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# Electrophysiological assessment of water stress in fruit-bearing woody plants

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ABSTRACT

Development and evaluation of a real-time plant water stress sensor, based on the electrophysiological behavior of fruit-bearing woody plants is presented. Continuous electric potentials are measured in tree trunks for different irrigation schedules, inducing variable water stress conditions; results are discussed in relation to soil water content and micro-atmospheric evaporative demand, determined continuously by conventional sensors, correlating this information with tree electric potential measurements.

Systematic and differentiable patterns of electric potentials for water-stressed and no-stressed trees in 2 fruit species are presented. Early detection and recovery dynamics of water stress conditions can also be monitored with these electrophysiology sensors, which enable continuous and non-destructive measurements for efficient irrigation scheduling throughout the year.

The experiment is developed under controlled conditions, in Faraday cages located at a greenhouse area, both in *Persea americana* and *Prunus domestica* plants. Soil moisture evolution is controlled using capacitance sensors and solar radiation, temperature, relative humidity, wind intensity and direction are continuously registered with accurate weather sensors, in a micro-agrometeorological automatic station located at the experimental site.

The electrophysiological sensor has two stainless steel electrodes (measuring/reference), inserted on the stem; a high precision Keithley 2701 digital multimeter is used to measure plant electrical signals; an algorithm written in MatLab®, allows correlating the signal to environmental variables. An electric cyclic behavior is observed (circadian cycle) in the experimental plants. For non-irrigated plants, the electrical signal shows a time positive slope and then, a negative slope after restarting irrigation throughout a rather extended recovery process, before reaching a stable electrical signal with zero slope. Well-watered plants presented a continuous signal with daily maximum and a minimum EP of similar magnitude in time, with zero slope. This plant electrical behavior is proposed for the development of a sensor measuring real-time plant water status.

## Introduction

Plant bioelectric activity in response to different stimuli was originally reported by Burdon-Sanderson (1872) and Darwin (1896). Plants have developed several paths for electric signal transmission between individual cells, tissues and organs, in order to adapt and optimize its physiological processes to sudden changes in environmental stress conditions (Fromm, 2006; Mancuso and Mugnai, 2006; Fromm and Lautner, 2007; Wang et al., 2009; Yan et al., 2009; Sukhov et al., 2011; Gurovich, 2012; Volkov, 2012).

Sibaoka (1966), working with *Mimosa pudica*, reported the onset of electric activity, equivalent to an action potential (AP), and Pickard (1973), experimenting with higher plants, described similar results. Different studies have been published on electric signaling in plant cells and tissues, resulting from biotic and abiotic stimuli, resembling an electrical replication of the specific stimulation (Fromm, 2006). AP is generated in plants in response to a stimulus larger than a certain threshold, in the form of an *all or nothing* response, as documented by several authors (Datta and Palit, 2004; Lautner et al., 2005; Gil et al., 2008; Volkov et al., 2012; Wang et al., 2009; Oyarce and Gurovich, 2010). Also, variation potentials (VPs) have been reported in plants, characterized as an electric response to stimuli smaller that the specific AP threshold, being

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*Abbreviations:* AP, action potential (mV); EP, electric potential (mV); SD, standard deviation; VP, variation potential (mV); $\theta$, volumetric soil water content (%).

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its magnitude a function of its intensity (Yan et al., 2009). It has been suggested that VP transmission rates are related to xylematic turgor conditions and VP is also known as "short wave potential" (Stahlberg and Cosgrove, 1995; Gensler and Yan, 1998; Stahlberg et al., 2005).

Electrical signal generation and transmission in plants has been documented in relation to modifications in light intensity (Volkov and Haack, 1995; Trebacz et al., 2006; Cabral et al., 2011; Volkov et al., 2012), osmotic pressure (Schroeder and Hedrich, 1989), temperature (Volkov et al., 2007; Wang et al., 2009; Cabral et al., 2011; Kai et al., 2011), mechanical wounds and insect activity (Brenner et al., 2006), changes in cytoplasmic $Ca^{2+}$ concentrations (Knight et al., 1991; Fromm and Spanswick, 1993; Shepherd, 2005), changes in respiration, photosynthesis and phototropism (blue light) (Lautner et al., 2005; Pavlovic and Mancuso, 2011), flower induction (Parimalan et al., 2005; Wagner et al., 2006,2012), defense mechanisms to herbivore damages (Volkov and Ranatunga, 2006; Fromm and Lautner, 2007; Heil and Ton, 2008), mechanical stimulation (Braam, 2005; Volkov and Ranatunga, 2006) and soil water availability (Fromm and Fei, 1998; Brenner et al., 2006; Gurovich and Hermosilla, 2009; Oyarce and Gurovich, 2010).

Several reviews related to plant electric responses to different stimuli have been published by Fromm and Lautner (2007), Baluška and Mancuso (2009) and Gurovich (2012). Electric signals can be transmitted between plant organs and tissues because changes in the trans - membrane potential induced by a specific stimulus create a depolarization wave, propagating through plasmatic membranes of excitable adjacent cells (Volkov and Ranatunga, 2006), enabling plants to generate and coordinate physiological activity at significant distances (Lautner et al., 2005). Transmission rates up to 40 m $s^{-1}$ for AP have been reported by Volkov and Brown (2004), Oyarce and Gurovich (2010). For VP transmission, rate ranges between 0.1 and 10 mm $s^{-1}$ have been measured (Stahlberg and Cosgrove, 1995, 1997; Koziolek et al., 2003; Stahlberg et al., 2005).

Electric signal transmission in plants takes place mainly through cellular connections in the symplastic phloematic continuum (Bose, 1926; Cosgrove and Hedrich, 1991; Fromm and Fei, 1998; Lautner et al., 2005; Van Bel and Ehlers, 2005). Essentially, it is an electrochemically activated communication system, operating as a simple neuronal net (Mancuso, 1999; Volkov et al., 2007; Zimmermann et al., 2009).

Evidences of electric signal transmission between distant plant organs have been documented by Fromm and Fei (1998), reporting electric potential (EP) rates of 1 cm $s^{-1}$, which induced stomata closing before a reduction of leaf turgor could be measured in *Zea mays* plants under water stress. Also, Grams et al. (2007) reported EP differences of 50 mV and electric signal transmission rates of 1 cm $s^{-1}$, associated to an increment in stomata conductance and $CO_2$ absorption, which starts 60 s after rehydration. These results indicate that signals different to hydraulic (Mullendore et al., 2010) or hormone signaling (Rodríguez-Gamir et al., 2011), must be present as an information pathway between plant organs, to account for these fast physiological responses to changes in water availability, defined as a dynamic *steady state* of the soil water flow to roots and the evapo-transpirative leaf water flow to the atmosphere (Koziolek et al., 2003; Fromm, 2006; Gibert et al., 2003).

Most studies on EP in plants have been carried on under laboratory or greenhouse controlled conditions, mainly in succulent plants (Mwesigwa et al., 2000; Volkov et al., 2007, 2009, 2012). In woody species, like *Vitis vinífera*, Mancuso (1999), measured significant EP changes as a result of leaf and shoot flaming injury and mechanical wounds, reporting also a detection of the resulting electric signal in distant plant organs, a short time after the injury event. Gibert et al. (2006) reported a significant correlation between EP and sap flow in *Populus trichocarpa* trees, concluding that the electric signals measured are propagated through the phloem, at short or long distances, modifying photosynthetic rates. Systematic electric signaling in fruit-bearing woody species, induced by alternate light/dark cycles and soil water deficits, have been reported by Gil et al. (2008, 2009), Gurovich and Hermosilla (2009), Oyarce and Gurovich (2011).

This research presents evidence on systematic EP variations in *Prunus domestica* and *Persea americana* trees for alternate cycles of full irrigation and water restriction periods, in association with alternate micro environmental conditions (soil water content depletion and evapotranspiration rates). Electrophysiological sensors developed for this research enable real-time EP measurements, to be used as a non-destructive and continuously agronomic tool for irrigation scheduling in fruit-bearing woody species, as an alternative to discrete xylematic pressure measurements or other soil and plant stress assessment techniques available, which are time consuming and highly dependent on the operator's skill, to accurately represent the actual plant water status.

## Materials and methods

### Experimental setup and plant material

The experiment was located at the greenhouse area adjacent to the School of Agriculture and Forestry, Pontificia Universidad Catolica de Chile in Santiago, Chile. Two independent Faraday cages are isolated from environmental electromagnetic fields, using a tested grounded connection. In each cage, ten 3-years-old avocado plants (*Persea americana* Mill., cv. Hass, grafted on *Mexicola* rootstock) and plum (*Prunus domestica* L., cv. D'Agen, grafted on *Mariana 2624* rootstock), are grown in individual 20 L containers in easily drainable 50% peat moss/50% perlite (*Prunus domestica* L.) and 100% perlite (*Persea americana* Mill.) substrates respectively, enabling to create water deficit conditions in short periods of time. An independent, programmable irrigation system is available on each Faraday cage, to create differentially controlled water supply treatments, in order to evaluate plant EP responses to alternated water availability conditions.

### Electric connections in plants

Two electrodes are inserted on each lignified trunk at 20 cm (reference electrode) and 30 cm (measuring electrode) above the graft (Fig. 1), according to our previous experimental results (Gurovich and Hermosilla, 2009). Each electrode consists of a solid stainless steel 304 SS type 316 rod, 3.18 cm long and 0.2 cm diameter, connected to a 0.5 mm cooper conductor, shielded from air relative humidity fluctuations by a flexible self-adhesive plastic ribbon.

Each electric circuit is connected to an amplifier–multi voltmeter (Keithley, model 2701), provided with two 20 channel multiplexer plates model 7700 (Fig. 2), with the positive terminal for the measurement electrode and the negative terminal for the reference electrode, on its 'high' (H) y 'low' (L) insertion points, respectively. Registration of EP measurements, using the EXCELinx® utility software, is made every 10 s and each measurement lasts 0.1 ms. EP information registered is analyzed in graphic format using a MatLab® algorithm, developed for this research.

Simultaneously to EP measurements, electric capacitance sensors (Decagon model 10HS), located within each plant container peat moss/perlite or perlite substrate, enables real-time volumetric soil water measurements ($\theta$, %); also, micro-environmental parameters are registered using sensors integrated into an automatic meteorological station (radiometer, hygrometer, thermometer, wind speed and direction sensors), located between the two