Seasonal dynamics of electrical impedance parameters in shoots and leaves relate to rooting ability of olive (*Olea europaea*) cuttings

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**Summary** Electrical impedance parameters were measured in shoots and leaves of *Olea europaea* L. for 18 months to determine seasonal variations in intracellular and extracellular resistances and in the state of membranes; these factors were related to rooting ability. Double- and single-DCE (ZARC) models were used as equivalent circuits for shoots and leaves, respectively. Seasonal variations were observed in all of the impedance parameters measured. Intracellular resistance of the shoots increased during the winter resting period, whereas intracellular resistance of the leaves decreased. Relaxation times for both leaves and shoots decreased during the winter. Close relationships were found between rooting ability and intracellular and extracellular resistances and relaxation times of shoots and leaves.

**Keywords:** cell membranes, extracellular resistance, intracellular resistance, vegetative propagation.

**Introduction**

Vegetative propagation is an important method for the commercial production of horticultural crops (Davies et al. 1994). In the USA, sales of greenhouse- and nursery-produced plants total 9.7 billion dollars annually (Anon. 1991). Because 70 to 90% of plant production depends on successful rooting of cuttings (Anon. 1991), there is a need to identify all of the factors that control rooting.

Although some easily rooted species including quince, fig, poplar and privet root well at any time of year, others including cherry (Hartmann and Brooks 1958), carob (Fadl et al. 1979) and olive (Gellini 1964, Hartmann and Loreti 1965) root successfully only at particular times of year. Many factors including nutritional status, phenological stage and physiology of mother plants interact with environmental conditions (Loreti and Pisani 1982, Hartmann and Kester 1989) to determine seasonal variations in rooting ability. Despite numerous studies on plant propagation from cuttings, there has been no attempt to relate the seasonal dynamics of electrical impedance (EI) parameters of the mother plants to rooting ability.

Electrical impedance comprises two components: a resistive (real) part and a reactive (imaginary) part. The conductive characteristics of tissue fluids provide the resistive component, whereas the cell membranes, acting as imperfect capacitors, serve as the frequency-dependent reactive component. When a low-frequency alternating current is applied to plant tissues it flows through the apoplast, whereas its passage through the symplast is limited by the high impedance of the membrane. With increased frequency, membrane impedance decreases and the amount of current that passes through the symplast increases (Cole 1968). Impedance measurements made over a range of frequencies therefore reveal information about the extra- and intracellular fluids.

Electrical impedance measurements have been used to estimate general plant health (Macdougall et al. 1987), nutrient status (Greenham et al. 1972, 1982), presence of viruses (Greenham et al. 1978), fruit damage (Cox et al. 1993), frost hardiness (Stout 1988a, 1988b, Repo 1992, 1993, Zhang et al. 1992, Zhang and Willison 1992a, 1992b, Repo et al. 1994), structural variation of cells following ethylene induction by electric currents (Inaba et al. 1995) and sensitivity to salinity (Mancuso and Rinaldelli 1996). In all of these studies, electrical impedance measurements provided a means of nondestructively analyzing variations in intra- and extracellular resistances and the condition of the membranes.

The aims of the present study were: (1) to study seasonal variation in EI parameters of shoots and leaves of mother plants; and (2) to correlate EI parameters to the rooting ability of cuttings collected at various times during the year.

**Material and methods**

**Plant material**

Thirty mother plants of olive “Minerva” (*Olea europaea* L.), a clone of the Leccino variety, were chosen from a 5-year-old orchard located in Pescia, Italy (43°54′ N, 10°41′ E, 30 m asl). Hardwood cuttings were taken from 1-year-old shoots at 20–30-day intervals between January 12, 1996 and August 10, 1997. On each sampling date, 250 cuttings of 1–2.5 cm in length and with six leaves were collected. To promote rooting, 5 mm of the basal portion of each cutting was immersed for 5 s in an aqueous solution containing 3.6 mg ml⁻¹ of 3-indolebutyrate (potassium salt, KIBA) (Mura et al. 1995, Mancuso et al. 1997). Of the 250 cuttings, 25 were not treated with KIBA, but were subjected to impedance analysis. The remaining 225 cuttings (in three replicates) were placed in an inert perlite
medium and maintained in a greenhouse at a high relative humidity (80–85%), a 12-h photoperiod at an irradiance of 130 μmol m⁻² s⁻¹ and a mean day/night temperature of 22/15 °C. After 8 weeks, plants were removed from the perlite medium and rooting percentage was calculated from the number of cuttings with at least one root.

**Impedance measurements**

For the 25 cuttings per sampling date that were not treated with KIBA, one 10-mm long stem section, removed from the basal region of each cutting and one 7 × 7 mm leaf sample, removed from the central area of one completely open leaf of each cutting, were measured for electrical impedance as described by Repo et al. (1994). Briefly, two Ag/AgCl electrodes were placed in contact with each sample using conductive paste (of the type commonly used for electrocardiograms) to keep the electrode–tissue interface polarization to a minimum. The device was calibrated by using OPEN/SHORT circuit correction to eliminate the polarization impedance of the electrode–paste interface. All samples were kept parallel to the direction of electrical current. Input voltage of the sine signal was 30 mV (rms). The absolute impedance value and phase angle were then measured within a frequency range from 100 Hz to 1 MHz at 62 frequency points with an HP 4284A LCR meter (Hewlett-Packard, Palo Alto, CA). The impedance was separated into its resistive and reactive components according to the formulae described by Mancuso and Rinaldelli (1996).

**Modeling of impedance in shoot and leaf tissue**

Mathematical models illustrated by the circuit diagram in Figure 1 were fitted to the data. For shoots, a model made up of two ZARCs (each ZARC comprises a distributed circuit element (DCE) which describes a symmetric impedance arc in the complex plane; for a detailed discussion of ZARC, see Macdonald 1987) in series with a resistor (Figure 1A) was used, whereas for leaves a model with a single ZARC in series with a resistor (Figure 1B) was used. Previous studies have shown that different models for shoot and leaf are necessary to describe the different tissues (see Zhang and Willison 1993, Mancuso and Rinaldelli 1996). The impedance of each ZARC in Figure 1A is defined by:

\[ Z_{\text{ZARC}} = \frac{r_1}{1 + (i\tau_1 \omega)^\psi_1} \text{ and } Z_{\text{ZARC}} = \frac{r_2}{1 + (i\tau_2 \omega)^\psi_2}, \]

where \( Z_{\text{ZARC}} \) is the impedance of the element, \( r_1 \) and \( r_2 \) are resistances, \( \omega \) is angular frequency, \( i \) is the imaginary unit, \( \tau_1 \) and \( \tau_2 \) are relaxation times and \( \psi_1 \) and \( \psi_2 \) are the distribution coefficients of the relaxation times.

In the model used for shoots (Figure 1A), the extracellular resistance \( r_2 \) was calculated as:

\[ r_2 = r + r_1 + r_2, \]

where \( r \) is the resistance in Figure 1.

The intracellular resistance \( r_1 \) was calculated as:

\[ r_1 = r \left[ 1 + \frac{r}{r_1 + r_2} \right]. \]

For the leaf model (Figure 1B), the extracellular resistance was calculated as:

\[ r_e = r + r_1, \]

and the intracellular resistance as:

\[ r_i = \left[ r \left( 1 + \frac{r}{r_i} \right) \right]. \]

The parameters were estimated by complex nonlinear least squares (CNLS) curve fitting (Macdonald 1990) performed with the LEVM 6.1 program (Macdonald and Potter 1987). Unit weighting was used in all the fittings.

All resistances were normalized with respect to the cross-sectional area and the length of the sample. Relaxation times (\( \tau \)) and relaxation time coefficients (\( \psi \)) were not normalized.

**Results**

There was marked seasonal variation in rooting ability (Figure 2). Rooting percentages were highest (80%) during the spring–summer period and minimum values (20–30%) were observed in winter.

**Impedance spectrum of the shoots**

The double-ZARC model (Figure 1A) fits the electrical impedance data obtained for shoots satisfactorily. The estimated relative standard deviation (ERSD = SD/Parameter estimates × 100) of the parameters remained below 3% (Table 1).

A Cole plot of the impedance spectrum of olive shoots yielded two partially overlapping arcs (Figure 3), with the low-frequency arc dominant over the high-frequency arc. The extent of the overlapping of the arcs varied during the year and was more marked during the winter. Overlapping became more evident on examination of the characteristic frequency, \( f_c \) (\( f_c = 1/(2\pi\tau) \)), which was 3.6 kHz for the low-frequency arc and 76.5 kHz for the high-frequency arc during the summer period. The corresponding values rose to 6.6 and 117.9 kHz during the winter resting period.
Impedance parameters of the shoots

The annual time courses of parameters \( r_e, r_1 \) and \( r_2 \) (Figure 4) were inversely related to those of parameters \( r_i \) and \( r \) (Figure 4). Extracellular resistance reached values around 29 \( \Omega \) m (i.e., specific resistance, \( \Omega \) m\(^{-1}\) cm\(^{-2}\)) in July–August and fell to 22 \( \Omega \) m in the winter months. Resistances \( r_1 \) and \( r_2 \) showed a similar seasonal trend with winter values approximately 35% lower than summer values. Intracellular resistance values were approximately twice as high in winter (approximately 5 \( \Omega \) m) as in summer. A similar trend was observed for resistance parameter \( r \).

The annual time courses of the relaxation times \( \tau_1 \) and \( \tau_2 \) (Figure 5) paralleled those of \( r_e, r_1 \) and \( r_2 \) (Figure 4), with highest values in July and August and a gradual decline to minimum values in winter. The distribution coefficients of relaxation time \( \psi_1 \) and \( \psi_2 \) were more or less constant throughout the year.

With the exception of coefficients \( \psi_1 \) and \( \psi_2 \) (data not shown), all other impedance parameters for shoots showed a sigmoidal relationship with rooting (Figure 6), with \( r^2 \) values ranging from 0.83 (\( \tau_2 \)) to 0.92 (\( r_1 \)).

Impedance spectrum of the leaves

The single-ZARC model closely fit the electrical impedance data obtained for leaves. The ERSD values were always less than 4% (Table 2). A Cole plot of the impedance spectrum for olive leaves yielded a single arc with a slightly depressed center (Figure 7). The characteristic frequency varied during the year from 31.2 kHz in the winter months to 18.3 kHz in the summer months.

Table 1. Parameter estimates, standard deviations (SD) and estimated relative standard deviations (ERSD) of parameters obtained when model was fitted to shoot data. Resistances are not normalized.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (k( \Omega ))</th>
<th>SD (k( \Omega ))</th>
<th>ERSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_e )</td>
<td>6.02</td>
<td>0.12</td>
<td>2.02</td>
</tr>
<tr>
<td>( r_1 )</td>
<td>37.52</td>
<td>0.67</td>
<td>1.78</td>
</tr>
<tr>
<td>( r_2 )</td>
<td>39.76</td>
<td>0.73</td>
<td>1.83</td>
</tr>
<tr>
<td>( \tau_1 )</td>
<td>2.08</td>
<td>0.06</td>
<td>2.84</td>
</tr>
<tr>
<td>( \tau_2 )</td>
<td>44.02</td>
<td>0.93</td>
<td>2.12</td>
</tr>
<tr>
<td>( \psi_1 )</td>
<td>0.70</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>( \psi_2 )</td>
<td>0.87</td>
<td>0.01</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Impedance parameters of the leaves

The annual time course of the leaf impedance parameters was the opposite of that observed for the impedance parameters measured in the shoot. Extracellular resistance (Figure 8) reached about 180 \( \Omega \) m during the winter, decreased in the spring, and declined to a low of 120 \( \Omega \) m in July and August. Resistance \( r_1 \) followed a similar pattern. In contrast, intracellular resistance (Figure 8) reached a maximum of about 30 \( \Omega \) m during summer, decreased during autumn, and declined to a minimum during winter. Resistance \( r \) exhibited a similar trend.

Relaxation time \( (\tau) \) values were maximal during summer and decreased to a minimum during winter (Figure 8). Values

Figure 3. Impedance spectra of olive shoots \((n = 25)\) during resting stage (■); vegetative growth (□); ripening (○); and drupe growth (●). The spectra are composed of 62 frequencies from 100 Hz to 1 MHz; not all of the frequency points are indicated. Characteristic frequencies for the resting stage and drupe growth are indicated.

Figure 4. Seasonal variations in the best fit parameters of the double-DCE model for olive shoots during 1996 and 1997. Abbreviations: \( r_e \), \( r_1 \) and \( r_2 \) are the resistances of the double-DCE model; and \( r_1 \) and \( r_2 \) are the extracellular and intracellular resistance, respectively. Bars indicate standard error.
of the distribution coefficient of relaxation time $\psi$ showed a rather irregular pattern throughout the year with a minimum during the winter months (Figure 8). As in shoots, relationships between $r_e$ and $r_1$ and rooting ability were sigmoidal (Figure 9), whereas the relationship between $r_i$, $r$ or $\tau$ and rooting was linear. The correlation coefficient $r^2$ was lower for leaves than for shoots, ranging from 0.60 ($\tau$) to 0.74 ($r_1$ and $r$).

Discussion

Impedance parameters of the shoots

The presence of two arcs in the impedance spectrum of olive shoots confirms previous findings for olive shoots (Mancuso and Rinaldelli 1996) and Scots pine shoots (Repo et al. 1994). The greater overlapping of the two arcs in the impedance spectrum for olive shoots compared with Scots pine shoots is probably associated with differences in tissue composition (e.g., differences in the proportions of woody to non-woody tissue) between the two species. The impedance parameters showed seasonal variations. Increases in intracellular resistance during the autumn–winter period have previously been found in Salix (Repo et al. 1997) and have been attributed to cold acclimation (Repo et al. 1994). The increase in $r_i$ reflects an increase in symplast viscosity as a result of an increase in intracellular sugar concentration (Zhang and Willison 1992) during cold acclimation of olive (Priestley 1977). In addition, cell compartmentalization increases during cold hardening (Toivinen et al. 1991) and this effect, together with the increase in viscosity, results in the reduction of both ion movement and the flow of electrical current.

In the present study, extracellular resistance decreased during autumn–winter. Previous studies have yielded conflicting data on the effects of cold acclimation on extracellular resistance. Wilner (1967), Glerum (1973), and Greer (1983) have each reported that extracellular resistance increased during autumn–winter. However, the present study found that extracellular resistance decreased during this period. This may be due to differences in the species used or in the methods employed.

Table 2. Parameter estimates, standard deviations (SD) and estimated relative standard deviations (ERSD) of parameters obtained when model was fitted to leaf data. Resistances are not normalized.

<table>
<thead>
<tr>
<th></th>
<th>$r$ (k$\Omega$)</th>
<th>$r_1$ (k$\Omega$)</th>
<th>$\tau_1$ ((\mu)s)</th>
<th>$\psi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>73.16</td>
<td>350.30</td>
<td>8.702</td>
<td>0.72</td>
</tr>
<tr>
<td>SD</td>
<td>1.64</td>
<td>8.73</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>ERSD (%)</td>
<td>2.24</td>
<td>2.49</td>
<td>3.16</td>
<td>2.08</td>
</tr>
</tbody>
</table>
cold acclimation, whereas Stout (1988a, 1988b) found no change in extracellular resistance. It is not known if these discrepancies are related to differences among species or to differences in technique.

The extracellular and intracellular resistances were inversely related; however, the magnitude of variation differed. Intracellular resistance reached a maximum in winter (February 26) that was 121% greater than the minimum in summer (August 9); whereas the maximum extracellular resistance (August 9) was only 27% greater than the minimum in winter (December 10). Examination of the seasonal trends in \( r_i \) and \( r_e \) indicated that these changes were probably mediated by seasonal variations in membrane properties and by the quantity of energy supplied from respiration (Higinbotham 1973, Carey and Berry 1978). In olive, the transition temperatures for membrane potential and respiration, are 10 and 14 °C, respectively (Rinaldelli and Mancuso 1994). These temperatures represent threshold values below which there is a slowing down of normal physiological processes. The mean temperature was below these limits (data not shown) for the entire winter period (from the end of October to the middle of March) of the present study. The temperature data, together with the
winter relaxation time $\tau_1$ and $\tau_2$ values, suggest that the winter increase in intracellular resistance was a result of modifications in cell membrane functionality. Because the value of $\tau$ can be considered approximately equal to $r_c$, where $c$ represents membrane capacitance, the finding that a decrease in $\tau$ during the winter period did not correspond to a similar marked decrease in $r$, indicates that membrane capacitance influenced the decrease in $\tau$. In addition, the decrease in $\tau$ was in part caused by (1) a reduction in cell size and (2) an increase in the proportion of xylem and sclerenchymatous tissue (Glerum and Krenciglowa 1970).

**Impedance parameters of the leaves**

The impedance spectrum of leaves formed a single arc that was not substantially modified during the annual cycle of the plant (cf. Mancuso and Rinaldelli 1996). There were seasonal variations in all of the impedance parameters for leaves. Intracellular resistance decreased during the winter as a result of an increase in electrolytic content (Crescimanno et al. 1976, Palta et al. 1977) and a decrease in cell sugar concentration, whereas extracellular resistance of leaves increased in autumn–winter (cf. Repo et al. 1994).

Examination of the relaxation time $\tau$ values of leaves indicated that the inverse relationship between $r_c$ and $r_1$ was mediated by variations in plasmalemma functionality. In leaves, $\tau$ decreased during the autumn–winter period as a result of a decrease in membrane capacitance of cells ($c$). Decreasing $\tau_1$ ($t_1 = r_c c$) and increasing $r_1$ could indicate either the presence of smaller cells or membrane component changes; however, further studies are necessary to elucidate the relationship between membrane capacitance and membrane component changes.

**Impedance parameters and rooting ability**

The rooting of cuttings increased in a sigmoidal manner with increasing $r_e, \tau_1$ and $\tau_2$ and decreasing $r_1$. In general, the highest rooting percentages were attained when intracellular resistance of the shoot was low and relaxation times were high (high membrane permeability and high electrolyte concentration in the cell), conditions characteristic of high metabolic activity.

Strong positive relationships were found between $r_1$ and $r$ of the leaves and rooting ability. Because the increase in intracellular resistance has been attributed to an increase in sugars, and olive leaves are important both for producing and storing photosynthates (Priestley 1977), an increase in $r_1$ could influence rooting by making available a greater quantity of carbohydrates (Davis and Potter 1981, Eliasson 1978).

I conclude that various impedance parameters are sensitive to physiological variations in the plant that are associated with seasonal changes in rooting ability. Moreover, the impedance parameters can provide information about structural changes in the cell during the annual cycle of the plant. Further studies are necessary to determine the sensitivity of this technique to change in membrane components and to obtain a more comprehensive biological interpretation of impedance parameters.

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**References**


