Assessment of Physiological States of Plants *in situ*

An Innovative Approach to the use of Electrical Impedance Spectroscopy

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Abstract— **The fast spread of asymptomatic forest diseases, with no cure available to date, constitute a problem of economical and forestall huge proportions. Furthermore, there is a lack of equipments and systems able of assessing and characterizing the physiological state of plant organisms, both in the diagnosis of disease and as a mean for supporting physiological studies. It is known that electrical impedance measurements have been proving its value in the characterization of vegetal tissues. However, the available commercial solutions are expensive, unfeasible for *in vivo* and *in situ* measurements and unspecific for biological applications. Herein is proposed the usage of impedance techniques to assess the physiological state of plants. Emphasis is given to the assessment of the hydric stress level of plants and its relation with the disease condition. To accomplish the study, a portable electrical impedance spectroscopy system was designed attending the biological application purpose. In order to show the potential of this technique and system, the procedure and the results obtained for three different species (*Pinus pinaster* Aiton, *Castanea sativa* Mill and *Jatropha Curcas* L), with economical and/or forestall interest, is also presented.**

Keywords-plant disease; physiological state; hydric stress; biodiesel; impedance techniques

I. INTRODUCTION

Plant organisms, being a plant organism any kind of living plant (tree, bush, or other), are affected by numerous diseases promoted by biological agents (such as fungus, virus, bacteria, nematodes, insects, and others) and/or inhospitable environmental conditions (such as drought, fires, extreme heat, contamination of soil and air, and others) [1]. It is worth adding that the knowledge of the health state of the plant organisms is important, particularly when diseases affect crops with economic and/or forestall impact. Currently there are some diseases strongly affecting specific crops with significant economical relevance in specific countries or regions. Such cases include, for instance, the pinewood nematode, affecting mostly the *Pinus pinaster* Aiton specie, the ink disease in the chestnuts and the esca disease in the grapevines. Whether these diseases are caused by fungus, nematodes or other biotic or abiotic agents, they are mostly asymptomatic, exhibit fast spread rate and currently have no cure properly developed and commercialized [1, 2].

The standard method to diagnose diseases in plant organisms is the symptomatology visualization by skilled personnel [1, 2]. However, usually, the external symptoms are only able of being visually accessible during the terminal stages of the diseases [1]. The plant organism that is considered affected is cut and destroyed. In order to avoid the fast spreading, the neighbor plant organisms are also cut and burned, even if visual symptoms are not accessible [2]. This preventive act poses a problem: the deforestation, and the resulting economic losses, caused by the massif felling.

In a similar way, but in the perspective of marketing and consumption, the characterization of plant organisms, which are important in processes like, for instance, the production of biodiesel [3] and the physiological studies of plant organisms for new applications [3, 4], also lacks a detailed and similar technical analysis [4]. The techniques available for characterizing plant organisms require expensive laboratory equipments and materials, are time consuming and hard to implement [1, 5].

Hereupon, it can be said that, in general, there is a lack of equipments and systems able of assessing and characterizing the physiological state of plant organisms. This overall described panorama motivated the present work. Herein, the authors propose an Electrical Impedance Spectroscopy, EIS, system and the usage of impedance techniques to assess the physiological states of plant organisms. Emphasis was giving to the assessment of the hydric stress level and its relation with the disease condition.

The hydric stress refers the internal hydration condition of a plant organism and it is one of the most relevant parameters to assess physiological states [5]. This parameter takes special significance in the assessment of diseases, since water absorption by the plant is one of the physiological processes firstly and strongly affected during a biotic or abiotic disease condition [5].

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EIS has been proving efficacy and utility in a wide range of areas, from the characterization of biological tissues to living organisms [6]. The electrical impedance of a biological material is a passive electrical property that measures the opposition relatively to an alternating current flow applied by an external electric field. The current I, as it passes across a section of a material of impedance Z, drops the voltage V, established between two given points of the same section, yielding the well-known generalized Ohm's law: V=IZ, where V and I are complex scalars and Z is the complex impedance. Hence, the result of the EIS measurements is a set of complex (magnitude and phase) of impedance versus frequency.

Cell membranes, intracellular fluid (cytosol) and extracellular fluid are the major contributors of the impedance of biological tissues [6]. A commonly used circuit to represent biological tissues consists of a parallel arrangement between a resistor, simulating the extracellular fluid, and a second serial arrangement connecting a resistor, this one of the cytosol, and a capacitor, of the membrane [7, 8].

The model commonly used to represent impedance values is the Cole bioimpedance model, in which the bioimpedance spectrum is represented by means of a Cole-Cole plot that explores resistance versus reactance, allowing the determination of the ohmic values of the cytosol and the extracellular fluid [9].

The physiological changes, due to diseases and nutritional or hydration levels, have direct influence in the impedance spectrum. The phase angle and other interrelated indices, such as Z_0/Z_∞ [6] and Z_0/Z_{50} [10], have been used to extract information about the physiological condition of biological materials.

The nature of the impedance excitation signal varies depending on the application. It is possible to excite the sample with a current and measure a voltage or to do the exact opposite. The discussion on what source, voltage or current, is the most convenient remains. Current sources, CS, provide suitably controlled means of current injection [11] and present reduced noise due to spatial variation when compared with voltage sources, VS [12]. However, CS accuracy decreases with high frequency [13], especially due to their output impedance degradation [12]. Since the impedance measurements are limited to field strength where the current is linear with respect to the voltage applied [8], or vice-versa, CS need high-precision components [14] and a limited bandwidth operation range [13, 14] to overcome the stated limitation. On the other hand, VS, although producing less optimal electrical impedance spectroscopy, EIS, systems [14], can operate over a sufficient broad frequency range [13, 14] and are built with less expensive components [14].

Nowadays, instruments with high precision, high resolution and frequency ranges extending from some Hz to tens of MHz are commercially available [6]. However, in what concerns to the range of low or high frequencies (already above 100 kHz), the degradation of the excitation signal affects the accuracy of the measurements [6]. Besides, the typical solutions consist in impedance analyzers and LCR meters which are desktop instruments [6], unfeasible for in vivo [6] and in field applications.

The EIS system presented herein is able to perform AC scans within a selectable frequency range. The system implements the phase sensitive detection, PSD, method and can drive either a current or a voltage signal to excite a biological sample *in situ* or *in vivo*. The instrumentation was designed to be cost-effective and usable in several applications.

TABLE I. SUMMARY OF SPECIFICATIONS OF THE EIS SYSTEM

	Range	
Parameter	***Current Mode***	***Voltage Mode***
Measuring method	2 electrodes	
Frequency	1 kHz to 1 MHz	
Signal amplitude	25 uA	4.6 V
Impedance magnitude	100 Ω to 100 kΩ 1	1.5k Ω to 2.2 MΩ1
Impedance phase	-π rad to π rad	-π rad to π rad
Mean absolute magnitude error	1675.45 Ω	709.37 Ω
Mean absolute phase error	2.45 %	2.06 %
Mean distortion	0.29 %	0.48 %
Mean SNR	117.0 dB	118.8 dB
Calibration	Automatically calibrated by software	

In this paper it is also resumed the most relevant studies obtained for three different plant species, with economical and/or forestall impact: pine *(Pinus pinaster* Aiton), cheastnut (*Castanea sativa* Mill) and *Jatropha Curcas* L.

The following sections of this paper are: 1) *System Design*, where the developed EIS system is presented in detail; 2) *Assessment of the Hydric Stress Level*, which presents the method and the results obtained for the hydric stress assessment for the three studied species; 3) *Study of Disease Condition*, which presents the method and the results obtained for the study of the nematode disease in *Pinus pinaster* specie; and 4) *Conclusions*, which resumes the main obtained results.

II. SYSTEM DESIGN

A. General Description

The developed EIS system employs two electrodes and consists of three main modules: signal conditioning unit, acquisition system (PicoScope® 3205A) and a laptop for data processing (Matlab® based software).

The electrodes being used are beryllium cooper gold platted needles with around 1.02 mm in diameter. The bioimpedance measurement requires the most superficial possible penetration of the electrodes in order to reduce the dispersion of the needles surface current density [9], and also to reduce damage on the biologic sample.

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The digital oscilloscope PicoScope® 3205A has dual functionality: 1) synthesizes and provides the excitation AC signal to the conditioning unit (ADC function); 2) digitizes both excitation and induction signals at high sampling rates (12.5 MSps) and transfers data to the computer via USB where it is stored. The signal conditioning unit receives the exciting AC signal, coming from the PicoScope®, and amplifies it to be applied, through an electrode, to the specimen under study. The induced AC signal is collected by a second electrode and is redirected to the conditioning unit where it is also amplified. Both excitation and induced signals are conduced to the PicoScope® to be digitized.

The features of both excitation modes are described below.

B. Design Specifications

The current mode circuit employs the current-feedback amplifier AD844 in a non-inverting ac-coupled CS configuration (see Figure 1), already studied by Seoane, Bragós and Lindecrantz, 2006 [15].

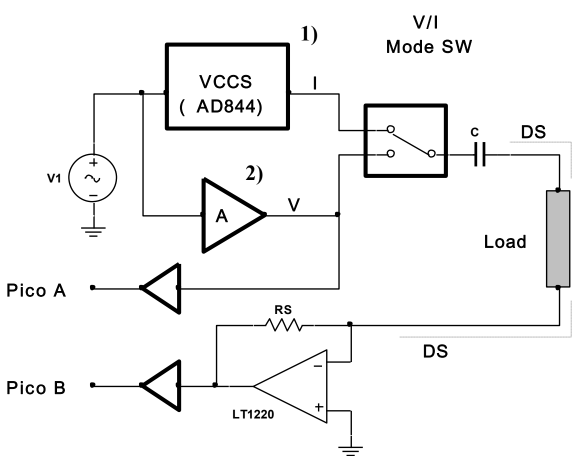

Figure 1. Schematic of the EIS system conditioning unit - 1) AC current source; 2) AC voltage source; 3) current/voltage sense.

A common problem inherent to bioimpedance measurements is the charging of the dc-blocking capacitor between the source and the electrode due to residual DC currents [15]. This effect lead to saturation of the transimpedance output of the AD844. The DC feedback of the implemented configuration maintains dc voltage at the output close to 0V without reducing the output impedance of the source. Subsequently, the output current, is maintained almost constant over a wide range of frequencies.

The high speed voltage-feedback amplifier LM7171 is employed in the voltage mode circuit (see Figure 1). This behaves like a current-feedback amplifier due to its high slew rate, wide unit-gain bandwidth and low current consumption. Nevertheless it can be applied in all traditional voltage-feedback amplifier configurations, as the one used. These characteristics allow the maintenance of an almost constant voltage output over a wide range of frequencies.

Current or voltage signals resulting from voltage or current excitation modes, respectively, are sensed by a high speed operational amplifier, LT1220 (see Figure 1), which performs reduced input offset voltage and is able of driving large capacitive loads.

Gain values of both current excitation source and voltage excitation source can be changed in order to extend the range of impedance magnitude. The transductance gain of the LT1220 is currently set to 5.1 kΩ and defines the gain of the system. Since the gain values are known and also the amplitude of the AC excitation signal, V_1, from the PicoScope®, the EIS system is calibrated automatically by software.

C. Cables Capacitance

For an optimized signal-to-noise ratio, coaxial cable must be used. Nevertheless, this type of cable is prone to introduce high equivalent parasitic capacitances, which translate in errors in the bioimpedance measurements, especially at high frequencies.

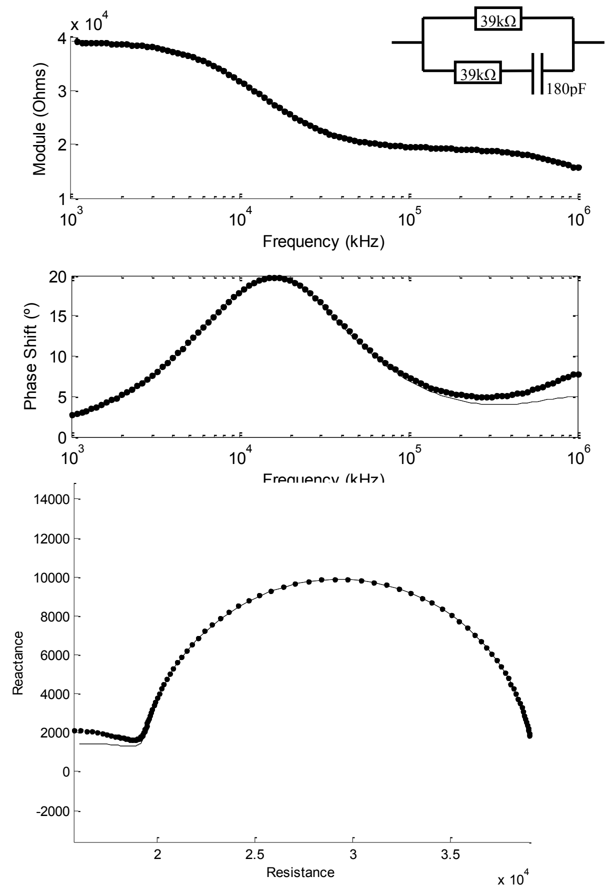

Figure 2. Bode and Cole-Cole diagram showing the reduction of cables capacitive effect by the application of the driven shield technique. The voltage mode excitation was used to analyze the circuit at the right top. The reduction is more noticeable at high frequencies where the capacitive effects have more influence.

When assessing bioimpedance, the capacitive effects from cables are not the only exerting influence. In fact, phase shift effects, perceptible especially in the high frequencies range, are introduced mainly by the amplifiers. The influence of phase shift errors has a cumulative effect that is translated, in the impedance spectra, as an inflexion that occurs at high frequencies (see Figure 2).

This behavior can be simulated by an equivalent circuit as it is like the system analyzes any load always in parallel with a capacitor.

The impedance magnitude, at high frequencies, is also affected. It presents a characteristic decline as the bode diagrams of the Figure 2 show. In the developed EIS system, the slight decline of the impedance magnitude is due to the loss of the product gain-bandwidth of the LT1220 for high frequencies.

Since stray capacitances are considered systematic errors of the system, thus affecting all the measurements, theirs influence doesn't directly affect the results. Although, it is convenient to have an approached sense of the real equivalent circuit (see Figure 3), in such a way that the effect of all the parasitic elements can be considered and/or discounted where justified.

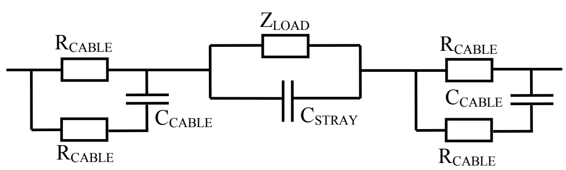

Figure 3. Equivalent electric circuit of all parasitic elements affecting impedance measurements of a load, Z_{LOAD}. The effect of the stray capacitances from cables, C_{CABLE}, is minimized by the driven shield. Other stray capacitance effect, C_{STRAY}, due primarily to the phase shift of amplifiers, can be minimized by software.

III. ASSESSMENT OF HYDRIC STRESS LEVEL

A. Materials and Methods

Assessing physiological states of plants, using impedance techniques, implies the knowledge of the typical EIS profiles of the species under study, i.e., the EIS profiles for healthy individuals under controlled environment conditions. For this reason, the studies presented herein required an exhaustive EIS assessment and monitoring, performed over months, and extensive data analysis. In addition, the populations of different plant species were kept under controlled environment conditions (temperature, luminosity, soil content and watering), in order to reduce the quantity of variables that may change EIS profiles.

For assessing EIS profiles and studying the hydric stress level there were used: 1) eight young healthy pine trees (*Pinus pinaster* Aiton), with about 0,8 meters tall and 1 to 2 centimeters in diameter; 2) eight young healthy chestnuts trees (*Castanea sativa* Mill), with about 0,5 meters tall and 1 to 2 centimeters in diameter; and 3) four young healthy *Jatropha Curcas* L. trees, with about 0,2 meters tall and 3 to 4 centimeters in diameter.

The choice of the plant species under study is substantiated by the economic and/or forestalls relevance they have. Chestnuts and pines have a crucial economic impact in our country and are currently affected by uncontrolled diseases: the nematode disease, in the case of pine trees, and the ink disease, in the case of chestnut trees. *Jatropha curcas*, by other hand, is a tropical species, lacking physiological studies, which seed are used for biodiesel production.

To perform the EIS measurements, the electrodes were placed in the trunk of each tree, in a diametric position, and about 20 cm above the soil, in the case of the pine and chestnut trees, and about 10 cm above de soil, in the case of *Jatropha curcas*. It was used the portable EIS system version in the voltage mode of excitation and a frequency range between 1 kHz and 1 MHz. Routine acquisitions took place between 11 a.m. and 13 p.m. since it was already verified in previous studies that at this time period the trees impedance is higher and presents few variation (see Figure 4).

To study the hydric stress level variation, there was performed EIS monitoring over two months for one individual of each plant species. Plants were kept one month without watering and, during the remaining month, plants were watered regularly. This process was repeated three times for each case.

B. Results

For each obtained impedance spectrum, there were assessed several impedance parameters. Due to paper space limitation and also because it is a well-known impedance parameter, it will only be presented the results obtained for the ratio Z_1/Z_{50}. Note that it is used the index 1, that corresponds to the lowest analyzed frequency (1 kHz), instead of the index 0, as explained in the *Introduction* section.

The EIS measurements revealed that EIS profiles have a daily oscillation. To analyze this behavior it was calculated the R_1/R_{50} ratio (R represents module) for a period of 4 days.

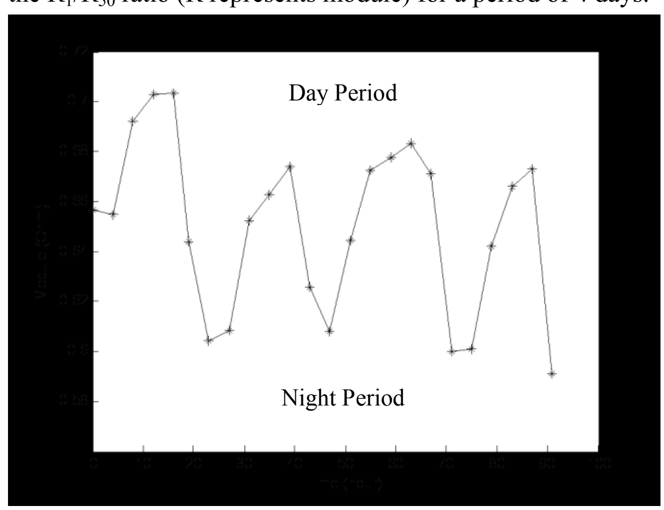

Figure 4. Variation of the R_1/R_{50} ratio during the monitoring of a healthy pine tree. The impedance values show a daily oscillation that is characteristic of the studied trees.

To confirm the daily oscillation it was calculated the Fast Fourier Transform of this ratio. A frequency of 11,57 μHz was clearly founded, which corresponds to a frequency of 24 h. The lower values of the ratio R_1/R_{50} correspond to the night period, while the higher values correspond to the day period where the temperature and luminance are higher (between 11 a.m. and 15 p.m.). Previous studies on plants also shown that, during the day period, the variation of impedance values is lower than the one observed at the night period.

Data obtained for the EIS monitoring and the study of the hydric stress level, revealed a consistent behavior for all the studied species. The Z_1/Z_{50} ratio tends to increase with higher values of hydric stress, for the pines and chestnut trees. After introducing regular watering, the same parameter progressively tended to the typical values of hydrated trees.

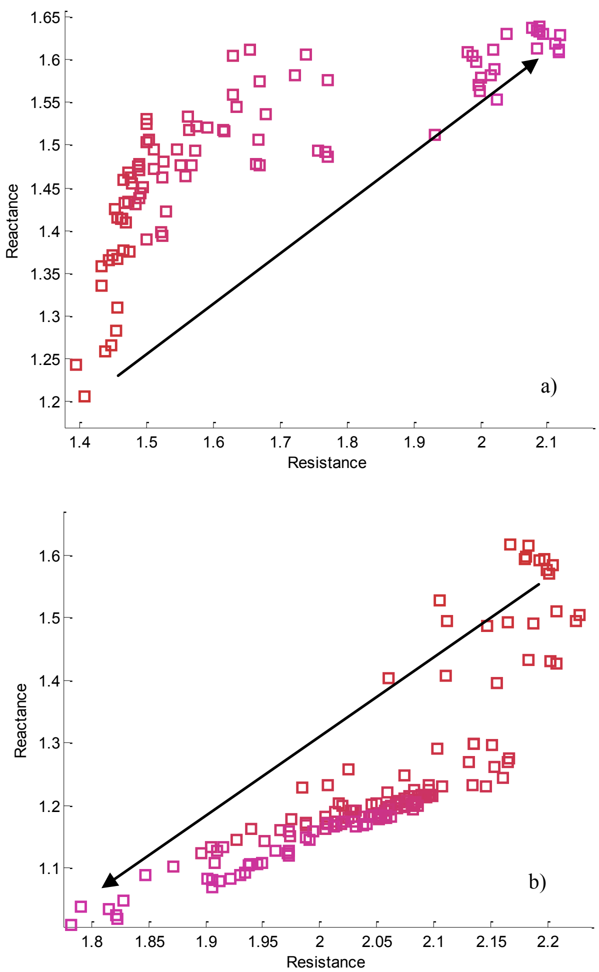

Figure 5. Evolution of the Z_1/Z_{50} ratio during the monitoring of a healthy chestnut tree a) while kept without watering and b) with regular watering. The arrows indicate the direction of the Z_1/Z_{50} ratio evolution.

In the case of *Jatropha curcas*, the impedance behavior with the hydric stress level variation was completely different. The found different results could be explained due the fact that this species contain latex vessels, while pine and chestnut trees do not. In this case, the Z_1/Z_{50} tend to decrease for higher values of hydric stress level and, after introducing regular watering, the ratio abruptly assumed the typical values.

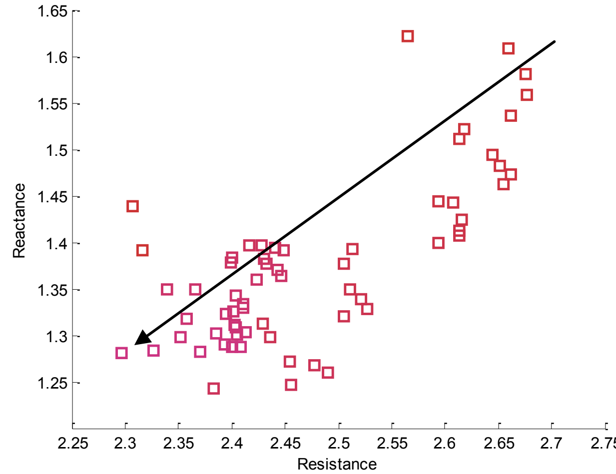

Figure 6. Evolution of the Z_1/Z_{50} ratio during the monitoring of a healthy *Jatropha curcas* tree while kept without watering. The arrow indicates the direction of the Z_1/Z_{50} ratio evolution.

IV. STUDY OF DISEASE CONDITION

A. *Materials and Methods*

Twenty four healthy pine trees (*Pinus pinaster* Aiton), with about 2,5 meters tall and 2 to 3 centimetres in diameter, constituted the population for the conducted study. The pine trees were placed in vases in a controlled water environment at a greenhouse. Half of the tree population was watered during 5 minutes per day (~ 133,37 mL/day), while the other half were watered during only 2 minutes per day (~ 66,67 mL/day). This second half was less watered to maintain a relevant level of hydric stress.

After one month elapsed since the pine trees were placed in the greenhouse, the inoculations with pinewood nematode, PWN, (*Bursaphelenchus xylophilus* Nickle) and with the bark beetle (*T. destruens* Wollaston) were performed. Six pines were inoculated with PWN, other 6 pines were inoculated with bark beetles, other 6 pines were inoculated simultaneously with PWN and bark beetles, while the remaining 6 were kept under normal conditions, i.e., healthy. The position of the pines in the greenhouse was made so that each sub-group had the same number of pines with normal watering (5 min/day) and with reduced watering (2 min/day).

To perform the inoculations with bark beetles, callow adults were collected immediately after emergence. In each tree, a box containing 15 beetles were placed in the middle and the device was covered using Lutrasil tissue to avoid beetles escape.

The inoculation with the PWN followed an innovative approach. Firstly, three 2 x 2 cm rectangle of cork were removed from the first tiers of the trunk (about 1,80 m above the soil) and exposed phloem was erased with a scalpel in order to increase the adhesion of the PWN. Afterward, 0,05 mL of of a PWN suspension was placed on in each incision. In the total, 6000 nematodes were inoculated per tree. To finalize the task, the removed rectangle of cork was fixed in the respective place and wrapped with plastic tape.

Seventy days after the inoculations, the EIS measurements were performed in all the tree population. At this time, the pine trees inoculated with PWN presented some visually symptoms of the PWD. The decay of those trees, rounded 40 %. Two of the healthy pines died (decay of 100 %) due to hydric stress. All remaining individual appeared healthy.

To perform the EIS measurements, the electrodes were placed in the trunk of each tree, in a diametric position, and about 30 cm above the soil. It was used the portable EIS system version in the voltage mode of excitation and a frequency range between 1 kHz and 1 MHz. There were taken two measurements for each tree. The acquisitions also took place between 11 a.m. and 13 p.m.

In order to relate the EIS data with the PWD and the stage of the disease, the trunk of the pine trees inoculated with PWN were cut in three distinct regions to perform a count of nematodes. The cuts were executed: a) immediately below the inoculation incision (180 cm above the soil); b) 30 cm above the soil (where EIS measurements took place); and c) in the middle of the previous two cuts (approximately 80 cm above the soil).

After the EIS measurements, two healthy pines were monitored by two independent portable EIS systems. After a week of monitoring, the same pines were inoculated with PWN, and the measurements continued during 7 more weeks. The main purpose of this last experiment was to study the variation of the pine EIS profiles during the decay due to the PWD.

B. *Results*

In order to compare results between the different physiological states of the trees, several impedance parameters were assessed. The impedance parameter that showed better results was the Z_1/Z_{50} ratio.

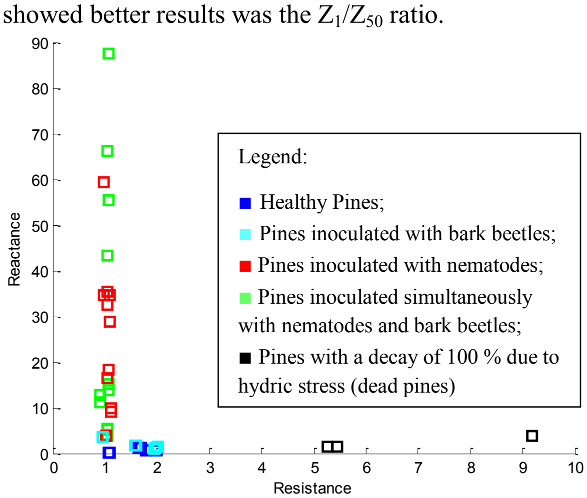

Figure 7. Values of the impedance parameter Z_1/Z_{50} for each of the 24 pine trees. Note that there are represented two values for each pine.

The analysis of the obtained results shown that the healthy pines and the pines inoculated with bark beetles have similar Z_1/Z_{50} values. In fact, the bark beetles doesn't damage the inner structure of the trees, therefore it was expected that the impedance profiles were similar between healthy pines and pines inoculated with bark beetles.

On the other hand, Z_1/Z_{50} values for the pines inoculated with nematodes and also, for those inoculated simultaneously with nematodes and bark beetles, locates in the same region, different from the previous one, of the graph of Figure 7. Those values present a relatively high dispersion in terms of reactance. It was later confirmed that higher reactance Z_1/Z_{50} values correspond to higher number of nematodes in the tree (see Figure 8).

The pines that died due to hydric stress (decay of 100%) were also studied and the Z_1/Z_{50} parameter present high resistance values in relation to all the other pines.

The counting of nematodes in the several cut sections revealed that the concentration of nematodes was higher in the cut sections b) and c) for the pines less watered (pines 1, 2 and 3) – see Table II. It is known that the nematodes move toward watered regions along the trunk. For this reason, the concentration of nematodes in the lower parts of the trunks was much higher for the pines with less watering than for those with regular watering (pines 4, 5 and 6).

TABLE II. NUMBER OF NEMATODES IN THE TRUNKS OF PINE TREES PER CUT SECTION

Tree	Cut Section	Number of nematodes in 0,05 mL
1	*a*	1
	b	0
	c	133
2	*a*	0
	b	43
	c	1
3	*a*	0
	b	0
	c	112
4	*a*	4
	b	20
	c	0
5	*a*	0
	b	17
	c	0
6	*a*	0
	b	0
	c	14

These results for the nematodes counting support the already referred results obtained for the Z_1/Z_{50} impedance parameter. In fact, it is observed a clear relation between the number of nematodes and the reactance dispersion for the Z_1/Z_{50} parameter, as Figure 8 shows. The higher the number of nematodes is, the higher is the reactance value of Z_1/Z_{50}. It is considered that the dispersion in terms of resistance is not significant when compared with values from pines in other physiological condition – see Figure 7.

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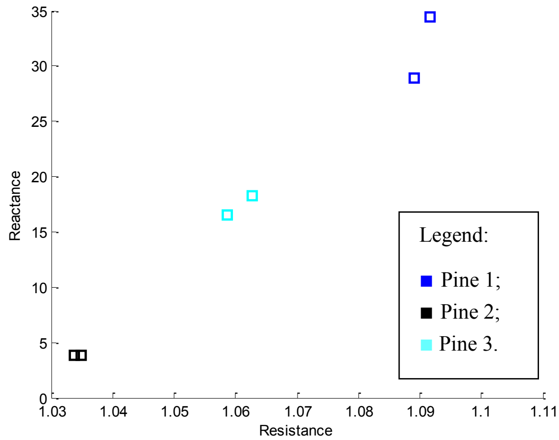

Figure 8. Values of the impedance parameter Z_1/Z_{50} for the pines inoculated with nematodes and with low watering (pines 1, 2 and 3 from the Table II). Note that there are represented two values for each pine.

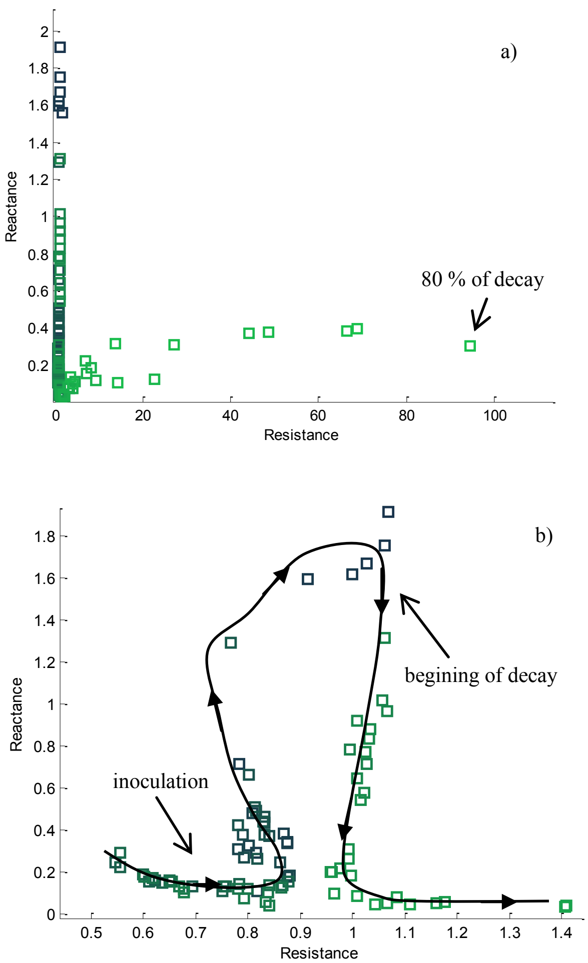

Figure 9. a) Evolution of the Z1/Z50 during the monitoring time (8 weeks). b) Closer view from the Z_1/Z_{50} evolution, showing a hysteresis-like behaviour.

There were monitored two healthy pines, one with low watering (2 min/day) and another with regular watering (5 min/day). After one week from the beginning of the monitoring, both pines were inoculated with nematodes. It was shown again a dispersion of the reactance values of the Z_1/Z_{50} parameter, as Figure 11 shows. As time passed the reactance values became higher. The higher values of reactance were achieved for the pine with less watering. According to the previous presented results, it was expected that the number of nematodes increase in the below part of the trunk for the pines with less watering; and consequently, to observe a higher rising of the reactance of the Z_1/Z_{50} parameter. After the 6th week, pines start to decay strongly and it was observed a relevant decrease of the reactance and a significant increase of the resistance for the same parameter – see Figure 9. The higher values of resistance were achieved for the pine with less watering, and also in a shorter period of time. At the end of the monitoring, the decay of the pines, evaluated by an expertise, was about 80 % for the pine with regular watering and 100 % for the pine with less watering.

From the Figure 9 b), that represents a closer view of the Z_1/Z_{50} values for the monitoring, it is possible to observe that the path followed during the period of nematodes population increasing is different from the path followed during the period of decay, i.e., it is observed an hysteresis-like behavior.

V. CONCLUSIONS

The EIS system was developed in order to ensure a robust, efficient and fast bioimpedance analysis. The adaptability to different biological applications, the portability and the usage of easily accessible and affordable components, were preferred aspects taken into account. In this manner, the system allows the user to choose the settings of the analysis that best fit to a specific application.

The system is able to perform AC scans within a frequency range from 1 kHz to 1 MHz. The frequency limits and the number of intervals of the scan can be selected at the user interface (developed with Matlab® tools). The type of signal used to excite de sample, voltage or current, can be preselected by an external switch. This allows the usage of the source with the best behavior in a concrete application.

To overcome problems inherent to stray capacitive effects from cables, a driven shield technique is applied. The maximum phase shift reduction is estimated at 20.4 % for the current excitation mode and at 35.8% for the voltage mode.

The biological application study aimed at discriminating between different physiological states of three plant species: pine *(Pinus pinaster* Aiton), cheastnut (*Castanea sativa* Mill) and *Jatropha Curcas* L.

The obtained results suggest that the implemented method may constitute a first innovative approach for the assessment of physiological states of plant organisms and to the early diagnosis of plant diseases. The consistency of the results obtained for the three studied species reveals the transversality of the method. Since EIS profiles are obtained

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for healthy individuals of a given plant organism it is possible to assess and study the physiological states.

EIS profiles showed a consistent behavior whit the hydric stress level of the three studied species. The Z_1/Z_{50} impedance parameter presents increased values of both reactance and resistance when the hydric stress is high, for the pines and chestnuts. In the case of *Jatropha curcas*, this parameter presented decreased values of both reactance and resistance when the hydric stress was high. This inverse behavior may be explained due to the presence of latex vessels in this species.

The evolution of the Z_1/Z_{50} impedance parameter may be used to predict risky hydric stress level of a plant organism.

In addition, the achieved impedance parameters allow discriminating three different physiological states for pine trees: healthy trees, trees with PWD and trees in hydric stress.

The trees with PWD present Z_1/Z_{50} ratio with high values of reactance, suggesting that the current flows preferably trough the cytosol. In fact, the action of the nematodes inside the tree may destroy cell membranes. This means that membranes capacitor effect becomes less significant in the impedance measurement.

It was also shown that the number of nematodes and Z_1/Z_{50} impedance parameter are related. The higher the number of nematodes is, the higher the reactance of the ratio is.

The action of bark beetles seems not to interfere, at least in measurable terms, in the level of hydric stress of pine trees.

Healthy trees, with high values of hydric stress (decays above 80 %), and also trees with PWD at advanced stages, revealed low reactance and high resistance for the same studied parameter. The high values of resistance are justified due to the water loss in the tree. Consequently, it means that for this specific case, the method cannot distinguish between trees with PWD or trees with high level of hydric stress but with no disease. However, it is known that advanced stages of PWD promote high levels of hydric stress. This means that both cases represent, in practical terms, the same situation, i.e., the tree presents high probability to die. In addition, in the stages where the method is able to distinguish between healthy trees and trees with PWD, the decay was determined to round the 40 %. Therefore, if a cure is available, this diagnosis could help to administrate a treatment and reverse the disease evolution.

Hence, the main conclusion of the developed study is that the implemented method could be used to assess physiological states (such as the hydric stress) of living trees, and that the Z_1/Z_{50} impedance parameter could be applied as a risk factor.

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