PART E

BIOPHYSICAL TECHNIQUES

ELECTRICAL IMPEDANCE TECHNIQUES IN PHYSIOLOGICAL STUDIES

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(Received for publication 18 January 1980)

ABSTRACT

The use of electrical impedance techniques with plant tissues is briefly reviewed, after which several annual electrical impedance trends in the stems of several Ontario coniferous species at three different locations are examined. The trends in spruce are always more pronounced than those in pine. The impedance trends of cold stored stock are significantly higher than those trends of the outdoor stock. It is inferred from the multiple regression analyses that to some degree impedance measures dormancy and frost hardiness.

INTRODUCTION

Measurements of the electrical impedance of plant tissues have been used in a variety of physiological studies but mainly in determining the extent of injury after freezing in frost hardiness studies. Annual trends in electrical impedance have received little attention. It is intended here to briefly review the various aspects of the electrical impedance method and then to examine several annual electrical impedance trends in the stems of some coniferous species.

ELECTRICAL IMPEDANCE METHOD IN REVIEW

When measuring the electrical resistance of plant tissues it is necessary to use an alternating current because electrode polarisation occurs when direct current is used and this produces a spurious signal and hence an incorrect reading. Electrical resistance is associated with direct current (DC) where Ohm's law $R = E/I$ gives the relationship between resistance (R), voltage (E) and current (I). Electrical impedance on the other hand is associated with alternating current (AC). However, Ohm's law still holds true for alternating currents except that the R is replaced by impedance (Z). In plant tissues there is no significant inductive reactance (X_L) and thus Z is the resultant of R and the capacitive reactance (X_c), which is frequently illustrated as in Figure 1. Plant cells

N.Z. J. For. Sci. 10(1): 196-207 (1980).

are leaky capacitors, that is, electrically each cell can be considered to be a capacitance (c) in parallel with a resistance (R_C). Therefore Z is not simply a combination of X_C and the series resistance (R), but includes the component R_C, the presence of which affects the phase angle (θ). The capacitive reactance varies inversely with the frequency of the current so that impedance is lower at higher frequencies. Generally, only the resistive component of the impedance is measured by balancing out the capacitive component. The theoretical aspects of impedance are discussed in detail by Remington (1928), Rothschild (1946) and Hearle (1953).

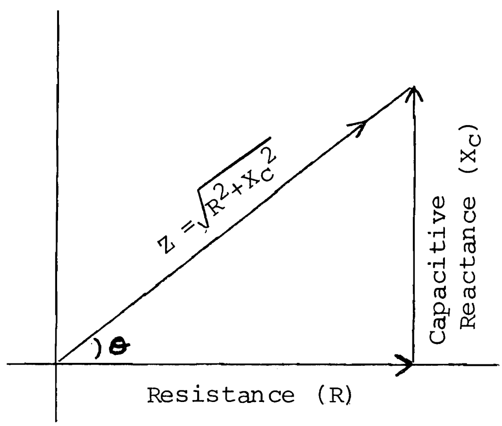

FIG. 1—Diagrammatic illustration of impedance. In an a.c. circuit resistance (R) and capacitive reactance (X_C) are not additive, but the resultant produced is impedance (Z), which is a complex number. The angle θ is the phase angle between current and applied voltage because of the presence of R and X_C together in the circuit.

Electrical impedance measurements are influenced by a number of physical factors, such as tissue size, temperature and moisture content, each of which has to be taken into consideration when measurements are taken.

The influence of diameter on impedance has been noted by many investigators (Fensom, 1966; van den Driessche, 1969; Glerum and Krenciglowa, 1970; Wargo and Skutt, 1975). Seedlings with small stem diameters usually have higher impedance readings than those with large diameters. However, I have found that the influence of diameter becomes minimal at about 0.5 cm and above. This is attributed to the fact that most of the AC current will pass through the tissue that lies directly between the electrodes, but some of the current will follow a less direct curved path between the electrodes. When the cross-sectional area of the tissue is increased, a wider pathway for the current is produced, which causes a decrease in electron density between the electrodes. Consquently, beyond a certain stem diameter, which is characteristic for a particular electrode spacing, tissue and current, increasing the diameter will have a negligible effect on the impedance.

The influence of temperature on impedance has also been noted by many investigators (Rothschild, 1946; Davidson, 1958; Fensom, 1966; Glerum, 1969). Impedance increases when temperature decreases, which is in agreement with the behaviour of ionic conduction in an electrolytic solution.

Although in some tissues it can be easy to measure changes in impedance with changes in moisture content, the results are not always easy to interpret (Meleshchenko, 1965; Sheriff and Sinclair, 1973). It is well established that in wood there is little change in resistivity and capacitance with moisture contents (mc) above the fibre-saturation point (fsp) which is between 25-30% mc (Brown *et al.,* 1963; Venkateswaran, 1971). Tatter *et al.* (1974) found that electrical resistance and capacitance properties of woody tissues above fsp appear to be similar to those of dilute solutions of mobile ions. I was unable to establish a relationship between moisture content and impedance with tissues that had moisture contents in excess of 50% (unpublished data). I suspect, therefore, that moisture content has little effect on impedance when the moisture content is above the fibre saturation point, and in living tissues the moisture content is usually well above that point.

Other aspects to be considered when taking impedance measurements are the type of electrodes and the frequency of the current. Nearly any type of steel pin can be used for an electrode. The claim that chloridised silver electrodes have to be used, which is based on their use in biopotential measurements, appears to have little validity (Glerum and Zazula, 1973). As mentioned earlier, the frequency of the electrical current is important in impedance measurements (Osterhout, 1922; Luyet, 1932; Glerum and Krenciglowa, 1970). In most studies a frequency of 1000 Hertz (hz) has been used. The suggestion that it is better to use direct current pulses than alternating current (Fensom, 1966; Hayden *et al.,* 1969; Dixon *et al.,* 1978) has, in my opinion, not yet been substantiated. This suggestion is mainly based on theoretical grounds that the path of the pulsed direct current in healthy tissue is known to be almost entirely in channels of the cell walls, while at high frequencies that path is unknown. Certainly it seems advisable not to use frequencies higher than 1 kHz and at times a frequency of 100 Hz might be preferable (Glerum and Krenciglowa, 1970).

The dependence of impedance on frequency resulted in the development of the kilohertz (kHz)-megahertz (MHz) ratio. Luyet (1932) found that the impedance at a frequency of 1 kHz decreased with increasing injury until the death of the tissue, while at 1 MHz the impedance of living and dead tissue was approximately the same. These measurements were similar to those at 1 kHz for dead tissue. Subsequently, Greenham and Daday (1957) suggested a ratio of impedance at 1 kHz over 1MHz as a criterion of vitality of injury. This ratio should come close to 1.0 when injury is fatal and should be above 3.0 when healthy, which is in agreement with other investigators (Glerum, 1970; van den Driessche, 1973). The advantage of this method is that only after-treatment measurements are required while 1 kHz impedance can be used to detect injury only, if measured both before and after treatment.

The fact that electrical impedance of plant tissue decreases significantly upon injury has made impedance extremely useful in frost hardiness studies, because in most cases frost hardiness is determined by subjecting tissue to freezing temperatures and subsequently determining the extent of injury (Wilner, 1962; van den Driessche, 1969; Glerum, 1973; Bialobok and Pukacki, 1974). Impedance is also quick, easily used and non-destructive. It has been suggested that impedance can measure frost hardiness directly (Wilner, 1967; Weaver *et al.,* 1968; van den Driessche, 1970; Glerum, 1973).

A high impedance reading indicates that the tissue is frost hardy, whereas a low reading suggests that the tissue is not hardy. Svejda (1970) disagreed with this, because she found little difference in the impedance of hardy and non-hardy rose tissue. The limitations of this approach have not been established as yet and requires more investigation.

Electrical impedance has also been found useful in the field of pathology, as is evident by the development of the "Shigometer", which detects discoloration and decay in trees (Shigo and Shigo, 1974). The role of bioelectrical measurements in that field has recently been reviewed by Tattar and Blanchard (1976).

Electrical impedance has been used in water potential studies. Karmanov *et al.* (1964, 1965) and Meleshchenko (1965) suggested that impedance is closely connected with water metabolism of the plant. Kitching (1966), on the other hand, found impedance an insensitive index of moisture stress in woody tissue. This does not seem surprising since impedance cannot measure moisture content reliably above fibre saturation point. Sheriff and Sinclair (1973) pointed out that considerable confusion exists in interpreting impedance with relation to water metabolism. However, they found that a relationship existed between impedance and changes in leaf water content. Recently, Dixon *et al.* (1978) suggested that water potential could be measured by impedance, which had been corrected for temperature. They measured impedance and expressed it as a percentage of a measured maximum impedance and the resulting percentage impedance varied linearly with water potential. It is evident, that considerable investigation is required before the role of impedance in water potential studies is clarified.

Another development in electrical impedance studies is the use of an oscilloscope with a square-wave current. The square-wave becomes distorted when the current passes through tissue, live tissue giving a different distortion than dead or injured tissue (Zaerr, 1972). Some investigators think that these traces can be used to determine dormancy (Ferguson *et al.,* 1975), but others have their reservations (Askren and Hermann, 1979). The usefulness of this approach is not yet clear and requires further testing.

In the last few decades interest has increased in electrical impedance and the literature on the subject has expanded rapidly. Hayden *et al.* (1972) pointed out that the theory of electrical resistance measurements in plants has been extended considerably by Fensom (1966) and by Hayden *et al.* (1969). In their earlier paper, Hayden *et al.* proposed a model to determine the impedance of potato tuber and alfalfa stems and roots, in which the cellular components such as cell membrances, cell walls, cytoplasm and vacuoles were used. This model has proven to be inadequate for highly differentiated woody stem sections because it does not represent their observed impedance properties (Glerum and Krenciglowa, 1970; Evert, 1973). It is unlikely that one model can be constructed that will be applicable to all plants because it should be realised that each type of tissue has its own impedance characterstics.

ANNUAL ELECTRICAL IMPEDANCE TRENDS IN CONIFEROUS NURSERY STOCK

Annual electrical impedance trends were established for some coniferous species at three tree nurseries in Ontario, because of the seasonal trends in electrical impedance observed earlier: high impedance in winter and low impedance in summer (Glerum, 1973). Electrical impedance was measured at weekly intervals for three years at Kemptville (Lat. N. 45° 01', Long. 75° 38') and Swastika (Lat. N. 48° 06', Long. 80° 06') nurseries and for two years at Orono (Lat. N. 43° 59', Long. 78° 37') nursery. At Orono and Kemptville, 25 trees per species per week were measured and 20 trees per species at Swastika. All measurements were taken on regular nursery stock, which was in most cases 3 + 0 or rising 3 + 0 stock, with the exception of jack pine (*Pinus banksiana* Lamb.) where the age-class was 2 + 0 or rising 2 + 0. The species used at Orono were red pine (*P. resinosa* Ait.), white pine (*P. strobus* L.), white spruce (*Picea glauca* (Moench) Voss); at Kemptville red, white and jack pine and white spruce; and at Swastika jack pine, white and black spruce (*P. mariana* (Mill.) B.S.P.).

All impedance readings were taken at room temperature (20°C ±2) so as to eliminate the need for temperature correction. Readings were taken on the stem just above the root collar with a 1 kHz impedance bridge, which measured the resistive component of the impedance. Steel pin electrodes were used, which were spaced 1 cm apart and 8 mm long. The electrodes were inserted all the way through the stem after the trees were brought indoors and their tissue temperature had reached room temperature.

The impedance data were analysed using a stepwise multiple regression technique with the regression equation of the form

$$Y = b_0 + b_1X_1 + b_2X_2 + \ldots\ldots + bnXn$$

where Y= dependent variable i.e. impedance (Z)

$X_1, X_2 \ldots\ldots Xn$ = independent variables

$b_0, b_1, b_2, \ldots\ldots bn$ = partial regression coefficients

The number of terms permitted in the equation was determined according to the following criteria: 1) a decrease in standard error (SE) or mean square error (MSE) and, 2) an increase in r^2. Those variables contributing significantly according to these criteria were permitted into the equation. The best equation obtained was

$$Y = b_0 + b_1 \text{Cos}(\text{Day—14}) + b_2\ 1/\text{Diam} + b_3\ \text{Cos}(\text{Day—14}) \times 1/\text{Diam} + b_4\ \text{Cos}(\text{Day—14}) \times \text{Tmin}$$

$$\text{where Cos(Day—14)} = \text{Cos}\left[(\text{Day—14}) \times \frac{360}{365}\right]$$

Day = day number in the year, where Jan. 1 = Day 1

Diam = diameter in cm

Tmin = minimum temp. in Fahrenheit

The equations for each species at each nursery were used to calculate the curves that are plotted together with the average impedance for the several years in Figures 2 to 4. These calculated curves fit the actual data reasonably well. Not all terms contributed significantly to regression in every case. The Cos(Day) and 1/Diam terms were common to all species and nurseries and are associated with most of the variation between and within measurements. The Cos (Day) × 1/Diam term was particularly associated with the spruces at all locations, while the Cos(Day) × Tmin term was associated in all species at the northerly latitude of the Swastika nursery. Temperature was selected as a variable because it influences the frost hardiness process. The multiple regression selected Tmin and not Tmax. It should be realised that time of year Cos(Day) is strongly correlated with such factors as temperature and light. Consequently, Cos(Day) overrides the effect of many variables, which makes the selection of Tmin even more significant.

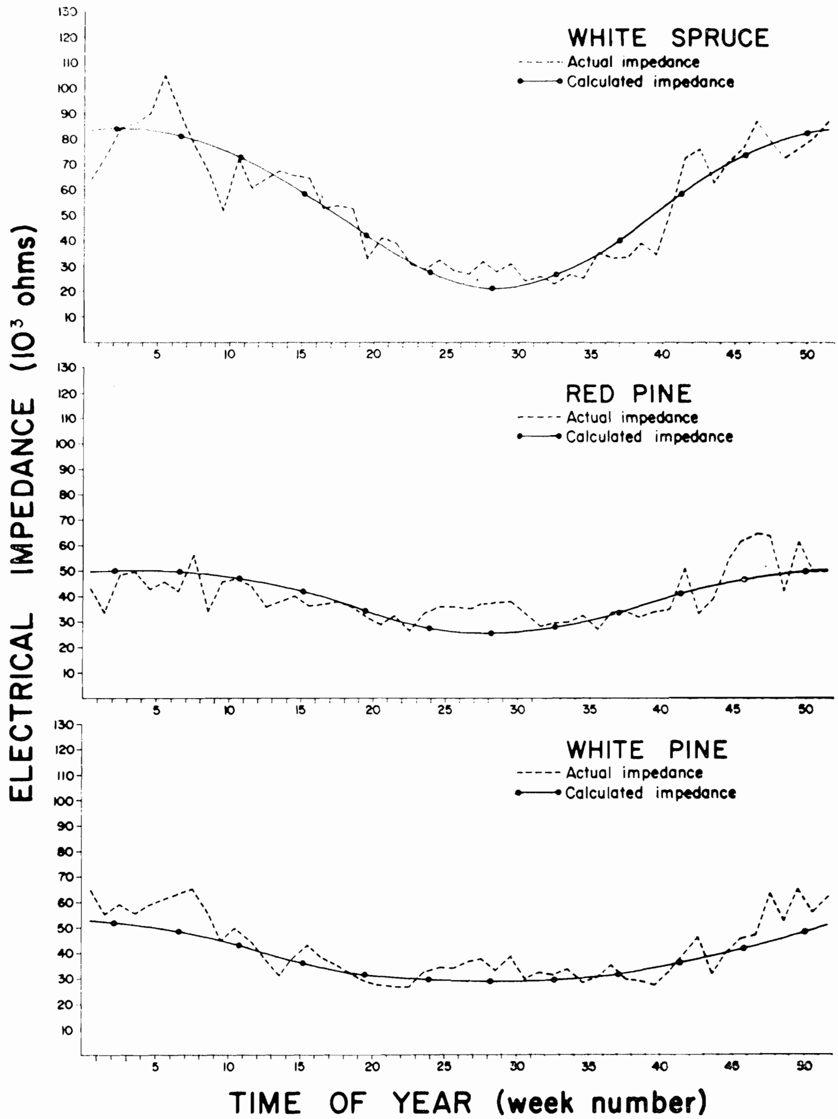

FIG. 2—Two-year average of weekly electrical impedance measurements on three conifers at Orono nursery. The calculated curves for each species are also shown. The slight rise in impedance around week 28 is due to a decrease in diameter when the regular 3 + 0 age-class was shipped and the switch was made to the rising 3 +0 age-class. The r values for red and white pine and white spruce were .72, .83 and .87 respectively.

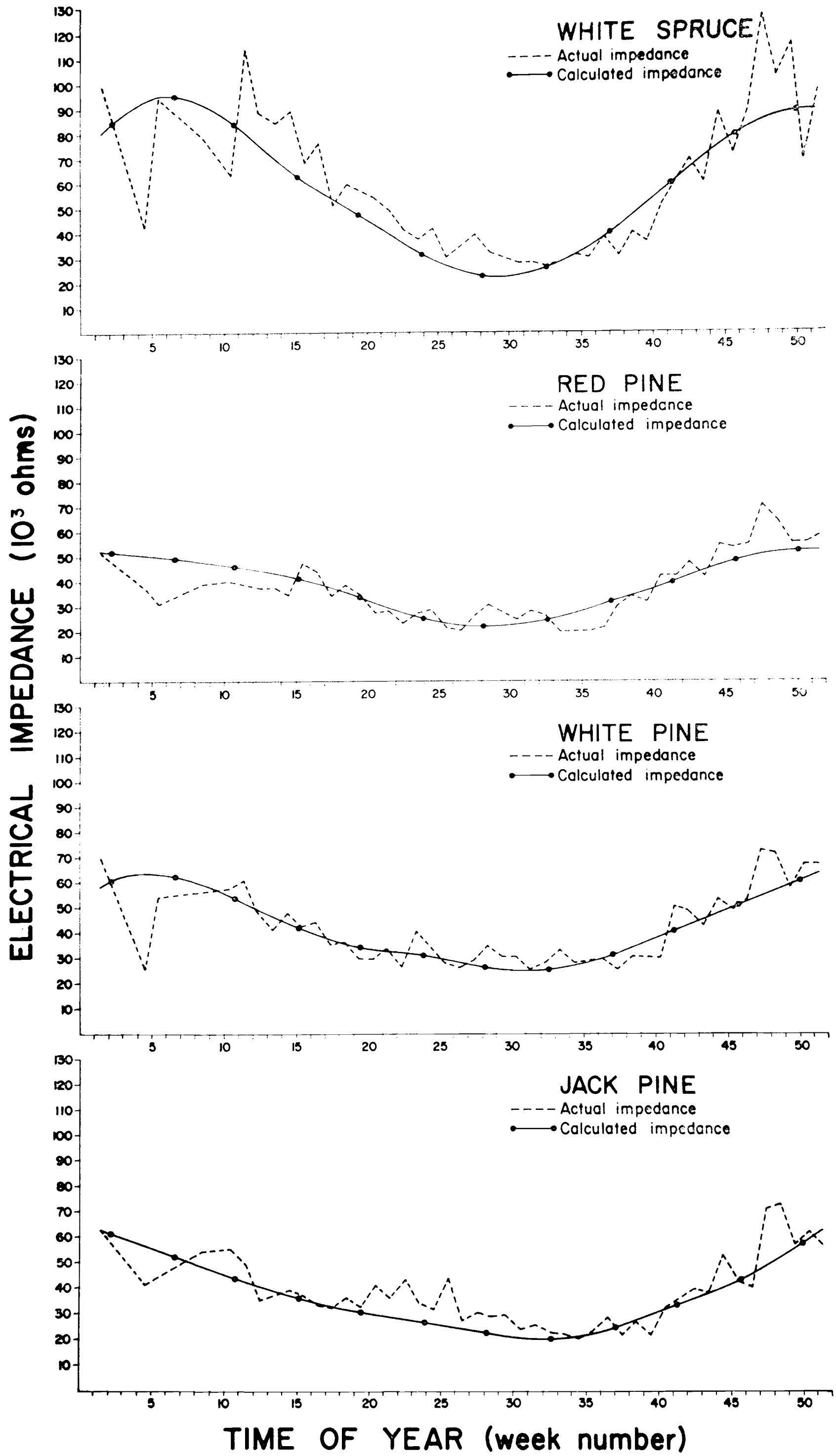

FIG. 3—Three-year average of weekly electrical impedance measurements on four conifers at Kemptville nursery. The calculated curves for each species are also shown. The slight rise in impedance around week 25 is due to a decrease in diameter when the regular age-class was shipped and the switch was made to the rising age-class. The r values for jack, red and white pine and white spruce were .77, .69, .78 and .85 respectively.

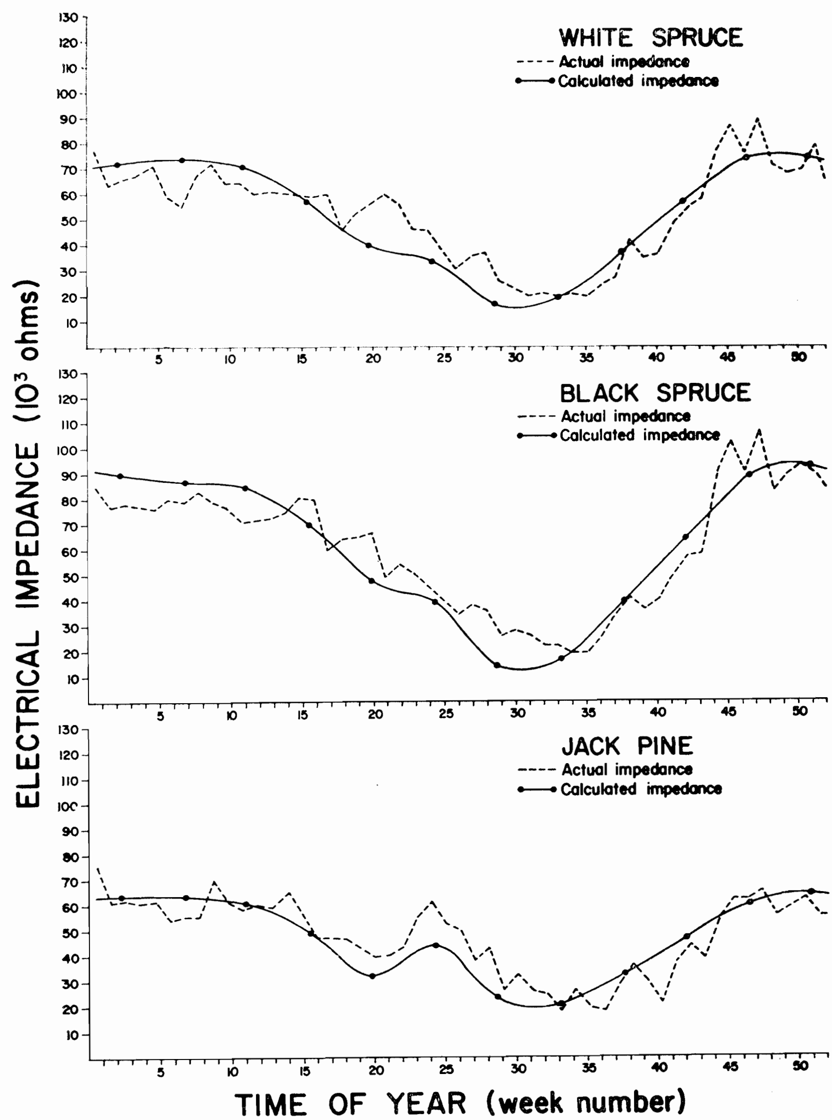

FIG. 4—Three-year average of weekly electrical impedance measurements of three conifers at Swastika nursery. The calculated curves for each species are also shown. The rise in impedance around week 25, particularly in jack pine, is due to a decrease in diameter when the regular age-class was shipped and the switch was made to the rising age-class. The r values for jack pine and black and white spruce were .78, .81 and .81 respectively.

When the impedance measurements were pooled by species, a fifth term relating to geographic location had to be added to the equation. The geographic factor, which is assumed to be latitude, had both an additive and interactive effect on the equation.

This factor was included in the regression as a dummy index, which corresponded to increasing latitude from Orono to Swastika.

Since I am mainly interested in the autumn and spring periods of the year, which correspond to the frost hardening and dehardening period of nursery stock respectively, seasonal impedance regressions were performed on the autumn (1 Aug.-30 Nov.) and on the spring (1 March-30 June) data. Overall correlations were high in the autumn (.81-.92) and considerably lower in the spring (.61-.85). Understandably, the annual correlation was somewhere between those of the autumn and spring.

Stock is lifted every autumn at the nurseries in Ontario and placed in cold storage until spring. At Orono the stock is generally lifted around mid-November and placed in cold storage for overwintering at about —5°C. To determine how the electrical impedance trend was affected when the trees were held in cold storage, impedance of cold stored red pine was monitored for three winters while that for white spruce for two winters. Twenty-five trees of each species were measured in the same manner as mentioned earlier and the monitoring was done simultaneously with the outdoor stock.

Multiple regression equations were calculated for the cold stored stock and for the outdoor stock for the period of November to May. These equations were used to calculate the curves that are plotted together with the actual measurements of both cold stored and outdoor stock, for each species and treatment in Fig. 5. The null hypothesis of no significant difference between impedance trends of the two treatments was applied. The impedance trends for both cold stored red pine and white spruce differed significantly at the 99% level from the impedance trends of the respective outdoor grown species.

Annual impedance trends do exist, with those in the spruces always being more distinct than those in pine. I do not know the reason for this difference between genera but I feel that it warrants further investigation and certainly illustrates the complexity of impedance and the plant tissue system.

I have hopes that electrical impedance can be used for determining frost hardiness and degree of dormancy, because some relationship exists between frost hardiness, dormancy and electrical impedance. For instance, Rietveld and Williams (1978) have also found that increasing resistance appeared to coincide with the onset of dormancy. However, the difficulty lies in trying to determine the relationships. An oversimplification of these relationships might serve the purpose here. Electrical impedance reflects the ionic or electrolytic content of the tissues. This in turn reflects their metabolic activity, which is high when the trees are growing actively and low when the trees are frost hardy and dormant. Low impedance means high ionic content and high metabolic activity while high impedance means low ionic content and low metabolic activity.

This oversimplification holds true for the data presented here. Differences in metabolic activity are indicated by the geographic term which had to be inserted in the regression equation to obtain a better fit. Nursery stock from the more northerly latitudes will remain dormant and frost hardy longer in the spring than the stock of southerly latitudes. Conversely, the stock will also become dormant and frost hardy earlier in the fall in the northerly latitudes than in the southerly ones. This is suggested by the impedance trends. Another indication is provided by the cold stored stock which retained its dormancy and frost hardiness longer than the outdoor grown stock. The

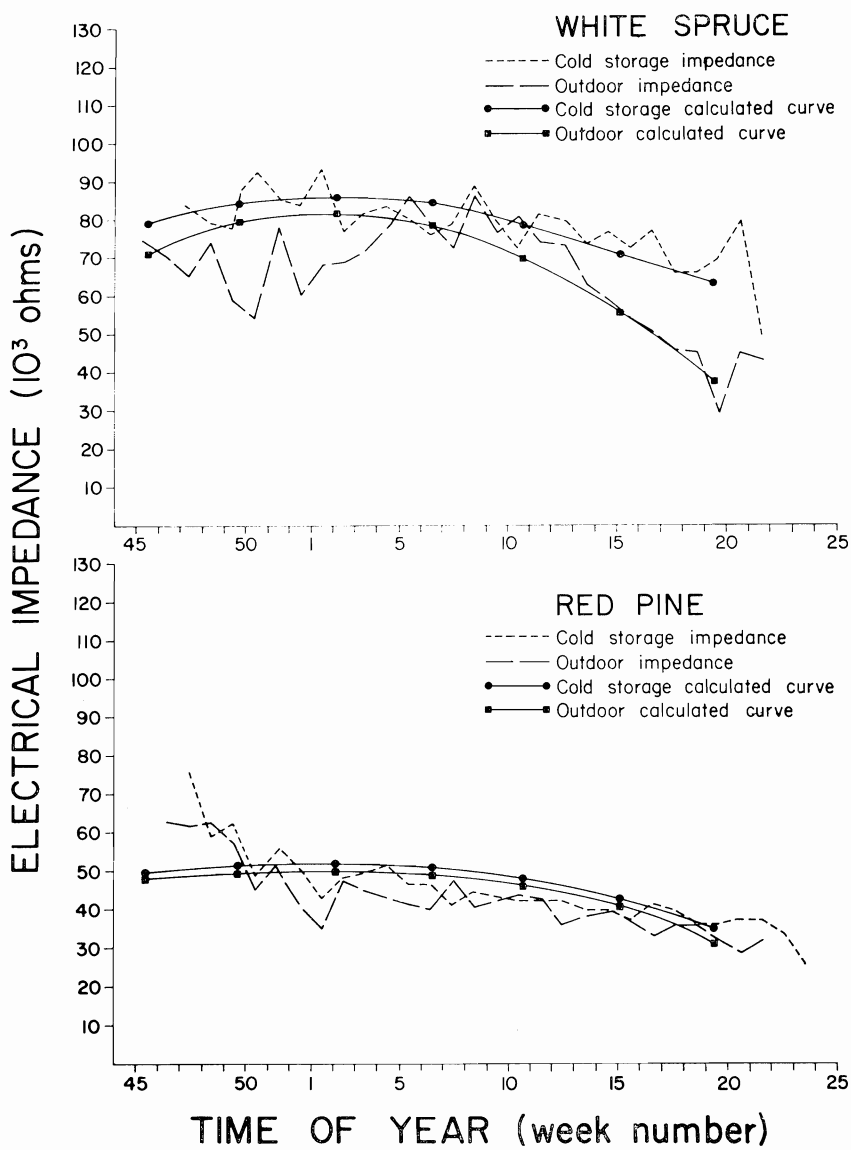

FIG. 5—Average weekly electrical impedance measurements on both cold stored and outdoor white spruce (2-year average) and red pine (3-year average) at Orono nursery. The cold storage impedance is generally higher than the outdoor impedance. The calculated curves accentuate these significant differences. The r value for red pine was .68 and for white spruce .72.

impedance of the cold stored stock remained significantly higher than that of the outdoor stock.

These observations appear to indicate that electrical impedance does measure dormancy and frost hardiness, but presently we can only make this statement in broad terms. It is hoped that we can refine the impedance technique so that ultimately one set of readings will tell us the state or degree of dormancy and frost hardiness.

ACKNOWLEDGMENTS

I am indebted to Professor V. G. Smith for his guidance in the statistical analyses and to Mr R. Bes for his dedication while doing all the multiple regression analyses. The technical assistance of Mr J. G. Boufford in collecting all the Orono data and the assistance of the employees of the Kemptville and Swastika nurseries is gratefully acknowledged.

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