

In addition to the runs made under standard conditions, we made three runs in which the petiole tissue between the cut and the sensing electrode was placed in contact with ice and water until just before the run was started. Although the onset of the voltage response was just as prompt as it was in earlier runs, in each of these three cases, the direction of the change was predominantly positive, rather than negative.

On two separate occasions we stopped the cutting arm after severing the petiole such that the razor blade blocked the severed end for a few seconds. A sharp voltage drop was observed immediately after the blade was lifted out of the wound.

Two more runs were made which compared the voltage response of a live and dead petiole. In each case the entire leaf was first broken off at the basipetal end of the petiole. One leaf was immersed in liquid N_2 and then removed and allowed to return to room temperature. Each leaf was then placed in the cutting apparatus and the leaf blade was excised. The dead petiole showed no response to excision, while the control gave a clear voltage change.

In addition, five measurements were made on detached geranium leaves using a Scholander pressure bomb. These leaves had not been used in the excision experiments. Bomb pressures ranging from 100 to 300 kPa were required to bring the xylem fluid back to the surface of the cut.

DISCUSSION

Mechanical vibrations, having sufficiently large amplitudes, are capable of generating spurious voltage signals in the electrode system used in this experiment. Since it is not possible to cut through the petiole without vibrating the electrodes to some extent, it is important to establish that the voltage changes which were observed following the cuts were not merely electrode artefacts produced by the vibrations. Consequently, prior to each of the first 20 runs, test vibrations were generated by going through all the motions of a cutting operation, without having a blade mounted in the cutting arm. These test vibrations were recorded and their amplitude compared to the vibrations generated by the leaf excision. In any case where the excision vibrations had a larger amplitude than the test vibrations, a second vibration test run was made where the vibrations generated had comparable amplitudes to those associated with the cut. In all runs reported, the electrical

FIG. 2. A. A vibration test run (no cut) showing the vibration amplitude in the top channel (ch 1) and the electrode response in the bottom channel (ch 2). The vertical scale is 20 mV/large division in both ch 1, and ch 2. The horizontal sweep rate is 50 ms/large division. The amplifier gain is 25. B. The voltage response to the first cut through the petiole. The horizontal and vertical scales and amplifier gain are all the same as in A. The peaks at ~10 ms and 80 ms are small capacitive discharges which mark the beginning and ending of the cutting process. C. A chart recording of the same vibration test and first cut as were shown in A and B. The vertical scale is 5 mV mm^{-1} in channel 1 and 20 mV mm^{-1} in channel 2. The chart speed is 5 mm s^{-1}. The amplifier gain is 25.

FIG. 3. A. The second cut response on the same petiole used in Fig. 2. (All scales are those given in Fig. 2A and B.) B. A final vibration test to check the electrode stability under mechanical shock. C. A chart recording of the second cut response. (Scales are the same as in Fig. 2C.)

response to the test vibrations was negligible compared to the voltage changes observed in response to wounding.

As a result of these observations, we conclude that the observed voltage changes were genuine responses to the cut, and not vibrational artefacts.

Two striking features of the wound response voltage are: (1) the rapidity with which the onset of this response follows the cut (we have detected a voltage change as far as 50 mm from the wound site, that began within 10 ms of the time that the blade started cutting through the petiole); and (2) there is no delay in the onset time as a result of chilling the petiole tissue with ice water.

These features have a number of important implications:

1. The very short delay time associated with the initial onset of the wound response voltage makes it very unlikely that any chemical substance (i.e. Ricca's factor) could travel from the wound site to the electrode, in the observed time interval. Thus the wound response mechanism reported by Van Sambeek and Pickard (1976a) does not seem able to account for the voltage changes observed in this experiment.

2. Since action potentials have been reported not to travel through chilled tissue in mimosa (Houwink, 1935), the ease and rapidity with which these voltage responses passed through chilled tissue strongly suggests that the cell-to-cell propagation associated with an action potential is not involved here.

3. The speed of response, and relative independence of the biological state of intervening tissue, strongly suggest that a physical rather than a chemical or biological mechanism is basically responsible for the observed voltage changes. A reasonable first step toward clarifying such a mechanism would be to look for a physical driving force which would be capable of setting the observed effects into motion.

Our pressure experiments indicate that the xylem fluid in the petiole is under considerable tension prior to the excision of the leaf. Other forces, such as capillarity and viscous drag forces, are also acting on this fluid. In the steady state operation of the plant these forces are all balanced. However, when the xylem vessels are severed, the forces acting on the vessel fluid are immediately unbalanced. In addition, if the normal operating pressure in the xylem vessels is below atmospheric pressure, the air will then push on the freshly exposed ends of the vessel fluid, and will act as a new unbalancing force. The fluid will then be accelerated, under the combined action of these forces, until a new balance is achieved. It is well known that a moving fluid is capable of generating electrical potential differences (Tyree and Fenson, 1968). This fluid flow hypothesis is certainly supported by our two observations where lifting the cutting blade, and unblocking the end of the petiole, produced a sharp drop in the observed voltage. Furthermore, there appears to be no reason to believe that the presence of a chilled region in the petiole would, necessarily, reduce the promptness with which a voltage change would be initiated in an unchilled portion of the petiole.

The question arises, however, how could such a mechanism account for the observed second cut responses? If the petiole vessels contain blocking structures, such as end walls, which would prevent any rapid change in the motion of the

petiole fluid, then a number of vessels could have one of these structures lying within 3 mm of the first cut. A second cut, 3 mm downstream, could then unblock these vessels and open the way for the observed second cut response.

Thus we consider that all of our data are consistent with the hypothesis that the excision process produces a sudden movement of xylem fluid away from the cut, and that this movement is responsible for the petiole voltage changes observed immediately after wounding.

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