Video Article

A New Application of the Electrical Penetration Graph (EPG) for Acquiring and Measuring Electrical Signals in Phloem Sieve Elements

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Abstract

Electrophysiological properties of cells are often studied *in vitro*, after dissociating them from their native environments. However, the study of electrical transmission between distant cells in an organism requires *in vivo*, artifact-free recordings of cells embedded within their native environment. The transmission of electrical signals from wounded to unwounded areas in a plant has since long piqued the interest of botanists. The phloem, the living part of the plant vasculature that is spread throughout the plant, has been postulated as a major tissue in electrical transmission in plants. The lack of suitable electrophysiological methods poses many challenges for the study of the electrical properties of the phloem cells *in vivo*. Here we present a novel approach for intracellular electrophysiology of sieve elements (SEs) that uses living aphids, or other phloem-feeding hemipteran insects, integrated in the electrical penetration graph (EPG) circuit. The versatility, robustness, and accuracy of this method made it possible to record and study in detail the wound-induced electrical signals in SEs of central veins of the model plant *Arabidopsis thaliana*[1]. Here we show that EPG-electrodes can be easily implemented for intracellular electrophysiological recordings of SEs in marginal veins, as well as to study the capacity of SEs to respond with electrical signals to several external stimuli. The EPG approach applied to intracellular electrophysiology of SEs can be implemented to a wide variety of plant species, in a large number of plant/insect combinations, and for many research aims.

Video Link

The video component of this article can be found at http://www.jove.com/video/52826/

Introduction

The ability to produce long-distance electrical signals is an advantageous trait of multi-cellular organisms that allows for efficient responses to external stimuli. This trait has evolved independently in plants and animals, and thus represents a case of convergent evolution. Given that electrical signals are coupled with important functions in animals such as neural transmission and muscle contraction, the molecular basis, mechanism of transmission, and function of stimulus-induced electrical signals in animals are subjects of intensive research. In contrast, stimulus-induced electrical signaling in plants has received little research attention. Although plants have no nerves or muscles, there seems to be enough evidence to assume that stimulus-induced electrical signals in plants play a key role in their responses to environmental factors.

The phloem, the living component of the plant vasculature, has been postulated as a major substrate for the transmission of stimulus-induced electrical signals, from stimulated/damaged to non-stimulated/undamaged areas[2]. The main cells in the phloem are the sieve elements (SEs), relatively simple, elongated cells. The ends of SEs are connected to other SEs, forming a continuous, low-resistance, sieve tube system that is spread throughout the plant. There are, however, very few studies on the electrical properties of these highly specialized cells. In these previous studies, researchers accessed SEs with either glass micro-electrodes[3] or with glass electrodes that were coupled to plant-inserted stylets of aphids, after stylectomy (cutting)[4]. Glass microelectrodes are made from glass capillaries that are pulled at one end with heat into a fine tip of less than 1 µm in diameter, and then filled with a KCl solution. A Ag/AgCl or platinum wire, inserted into the KCl-filled glass electrode is then connected to the amplifier input, and a referent electrode is inserted into the bath surrounding the cell of interest, completing the circuit. This setup records the difference in potential between the extracellular referent electrode and the intracellular measuring electrode, *i.e.,* the membrane potential of the cell[5]. With this method, Umrath made the first intracellular recording from a plant cell, using the algae *Nitella*[6,7]. *Nitella* is a relatively simple organism with large cells, and therefore amenable to intracellular electrophysiology experiments. In contrast, the insertion of intracellular glass electrodes into the small cells of multi-cellular, three-dimensional terrestrial plants is technically demanding, requires a highly skilled researcher, as well as sophisticated visualization, micromanipulation, and anti-vibration equipment. Although glass electrodes are suitable to record from superficial cells in plants, such as root epidermal cells[8], intracellular recordings from cells deeply embedded in the plant's tissue, such as SEs, very likely cause damage-induced responses, confusing the results. In 1989, Fromm and Eschrich reported the use of an alternative method, called the 'aphid method', in which glass electrodes are coupled to aphid stylets after stylectomy[4]. The aphid method is minimally invasive, because flexible stylets do not cause tissue or cell damage as glass electrodes do. Aphid stylets are nature's great invention for plant penetration, and aphids are considerably more skilled than humans in finding SEs. Unfortunately, this aphid method is also highly

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demanding in terms of technical expertise and equipment. In addition, the success of each experiment that implements this technique depends entirely on the aphid being in feeding mode — with the stylet stably inserted into a SE, at the time of stylectomy. Thinking in retrospective, one can see that the odds of success of this technique could have been improved by adding to the experimental setup an instrument that allows identifying whether or not the aphid stylet is in the SE when applying stylectomy.

In 1964, McLean and Kinsey described an 'electronic monitoring system' for the study of the feeding behavior of aphids in real time[9,10]. In this system, the aphid and the stylet-penetrated plant were integrated into an electrical circuit. Later, in 1978, Tjallingii devised a modified version of the system, called the 'Electrical Penetration Graph' (EPG) system[11,12]. Whereas the original electronic monitoring system was sensitive to the resistance-originated potentials only, with the EPG system, the electromotive force (emf) originated potentials, *i.e.,* generated in the plant or in the insect, could be recorded in addition to potentials arising from resistance (R) in the insect. This represents an important improvement, because both signal components, emf and R, provide biological relevant information on events during plant penetration by aphids. What makes the EPG pre-amplifier sensitive to the R-components is its relatively low input resistance of 1 GΩ, which is close to the average of the plant/aphid resistance. A small offset voltage (**Figure 1**, V) of approximately +100 mV is applied to the plant, which then is divided across plant and insect on one side, and the input resistance on the other. The voltages and their changes are measured at a point (**Figure 1A,B**) between the insect and the input resistor. Therefore, the R-components represent plant-aphid resistance modulations of the offset voltage, whereas the emf-components are a certain fraction of plant potentials at the stylet tip and potentials caused in the insect. The plant potentials — most relevant here — are mainly membrane potentials of the plant cells punctured by the aphid stylets. The insect potentials appear to be mainly streaming potentials caused by fluid movements within the two stylet canals, *i.e.,* the food and the salivary canals; no internal nerve or muscle potentials are recorded in the EPG. In practice, the stylet tip functions as an electrode tip. All plant cells are negatively charged inside relative to the positive outside of the cell. The electrical current (*i.e.,* the movement of charged ions in watery solution) flowing from the inside to the outside and *vice versa* is very limited due to the high resistance of the cell membrane. Normally the resting potential is kept constant. However, when negative ions move out or positive ions move in through the cell membrane, the membrane potential is reduced, *i.e.,* it 'depolarizes'. Depolarization occurs in case of cell excitation. Ions then move in or out when specific ion channels in the membrane are opened or when the membrane is damaged and ions leak in and out. All cells have ion channels and pumps in the plasma membrane that bring the membrane potential to its resting level by restoring the original concentration of various ions inside the cell. The resting potential and its changes are emf components, and therefore, the EPG technique is suitable to measure them.

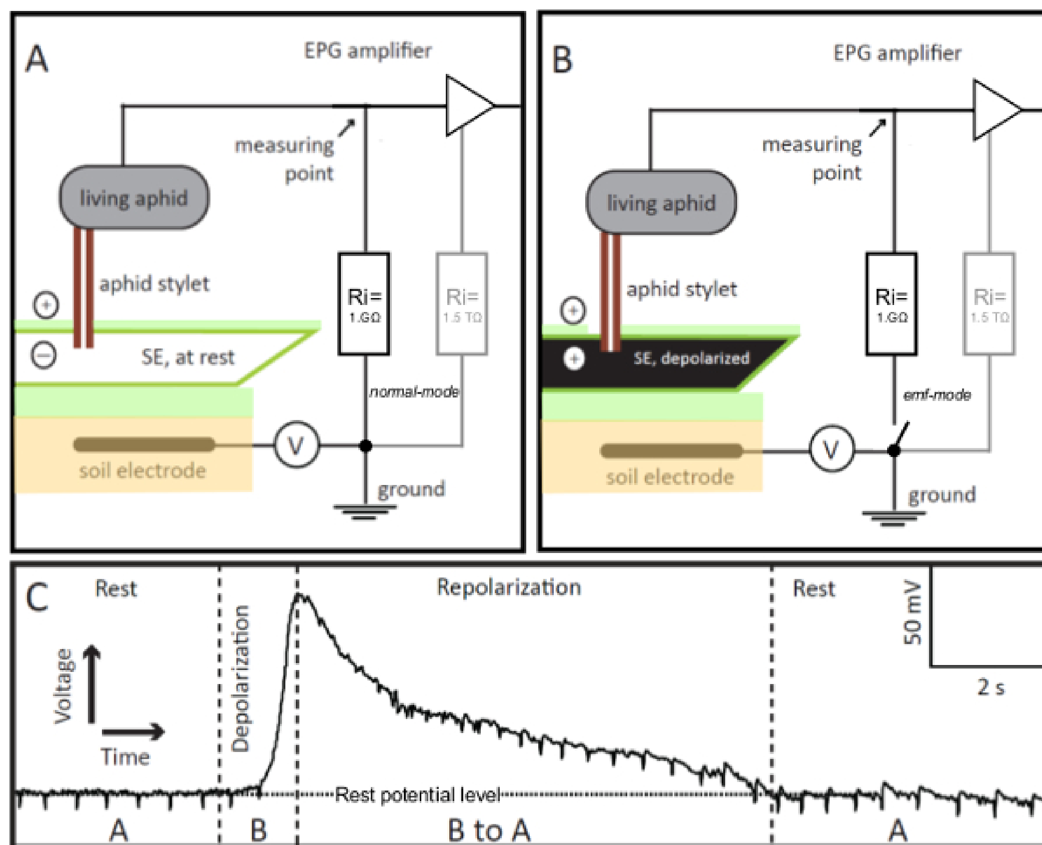

Figure 1. EPG-electrodes. The EPG-electrode is a living aphid integrated into the Electrical Penetration Graph (EPG) circuit, whose stylet is inserted into a sieve element (SE) in stable feeding mode. If the stylet-impaled SE is at rest (panel A), the voltage in the circuit, recorded by EPG, is stable and at the resting potential level (Panel C, Rest). If the SE is excited, its membrane depolarizes (panel B), which is visualized in the EPG as a gradual increase in voltage (panel C, Depolarization). As the ionic balance in the SE returns to rest, *i.e.,* it repolarizes, the voltage recorded by EPG gradually decreases to the rest potential level (Panel C, Repolarization). In panel C, “A” and “B” refer to the scenarios shown in panels A and B, respectively. V = Adjustable offset voltage source. Ri = Input resistor. In parallel to the 1 GΩ external resistor, the amplifier has an internal (in the OpAmp) high 1.5 TΩ resistor (panels A and B, in gray). By remote control of the switch the EPG pre-amp can be changed from normal to emf-mode, which allows obtaining highly accurate voltage values. Please click here to view a larger version of this figure.

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In the next section, we provide the reader with a basic protocol for performing EPG experiments that is valid for both insect-focused and plant-focused studies.

Protocol

1. Aphid Rearing

Note: The choice of plant and aphid species for EPG recordings depends on the research aim. For studies on *Arabidopsis thaliana*, the aphid *Brevicoryne brassicae* is appropriate.

1. Rear *B. brassicae* aphids in a greenhouse on *Brassica oleracea*. Keep the plants used for aphid rearing in cages, in order to avoid contaminating other plants. Keep aphid-rearing plants and experimental plants (in our case *B. oleracea* and *A. thaliana*) in separate rooms, in order to avoid contamination of experimental plants with aphids.
2. Transfer aphids to fresh plants about every 2 weeks, before causing significant plant damage, or reaching overpopulation. Transfer 10-20 adult aphids to a fresh rearing plant to initiate a new colony.
3. Monitor rearing plants regularly for contamination by unwanted aphid species, other insect herbivores, aphid parasitoids, and fungi that may affect the health of the aphid colony.
4. Collect adult, wingless aphids up to one week after their final molt for EPG recording.
5. After the experiments, return the experimental plants that were not used to the growth chamber, as they often have some offspring that has been produced during the recording, which could inadvertently contaminate other plants.

2. Insect Wiring for EPG Recording

1. To make insect electrodes, obtain brass connector pins (nails, Ø 1.2 mm), thin copper wire (Ø 0.2 mm), very thin gold wire (Ø ca. 20 µm), water-based silver glue, a simple small soldering bolt with soldering fluid and resin-cored soldering wire, stereomicroscope with 10X magnification, small scissors or scalpel, two fine forceps, and a Styrofoam sheet or box. Note: A vortex mixer might be useful. Note: The step-by-step protocol to make electrodes is indicated in **Figure 2**.
2. For aphid handling and glue application, obtain: a small and soft watercolor camelhair brush (size 2 or smaller) and insect pins such as those used for insect collections, although a fine sewing needle or toothpick may work as well. Step 4 shows how to start EPG recording.
3. Step 1.
   Note: Steps 1 and 2 below show how to prepare insect electrodes minus the aphid. Step 3 shows how to connect an aphid to the electrode. Vacuum fixation of the aphid is recommended during wiring, but not always required for slow moving species (*e.g., B. brassicae*).
    1. Switch on the soldering bolt and melt some soldering wire at its tip (**Figure 2A**). Moisten the head of the brass connector pin with some soldering fluid (**Figure 2B**) and dip it into the melted soldering metal (**Figure 2C**).
    2. Apply a sheath of melted solder metal on one end of a 1-2 cm long piece of the thin copper wire (**Figure 2D**). Then bring the pin and copper wire together against the hot bolt (**Figure 2E**) and move them together away to cool and solidify (**Figure 2F**).
4. Step 2.
    1. Thoroughly shake (or vortex) the vial with silver glue for several minutes until a smooth emulsion is shown. Cut (scissors or scalpel) a few pieces of the gold wire (of approximately 1.5 cm in length) on the object plate of the stereomicroscope (**Figure 2G**).
    2. Take a brass pin with soldered copper wire (made in section 2.3) and dip the free end of the copper wire into the small silver glue reservoir that will have gathered at inside the lid of the vial after opening it (**Figure 2H**). Note: Only a small droplet is needed.
    3. Move the glue-dipped end of the copper wire to the piece of gold wire, while lifting one end to avoid smearing the glue onto the stereomicroscope object plate. Try to overlap copper and gold wire for a few mm (**Figure 2I**), distributing the glue along the overlap of the two wires.
    4. Wait until the glue has been dried enough to keep the wires united. Check the glue contact after drying and add some fresh glue with a small pin or other piece of copper wire if some parts of the joined wires show glue-free parts.
    5. After the insect electrode is ready, store it, for example inserted into a piece of Styrofoam.
       Note: The length of the gold wire will determine the freedom of movement of the aphid: if it is too short (less than 5 mm), the aphid may feel constrained and will not behave normally; if it is too long (>2 cm), the aphid will move freely. Aphids tend to move to the adaxial side of leaves, if allowed to. If the gold wire touches the leaf, the signal will be short-circuited.
5. Step 3.
    1. The aphid may be kept in place by means of light suction, using a vacuum; in this case, install the suction device under the stereomicroscope. Place the suction opening in the center of the field.
    2. Thoroughly shake the vial with silver glue for several minutes (or vortex) until a smooth emulsion is formed. Collect an aphid with the small brush.
    3. Switch on the suction device and mount the aphid on the suction opening (**Figure 2J**), with the back of the abdomen turned to the experimenter. With the fine brush, remove any surface wax from the abdomen (abundant in cabbage aphids).
    4. Open the glue vial and wet a pin with a very small droplet of silver glue (**Figure 2K**). Apply the droplet of the silver glue onto the back of the aphid's abdomen (**Figure 2L-M**). Let this droplet completely dry during several minutes, vigorously shake the glue vial again and add a second droplet of silver glue on top of the first. Note: While the silver glue is an electrical conductor, it does not cause significant damage to the insect's cuticle.
    5. After closing the glue vial, insert the free end of the gold wire into the wet droplet and keep the wire still while allowing the glue to dry completely (**Figure 2N**). Avoid smearing glue onto legs or antennae and discard an aphid if this has happened.
    6. Switch off the suction fixation device and carefully lift the insect (**Figure 2O**). If needed, use a fine brush to assist in the lifting of the aphid from the suction device.

Note: Wiring *B. brassicae* does not require a vacuum, as they can be wired on a piece of a precision laboratory tissue, the rough surface of which provides the aphid with enough grip so that it will not be lifted after applying a drop of wet glue to its abdomen. After glue drying one can lift the aphid from the tissue with the help of a fine brush.

7. Insert the brass pin with the wired insect into the Styrofoam and if needed, continue wiring all other insects to be used for the EPG recording session.
   Note: These protocols for wiring aphids work well for us. The user may find his/her own method for wiring aphids.

6. Step 4.
   1. Put plants in the Faraday cage (**Figure 2P**) on a non-conductive support: use Petri dishes or a plate of glass or plastic.
   2. Insert a plant electrode into the soil of each pot. Insert the brass pin of the wired insect into the input connector of the EPG pre-amplifier (**Figure 2Q**). Note: the soil electrode does not correspond to the ground electrode used in other electrophysiological techniques. It has the offset voltage needed to adjust and compensate for electrode polarization voltages.
   3. On the interface of the acquisition software Stylet+, with fixed sample frequency of 100 Hz, enter a filename, specify the recording time, and write text to specify details of the experiment (treatment, plant/insect species, *etc.*) in Comment lines 2 and 3.
   4. Lower the insects onto a suitable landing area of the plant and start the recording session by clicking on the Start button of the acquisition software (Stylet+) interface.
      Note 1: a maximum of 8 channels can be used simultaneously in an EPG set up. One EPG-electrode or several EPG-electrodes per plant can be used.
      Note 2: when the focus of the study is the aphid behavior, start the recording before plant access of the aphids to avoid missing the first plant penetration activities.
   5. For studying the electrophysiological responses of SEs to stimuli, wait at least 10 min after the aphid has entered into phloem phase, in order to ensure that the aphid is in a sustained phloem ingestion phase, and that the signal baseline is stable. Only then, start any plant stimulation experiment.

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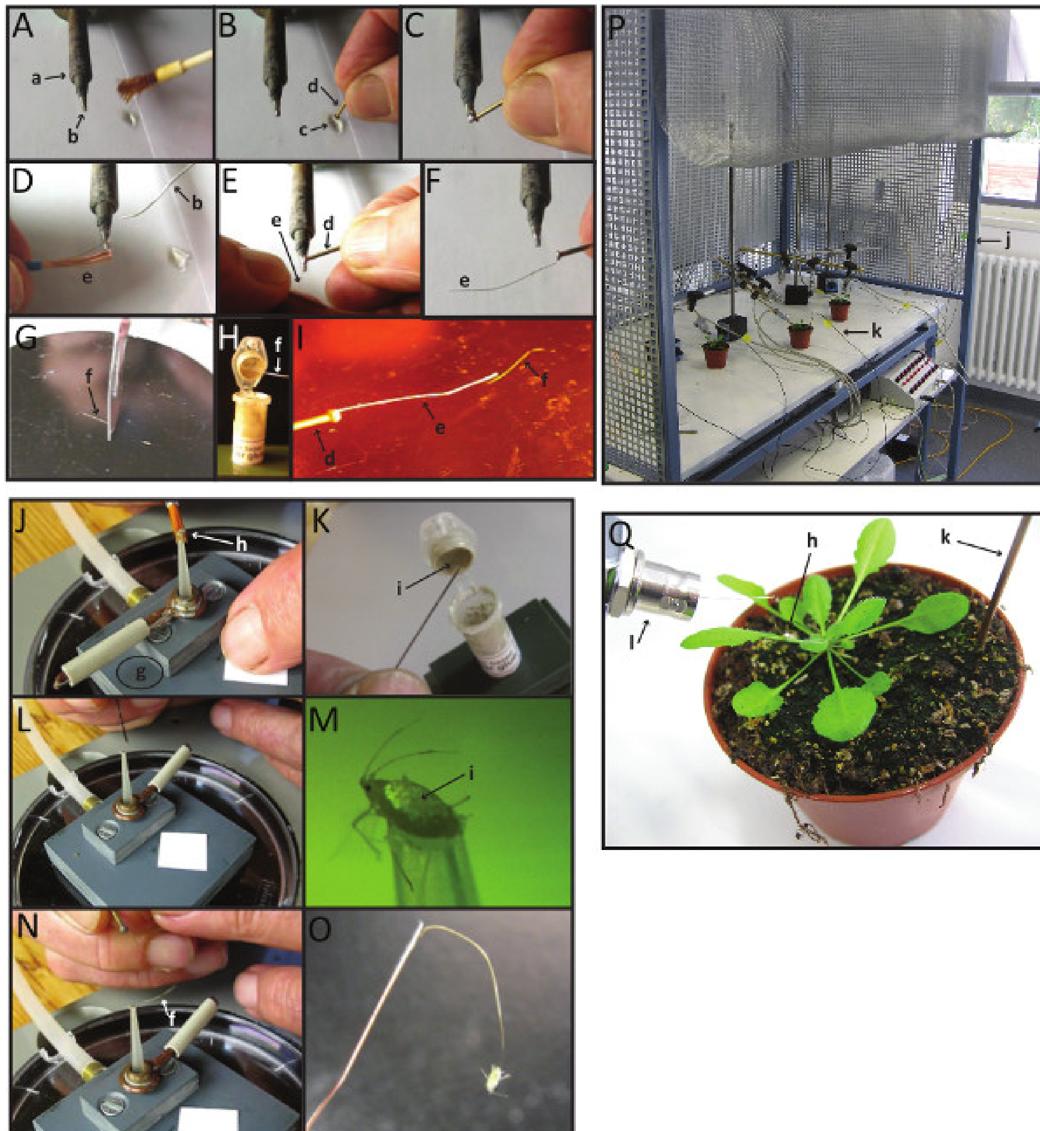

Figure 2. **Making EPG-electrodes with aphids or other hemipteran insects forelectrical penetration graph (EPG) recordings.** Please click here to view a larger version of this figure.

Panels **A-I**, steps required to prepare EPG-electrodes minus the aphid. First, melt a piece of soldering metal on the tip of a soldering bolt (**A**). Then, dip the head of the brass pin into a drop of soldering fluid (**B**), and contact it with the melted metal at the soldering bolt tip (**C**). Immediately after this step, contact the end of a copper wire to the tip of the soldering bolt, in order to glue it to the head of the brass pin (**E-F**). With a scalpel or a blade, cut a piece of the gold wire (**G**). Dip the free end of the copper wire (joined at the other end to the brass pin) on the silver glue (**H**), and quickly join the gold wire to it (**I**) before the silver dries up. The gold wire is an excellent conductor, and can be polarized. In reality, in the majority of the cases the polarization is too small to be detected, and if so, it can be compensated for with the offset voltage (**V**).

Panels **J-O**, steps needed to connect an aphid (or other hemipteran insect) to the electrode. First, carefully lift an aphid with a fine watercolor brush and place it on the opening of the vacuum suction device (**J**). Turn on the vacuum pump and cover the air valve hole with a piece of paper to apply suction. Dip the tip of the insect pin into the silver glue (**K**), and put a small glue droplet on top of the aphid's abdomen, under a stereomicroscope (**L-M**). Within the next ~20 sec, before the silver glue droplet on the aphid dries, insert the end of the gold wire of the insect electrode into the wet droplet of silver glue, and keep it in place for 1-3 min, until the silver glue has completely air-dried (**N**). At this point, disable suction by removing the piece of paper that covers the air valve hole of the suction device and carefully remove the aphid, from the suction device: lifting the aphid after wiring often requires some help by a fine brush (**O**).

Panel **P** shows an overview of the entire EPG set up inside the Faraday cage, and Panel **Q** shows an overview of the plant-aphid combination for EPG. See section 2 above for a more detailed explanation of this process.

Small letters are labels referring to the items that one needs to make EPG-electrodes: **a**: soldering bolt; **b**: melted soldering metal; **c**: soldering fluid; **d**: brass connector pin (nail); **e**: copper wire; **f**: Ø 18µm gold wire; **g**: suction device; **h**: aphid; **i**: water-based silver glue; **j**: Faraday cage; **k**: plant electrode; **l**: input connector (BNC) of the EPG pre-amplifier.

Representative Results

In a previous study, we implemented the EPG-electrode technique with the purpose of characterizing the electrical signals produced in SEs of the midvein during caterpillar attack[1]. The midvein is a preferred insertion site for conventional glass electrodes, as well as for glass-stylet electrodes, because it is SE-dense, and relatively robust, hence amenable to the fixation needed for implementing these techniques. Here, we took advantage of the versatility of the EPG electrode with the purpose of gathering electrophysiological information from more difficult to access SEs, in particular those in the marginal veins of leaves. **Figure 3** shows a typical EPG recording from a SE in a marginal vein of *A. thaliana* plant, which contains an electrical signal induced by distal wounding. Differently from SEs in major veins, SEs in marginal veins responded to remote damage with a single, slow depolarization wave that may correspond to the slow depolarization wave in central SEs. On average, this remotely induced slow depolarization in the SEs of the leaf margin had an average duration of 61 ± 27 sec, and average amplitude of 37 ± 2 mV (n = 3, mean ± S.E.M.). These data, easily obtainable with EPG-electrodes, suggest that wound-induced electrical signals in unwounded leaves do spread from the major vascular bundle to the phloem in minor veins.

Here we also exploited the robustness of EPG electrodes, to investigate whether a SE can respond to various damaging stimuli, delivered within a time interval of the order of minutes. When two leaves were cut with scissors at the petiole-lamina junction, an EPG-electrode placed in an intact leaf detected similar responses from the same SE to these wounds (**Figure 4A**). In another experiment, a caterpillar was used as the wounding agent. The caterpillar first cut a non-neighbor leaf, and then, after a few minutes, it moved to a neighbor leaf and cut it as well. Whereas the first wound in the non-neighbor leaf induced only a slow transient depolarization, the second wound in the neighbor leaf induced the complete electrical signal that contains a slow and a fast depolarization, consistently with earlier experiments[1]. These data show that a sieve element can detect multiple wounding events inflicted to other leaves, delivered within minutes from each other.

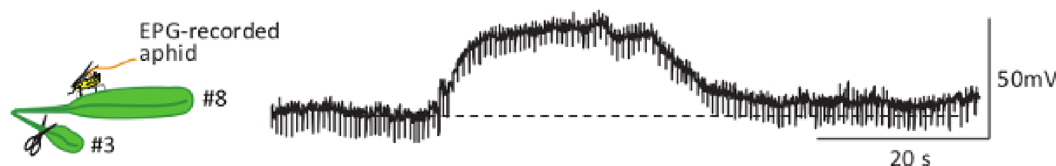

Figure 3. **Intracellular recordings of wound-induced electrical signals from sieve elements (SEs) in marginal veins with EPG-electrodes.** Electrical Penetration Graph (EPG) signal segment of the phloem-feeding phase of the aphid *Brevicoryne brassicae*. The EPG-recorded aphid was feeding from a SE located in a marginal vein of the leaf #8 (*Arabidopsis thaliana*, wild type), shown in the cartoon on the left. The EPG signal shows a slow depolarization wave in the marginal SE shortly after cutting a proximal neighbor leaf (leaf #3). The rhythmic, small, downward fast signal components represent the streaming potentials arising from the rhythmic ascension of sap along the food canal of the stylet during the ingestion phase (waveform E2). Please click here to view a larger version of this figure.

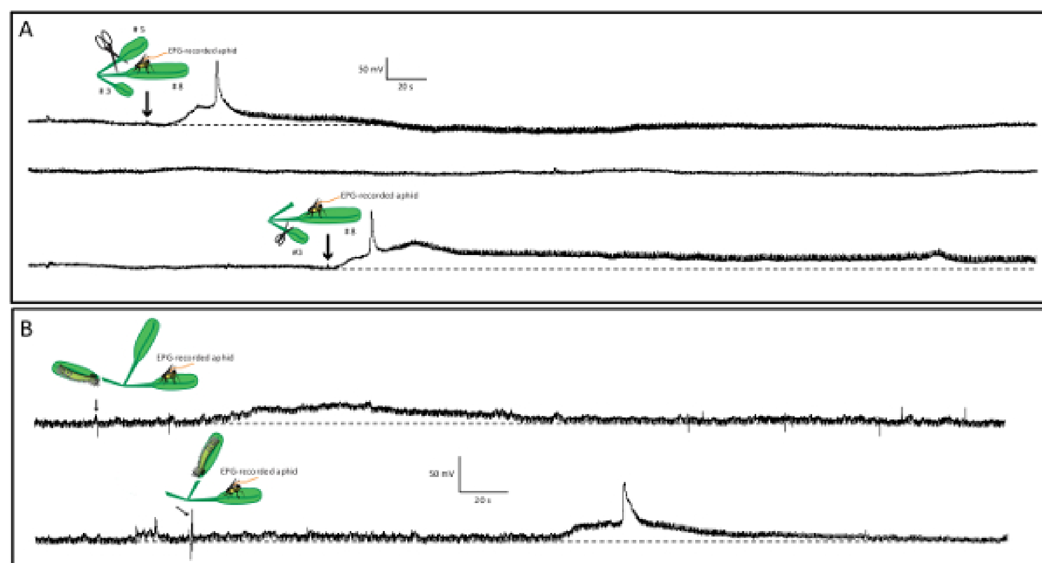

Figure 4. Multiple electrical responses to wounding stimuli in sieve elements (SEs) acquired with EPG-electrodes. The robustness of the aphid-plant (*Brevicoryne brassicae-Arabidopsis thaliana*) interaction and the stability of the SE-inserted aphid stylets are important properties of EPG-electrodes that allow acquisition of long EPG recordings (hours). Here we took advantage of these properties to investigate whether a SE can respond to two remote wounding stimuli. Panel A shows the responses of a SE to two artificial wounding events (leaf cutting by scissors) consecutively inflicted on two different neighbor leaves. There is an interval of approximately 17 min between the two stimuli. Panel B shows the responses of a SE to two consecutive natural wounding events. A 4th instar caterpillar of the cabbage butterfly (*Pieris brassicae*) was used in this experiment. The caterpillar first cut a non-neighbor leaf (in relation with the EPG recorded leaf), inducing a slow, transient depolarization. Then, approximately 7 min later, the caterpillar cut another neighbor leaf, which triggered a double depolarization signal (*i.e.,* containing a slow and a fast transient depolarizations) in the aphid-recorded SE. Arrows indicate the time where the wounds were inflicted. Please click here to view a larger version of this figure.

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Discussion

This article provides a detailed protocol for making Electrical Penetration Graph (EPG) recordings. The EPG technique is well established, with 100-200 active users worldwide, and it has been implemented for many studies on different topics, for example: a) host plant resistance to aphids and other stylet-bearing insects[13]; b) plant virus and pathogen transmission mechanisms[14]; c) insecticide mode of action, (toxicity and behavior changes)[15]; d) EPGs have even been useful to demonstrate that aphid fights are advantageous for the winner as it increases its feeding efficiency[16].

Learning to wire insects for EPG recording is not difficult but requires patience and practical experience to master. From making electrodes to the final wired insect, a practice period of one or two weeks is recommended. During this time, the researcher will familiarize herself/himself with the handling of the chosen insect species and to proceed through all steps successfully. Critical steps are: soldering properly, thoroughly shaking the silver glue vial before taking a glue droplet of the right size — neither too small nor too large — ensuring proper electrical and mechanical connection, and placing the gold wire within the silver droplet on the insect's abdomen in a manner that will not constrain the insect movements. Failing to perform these steps correctly will result in poor electrical connectivity, which will result in data of poor or unacceptable quality. When giving the wired insects access to the plant, it is important to visually monitor them during the first half hour of the recording. During this period, aphids are becoming accustomed to the wire, and may walk away from the desired recording place, or drop off the plant. Therefore, one may need to re-position the aphids during this period. In case of behavioral aims, no re-positioning should be done after the first half hour to prevent large differences between replicates. Poorly positioned or dropped-off individuals should be discarded.

In addition to providing a protocol for EPG recording, this article recapitulates the features of the EPG circuit that are relevant for its application as a method in plant intracellular electrophysiology (**Figure 1**). The major feature of EPG-electrodes is that they are highly cell-specific electrodes that allow for accurate intracellular recordings from SEs. In the regular EPG amplifier, the input resistance is relatively low, 1 GΩ (10^9 Ω). In practice, this means that the resistance changes during recording affect the measured potential. This is not an issue in conventional intracellular electrophysiology with glass electrodes, which typically use higher input resistance amplifiers. One can correct for the changes in resistance arising from the plasma membrane and from other sources by using calibration pulses, as in Salvador-Recatalà *et al.*[1]. Another option is to increase the regular EPG preamplifier's input resistance, from 1 GΩ to 1.5 TΩ (1.5 x 10^{12} Ω) or even to 1 PΩ (10^{15} Ω, depending on the pre-amp's OpAmp), which corresponds to the input resistance in regular amplifiers for intracellular recording. The EPG amplifier with higher input resistance is referred to here as the "emf-mode EPG" system. In this amplifier, a switch disables the normal-mode EPG amplifier (see **Figure 1**). In emf-mode, the EPG-electrode method records the plant cell potentials as accurately as the regular amplifiers used in intracellular electrophysiology. The only disturbance left in the EPG in emf-mode arises from the emf components of the aphid during SE feeding, but these are fairly low and do not compromise the accuracy of the measurements. If the researcher wishes to simultaneous record the reaction of the aphid to environmental stress factors (for instance, changes in salivation and ingestion) and the information of the SE membrane potential, then the normal-mode of the EPG is recommended. In that case, applying the calibrated pulse provides acceptable, approximate values of the membrane potential.

The lack of appropriate methods for acquisition of electrical responses of the phloem vasculature *in vivo* is currently limiting the type and number of questions related to environmental stress responses of plants. Researchers have implemented glass electrodes[3] and glass-stylet electrodes[4] for acquisition of intracellular recordings in SEs in the midvein. In contrast with these two types of electrodes, EPG-electrodes can be positioned on virtually any aerial part of the plant (and even on roots, using root aphids). Therefore, the EPG-electrodes will facilitate more comprehensive studies in plant electrophysiology. Another advantage of EPG-electrodes over conventional electrodes is that the former allow for extended recording periods, which makes possible investigating the responses of single SEs to various stimuli. This is an important biophysical feature, and may provide interesting information on how SEs integrate information from different environmental stimuli. Indeed, the data shown here proves that SEs do respond to various damaging stimuli (**Figure 4**), which justifies further research on this important biophysical feature of SEs.

The number of electrophysiological studies of SEs is too small to make comparisons between the data acquired with EPG-electrodes and the data obtained with traditional glass electrodes. In fact, to our knowledge there is no data in the literature on stimulus-induced electrical signals in SEs of Arabidopsis, acquired with a traditional method, be glass electrodes or glass-stylet electrodes. Using glass electrodes, Rhodes and collaborators[3] found that heat induces action potential-like spikes in the SEs of tomato plants. The fast depolarization that they showed had a magnitude of about 70 mV, occurring on top of a small, slower depolarization. This is consistent with our electrical signals acquired by EPG-electrodes[1], although care should be taken when comparing electrical signals from two different plant species, and induced by different types of stimuli.

The EPG electrode is a versatile and elegant tool for studying the electrophysiological responses of phloem cells to various types of stimuli.

Disclosures

WFT launched EPG Systems as a retirement activity, and is affiliated with it financially.

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