On the Interpretation of Four Point Impedance Spectroscopy of Plant Dehydration Monitoring

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***Abstract*—In this paper, we present a study of in-vivo four-point impedance spectroscopy (FPIS) for plant monitoring. FPIS was measured vs. time under hydration/dehydration cycles in tobacco plants. The plants were set up in a gravimetric system and grown in sand for better control of water drainage. Data was gathered at fixed time intervals, every few minutes, collecting the complex impedance values at a few hundred frequencies in the 40 Hz to 2MHz range. Due to the large amount of the collected data, the issues of data representation and interpretation became a challenge. These two issues are discussed examining system orientated approaches, along with possible methods representing and interpreting the data. The results of the electrical measurements and the various analysis methods were compared to those of the well-established agriculture gravimetry measurements. The FPIS results show that the impedance depends on the plant status, similar to the value expressed in the gravimetry system, thus the plant status can be interpreted from the electrical measurements. Each of the data interpretation methods examines the change and is suggested for further device development.**

***Index Terms*—Plant impedance, in-vivo impedance spectroscopy, plant sensor system.**

I. Introduction

THE rapid growth in world population has created an increased demand for agricultural produce [1], [2] while the limited availability of food production resources, (i.e. land, water and energy) threatens to decrease global crop yields [3]. There is, therefore, a dire need for sustainable management of land and water, alongside technological developments to improve crop yields and all other parameters throughout the food chain. There is a need to generate as much data as possible about the crop from the "farm to the fork", to achieve this goal. One important aspect is on-field monitoring. Thus, development of low cost, reliable, robust, and simple devices, systems and methods for sensing and data management for precision agriculture is required. In this paper, we focus on direct sensing, e.g. sensors that are directly mounted on the plants as one possible solution and the interpretation of the obtained data.

Precision agriculture depends on the ability to collect accurate data from the field, in a simple and continuous manner. The accuracy and timing of the collected data are crucial to support effective monitoring and ongoing adjustments of resources (such as irrigation). Once reliable data is available, further data studies, such as deep learning algorithms, can be implemented. This will result in overall improvements of the food-chain yield and efficiency.

Data collection in the field depends directly on available sensing technologies. This includes the direct interface of the sensors with different crops and resources in the field. Today, a range of sensing technologies are used to monitor crops [4], [5]. Nowadays, monitoring in the field uses a range of diverse technologies, yet the sensing is mainly focused on the surrounding environment of the crop and the top external pheno-typing expressions. This includes the monitoring of environmental conditions, such as temperature, air humidity and lighting, as well as the closer monitoring of soil moisture and nutrient content [4], [5]. Additional methods include imaging of plant fields, using satellite and drone technologies, where visible or near infra-red image changes are correlated with physiological plant changes [4]–[7]. These methods are mostly based on data collected from the plant's surrounding environment and external expressions, rather than collecting data signals produced internally within the plant itself, directly. Direct measurement methods for sensing are available, and new methods are evolving. Some methods use standard available sensors, offering measurements such as, change in luminescence or leaf shape, while being exposed to a certain area or part of the plant. Other methods include the study of cell signaling between cells, and across membranes at cell level, which could be based on bio-electrochemical changes [8]–[12]. Other approaches study plant tissue, yet these are mainly destructive or localized methods [13].

Electronic impedance spectroscopy methods for plant study have been used in the past [14], yet few are able to provide an indication of overall plant well-being in a non-destructive manner. Some of these works focused on material characterization, rarely showing indications of the overall plant and rather are an expression of a localized reaction. These have offered characterization of different tissue areas as an estimate of local physiological change, such as fruit ripening or resistance changes in the cell membranes [13], [15]. Prior to these studies, resistivity and impedance measurements were reported for wood [16], [17]. The need for direct continuous

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monitoring and field adaptable systems is clear, while the presented approach will allow this versatility.

Continuous impedance measurements of living plants, in a non-destructive manner, have only recently been presented [18]–[21]. These works suggest use of a four-point probe configuration, coupled to the plant stem, to acquire continuous timed readings as an indication of overall plant well-being.

These works [20]–[22] of monitoring plant impedance have shown feasibility for detecting changes in plant impendence, while monitoring environmental conditions. These experiments provide a basis for this paper, showing the need for a system orientated approach towards the data interpretation across the spectrum collected. The significance and correlation of impedance change has been demonstrated [21]. However, the amount of data generated for this purpose requires refinement and points to the need for a system orientated approach. In this paper we inspect methods to interpret the data using an electronics system approach. We take the plant stem impedance data and attempt to interpret the overall change across time using various methods. In the presented continuous experiment, the environment is controlled and physiological changes are induced under well controlled and monitored conditions. In this manner the response to non-intended physiological changes is eliminated.

This seemingly "straightforward" application of a well-known measurement method in electronics [25] is applied to on-field plant monitoring. In order to create an effective measurement system, that will provide significant data on the plant status as well as minimize the cost of the hardware for the sensor device, supporting circuitry and communication, there is a need for an in-depth study of this newly developed measurement method. This work attempts to address some of these issues by showing feasibility of change detection using different system analysis methods across a time series.

"Broad-band sensing" due to the use of a wide frequency range (Δf), yields very useful information about various changes in the internal interactions affecting the plant's electrical properties. The main assumption is that changes in the plant's vascular cambium, water content and electrolyte composition and concentration, will have an effect on the impedance. In addition, the wider the frequency range, the more effects can be monitored in a simple sum yielding a number of useful parameters, while increasing the methods' accuracy and reliability. Note that we assume that due to the random nature of the electrode positioning on the plant, we focus on the relative change of the measured impedance, rather than on its absolute value. Although one should note that after few experiments, the impedance values between various runs do not change much. Multi-frequency measurement and analysis differs substantially from a single frequency method that estimates a single "raw" value [25]. Since the spectrum of measures provides insights regarding the actual changes in relation between the different components within the vascular cambium, it offers an indication of the overall plant status, rather than a localized momentary evaluation.

A cost-effective solution will include a "frequency tailored" impedance analyzer device as the sensor. This is due to the fact that high-end impedance analyzers, which provide a wide frequency band sweep, are costly. Here, a study of the best fitted impedance analysis for evaluation of the plant is needed, to establish the correct demands for the sensor device design. The hardware required to support impedance measurement across a broad range of frequencies will be a defining measure of the device pricing and affordability.

In this paper we present a study of in-vivo four-point impedance spectroscopy for plant monitoring in comparison to gravimetry. Electrical monitoring methods are expected to be low cost, easy to install, low maintenance and relatively straight forward data interpretation, making it useful for farmers. A four-point impedance measurement (FPIM) was devised, eliminating contact impedance effects which may affect a two-point measurement. In FPIM the current is injected by the two external electrodes, which are collinear with two internal electrodes where the voltage is measured. The effective impedance is defined as the ratio between the measured voltage to the induced current [26]. At each time spot the impedance is measured as a function of the frequency. Since for every measurement in time, the impedance vector includes a few hundreds of complex values, the data representation and interpretation are a challenge. In this paper, we present electrical impedance spectra and analysis of tobacco plants grown in sand in a special gravimetry setup. The data is presented under a unique predetermined sequence of hydration-dehydration cycles. The plants were exposed to short cycles of daily hydration-dehydration where every few days a draught function was applied. The data shows that the impedance data depends on the plant status, while the various analysis methods for such continuous time-dependent monitoring of the tobacco plants are presented and discussed. The impedance results are compared to simple gravimetry that was performed in parallel.

This type of approach and analysis will be significant for future system design, as the choice of frequency bandwidth will affect overall system complexity and pricing. Furthermore, the analysis method will allow for future determination of the significance of monitoring the different spectral ranges. This will allow for cost effective planning and establishing the accuracy needed from each measured crop.

The paper is structured to initially establish a clear link between the impedance data collected and the gravimetric method, showing that impedance data expresses physiological water stress response. Next it explores three system orientated approaches for analysis of the collected impedance data across time. These are crucial for future system design and supporting circuitry planning. The significance and sensitivity of a case study of an introduced physiological stress is presented. Finally, a qualitative comparison to a well known plant physiology monitoring method is shown.

II. Experimental Methodology

The measurement method presented applies an in-vivo technique, where the electrodes are placed in direct contact with the plant. Furthermore, contact is made with the inner vascular cambium of the plant, suggesting the acquired readings

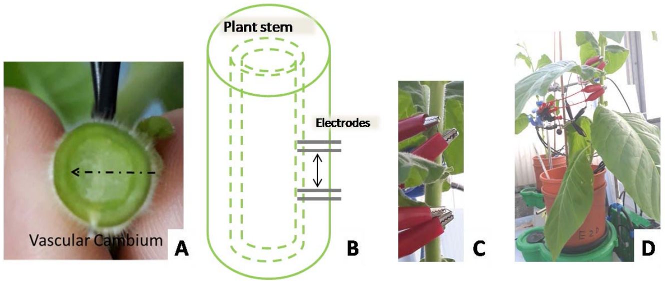

Fig. 1. Plant stem structure and four-point probe configuration. (A) Nicotiana Tabacum plant stem cross section showing the vascular cambium area assumed to conduct electrical signals. (B) Schematic description of the plant stem showing electrodes inserted for measurement. (C) Close up image of 4 probes connected to the plant stem. (D) A full tobacco plant in the system during measurements.

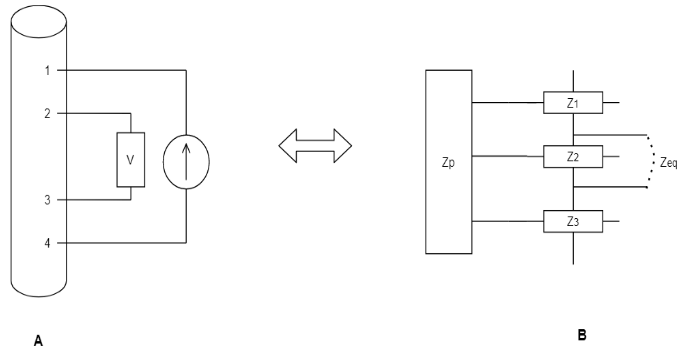

Fig. 2. Schematic description of the of the four-point probe impedance setup (Left) and it's schematic lumped model (right).

indicate the internal status of the water and nutrients transport vessels of the plant.

(Figure 1 shows the plant stem structure and electrode placement). In addition, the monitoring and data is collected in a continuous manner across time to allow actual ongoing measurement of plant changes. Furthermore, the environment of a greenhouse controlled during summer months, as in the presented data, is a true example of actual application of crop monitoring as intended. We use the four point impedance measurement data to examine the different plant responses to changes like water stress cycles and how they can be identified at system level.

In the presented work, the measurements are shown using *Nicotiana tabacum (tobacco)* plants, as a representative plant for the study. The method can be adapted to different crop types, as tobacco is an example standard physiological structure [23], [24].

A. Four Point Electrical Impedance Spectroscopy

Electrical impedance measurements can be carried out in a range of probe connections. These include two, three and four probe connection. Each configuration is suitable for specific measurements and specimens under test [25]–[27] i.e. two probe measurements are used often for linear, well characterized Ohmic contacts; 3-point measurements are prevalent in electrochemical measurement, where a reference electrode is often required. The four probe configuration is used in cases where there is question regarding the contact linearity, and therefore neglecting contact effects by measurement setup is advised. This is the case using in vivo connections to a plant stem. Use of the four point probe connection, allows to eliminate contact effects as well as neglect the differences between plants and electrode placement, in a biological specimen. In the case of field devices for agriculture, removal of electrode variation dependencies simplifies the setup and allows higher accuracy of overall acquired data.

The setup for impedance measurements applies a four-point probe configuration. This approach is based on the connection of the electrodes to the device under test, while considering the exciting current and measured the voltage across the specimen. Here the four-point probe connection is examined as a collection of effective voltage dividers, while series and parallel connections are considered. A schematic diagram in Figure 1 shows the experimental connection to the plant and a schematic lumped model layout of the four-point probe connection. This connection yields output impedance equal to

$$Z_{eq}\frac{V_{out}}{I_{in}} = \frac{V_{23}}{I_{14}} \quad (1)$$

This connectivity approach reduces to a minimum the contact effects on the measurement, allowing a more accurate and robust assessment regardless of the contact formation. As shown in the schematic description in Figure 2, the equivalent impedance can be divided into components, including parallel impedances, and three series impedances.

B. Measurement Setup

Impedance spectroscopy was measured using a four-point probe configuration. Four metal probes, made of Au wires, were inserted at a depth of approximately 5mm into the stem, thus connected galvanically to the plant under test. The designated distance between the two pairs of probes was set to 5cm. The measurement was done using a HIOKI IM3570 impedance analyzer. The frequency was swept across 40Hz- 2MHz logarithmically across 801 points at 500mVrms, and averaged with a factor of 4 for each measurement across 10msecs. Error in impedance magnitude and phase are estimated at 0.12% and 0.08% respectively [28]. Calibration for measurements and data acquisition was completed using the connecting cables, excluding the electrode inserted in the plant. Stray capacitances and measurement noise were reduced in this manner. The impedance measurements were collected continuously across time using our dedicated software, written in Python® that was required to customize the interface with the equipment specifically to our plant sensing setup. All four connections were similar, allowing optimal calibration and minimization of stray coupling effects from the system, enhancing the accuracy of the measurement of the device under test. Plant readings were acquired every 24 minutes to allow synchronization with the gravimetry method (explained in section D). A block diagram of the plant measurement setup is shown in Figure 3. (The actual connection to a monitored plant is displayed in Figure 1).

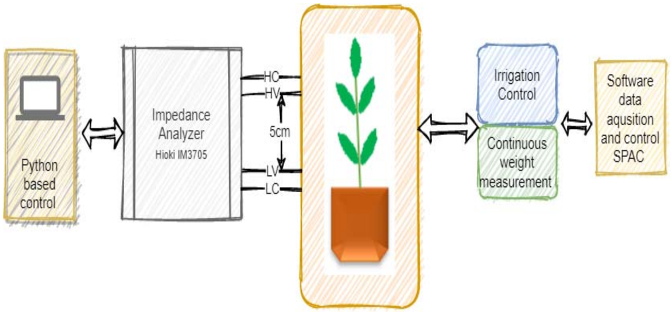

Fig. 3. Block diagram of four point impedance setup alongside gravimetry measurements.

C. Plant Choice and Preparation

Young *Nicotiana tabacum* plants were grown for 3-5 weeks in a greenhouse environment. Once plants reached a stem diameter of approximately 0.8cm, electrodes were inserted in to the plant, secured, and measurements began. Measurement periods were across periods of 2-3 weeks at a time. Verification regarding stability of both impedance and gravimetric measurement systems were verified across 3-5 days prior to conducting experimental procedures.

D. Plant Array Gravimetry Setup

The whole-plant physiological performance was monitored with the functional pheno-typing system platform, as described in detail in [29]–[33]. The system includes combined weights sensors. This allows for monitoring the water content in the monitored plant at any time point, providing assessment of changes in plant physiological status across the day, and across longer time periods. In addition, the system is connected to a controlled irrigation system, where the plant can be hydrated according to experimental requirements. Alongside the local monitoring of the plant, the greenhouse environment is continuously monitored and maintained. This includes set day and night temperature control and controlled vapor pressure deficit (VPD) in the environment, alongside measurements of temperature, light conditions and air humidity [32].

E. Comparative Study

An assessment of the ability of impedance measurements to detect physiological changes was completed in a comparative study manner, where a known stable condition was measured and compared to the same specimen under physiological change (such as water stress).This approach is commonly used for biological specimen experimental evaluation. In order to verify the accuracy of these occurrences, the experiments were carried out across a number of plants. Here, a series of experiments to evaluate changes due to water stress were completed. The presented data is of a representative experiment across time.

III. Results

A. Impedance Spectra

A typical impedance measurement, across the frequency range of 40Hz-2MHz, is shown in Figure 4. The impedance data is displayed in a log-log plot (Bode diagram representation) demonstrating a resistive characteristic, with a phase close to zero up to a few KHz and monotonous decrease of the amplitude at higher frequencies. It was noticeable that the phase dropped to value in the range of -40 degrees, demonstrated a negative double hump and above $\sim$200-300 KHz starts to climb monotonously back to more resistive values. This behavior can be roughly approximated by a simple resistor in series to a leaky capacitor that is characterized by the leakage conductance in parallel. Trying to fit such 3-elements (2 resistors and a capacitor) model to the data yields a large error, especially in the phase plane. Nevertheless, we can use the dominant pole of this model as a figure of merit. An improved fit is achieved when using a physically derived model taking into consideration the signal transport along the conducting channels in the plant. The use of a lumped element model approach allows for better representation of the plant hydration/dehydration status, including its daily recovery from the stressed condition.

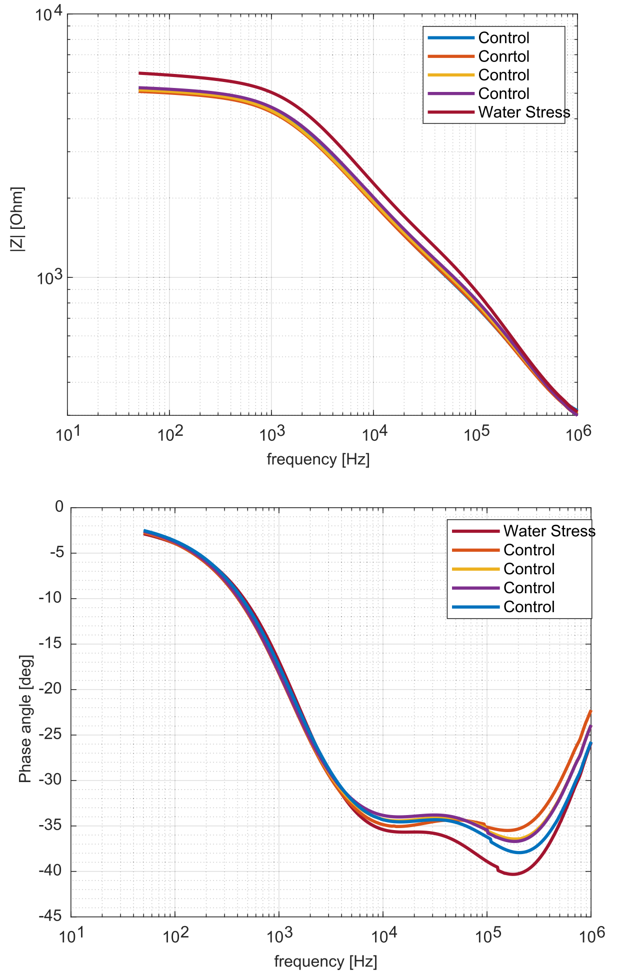

Fig. 4. Four Point Impedance Spectra (FPIS), magnitude (top) and phase (bottom) of the tobacco plant at a single time measurement. The control examples are shown across various daylight hours. The "Water Stress" curve is a representative curve taken at midday.

The specific impedance characteristics were consistent as a function of time, for different plants in different seasons,

TABLE I
MEAN AND STANDARD DEVIATION (STD) OF IMPEDANCE UNDER CONTROL AND STRESS CONDITIONS

Frequency	Control [mean ±std]		Stress [mean ±std]		Δ% (Relative change)	
	|Z| *[Ohm]*	*Phase angle [deg]*	|Z| *[Ohm]*	*Phase angle [deg]*	*Δ |Z|*	*Δ Phase angle*
100Hz	5400± 750	-3.9 ±0.4	7960	-5.3	~47%	~34%
10KHz	2080± 220	-34.2 ±1.0	2800	-36	~35%	~5%
100KHz	850± 74	-36.5 ±1.4	1100	-40	~30%	~10%
1MHz	316± 21	-24.1 ±1.4	400	-27	~25%	~12%

under various temperature, humidity and lighting conditions. For the same plant, the variations vs. time, under controlled physiological state, were small although they demonstrated changes vs. time of the day and other extrinsic ambient parameters. However, in the case of an induced water stress the impedance values across the spectrum, up to a few hundred KHz, change substantially (see Figure 4).

There were two types of changes:

a. Daily changes in correlation with the daily (normal) hydration/dehydration sequence
b. The change to a long period of stress (48 hours) that was induced after 3 days of a controlled normal sequence.

An estimation of the impedance change across a day for the different physiological states and the measured variations are presented in Table I. The data includes an averaging of different experiment days, across the different times of the day for each case (control vs. stress).

The results presented show that a change is detected with impedance measurements by comparing drought and control status. It is visibly clear that the impedance magnitude across the lower frequencies show a change in value due to different physiological status, whereas the phase values differ only across the higher frequency values. The estimation of change is done by comparing the impedance under the controlled situation at a fixed time of the day to the impedance under induced stress factor, at the same time of the day. We observe that the change due to stress occurs across the frequency spectrum and is higher at lower frequencies, reaching a value of almost 50% at 100Hz and below. The changes at higher frequency are significantly lower and are in the range of 30% at 100 KHz and 25% at 1MHz. These changes were significantly higher than the measurement of noise that was less than 2% and also much higher than the daily increase under normal cycles that were in the range of 10 % and less (as can be seen in Figure 4). All showing that the impedance changes could be good indicators for the plant stress.

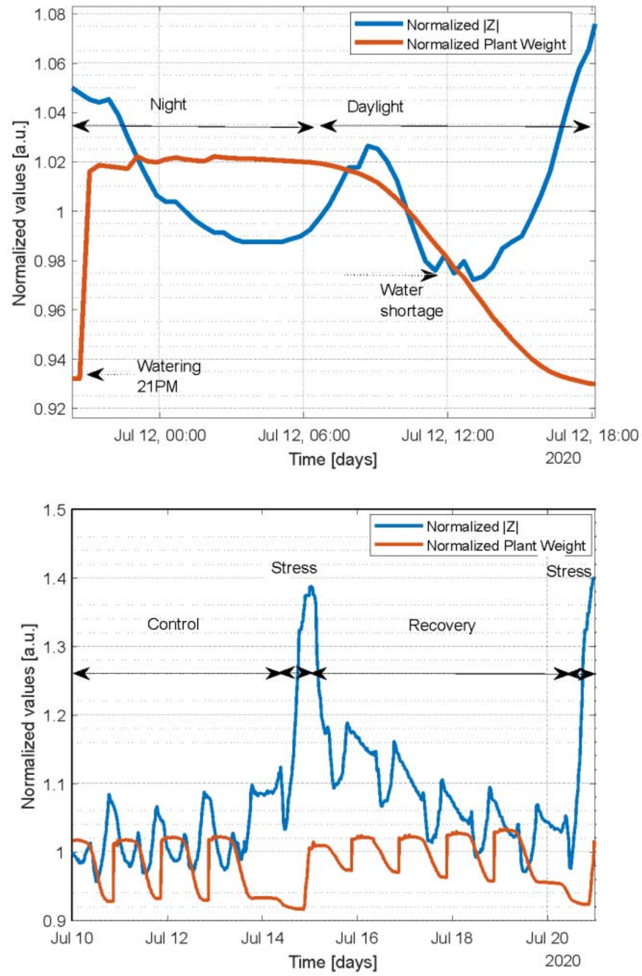

Fig. 5. Normalized plant weight and normalized impedance magnitude across time: (Top) 1-day cycle, where watering begins at 21PM, while by midday, the plant is dehydrated, (Bottom) 10 days period while the plant is watered regularly each day and then dehydrated for a 24 hours period.

B. Validation With a Known Plant Monitoring Method

In order to verify the validity of changes monitored using this newly established approach for plant monitoring, the plant was set up in a greenhouse environment using a continuous gravimetric measurement system. Our specific experiment was performed in sandy soil in order to aggravate the plant dehydration due to the fast percolation of the water inducing dehydration stress in a matter of hours. It should be noted that the actual dehydration state depended also on the temperature and humidity in the greenhouse. The gravimetric system allows for controlled plant irrigation, and measures changes in plant weight across time, keeping the environment conditions stable. The plant is watered regularly during the night, and during the day follows a well-established transpiration curve. Here, we show the normalized plant weight changes across the day alongside the changes in impedance magnitude. The impedance values have been normalized to the initial measured value, for convenience. The results are shown in Figure 5.

Inspecting the plant behavior across the day we observe fluctuations within the gravimetric system that indicate weight loss. This is expected, as during the night the plant is watered to saturation, and once daylight starts, a transpiration cycle begins i.e. water is evaporated through the plant leaves in the photosynthesis process. In the given example, conducted during the summer months, daylight begins at 6am and ends around 6pm, while maximal temperatures are at mid-day. At this point of the day, according to the weight decrease we observe that the plant lacks water resources, and experiences a slight drought. Looking at the impedance magnitude, we can qualitatively see that an increase in impedance is apparent until the night hours.

It can be seen that the impedance values show more fluctuation across the day, yet are repetitive, as the weight changes. Once severe stress is induced, and the plant weight is decreased, the impedance value shows a peak in its values. This shows that the changes measured in the impedance spectrum coincide with measurements completed using known plant physiology monitoring method.

Furthermore, this example shows high sensitivity of the in-vivo impedance reading to the plant physiological status across a number of days, as well as within the day itself.

C. Methodological Analysis Across Time

Following the data collection, analysis is completed over the inspected time period. Three different methods for analysis are suggested:

I. Analysis of the impedance at pre-chosen representative frequencies;

II. Spectral analysis using the dominant pole parameters as indicators;

III. Fitting the spectrum, at each time point, to a physically based lumped element circuit model; using the model parameters as indicators.

The three analysis methods are presented in the following sections, followed by a comparison to gravimetry and a discussion. The results were analyzed from the plant electrical response each day across a number of days. Across those days, a series of hydration-dehydration cycles were preformed daily. Every few days, a longer dehydration period (about 24 hours) was applied followed by a return to the daily hydration/dehydration sequence. The longer dehydration period was applied to study the longer term characteristics of the plant such as post dehydration recovery time. The results were found to depend on the dehydration/hydration cycles as well as the plant ambient conditions, i.e. temperature, time of the day, humidity and lighting. Therefore, the effect of the dehydration/hydration cycles on the electrical measured parameters was studied at the same time daily where the temperature and lighting provide similar comparative conditions.

Method I– Analysis at predetermined frequencies

Initially we examine the raw data across time. We present impedance data across time for a choice of a few representative frequencies, while we examine the change in values expressed at each frequency. It can be clearly seen that across the control period, the values of impedance magnitude fluctuate yet within a constant window (see Table I for mean and standard deviation values). In contrast, when a water stress is introduced, a large change in values is clearly seen throughout

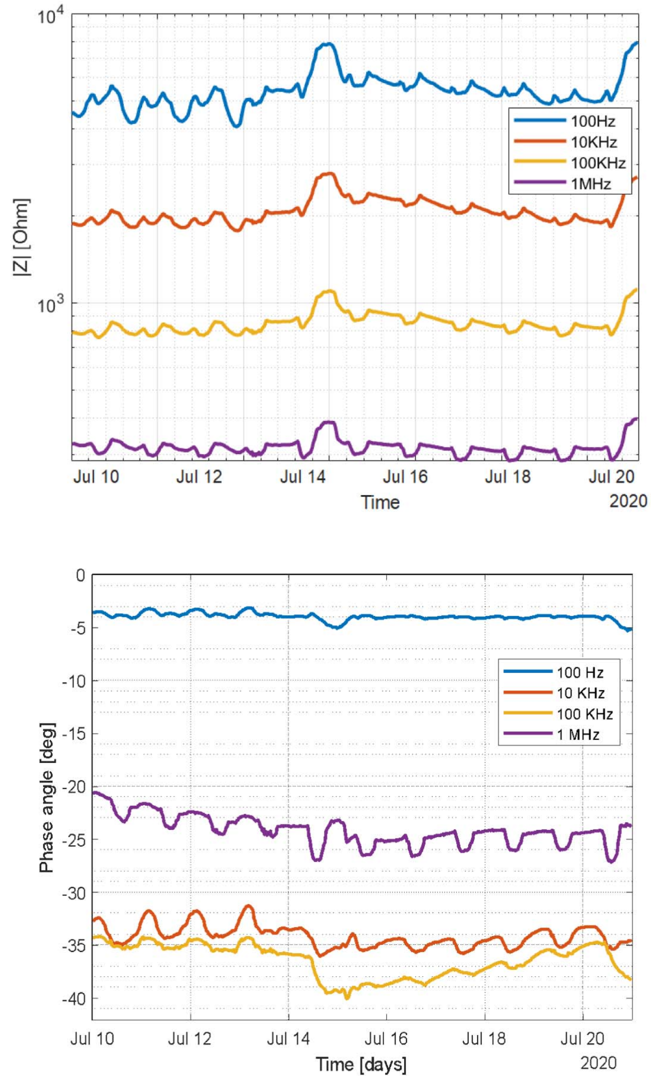

Fig. 6. Impedance vs. time measured at 100Hz, 10KHz, 100KHz and 1MHz.Top: magnitude, Bottom: phase.

the frequencies shown. Although for each frequency the magnitude of impedance values varies, the overall trend is visible across the full frequency range measured.

Inspecting the phase changes across time for the different frequencies yields a different perspective. The phase changes are smaller in percentage, yet also apparent across the different frequencies measured. The phase angle in the impedance measurements indicates transitions and relations between capacitive and resistive components (in our analysis of the plant using lower frequency ranges, we neglect any inductance effects) across the measured specimen. For lower frequencies, we expect a resistive response from the system, and therefore a phase angle near zero.

For higher frequencies, we expect a maximal angle of -90^0, which indicates a purely capacitive response. In the results presented, the phase angle fluctuates within these values, however reaching a maximal angle of approximately -45^0(Figure 6) [25], [27]. From the phase data presented, we can establish that for a frequency of 100 KHz, we observe that higher sensitivity to the induced water stress exists.

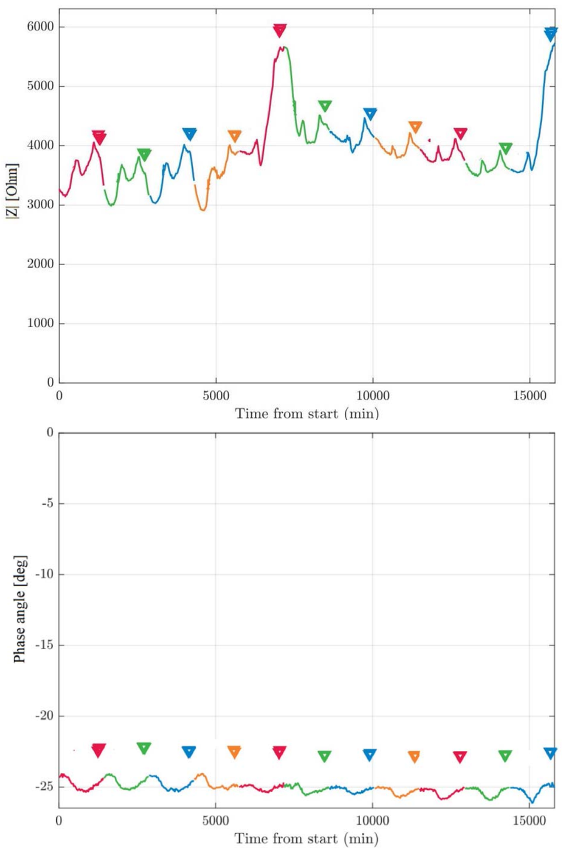

Fig. 7. Impedance values across time at the -3dB frequency. Top: Impedance magnitude, Bottom: Impedance phase. The arrows indicate watering of the plant.

Method II –Dominant Pole Analysis

In system engineering, very often the transfer function is characterized by a dominant pole, which in our case occurs in the range of 10-100 KHz. The pole is characterized by the -3dB (or the "knee") frequency which is suggested here to be a figure of merit. This frequency is related to the dominant time constant of the system's impulse response, which assumes the plant is represented as a linear system. Since we use a four-point configuration, we reduce the contact effect which can be non-linear. Hence, we can assume that the electrical measurement setup behaves as a linear system. This has been verified experimentally [18]. The characteristic time constant relates directly to the physical behavior of the system, i.e. the signal attenuation within the system. Examining the plant stem in the suggested impedance measurement setup, this approach to understanding the system frequency response is clear. It may be expected that it will indicate a transition between the resistive and capacitive areas of conduction within the measured plant stem. While following the phase values will provide this information. Here this frequency was taken as the attenuation by -3dB relative to the lowest frequency measured for each measurement. The frequency fluctuates across a few KHz up to 10KHz, for extreme stress, while the given bandwidth is derived from the logarithmic sweep, and is approximately 50Hz. The impedance magnitude and phase at the (-3dB) frequency, as a function of time, are presented in Figure 7.

It can be seen that the impedance magnitude shows daily fluctuations, and sensitivity to the induced water stress. Mean values with deviations of the impedance magnitude can be estimated at 3.5$\pm$0.5KOhms for controlled conditions. During drought these values increase substantially reaching 5.5KOhms, showing a change of over 50% in value. Examining the phase data, we are able to see that the phase values change minimally, as expected of the system response at the knee frequency. Furthermore, the phase values at the – 3dB points are around -20 to -25^0. Expecting a transition between effective time constants and the relation between resistive and capacitive components at the knee frequency, should have been near -45^0 [34]–[37]. The lower measured phase value can be interpreted in two ways: (a) It could have been affected by a nearby zero at higher frequency, or (b) the dominant conduction mechanism is dominated by diffusion limited transmission line model where the impedance depends on $1/(j\omega)^{1/2}$. Since no significant zero is observed at higher frequency option (b) seems to be more reasonable.Another notable characteristic in the bode plot, both amplitude and phase, of the impedance is the appearance of a "double hump" in the 10kHz – 1 MHz range. This indicates that the actual conductivity includes at least two possible conducting paths in parallel, maybe three that require a more rigorous model that is shown and discussed in the next section.

Method III- Physically based Lumped element model

Commonly used to analyze a biologically based system using electronics, is the lumped element modelling approach [34]–[36]. An equivalent circuit using the lumped element modelling (Figure 8A) has been suggested for this measurement setup [22]. A lumped element circuit approach, attempts to consider the different physical components in the device under test, and represent each of their contributions across the collection of frequencies used. A range of tools for fitting impedance data exist [38], here Matlab® fitting was used iteratively to fit the experiment data and extract the values for each component in the equivalent circuit across time.

The model parameters graphs across time are shown in Figure 8. In our case, a collection of resistors, constant phase elements and capacitors, have been arranged based on the measurement setup and known plant physiology. The fitting accuracy was estimated to be higher than 95%. The circuit assumes current flow across a collection of channels, due to the plant stem physiology. Each channel is represented by a resistor and a CPE (constant phase element), while the different channels are capacitively coupled to each other. Inspection of each of the elements in such a circuit across time, will provide an indication of their contribution to conduction across the specimen, and the sensitivity of the system to change. In our case, this allows assessing significance of different components and estimating the system response to induced physiological stress. The presented parameters of the lumped element circuit are grouped by type in each graph, i.e. resistive, capacitive and CPEs.

Inspecting the different parameters, we may notice different behaviors. The different resistive components all show a change due to the stress. In addition, across the presented control period, they show slight fluctuation. These changes

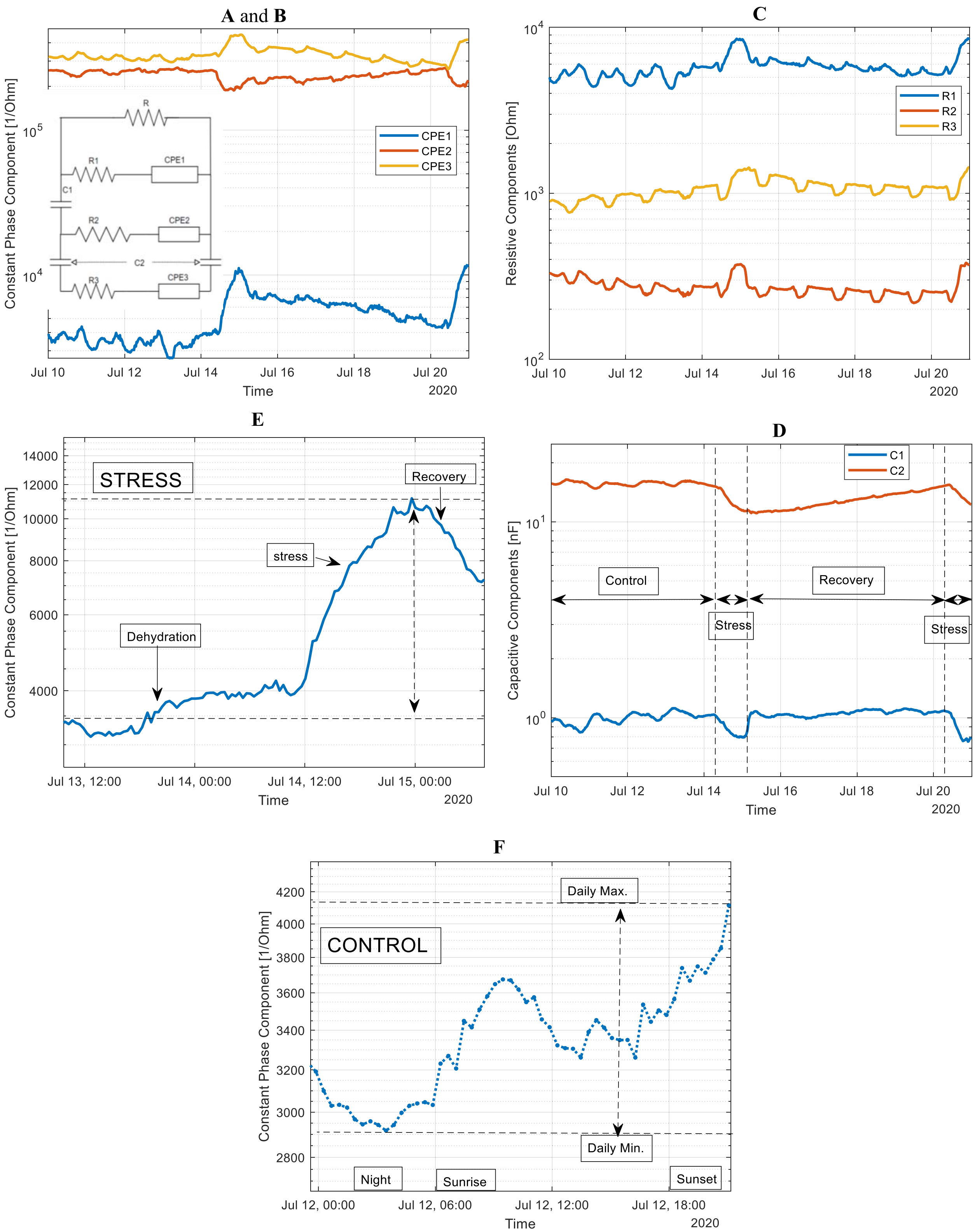

Fig. 8. Lumped element circuit parameters across time. **A:** the insert: The plan physically based lumped electrical model, **B:** the constant phase elements (CPE1, 2 &3), **C:** Resistive components, **D:** Capacitive elements vs. time, over 10 days. **E&F**: CPE1 of the plant as a function of time for normal conditions (e.g. daily watering) or under a draught induced stress (E).

may in the future be correlated to daily changes within the plant. Yet all resistive components behave similarly.

Looking into the capacitive and CPE components, we find that all parameters show an absolute value change due to the water stress introduced. Yet the direction of the change differs. This may be an indication of the actual physical mechanism that the parameter represents within the plant.

The most significant change that we observe using this method seemingly occurs in the first CPE component (CPE1). The daily fluctuations are enhanced, while the response to stress (observed as a peak both in the middle and at the end), express an increase by order of magnitude. Furthermore, it clearly can be seen that after the stress, during recovery the baseline of values is shifted.

In a modelling of a system as in the plant stem, a constant phase element represents transport and diffusion within the biological specimen and can be expected to show higher sensitivity to a physiological change such as the water stress tested here. Comparing the change observed across the resistive elements, it is can be assumed that they are more responsive to changes in ion concentration and therefore indicate smaller deviations.

A better understanding of these changes requires a combined study with known plant physiology measurement methods.

In addition, these qualitative changes shown indicate the added value of the presented continuous impedance measurement method, as it allows for data sensitivity to more than a single change across the plant. Although the lumped element approach with different parameters requires further study of the relations between the parameters and their physical meaning. The ability to detect variations across the different components is clearly apparent.

IV. Discussion

The in-vivo four-point method presented shows that the impedance measurements are sensitive across frequencies to physiological plant changes, e.g. water stress (as shown in previous publications [18]-[22]). There is a question regarding the application and analysis of the acquired data for actual system field realization. We have presented three system orientated approaches for extracting information regarding the monitored changes in the plant. We can see (method I) that across the frequencies, collecting single frequency data is a possibility. Yet we cannot be certain of the accuracy and signal to noise ratios that a field system may encounter.A clear evaluation of response to water stress can be detected using solely the lower frequencies. A single frequency measurement is not advisable, yet a narrow bandwidth including frequencies around 100KHz, where sensitivity is apparent across the phase too, may be possible. Using a single frequency will not provide a reliability or "sanity check" across the measurement period, and would not be advisable as a single stand-alone monitoring tool.

The second suggestion (method II), relies on the overall system response, under the assumption that across the measured frequency range, the plant stem behaves in a linear manner [25]. Here, changes monitored are expressed in a frequency shift, as well as change in impedance values. The overall spectrum data is still required. The phase data will provide a measurement accuracy and reliability factor, while the changes in impedance magnitude can be monitored for evaluation of the plant physiological status.This provides a more reliable and accurate estimate of the system situation, and allows higher quality measurements of the overall plant status.

The last method presented, using a lumped element model, yields high accuracy, and enhanced sensitivity to changes resulting from different areas within the plant stem. As this is an analysis across the measured spectrum, the measurement is an indication of the different interactions between the model elements, and can be linked to biological phenomena within the plant stem in the future. Available plant models using the lumped element modelling (as shown in [11]–[15]) have been used to characterize single data rather than continuous monitoring of the plant fluctuations. Therefore the study of the each element across time is not addressed. Here we have shown that examining the change in each element across time could be used as a monitor of change in plant status. Further studies combined with plant physiological research are needed. The work presented will allow reduction of the acquired data as well as offer an excellent option for better understanding of inner plant electrical conductivity as an expression of different stress phenomena. It offers a broader evaluation of the change across the spectrum and further study will allow determination of the different physiological attributes that each parameter signifies. Analysis of the spectrum data in this manner, is expected to provide a high accuracy measurement of the overall plant health status at any given time point, rather than a vague estimation based on single value.

This may be a costly approach, as together with the hardware for the full spectrum measurement computing the results will demand extra expenses. Each data point in the experiment decided in this paper, included about 800 impedance datapoints where the measurement was repeated every 20 minutes, yielding a very large database. It is anticipated that using four-point impedance spectroscopy will generate a larger data base than simple single frequency impedance data, which is available in some mobile equipment already used. However, it is clear that the four-point impedance spectroscopy described here, provides significantly more information about plant response to a dehydration step. In general, any phenomena that will affect the conducting and dielectric properties of the plant will be detectable using the impedance spectroscopy methods. The main hypothesis is that plant electrical properties depend on the electrolyte concentration in the conducting channels, as well as the dimensions of those channels, their absolute number and the interaction between the channels and the impedance. However, a single frequency impedance data is very limited. Therefore, we propose to use a multi-frequency impedance data and analyze it using different methods. Due to the large data base generated in this method, the data representation should be taken care of before applying the algorithms for the data interpretation. Applying one of the presented methods will allow further reduction of the amount of data for monitoring alongside improved understanding of the data. This will form a basis to apply new available techniques such as machine learning, deep learning and furthermore.

The significance of the four point in-vivo impedance spectroscopy measurement shown, using the different system analysis methods incorporates the direct monitoring of change within the plant, using well established electrical measurement methods for analysis of overall plant condition. Throughout the methods shown, we are also able to observe sensitivity to daily fluctuations due to changes in the measured values. These fluctuations require further study, in order to estimate their significance in detection of plant physiological transitions. Such a study may be conducted alongside well-established plant physiology measurement methods, such as the described gravimetric method [29]–[33]. This will in the future provide further insights regarding the relations between the different methods and parameters. These topics require further research. However, the feasibility of a system approach to data interpretation has been presented, showing that all the impedance data analysis practices allow detection of water stress situations.

V. Conclusion

In this paper, we demonstrate the applicability of the newly suggested in-vivo four-point impedance spectroscopy for plant monitoring. To test this, a special experiment was devised, where tobacco plants planted in sand in a special gravimetry setup were monitored continuously under predetermined hydration/dehydration cycles and step-functions. It is shown that a continuous monitoring is possible where the impedance is measured over a long time and the spectrum is affected by the external excitations applied to the plant. Time analysis of the data has been applied, indicating a significant change due to the external conditions well above the noise level of the system. The validity of changes detected using electrical impedance measurements has been confirmed using a gravimetric measurement of plant status in parallel. The comparison to the well-established gravimetric method for plant monitoring is shown, indicating that the plant impedance response is indeed a representation of the plant's response to an induced stress factor.

The four-point impedance data collected has been analyzed using three different system analysis methods under control and stress situations. Analysis per single predetermined frequencies was presented, the system response at the -3dB frequency, and a lumped elements approach. All methods indicate that changes in plant status can be monitored using the suggested electrical method. The feasibility of analysis using the three suggested methods shows the versatility and possibilities for deploying a field system. In order to deploy this method into a field sensing device, a choice of frequency range and data analysis is required. This paper presents a few suggestions to pave the way towards a solution. This method can be implemented by a very simple field deployable setup. Note, that although the database is large, typically in the range of 10^5 to 10^6 values for a 2-3 weeks monitoring time, it still requires a significant small storage (few tens of megabytes). Since the analysis is rather straightforward and as there is no need for very fast computation, the data processing is rather simple and can be performed by a very simple low-cost and low power microprocessor. A field deployable system, equipped with a communication capability, will allow farmers a simple way of monitoring any effect that affects the electrical properties of plants. Here hydration/dehydration states monitoring is demonstrated. It can however be extended to a pathological event affecting the conducting channels in the plant and/or the electrolyte concentration in those channels.

The presented analysis methods will allow future design of a device with a range of sensitivities providing systems at different cost, which will be adaptable for the monitoring of different crops. For example, trees monitoring may be completed with more expensive equipment, while small perennial plants would require cheaper devices. We have shown that there are different analysis options for the presented impedance measurement, while all indicate a response to plant stress, use of the method needs to be tailored to the plant type, hardware and cost limitations.

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