

Chapter 7

Concluding Remarks

The main objectives of this thesis were to determine the physiological responses of avocado (*Persea americana* Mill.) to different soil water-to-air ratios and to elucidate the possible mechanism of root-to-shoot communication in response to water stress or root hypoxia. The proposed specific objectives related with the main aims of this thesis were : 1: to compare the physiological, morphological and growth responses of avocado trees to different soil air conditions, 2: to determine the effect of hypoxia on plant water status, leaf gas exchange and biomass of avocado, 3: to determine if the negative effects of low air content in soil on physiology, vascular anatomy and growth of avocado trees can be mitigated by the injection of hydrogen peroxide into the soil, and 4: to determine the possible physiological mechanisms involved in root-to-leaf signal transmission that triggers stomatal closure in response to soil drying and root hypoxia. To reach these objectives several experiments were conducted outdoors, in a greenhouse and in laboratory conditions where avocado trees were subjected to root zone hypoxia, drought, or different soil water-to-air ratios (W/A) and physiological, biochemical, anatomical and growth responses were assessed.

The water-to-air ratio in soil irrigated at field capacity, non-*Phytophthora*-infested soils affected avocado physiology, growth and productivity. In soils with low water-

to-air ratios (T5 and T4) stomatal conductance (g_s), transpiration (T), stem water potential (SWP), net CO₂ assimilation (A), water use efficiency expressed as total plant dry matter produced in relation to the amount of water applied (WUE_b) and tree growth were higher than in soils with high water-to-air ratios (T2 and T3). During the first season after planting, avocado trees in soils with high soil water-to-air ratios (T1, T2 and T3) had lower g_s , T and growth than trees in soils with low soil water-to-air ratios (T4 and T5), but soil oxygen content was apparently not low enough to severely stress the trees. Trees in T1 apparently acclimated to the high water-to-air ratio during the following season, possibly by an apparent alteration of the vascular anatomy. Thus, physiological responses of trees in soils with a high soil water-to-air ratio improved during the second season, reaching similar values of g_s , A and T than trees under low soil water-to-air ratios (T4 and T5). However, the effect of the first season of low water-to-air ratio in the soil in plants under T1 affected tree growth and thus the final biomass and WUE_b, which were significantly lower than the other treatments.

Physiological and growth responses of two different avocado cultivars grafted onto the same ('Waldin') seedling rootstock differed in response to long-term (several days) waterlogging. 'Hass' avocado (a hybrid of Guatemalan x Mexican avocado races) showed less susceptibility to flooding than 'Beta' (a West Indian avocado race). For 'Hass' there was a significant reduction in g_s and T after 10 days of flooding, a reduction in A after 12 days of flooding, and no differences in SPW during the evaluation period. 'Beta' avocado was more susceptible to flooding than 'Hass', with significant reductions in g_s , T, and A eight days after the flooding

treatment was initiated, whereas SWP was significantly reduced 14 days after flooding commenced. Also, flooding resulted in a greater reduction in biomass of 'Beta' than 'Hass'. Stomatal closure in avocado as a result of flooding was significant when g_s decreased below $50 \text{ mmol m}^{-2} \text{ s}^{-1}$. However, there was no correlation between SWP and the percentage of closed stomata of flooded plants.

Injecting hydrogen peroxide (H_2O_2) into a heavy clay soil with the water content maintained near field capacity and average soil air content of 15.6%, significantly increased the biomass of the aerial portions and water use efficiency (WUE), but had no significant effect on A, T, g_s , or SWP of avocado trees. Xylem vessel diameter and the xylem to phloem ratio tended to be greater for trees in soil injected with H_2O_2 than for trees in the control treatment. Thus, injecting H_2O_2 into the soil through the irrigation system may have potential as a method for improving soil oxygen content in a heavy clay loam soil. However before this method is used to mitigate damage caused by low soil aeration in avocado orchards, further studies are needed to evaluate the practical and economical feasibility of using H_2O_2 on a larger scale in orchards.

The existence of a fast electrical signal conducted from the root to the leaf in response to soil wetting or drying can be measured and correlated with stomatal control. This opens the possibility for developing a new phytomonitoring technique and/or artificially modifying plant responses by imposing agronomic management strategies aimed at rapid stomatal adaptation to changes in soil water content. Changes in voltage between the base of the stem and the leaf petiole (ΔV_{L-S}) may play a role in root to shoot communication when avocado plants are water

stressed. It was determined that the electrical signal in avocado in response to soil drying was transmitted through the phloem. However it is necessary to separate true electrical signaling phenomena from conductivity changes arising from water and/or ion handling by the plant tissue. However, even if the change in electrical potential was generated by a shift in the water or ionic balance in the roots and xylem resulting from soil drying, it does not negate the fact that changes in the extracellular electrical potential may be the mechanism for root to leaf communication resulting in stomatal closure in response to soil drying.

Experiments were conducted to elucidate the mechanism for a root-to-leaf signal in response to short-term (hours) and long-term (days) drought and root hypoxia. Short-term and long-term drought caused an increase in root to leaf electrical potential differences, whereas there was no significant effect of root hypoxia on root-to-leaf electrical signals. There was no effect of short-term drought or root hypoxia on root or leaf ABA concentrations. Long-term root hypoxia resulted in increased ethylene concentration in the leaves and increased leaf abscission. The results indicated that an extra-cellular electrical signal transported from the roots to the leaves in avocado trees may be the root-to-shoot communication pathway eliciting stomatal closure in response to drought stress. For avocado plants exposed to root hypoxia, typically encountered in flooded or poorly drained soils, electrical signals did not appear to be the primary root-to-shoot communication pathway. Also, in avocado trees, ABA did not appear to be involved in rapid signaling for stomatal closure as a result of stress caused by either drought or reduced oxygen concentration in the root zone.

Attachments

1. Abstract of Works presented at Symposium, Congress and Brainstorming

Root-to-leaf electrical signaling in avocado (*Persea americana* Mill.)

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Abstract

Our work using phytomonitoring techniques for Avocado (*Persea americana* Mill) tree irrigation indicates that plant response to changed soil water availability is a very fast process. Root-to-leaf ABA transport or hydraulic processes cannot fully explain the almost immediate stomata physiological response (less than 15 minutes) to either water application and/or sudden ETp increments or reductions. Some studies in Avocado physiology report that root-to-leaf ABA transport is a transpirative mass-flow process. In other tree species a decline in stomatal conductance (gs) was detected when volumetric soil water content declined below

0.12, but the decline in g_s , could not have been mediated by increasing [ABA(xyl)] because stomatal closure appeared to precede any increase in [ABA(xyl)]. Sap flow velocity in the range of 30 to 35 cm/h has been reported in avocado, indicating that hydraulic forces cannot either fully explain the fast stomatal response to soil water availability. This work is aimed to study the eventual existence of an electrical signaling process regulating stomata behavior. Two year old avocado trees were subjected to several drying and re-watering cycles, as well as to modifications on some ETp parameters, as incident radiation and air flux conditions. Extracellular electrical potential was continuously recorded between trunk and leaf petiole; leaf stomata conductance was also registered. Drying the root system with a continuous air flow at room temperature generated the arrival of an electrical signal to the leaf petiole; the same signal was detected when the root system was re-watered. Our results indicate that a sudden change in soil water availability creates a significant electrical signal, which reaches leaf petiole in 10 to 50 min. Other measurement were made on girdled plants, in several cycles, re-watering plants after 4 drying days, indicating that the electrical signal detected is possibly conducted by the xylem, and not by the phloem tissue. The eventual existence of root-to-leaf electric information exchange mechanisms opens interesting possibilities to artificially modify plant response to environmental or agronomic management strategies, aimed to increment water use efficiency.

Work submitted and presented (poster session) at the First Symposium of Plant Neurobiology, Florence, Italy, May 2005.

Electrical signal measurements as a tool for monitoring responses of avocado
trees to soil water content

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Abstract

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 orchards, but options for measuring plant water status are more limited. The objective of this study was to determine if measuring changes in electrical voltage differences between roots and leaves can be used as a phytomonitoring technique to measure plant water status related to soil water content. Root and

shoot voltage differences were monitored in two-year-old 'Hass' avocado trees grafted onto Duke 7 rootstocks in a laboratory. Root and shoot voltage differences were initially measured for about 2 hours 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 phloem. Thus, the possibility exists to use these root to shoot electrical voltage differentials as a phytomonitoring technique to relate physiological responses of avocado trees to soil moisture content.

Key words: Phytomonitoring, electrical signal, soil water content

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Effect of waterlogging on plant water status, leaf gas exchange and biomass of
avocado

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Abstract

Avocado trees (*Persea americana* Mill.) are highly sensitive to soil flooding. An early physiological response of avocado trees to soil flooding is reduced stomatal conductance and net photosynthesis. The objective of this study was to determine the effect of flooding on plant water relations, leaf gas exchange, growth and survival of avocado and to relate stomatal conductance to stomatal closure and stem water potential in response to soil flooding. In August 2006 in Homestead, Florida, USA, one-year-old 'Beta' and 'Hass' grafted onto 'Waldin' seedling rootstocks were either flooded continuously for 11 days or not flooded (control plants). Net CO₂ assimilation (A), stomatal conductance (gs), transpiration (T) and stem water potential (SWP) were measured every two days during the flooding period and periodically after the flooding period. During the flooding period, stomatal impressions were made for plants in each treatment and stomatal closure

was related to leaf gas exchange. 'Hass' avocado showed less susceptibility to flooding than 'Beta', with a significant reduction in g_s and T after 10 days of flooding, a reduction in A after 12 days, and no differences in SWP during the evaluation period. Avocado cv. Beta showed more susceptibility to flooding than 'Hass', with significant reductions in g_s , T and A eight days after treatment initiation, whereas SWP was significantly reduced 14 days after flooding commenced. Also, flooding resulted in a greater reduction in biomass for 'Beta' than 'Hass'. Stomata closed as a result of flooding when g_s values decreased from $50 \text{ mmol m}^{-2} \text{ s}^{-1}$. However, there was no correlation between SWP and the percentage of closed stomata of flooded plants.

Effect of the soil water-to-air ratio on water status, net CO₂ assimilation, biomass
and vascular anatomy of avocado trees

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Abstract

Avocado (*Persea americana* Mill.) is one of the most sensitive tree fruit species to flooded or poorly drained soil conditions. In Chile, avocado orchards are often planted in poorly drained soils that are low in oxygen resulting in tree stress. Understanding the relationship between the water-to-air ratio of different soils and avocado tree physiology, growth and yield should be helpful for irrigation management of this crop. The objective of this study was to evaluate the effect of the water-to-air ratio in five different soils, kept near field capacity, on water status, net CO₂ assimilation, biomass and anatomy of avocado trees. The experiment was conducted during the Spring and Summer seasons of 2005-2006 and 2006-2007, starting with two-year-old 'Hass' avocado trees planted outdoors in containers filled with one of five different soils typically found in Chilean avocado production

regions. At field capacity, the soil water-to-air ratio (W/A) of each soil was 1.7, 1.3, 0.6, 0.4 and 0.3. In addition to determining physical characteristics of each soil, net CO₂ assimilation (A), transpiration (T), stomatal conductance (gs), stem water potential (SWP), shoot and root fresh and dry weights, leaf area, water use efficiency (WUE), root ACC content, leaf ABA content and vascular anatomical characteristics of stems and roots were evaluated for trees in each soil type. Although plants were kept at non-stressing soil air conditions, treatments with lower soil W/A presented higher A, T, gs, WUE and higher SWP. Also, they presented higher biomass, and longer autumn leaf retention. During the first season of measurements, the W/A=1.7 treatment presented the lower values for all the measured parameters, but at the second season this changed to reach a similar performance with the lower soil W/A treatments, possibly due to an anatomical acclimation showed by those plants, where a higher number of root xylem vessels was observed. Another interesting result was that the W/A=1.2 treatment presented the higher flower and fruit production during the second evaluated season. In conclusion, soil water-to-air ratio in well irrigated soils is an important factor that affects plant physiology, biomass and thus productivity of 'Hass' avocado trees.

Key words: soil aeration, stomatal closure, net photosynthesis, root histology, root hypoxia.

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2. Online version of the *in press* original article

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