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Apoplastic Electropotentials in Plants: Measurement and Use

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INTRODUCTION

The object of this Chapter is to describe the characteristics and use of the electropotentials obtained by *in vivo* invasive sensors placed directly in plant tissue under laboratory or field conditions. First, the existence and possible origin of different types of electropotential variations will be described. Then, a specific use of the potential variations in agriculture will be presented.

BASIC TECHNIOUE AND TYPE OF POTENTIAL VARIATIONS

The basic technique is to place a palladium electrode invasively in the stem, petiole, or peduncle of the plant, and a reference electrode in the root environment (Figure 1). The measuring electrode consists of a palladium rod 150 μm in diameter and 10 mm long. The reference electrode is a conventional silver-chloride electrode, modified for long-term burial in the field. The two electrodes and the plant form a galvanic cell which yields a DC electrical potential which is coherent, reproducible, and which changes with environmental conditions. The fundamental problem is to explain the origin of the potential and the physiological reasons for the changes.

Several definitive changes in potential are observed. Immediately after the insertion of the probe, the potential rises towards more positive values. The change is termed a healing potential and is similar to the response in human tissue that occurs under the same circumstances (1, 2). In virtually hundreds of electrode insertions, the author has never seen this direction of potential change violated. This suggests that the mechanism that causes the potential reaction is exceedingly basic. The timing of the rise varies widely. Tomato plants in environmental growth chambers take three days to reach a steady-state potential value (3), but cotton plants under field conditions take only a few hours.

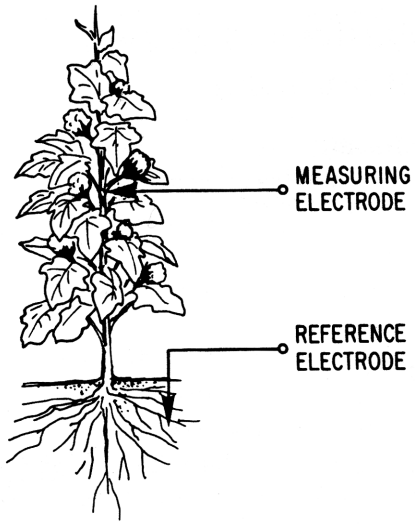

Figure 1. The basic measurement technique.

After healing, disturbance of the probe causes a rewounding and a repeat of the insertion response. The potential under such circumstances drops precipitously and then rises in a roughly exponential. manner to the original level (2), The rewounding potential is similar in form but smaller in magnitude than the original healing potential.

The steady-state or homeostatic potential level is 100–400 mV relative to the saturated silver-chloride reference electrode in the root zone. There is considerable variation from electrode to electrode, but the average value of the potential is similar in different plant types and in different soil conditions. The values obtained with a hydroponic root environment are the same as with soil under normal field conditions. The origin of the potential variation between individual electrodes is an unsolved problem.

The homeostatic electropotential value in cotton changes during the 24-hour cycle, but under field conditions it settles in the mid-afternoon at approximately +230 mV. The subsequent variations are basically movements around this level.

A recent study by Ladezma-Rascon indicated that the plant attempted to maintain a homeostatic potential even in the presence of external attempts to change the level (4). He applied external loads to the measuring electrode to perturb the potential from equilibrium. The plant deviated from the homeostatic level while the disturbance was applied, but returned when the disturbance was removed. This same situation ensued if the plant potential was perturbed by the addition of external charge. The plant would accept the charge and then return slowly to the homeostatic level.

Internal plant processes, however, can readily change the homeostatic level. In water-stressed cotton under field conditions, there is a potential drop of as much as 300–400 mV upon irrigation or rainfall (5). If the drop occurs in the late afternoon, the potential remains low until about midnight when it rises for about an hour and then drops

again until dawn. The potential remains high until the next late afternoon, at which time the entire sequence is repeated. This phenomenon repeats for three or four days with decreasing amplitude. Simultaneous with the drop in potential, there is an expansion of the main stem of the plant.

When cotton is not under water stress, the potential rises rapidly in the morning, and then exhibits a small drop around 0930 hours. The midmorning adjustment is not present if the rise is more moderate. Examples of this phenomenon are shown in Figure 6. The plant potential also changes at night (Figure 2).

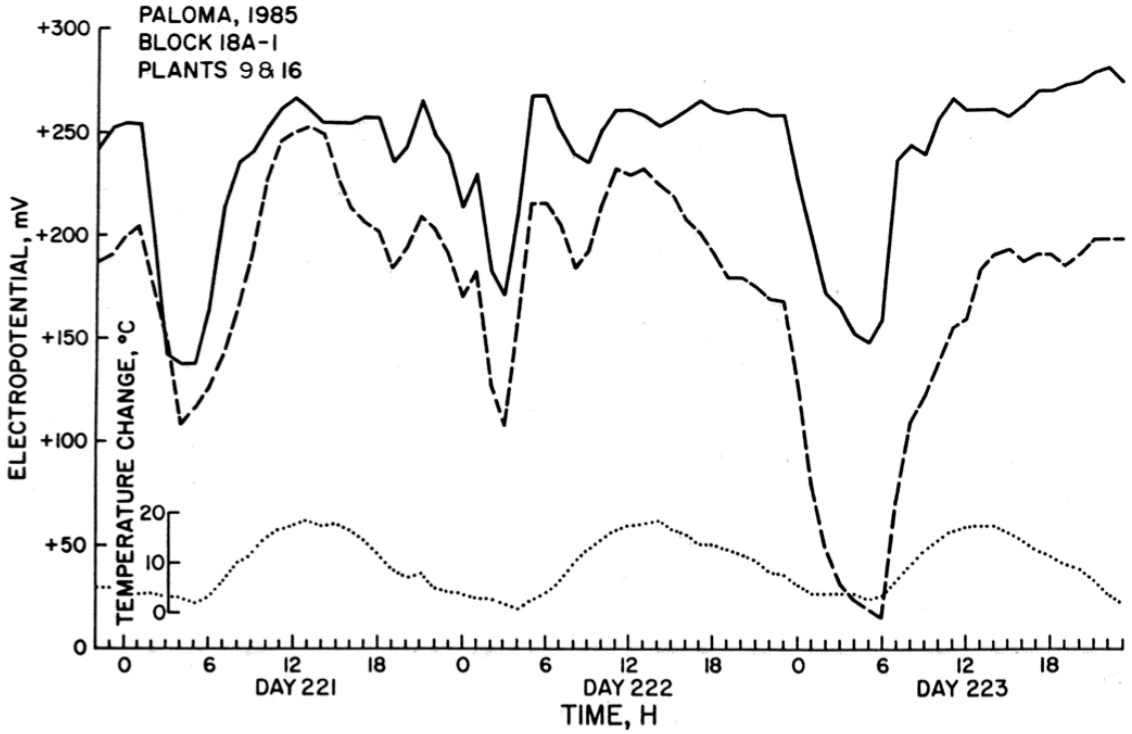

Figure 2. Cotton nocturnal potential. Electrode 9 and 16, Paloma Ranch, Gila Bend, Arizona, PDL90, 1985.

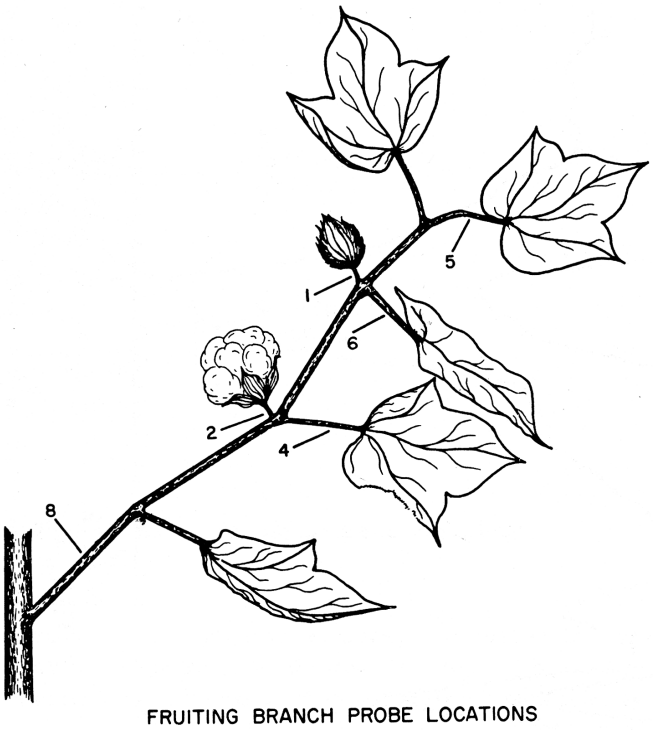

Figure 3a. Multiple electrode placement in one plant.

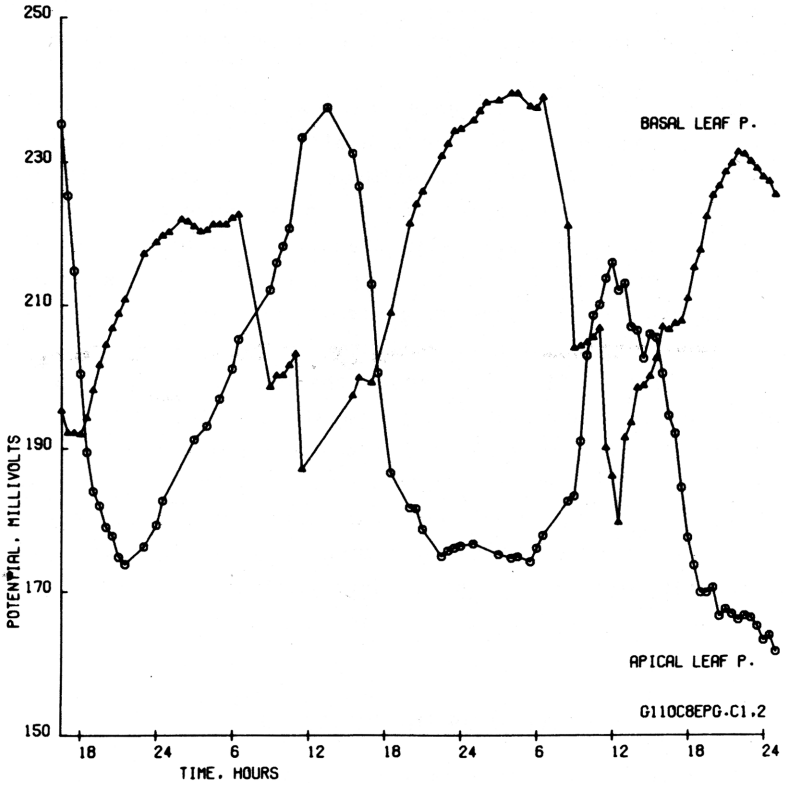

Figure 3b. Simultaneous electropotential variations in apical and basal petioles of the same side branch.

By placing several probes in various points along a single branch, it is possible to observe systemic relations between the various regions of the plants. Figure 3a shows an example of the probe placement. Figure 3b shows the observed simultaneous potential changes. Additional experiments indicate that there is a simultaneity of potential change between the bottom and the top of the plant main stem. Whether this simultaneity implies some form of communication has not been determined. The timing of the potential change and the distance involved requires that the mode of transmission be rapid. Such rapidity is possible by hydraulic or electrochemical information transfer.

LOCATION OF THE POTENTIAL

The question that immediately arises when a galvanic circuit of this complexity is formed is the exact location of the potential changes. The DC level is an algebraic sum of the potentials of the string of interfaces that occur between the two pieces of wire that terminate the galvanic cell. It is not possible to determine the value of any individual contributor to this sum. The changes in potential can be localized, however, if one places two electrodes in the stem of the plant in a vertical direction and if the electrodes are relatively close, 1 cm, for example, then one can assume that there exists a common path from both measuring electrodes back to the reference electrode. If this is the case, a change in this common path will show up at a simultaneous change in the potential of both measuring electrodes. A change in the reference electrode itself would be such a disturbance. Non-simultaneous changes in potential can then be attributed to the interface of the measuring electrode. Additional evidence to support this contention occurs when one purposely causes a mechanical disturbance at one of the measuring electrodes. A rewounding potential occurs as described above. The conclusion is that the changes of potential that occur arise from the interface between the measuring electrode and the fluid in which it bathes. The fluid is extracellular or apoplastic which means that the measurement itself is extracellular.

The location of the interface of the measuring electrode and the plant tissue is an important question. The electrode penetrates into the plant tissue 2–3 mm. In most plant tissue, this means it passes through the dermal and vascular layers, and into the pith. Since. the surface of the electrode is equipotential, electron transfer can occur anywhere along the length of penetration. It is therefore not possible to isolate the location of the potential-determining region. Electron and light microscopy studies have indicated that there is normal vascular tissue within 20 μm of the electrode surface (6). Studies of the anatomical reaction of plant tissue to probe penetration indicate that if the probe is placed at the upper shoulder of the S-shaped growth curve, almost no scar tissue forms (7). If the probe is placed in juvenile tissue, the reaction is such that scar tissue will form as an invagination of the outer dermal layers (8).

PLANT ACCEPTANCE

The question of plant acceptance of the electrode must also be considered from a materials viewpoint. Glass, plastic, silicon, silicon dioxide and tungsten carbide are not biocompatible, and result in necrosis of the tissue contiguous to the probe. Titanium, palladium, aluminum, carbon and stainless steel are readily accepted. The acceptance takes the form of healing and subsequent sealing off of the electrode entry point. It is essential that sealing occurs. This insures the presence of a normal fluid at the surface of the measuring electrode.

The tissue reaction has been extensively studied in tomato, cotton, and pecans (9-12); it appears to be similar in tomato and cotton. In pecans, the hardness of the tissue permits placement of the electrode only during the spring, in the region of new buds. If placement occurs at that time, the bud expands and substantial hardening occurs within a five-week interval. At that same time, scar tissue, referred to as a bolsa or pocket, forms around the electrode. When the electrode is removed for inspection, the pocket comes with it, indicating a substantial adhesion between the tissue and the electrode. This indicates that the scar-tissue layer renders the potential changes suspect, and further investigation is required before definitive conclusions can be reached. Nocturnal potentials in pecan have been seen, but the compartmentalization of the electrode obscures their meaning.

ORIGIN OF THE POTENTIAL

The electrochemical origin of the potential has been examined both theoretically and experimentally. Goldstein listed three possibilities for the origin of the potential: a redox couple or couples, image charge on the electrode due to charge in the contiguous tissue, and unbalanced charge in the region of the electrode (13). The first possibility is electrochemical while the two other possibilities are physical. Silva-Diaz used cyclic voltammetry to determine the presence of redox couples in the apoplast fluid, and found that no redox couples were discernible (13). The study did indicate that oxygen may be a factor in setting the potential. It also pointed out the main experimental limitation of *in vivo* cyclic voltammetry, namely, solution resistance. It was not possible to place the reference electrode physically close enough to the measuring electrode to achieve a low resistance in the path between the two electrodes. A low resistance is necessary to discern the presence of weak couples, and to facilitate interpretation of the voltammograms. Ledezma-Rascon compared carbon and palladium electrodes *in vivo* in cotton subjected to active and passive loads (4), and found that carbon electrodes had a consistently lower homeostatic potential. He also observed differences in the transient and steady-state response of the two electrode types. The responses were analyzed in terms of the circuit model in which the interface is characterized by a parallel resistance and capacitance, RI and CI respectively (Figure 4). The resistance is the conventional charge-transfer

resistance derived from the Butler-Volmer equation for small perturbations about the Nernst potential. The capacitance is a result of the double layer of charge at the interface. The carbon electrode likely has a large active electrochemical surface area per unit of geometrical surface area, leading to a large surface capacitance and a small surface resistance. The opposite situation prevails for the palladium electrode in which there is a relatively small electrochemically active surface area per unit of geometrical surface area. The results were in agreement with the experimentally observed response, suggesting that the origin of the potential is electrochemical, and that surface characteristics of the electrode figure prominently in the change in the potential of plants subject to external disturbances.

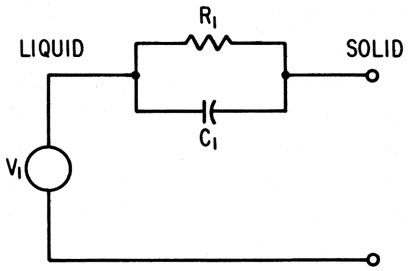

Figure 4. Single energy storage model of the electrode-apoplast fluid interface.

The modified coulostatic response of carbon versus palladium also lends insight into the origin of the potential. This response is obtained by discharging a capacitor into two palladium or carbon electrodes placed within 1 cm of each other in the stem of the cotton plant. The carbon pair yields a much lower magnitude of response, and it recovers more quickly. This is in agreement with the theoretical model of carbon involving a relatively high interfacial capacitance and a relatively low charge-transfer resistance.

The difference in the steady-state potential between carbon and palladium electrodes also supports the thesis that the potential is electrochemical and not physical in nature. In the latter case, the potential would arise as a response to external charge.

If a palladium electrode and a reference electrode are placed in a beaker of tap water and the solution sparged with nitrogen the oxygen is driven off and replaced by nitrogen, and the potential drops precipitously. This implicates oxygen in the origin of the potential, and thus also supports the electrochemical theory of origin.

These results have led to the hypothesis that the potential changes are due to varying concentrations of oxygen in the tissue (14). A low potential is associated with a low oxygen concentration, and a high potential with a high oxygen concentration. The healing potential can be explained in terms of this hypothesis by suggesting that the wounding and healing result in an initial precipitous consumption of oxygen and then a gradual increase in the oxygen concentration in the healing site. Under this hypothesis, the potential drop associated with the change in stem diameter would be a result of an expenditure of energy and concomitant consumption of oxygen. This energy is required

to implement the transfer of water associated with the expansion of the stem. The potential rise in the morning is a result of an increase in the oxygen level in the tissue over the level caused by diffusion. The drop of potential under water stress conditions is a result of a net decrease in the oxygen level in the tissue during the morning hours. The nocturnal potentials are a result of a sudden increase in metabolic activity on the part of the plant.

The fact that the carbon electrode produces a homeostatic potential and acts basically similar to the palladium electrode in its response would require oxygen to be involved in a complex form. A possibility is that adsorbed oxygen is presented at the surface of the electrode in a non-charge neutral complex.

ELECTRODE LOCATION AND PLANT ARCHITECTURE

The location of the electrode in the plant has received considerable attention. There are four possibilities: the main stem, side branches, petioles, and peduncles. Experimental results indicate the electropotentials from the petioles pass through a specific sequence as the leaf itself moves from juvenility to senescence. For example, a senescent leaf maintains an almost constant petiole potential until a change occurs in the water status of the root zone. At that time, the potential changes vigorously for a few days before returning to the predisturbance level. The peduncle potential is relatively stable compared to the potentials in the other parts of the plant, At the termination of the fruit development process in cotton, the potential of the peduncle drops precipitously. For long-term sensing of plant electrical activity, the main stem is the most desirable location. There is evidence that the age of the tissue has an influence on the level of potential variations, but in cotton over an entire growing season, the decrease in activity has not been significant.

The electrode placement described above is amenable to plants in which an anatomical region of sufficient size to insure long-term undisturbed placement is present. Cotton, tomatoes, soybeans, safflower, alfalfa, grapes, and potatoes are examples of plants with a stem architecture that permits placement of the probe. Sugar beets, lettuce, carrots, and cabbage are examples of plants not easily amenable to electrode placement. Several commercially important monocotyledons such as corn, wheat and rice are also not easily amenable to electrode placement. A distinct petiole is not present and the main stem in wheat consists of a series of concentric tubes which move relative to each other in the course of growth. An electrode placed in the stem passes through several of these tubes simultaneously. The relative movement then causes a tearing of the tissue around the probe.

REFERENCE ELECTRODE

The reference electrode is shown in Figure 5. It is basically a silver-chloride

electrode with a connection to the root zone consisting of a polyacrylamide layer and a porous ceramic plug. The purpose of the latter two items is to prevent loss of the filling solution and maintenance of a constant junction potential. One of the major electrode design problems is to seal the wire connection from water penetration. The present method is to use a barrier of fiber-impregnated tar. This material will wet the polyvinylchloride or polyethylene insulation covering the copper wire, and thus prevent water encroachment into the soldered junction.

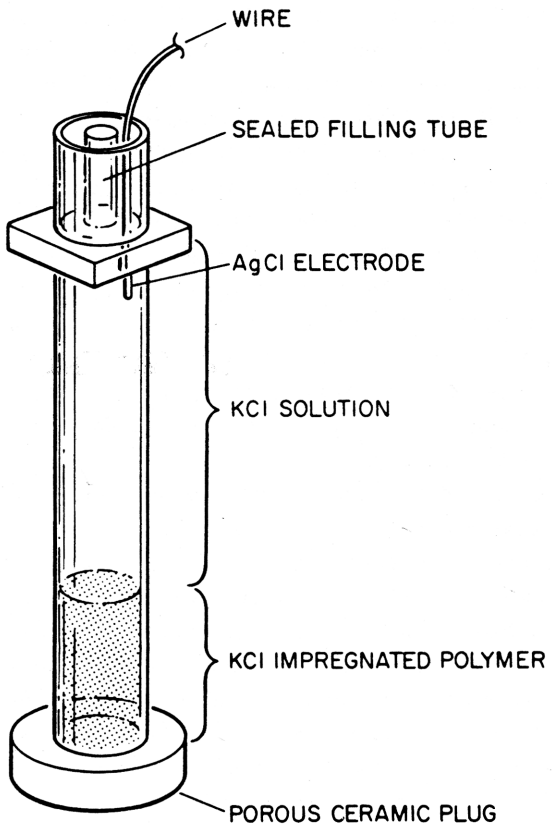

Figure 5. Electrical model of the reference electrode.

An auxiliary reference electrode is always employed to test the junction of the main reference electrode. The two electrodes are placed side by side in the root zone and form a concentration cell. A change in the potential of this concentration cell indicates a change in the reference electrode potential. A constant potential indicates that the potential between the bulk root zone and the wire leading from the main reference electrode is constant. Electrodes of the design shown in Figure 5 are stable to within a few millivolts for months at a time.

The distance between the reference electrode and the plant under measurement has not been a problem. The furthest separation employed has been about thirty meters. In addition, a common set of reference electrodes can be transported from site to site and used to determine absolute potential levels. For example, cotton electropotentials in fields

125 km apart have been compared by this technique.

APPLIED ASPECTS OF THE PHYTOGRAM TECHNIQUE

A major emphasis has been directed towards relating the variations in potential to the water status of the cotton plant under field conditions (15). The application begins with the analog plots of potential versus time (phytograms). An analysis of the phytogram patterns combined with an empirical knowledge of the water status of the field has led to general conclusions concerning the potentials that one can expect when a crop is in a water-stressed or non-stressed condition. A field not under stress will yield an average potential that will rise in the morning. A field under mild stress will yield a potential that rises only moderately. A field under serious water stress will yield a potential that falls in the morning. The method of using the potentials to determine water status is to quantify the average potential variation from 0500 hours to 1300 hours. The present method of operation is to place the electrodes in the main stems of the cotton plant at approximately node nine. Forty-four plants are monitored over an area approximately 31 meters by 20 meters. Data is transferred by wire from the electrode to a central acquisition canister (pod), buried in the field. The pod contains the electronics required to time the acquisition, multiplex the electrodes, process and store the data, and transfer the data to a central site by wireless telemetry.

The voluminous electropotential data is processed through the use of a cardinal point. The cardinal point is defined as the potential of each probe at 0500 hours each day. The potential is measured at later times and an increment formed by algebraically subtracting the value of the cardinal point from the potential at these particular times. These incremental values are then summed and averaged to yield a potential increment. This average potential increment is termed a phytogram index. The index can be plotted at various times after 0500 hours to yield a trend in the potential between sunrise and solar noon. An example of this daily trend is plotted in Figure 6.

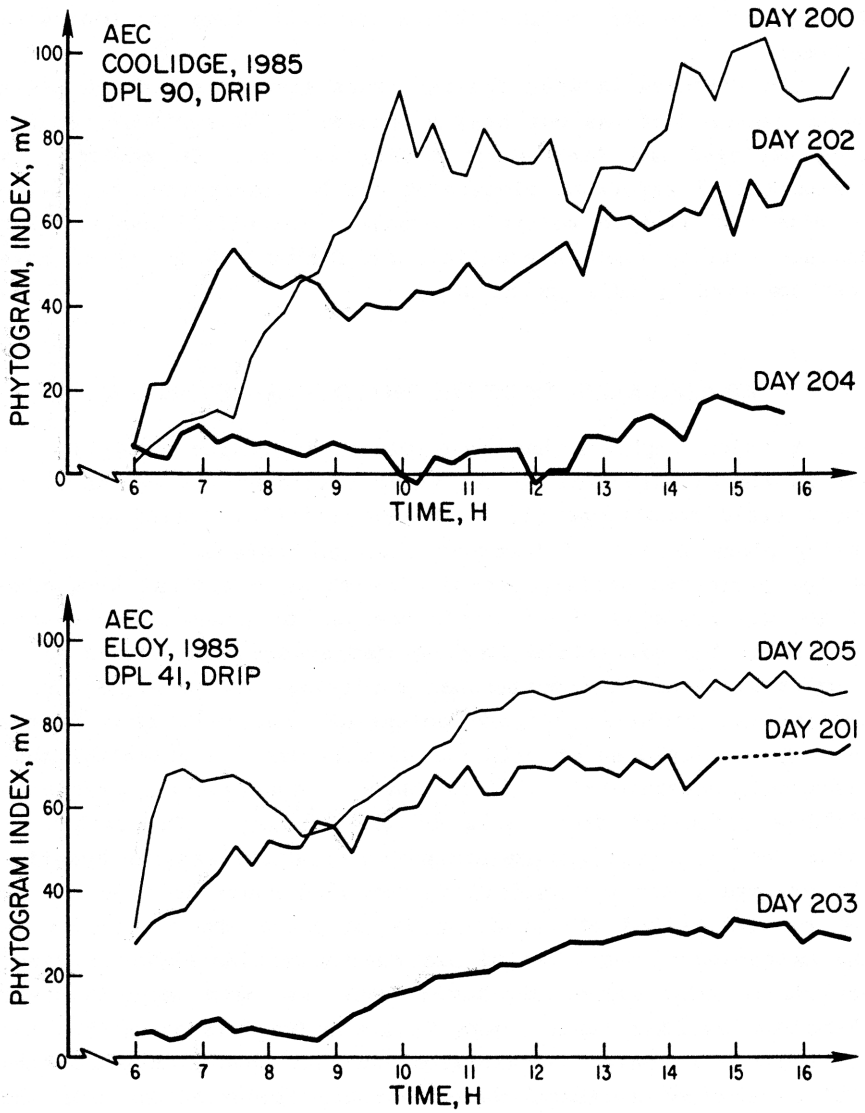

Figure 6. Daily trend in the phytogram index in cotton, Regal Farms, Eloy, AZ, and Sundance Farms, Collidge, AZ, 1985. Numbers are in Julian days.

The phytogram index can be used as the essential measurement in a complete network wherein the bioelectrochemical status of the crop is monitored every 15 minutes throughout the 24-hour period. This status is transferred by radio telemetry to the farm headquarters where it is processed into a daily numerical index. The index is used to set a water application rate for the field.

ACTIVE BIOELECTROCHEMICAL MEASUREMENTS

The previous discussion has centered on the measurement and use of an electropotential obtained by purely passive means. No external energy was employed to make the measurement. Active bioelectrochemical measurements are also possible wherein energy is supplied externally to achieve a particular assay of some desired

bioelectrochemical condition. For example, *in vivo* cyclic voltammetry can be performed by placing electrodes in the plant and sweeping the potential of the electrode above and below the homeostatic potential level in the same manner as *in vitro* voltammetry (16). Various other microelectroanalytical methods can be similarly applied. One method that circumvents the problem of solution resistance encountered in cyclic voltammetry is to use coulostatic impulses. In this method a pair of electrodes are placed very near each other in the main stem of the plant and electrical charge is precipitously transferred into and out of the tissue adjacent to the electrode. The potential changes that occur relative to the third .reference electrode can be used to assay the condition of the plant electrolyte.

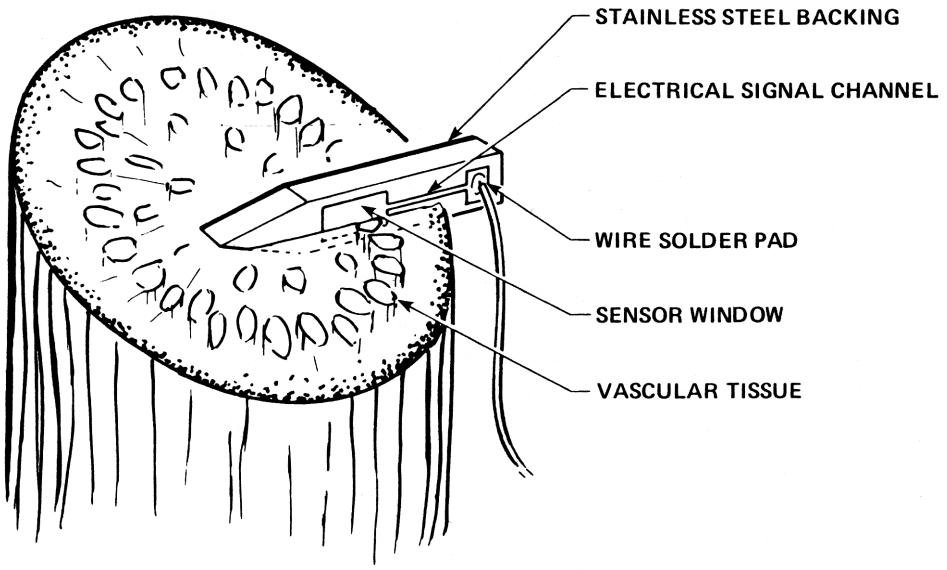

Figure 7. Invasive planar microelectronic electrode (not to scale).

The active methods described above can use conventional macroelectrodes such as round rods placed invasively in the tissue. Alternately, planar microelectronic electrodes can be employed in the manner shown in Figure 7. The electrode consists of a rigid substrate, a surface or window to achieve the assay, a channel to transfer the signal out of the plant and a bonding pad to connect the electrode to a lead wire. These electrodes are fabricated in the same manner as conventional chip production. A stainless steel rather than a silicon substrate is used to achieve biocompatibility, and polymide layers are used for insulation. Multiple-window electrodes with specific ion-sensitive layers can also be produced. There are myriad possibilities for *in vivo* electrochemical analysis of the plant electrolyte.

SUMMARY

This discussion has considered the basic method of determining the *in vivo* electropotential of the plant apoplast. Various characteristics of this potential have been presented. The physiological interpretation and use of the patterns of potential variation

have just begun. Determination of crop water status is an initial application of these studies. Active electrochemical techniques can also be employed using both conventional macroelectrodes and planar microelectrodes.

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