

# Factors Affecting Electrical Impedance of Internodal Stem Sections<sup>1</sup>

Received for publication August 21, 1972

DEAN R. EVERT

Department of Plant and Soil Science, University of Vermont, Burlington, Vermont 05401

## ABSTRACT

A sample holder was designed and built to facilitate measuring the magnitude and phase angle of the electrical impedance of internodal stem sections from *Cornus stolonifera* Michx. A nonpolarizing, electrically conducting manganese dioxide-carbon paste used between the stem sample and the electrodes of the sample holder allowed measurement of impedance at frequencies from 50 hertz to 500 kilohertz without electrode polarization or electrical interference. The impedance magnitude was linearly dependent on the sample length, but this dependence was minimized by computing a normalized impedance magnitude. The normalized impedance magnitude ( $Z_{nf}$ ) was calculated using the impedance magnitude ( $Z$ ) at any specified frequency ( $f$ ) and the impedance magnitude at 500 kilohertz ( $Z_{500 \text{ kHz}}$ ) in the following formula:  $Z_{nf} = (Z - Z_{500 \text{ kHz}}) / Z_{500 \text{ kHz}}$ . The normalized impedance magnitude was sensitive to injury produced by boiling and peeling the sample. Electrical impedance measurements on the bark and wood separately demonstrated that they have different electrical properties.

## MATERIALS AND METHODS

Electrical impedance measurements, both of magnitude and phase angle, were made on stem sections cut from 1 year internodes of *Cornus stolonifera* Michx. A vector impedance meter (Hewlett Packard model 4800A) provided a rapid direct reading of impedance magnitude, 1 ohm to 10 megohm, and phase angle,  $-90$  to  $+90$  degrees, at any frequency from 5 Hz to 500 kHz.

Three sample holders were designed and tested to determine their suitability for routine electrical impedance measurements. The sample holder giving the best results measured impedance along the longitudinal axis of sections cut from a stem. This sample holder had an aluminum base and an aluminum cover to shield the stem section completely from electrical interference (Fig. 1). Stainless steel electrodes were cemented to plastic insulators, and electrical contact with samples 0.4 to 2.4 cm long was made by sliding one insulator along a slot cut in the aluminum base of the holder. Electrical contact over the entire cut ends of the sample reduced the electrode impedance and made removal of the cuticle unnecessary. With the sample completely enclosed, a tissue paper saturated with water and placed in the cover of the sample holder minimized drying at the cut surfaces. The paste on the sample was still moist 48 hr after the sample was placed in the holder.

Several electrically conducting compounds were evaluated to determine if they would reduce electrode polarization. In addition to reducing electrode polarization, the conducting compounds, if flexible or in paste form, should improve the electrical contact by filling in irregularities in the cut surfaces of the sample. Also, the compound should be nontoxic to plant cells.

A  $\text{MnO}_2\text{-C}$  paste originally used for electrical measurements on animal cells met the requirements and was the best of those evaluated in this study (10). Impedance magnitudes at several frequencies as a function of sample length are shown in Figure 2 and were used to evaluate electrode impedance. The impedance magnitude measured the combined impedance of the sample and the electrodes. Electrode polarization would cause the impedance magnitude to increase as the frequency decreased and would mask the frequency-dependent properties of the sample. Extrapolating the impedance magnitude to a sample length of zero indicated electrode impedance. Because the extrapolated impedance magnitudes (Fig. 2) actually decreased at low frequencies, the paste was nonpolarizing. This conclusion was supported by noting that the impedance phase angle at 50 Hz was less than 2 degrees for all samples greater than 0.5 cm (Fig. 3). Also, the phase angle was small at all frequencies for a killed sample (Table I). The  $\text{MnO}_2\text{-C}$  paste was apparently nontoxic. Callus grew through paste applied to the ends of samples that were incubated in the dark at high humidities at room temperature.

The  $\text{MnO}_2\text{-C}$  paste was separated from a new D cell flashlight battery and made into a slurry using distilled water. The

---

Electrical impedance measurements on woody stems provide information about the physiological conditions of cells and tissues (1, 12). Whether electrical impedance measurements can be combined to determine injury rapidly depends on what factors affect the measurements. Numerous workers have shown that electrical measurements depend on the frequency of the applied electric current (2-9, 11). These investigations have some limitations: (a) measurements were made at only two frequencies, (b) distance between electrodes was fixed, and (c) electrode polarization at low frequencies was not investigated or minimized.

This research was undertaken to evaluate the possibility of using electrical impedance measurements at several frequencies as a rapid, nondestructive measure of plant injury. The purposes were: (a) to determine how stem length affects electrical impedance and if measurements at several frequencies can be combined to minimize length effects while still retaining sensitivity to tissue vitality, (b) to determine the relative contributions of the living bark and wood to the measured electrical impedance, and (c) to determine how injury produced by peeling the bark or boiling the stem affected electrical impedance measurements.

---

<sup>1</sup> University of Vermont Agricultural Experiment Station Journal Article 293.

slurry was vacuum-filtered using a Buchner funnel, and the cake was washed with distilled water—95% ethanol—0.05 M phosphate buffer, pH 7.5, and 95% ethanol in that order. The resulting cake contained pieces of plastic or wax that were removed by passing the material through a stainless steel screen. The resulting wet paste was dried at 50 C overnight in a vacuum oven connected to an aspirator. For electrical contact studies and routine use, 5 ml of distilled water were mixed with 5.5 g of the powder to form a thick paste. The exact proportions were not critical.

To measure the variation of electrical impedance with sample length, stem sections were cut from internodes with a razor blade and the sample ends were covered with the MnO<sub>2</sub>-C paste. The electrical impedance, both magnitude and phase angle, measured at 500 kHz, 50 kHz, 500 Hz, and 50 Hz, revealed the general frequency-dependent properties of the sample. After measurements, an end of the section was cut off, the sample length was remeasured to the nearest 0.0025 cm using a micrometer, the paste was reapplied to both ends, and the measurements were repeated.

The electrical impedance of the bark and wood were measured by peeling the bark from the wood. Electrical impedance was measured along the longitudinal axis of an intact stem section, then of the bark and wood together after peeling, and of the bark or wood individually.

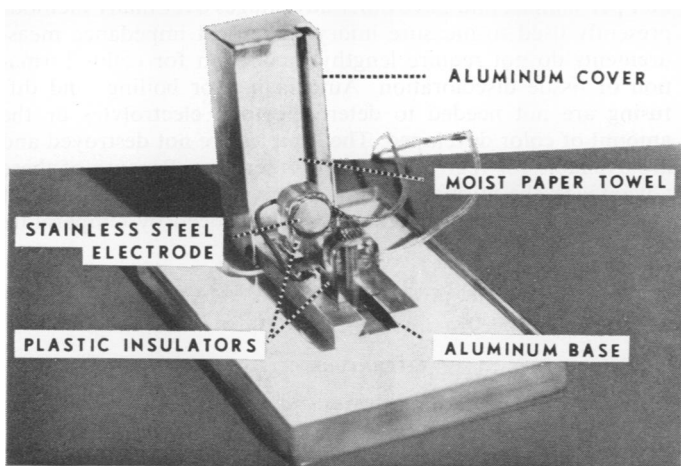


FIG. 1. Sample holder for measuring electrical impedance along the longitudinal axis of internodal stem sections.

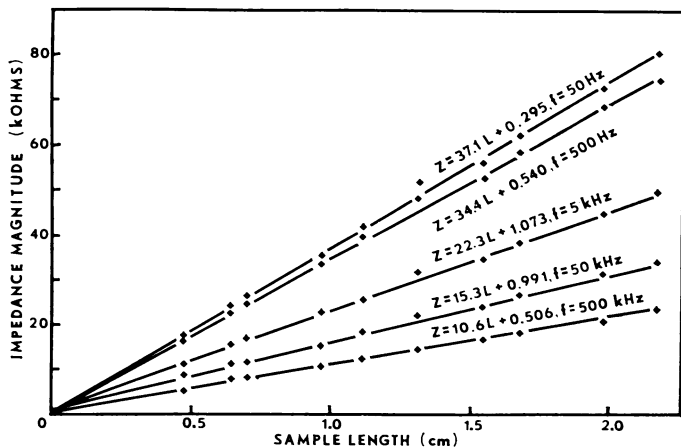


FIG. 2. Electrical impedance magnitude ( $Z$ ) at five different frequencies ( $f$ ) as a function of sample length ( $L$ ). Regression equations are the least squares straight line for each frequency.

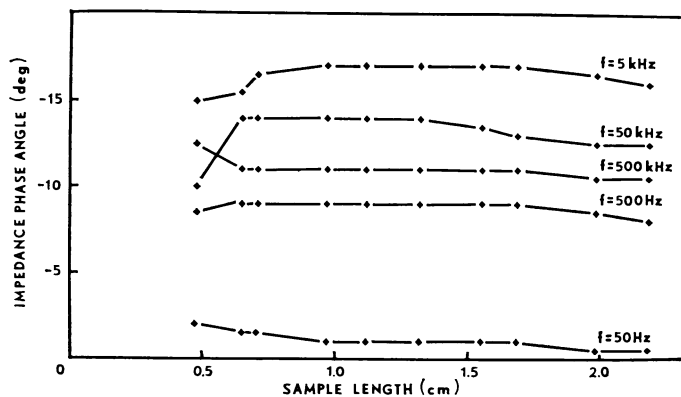


FIG. 3. Impedance phase angle as a function of sample length at five different frequencies ( $f$ ).

Table I. Magnitude and Phase of Impedance and Normalized Impedance of an Internodal Stem Sample as a Function of Frequency before and after Boiling for 30 sec

Impedance	Frequency				
	500 kHz	50 kHz	5 kHz	500 Hz	50 Hz
Magnitude	<i>kohms</i>				
Before boiling	12.0	17.9	27.0	39.8	42.1
After boiling	11.0	11.5	11.6	11.9	12.9
Normalized magnitude	<i>Change relative to magnitude at 500 kHz</i>				
Before boiling	0.00	0.49	1.25	2.32	2.51
After boiling	0.00	0.05	0.05	0.08	0.11
Phase angle	<i>degrees</i>				
Before boiling	-12.5	-14.5	-19.0	-7.5	-0.5
After boiling	-1.0	-0.5	-1.0	-1.0	-0.5

Further measurements were made on sections killed by a 30-sec immersion in boiling water.

Temperatures during impedance measurements ranged between 21 and 25 C. Variations during any particular experiment were less than 2 C.

RESULTS AND DISCUSSION

The impedance magnitude depended on the length of the sample (Fig. 2). The correlation coefficient between sample length and impedance magnitude was greater than 0.998. A number of reports have shown that impedance magnitude ratios or ratios of other related electrical quantities can be used to minimize the effects of stem length, diameter, and moisture level (2-4). For stem sections 1 to 2 cm long, the sample length was unimportant in determining the normalized impedance magnitude (Fig. 4). The decrease in the normalized impedance magnitude for samples less than 0.75 cm may be due to injured cells making up an increasing percentage of the total number of cells in the sample as length decreases.

The impedance phase angle was relatively independent of sample length without normalization (Fig. 3). The variations at any frequency are relatively unimportant because phase angle was measured only to the nearest 0.5 degree. The variation of impedance phase angle with frequency is characteristic of living cells (1). At low frequencies, 50 Hz and below, the phase angle is nearly zero. Any electrode polarization at low frequencies will cause a negative rather than zero phase angle. As the frequency increases, the phase angle becomes more negative until a minimal value is reached. Further increases in frequency cause the phase angle to again approach zero, but

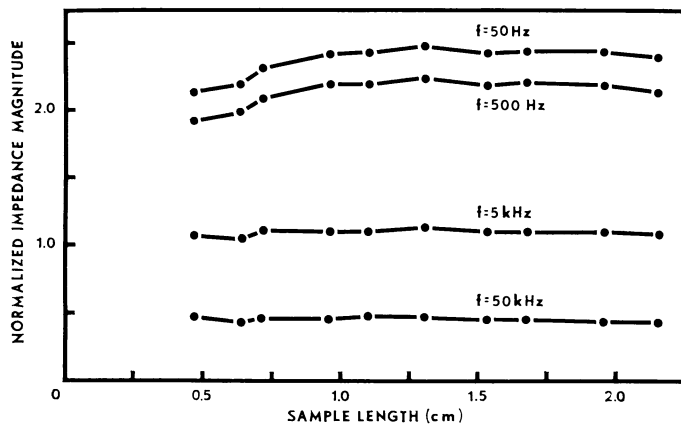


FIG. 4. Normalized impedance magnitude at four different frequencies as a function of sample length. Normalized impedance magnitude ( $Z_{nf}$ ) was calculated using impedance magnitude ( $Z_f$ ) at any specified frequency ( $f$ ) and impedance magnitude at 500 kHz ( $Z_{500 \text{ kHz}}$ ) in the following formula:  $Z_{nf} = (Z_f - Z_{500 \text{ kHz}})/Z_{500 \text{ kHz}}$ .

Table II. Magnitude and Phase of Impedance as a Function of Frequency for Intact and Peeled Internodal Stem Sections and for the Bark and Wood Separately

Impedance	Frequency				
	500 kHz	50 kHz	5 kHz	500 Hz	50 Hz
Magnitude	<i>kohms</i>				
Intact	6.1	8.8	14.7	32.5	36.5
Peeled (bark and wood)	6.9	9.4	14.6	24.4	26.0
Bark	18.1	33.8	50.7	60.5	62.0
Wood	9.8	12.0	18.8	31.7	37.5
Phase angle	<i>degrees</i>				
Intact	-11.5	-11.5	-29.5	-16.0	-2.5
Peeled (bark and wood)	-11.0	-10.0	-24.0	-9.5	-1.5
Bark	-20.0	-18.5	-12.5	-4.0	-0.5
Wood	-6.0	-11.5	-26.0	-10.5	-2.0

frequency must be well above 500 kHz before the impedance phase angle approaches zero.

Both the normalized impedance magnitude and the impedance phase angle depend on cell viability (Table I). The magnitude of the impedance at high frequencies was relatively unchanged by placing the sample in boiling water for 30 sec. Frequencies higher than 500 kHz should reduce the change even further, but 500 kHz was the maximum obtainable with the vector impedance meter. After boiling, the impedance magnitude was nearly independent of frequency, and the normalized impedance magnitude dropped to near zero at all frequencies. These changes are consistent with the theory that cellular membranes behave like leaky capacitors and represent a major barrier to electric current and ion movement at low frequencies but do not impede high frequency currents. Destruction of the membranes by boiling would make the impedance, both magnitude and phase angle, independent of the frequency. Because the impedance phase angle does not depend on sample length but does depend on the condition of the membranes, it may be possible to use phase angle measurements at a single frequency to determine injury.

Measurements of the bark and wood separately show that they have different electrical properties (Table II). This supports the observations of Glerum and Krenciglowa (5) that the simple model proposed by Hayden *et al.* (8) does not ade-

quately represent the observed impedance properties of highly differentiated stem sections.

No detailed studies were made of the effects of stem diameter on the normalized impedance magnitude. However, the measurements made did provide some information about the relationship between stem diameter and the normalized impedance magnitude. Because the bark and wood have different electrical properties (Table II), the normalized impedance magnitude will vary as the relative proportions of bark and wood change. With large diameter samples most of the stem will consist of wood, and the normalized impedance magnitude should be independent of diameter. A similar problem occurs with any method of measuring injury that evaluates the bulk properties of a sample. For example, the electrolyte test is a function of stem diameter if the bark and wood release different amounts of electrolytes.

The impedance of a peeled sample, bark and wood combined, was not identical with the impedance of the intact sample, but the difference was probably due to injury from peeling (Table II). The bark and wood apparently are electrically connected in parallel in the intact sample; this conclusion is consistent with that of Glerum and Krenciglowa (5).

In summary, the normalized impedance magnitude estimates vitality of a stem section. The estimate does not depend on sample length, but on the combined properties of the bark and wood. Impedance measurements are rapid, less than 5 min per sample, and have other advantages over many methods presently used to measure injury. Electrical impedance measurements do not require lengthy incubation for callus formation or tissue discoloration. Autoclaving, or boiling, and diffusing are not needed to determine total electrolytes or the amount of color developed. The samples are not destroyed and are available for regrowth tests or other uses. Because of these advantages, the normalized impedance magnitude should be of particular value in measuring injury produced by temperature and moisture stresses.

*Acknowledgment*—I am grateful to Dr. N. Pellett for critically discussing the research.

#### LITERATURE CITED

1. COLE, K. S. 1933. Electric conductance of biological systems. Cold Spring Harbor Symp. Quant. Biol. 1: 107-116.
2. DE PLATER, C. V. AND C. G. GREENHAM. 1959. A wide-range A. C. bridge for determining injury and death. *Plant Physiol.* 34: 661-667.
3. EVERT, D. R. AND C. J. WEISER. 1971. Relationship of electrical conductance at two frequencies to cold injury and acclimation in *Cornus stolonifera* Michx. *Plant Physiol.* 47: 204-208.
4. GLERUM, C. 1970. Vitality determinations of tree tissue with kilocycle and megacycle electrical impedance. *Forest. Chron.* 46: 1-2.
5. GLERUM, C. AND E. M. KRENCIGLOWA. 1970. The dependence of electrical impedance of woody stems on various frequencies and tissues. *Can. J. Bot.* 48: 2187-2192.
6. GREENHAM, C. G. AND D. J. COLE. 1950. Studies on the determination of dead or diseased tissues. I. Investigations on dead plant tissues. *Aust. J. Agr. Res.* 1: 103-117.
7. GREENHAM, C. G. AND H. DADAY. 1960. Further studies on the determination of cold hardiness in *Trifolium repens* L. and *Medicago sativa* L. *Aust. J. Agr. Res.* 11: 1-15.
8. HAYDEN, R. I., C. A. MOYSE, F. W. CALDER, D. P. CRAWFORD, AND D. S. FENSOM. 1969. Electrical impedance studies on potato and alfalfa tissue. *J. Exp. Bot.* 177-200.
9. LUYET, B. J. 1932. Variation of the electric resistance of plant tissues for alternating currents of different frequencies during death. *J. Gen. Physiol.* 15: 283-287.
10. NENCINI, R. AND E. PASQUALI. 1968. Manganese dioxide electrodes for stimulation and recording. *Med. Biol. Eng.* 6: 193-197.
11. POLOZHENTSEV, P. A. AND L. A. ZOLOTOV. 1970. Dynamics of electrical resistance of bast tissues in pine trees as an indicator of changes in their physiological condition. *Soviet Plant Physiol.* 17: 694-698.
12. ROTHSCHILD, L. 1946. The theory of alternating current measurements in biology and its application to the investigation of the biophysical properties of the trout egg. *J. Exp. Biol.* 23: 77-99.