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ELECTRICAL SIGNAL MEASUREMENTS AS A TOOL FOR MONITORING RESPONSES OF AVOCADO (*Persea americana* Mill) TREES TO SOIL WATER CONTENT

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Monitoring soil water content together with plant-monitoring techniques have proved to be very good management tools for making irrigation decisions in avocado orchards. There are many well-tested devices for monitoring soil moisture content in orchards, but options for measuring plant water status are limited. The objective of this study was to determine if measuring variations in electrical voltage differences between roots and leaves can be used as a plant-monitoring technique related to soil water content. Root and shoot voltages differences were monitored in two-year-old 'Hass' avocado trees grafted onto Duke 7 rootstocks in a laboratory. Root and shoot voltages differences were initially measured for about 2 hours in unaltered trees to determine steady state (control) conditions. Plants were then exposed to cycles of soil (root) drying and re-watering. The extracellular electrical potential difference between the base of the trunk and the leaf petiole was continuously monitored after exposure to soil drying or re-watering. Results indicated that a change in soil water content induced by root drying and re-watering was accompanied by a slow significant change in the electrical signal at the leaf petiole which was greatest after 52 and 32 minutes for root drying and re-watering, respectively. Measurements in girdled plants suggest that the electrical signal is propagated in the xylem. Therefore, it is possible to use electrical voltage differences between roots and shoots as a plant-monitoring technique to relate physiological responses of avocado trees to soil moisture content.

Key words: Phytomonitoring, electrical signal, soil water content

MEDICIÓN DE SEÑALES ELÉCTRICAS COMO HERRAMIENTA DE MONITOREO DE RESPUESTAS DEL PALTO (*Persea americana* Mill.) ANTE EL CONTENIDO DE AGUA EN EL SUELO.

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El monitoreo del contenido hídrico del suelo junto con técnicas de fitomonitoreo han demostrado ser herramientas útiles para tomar decisiones de riego en huertos de palto. Existen muchos sensores para medir contenido de agua del suelo, pero opciones para la medición del estatus hídrico de la planta son limitadas. El objetivo de este estudio es determinar si la medición de la variación de voltaje entre raíces y hojas se puede utilizar como técnica de fitomonitoreo relacionada con el contenido en agua del suelo. La diferencia de voltaje entre raíces y hojas fue registrada en paltos Hass de 2 años injertados en patrón Duke 7, bajo condiciones de laboratorio. Se midieron diferencias de voltaje inicialmente por cerca de 2 horas en árboles sin alteraciones para determinar condiciones basales (control). Las plantas luego fueron expuestas a ciclos de desecamiento y rehidratación del suelo. La diferencia de potencial eléctrico extracelular entre la base del tronco y el pecíolo de una hoja fue registrada continuamente después de desecamiento y rehidratación. Los resultados indican que cambios en el contenido en agua del suelo son acompañados por un cambio lento pero significativo en la señal eléctrica medida en el pecíolo de la hoja, el cual es mayor luego de 52 y 32 minutos para desecamiento y rehidratación del suelo respectivamente. Mediciones realizadas en plantas anilladas sugieren que la señal eléctrica es propagada en el xilema. Existe entonces la posibilidad de utilizar diferencias de voltaje entre raíces y brotes como técnica de fitomonitoreo en plantas de palto

Palabras claves: fitomonitoreo, potencial eléctrico, contenido de agua en el suelo.

1. Introduction.

Avocado (*Persea americana* Mill.) is very sensitive to drought and waterlogging. Some reasons for the relatively low productivity of this species may be related to the water status of the crop, which at times is under irrigated causing water stress and at other times is over irrigated resulting in root asphyxiation. In Chile, root asphyxiation is an increasing concern to avocado growers because trees are very sensitive to lack of soil oxygen and commercial production has expanded to areas with poorly drained soils that are low in oxygen.

Proper irrigation management in avocado orchards is necessary to insure adequate yield and fruit quality (Lahav and Whiley, 2002). An excess or lack of water during growth limits fruit production and quality, particularly if stress occurs between Spring and the beginning of Summer (Whiley et al., 1988a; 1988b).

Monitoring soil water content, coupled with phytomonitoring techniques have been shown to be very good management tools for making irrigation decisions in avocado orchards. There are many well-tested devices for monitoring soil moisture content in fruit tree orchards, but options for measuring plant water status are more limited. Phytomonitoring techniques have been tested with varying degrees of success for irrigation scheduling in avocado orchards. These have included measuring leaf thickness, leaf water potential, sap flow velocity and diurnal differences in trunk or fruit diameter (Lahav and Whiley, 2002). Measuring diurnal differences in trunk and fruit diameter, known as dendrometry, as an indicator of plant water status has been used recently in Chile for fine tuning irrigation scheduling in avocado orchards (Gurovich et al., 2006). Real-time, continuous dendrometric measurements of tree water status indicated that avocado trees respond very rapidly to fluctuations in soil water content. Dendrometry has been a useful technique for fine tuning irrigation scheduling and can help to improve fruit yield and size in avocado orchards. However, this technique is still very expensive and installing a sufficient number of dendrometers to provide an adequate number of replications in an orchard can be costly. Thus, there is a need to explore alternative methods for real-time, continuous phytomonitoring of plant water status related to soil moisture content in avocado orchards.

The presence of fast conducting signals generated in the roots and conducted through the vascular system to the leaf has been studied in several annual plant species. Stimulation of roots in *Salix viminalis* by the application of nutrients, hormones or changes in the pH, caused changes in electric potential difference between the roots and the leaves. These changes were followed by a modification of leaf respiration and photosynthetic rates within three minutes after treatments were applied, indicating that the changes in the electrical signals might reflect or be a direct mechanism of communication between the roots and the leaves (Fromm and Eschrich, 1993; Mishra et al, 2001). Similarly, osmotic stress suddenly applied to sunflower roots generated an electric potential difference between the roots and the leaves that accompanied decreases in stomatal conductance (Mishra et al., 2001). It has been postulated that electric signals could be a communication pathway between roots and shoots under water stress (Fromm and Fei, 1998). Thus, stomatal response to soil water content may be triggered by an electrical voltage differential between the roots and the leaves that can be easily measured via electrodes connected to an amplifier. This opens the possibility for developing a new phytomonitoring technique for measuring plant response to soil water content based on measuring voltage differences between roots and leaves.

The objective of this study was to determine if changes in voltage between roots and leaves of avocado trees can be measured and related to changes in soil water content.

2. Materials and Methods

This study was part of a more in-depth study to investigate root to leaf electrical signaling in avocado in response to various environmental stimuli (Gil et al., 2007). Thus, the techniques used in this study were the same as those used in that more in-depth study.

Plant material.

Two-year-old 'Hass' avocado trees on clonal Duke 7 rootstock were used in this study. The plants were grown in a commercial nursery in a medium composed of peat, pearlite, compost and sand and fertilized according to standard nursery practices. The plants ranged in height from 1.2 to 1.4 m with a variable number of leaves (22 - 45) per plant.

Experimental design.

Control.

In a laboratory, electric voltage signals were measured for about 80 minutes in eight avocado plants (replications) under stable environmental conditions to determine voltage differences between the base of the stem and the leaf petiole (ΔV_{L-S}) in the absence of environmental alterations (control or baseline).

Treatments: root drying and root wetting.

The same eight plants were exposed to the following treatments: 1) desiccation of the roots by exposure of the root system to a directed air current at ambient temperature (20ºC) for about 80 minutes, and 2) re-wetting of desiccated plant roots by adding 500 cc of distilled water to the soil and monitoring the ΔV_{L-S} response for 120 minutes. The ΔV_{L-S} was continuously recorded during each of the experiments.

Air temperature during the measurement period was between 22.5 and 23.3ºC and leaf temperature ranged from 22.4 to 23.7ºC. The photosynthetic photon flux (PPF) (Quantum sensor QSS-01 light meter, Lehle Seeds, Round Rock, Texas, USA) directly above the abaxial leaf surface was about 85 µmol photons $m^{-2} s^{-1}$, which is above the light compensation point of this species which is 30 µmol photons $m^{-2} s^{-1}$ (Whiley, 1994).

Measurements

Measurement of voltage.

Surface contact electrodes were placed on the stem 20 cm above the soil surface and in the petiole of a leaf located in the lower third of the plant canopy (Fromm and Fei, 1998). The electrodes consisted of a thick cotton thread absorbed with KCl 0.1 M dipped in a 2.0 mL Eppendorf tube containing KCl 0.1 M. Ag/AgCl electrodes (0.4 mm of diameter) were immersed in the Eppendorf tubes and were connected to an amplifier (M-707 Microprobe System; World Precision Instruments, Sarasota, Florida, USA), and the output was recorded with a Powerlab analog digital acquisition system (AD Instruments, Castle Hill, Australia). Prior to plant measurements, the electrodes were placed in KCl 0.1 M to compensate the junction potential. To record $\Delta V_{L\text{-}S}$ the electrode located on the leaf petiole acted as the recording electrode while the electrode located on the stem served as the reference.

Measurement of stomatal conductance.

Stomatal conductance (gs) was measured with a steady state porometer (Li-Cor 1600, Lincoln, Nebraska, USA) as described by Prive and Janes (2003) and Raviv et al. (2001). Stomatal conductance was measured on the same leaf on which the electrode was placed (Fig. 1). Stomatal conductance was measured on each of the 8 replications before and after the voltage was recorded during each treatment (root drying and root wetting).

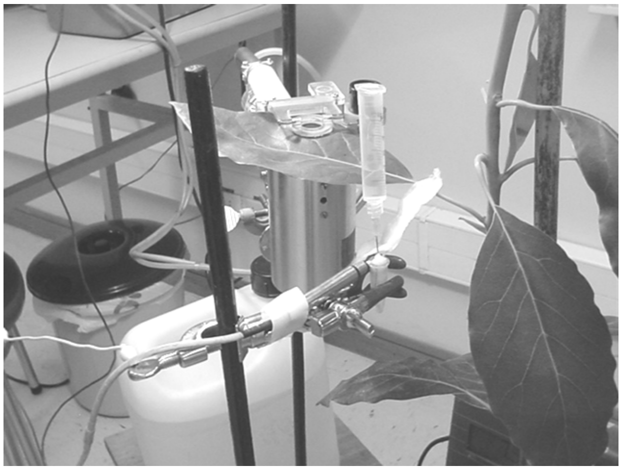

Figure 1: Measurement of stomatal conductance and voltage in an avocado leaf.

Measurement of soil moisture.

Plants in each treatment were placed on a digital balance (Mettler Toledo, Hispanic Precision, Model Wildcat, Columbus, Ohio, USA), and the total weight of the plant, soil and pot was determined before and after each treatment. Plants were then excised at the soil surface, and pots and soil were reweighed. Gravimetric soil water content (ω) was then determined with the formula:

$$\omega = ((\text{wet soil weight} - \text{dry soil weight}) / \text{dry soil weight})*100$$

Data Analysis

The effect of treatments on the Maximum Different Voltage – Initial Voltage (Voltage Differences) was analyzed by one-way analysis of variance (ANOVA) and Tukey´s studentized ($P \leq 0.05$). Effects of changes in ΔV_{L-S} on Δgs were analyzed by linear regression analysis. Data were analyzed using the SAS statistical package (SAS Institute, Cary, North Carolina, USA).

3. Results and Discussion

Control plants

Although, plants showed different initial ΔV_{L-S} values, they remained fairly constant and there was little modification in ΔV_{L-S} in control plants kept in stable environmental conditions for about 80 minutes (Fig. 2).

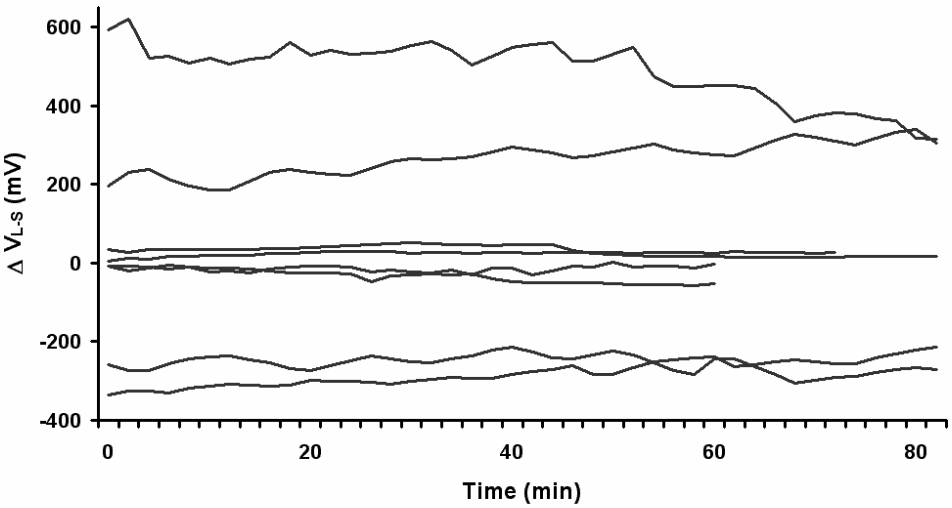

Figure 2: Voltage difference between the leaf petiole and the base of the stem (ΔV_{L-S}) in 8 control plants within 80 minutes.

Plants subjected to root desiccation and rewatering.

The root desiccation treatment produced a statistically significant change in ΔV_{L-S} (Voltage Difference) compared to the control. Drying the soil to -1.3% of field capacity (1/3 atm) resulted in a significant reduction ($P \leq 0.05$) of ΔV_{L-S} (-214.3 mV) after 52 minutes. In contrast, adding water to the dry soil resulted in an increase of 85.3 mV in ΔV_{L-S} after 32 minutes of re-watering, but this value was not significantly different ($P > 0.05$) from the control one (Table 1 and Fig. 3). Both treatments produced changes in the stomatal conductance (Table 2), which was often directly related to stomatal opening and closing (Cowan, 1972, Tinoco-Ojanguren and Pearcy, 1992, Buckley et al., 2003). However, there was just a slight correlation ($R=-0.56$, $P< 0.05$) between change of ΔV_{L-S} and stomatal conductance difference measured before and after the soil drying treatment (Data not shown).

Table 1: Effect of soil drying and soil wetting on the root to leaf voltage difference in avocado plants.

Treatments	Initial Voltage (mV)	Maximal different Voltage (mV)	Time to maximal voltage difference (min)	Voltage Difference (mV)
Control	27.3	21.1	58.0	**-6.2 (a)**
Drying	436.8	222.5	52.0	**-214.3 (b)**
Wetting	77.4	162.6	32.0	**85.3 (a)**

Each value of voltage difference represents the mean (n = 8 plants). Maximal $\Delta V_{L\text{-}S}$ is related to the initial value of the voltage curve for each replication within 80 minutes. Different letters indicate significant differences ($P \leq 0.05$) among treatments (one-way ANOVA and Tukey´s studentized range test).

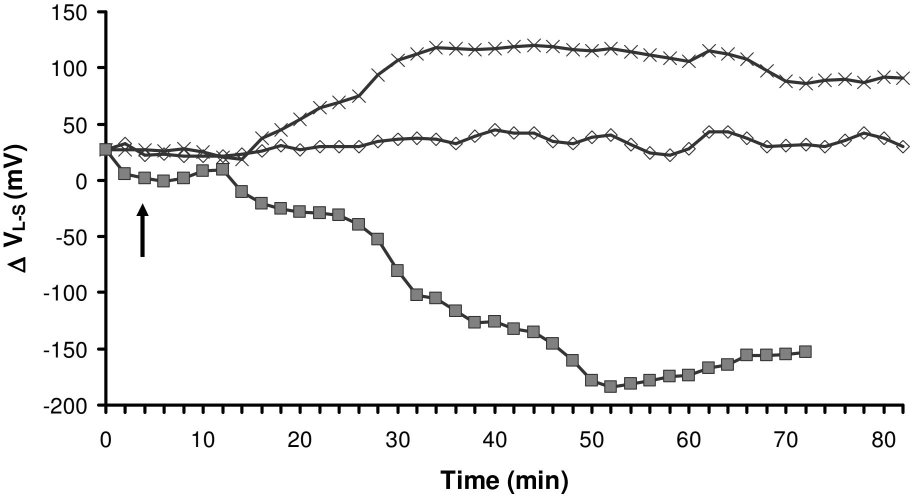

Figure 3: Effects of different treatments on $\Delta V_{L\text{-}S}$ (mV). Each data point represents the mean value (n= 8). —◇— Control, —×— Wet, —■— Dry. The arrow indicates the beginning of each treatment.

Table 2: Stomatal conductance difference after soil drying and re-wetting treatments were imposed.

Treatments	Δgs (cm s^{-1})
Soil Drying	-0.021 ± 0.03
Soil Wetting	0.017 ± 0.005

Both treatments had an effect on stomatal conductance. Positive numbers for the soil wetting treatment indicate stomatal opening, whereas negative numbers for the soil drying treatment indicated stomatal closure. Values represent means ± SE (n = 8).

Environmental changes generate bioelectric potentials in plants (Datta and Palit, 2004). In this study with *Persea americana* Mill., the shape, magnitude and duration of $\Delta V_{L\text{-}S}$ was dependent on the stimulus. In control plants that received no sudden environmental stimulation, changes in $\Delta V_{L\text{-}S}$ were small and non-significant. However, drying the roots resulted in a large and significant decrease in $\Delta V_{L\text{-}S}$ after a slight decrease in gravimetric soil water content (1.3%). Similar changes in electrical signals were reported by Fromm and Fei (1998). Soil drying resulted in a change in $\Delta V_{L\text{-}S}$ that was observed within 28 to 56 minutes after forced-air soil drying was initiated, with an average change in $\Delta V_{L\text{-}S}$ of -214.3 mV, which corresponds to a 96.3% variation of the initial value. Thus, as a result of plant stress induced by decreased soil water content, there was a significant modification of the variation potentials or slow wave potentials which appeared to be transmitted at a speed of 2.4 cm min^{-1} or 144 cm h^{-1}. The modification of $\Delta V_{L\text{-}S}$ was positively correlated with a reduced stomatal conductance, suggesting that there may be a cause and effect relationship between these two processes.

The results reported here demonstrate the existence of an electrical potential difference between avocado roots and shoots that can be readily measured and is correlated with soil moisture content. The response to soil desiccation was a decrease in voltage, whereas the response to soil wetting was an increase in voltage. Thus, the potential exists to use root to leaf voltage differences as a phytomonitoring technique for measuring plant response to soil water content. It is important to underscore that this study was intended as a “first step” aimed at determining if such a signal exists in avocado and if it could be easily measured. The specific laboratory equipment used for this study is too cumbersome to be practical for field use. Developing a practical phytomonitoring technique based on this concept would require refinement of the instrumentation for field use. Furthermore, substantially more work is needed to relate the magnitude of this signal to soil water content over a wide range of soil moisture contents and varying edaphic and environmental conditions. However, this study does suggest that developing a practical phytomonitoring technique for measuring plant water status based on root to leaf electrical potential difference may be possible.

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