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The Effect of Bark Stripping on the Electro-Physiological Properties of Cork Oak (Quercus Suber L.) Leaves Evidenced by Electrical Impedance Spectroscopy

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Abstract
The present paper is an investigation of the influence of bark stripping on the electrical impedance parameters and on the moisture content of cork oak leaves. To this end, moisture content and current electro-physiological parameters were determined as functions of stripping coefficients on young leaves after bark stripping in July and August 2009. These biophysical parameters were measured using electric impedance spectroscopy. The analysis of the experimental results revealed that bark stripping at several times may lead to a decrease in moisture content and an increase in intracellular resistance during the bark stripping period, while the relaxation time seems to remain fairly constant. Finally, the statistical analysis of the mean-values of different parameters point-out a significant correlation between water content, stripping coefficient and electrical parameters.

1. Introduction

Industrial cork, mainly based on *Quercus suber* L., might be normally expected to be collected every 9 years. Since *Quercus suber* trees are submitted to recurrent cork extractions, excessive stripping is an important stress factor that may affect their productivity. The effects of bark stripping are very complex; the large amount of water lost from the stripped surface is certainly expected to influence the water balance and consequently limit the physiological processes (Correia et al., 1992). Up to now, bark stripping has been controlled in term of periodicity and quantity of extracted cork as the international regulations limit up to 9 year-period of extraction (Pinto at al., 2006). Moreover, the stripping size must not exceed 3 times the trunk at human breast height perimeter in full maturity and yielding trees (i.e., 3 is the maximum stripping coefficient allowed). According to the Morocco legislation, it is reduced to 2. This bark stripping coefficient, currently adopted in Morocco, is only based on some empirical considerations (Mourad, 1997). The effects of cork removal on adult tree account for an increase in radial growth of cork, mainly observed in the first 2 or 3 years following cork removal (Natividade, 1950, Costa et al., 2004). The growth of the tree diameter and its seasonal pattern do not seem to be affected either in very young cork oak trees (Fialho et al., 2001) or in mature trees under production (Costa et al. 2003). The operation of cork removal is extremely traumatic for cork oak trees (Natividade, 1950) since excessive stripping is an important stress factor which may lead to a considerable water losses and consequently affect the tree productivity (Correia et al., 1992).

Despite numerous studies on *Quercus suber* trees after stripping, there is no noticeable tree's study which attempts to relate the effect of bark stripping on electrical impedance parameters of their leaves. Electrical impedance spectroscopy (EIS) is a non destructive method allowing for the study of the behaviour of a material undergoing various stresses. This technique has been widely used to assess the in vivo conditions of animal and plant tissues since it is fast and easy while, at the same times performing measurement (Muzukami et al., 2006). Owing to the aforementioned reasons, EIS appears as a suitable method devoted to materialize how bark stripping can cause the stress of the cork oak tree. In biological samples, the part of the electric current flowing through the apoplastic and symplastic spaces in tissues depends on the AC frequency. At low frequency, AC current passes through the apoplast. The cell membranes become more conductive as the frequency of the current increases (Repo et al, 2004), and accordingly the amount of symplastic current increases. Therefore, information about different tissues features, i.e., intra and extracellular fluids may be revealed by EIS (VÄINÖLÄ and Repo, 2000).

The main aim of the present paper is to investigate the influence of bark stripping on the electrical impedance parameters and on the moisture content of cork oak leaves. A further attempt of this study is to promote EIS as an alternate method that can be efficiently used for the prediction of the effect of bark stripping to achieve optimal production of cork.

2. Experimental Framework and Method

2.1. Plant Material

Fig. 1. Oak cork leaves.

The experiments were carried out on young leaves (figure 1) of mature *Quercus suber* L. trees at the Mamora forest (6°45'O, 34°2'N, 30m of altitude), Rabat, Morocco. Mamora soil is made up a clay layer on which we have red or pink colour sand layer. The average annual precipitation is 500 mm and the maximum temperature is 17°*C*. Six mature and productive cork trees (representative of the dominant and typical tree in that field) were selected and labelled T1 to T6. In this collection, two (2) trees were not stripped while the 4 others were stripped with different coefficients. All these trees were regenerated by reject of stump after ablation of the trunk. Trees T1 and T2 (not stripped) were used for control purpose meanwhile trees T3, T4, T5 and T6 were stripped at the same day with stripping coefficients 1, 1.5, 2 and 2 respectively. Trees T5 and T6 (about 40 years old) were once bark-stripped previously, while trees T2 and T3 (30 years old) were bark-stripped for the first time. The bark stripping characteristics of the studied trees are displayed in Table 1.

Table 1. *Stripping Characteristics of the trees under study. ND: number of bark-stripping, it is the number of time that the tree was stripped, CD: stripping coefficient calculated as the ratio of the maximum length by the trunk perimeter at breast human height (Natividade, 1950).*

Trees	ND	CD	Remarks
T1	0	0	Non-stripped
T2	0	0	Non-stripped, same stump as T5
T3	1	1	same stump as T4 and T6
T4	1	1.5	same stump as T3 and T6
T5	2	2	same stump as T2
T6	2	2	same stump as T3 and T4

2.2. Electrical Impedance Spectroscopy of Leaves

In this technique, Alternating Current (AC) causes polarization and relaxation in the sample leading to a change of the amplitude and phase of the applied AC signal. Based on this change, the impedance of the sample, which comprises a real and an imaginary parts in a complex plane can be determined. If the real and the imaginary parts are measured at different frequencies, an impedance spectrum will result. Electrical impedance spectroscopy (EIS) of leaves was performed in summer 2009 (before and after the bark-stripping period). There were five sampling dates, i.e., on the 8th July (few hours before stripping), 14th July, 22th July, 7th August and 27th August. From each tree and at each sampling date, one short-shoot leave was sampled each time from branches located at the central crows of the trees. The leaves were immediately placed in plastic bags after sampling and transported to the laboratory. Impedance spectra were measured with an Ag/AgCl-cell connected to an HP3330LCZ meter. The Ag/AgCl electrodes were kept in contact with the samples using a conductive paste (of the type commonly used for electrocardiograms) to maintain minimum electrode tissue interface polarization. Further, the device was calibrated by using OPEN/SHORT circuit correction to eliminate the polarization impedance of electrodes-sample-paste surface. The real (*Zr*) and imaginary (*Zi*) values of impedance were then measured in the frequency range of 40 Hz-100 kHz and the input voltage of the signal was 30 mV (rms). The section of the conductive part of electrodes was 0.78 cm^2 corresponding to the one of a disk of *1cm* of diameter. From each short-shoot, three or four young leaves were tested and the mean-value was calculated. All the tested leaves had a surface area of more than *0.78* cm^2 and with naked eye they presented no sign of oldness as Laarabi et al. (2005b) characterises by desiccation.

2.3. Impedance Model of Leaves

To connect the data provided by EIS analysis, the plant tissue under test must be represented by an equivalent electrical circuit. In this study, the measured values for the real and imaginary parts of the impedance by EIS were fitted to the simple model as depicted in fig.1. This model gives information on changes that a trial can undergo after a control and different preparations (Laarabi, 2005b). The impedance model relies simply on a *RC* parallel circuit in series with a resistor R_∞.

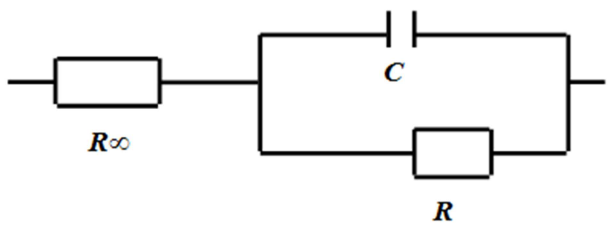


Fig. 2. *Equivalent Electrical circuit model (simple circuit) for samples of cork oak leaves.* R_∞ *and R are resistors, C is the capacitance of the cells membranes.*

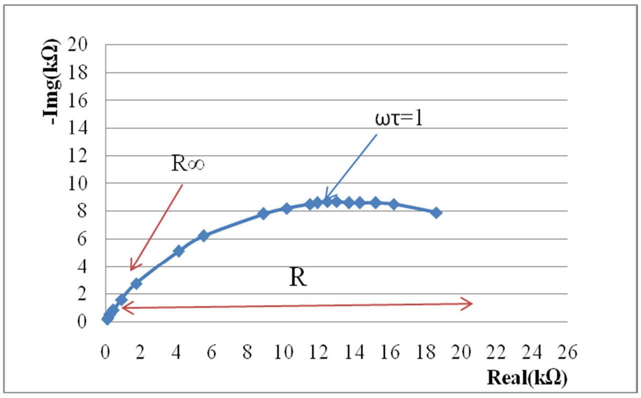


Fig. 3. *Determination of EIS parameters of an impedance spectrum of cork oak leave. Real part of impedance is labeled by the x-axis while the imaginary part of impedance by the y-axis. Frequency increases from right (40Hz) to left (100kHz).*

The impedance Z of the resulting circuit has a real part Zr and an imaginary part Zi which are functions of the frequency of the alternative current. The graph representing $Z_i = f(Z_r)$ seems to fit the form of a parabola where the top corresponds to the frequency value called characteristic frequency of the material. The impedance of the equivalent circuit (fig.2) is derived as:

$$Z = R_\infty + \left(\frac{R}{(1 + jRC\omega)}\right) \tag{1}$$

where the decomposition allows one to deduce the real and imaginary parts written respectively:

$$Z_r = R_\infty + \left(\frac{R}{(1 + R^2C^2\omega^2)}\right) \text{ and } Z_i = -\left(\frac{(R^2C\omega)}{(1 + R^2C^2\omega^2)}\right) \tag{2}$$

$\omega = 2\pi f$ is the angular frequency where f is the frequency of the input signal (Laarabi, 2005a).

A Cole plot of the impedance spectrum for leaves yield a single arc with a slightly depressed centre (Mancuso, 1997; Repo et al., 2005, Mizukami et al., 2007) as presented in fig.3.

The intracellular resistance is calculated as:

$$R_i = R_\infty \left[1 + \left(\frac{R_\infty}{R}\right)\right] \tag{3}$$

where resistances R_∞ and R are measured according to the intersection of the circle with x-axes respectively at high and low frequencies (fig.2)

The relaxation time can then be determined:

$$\tau = \frac{1}{(2\pi f_c)} \tag{4}$$

where f_c is the characteristic frequency and is graphically measured at the apex of the arc (Repo et al., 2000). At high frequencies, the current may pass through the cell membranes and accordingly flows in both the apoplastic and the symplastic spaces.

2.4. Moisture Content of Leaves

The sample were weighed and dried at 100°C ±3 for 24*h* just after the impedance measurements were performed. The scale of 1*mg* accuracy was used in weighing and the moisture content was calculated as:

$$H = \frac{100 \times (M_H - M_O)}{M_O} \tag{5}$$

where M_H is the weight of fresh leaves and M_0 is the in oven dried weight after drying at 100°*C* for 24*h* duration.

2.5. Analysis of the Experimental Data

The extracted data from the EIS measurements of leaves samples have been subject to statistical analysis including correlation tests. The relations between the leaves's properties and electrical parameters were studied while using multiple correlations. The Pearson correlation coefficient (r) was calculated and the t-test was used to estimate the significance of correlation, meanwhile the statistical analysis was carried out with the aid of SPSS v.11.0 software.

3. Results and Discussions

Independent of bark stripping, there was a gradual lowering in moisture content for all trees during the period of measurements, that is from July to August (figure 4). Such situation might be explained by major dehydration in August mainly due to the prolonged summer's stresses since no rainfall occurred during this period. This result was in accordance with the previous study on *Quercus robur* L. Leaves (Repo et al, 2007). In spite of the general decline in water, additional stress caused by the striping led to a further decrease in water content (figure 4). The decrease in the moisture content, which is more accentuated for bark-stripped trees (from 12 to 17% according to the stripping height) could be explained by the fact that as well as stripping height increases, there was also the lost of natural water by evaporation at the level of disclose area after stripping. Indeed, as mentioned by Correia et al (1992), one of the main effects of stripping is the excessive water lost from the exposed surface. This water is thus deviated from the root-to-leaf pathway during the dry season, with consequences at the industrial profit level.

An increase of the stripping coefficient contributed to the decrease in the moisture content, with a good correlation ($r=0.741$, $p<0.01$); as mentioned by Correia et al.,(1992), the quantity of the water lost of the stem surface depends on the stripped area.

However, the difference between moisture content data of trees 5 and 6 with the same stripping coefficient could be due to the physiological difference between the trees since one of the relevant aspects of the effect of stripping on water relations is the phloemic tissue composition of each tree, which will determine the extent of water lost by evaporation (Correia et al, 1992).

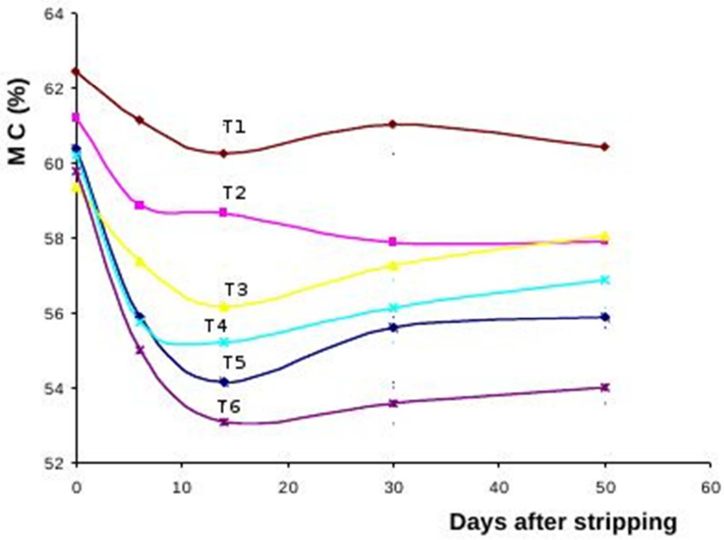


Fig. 4. *Dependence of moisture content of control and bark stripped trees leaves during July-August 2009. The day 0 corresponds to the data before stripping Trees T1 and T2 were non-stripped; Trees T3-T6 were stripped with different coefficients (as given in table 1).*

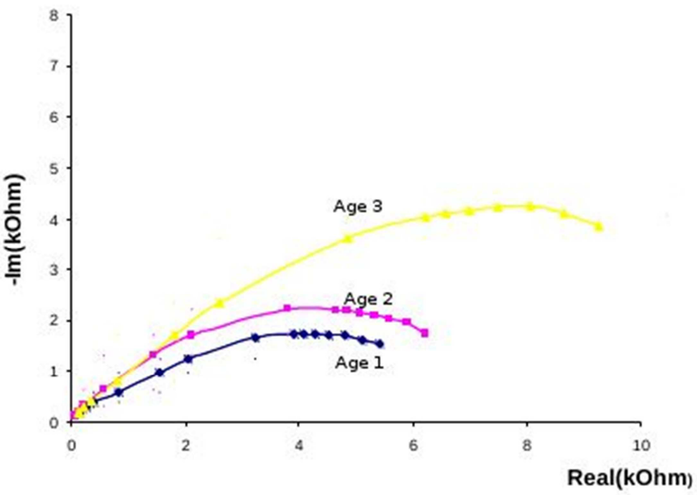
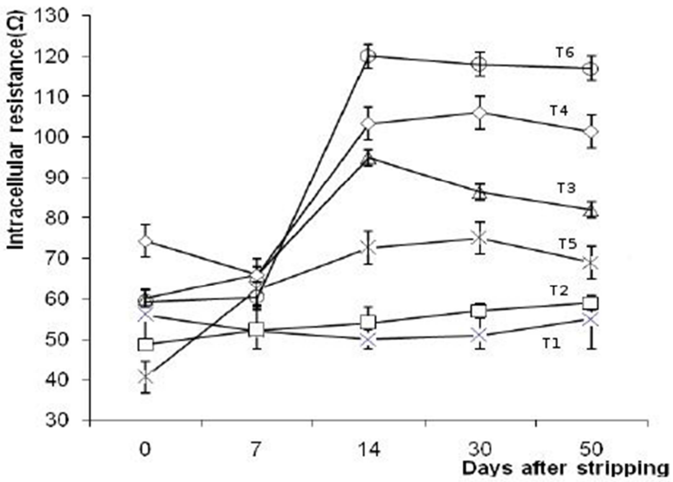


Fig. 5. *Typical impedance spectra of cork oak of young leaves sampled at different stages of growth, ages 1, 2 and 3 are taken in the order of growing maturity of leaves. The spectra are composed of 35 different frequencies ranging from 80Hz to 100 kHz (from right to left respectively).*

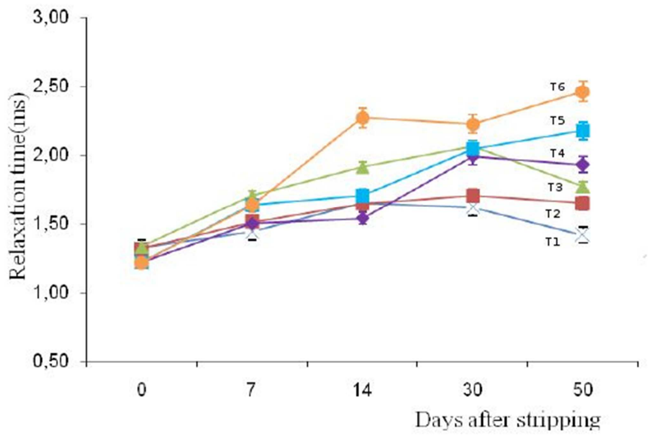
To clarify the differences among the EIS spectra with maturity origin of leaves, the real and imaginary parts of the impedance of the leaves at 3 different stages of maturity are illustrated in fig.5. The impedance spectrum of cork oak leaf increased as anatomical growth progresses and this, in accordance with the earlier study in tea leaves (Mizukamy, 2007). There was no clear change in the shape of the spectrum with the age. However, the radius of arc corresponding to the impedance plot (figure 5) strongly depends on the level of leaf maturity. Contrary to the case of mature leaves, the spectroscopic data of young leaves change. At high frequencies (left in fig.5), the real part of the impedance at each age presents identical values. Besides, at low frequencies (right in fig.5) the real part of the impedance follows up the age.

Data corresponding to EIS parameters of the leaves sampled on control and stripped trees are plotted in fig.6. Progressive increase of the intracellular resistance the stripped trees leaves were observed during this study (fig.6A). This behaviour can be attributed to the losses of excessive water lost by trees after bark stripping by evaporation at disclosed parts; those losses could limit directly or indirectly physiologic processes of the tree (Correia et al., 1992).

However, no change regarding the intracellular resistance of the control trees could be explained by the weak decrease in the moisture content (6%) of those trees which was not sufficient to influence intracellular resistance.



A



B

Fig. 6. *Behaviour of EIS parameters of young leaves of cork oak after stripping. A: Intracellular resistance; B: Relaxation time. The day 0 corresponds to the data before stripping. Trees T1 and T2 were non-stripped; Trees T3-T6 were stripped. Points represent ± means standard error for three leaves.*

In mathematical terms, the relaxation time τ is obtained from the apex of high frequency arc of the impedance spectrum (fig.3). The parameter τ did not know a huge change when comparing its value corresponding to tree being stripped to those of the trees with bark stripping coefficient 2 (an increase from 1.22 to 2.27*ms* can be observed on fig.6). The biological interpretation of τ is not yet known. However it has been proposed that the properties of depleted cell membrane affect the relaxation time of leaves and stem of olive trees, *Olea europaea* L., (Mancuso and Rinaldelli, 1996). The relaxation time τ grows with an increase in dry matter content (Repo et al., 2000). Thus, the water content could be partially associated to the behaviour of τ.

Moisture content measurements as those published by Hakam et al., 2012 and all extracted electrical parameters curves rise to an asymptotic value e.g. 21 days after bark stripping. There are many explanations of this fact: either the reaction of the tree to balance its wet gap by progressive decrease of transpiration at the level of the leaves (Correia et al., 1992), either by stomata regulation (open and close stomata), decreasing therefore losses of water to outside, or finally, as mentioned by Natividade (1950), by the formation of a new phellogen layer with immediate cork regeneration function which, due to cork's impermeability, limits the water loss by evaporation.

According to the Pearson correlation coefficient, all electrical parameters are correlated with the trees parameters and water content with good significance ($p<0.01$) (table 2). However, the water content had the highest Pearson correlation coefficient($r=-0.788$, $p<0.01$) with *Ri* whose growth is accompanied by a decrease in the water content (fig.6). Since the water content in the leaf can be used to assess water losses after stripping on the one hand and had a good correlation with stripping coefficient and trees age ($p<0.01$) on the other hand, *Ri* would be useful for damage assessment after stripping.

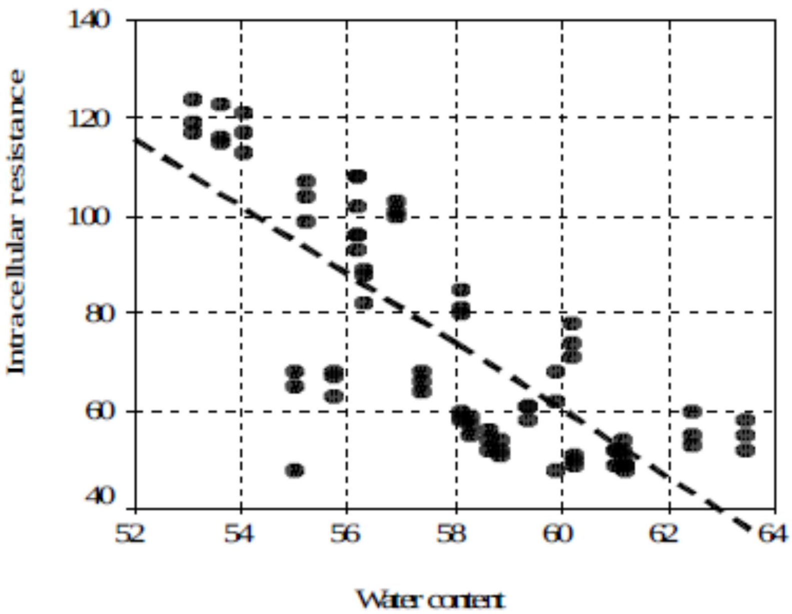


Fig. 7. *Relationship between intracellular resistance and water content in the leaf (n=90). Points represent water content (%) and intracellular resistance (Ω).*

Table 2. *Pearson's correlation coefficient (r) between trees parameters (leaves water content, trees age, stripping coefficient) and electrical characteristics (intracellular resistance and relaxation time).*

	Ri(Ω)	**τ(ms)**	**WC(%)**	**ND**	**CD**
Ri(Ω)	1	0.707**	-0.788**	0.688**	0.728**
τ(ms)		1	-0.697**	0.438**	0.394**
WC(%			1	-0.735**	-0.741**
ND				1	0.969**
CD					1

**correlation is more significant at the 0.01 level (2 tailed)

4. Conclusion

Intracellular resistance seems to be an adequate electrical parameter for the study of physiological reaction of *Quercus suber* L. to bark stripping. The increase of intracellular resistance which is strongly pronounced 14 days after cork extraction reflect the stress caused by the stripping of the cork oaks.

We suggest to pursue the researches in this direction because throughout this technique (spectroscopy impedance), it is possible to determine optimal size that the tree could be stripped without fear to harm its health by increasing the quantity of cork made.

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