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Physical and physiological aspects of impedance measurements in plants

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TIIVISTELMÄ: KASVIEN IMPEDANSSIMITTAUKSIIN LIITTYVIÄ FYSIKAALISIA JA FYSIOLOGISIA NÄKÖKOHTIA

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The electrical impedance characteristics of the plant cells are dependent on such physiological factors as physiological condition, developmental stage, cell structure, nutrient status, water balance and temperature acclimation. In the measurements also such technical and physical factors as type of the electrodes, frequency, geometry of the object, inter-electrode distance and temperature have an effect. These factors are discussed especially with respect to the impedance method in frost resistance studies of plants.

Kasvisolujen impedanssiominaisuuksiin vaikuttavia fysiologisia tekijöitä ovat kasvin fysiologinen kunto, kehitysvaihe, solurakenne, ravinnetila, vesitasapaino ja lämpötila-akklimaatio. Lisäksi vaikuttavat sellaiset mittaustekniset ja fysikaaliset seikat kuin elektrodityyppi, taajuus, mittauskohteen geometria, elektrodien välimatka ja lämpötila. Tutkimuksessa tarkastellaan näitä tekijöitä erityisesti, kun impedanssimenetelmää sovelletaan kasvien pakkaskestävyyden määritykseen.

Keywords: frost resistance, cross-sectional area, developmental stage, temperature acclimation
ODC 812.15+811+161.7+181.221.1

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1. Introduction

Impedance technique is one method used in frost resistance studies of plants (e.g. Wilner 1961, van den Driessche 1969, 1973, Glerum 1973, Greer 1983, Pelkonen and Glerum 1985, Repo and Pelkonen 1986, Rikala and Repo 1987). It is based on the phenomenon that frost causes structural changes in the cells which are either irreversible or partially reversible and which change the electrical properties of those cells.

The structure of plant cells brings about difficulties in measurements of impedance characteristics. Due to this fact, and maybe the lack of interest of the electrophysiologists and engineers, there are not as sophisticated and widely used methods for studying plant cells as there are for animal cells. Microelectrode techniques have been used with large thin-walled cells such as in algae (Williams et al. 1964, Bernhardt and Pauly 1974, Coster

and Smith 1977), but not with higher plants.

In most of the studies with plant cells the impedance modulus of the tissue at a selected frequency or the ratio of the modulus at two frequencies is used to describe the condition of the cells. The parameter values of a simplified electrical model for cell membranes (Coster and Smith 1977, Smith 1983) and tissues (Tattar et al. 1974, Pukacki 1982, Piene et al. 1984) have been reported in few papers.

Changes in impedance can be considered indicators of frost damage in plant tissue. Adequate observations and interpretation of impedance values necessitate sound knowledge of physical and physiological phenomena involved. This text is a short review on the area. It includes also some unpublished results. This report attemps to elucidate the matter from the biophysical point of view.

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2. Physical aspects of impedance mesurements

2.1 Plant tissue as an electrical circuit

Impedance is an alternating current resistance which is composed of resistive and reactive components (Appendix). In plant tissues the components are determined by the liquids in the cells, cell membranes and cell walls. The boundary layers between liquids and "solid" components such as cell membranes and cell walls (Blanck 1984), as well as the layers between tissue and electrodes also have resistive and reactive characteristics (Cobbold 1974) (Fig. 1).

Due to the reactive nature of the plant tissue the impedance is dependent on the frequency. When measuring with low frequencies up to about 1 kHz, the reactive component of the tissue is ignored. Thus the impedance modulus describes the resistive component only. At high frequencies current flows through the capacitive component. This decreases the overall impedance. Frequency also has an influence on the boundary layer impedance between tissue and electrodes (see chapter 2.2) and on the layer impedances of the cellular components. The polarization of the electrodes sets the lower limit of useful frequencies to about 20 Hz (Cobbold 1974).

It is evident from the phase angle measurements (see Appendix) that the plant cells have capacitive characters that result in phase angles with negative values. The phase angle of Magnolia shoots (*Magnolia* × *soulangiana, Magnolia* × *kobus*) (Pukacki 1982) and the stem sections of red-osier dogwood (*Cornus stolonifera* Michs.) (Evert 1973) have a parabola shape dependency on the frequency.

In some studies the plant tissue has been described as a simple resistance-capacitance-circuit (RC-circuit) (Fig. 1a) or a RC- circuit with one additional resistance in series (Fig. 1 b, Appendix) (Tattar et al. 1974, Pukacki 1982, Piene et al. 1984, MacDougall et al. 1987). The resistance has been found to be

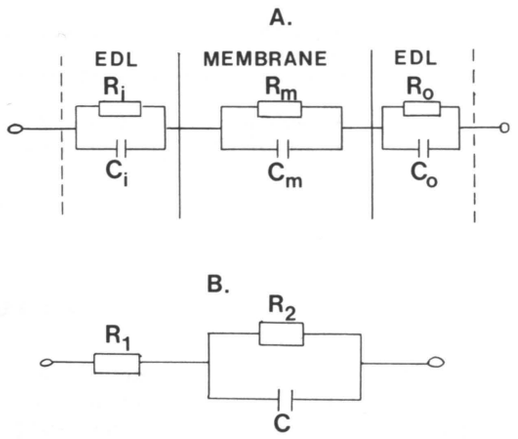

Fig. 1. A: Electrical model of the cell membrane and the boundary layers between cell membranes and the surrounding liquids. EDL means electrical double layer. Subindices (i), (o) and (m) mean inside, outside and membrane. The resistive and the capacitive components are indicated with (R) and (C). B: A simplified electrical model of the plant tissue.

inversely proportional to the capacitance. The resistance of tree shoots are generally between 10−100 kΩ and the capacitances from picofarades to nanofarades (Tattar et al. 1974, Pukacki 1982, Piene et al. 1984, MacDougall et al. 1987). The resistivity is the specific impedance at a low frequency, and for needles and shoots of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies*) is in the range of 30−80 Ωm (Repo et al. 1984, cf. Chapter 2.4.).

The measurement of the impedance characteristics of the tonoplast and plasmalemma of higher plants (e.g. forest trees) has not succeeded. On the contrary with lower plants e.g. *Chara australis* (R. Br.) the area-specific capacitance and resistance of tonoplast are about 60 mF m^{-2} and 0.15 Ω m^{-2} (f=5 Hz), respecitively. The corresponding values of plasmalemma are 24.3 mF m^{-2} and 0.55 Ω m^{-2}, and those of the series combination of plasmalemma, cytoplasm and tonoplast 16.5 mF m^{-2} and 0.69 Ω m^{-2} respectively (Smith 1983). Both the capacitance and the resistance of the membranes are dependent on the frequency at the range of 1−100 Hz. At very low frequencies (f<1 Hz) the membranes become inductive (Coster and Smith 1977).

The impedance locus representation is one way to analyse the electrical model and the properties of the tissues (e.g. Rothschild 1946, Schanne and Ruiz-Ceretti 1978). In this representation, the imaginary part of the impedance is a function of the real part as shown in the Appendix. Monitoring the changes in the locus with respect to the functional changes of the tissue has been called impedance spectroscopy (Gersing 1982).

2.2 Type of electrodes

The contact between the electrode and the tissue is important in impedance measurements at low frequencies. According to the electrode theory the so called Warburg impedance, or diffusion impedance appears at the boundary layer. At high frequencies the effect of the Warburg impedance diminishes and the polarization of the electrodes is reduced. When the tissue impedance is large compared to the diffusion impedance, the effect of polarization can be ignored (Cobbold 1974, Schanne and Ruiz-Ceretti 1978). The contact can be improved with pastes. In one study the phase angle of the diffusion impedance at low frequencies could be decreased to nearly zero with manganese dioxide-carbon paste between electrode and tissue (Evert 1973).

The polarization is dependent on the type of the electrodes. The capacitance of the electrode impedance was significantly higher at the frequency of 80 Hz when measured with platinum or nickel-plated steel electrodes than with chloridized silver (Ag/AgCl) electrodes (Pukacki 1982). Ag/AgCl electrodes can not be used in the woody stems, however, because they decay too rapidly. The type of the electrode had no significant influence on the phase angle and impedance modulus with frequencies higher than about 100 Hz (Glerum and Zazula 1973, Pukacki 1982).

When the plant tissue is measured with needle-electrodes, the cells are damaged at the contact site. This probably causes the leakage of the electrolytes into the contact layer improving the contact. If there is not enough liquid around the electrodes, air can diffuse to the contact layer which can cause instability of the readings. The damage caused by the electrodes can also have harmful effects because they increase the risk of infections by fungi during long experiments.

2.3 Temperature

The impedance of the plant tissue increases as the temperature decreases (Glerum 1969) (Fig. 2). At the temperature range from 25 °C to 0 °C the impedance increases about 2.7 kΩ $°C^{-1}$. When extracellular freezing occurs a few degrees below zero, the temperature of the tissue increases 1−3 °C (HTE=high temperature exotherm). During this phase the impedance increases with increasing temperature. When all easily freezeable extracellular water is frozen, the impedance value increases again with decreasing temperature at the rate of 80 kΩ$°C^{-1}$. There is some hysteresis in the curve of Fig. 2 during the warming phase which results in the impedance at room temp-

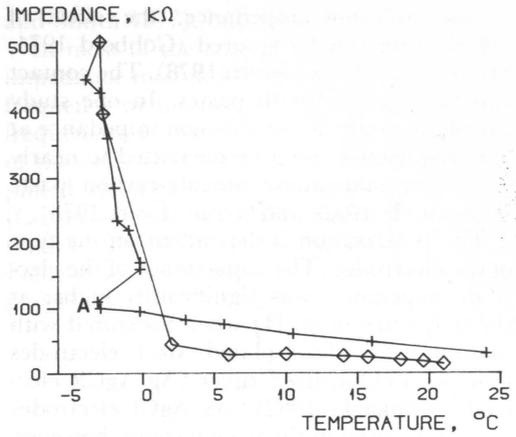

Fig. 2. The effect of the tissue temperature on the impedance (f=1 kHz) in red pine during the cooling (−+−) and warming. (−◇−) phases. Point A indicates the freezing of the extracellular liquid (Glerum 1969).

erature being lower after freezing than before. This usually reflects cell injuries but may be due to the endotherm or acclimation (chapter 3.6). No changes resulting from the low temperature exotherm (LTE) in the impedance curves have been reported.

There are three possible explanations for the temperature dependency of impedance. Firstly, it may be due to the higher mobility of the ions at high temperature (e.g. Tattar and Blanchard 1976). The slope of the temperature dependency of the impedance is not the same during the cooling and warming phases, however (Fig. 2). Secondly, it may be due to the change in membrane resistance and capacitance. Paszewski and Spiewla (1986) found significant temperature dependence of the membrane resistance in *Characeae* cells. In the temperature range 4−48 °C the electrical resistance of the cell membranes underwent from 5 to 9 distinct changes. Thirdly, the apparent stationary state between intra- and extracellular liquids corresponding to a given temperature may be disturbed when the temperature is changed. A change in the water balance would be reflected in the impedance (chapter 3.6).

2.4 Cross-sectional area

Seedlings with small diameters have higher impedances than those with large ones (van den Drienssche 1969, Wargo and Skutt 1975, Carter and Blanchard 1978, Pukacki 1982). If the material was isotropic, the dependency of the impedance on the cross-sectional area would be linear. The non-homogeneity of plant tissue caused by different cell-layers and also the variation in the composition of the cell structure within a population contribute to the nonlinearity.

The impedance (f=1 kHz) of the shoots of Scots pine and Norway spruce decreases exponentially as a function of the cross-sectional area (Fig. 3). The shape of the curves is about the same in different developmental stages although the level is changed. When the area is greater than about 15 mm^2 (i.e. d>4.4 mm) the impedance modulus is independent of the area. Pukacki (1982) found a linear correlation between impedance of uninjured Magnolia shoots and diameter (2 mm <d< 4 mm) but no relationship in the case of injured shoots. The influence of diameter becomes minimal at about 5 mm and above (Glerum and Krenciglowa 1979). With large diameter samples most of the stem will consist of wood, and the normalized impedance magnitude is thus independent of diameter (Evert 1973).

To further explore this relationship, a study using Scots pine and Norway spruce seedlings was done in our laboratory. It was assumed that the shoot cross-section was circular. The following evaluation was used to calculate the specific impedance with equation

$$z = \frac{\pi d^2}{4\,l}\,|Z| \qquad (1)$$

where z = specific impedance, Ωm
d = diameter of the shoot, m
l = distance between the electrodes, m
$|Z|$ = impedance modulus, Ω

It was found that this area normalization of impedance decreases the dependency on the area significantly. Specific impedance has a rather constant value when the cross-sectional area exceeds 5 mm^2 (Fig. 4). With small diameters significant measurement errors of the impedance and the diameter are possible

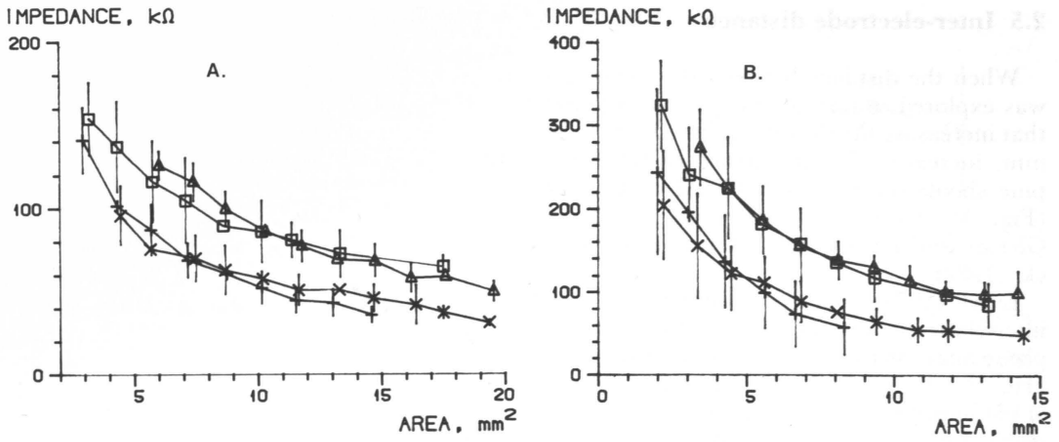

Fig. 3. The impedance (f=1 kHz) of the previous year's shoots of Scots pine (A) and Norway spruce (B) in different growth stages as a function of the cross-sectional area of the shoot. The samples were taken in March and April (−□−), in May and June (−+−), in July and August (−×−), and in September and October (−△−). Impedance measurements were done at room temperature.

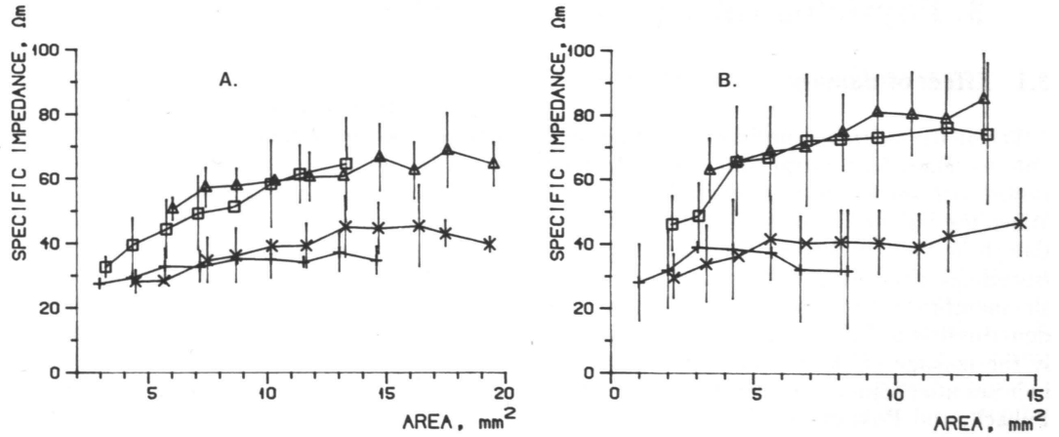

Fig. 4. The specific impedance (f=1 kHz) of the previous year's shoots of Scots pine (A) and Norway spruce (B) as a function of the cross-sectional area. Same material as in Fig. 3.

which may cause the change of the specific impedance in that range.

Specific impedance of the shoots and needles is dependent on the developmental stage (Repo et al. 1984, Fig. 4). It is generally high in winter and low in summer. There are also slight seasonal changes in the area-dependency of the shoots. Presumably the changes are more obvious in developing shoots where cell differentiation is occurring than in the previous years' shoots.

There is controversy about the usefulness of the area normalization. It did not eliminate diameter dependence of the impedance measurements in woody stems (Fensom 1966, Glerum and Krenziglowa 1970, Glerum 1980) or shoots of one-year-old Magnolia (2 mm <d< 4 mm) (Pukacki 1982).

2.5 Inter-electrode distance

When the distance between the electrodes was explored in our laboratory it was found that increasing the distance from 10 mm to 50 mm, increased the impedance of the Scots pine shoots linearly from 40 kΩ to 190 kΩ (Fig. 5). Similar linearity was found by Glerum and Zazula (1973) but not by Pukacki (1982).

Evert (1973) found no influence of the inter-electrode distance (> 7 mm) on the phase angle at the frequencies of 50 Hz−500 kHz. He also proposed that the extrapolation of the impedance magnitude to an inter-electrode distance of zero would indicate electrode imperance.

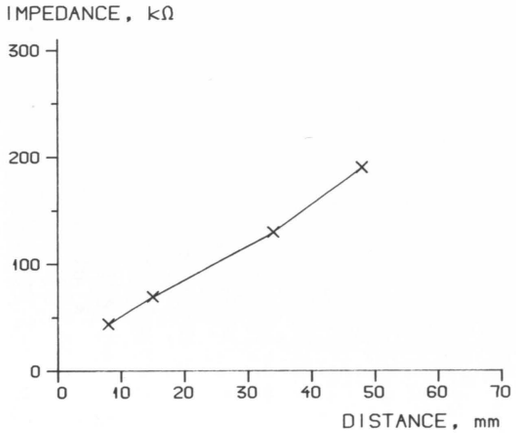

Fig. 5. The impedance (f=1 kHz) of a one-year-old shoot of Scots pine as a function of inter-electrode distance.

3. Physiological aspects of impedance measurements

3.1 Effect of damage

When the cells are undamaged, they are able to retain high intracellular ion concentration with respect to the intercellular space. When the cell membranes are injured by frost they lose their ability to maintain high intracellular concentration because the intrinsic membrane proteins (e.g. ATPases) are denatured (e.g. Palta and Li 1978). Especially the leakage of the K^+-ions from the cells increses after injury (van den Driessche 1969, Pukacki and Pukacka 1987). As a result the concentration gradients between the intra- and extracellular spaces disappear. The damage can be seen as a decreasing of the low-frequency impedance.

The impedance of the undamaged tissue is high at low frequencies. When the cells and especially the cellmembranes are damaged by frost, the low-frequency impedance decreases (e.g. Wilner 1961, Glerum 1962, van den Driessche 1969). As an example the impedance modulus of the uninjured one- and two-year-old shoots of Scots pine and Norway spruce is in the range 100−300 kΩ (Fig. 3) (f=1 kHz), whereas the corresponding values of the seriously damaged shoots are 10−50 kΩ.

When the frost resistance is estimated using the impedance method, it is useful to compare the impedance values of the same plants before and after frost exposure. If the plants have suffered no damage their impedances should be equal. The difference between impedances after and before frost treatment should be lower with damage than without. With this relationship in mind we calculated the impedance difference and normalized it with respect to the cross-sectional area and interelectrode distance as in equation 2 and for comparison as in Eqn. 3. The result of Eqn. 2 is called specific impedance difference and that of Eqn. 3 difference of specific impedances.

$$\Delta z_1 = \frac{\pi d^2}{4l}\left(|Z_a| - |Z_b|\right) \qquad (2)$$

$$\Delta z_2 = \frac{\pi d_a^2}{4l}|Z_a| - \frac{\pi d_b^2}{4l}|Z_b| \qquad (3)$$

where Δz_1 = specific impedance difference Ωm
Δz_2 = difference of specific impedances Ωm
d = diameter, mean of six measurements (three before and three after frost treatment) m
d_a = diameter after frost treatment, mean of three measurements m

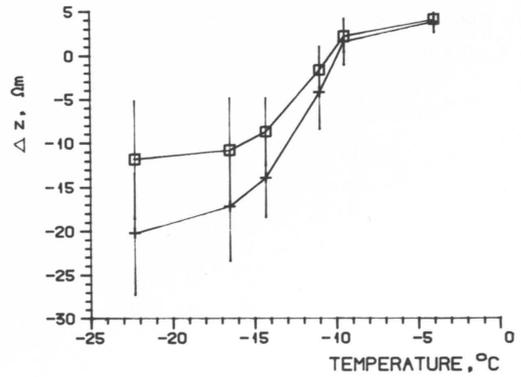

Fig. 6. The specific impedance difference (−□−) and the difference of specific impedances (−+−) (f=1 kHz) of the shoots of one-year-old Scots pine seedlings as a function of frost treatment temperature. The experiment was carried out during the dehardening phase. The dots represent the mean of 10 seedlings. The standard deviations are indicated with bars.

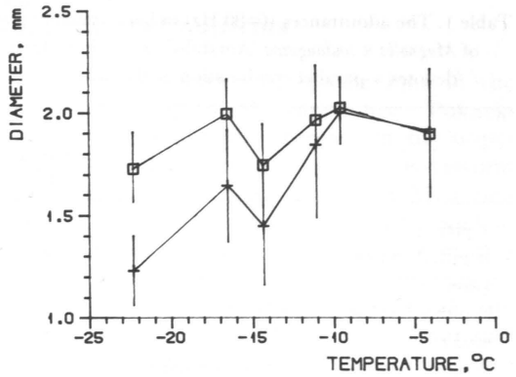

Fig. 7. The diameter of the shoots of the same seedlings as in figure 6 before (−□−) and after (−+−) frost treatment as a function of frost treatment temperature. The diameter was measured with micrometer (Mitutoyo, accuracy ± 0.01 mm) three times before and three times after frost treatment from random directions on the shoot. The needles of the shoots were removed from the measurement point.

d_b = diameter before frost treatment, mean of three measurements m
l = distance between electrodes m
$|Z_a|$ = impedance modulus after frost treatment Ω
$|Z_b|$ = impedance modulus after frost treatment Ω

When the Δz (Δz_1 and Δz_2) is expressed as a function of the frost treatment temperature the curves as calculated with Eqn. 2 and 3 differ somewhat. The phenomenon is more obvious in young current year's shoots than in older ones but can be found also in naturally hardened one-year-old Scots pine seedlings during the dehardening phase (Fig. 6).

The difference between the curves in Fig. 6 is caused by the change of the diameter of the shoots during the frost treatment (Fig. 7). The average difference between undamaged and damaged shoots is as high as 0.5 mm which has a signifcant effect on the Δz-value if either Eqn. 2 or Eqn. 3 is used. The contraction is propably partly due to the damage of the cells, partly due to compressing the stem when measuring the diameter.

3.2 Effect of different cell-layers

There are few studies on the impedance characteristics of separate cell-layers, except in the case of bark and wood of red-osier dogwood (Evert 1973), xylem of red pine (*Pinus resinosa* Ait.) and poplar (*Populus deltoides* Marsh.) (Glerum and Krenciglowa 1970, Glerum and Zazula 1973) and pith of Magnolia shoots (Pukacki 1982). Bark, which is regarded as a combination of periderm, phloem and cambium, and xylem were measured by inserting the electrodes in a step by step procedure through different cell-layers (Glerum and Krenciglowa 1970, Glerum and Zazula 1973).

Pukacki (1982) estimated the effect of pith, xylem, cambium and bark on the impedance of Magnolia shoot by peeling the shoot layer by layer without elimination of the effects of the inside layers. Using these results the author calculated the approximate values of the impedance modulus (f=80 Hz) of different intact and injured layers (Table 1). Pith, xylem, cambium and bark were assumed to form a parallel circuit of electrical resistances.

The impedance of the thin cambium layer is high and injuries there cause a drastic decrease of impedance. Uninjured pith, xy-

Table 1. The admittances (f=80 Hz) of bark, cambium, xylem and pith of one-year-old uninjured and injured shoots of *Magnolia* x *soulangiana* 'Amabilis' (Pukacki 1982) and the corresponding impedances calculated by the author. / /denotes a parallel combination of the layers.

	Uninjured		Injured	
	admittance μS	impedance kΩ	admittance μS	impedance kΩ
½ *pith*	4.8	207	5.7	175
½ pith / / xylem	9.2	109	10.5	95
xylem	–	228	–	207
½ pith / / xylem / / cambium	10.5	95	18.9	53
cambium	–	760	–	120
½ pith / / xylem / / cambium / / bark	15.1	66	25.9	39
bark	–	219	–	143

lem and bark have about the same impedance values, but injuries cause the most remarkable change in the values of bark, and the smallest one in xylem.

The calculated results in Table 1 can be questioned, however, because the peeling may be injurous. For example the impedance of the peeled bark and wood, when measured together, was lower than that of the intact stem of red-osier dogwood (Evert 1973). Also when the stem impedance is calculated using the separately measured values of bark and wood, the calculated stem impedance is lower than that of the intact stem (Glerum and Zazula 1973, Evert 1973). Maybe the application of the circuit theory of parallel resistances is not reasonable in this case.

There is a strong inverse correlation between the electrical resistance of combined bark and wood of red maple (*Acer rubrum* L.), and the phloem width (Carter and Blanchard 1978). Similar results were found in lodgepole pine (*Pinus contorta* Dougl.) (Cole and Jensen 1979). Cambial electrical resistance has been related to the number of cells per radial file of vascular cambium in balsam fir (*Abies balsamea* (L.) Mill.) during the growing season (Smith et al. 1984).

The differences in the impedances of the cell-layers can be explained with the structural differences of the cells and the thickness of the layers. The central pith and xylem have few living protoplasm and membranes to maintain high intracellular ion concentration, in contrast to the cambium and phloem. The studies of Glerum and Zazula (1973) and Pukacki (1982) showed, that if the electrolyte content of the tissue is high, as in living tissues, the impedance is low. There are also more membranes in the living tissues which contribute to the reactive part of the impedance through the permittivity of the membrane material and through the boundary layers associated with the membranes and the electrodes.

3.3 Effect of growth stage

The specific impedance of the current year needles and shoots are lower than the previous year's corresponding parts until September. Also the values of the previous year's shoots and needles are lower in summer than in winter (e.g. Glerum 1973, 1980, Repo et al. 1984, Fig. 4). This may be due to the seasonal changes in vacuolization and wall thickness in the cells (Sennerby-Forsse 1986). Also concentration of the liquids and storage materials in the cells, and the thickness of the layers change from one growth stage to another (e.g.. Carter and Blanchard 1978, Cole and Jensen 1979, Levitt 1980, Sennerby-Forsse 1986). The cambial zone is thinner (Sennerby-Forsse 1986), and the phloem is thicker (Cole and Jensen 1979) during dormancy than during the active period.

Structure and compartmentalization of water is different in dormant than in active cells (Tumanov 1967). The water content of the tissues, and the distribution between the intra- and extracellular spaces are also characteristics which change during the year (e.g. Scarth 1936, Levitt 1980). In addition, there is a slight within-day fluctuation with the specific impedance of the needles of Scots pine in summer (Repo et al. 1984), which may be connected with changes in water content (Scarth 1936), and water potential of the tissue (e.g. Dixon et al. 1978, Unger 1980, Hillerdal-Hagströmer et al. 1982), or the water potential of the ambient air (Gagnon et al. 1987). The rapid acclimation of hardened samples (shoots, needles), when transferred to room temperature can be a source for the higher impedance values in winter than in summer (chapter 3.6).

3.4 Effect of the moisture content

The effects of water content on impedance are inconsistant. Tattar et al. (1972) found that the electrical resistance of plant tissues will be relatively independent of small changes in tissue water content as long as sufficient interstitial free water is available (moisture content > 30 %). The resistance of the undamaged tissue of red maple changed by 50 kΩ/% change in water content in the moisture range 20 %−35 %. Glerum (1980) proposed that moisture content has little effect on impedance when the moisture content is above the fibre saturation point. The impedance of the Magnolia shoots decreased from 400 kΩ to 50 kΩ, however, when the moisture content increased from 30 % to 50 % (Pukacki 1982, cf. also Kucera 1986). In living tissues the moisture content is usually well above the fibre saturation point (about 30 %).

Karmanov et al. (1964, 1965) and Meleshchenko (1965) suggested that impedance is closely connected with water metabolism of the plants. The observation is supported by the correlation of the impedance with water potential (e.g. Dixon et al. 1978 and Unger 1980).

3.5 Effect of nutrients

The accumulation of mobile ions in the tissues can decrease impedance. Pukacki (1982) found a slight decrease in the impedance (f=80 Hz) with increasing potassium and sodium content in uninjured Magnolia shoots. A greater response was observed in frozen shoots. Tattar et al. (1972) found a correlation between resistive impedance (f=100 Hz) of the shoots of sugar maple and the concentration of potassium and calcium ions. High concentrations of ions in solutions taken up by the shoots resulted in a large decrease in resistive impedance. The impedance (f=1 kHz) of birch (*Betula pendula*) seedling shoots was low when the potassium concentration of fertilizer was high (Jozefek pers. comm.).

3.6. Effect of acclimation

Recent studies in our laboratory have shown that the specific impedance of hardened shoots of Scots pine and Norway spruce changed when transferred to room temperature (Fig. 8). In the first phase, which was over in a few minutes, the specific impedance decreased as a result of melting of the extracellular ice. At the beginning of the second phase, there occured about a half hour long latent period after which the specific impedance increased. An exact stationary level was not reached in either species within eight hours, although the steady state is more obvious in spruce than in pine. After the frost treatment (–15 °C for 3 h), which was suspected not to be injurious to the cells in this phase, no significant acclimation was observed (Fig. 8).

This acclimation phenomenon causes problems in the frost resistance estimation when the plants' growing conditions differ significantly from the measuring conditions. Due to the acclimation, the specific impedance difference at the high temperature level as calculated with equation 2 is below 0 Ωm although no cell injuries are suspected during the frost treatments. In this case the estimation of the temperature equivalent of –10 Ωm is inaccurate, or it is impossible if the high

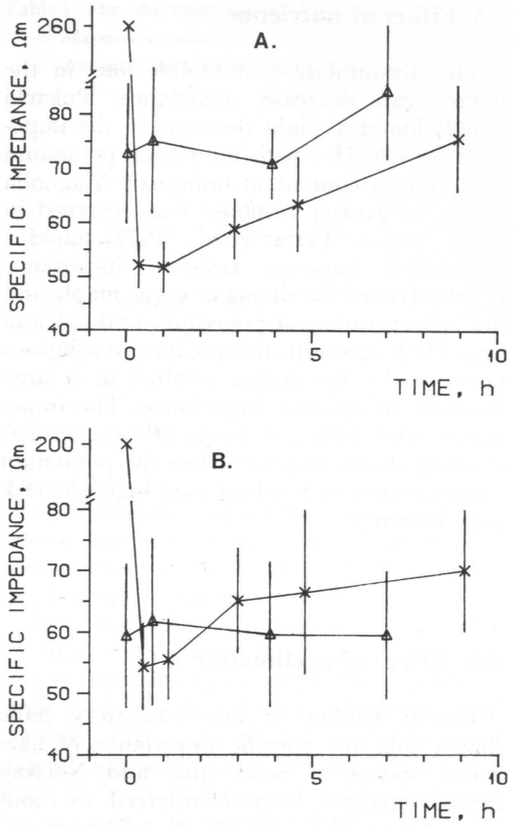

Fig. 8. The specific impedance of the wintertime (March 18) shoots of Scots pine (A) and Norway spruce (B) before (−×−) and after −△−) frost treatment as a function of time at room temperature (mean of 15 shoots). All measurements were made on the same shoots. The frost treatment consisted of freezing from 20 °C to −15 °C and remaining at the low temperature for 3h. The cooling and the warming rates were 6 and 7 °C h^{-1}.

temperature level of specific impedance difference is <−10 Ωm.

Contrary to the results in Figure 8, the specific impedance of the one-year-old Scots pine seedlings, when stored about 2 months in cold storage, decreased from about 45 Ωm to 30 Ωm after 20 hours at room temperature. Because the time constant for temperature stabilization of the tissue with diameter 2−3 mm is a few minutes, the phenomenon can not be explained by temperature changes in the tissue (cf. chapter 2.3).

The explanation of wintertime shoot acclimation at room temperature is unclear. It may be coupled with water flows between intra- and extracellular spaces. The specific impedance (f=1 kHz) is supposed to represent the resistive nature of the extracellular space. Because there is an imbalance in the vapour pressure between intra- and extracellular spaces after thawing of the extracellular ice, water flows inside the cells causing swelling. When the amount of the extracellular water diminishes the specific impedance value increases. The swelling would not be strong enough, however, to plasmolyse the cells and thereby decrease the impedance.

This acclimation phenomenon necessitates that much more accuracy is required with the measurement temperature, when impedance measurements are carried out at different growth stages. To eliminate the acclimation error the temperature where the measurements are carried out may be necessary to change according to the temperatures of the growing conditions. The measurement temperature has to be > 0 °C, however, because the impedance of the frozen tissue is very high. When the measuring conditions are changed, the error caused by the temperature dependency of the impedance of the cell liquids, and maybe also that of the cell membranes, increase. On the other hand the error may be small after the specific impedance difference is calculated.

If the seasonal fluctuation of the specific impedance is due to the acclimation phenomenon, it's elimination may make it unnecessary to calculate the specific impedance difference. Thus the frost resistance estimation would be possible only after the temperature response of the specific impedance following frost treatment has been measured. The elimination of the temperature dependency error of the impedance would be possible by using a correction coefficient, as is generally done in the conductivity measurements of liquids.

4. Concluding remarks

Although there are many factors which have an effect on the impedance characteristics of the plant tissue, the use of the impedance method in frost resistance studies is not as cumbersome and unreasonable as it may seem (cf. Greer 1983, Repo and Pelkonen 1986). Once we are satisfied with measuring the impedance modulus of the whole shoot with a relatively low frequency (100−1000 Hz), the technical factors do not present large difficulties. Many of the factors can be standardized. For example by using a given type of electrodes, a constant frequency, temperature and inter-electrode distance, the error caused by these factors can be minimized. The effect of the nutrient status and the water content can be normalized when the same material is measured both before and after frost treatment and the results compared.

The accurate measurement of the cross-sectional area of the stem and especially of different cell-layers separately is a problem. For the present there are no electrodes available to measure the impedance modulus and phase angle of separate cells or cell-layers in higher plants. The studies of the effect of the temperature acclimation on impedance is just beginning and so far the phenomenon is unexplained. Many technical problems appear when the impedance phase angle is measured at high frequencies (above 100 kHz). The solving these problems is necessary for further developing of the method.

When the impedance method is further developed for frost resistance studies of plants, the effort should be concentrated on developing the method in the direction of impedance spectroscopy. The estimation of the electrical model for different plant tissues is also important. This necessitates improvement of the measuring technique including the electrodes, and both measuring and analysing devices so that more details of the plant cells and tissues can be measured. Then the effect of different stress factors on the cell constituents and, thereby, on the parameters of the electrical model could be predicted more accurately. The analysis of noise input voltage response may be a future tool in impedance spectroscopy. Also the analysis of the electrical noise generated by the membranes is an "unploughed" area of cryobiological electrophysiology in higher and lower plants

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Total of 50 references

Appendix. Impedance locus

One of the commonly used circuits to describe the biological structure is that shown in Fig. A1.

Impedance of this circuit can be written in a complex notation:

$$Z = (R_1 + \frac{R_2}{1+\omega^2C^2R_2^2}) - j\,(\frac{\omega\, CR_2^2}{1+\omega^2C^2R_2^2}) \qquad (1')$$

where R_1, R_2 = resistances
C = capacitance
ω = angular velocity
= $2\pi \cdot$ frequency
$j = \sqrt{-1}$

The first term in the parentheses of Eqn. 1' is the real or resistive component and the second term the imaginary or reactive component of the impedance. Indicating these terms as R and X, respectively, the impedance modulus of the considered circuit simplifies to

$$|Z| = \sqrt{R^2 + X^2} \qquad (2')$$

and the phase angle of the impedance becomes

$$\tan\varphi = \frac{X}{R} \qquad (3')$$

The low frequency impedance of the circuit in the inset of Fig. A1 can be expressed as

$$R_0 = R_1 + R_2 \qquad (4')$$

and the high frequency impedance

$$R_\infty = R_1 \qquad (5')$$

Indicating the time constant $R_2C=\tau$, the resistive component can then be written

$$R = R_\infty + \frac{R_0 - R_\infty}{1+\omega^2\tau^2} \qquad (6')$$

and the reactive component

$$X = -\frac{\omega\tau\,(R_0 - R_\infty)}{1+\omega^2\tau^2} \qquad (7')$$

Impedance modulus, phase angle, real part and imaginary part of the given circuit are all dependent on frequency. When the frequency is changed between 0 and ∞, Z will change continually along the curve shown in Fig. A1. The corresponding change in the RX-plane will be as in Fig. A2, which is called the impedance locus of the circuit.

The interception of the locus, or more precisely the interception of the hemisphere or -spheres with the real axis, and the center of the hemisphere characterize the electrical circuit under consideration.

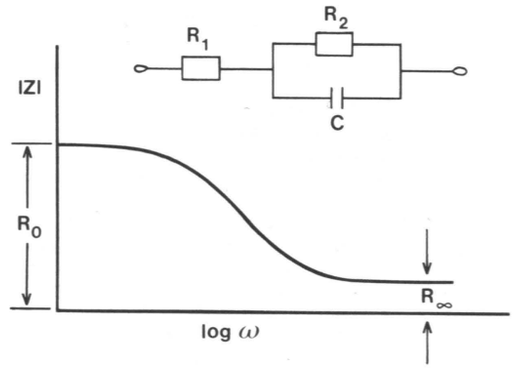

Fig. A1. Impedance modulus of the circuit in the inset as a function of logarithm of angular velocity.

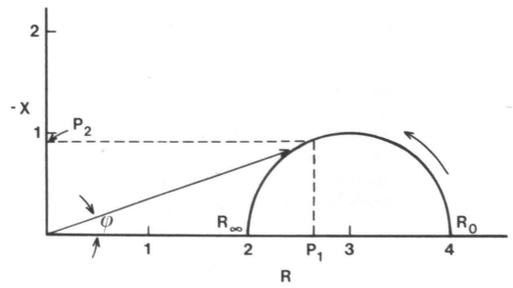

Fig. A2. Impedance locus, it is imaginary part of impedance as a function of real part, of the circuit in Fig. A1. P_1 and P_2 indicate resistive and reactive component of impedance at a given frequency. The direct current impedance $R_0 = R_1 + R_2$. The impedance at very high frequencies is $R_\infty = R_1$.