Chapter 2

Original Article

Effect of the soil water-to-air ratio on water status, net CO_2 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 relate the water-to-air ratios in different soils to water status, leaf gas exchange, biomass and anatomy of avocado trees. Avocado trees were grown in one of five soils each collected from a different area of the Chilean avocado growing region with different physical properties and hence different water to air ratios. Thus, there were five treatments (T1-T5) corresponding to each of the five soils. The experiment was conducted during the spring and summer of 2005-2006 and 2006-2007 starting with two-year-old 'Hass' avocado trees planted outdoors in containers filled with one of the five soil treatments. At field capacity, the two-season average soil water-to-air ratio (W/A) was 1.7, 1.3, 0.6, 0.4 or 0.3 for treatments T1, T2, T3, T4, or T5, respectively. In addition to determining soil physical characteristics and monitoring W/A, net CO_2 assimilation (A), transpiration (T), stomatal conductance (gs), stem water potential (SWP), shoot and root fresh and dry weights, leaf area, water use efficiency [plant biomass per applied water (WUEb)], root ACC content, leaf and xylem ABA content and vascular anatomical characteristics of stems and roots were evaluated for trees in each treatment. Although aerobic soil conditions were maintained in all treatments, trees in soil with lower W/A had higher A, T, gs,

WUEb and SWP than trees in the treatments with higher W/A. Also, trees in treatments with lower W/A had more biomass, and longer autumn leaf retention than trees in treatments with higher W/A. There was no effect of treatment on root ACC content during either season. Leaf xylem ABA content was higher for T1 than the other treatments during the second season, but ABA level did not reflect plant stress. During the first season, the trees in T1 had lower values for all the measured variables, but during the second season there were no differences in plant responses among T1 and T4 and T5, possibly due to an anatomical acclimation by plants in soils with a high W/A, where a large number of root xylem vessels was observed at the end of the second season. Plants in T2 had more flowers and fruit than plants in the other treatments during the second season. The results of this study indicate that the soil water-to-air ratio significantly affects plant physiology, growth and thus productivity of 'Hass' avocado trees.

Abbreviations: gs = stomatal conductance; A = net CO_2 assimilation; ω = gravimetric soil water content; □= volumetric soil water content; ODR = oxygen diffusion rate; BD = bulk density; FC = field capacity; T = transpiration; SWP = soil water potential; ABA= abscisic acid; ACC= 1-aminocyclopropane-1-carboxylic acid.

INTRODUCTION

Chile is the second largest exporter of avocado (*Persea americana* Mill.) fruit in the world, accounting for 18.8 percent of the total world's avocado exports (Schwartz *et al.*, 2007). Commercial avocado production in Chile has expanded to areas with

poorly-drained soils that are low in oxygen. In many of these areas, irrigation management is difficult because new plantations are often placed on slopes of hills. Poorly aerated soils combined with irrigation design and management problems can limit avocado fruit production and quality due to an excess of water in the root zone. Avocado trees are very sensitive to waterlogging (Schaffer *et al.*, 1992; Schaffer and Whiley, 2002; Whiley and Schaffer, 1994) and the relatively low productivity of this species may be related to the water status of the crop, which at times is over irrigated resulting in root asphyxiation. However well-irrigated soils can have different water-to-air ratios which could also influence the productivity of avocado trees, because the relationship between the production potential of avocado trees and soil type is most likely related to the water-to-air ratio in the soil (Shein and Mizury, 1998; Zhou and You, 2005; Ferreyra *et al.*, 2007a; Ferreyra *et al.*, 2007b).

The soil water-to-air ratio is a result of water management as well as the physical properties of the soil. Factors that most affect soil aeration are soil water content, texture and structure. The higher the soil water content, the lower the air volume and therefore the greater the limitation to aerobic metabolism of the roots (Letey, 1961; Blokhina *et al.*, 2003). Fine textured soils have a greater capacity for water retention than coarser textured soils. Therefore, a slight error in the irrigation rate or frequency, due to a lack of understanding of soil properties, may lead to continuous anaerobic conditions in the root zone (Letey, 1961; Blokhina *et al.*, 2003). Previous studies (Ferreyra *et al.*, 2007a) have shown that the soil air content affects avocado water relations. Ferreyra *et al.* (2007a) reported that low

soil air contents (5% to 18%) negatively affect stomatal conductance (gs) in avocado trees. The same authors established that soil air content lower than 17% restricts the oxygen diffusion rate to less than 0.2 µg cm^{-2} min^{-1} and that macroporosity values were correlated with soil O_2 and CO_2 contents.

In heavy clay, compacted, or saturated soils or when subsurface drainage is impeded, an inadequate oxygen concentration in the root zone can negatively affect the biological functioning of plants (Letey, 1961). For avocado trees, root hypoxia or anoxia usually results in reductions in gs, transpiration (T), net CO_2 assimilation (A) and root and shoot growth, inhibition of leaf expansion, moderate to severe stem and leaf wilting, leaf abscission, and root necrosis (Schaffer and Ploetz, 1989; Schaffer *et al.*, 1992; Schaffer, 1998; Schaffer and Whiley, 2002). Moreover, Ploetz and Schaffer (1987, 1989) reported a synergistic relationship between *Phytophthora* root rot and root hypoxia of avocado, resulting in considerably more root damage by both factors together than caused by either stress alone.

Optimum root growth of avocado trees occurs in well-drained soils with O_2 and CO_2 contents at 15% and 0.03%, respectively, whereas root growth is inhibited in poorly aerated soils with 1% O_2 and 16% CO_2 (Menge and Marais, 2000). Roots of avocado cultivars such as 'Scott', 'Duque' and 'Topa Topa' did not grow when the oxygen diffusion rate (ODR) in the soil was lower than 0.2 µg cm^{-2} min^{-1} (Valoras *et al.,* 1964). Similarly, Stolzy *et al.* (1967) reported that when soil ODR was lower than 0.17 µg cm^{-2} min^{-1}, there was 44 to 100% damage to roots of 'Mexicola' avocado trees. An excess or lack of water during growth limits avocado fruit

production and quality, can stimulate alternate fruit bearing, reduce fruit size and limit post-harvest storage life of fruit, particularly if stress occurs between spring and the beginning of summer (Wolstenholme, 1987; Whiley *et al.* 1988a, 1988b).

Root anoxia or hypoxia often results in increased concentrations of ACC (Bradford and Yang, 1980), ethylene and ABA in leaves (Bradford and Yang, 1980; Kozlowski, 1997). Elevated concentrations of ACC and ABA in leaves of flooded plants can accelerate abscission (Kozlowski, 1997). Additionally, an increase in leaf ABA concentration has been implicated as a stimulus for stomatal closure in flooded plants (Kramer and Boyer, 1995; Else *et al.*, 1995; Kozlowski 1997). Ultimately, low soil oxygen content can result in root tissue damage, inhibition of vegetative and reproductive growth, changes in plant anatomy and morphology (i.e., development of hypertrophic stem lenticels, development of adventitious roots, changes in the xylem to phloem or bark relationship, development of root and stem aerenchyma), premature senescence and plant mortality (Schaffer *et al.*, 1992; Drew, 1997; Kozlowski, 1997). Translocation of carbohydrates and macronutrients are also affected by root hypoxia due to metabolism suppression or death of the root system (Kozlowski, 1997; Schaffer and Whiley, 2002).

Although there are several reports of the effects of the flooding on net CO_2 assimilation and water relations of avocado, little is known about the effects of soil water-to-air ratios on physiology, anatomy, growth and yield of avocado trees. An understanding of the relationship between the soil water-to-air ratio and avocado physiology, growth and yield should provide valuable insight for irrigation management of this crop in different soils, particularly in areas with poor soil

aeration. The objective of this study was to evaluate the effects of the water-to-air ratio of five different soils, kept near to field capacity, on plant water status, net CO_2 assimilation, biomass and anatomy of avocado trees.

MATERIALS AND METHODS

Plant material

The experiment was conducted from the spring of year 2005 to the end of the summer of year 2007, beginning with two-year-old 'Hass' avocado trees grafted onto seedling 'Mexícola' avocado rootstock. Trees were planted in one of five different soils in approximately 200-L "containers" constructed by mounding field-collected soil and holding mounds in place with a white plastic mesh sustained by a structure of metal wire.

Climatic conditions

The study site was located outdoors at the Regional Research Center, INIA, in La Cruz, Region of Valparaíso, Chile. The region has a humid marine Mediterranean climate with an average annual temperature of 14.5 ºC, a minimum average temperature of 5.2 ºC (July) and a maximum average temperature of 29.3 ºC (January). The nine-month period from September to May is frost-free. The average total annual precipitation in the region is 328.5 mm with 80% of the precipitation occurring from May to August.

Experimental design

Five different soils were obtained from 5 different fallow fields and hills with characteristics typical of soils in avocado orchards in Chile. The different soil textures and their physical characteristics are shown in Tables 1 and 2. Soil was steam sterilized and periodically treated with Metalaxil and Fosetyl-Al fungicides to prevent root damage from *Phytophthora cinnamomi*, a common root pathogen in avocado orchards worldwide. Trees were drip irrigated with well water by 16 drippers (0.5 L h^{-1}) per plant. The irrigation frequency varied from 2 to 6 times per day (according to soil texture and daily evapotranspiration) to maintain relatively constant water content near field capacity (soil tension of -0.33 KPa). The volume of water applied daily was the same for all treatments. Irrigation water and soil analyses indicated no salt or carbonate problems. Trees were fertilized once each week from October to March with 145 g N applied as Urea, 10 g P applied as phosphoric acid, 63 g K applied as potassium nitrate and 14 g Mg applied as magnesium sulfate per plant.

Treatments. Each of the five soils was kept at soil water content near to field capacity during the experimental period; Each of the 5 soils had different physical characteristics and thus water content, air content and water-to-air ratio (W/A). Thus, there were five soil treatments (T1-T5) each with different average W/A ratios: T1, trees in fine loam clay soil irrigated frequently with water content near field capacity, average W/A=1.7 and an average seasonal soil air content of 17.4%; T2, trees in loam clay soil irrigated frequently with water content near field capacity, average W/A=1.3 and an average seasonal soil air content of 19.5%; T3, trees in

loam clay soil with higher silt content, irrigated frequently with water content near field capacity, average W/A=0.6 and an average seasonal soil air content of 35.0%; T4, trees in loam sandy soil irrigated frequently with water content near field capacity, average W/A=0.4 and an average seasonal soil air content of 32.8%; and T5, trees in sandy soil irrigated frequently with water content near field capacity, average W/A=0.3 and an average seasonal soil air content of 36.8%. All soils had a neutral pH ranging from 6.5 to 7.2. Soil textures were determined in a laboratory by the Bouyoucos hydrometer method (Day, 1965). The experimental design was a randomized complete block with 5 replications per treatment. A treatment block is illustrated in Figure 1.

As shown above, treatments were determined according the total experimental season average W/A, but probably due to soil and trees accommodation to the pot, there were some differences in the W/A of each treatment between the first and the second season. During the first season the mean and standard error (SE) of the W/A was 1.5 ± 0.04 for T1, 0.9 ± 0.05 for T2, 0.5 ± 0.02 for T3, 0.4 ± 0.01 for T4 and 0.2 ± 0.01 for T5. During the second season, soil and larger trees allowed the irrigation management to keep soil closer to field capacity and thus in slightly different W/A per treatment, so the mean W/A and SE were: 1.9 ± 0.04 for T1, 1.7 ± 0.3 for T2, 0.8 ± 0.09 for T3, 0.4 ± 0.02 for T4 and 0.4 ± 0.01 for T5. The soil water content, air content and W/A during each season are shown in Table 3; the average water content, air content and W/A ratios during the entire experiment are shown in Table 4.

Data Collection

Physical soil proprieties

Soil bulk density (BD) was determined by the cylinder method of Blake and Hartage (1986). Final BD values were obtained from the average of 3 *in-situ* measurements and one laboratory determination. Total soil porosity was calculated as described by Danielson and Sutherland (1986) using a soil Real Density value of 2.64 g cm^{-1}, which is a typical value in most mineral-originated soils (Blake and Hartage, 1986). Soil macroporosity (air capacity) *in situ* was calculated as described by Ball and Smith (1991). The *in-situ* value was averaged with a laboratory air capacity measurement obtained using the method described by Carrasco (1997). The soil water content at '*in situ* field capacity' (FC) was determined six times during the each season using the method described by Cassel and Nielsen (1986). The FC was also determined once in a laboratory by subtracting the percentage of macropores from the percentage of total pores; the percentage of pores that remained corresponded to the total microporosity which in saturated soil is the same as the water content at field capacity (Danielson and Sutherland, 1986). The six *in-situ* and the laboratory measurements were pooled to obtain an average FC value. The volumetric (θ) soil water content at field capacity was determined by multiplying the gravimetric water content (ω) by the BD value as described by Cassel and Nielsen (1986).

Soil air content

Volumetric air content of the soil was calculated as described by Benavides (1994). Volumetric water content was subtracted from total porosity and the remaining value was the percentage of air in the soil.

Soil water content

Soil water content was measured daily at a soil depth of 30 cm by frequency domain reflectometry (FDR) using a Diviner probe (Diviner 2000, Sentek Sensor Technologies, Stepney, Australia).

Soil water content was also determined gravimetrically (ω) and volumetrically (θ) at a soil depth of 30 cm. The ω was determined with the formula:

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

The θ was determined by multiplying ω by the BD value. The θ from saturation to the permanent wilting point was used to calibrate the FDR probe and for FC determination.

Soil oxygen diffusion rate (ODR) and CO_2 and O_2 content

The oxygen diffusion rate (ODR) in the soil was measured on 2 dates during the first season and at 3 dates during the second season with a Pt-electrode and oxygen diffusion meter (Eijkelkamp, Netherlands) as described by Letey and Stolzy (1964). Measurements were made during the morning with 2 irrigation pulses

applied during the measurement period; the Pt-electrode was inserted at 15-cm depth. Air in the soil was sampled at a 30-cm depth through “point-source soil atmospheric sampler” described by Staley (1980). Air samples were collected on two dates each season, during the morning before the irrigation started. Samples were analyzed for O_2 and CO_2 concentrations by injecting a 1 mL headspace sample into a AutoSystem XL gas chromatograph (Perkin-Elmer, Waltham, Massachusetts, USA) equipped with a TCD detector and a CTR-1 column.

Plant water relations

Stomatal conductance (gs) and transpiration (T) were measured with a Li-1600 steady state porometer (Li-Cor, Inc., Lincoln, Nebraska, USA) as described by Prive and Janes (2003) and Raviv *et al.* (2001). Both gs and T were measured at two-week intervals during the morning (9:00 - 11:00 hr) and at noon (13:00 - 16:00 hr). Measurements were made on 3 mature, sun-exposed leaves per plant.

Stem (xylem) water potential (SWP) was measured with the same frequency as gs and T. For SWP determinations, 3 sun-exposed leaves per tree were covered with plastic and aluminum foil and then excised 30 minutes after covering (Meyer and Reickosky, 1985). The SWP of the excised leaves was immediately measured with a pressure chamber as described by Scholander *et al.* (1965). Leaves were excised and SWP was measured during the morning (9:00 - 11:00 hr) and at noon (13:00 -16:00 hr).

Net CO_2 assimilation

Net CO_2 assimilation (A) was measured once each month during the second season with an open system portable gas analyzer Li-6400 (Li-COR Inc., Lincoln, Nebraska, USA). Measurements were made from 10:00 to 13:00 hr on 3 mature leaves per plant, of similar size, with similar light exposure located in the middle of a spring shoot. Measurements were made at a photosynthetic photon flux (PPF) ranging from 1300 to 1900 µmol m^{-2} s^{-1}, a reference CO_2 concentration in the leaf cuvette between 375 to 400 ppm, and an air flow rate into the cuvette of 200 µmol s^{-1}.

Plant water use efficiency (WUE)

Instantaneous plant water use efficiency (WUEi) was calculated by dividing A by T values, both obtained with the Li-6400 portable gas analyzer (Li-COR Inc., Lincoln, Nebraska, USA). Also, water use efficiency expressed as total plant dry matter produced in relation to the amount of water applied (WUEb) was calculated by dividing the final total plant dry weight by the volume of water supplied to the plants from the time of planting to harvest.

Biomass

At the end of the study period, potted plants were harvested, aerial parts were separated from the roots and the fresh weight of leaves, shoots and wood was determined with a digital balance (Shanghai SP-300, Shanghai Huade Weighing Apparatus Co., Shanghai, China). A “shoot” refers to the current seasons branches

and “wood” refers to the older trunk and branches. Tissues were then oven-dried at 70ºC for 3 days and leaves, shoots and wood dry weights were determined with an electronic balance (Transcell ESW-5M, Transcell Technology, Inc. Buffalo Grove, IL, USA). Root density was determined for 3 replications per treatment by subsampling roots with a 9-cm diameter, 1-m long tube sampler (Split tube sampler, Eijkelkamp, Netherlands) inserted into the soil as described by Ferreyra *et al.* (1984, 1989). The depth of soil sampled for root density ranged between 40 and 45 cm, depending on the depth of the soil in the sampled pot. Root samples were rinsed twice with tap water and once with deionized water, separated from the soil and fresh weights were determined. Roots were then oven-dried at 70ºC for 3 days and root dry weight and root density (g cm^{-3}) were determined for each plant. Total root dry weight was estimated by multiplying the root density by the total soil volume in each pot.

Leaf area

After detaching and weighing all the leaves of each tree, approximately 300 leaves from each tree were randomly sampled and leaf area was measured with a portable leaf area meter (model LI-3000C, Li-Cor, Lincoln, Nebraska, USA). Leaf samples were also weighed with an electronic balance (Transcell ESW-5M, Transcell Technology, Inc. Buffalo Grove, Illinois, USA) and the total leaf area per tree was estimated by multiplying the area/weight ratio of the 300 sub-sampled leaves per plant by the total leaf weight per plant.

Leaf area index

Leaf area index (LAI) was estimated every two weeks from the solar photosynthetically active radiation (PAR) intercepted by the tree foliage at noon (Suckel, 2001) which was measured with a linear PAR ceptometer (AccuPar model PAR 80, Decagon Device Inc., Pullman, Washington**,** USA).

Leaf size

Leaves were classified as large, medium or small, with mean areas and standard deviations of 205.1 cm^2 ± 40.6, 102.5 cm^2 ± 47 and 23.0 cm^2 ± 1.7 for large, medium and small leaves, respectively. The number of large, medium and small leaves was manually counted two times during the second season and the average leaf area was determined each time.

Spring shoot growth

Ten similar-size spring shoots were labeled and their lengths were measured once each month during the second season to determine the shoot growth rate and final shoot length. Measurements were made from December to March.

Autumn leaf retention

Ten similar shoots from the autumn vegetative flush were labeled and the total number of leaves per shoot was determined from January to March of the second season.

Number of flowers and fruit

During the blossom period of 2006/2007, 10 panicles were labeled and flowers were counted. The total number of panicles per tree was also counted. Two month later, the number of fruit per panicle and the total number of fruit per tree were counted after removing them from the trees. Fruit were harvested at an early stage of development to prevent the possibility of reproductive growth during fruit maturation from confounding differences in vegetative growth among treatment. At the time that fruit were harvested they were between 3-4 cm in diameter at the widest point.

Vascular anatomy of active roots and spring shoots

Three 2-mm diameter pieces of active roots and three 2-mm diameter pieces of spring shoots were sampled from 3 plants (replications) in each treatment at the end of the experiment. Finer roots were selected for histological examination because it has been suggested that these are the most active in direct uptake of water and minerals (Zilberstaine *et al.*, 1992). Samples were fixed in a formalin-acetic acid-alcohol solution (10 formalin: 5 acetic acid: 50 ethanol, by volume) (Ruzin, 1999). The tissue was embedded in a water-soluble wax. Wax blocks that were 6-18 µm thick were cut from the embedded shoot and root tissues and 5-µm thick sections were cut from the tissue and wax blocks using a rotary microtome (Spencer 820 Microtome, American Optical Co., Buffalo, NY, USA). Sections were stained with safranin and fast green.

Histological sections were observed at 100 X for roots and 40 X for shoots using a Leitz orthoplan optical microscope with an incorporated semiautomatic camera (Leitz, Wetzlar, Germany). Images were analyzed for average vessel area and total xylem area using Sigma Scan Pro 5.0 software (Systat Software, Richmond, California, USA). Scion Image for Windows Beta 4.02 (Scion Corporation, Frederick, Massachusetts, USA) was used to determine the average number of vessels per root xylem tissue. To determine the xylem/phloem ratio in shoots and roots, xylem and phloem areas were measured in each photomicrograph using the Sigma Scan Pro 5.0 software and the ratio was obtained by dividing the xylem area by the phloem area. To determine the bark/xylem ratio in shoots, bark and phloem widths were measured in three different randomly selected areas of each photomicrograph. The bark/xylem ratio was obtained by dividing the bark by the xylem width.

Leaf ABA, leaf xylem ABA and root ACC content

At the end of the first season (March 2006), a sample of mature leaves from 3 plants (replications) was harvested, rinsed two times in tap water and finally in deionized water, frozen in liquid nitrogen (-192ºC) and kept in a freezer at -82ºC until ABA concentrations were determined. For leaf ABA determination, about 1 g of leaf tissue was weighed, mashed and homogenized in 99.8% methanol in semi-darkness to avoid ABA photo-oxidation. The homogenized solutions were placed in Eppendorf tubes, incubated for 1 h at 4ºC and centrifuged for 7 min at 3000 g at 4ºC as described by Peña-Cortés *et al.* (1989). The ABA concentration was determined by an ABA indirect ELISA assay using an ABA Phytodetek kit (AGDIA

Inc., Elkhart, Indiana, USA). For leaf xylem ABA determination after the second measurement season, 8 leaves per tree (from each of 3 replications) were collected and immediately cooled. Sap was extracted from the leaf xylem with a pressure chamber at -1.2 MPa in a semi-dark room, collected with a sterilized Pasteur pipettes and placed in 0.2 ml Eppendorf tubes. Sap samples were frozen in liquid nitrogen (-192ºC) and kept in a freezer at -80ºC until the determination of sap ABA concentrations. The ABA concentration of the xylem sap was determined by an ABA indirect ELISA assay using an ABA Phytodetek kit (AGDIA Inc., Elkhart, Indiana, USA).

For ACC measurement, a sample of live root tissues from each replication was harvested and rinsed in tap water (two times) and in deionized water at the end of each measurement season (March). About 5 g of root tissue was collected from each replication, frozen in liquid nitrogen (-192ºC) and kept in a freezer at -82ºC until the ACC concentrations were determined. Leaf and root tissues were homogenized separately in 0.9% TCA. The homogenates were centrifuged for 20 min at 27,000 g at 4ºC. The ACC concentrations were determined separately in roots and leaves through the conversion of ACC to ethylene by a reaction with NaOCl using the method described by Lizada and Yang (1979) modified by Hoffman and Yang (1980). Samples and standards were measured from tubes with rubber serum caps that were hermetically sealed. The concentration of ethylene liberated during the reaction was determined by injecting a 1-ml headspace sample into a gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and alumina column.

Leaf nutrient content

After plants were harvested for biomass determination, 20 leaf samples per tree were rinsed twice in tap water and finally rinsed in deionized water, then dried at 70ºC in an oven for 48 hr until they reached a constant weight. Dry tissue samples were ground and N, P, K, Ca, Mg, Mn and C concentrations determined as described by according to Page (1982). Nitrogen and C concentration were determined with a LECO CNS-2000 Macro Elemental Analyzer (Leco, Michigan, USA). Phosphorous, K, Ca and Mg concentrations were determined by dry combustion at 500 °C until the total organic components were converted to ash. For P, ashes samples were analyzed with an atomic absorption spectrophotometer (Shimadzu Corporation, Kyoto, Japan) after forming a complex with molybdate-vanadate. For K, Ca, Mg and Mn, the ashed tissue samples were dissolved in dilute HCl (2 *M*) and concentrations were determined with an atomic absorption spectrophotometer (Varian SpectrAA 220 FS, Varian Techtron Pty. Limited, Victoria, Australia).

Soil pathogen determination

At the time that plants were harvested, a composite sample of each type of soil was taken to the Phytopatology Laboratory at the Pontificia Universidad Católica de Valparaíso and screened for the presence of soil fungi. The procedure involved diluting the soil with deionized water to 0.5×10^{-3} g ml^{-1} and placing 100 ml of diluted soil extract into three different selective media APD, SPS AND MSP (Brayford, 1992).

Climatic variables

Throughout the experiment, temperature and relative humidity were continuously monitored with a Hobo datalogger (Onset Computer Corporation, Pocasset, Massachusetts, USA) and vapor pressure deficit was calculated from these variables.

Data analysis

Data are expressed as means. The effects of treatment on ODR, soil CO_2 and O_2 concentrations, gs, T, SWP, A, WUE, WAE, leaf area, leaf area index, leaf size, number of fruit, autumn leaf retention, spring shoot growth, dry weights, xylem/phloem ratio, bark/xylem ratio, root xylem vessel diameter, ACC and ABA concentration, and leaf nutrient concentrations were analyzed by an ANOVA and mean differences were determined with a Waller-Duncan Multiple Comparison Test ($P \leq 0.1$). The effect of treatment on root density was also analyzed by an ANOVA and mean differences were determined with a Duncan's Multiple Range Test ($P \leq 0.1$). All statistical analyses were performed using the SAS statistical software package (SAS Institute, Cary, North Carolina, USA).

RESULTS

Physical soil proprieties

The physical soil characteristics measured are summarized in Tables 1, 2, 3 and 4. The soil water-to-air ratio (W/A) for seasons 2005/2006 and 2006/2007 were

obtained from total porosity (Table 2) and the average volumetric soil water content (θ during each experimental season (Table 3).

Soil water content

In each soil, the volumetric soil water content (Figure 2) was kept near field capacity throughout the experiment, although during the second season, the fine tuning irrigation management allowed for the soil water content to be maintained closer to the field capacity. Volumetric soil water content tended to fluctuate more in the T2, T3 and T4 treatments than in the T1 and T5 treatments (Figure 2).

Soil oxygen diffusion rate (ODR) and CO_2 and O_2 content

The mean ODR, CO_2 and O_2 contents throughout the experiment are shown in Table 5. Differences in ODR among treatments in season 2005/2006 indicate that T4 had the highest ODR, followed by the T5 and T3, with the lowest ODR in the T2 and T1. In the 2006/2007 season, T5 had the highest ODR, followed by the T4, and finally T3, T2 and T1, which did not show statistical differences among them. During both seasons, T2 had the highest CO_2 content and T4 and T5 had the lowest CO_2 contents. No significant differences were observed in soil O_2 content among treatments during the first measurement season, but there was a significant difference in O_2 content between the T4 and T3 during the second season. The soil O_2 content diminished considerably from the first to the second measurement season, probably because during the second season the soil water content was kept more close to field capacity than the first season (Figure 2).

Non-linear regression analysis showed an inverse exponential relationship between the average W/A of each soil per season and soil ODR (R^2=0.75) (Figure 3). There was a direct exponential relationship between the average soil air content and ODR (R^2 = 0.61, data not shown), but this relationship was not as strong as that between the W/A and ODR.

Percentage of days with soil air content below a critical level for avocado trees

The percentage of days with soil air content below 17% was determined for each soil treatment during each season because soil air content below that level restricts ODR to less than 0.2 µg cm^{-2} min^{-1} (Ferreyra *et al.,* 2007a) and can restrict root growth and cause root damage in avocado (Valoras *et al.,* 1964, Stolzy *et al.,* 1967). Taking into account the water content measured each day during both experimental seasons, the percentage of days with the soil oxygen content below the critical 17% level during season 2005/2006 was 40.78%, 18.78%, 0.96%, 0% and 0% for T1, T2, T3, T4 and T5, respectively. During season 2006/2007 the percentage of days with the soil air content below the critical level was 54.89%, 68.90%, 15.16%, 0% and 0% for T1, T2, T3, T4 and T5, respectively (Table 6).

Plant water relations

The effect of treatment on gs, T and SWP during seasons 2005/2006 and 2006/2007 are shown in Tables 7 and 8, respectively. There were no significant differences among treatments for any plant water relation variable measured during the morning (AM) in 2005/2006. During 2005/2006, gs and T measured in the afternoon (PM) were higher for trees in the T4 and T5 treatments than for trees

in the other treatments, with exception of gs of T2 which was not significantly different from that in T4 or T5. In the afternoon, SWP was higher for trees in T5 and T3 than the other treatments (Table 7).

During 2006/2007, there were no significant differences among treatments in gs or SWP measured during the afternoon, possibly due to the very high vapor pressure deficits observed during December and January, which reached a maximum of 4.5 KPa compared to a maximum VPD of 3.9 KPa during the summer of 2005/2006 (Figure 13). Transpiration measured during the afternoon was higher for trees in T5, T4 and T2. During the mornings of season 2006/2007 there was an effect of treatment on water relations. Trees in T5, T4 and T1 had significantly higher gs and T than those in the T2 and T3 and trees in the T3 and T5 had significantly higher SWP than trees in T2 (Table 8).

Net CO_2 assimilation (A) and instantaneous plant water use efficiency (WUEi)

Net CO_2 assimilation and WUE calculated from A and T [instantaneous WUE (WUEi)] are shown in Table 9. Trees in T5 had significantly higher A than trees in T3 or T2. There were no significant differences in WUEi among treatments.

Plant water use efficiency expressed as total plant dry mater produced in relation to the amount of water applied (WUEb). Trees in T1 had significantly lower WUEb than trees in any of the other treatments. Also trees in T4 had a significantly higher WUEb than trees in T3, T2 and T1 (Table 10).

Plant dry weight and root density

Trees in T1 had significantly lower total and wood dry weights than trees in the other treatments (Figure 4). Leaf and root dry weights were lower for trees in T1 than for trees in T4 or T5 (Figure 4). Root density was significantly greater for trees in T4 than for trees in T1 (Figure 5).

Leaf area index (LAI), leaf area, leaf size, spring shoot growth and autumn leaf retention

At the end of season 2005/2006, LAI was significantly lower for trees in T1 than those in T5, T4 or T3 (Figure 6). During 2006/2007, there were no differences in LAI among treatments (data not shown), although at the end of that season, the total leaf area was significantly higher for trees in T4 than for trees in T1 (Figure 7) and average leaf size was greater for trees in T5 compared to trees in T3 or T1 (Figure 8).

The length of the spring shoots was less for trees in T1 than trees in the other treatments during the first part of the second season, but at the end of that season there were no differences in spring shoot length among T5, T3 and T1, whereas trees in T4 and T2 had longer spring shoots than trees in the other treatments (Figure 9). Retention of autumn leaves was consistently longer on trees in the T5 and T4 treatments than trees in the other of treatments throughout the entire season (Figure 10).

Number of flowers and fruit

Although there was large variability in the number of flowers and fruit per tree during spring of season 2006/2007, the total number of flowers and fruit per tree were much higher in T2 than the other treatments. These differences were significant for the number of flowers per tree between T2 and T1, whereas the number of fruit per tree was significantly higher in T2 compared to all of the other treatments (Figure 11).

Vascular anatomy of active roots and spring shoots

Trees in T2 had a higher shoot xylem/phloem ratio than trees in all other treatments except T5. Also, trees in T2 had a lower shoot bark/xylem ratio than trees in all other treatments, except for T3. The number of root xylem vessels was higher in trees in T1 than in trees in T3. There were no significant differences among treatments in the root xylem/phloem ratio, the xylem vessel area and the total xylem area (Table 11 and Figure 12).

Leaf ABA and root ACC content

At the end of the 2005/2006 season, there were no significant differences in leaf ABA content among treatments (data not shown). However at the end the 2006/2007 season, trees in T1 had a significantly higher leaf xylem ABA concentration than trees in the other treatments. There were no significant differences in root ACC concentration among treatments at the end of the 2005/2006 or 2006/2007 seasons. For trees in each treatment, root ACC

concentration was higher the end of the second season than at the end of the first season (Table 12).

Climatic variables

Vapor pressure deficits during 2005/2006 and 2006/2007 are shown in Figure 13. The maximum VPD (4.5 KPa) was much higher in December and January 2006/2007 than during the same period in 2005/2006. The average VPD for the entire 2005/2006 season during the AM and PM hours was 1.7 and 2.7 KPa, respectively, while the VPD for the 2006/2007 season averaged 1.6 and 3.03 KPa for AM and PM hours, respectively.

Leaf nutrient content

Leaf N content was significantly higher in trees in T1 compared to the other treatments with the lowest N concentrations in T5, T3 and T2. Leaf K concentration was significantly higher for trees in T3 compared with all of the other of treatments. The Ca content was higher in leaves of trees in T5 and T3 than in leaves plants in the other treatments; trees in T2 had the lowest Ca content. Leaf Mg concentration was higher for trees in T1 and T5 compared to trees in the other treatments. Leaf Mn content was significantly higher in T1, T2 and T4 compared with T5 or T3. There were no significant differences among treatments for leaf P or C contents (Table 13).

Soil phytopathology analysis

No significant number of *Phytophthora sp*. colonies g^{-1} of soil were found in the analyzed soil samples, indicating good control of this genera of oomycete during both measurement seasons (data not shown).

DISCUSSION

In soils irrigated to maintain water content near field capacity, ODR of the soil was more closely related to the W/A than the soil air content. Therefore, differences in W/A due to different physical soil properties had a significant effect on leaf gas exchange, water relations, vascular anatomy and growth of avocado trees. In general, the lower the soil water-to-air ratio, the higher the gs, T, A and SWP. This resulted in higher WUEb and more vegetative growth in soils with low water-to-air ratios. Avocado roots are highly sensitive to lack of oxygen in the soil caused by flooding or excessive water in the root zone (Schaffer *et al.*, 1992; Whiley and Schaffer ,1994; Schaffer and Whiley, 2002). This sensitivity may, in part, be due to avocado´s root system which is extensively suberized with inefficient water absorption due to low hydraulic conductivity and few root hairs (Whiley *et al.*, 1987; Du Plessis, 1991). The soils where avocado originated are characterized by a low bulk density (0.5-0.8 g cm^{-3}), a high macroporosity (46%) and high organic matter content (Aguilera and Salazar, 1991). Thus, the high susceptibility of avocado trees to soil hypoxia may be the result of their evolution in soils with very good aeration. Consequently, in poorly-drained or saturated soils, the reduction of ODR affects the growth and productivity of avocado by inhibiting the development of stems and

roots (Valoras *et al*., 1964; Stolzy *et al.*. 1967; Schaffer and Ploetz, 1989; Ansorena, 1994).

In the *Phytophthora*-free soils examined in this study, the different water-to-air ratios were primarily due to differences in soil texture and bulk density as well as the ODR and CO_2 concentration in the soil. Soil ODR values of 0.2 µg cm^{-2} min^{-1} (Valoras *et al.*, 1964) and 0.17 µg cm^{-2} min^{-1} (Stolzy *et al.*, 1967) have been reported as limiting to avocado development. Menge and Marais (2000) reported that soil CO_2 concentrations of 16% inhibited avocado root growth and survival. Although none of the soils tested in the present study had limiting ODR values or CO_2 concentrations when those parameters were directly measured, the percentage of days that the soil air content was below 17% and thus reaching a critical ODR (Ferreyra *et al.*, 2007a) for normal avocado root functioning in T1 and T2 was 40.8% and 18.9%, respectively during season 2005/2006, and 54.9% and 68.9%, respectively during season 2006/2007. The soil air content in T4 or T5 never reached soil air contents below the critical and in T3 only was below the critical soil air content 0.9% of the time during season 2005/2006 and 15.2% during season 2006/2007.

In general, the lower the soil water-to-air ratio, the higher the ODR and the lower the CO_2 concentration. Oxygen concentrations were not significantly different among soils. However, O_2 content diminished considerably from the first to the second season, probably because the water content was kept closer to field capacity during the second season. This may also be the reason for the increased root ACC concentration during the second season compared to the first for all

treatments. Although O_2 and ACC concentrations increased by the second season, none of treatments had ACC or ABA contents high enough to indicate plant stress. In this study avocado root ACC concentration ranged from 0.38 to 0.94 nmol g^{-1}, while ABA concentration in the xylem sap ranged between 82 to 341.9 pmol ml^{-1}. Little is known about phytohormone concentrations in avocado trees as result of abiotic stress. In flood-stressed tomato plants leaf, ACC concentrations may reach 1.44 to 4.7 nmol g^{-1} (Hall *et al.*, 1993; Else and Jackson, 1998), while in xylem sap of water-stressed sunflower ABA concentrations ranged from 1,100 to 2,300 pmol ml^{-1}.

Root hypoxia or anoxia usually results in reductions in stomatal gs and T of avocado trees (Schaffer and Ploetz, 1989; Schaffer *et al.*, 1992; Schaffer, 1998; Schaffer and Whiley, 2002). During the first season of this study, gs, T and SWP were higher for trees in the T4 and T5 treatments than for trees in the other treatments. However during the second season, trees in the T1 treatment had similar gs, T and SWP as plants in the T4 and T5 treatments. Ferreyra *et al.* (2007a) reported that low soil air contents (5% to 18%) negatively affected gs, but not the SWP. The same authors established that a soil air content lower than 17% restricts the oxygen diffusion rate to less than 0.2 µg cm^{-2} min^{-1} and that macroporosity values were correlated with soil O_2 and CO_2 content.

Some authors have suggested that the stomatal closure in plants due to root zone hypoxia is related to a reduction of root hydraulic conductivity. However other studies have suggested that a reduction in gs as a result of root zone hypoxia is more related to changes in leaf ABA concentration (Kozlowski, 1997). In the

present study, anatomical analysis of avocado roots did not suggest a change in root hydraulic conductivity because no differences in root xylem vessel area and total xylem area were observed among treatments. However, histological analysis indicated a possible acclimation of plants in T1 to a high water-to-air ratio. This was based on the higher number of root xylem vessels observed in plants in this treatment at the end of the second season compared with other treatments, such as T3. Also, trees in T1 had similar gs, T and SWP as plants in T4 and T5 during the second measurement season. The ABA concentration in leaf xylem sap that was collected at about the same time as the histological samples was significantly higher in T1 than in the other treatments. Thus, of the high gs, T and SWP values for plants in T1 during the second season may have been caused by an anatomical acclimation of those plants. Kozlowski (1997) reported that the lack of oxygen often affects xylem and phloem production differently. In *Pinus halepensis* and *Thuja orientalis*, hypoxia accelerated tracheid production in stems (Yamamoto *et al.*, 1987). Although acclimation to hypoxic conditions in the root zone has not been reported for avocado trees, the results in this study suggest that the relatively high leaf gas exchange and water status of plants in T1 during the second measurement season, compared with plants in the other treatments and also compared with the physiological response for plant in this treatment during the first measurement season, in spite of the higher level of ABA found in the leaf xylem sap, was probably due to an increased number of root xylem vessels developed between the first and second seasons.

Net CO_2 assimilation and WUEb were also affected by the soil treatments. Trees in soils with a low water-to-air ratio had higher A which was highly correlated with gs (R^2=0.88, data not shown). For avocado trees, root hypoxia or anoxia usually results in reductions in gs, T and A (Schaffer and Ploetz, 1989; Schaffer *et al.*, 1992; Schaffer, 1998; Schaffer and Whiley, 2002). In other species including *Citrus sinensis*, continuous soil flooding reduced A 94% after 24 days (Vu and Yelenosky, 1991). Increasing the oxygen supply in the soil also has show to be effective for A and WUE increasing. For example, for zucchini, soybean and cotton in heavy clay soils kept at field capacity A and WUE were increased by air injection into the soil which increased the soil oxygen concentration (Bhattarai *et al.*, 2004).

Trees in T3 had lower values of gs, T and A during the second measurement period (AM time) than trees in the other treatments; this was reflected as lower biomass, total leaf area, leaf size and autumn leaf retention compared trees in T5 and T4. Generally, all the biomass results were similar to those of trees in T2. Although the water-to-air ratio was low in this case, due mostly to the low bulk density (1.1%), the high silt proportion in this soil (37.9%) probably affected the water relations of those trees. Silt causes in soil impermeability and low aeration, because those soil particles has not colloidal characteristics, do not form structural aggregates and also they are sufficiently thin to clog macropores (Feng *et al.*, 2002; Sun *et al.*, 2006). Studies made in organic-mineral horizons of soils (Aranda *et al.*, 2002) showed that higher content of silt in the soil signified a relative high organic carbon content that means a slow soil mineralization of organic matter, due to a lower aeration of the soil.

Trees in soils with lower water-to-air ratios had more biomass, a higher leaf area, greater root density and longer leaf retention in autumn that trees in soil with the higher water-to-air ratios. The effect of an increased soil oxygen content on plant biomass as a result of different oxygen sources applied to the soil, has been studied in different species. Zucchini, soybean and cotton exhibited increased biomass when clay soil was treated with hydrogen peroxide (H_2O_2) or injected with air to increase the soil oxygen content (Bhattarai *et al.*, 2004). In a recent study of avocado trees in a heavy clay loam soil, it was demonstrated that injecting H_2O_2 into the soil significantly increased the biomass of the aerial portions of the plant and WUE, but had no significant effect on A, T, gs, or SWP (Gil *et al.* 2008). Increased growth had also been observed in tomato plants in flooded conditions when H_2O_2 was added to the flood water (Bryce *et al.*, 1982). Lack of oxygen in the root zone can adversely affect the shoot growth of many woody plant species by suppressing formation and expansion of leaves and internodes, or causing premature leaf senescence and abscission (Kozlowski *et al.*, 1991; Kozlowski and Pallardy, 1997). Also, soil hypoxia reduces root growth of most plants by inhibiting root formation and branching, growth of existing roots and mycorrhizae, and by inducing root decay (Kozlowszi, 1984; Kozlowski and Pallardy, 1997). Leaf abscission is a common response of avocado trees to root hypoxia (Ploetz and Schaffer, 1989; Schaffer *et al.*, 1992). In many plants, root hypoxia stimulates ethylene production (Jackson, 1985; Kozlowski 1997); the conversion of ACC to ethylene is dependent upon an adequate oxygen supply (Bradford and Yang, 1980). Thus, in anoxic conditions, the total lack of O_2 in the root zone halts the conversion of ACC to ethylene. However, in hypoxic conditions, when partial

pressures of O_2 in the root zone are between 0 and that of air, conversion of ACC to ethylene is actually stimulated (Jackson, 1985). In the present study, the soil treatments did not affect root ACC content. In avocado trees, higher soil water-to-air ratios may result in increased production of ACC in the roots which could not be measured because it was quickly converted to ethylene and transported to the leaves, thus stimulating leaf abscission resulting in less leaf area and biomass.

The lack of soil oxygen often affects xylem and phloem production differently (Kozlowski, 1997). In some species such as *Pinus halepensis*, *Pinus densiflora* and *Cryptomeria japonica*, waterlogging resulted in increased bark thickening. In other species including *Fraxinus mandshurica*, flooded plants had an increased number and size of stem xylem cells (Yamamoto *et al.*, 1995) while in *Pinus halepensis* and *Thuja orientalis* flooding accelerated xylem growth because of more rapid tracheid production. In the present study, the different soil treatments had no clear effect on shoot xylem-to-phloem ratio or bark-to-xylem ratio, although trees in T2 and T3 had significantly lower bark/xylem ratios. Trees in T2 also had a higher xylem/phloem ratio.

The lower phloem area in T2 compared to the other treatments may have been related to the significantly greater number of flowers and leaves for that treatment compared to the others. A lower phloem section in shoots my affect carbohydrate translocation from the leaves to the root, causing a higher carbohydrate concentration in buds that may have increased flower induction and thus flowering and fruit set. There are indications that O_2 deficiency in the soil may cause a blockage of phloem transport of carbohydrates (Vu and Yelenosky, 1991).

Accumulation of starch has been reported in leaves of various flooded plants, e.g. *Helianthus annus* (Wample and Davis, 1983), *Citrus sinensis* (Vu and Yelenosky, 1991), *Momordica charantia* (Liao and Lin, 1994) and wax-apple trees (Hsu *et al.*, 1999). When hypoxic conditions in the root zone exist, starch accumulation in leaves has been attributed to a reduced rate of carbohydrate translocation from leaves to roots (Barta, 1987). In avocado, there exist a direct relationship between flower initiation and a high level of reserve carbohydrates in the wood (Scholefield *et al.*, 1985; Gazit and Degani, 2002). Thus, a higher concentration of starch accumulation in the aerial portion of the tree as a result of a high water-to-air ratio may possibly explain the high fruit production of trees in T2. The fact that trees in T1 did not show such high flower and fruit production during the second measurement season may have been due to the greater water stress for trees in T1 treatment than trees in T2 during the first measurement season, which clearly affected gs, T and finally growth. The stress during the first season of plants in T1 could have also altered carbohydrate accumulation in leaves and wood. According to Gazit and Degani (2002), flower bud formation may not proceed in shoots with inadequate carbohydrate levels.

Reduced absorption of macronutrients has been reported as a response to flooding (especially N, P and K) (Kozlowski and Pallardy, 1984). In this case soil water-to-air ratios were not related to leaf N, P and K concentrations, probably because soil never reached a flooded condition, and thus did not affect nutrient uptake. Trees in T5 treatment had higher leaf Mg and Ca concentrations than trees in the other treatments, which was unexpected because sandy soils have lower natural fertility.

Plant uptake and translocation of Ca is closely related to irrigation management because Ca is translocated in the xylem. Therefore, the high gs and T for trees in that treatment may explain the high leaf Ca concentration. The higher Mn concentration in leaves of plants in T1 and T2 may have been a result of the lower O_2 of soils in these treatments. It has been reported that lack of O_2 in soil enhances Mn uptake. When soil O_2 content is low, the manganic form of Mn is converted into soluble manganous forms which are more available for plant uptake (Kozlowski, 1997). In flooded soil, this can result in soluble Mn concentrations reaching toxic levels (Kozlowski, 1997). In this case, Mn uptake was greater in trees in the soils with the highest water-to-air ratios, but concentrations did not reach levels that could negatively affect tree metabolism.

In conclusion, the water-to-air ratio in non-*Phytophthora*-infested soils irrigated near to field capacity, is an important factor that affects avocado physiology, growth and thus productivity. In soils with low water-to-air ratios, gs, T, SWP, A, WUEb and growth of avocado are higher than in soils with high water-to-air ratios. During the first season after planting, avocado trees in soils with high soil water-to-air ratios had lower gs, T and growth than trees in soils with low water-to-air ratios, but soil oxygen content was apparently not low enough in those soils to severely stress the trees. Trees in the T1 treatment could apparently acclimate to these high water-to-air ratios during the following season by an alteration of their vascular anatomy, leading to improved physiological responses during the second season. Taking into account that avocado production in Chile and other places in the world is expanding to areas with marginal soils that are often poorly drained and low in

oxygen, soil water-to-air ratios should be an important consideration when assessing the potential productivity of an avocado orchard adequately irrigated. Based on relationship between soil physical properties and water-to-air ratio, it may be necessary to mitigate the effects of low oxygen content in the soil by methods such as air or hydrogen peroxide injection into the soil (Bryce *et al.*, 1982; Goorahoo *et al.*, 2001; Bhattarai *et al.* 2004; Gil *et al.*, 2008), improvement of soil physical proprieties through organic matter application to the soil (Hamblin and Davies, 1977; Walt and Dexter, 1997; López-Cervantes *et al.*, 2006), or regulating irrigation times and frequencies according to the water-to-air ratio of the soil (Gur *et al.,* 1979; Levin *et al.*, 1979; Laher and Avnimelech, 1980; Heiskanen, 1995; Dunbabim *et al.*, 1997; Sellés *et al.*, 2001).

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Table 1. Texture classes and composition of five soil treatments. Values represent means obtained from laboratory measurements.

Treatment	% Sand	% Silt	% Clay	Texture Class
T1	39.5	25.1	35.4	Loam Clay
T2	39.2	22.6	38.2	Loam Clay
T3	34.1	37.9	28	Loam Clay
T4	84.4	5.5	10.1	Loam sandy
T5	92	0.5	7.5	Sand

Table 2. Physical characteristics of five different soil treatments (Tmt). Values represent means obtained from *in situ* and laboratory measurements. FC = field capacity, BD = bulk density.

Tmt	FC ω %	BD g cm^{-3}	Porosity %	Microporosity %	Air capacity %
T1	20.0	1.43	46.0	28.6	17.5
T2	19.6	1.49	43.8	29.2	14.6
T3	20.9	1.14	57.0	23.8	33.2
T4	7.3	1.45	45.3	10.5	34.7
T5	12	1.38	47.9	16.5	31.4

Table 3. Average soil water content, soil air content and water/air (W/A) ratio of five different soil treatments during 2005/2006 and 2006/2007.

Tmt	Soil water content (θ%) 2005/2006 (mean ± SD)	Soil water content (θ%) 2006/2007 (mean ± SD)	Soil air content (%) 2005/2006 (mean ± SD)	Soil air content (%) 2006/2007 (mean ± SD)	W/A Season 2005/2006 (mean ± SE)	W/A Season 2006/2007 (mean ± SE)
T1	27.47 ± 2.7	29.98 ± 2.1	18.57 ± 2.7	16.02 ± 2.1	1.5 ± 0.04	1.9 ± 0.04
T2	21.11 ± 4.8	27.59 ± 5.9	22.66 ± 4.8	16.21 ± 5.9	0.9 ± 0.05	1.7 ± 0.3
T3	18.40 ± 5.3	25.55 ± 10.6	38.58 ± 5.3	31.45 ± 10.6	0.5 ± 0.02	0.8 ± 0.09
T4	11.82 ± 2.5	13.18 ± 3.2	33.46 ± 2.5	32.12 ± 3.2	0.4 ± 0.01	0.4 ± 0.02
T5	8.99±1.9	13.45 ± 2.5	38.93 ± 1.9	34.72 ± 2.5	0.2 ± 0.01	0.4 ± 0.01

Table 4. Average soil water content, soil air content and water/air (W/A) ratio of five different soil treatments (Tmt) during the entire experimental period. Treatments were based on the average water-to-air ratios (W/A) near field capacity.

Tmt	Soil texture	Average water content (θ%) 2005-2007	Average soil air content (θ%) 2005-2007	Average soil water/air ratio 2005-2007
T1	Loam Clay	28.8	17.4	1.7
T2	Loam Clay	24.4	19.45	1.3
T3	Loam Clay	22.0	35.0	0.6
T4	Loam sandy	12.5	32.8	0.4
T5	Sand	11.2	36.8	0.3

Table 5. Effect of treatment (Tmt) on soil oxygen diffusion and CO_2 and O_2 concentrations. Values correspond to means. Different letters (a, b, c) within columns indicate significant difference among treatments (Waller-Duncan Test, $P \leq 0.1$).

	2005/2006				2006/2007			
Tmt	W/A	ODR ($\mu g\ cm^{-2}\ min^{-1}$)	CO_2 (%)	O_2 (%)	W/A	ODR ($\mu g\ cm^{-2}\ min^{-1}$)	CO_2 (%)	O_2 (%)
T1	1.5	0.51 c	0.83 ab	16.60 a	1.9	0.34 c	0.44 b	5.02 ab
T2	0.9	0.51 c	0.94 a	18.46 a	1.7	0.38 c	0.74 a	4.63 ab
T3	0.5	0.70 b	0.74 abc	16.60 a	0.8	0.50 c	0.41 b	3.35 b
T4	0.4	1.05 a	0.55 c	18.90 a	0.4	1.05 b	0.32 bc	5.32 a
T5	0.2	0.83 b	0.66 bc	17.36 a	0.4	1.36 a	0.16 c	4.47 ab

Table 6. Percentage of days with soil air content below the critical level (<17%), which has been shown to limit oxygen diffusion in soil and availability to avocado roots (Ferreyra *et al.* 2007a). Values correspond to means ± standard error (SE).

	% of Days with soil Ea < 17% (mean ± SE)	
Tmt	2005/2006	2006/2007
T1	40.78 ± 19.18	54.89 ± 21.45
T2	18.78 ± 7.59	68.90 ± 8.41
T3	0.96 ± 0.96	15.16 ± 7.76
T4	0.00 ± 0.00	0.00 ± 0.00
T5	0.00 ± 0.00	0.00 ± 0.00

Table 7. Effect of treatments (Tmt) on water relations of avocado plants during 2005/2006. Values represent means. AM = 9:30 to 11:00 hr (gs and T); 5:30 to 6:30 hr (SWP), PM = 13:00 to 15:00 hr, gs = stomatal conductance, T = transpiration, SWP = soil water potential. Different letters (a, b) within columns indicate significant difference among treatments (Waller-Duncan Test, P ≤ 0.1).

Tmt	W/A	AM			PM		
	2005/2006	gs ($cm\ s^{-1}$)	T ($\mu g\ cm^{-2}\ s^{-1}$)	SWP (MPa)	gs ($cm\ s^{-1}$)	T ($\mu g\ cm^{-2}\ s^{-1}$)	SWP (MPa)
T1	1.5	0.30 a	2.88 a	-0.11 a	0.31 c	4.98 c	-0.77 b
T2	0.9	0.37 a	2.84 a	-0.10 a	0.38 ab	6.48 b	-0.75 b
T3	0.5	0.30 a	2.42 a	-0.10 a	0.34 bc	6.08 b	-0.71 ab
T4	0.4	0.46 a	3.46 a	-0.10 a	0.42 a	7.24 a	-0.72 b
T5	0.2	0.42 a	3.23 a	-0.11 a	0.43 a	7.22 a	-0.61 a

Table 8. Effect of treatments (Tmt) on water relations of avocado plants during 2006/2007. Values represent means. AM = 9:30 to 11:00 hr, PM = 13:00 to 15:00 hr, gs = stomatal conductance, T = transpiration, SWP = soil water potential. Different letters (a, b) within columns indicate significant difference among treatments (Waller-Duncan Test, P ≤ 0.1) during the AM or PM measurement time.

Tmt	W/A	AM			PM		
	2006/2007	gs ($cm\ s^{-1}$)	T ($\mu g\ cm^{-2}\ s^{-1}$)	SWP (MPa)	gs ($cm\ s^{-1}$)	T ($\mu g\ cm^{-2}\ s^{-1}$)	SWP (MPa)
T1	1.9	0.47 a	4.45 a	-0.62 ab	0.28 a	5.10 b	-0.91 a
T2	1.7	0.41 b	3.83 b	-0.65 b	0.28 a	5.24 ab	-0.88 a
T3	0.8	0.40 b	3.84 b	-0.55 a	0.26 a	4.91 b	-0.91 a
T4	0.4	0.44 ab	4.55 a	-0.59 ab	0.28 a	5.36 ab	-0.90 a
T5	0.4	0.47 a	4.74 a	-0.54 a	0.30 a	5.95 a	-0.83 a

Table 9. Effect of treatments (Tmt) on CO_2 assimilation (A) and instantaneous plant water use efficiency (WUEi). Values represent means. Different letters (a, b) within columns indicate significant difference among treatments (Waller-Duncan Test, P ≤ 0.1).

Tmt	W/A 2006/2007	A (µmol s^{-1} m^{-2})	WUEi(A T^{-1})
T1	1.9	4.71 ab	4.28 a
T2	1.7	4.44 b	4.30 a
T3	0.8	4.35 b	4.38 a
T4	0.4	5.40 ab	4.80 a
T5	0.4	6.07 a	4.28 a

Table 10. Effect of treatments (Tmt) on plant water use efficiency expressed as total plant dry matter produced in relation to the amount of water applied (WUEb). Values represent means. Different letters (a, b, c) within columns indicate significant difference among treatments (Waller-Duncan Test, P ≤ 0.1).

Tmt	W/A Average 2005-2007	WUE for biomass (g L^{-1})
T1	1.7	2.40 c
T2	1.3	3.23 b
T3	0.6	3.09 bc
T4	0.4	3.99 a
T5	0.3	3.70 ab

Table 11. Effect of treatments on root and shoot vascular anatomy. Values represent means (n = 3). Different letters (a, b, c) within rows indicate significant differences among treatments (Waller-Duncan Test, P ≤ 0.1).

	T1	T2	T3	T4	T5
Shoot xylem/phloem ratio	1.5 b	1.9 a	1.5 b	1.3 b	1.6 ab
Shoot Bark/xylem ratio	2.0 a	0.9 c	1.3 bc	1.7 ab	2.1 a
Root xylem/phloem ratio	1.5 a	1.1 a	1.1 a	1.5 a	1.0 a
Nº root xylem vessels	59.8 a	42.5 ab	32.5 b	45.2 ab	52.1 ab
Root Xylem vessel area (μm^2)	2392.5 a	2592.9 a	2757.3 a	2244.6 a	2545.9 a
Root Xylem total area (μm^2)	146,630 a	109,452 a	83,895 a	113,260 a	128,485 a

Table 12. Effect of treatments on leaf xylem sap ABA and root ACC content. Values represent means. ABA: n=3, ACC: n=5. Different letters (a, b) within columns indicate significant differences among treatments (Waller-Duncan Test P ≤ 0.1).

Tmt	2005/2006		2006/2007		
	W/A 2005/2006	Root ACC Concentration (nmol g^{-1})	W/A 2006/2007	Leaf xylem sap ABA concentration (pmol ml^{-1})	Root ACC concentration (nmol g^{-1})
T1	1.5	0.18 a	1.9	341.9 a	0.56 a
T2	0.9	0.17 a	1.7	136.9 b	0.44 a
T3	0.5	0.25 a	0.8	147.4 b	0.38 a
T4	0.4	0.14 a	0.4	104.5 b	0.71 a
T5	0.2	0.16 a	0.4	82.0 b	0.94 a

Table 13. Effect of treatments on macronutrients, Mn content and C content. Values represent means (n=5). Different letters (a, b, c) within columns indicate significant differences among treatments (Waller-Duncan Test P ≤ 0.1).

Tmt	W/A Average 2005-2007	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg Kg^{-1})	C (%)
T1	1.7	3.2 a	0.2 a	1.2 c	1.2 c	0.4 a	450.3 a	52.4 a
T2	1.3	2.7 c	0.2 a	1.3 c	1.0 d	0.3 b	378.7 a	53.2 a
T3	0.6	2.7 c	0.2 a	1.6 a	1.5 a	0.3 b	135.7 b	53.3 a
T4	0.4	3.0 b	0.2 a	1.3 c	1.3 b	0.3 b	337.3 a	52.7 a
T5	0.3	2.6 c	0.2 a	1.4 b	1.5 a	0.4 a	186.7 b	52.3 a

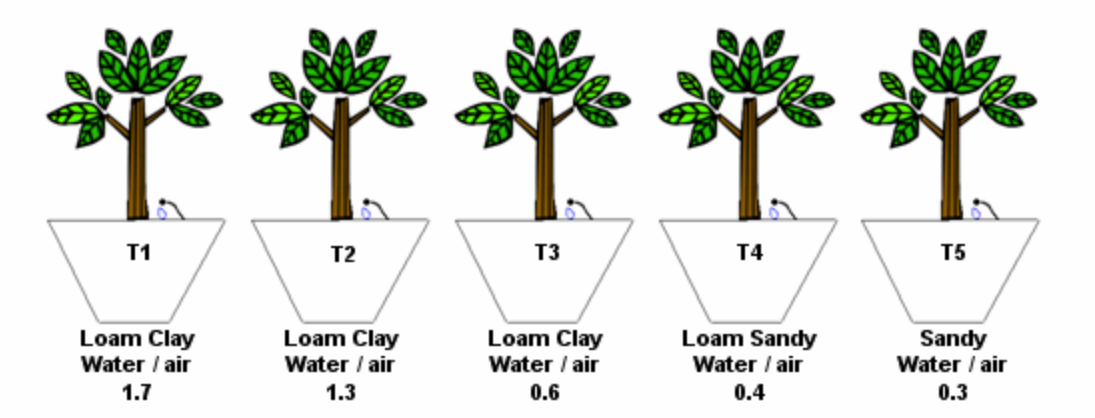
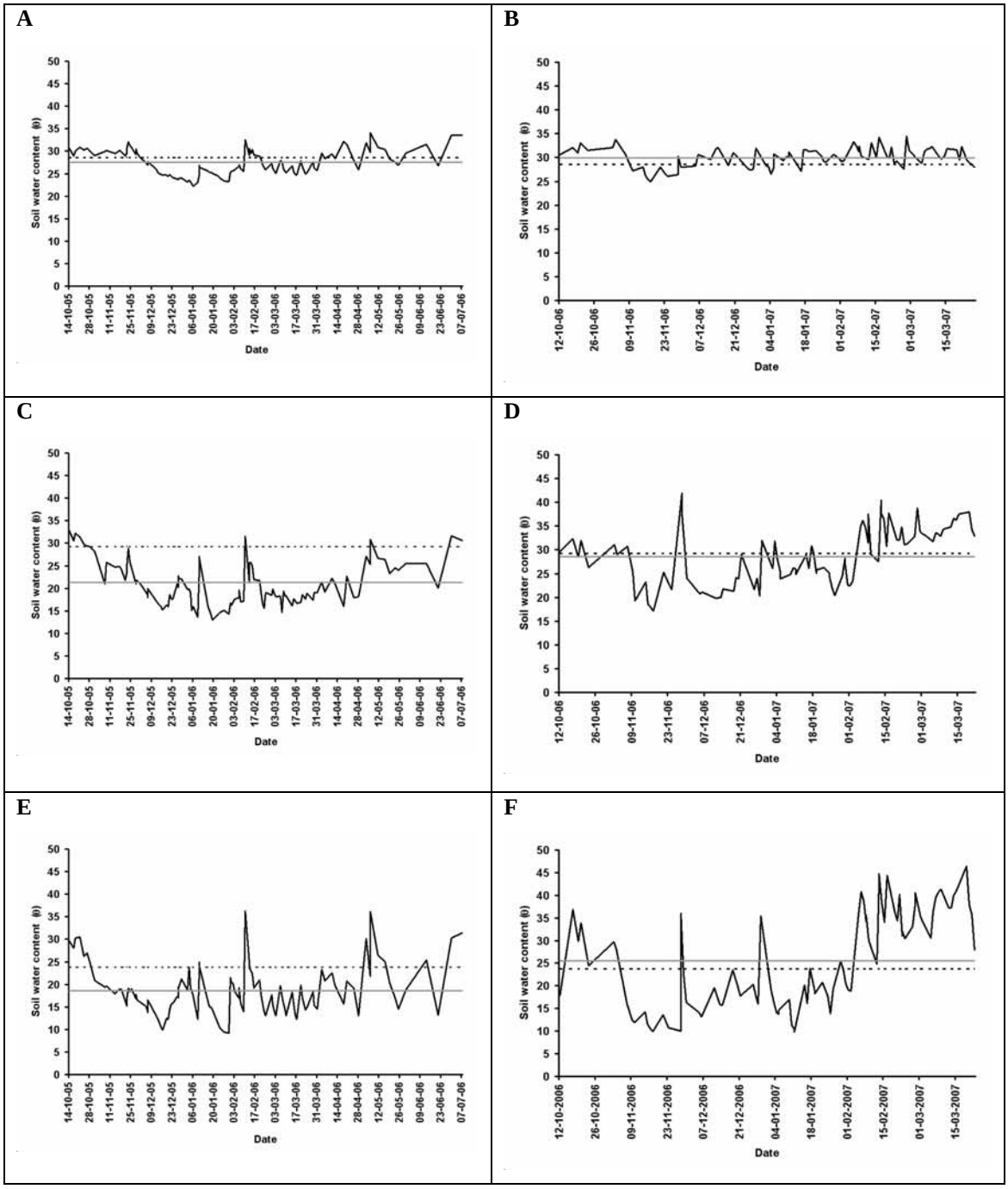


Figure 1. Illustration of a treatment block showing the texture and the average soil water-to-air ratio (W/A) of each soil.


A
B
C
D
E
F
Soil water content (θ)
Date

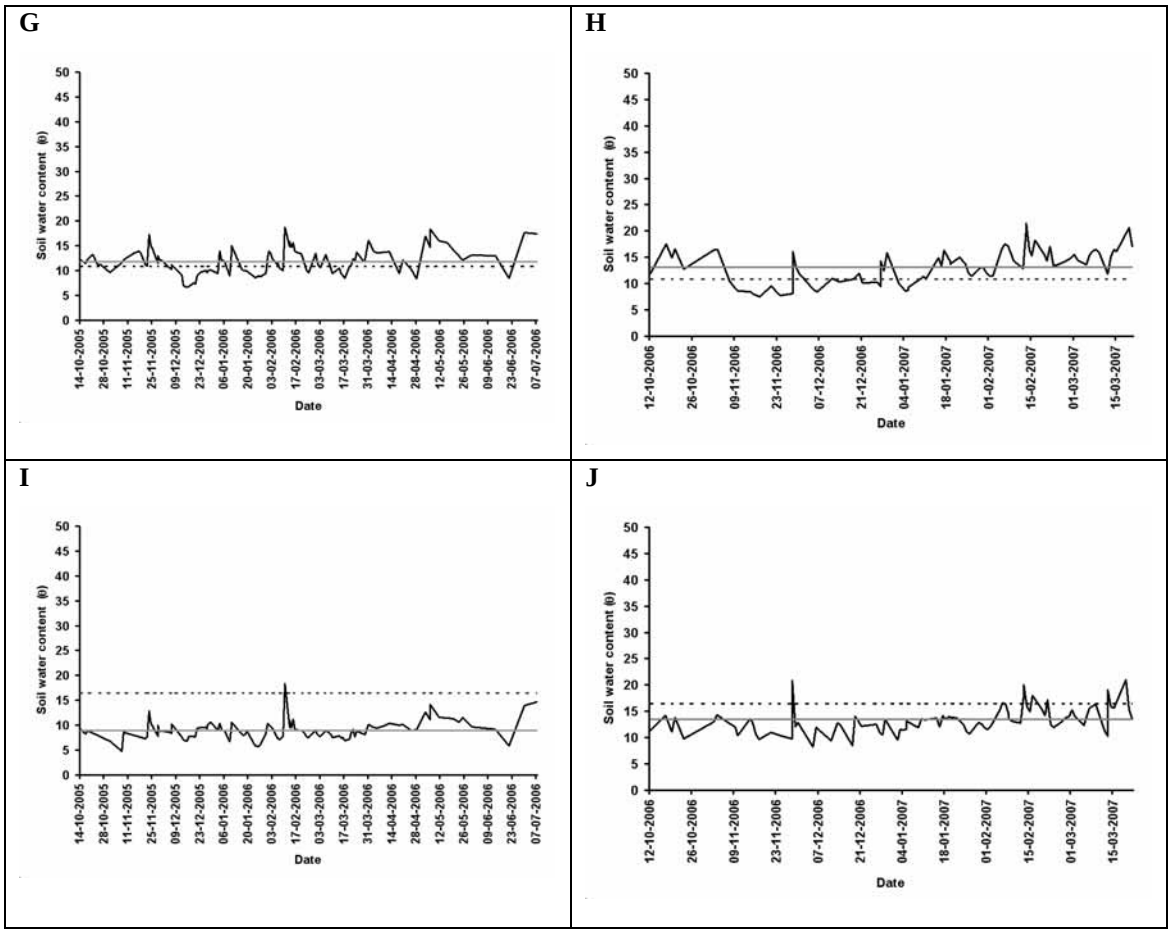


Figure 2. Volumetric soil water content (θ) at a 30-cm soil depth. A and B: θ of treatment T1 during the 2005/2006 and 2006/2007 seasons, respectively. C and D: θ of treatment T2 during the 2005/2006 and 2006/2007 seasons, respectively. E and F: θ of treatment T3 during the 2005/2006 and 2006/2007 seasons, respectively. G and H: θ of treatment T4 during the 2005/2006 and 2006/2007 seasons, respectively. I and J: θ of treatment T5 during the 2005/2006 and 2006/2007 seasons, respectively. Graphed values represent means (n=5).

— Average soil water content during the experimental season; ···· Soil moisture at field capacity value calculated from soil physical properties.

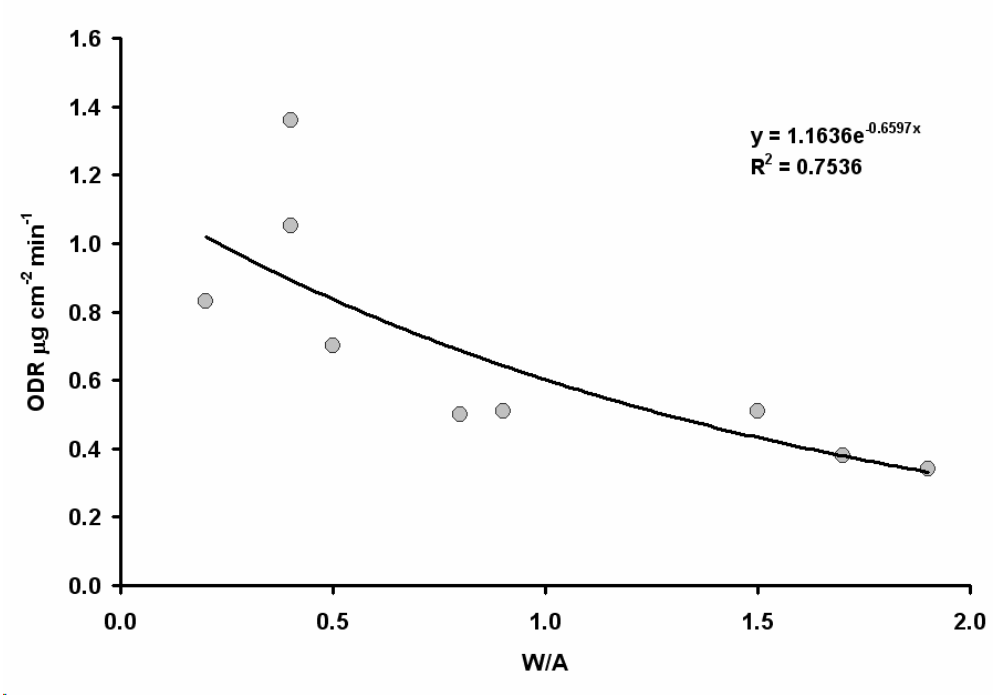


Figure 3. Relationship between the soil water-to-air ratio and the oxygen diffusion rate (ODR) in the soil. Mean W/A and ODR values of each of the five soils during each season (2005/2006 and 2006/2007, Table 5) were used in the regression analysis. W/A = 0.4 (T4) had a mean ODR of 1.05 µg cm^{-2} min^{-1} for both season, thus graphic appear to have 9 point instead of 10.

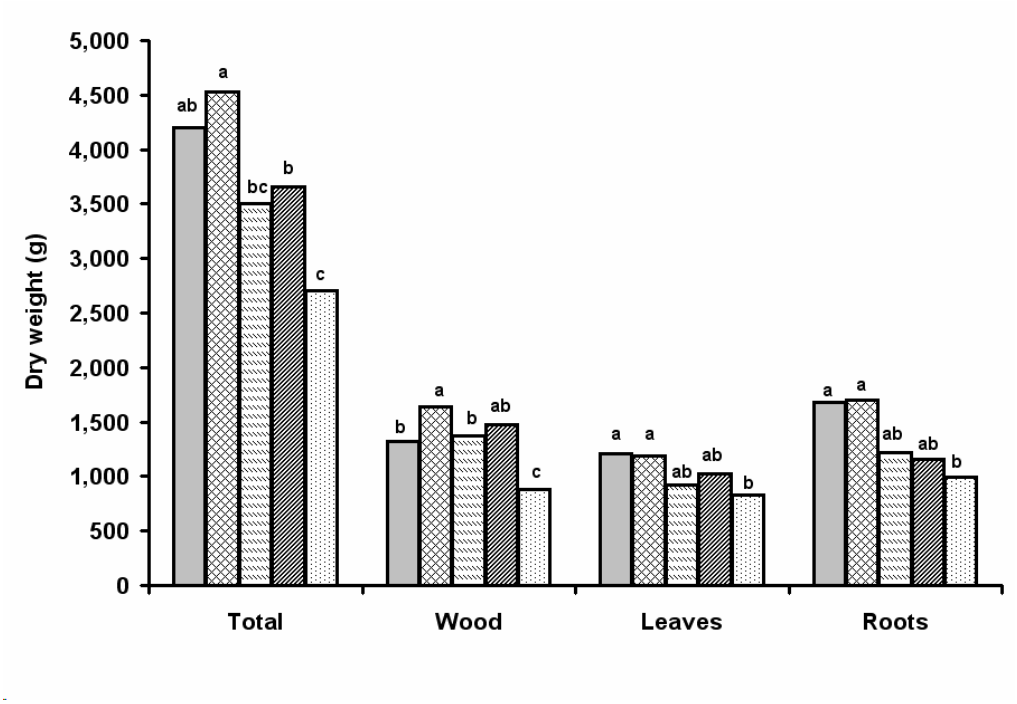


Figure 4. Avocado tissue dry weights at the end of the experiment. Bars indicate means. Different letters (a, b, c) indicate significant differences (Waller-Duncan Test, P ≤ 0.1). T5, T4, T3, T2, T1.

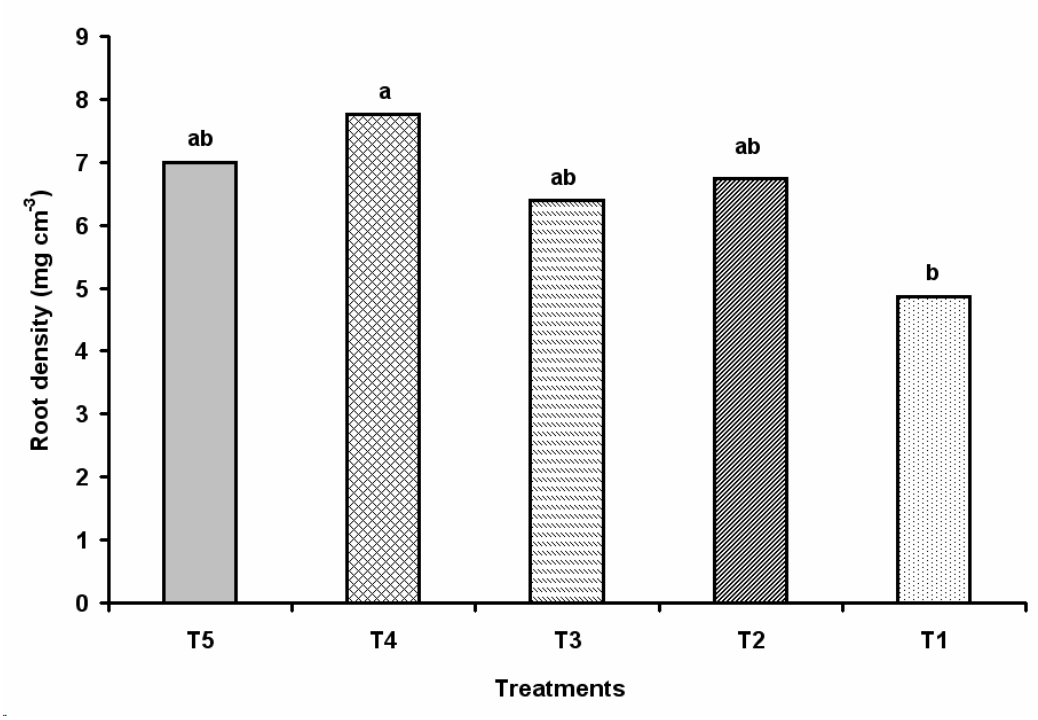


Figure 5. Avocado root density at the end of the experiment (n=3). Bars indicate means. Different letters (a, b) indicate significant differences (Duncan Test, $P \leq 0.1$).

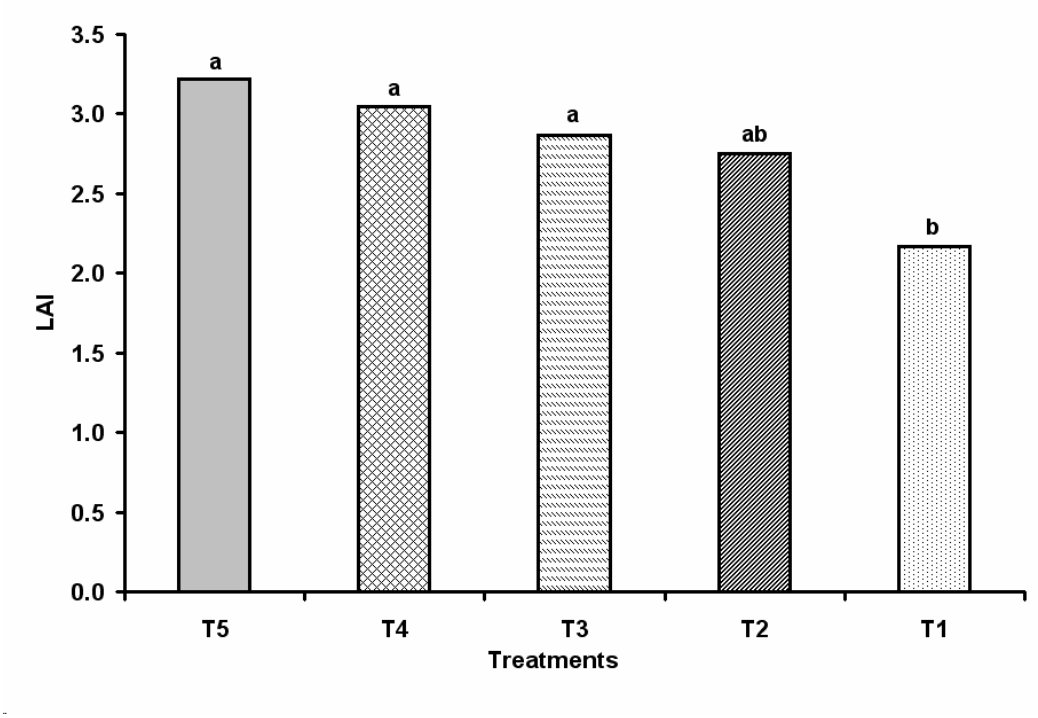


Figure 6. Avocado leaf area index (LAI), at the end of the 2005/2006 season. Bars indicate means. Different letters (a, b) indicate significant differences (Waller-Duncan Test, $P \leq 0.1$).

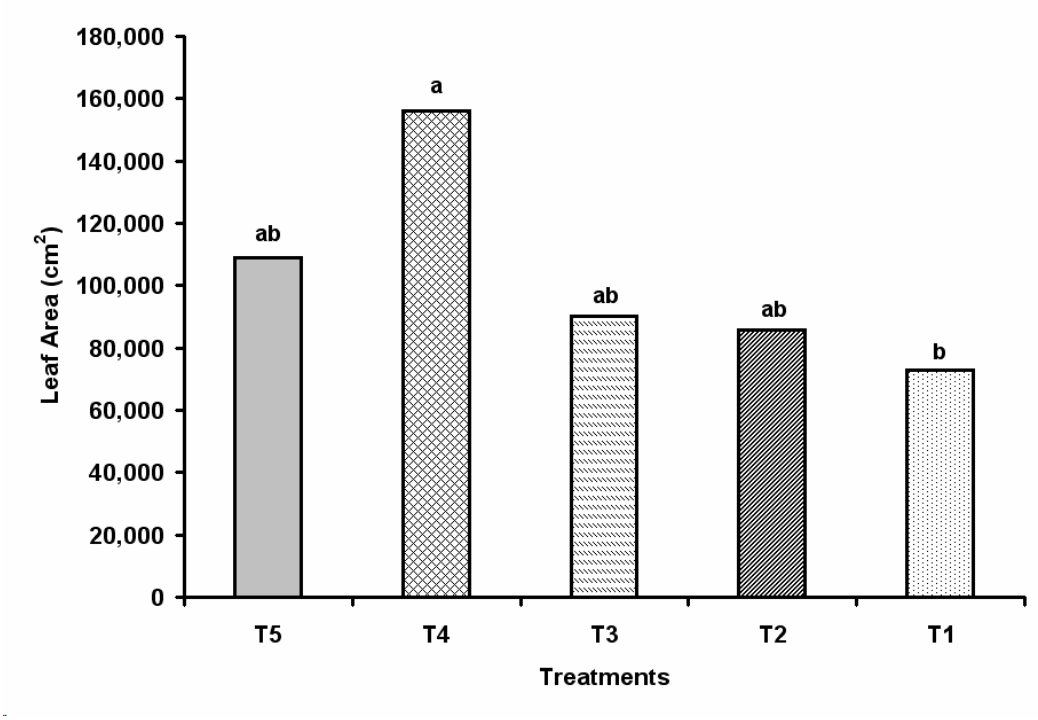


Figure 7. Avocado leaf area at the end of the 2006/2007 season. Bars indicate means. Different letters (a, b) indicate significant differences (Waller-Duncan Test, $P \leq 0.1$).

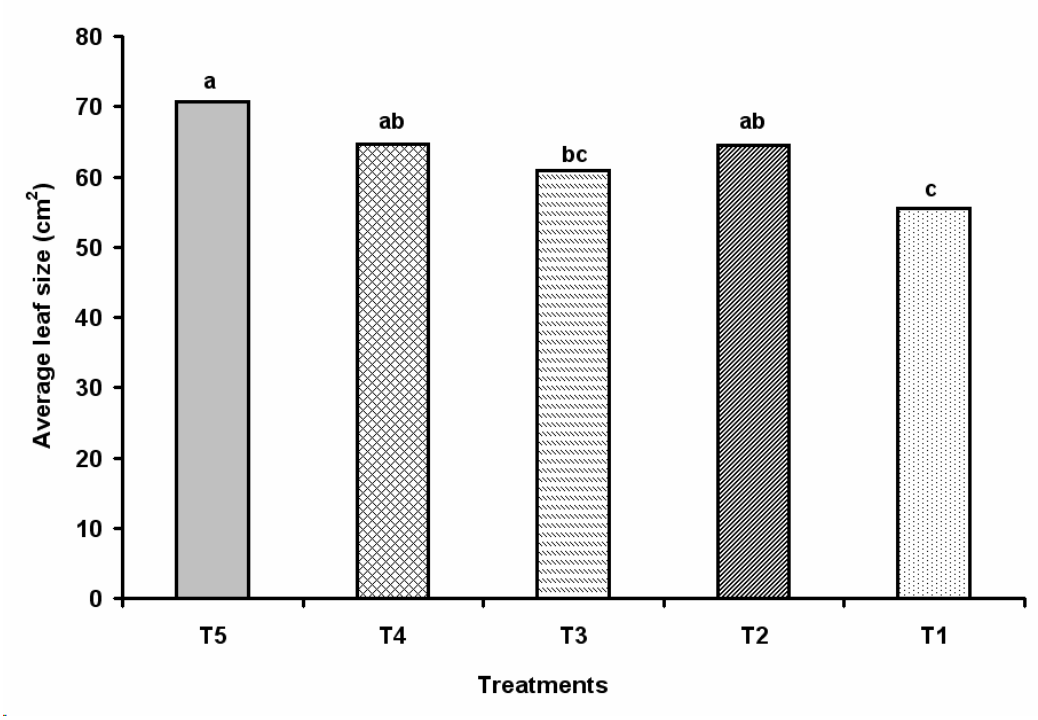


Figure 8. Average leaf size at the end of the 2006/2007 season. Bars indicate means. Different letters (a, b, c) indicate significant differences (Waller-Duncan Test, $P \leq 0.1$).

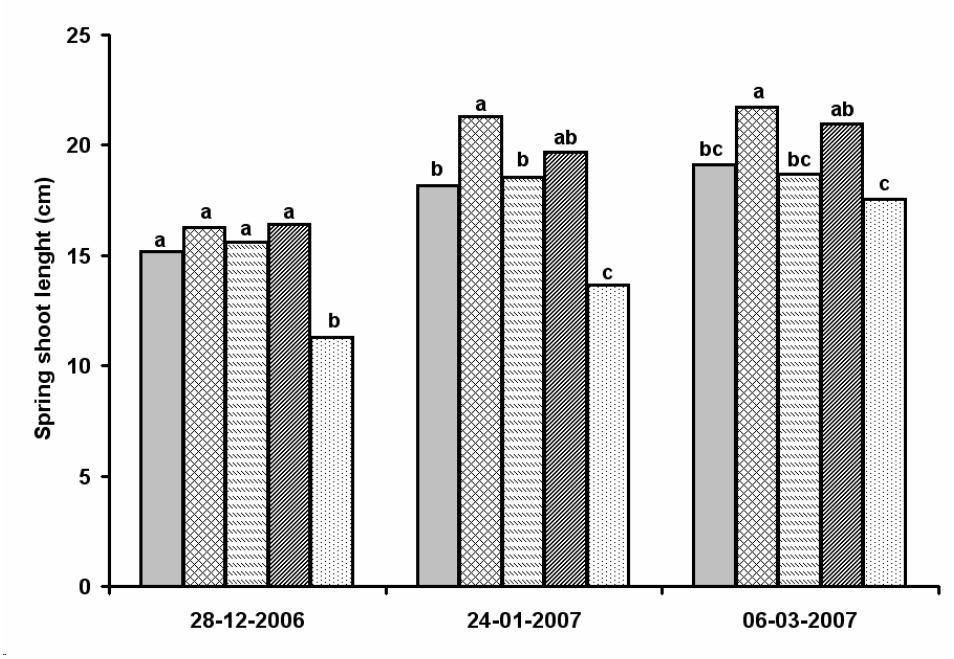


Figure 9. Length of spring shoots in December, January and March of season 2006/2007. Bars indicate means. Different letters (a, b, c) indicate significant differences (Waller-Duncan Test, P ≤ 0.1). T5, T4, T3, T2, T1.

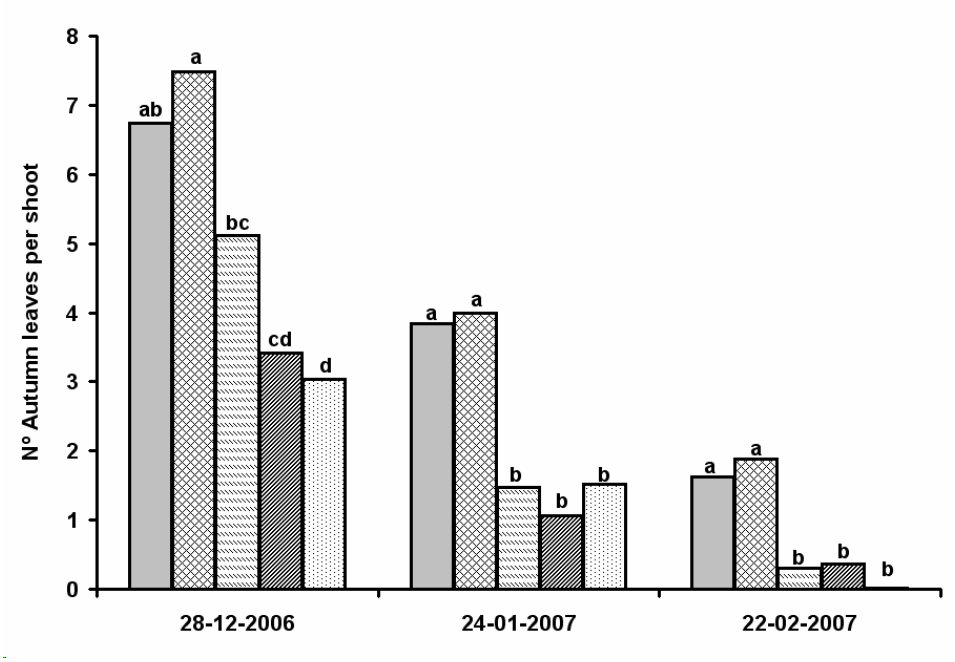


Figure 10. Number of leaves remaining on autumn shoots in December, January and February of 2006/2007. Bars indicate means. Different letters (a, b, c, d) indicate significant differences (Waller-Duncan Test, P ≤ 0.1). T5, T4, T3, T2, T1.

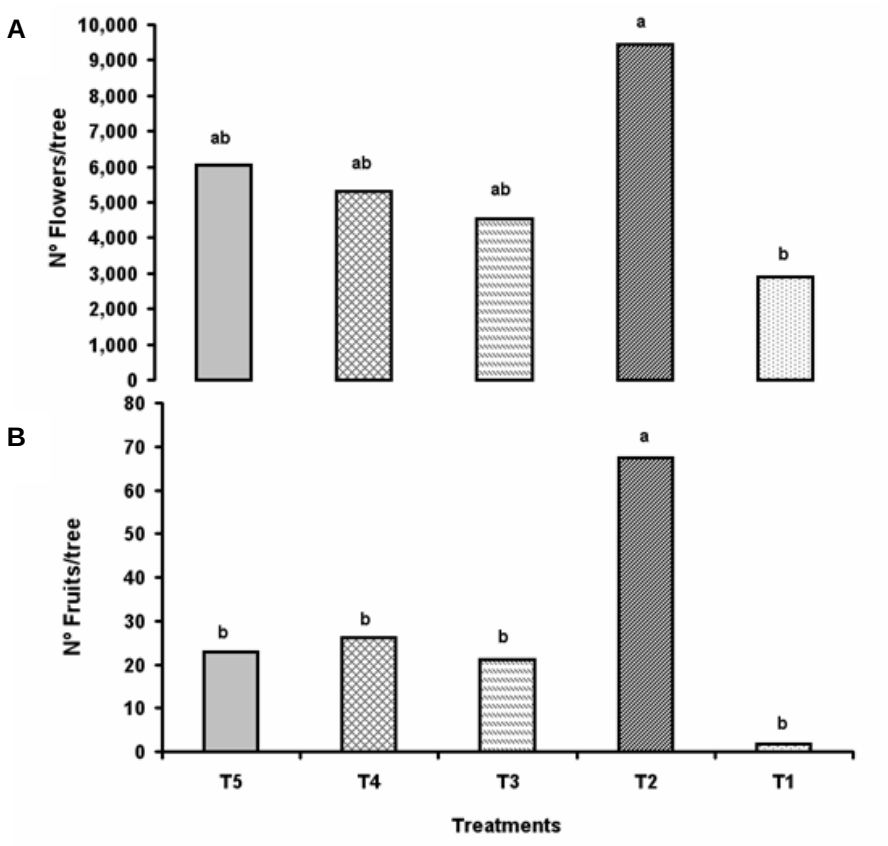


Figure 11. Number of flowers (A) and fruit (B) per tree at the end of the 2006/2007 season. Bars indicate means. Different letters (a, b) indicate significant differences (Waller-Duncan Test, P ≤ 0.1).

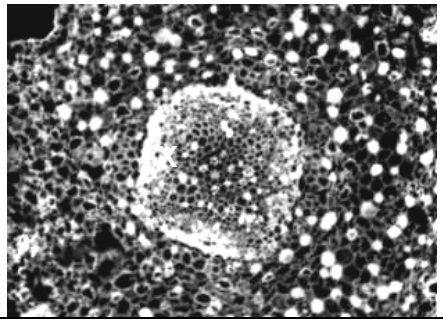
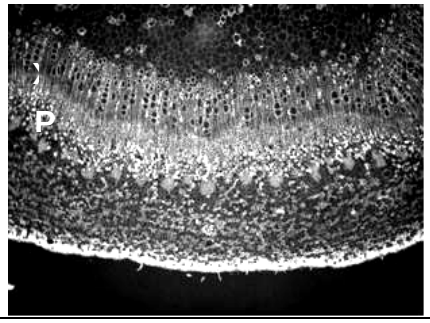
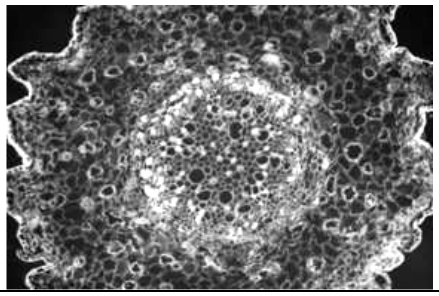
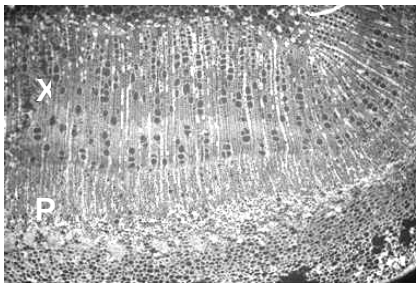
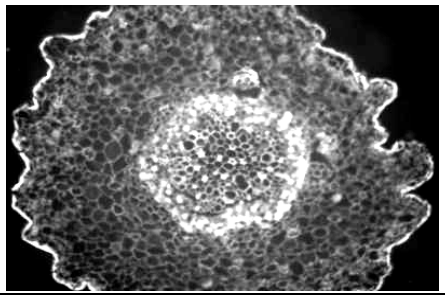
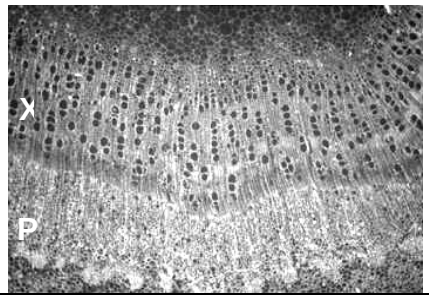
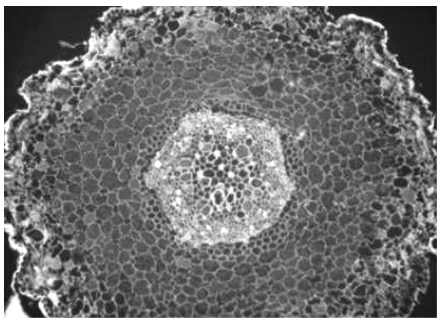
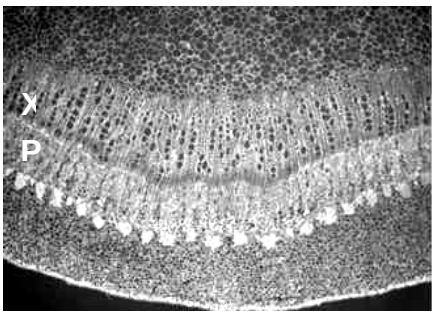
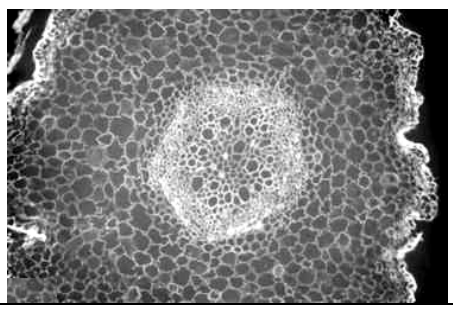
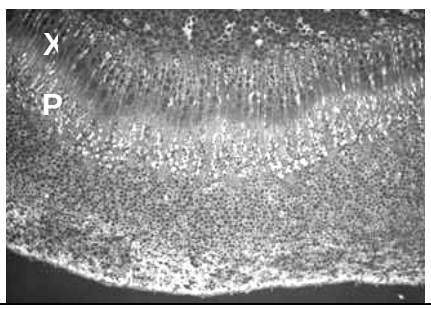
A 	**B** 
C 	**D** 
E 	**F** 
G 	**H** 
I 	**J** 

Figure 12. Vascular anatomy of root and shoot tissue. Photos A, C, E, G, I: Root sections of plants in treatments T1, T2, T3, T4 and T5 respectively. Photos B, D, F, H, J: Spring shoot sections of plants in treatments T1, T2, T3, T4 and T5 respectively “X” indicates xylem tissue; “P” indicates phloem tissue. Magnification was 100 X for root sections and 40 X for shoot sections.

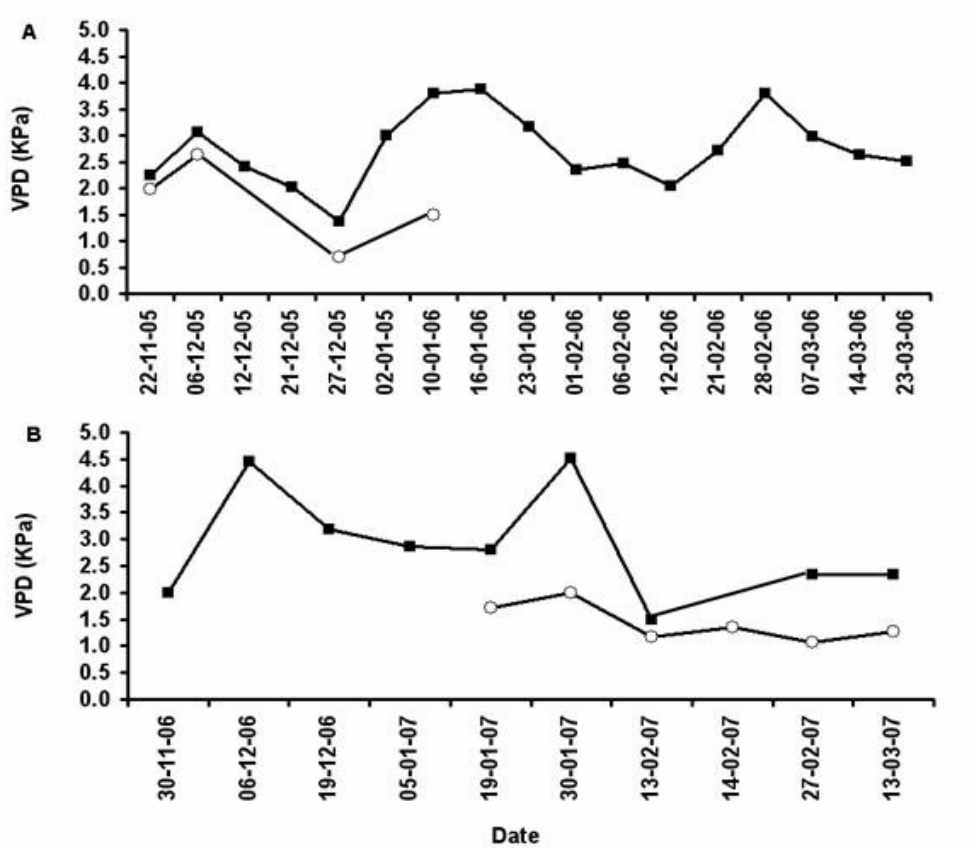


Figure 13. Vapor pressure deficit (VPD) during the 2005/2006 (A) and 2006/2007 (B) seasons. —■— VPD average at noon (13:00 – 15:00 hr) —○— VPD average during the morning (10:00 – 12:00).