Chapter 4

Original Article

Effect of hydrogen peroxide injection into heavy loam clay soil on plant water status, net CO_2 assimilation, biomass and vascular anatomy of avocado trees

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Abstract

In Chile, avocado (*Persea americana* Mill.) orchards are often situated in poorly drained soils that are low in oxygen and it is well known that an excess or lack of water during growth limits fruit production and quality of this species. The objective of this study was to evaluate the effect of the H_2O_2 injection into the soil on plant water status, net CO_2 assimilation, biomass and anatomy of avocado trees in a heavy clay loam soil with water content maintained at field capacity. Three-year-old 'Hass' avocado trees were planted outdoors in containers filled with heavy loam clay soil with soil moisture content kept at field capacity. Plants where divided into two treatments, those with H_2O_2 injected into the soil through subsurface drip irrigation to supply additional oxygen to the soil and plants in soil with no H_2O_2 added (control). In addition to determining physical soil characteristics, net CO_2 assimilation (A), transpiration (T), stomatal conductance (gs), stem water potential (SWP), shoot and root biomass, water efficiency use (WUE) and stem and root vascular anatomical characteristics were determined for plants in each treatment. 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. 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. The increased biomass of the aerial portions of plants in treated soil indicates that injection H_20_2 into heavy loam clay soils that may be a useful management tool in poorly aerated soil.

Key words: stomatal closure, net photosynthesis, root histology, oxygen injection, root hypoxia, subsurface drip irrigation.

INTRODUCTION

Avocado (*Persea americana* Mill.) 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. An excess or lack of water during growth limits avocado fruit production and quality, particularly if stress occurs between spring and the beginning of summer (Whiley *et al.*, 1988a; 1988b). Therefore, proper irrigation management in avocado orchards is necessary to insure adequate yield and fruit quality (Lahav and Whiley, 2002). In Chile, commercial avocado production has expanded to areas with poorly drained soils that are low in oxygen. Thus, root asphyxiation is an increasing concern to avocado growers when trees are planted on these marginal soils.

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 stomatal conductance (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). Stolzy *et al.* (1967) reported that when the oxygen diffusion rate (ODR) in the soil was lower than 0.17 μg cm^{-2} min^{-1}, there was 44 to 100% damage to roots of 'Mexicola' avocado trees. Ploetz and Schaffer (1987, 1989) observed a synergistic relationship between *Phytophthora* root rot and root hypoxia of avocado, resulting in considerably more root damage than caused by either stress alone.

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 the 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 relationship, development of root and stem aerenchyma), premature senescence and plant mortality (Drew, 1997; Kozlowski, 1997; Schaffer *et al.,* 1992).

At the present time, there are few methods to alleviate growth and production problems in avocado orchards due to lack of oxygen in the root zone. For other fruit tree species the use of flood-tolerant rootstocks (Schaffer and Moon, 1991; Schaffer, 1992; Striegler *et al.*, 1993; Nuñez-Elisea *et al.*, 1999, Gettys and Sutton, 2001) has been tested, but currently there are no flood-tolerant rootstocks

available for avocado trees. In Israel, few clonal rootstocks have been selected for soils with poor aeration (Ben-Ya´acov and Zilberstaine, 1999), but they have not been massively used in commercial orchards. Ben-Ya´acov *et al.* (2003) observed that West Indian and Mexican race rootstocks are more resistant than other rootstocks to poorly aerated soils. The 'Mexícola' rootstock, widely used in Chile, does not appear to be flood-tolerant.

In Chile, to reduce problems caused by root asphyxia in avocado orchards, trees are often planted on raised beds to increase the volume of soil occupied by roots and to improve drainage of irrigation and rain water. However, the use of raised beds can result in significant soil erosion of steep hillside orchards, and also can cause serious problems of water obstruction when heavy rains wash soil from raised beds into canals and streams.

Atmospheric enrichment of air surrounding the canopy with CO_2 has been used to enhance photosynthesis of several crop species, thereby stimulating plant growth and/or yield (Cave et al., 1981, Mortensen, 1984, Heij and van Uffelen, 1984). However, increasing soil gas concentration, especially the oxygen content of the rhizosphere to improve root metabolism is a less studied technique. For production of pepper plants, injecting air into the water through the subsurface drip irrigation (SDI) increased the oxygen concentration and partial pressure in the root zone as well as nitrogen absorption fixation and reduced plant stress leading to a 39% increase in fruit weight (Goorahoo *et al.*, 2001). Injection of air into the root zone also increased biomass and fruit production as well as water use efficiency of zucchini, soybean and cotton in heavy clay soils (Bhattarai *et al.,* 2004).

An alternative technique to the injection of oxygen into the soil is the use of hydrogen peroxide (H_2O_2). Hydrogen peroxide has been successfully utilized as an oxygen source for *in situ* remediation in a saturated aquifer (Zappi *et al.*, 2000). The natural decomposition of H_2O_2 provides molecular oxygen needed for aerobic metabolism of microorganism and roots. In the soil, H_2O_2 typically dissociates to produce one-half mole of dissolved oxygen per mole of H_2O_2 as shown by the equation:

$$H_2O_2 + H_2O \rightarrow 0.5\ O_2 + 2\ H_2O$$

This reaction can be catalyzed by iron or by the enzyme catalase, which is ubiquitous within aerobic organisms (Petigara *et al.*, 2002). Bhattarai *et al.* (2004) found that injecting H_2O_2 through the irrigation system into a heavy clay soil, that was saturated or at field capacity, increased biomass and yield of zucchini, soybean and cotton. Injecting H_2O_2 into the soil through the irrigation system may also be an effective method to alleviate potential root asphyxia of avocado trees in flood-prone or slow draining soils. If this method is successful for avocado, adequate productivity of this very flood-sensitive fruit crop may be sustained in areas where soils can become deficient in oxygen.

The objective of this study was to evaluate the effect of the injecting $H_2O_{2\ into}$ a low soil air content soil, through subsurface drip irrigation on plant water status, photosynthesis, biomass and anatomy of avocado trees.

MATERIALS AND METHODS

Plant material

The experiment was conducted with three-year-old 'Hass' avocado trees (*Persea americana* Mill.) grafted onto seedling 'Mexícola' avocado rootstock that were planted in a heavy clay loam soil in 200-liter containers. Containers were constructed to simulate raised beds by placing a white plastic mesh sustained by a structure of metal wire around a mass of soil. The soil was obtained from a fallow hillside with soil characteristics typical of avocado orchards planted on hillsides. Physical characteristics of the soil are shown in Table 1. The soil was steam sterilized and periodically treated with Metalaxil and Fosetil-Al fungicides to avoid *Phytophthora* root damage. Plants were irrigated with a localized irrigation system with 16 drippers (0.5 L h^{-1}) per plant. The irrigation frequency varied from 2 to 4 times per day (according to daily evapotranspiration) to maintain a relative constant water content near field capacity (-0.33 KPa). 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, 10 g P, 63 g K and 14 g Mg per plant.

Climatic conditions

The study site was located outdoors at the Regional Research Center, INIA La Cruz, in the la Cruz, Region of Valparaíso, Chile. The region has a humid marine Mediterranean climate with an average annual temperature of 14.5º C, a minimum average temperature of 5.2º C (July) and a maximum average temperature of 29.3º C (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 over 80% of this occurring from May to August.

Experimental design

Plants were divided into two treatments, a control treatment (T_0) and a H_2O_2 injection treatment (T_1). The To treatment consisted of trees irrigated frequently to maintain soil water content near field capacity and a seasonal average total soil aeration at 17%. The T_1 treatment consisted of trees irrigated frequently to maintain soil water content near field capacity and a seasonal average total soil aeration at 16%, plus a 1 ppm of H_2O_2 (50%) solution injected into the soil through the irrigation system at the end of the irrigation time. The injection time corresponded to the 10% of the total irrigation period. The total amount of H_2O_2 applied from November to March of the 2006/2007 summer season was approximately 700 ml per tree. The drippers were buried in the soil at a depth of 3 cm. The diluted H_2O_2 solution was injected from a 200-L container. The experimental set up is illustrated in Figure 1.

Data collection

Physical soil proprieties. Soil bulk density (BD) was determined by the cylinder method (Blake and Hartage, 1986). Final BD values were obtained from the average of 3 *in situ* measurements and one laboratory measurement. *In situ* soil measurements were complemented by laboratory measurement to confirm and to get a more accurate value. 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 compared with a laboratory air capacity measurement obtained using the methodology described by Carrasco (1997). The soil water content at *in situ* field capacity (FC) was determined six times during the experimental period using the method described by Cassel and Nielsen (1986); the six *in situ* obtained values and one laboratory measurements were pooled to obtain an average FC value. The FC was determined by subtracting of the percentage of macropores from the percentage of total porosity; the percentage of pores that remained corresponded to the total microporosity, which when full with water is the same as the water content at field capacity (Danielson and Sutherland, 1986). The soil water content at field capacity was determined by multiplying the gravimetric water content (□) 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; the remained space corresponds to the percentage of soil aeration.

Soil moisture 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).

The soil water content was also determined gravimetrically (ω) and volumetrically (θ) at a soil depth of 30 cm. The ω and θ water content were used to calibrate the

FDR probe and for FC determination. The ω of the soil was determined by the formula:

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

The θ of the soil was determined by multiplying ω with the BD value.

Soil oxygen diffusion rate (ODR), CO_2 and O_2 content. The soil oxygen diffusion rate (ODR) was measured on 3 dates throughout the experimental period (27/12/06, 24/01/07, 15/03/07) with a Pt-electrode (Oxygen Diffusion Meter, Eijkelkamp, Nederlands) as described by Letey and Stolzy (1964). Measurements were made during the morning hours with 2 irrigation pulses applied during that period; the Pt-electrode was inserted to a 15 cm depth in the soil. Soil air was sampled at a 30-cm depth by using a "point-source soil atmospheric sampler" as described by Staley (1980). Samples were collected on two dates (22/02/07 and 14/3/07) during the morning before the irrigation started. Samples were analyzed by injecting a 1 mL headspace sample into a Perkin-Elmer (Perkin-Elmer AutoSystem XL, Waltham, Massachusetts, USA) gas chromatograph 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 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} which is above the light saturation point for maximum net CO_2 assimilation of avocado leaves (Whiley and Schaffer, 1994). Reference CO_2 concentration in the leaf cuvette ranged from 375 to 400 ppm, and the air flow rate into the cuvette was set at 200 □mol s^{-1}.

Biomass. At the end of the study period, plants were harvested, aerial parts were separated from the roots and the fresh weights of leaves, shoots and wood were determined with a digital balance (Shanghai SP-300, Shanghai Huade Weighing Apparatus Co., Shanghai, China). 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 leaf shoot and wood dry weights were determined with an electronic balance (Transcell ESW-5M, Transcell Technology, Inc. Buffalo Grove, Illinois, USA). Root density was determined by subsampling roots with a 9.2-cm diameter, 1-m long tube sampler (Split tube sampler, Eijkelkamp, Netherlands) inserted into the soil (Ferreyra *et al.,* 1984; Ferreyra *et al.,* 1989). The depth of soil sampled for root density ranged between 40 and 45 cm, depending on the depth of soil in the sampled pot. Root samples were rinsed two times with tap water and finally with deionized water and 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 weighting all the leaves of each tree, 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). The sample was weighed with an electronic balance (Transcell ESW-5M, Transcell Technology, Inc. Buffalo Grove, Illinois, USA) and the total leaf area per plant was then estimated by multiplying the area/weight ratio of the 300 sub-sampled leaves per plant by the total leaf weight per plant.

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.

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 were expressed as means ± standard error (SE). The effects of treatment on ODR, soil CO_2 and O_2 concentrations, gs, T, SWP, A, leaf area, biomass, xylem: phloem ratio and root xylem vessel diameter were analyzed by a Bonferroni test using the SAS statistical software package (SAS Institute, Cary, North Carolina, USA).

RESULTS

Physical soil proprieties and soil air content

The physical soil characteristics measured are summarized in Table 1. From those data and the average . during the season (Figure 2) the average air content in the soil was determined, which is also shown in Table 1.

Soil moisture

Both volumetric soil water content ( ) (Figure 2) and gravimetric soil water content (□) (data not shown) were similar for each treatment throughout the experimental period. Volumetric soil water content tended to fluctuate more in the H_2O_2 injection treatment than in the control treatment (Figure 2).

Oxygen diffusion rate (ODR) CO_2 and O_2 concentrations in the soil

The average ODR throughout the experimental period is shown in Figure 3A. Although the ODR was higher in the T1 treatment, differences between treatments were not statistically significant ($P \geq 0.1$). There were no significant differences

between treatments in CO_2 or O_2 concentration in the soil (Figure 3B), although the soil O_2 concentration tended to be higher in the H_2O_2 treated soil.

Water relations and net CO_2 assimilation

There were no significant differences between treatments for gs T, A, or SWP ($P \geq 0.1$) (Table 2).

The higher gs and SWP observed during the morning than the afternoon may have been related to the VPD which averaged 1.6 KPa during the morning and averaged 3.03 KPa in the afternoon.

Biomass

Plants in the H_2O_2 injection treatment had significantly higher wood (shoots plus old wood) and leaf dry weights than plants in the control treatment ($P \leq 0.05$); the wood and leaf dry weights were 27% and 28% higher, respectively for the T1 than the To treatment (Figure 4). The T1 treatment also had a significantly higher in total plant dry weight than the control treatment ($P \leq 0.1$). There was no significant difference in root density (data not shown) or total root dry weight (Figure 4) between treatments.

Leaf area

Leaf area was significantly (43.1%) greater ($P \leq 0.05$) for plants in the T_1 treatment than for plants in the control treatment (Figure 5).

Water use efficiency

The WUE calculated from the total biomass divided to the total water supply showed statistical differences between treatments (Bonferroni test, $P \leq 0.05$).

Vascular anatomy of active roots and spring shoots

The xylem/phloem ratio in roots and shoots, the number of root xylem vessel, the average area of root xylem vessels and the total root xylem area is shown in Table 3. Although for almost all histological variables measured the roots and shoots of treated plants had a larger xylem system, the differences were only statistically significant for the spring shoot xylem/phloem ratio ($P \leq 0.15$).

An example of the anatomical differences in the xylem vessel features in roots and spring shoots of trees in both treatments is shown in Figure 7.

DISCUSSION

Injection of H_2O_2 into heavy loam clay soil kept at water content near field capacity during four months resulted in an increase in biomass of the aerial portion of avocado trees, and also a higher WUE. Similar results were reported for zucchini, soybean and cotton (Bhattarai *et al.*, 2004). A growth increase has also been observed in tomato plants cultivated in flooded conditions when H_2O_2 was added to the flood solution (Bryce *et al*., 1982). Similar results were also observed for maize, where there was a significant gain in biomass due to the application of H_2O_2 to soil with excellent structure and adequate irrigation rates (Melsted *et al.*, 1949).

Factors that most affect soil aeration are water content and physical soil properties such as texture and structure. The higher the soil water content, the lower the air volume and therefore greater limitations 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 may lead to continuous anaerobic conditions in the root zone (Letey, 1961; Blokhina *et al.*, 2003). Soils that are compacted or have limited drainage may also facilitate root hypoxia or anoxia, which negatively affects the normal metabolic functioning and growth of plants (Letey, 1961). Although in the present study, plants were established in a loam clay textured soil maintained near field capacity, and average soil air content lower than a critical level for avocado development of 17% (Ferreyra et al., 2007) (Table 1), measured ODR never reached limiting values for avocado (0.17 μg cm^{-2} min^{-1}, Stolzy *et al.,* 1967). Also, laboratory analysis of *Phytophthora sp* in soil samples taken at the end of the experimental period indicated no presence of this soil pathogen.

Although the H_2O_2 injection into the soil significantly increased biomass of avocado trees, there was no significant effect of H_2O_2 injection on gs, T, A or SWP. The measured values of SWP were very high compared to values of water stressed avocado trees, which have been reported to be from -1.2 to -1.0 KPa (Sterne *et al.,* 1977; Bower, 1978; Scholefield *et al.*, 1980; Whiley *et al.*, 1988a). Although it is well known that prolonged flooding causes stomatal closure and lowers transpiration in avocado trees (Schaffer and Ploetz, 1989; Schaffer *et al.,* 1992; Schaffer, 1998; Schaffer and Whiley, 2002), in this study it was not possible to

observe a significant improvement of gs and T as a result of a soil oxygen supply by the addition of H_2O_2 to the soil. The same phenomenon was observed by Bhattarai *et al.* (2004) for zucchini, soybean and cotton.

The greater biomass of plants in the H_2O_2 soil injection treatment than of plants in the control treatment should have been the result of greater net CO_2 assimilation by plants in the treated soil. However, there was no significant difference in the measured net CO_2 assimilate rate between the H_2O_2 soil injection treatment and control treatment (Table 2). However, it must be pointed out that in this study, net CO_2 assimilation was measured on a leaf area basis. Trees in the H_2O_2 soil injection treatment had a greater total leaf area than trees in the control treatment (Figure 5). Therefore, on a whole-plant basis, net CO_2 assimilation was most likely significantly higher for plants in the H_2O_2 injection treatment than for plants in the control treatment, presumably accounting for the greater biomass of plants in soil injected with H_2O_2. Also, an extra oxygen supply due to the hydrogen peroxide injection to the soil may enhance the ATP production in roots resulting in increased energy for plant metabolic processes, including growth. Although soil measurements of O_2, CO_2 and ODR did not show a significant effect of the H_2O_2 treatment, it have to be point out that measurement were not continues, and were performed three times during the measurement season (Figure 3). Thus it was not possible to see changes in O_2 and CO_2 composition or ODR, within a day with several irrigation pulses. For this it may be presume that changes would be momentary, and those specific changes in soil O_2 concentration could generate a better root oxygenation and thus ATP production.

For avocado trees in this study, it was observed that only the biomass of the aerial portion of the plant was increased as a result of H_2O_2 injection into the soil, whereas the root density and biomass were not affected by the treatment. In contrast, Bhattarai *et al.* (2004) reported that root biomass was significantly higher in plants in treated soil compared to control plants. Histological examination of avocado roots revealed larger xylem vessel average diameter for plants in the H_2O_2 injection treatment than in plants in the control treatment, which may indicate that H_2O_2 injection into the soil leads to an improvement of root anatomy in avocado more than growth of the root system; however the difference in xylem anatomy between treatments was not statistically significant, probably due to the high variability in the size of xylem vessels among individual plants.

Larger xylem vessels would increase the capacity for water conduction, allowing for better development of the aerial portion of the plant. According to Poiseuille's law, the water flow in a vascular conduit is related to its radius by a factor to the fourth power, meaning that a slight increase in xylem vessel diameter could result in a significant increase in water conductivity through the xylem. Further indirect evidence of increased water conductivity in avocado trees in the H_2O_2 injection treatment was also observed in the histological sections of the spring shoots, where a significantly higher xylem/phloem ratio was observed. According to several researchers (Kozlowski, 1997; Hsu *et al.,* 1999; Liao and Lin, 2001), plants in soils that are low in oxygen exhibit a reduction in the xylem/phloem ratio. Therefore, in the present study with avocado, the increased xylem/phloem ratio in plants in the H_2O_2 soil injection treatment may be an indirect indication that H_2O_2 increased the

oxygen content of the root zone. Although direct measurement of O_2 in the soil did not show differences between treatments (Figure 3), it must be pointed out that direct O_2 measurements were only made at a specific point in time and may not accurately reflect the cumulative O_2 concentration in the soil throughout the entire experimental period.

In summary, injecting H_2O_2 through the irrigation system into a soil with low air content may have potential as a method for improving soil oxygen content in a heavy clay loam soil as indicated by increased leaf area and aerial biomass for plants in treated soil. However, the present study was conducted with trees in containers. Therefore, 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 hydrogen peroxide on a larger scale in orchards.

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Table 1. Physical characteristics of heavy loam clay soil.

Tmt	Soil texture	FC ω (%)	BD g cm^{-3}	Porosity (%)	Microporosity (%)	Air capacity (%)	Average Air Content (%)
To	Loam Clay	20.0	1.4	46.0	28.6	17.5	17.4
T1	Loam Clay	18.3	1.5	43.8	27.3	16.5	15.6

Values represent means obtained from *in situ* and laboratory measurements. FC = field capacity, BD = bulk density.

Table 2. Effect of H_2O_2 injection into heavy clay loam soil at field capacity on water relations and physiological variables of avocado plants.

	AM		**PM**	
	T_0	**T_1**	**T_0**	**T_1**
gs (cm s^{-1})	0.49 ± 0.03 a	0.51 ± 0.03 a	0.29 ± 0.02 a	0.28 ± 0.02 a
T (µg cm^{-2} s^{-1})	4.5 ± 0.18 a	4.8 ± 0.25 a	5.3 ± 0.24 a	4.8 ± 0.22 a
SWP (KPa)	-0.6 ± -0.05 a	-0.63 ± -0.04 a	-0.92 ± -0.04 a	-0.88 ± -0.04 a
A (µmol s^{-1} m^{-2})	4.86 ± 0.48 a	5.38 ± 0.58 a		

Values are means ± SE. T_0 = the control treatment, T_1 = the soil H_2O_2 injection treatment, gs = stomatal conductance, T = transpiration, SWP = soil water potential, A = net CO_2 assimilation, AM = 9:30 to 11:00 hr, PM = 13:00 to 15:00 hr. Same letters (a) indicate no significant difference between treatments (Bonferroni test, $P \leq 0.1$) during the AM or PM measurement time.

Table 3: Effect of the H_2O_2 injection to heavy clay loam soil at field capacity on root and shoot vascular anatomy.

	T_0	**T_1**
Spring shoot xylem/phloem relationship	1.5 b	2.0 a
Root xylem/phloem relationship	1.5 a	1.4 a
Number of root xylem vessels	59. 8 a	63.6 a
Average xylem vessel area (μm^2)	2,392.5 a	2,418.4 a
Xylem total area (μm^2)	146,630 a	154,202 a

T_0 = control treatment, T_1 = soil H_2O_2 injection treatment. Values are means. Different letters indicate significant differences between treatments (Bonferroni test, $P \leq 0.15$).

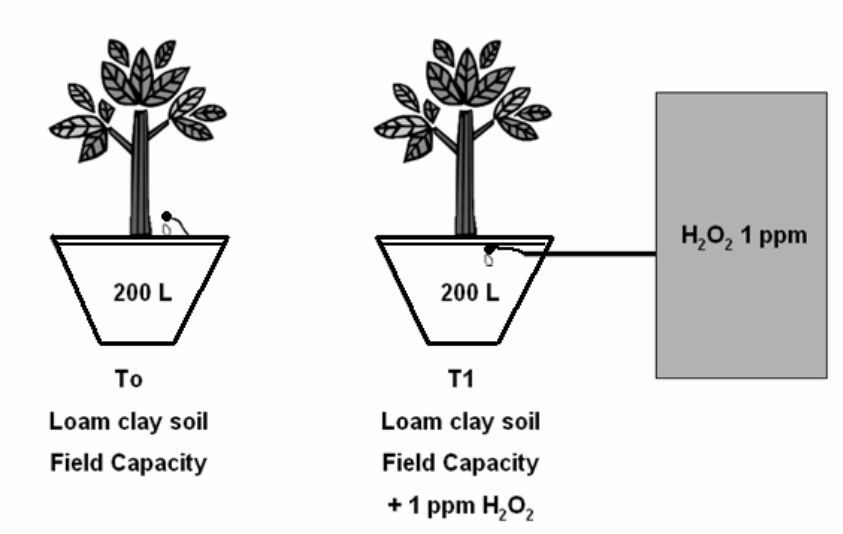

Figure 1. Experimental set up to test the effects of H_2O_2 injection into the soil on avocado trees

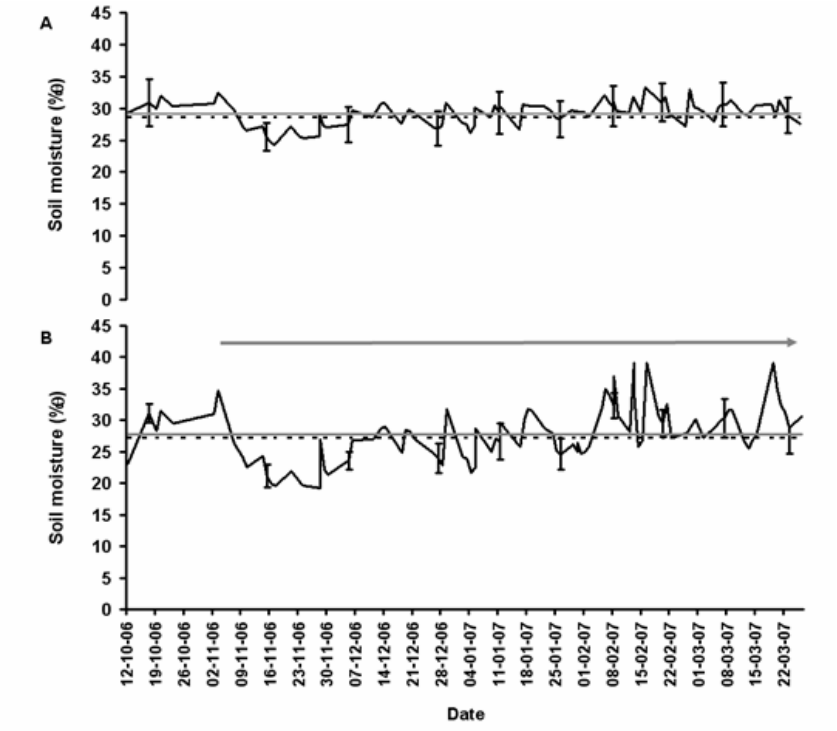

Figure 2: Volumetric soil water content (θ) at a soil depth of 30 cm during the experimental period. A) Control treatment (To). B) H_2O_2 injection treatment (T1). Graphed values correspond to means. ——— Average soil moisture during the experimental season, •••• Soil moisture at field capacity value calculated from soil physical characteristics measurements. Standard Error bars are plotted at 10-measurement (date) intervals. The grey arrow indicates the experimental period.

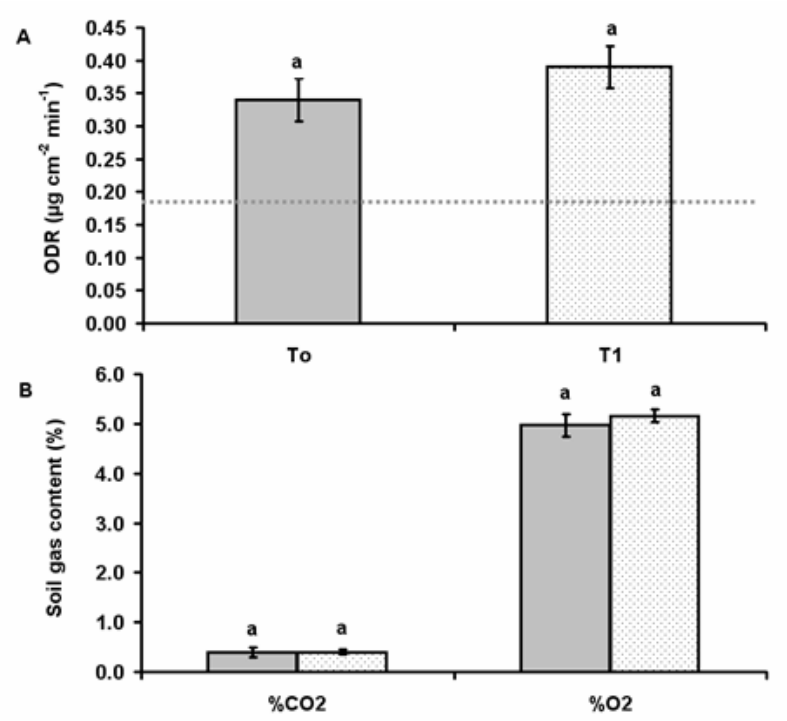

Figure 3. A) Oxygen diffusion rate (ODR) averaged from 3 measurement dates and B) CO_2 and O_2 soil content averaged from 2 measurement dates. ▭ Control treatment, (To) and ▨ H_2O_2 soil injection treatment (T1). Values represent treatment means ± SE. The dotted line indicates the limiting ODR value reported for avocado plants (Stolzy *et al*., 1967). The same letters between treatments indicate no significant differences (Bonferroni Test, $P \leq 0.1$).

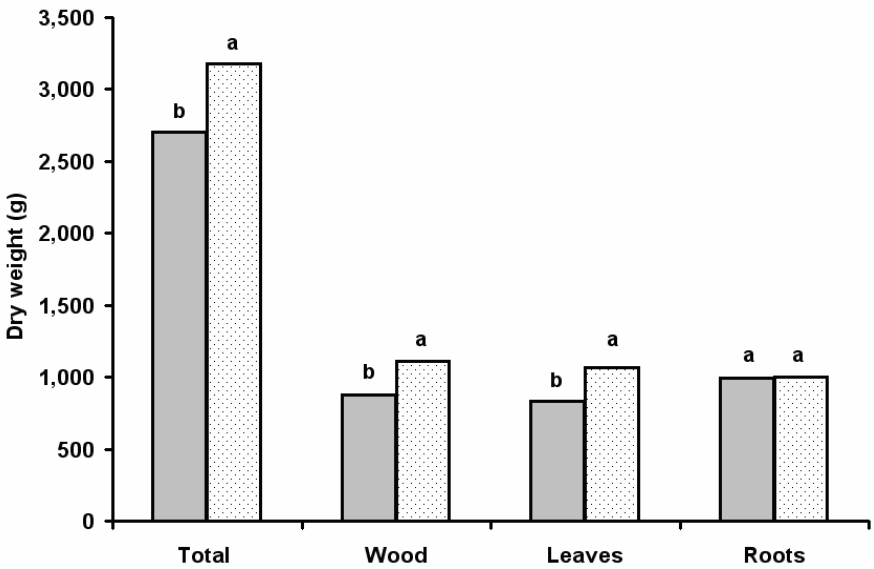

Figure 4. Avocado plant dry weight at the end of the experiment. Bars indicate means. Different letters indicate significant differences (Bonferroni Test, $P \leq 0.1$ for total and roots and $P \leq 0.05$ for wood and leaves). Control (To), Soil with H_2O_2 injection (T1).

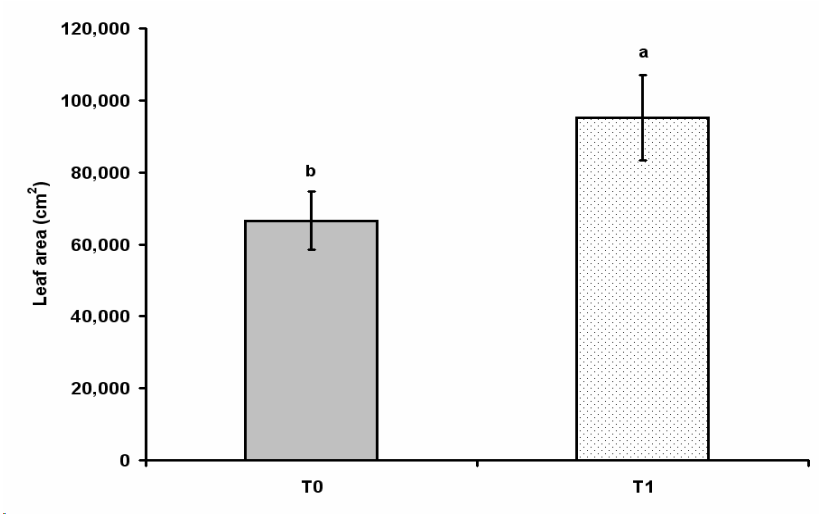

Figure 5. Leaf area at the end of the experiment. Bars indicate means ± SE. Different letters indicate significant differences between treatments (Bonferroni test, $P \leq 0.05$).

Control treatment (To) and soil H_2O_2 injection treatment (T1).

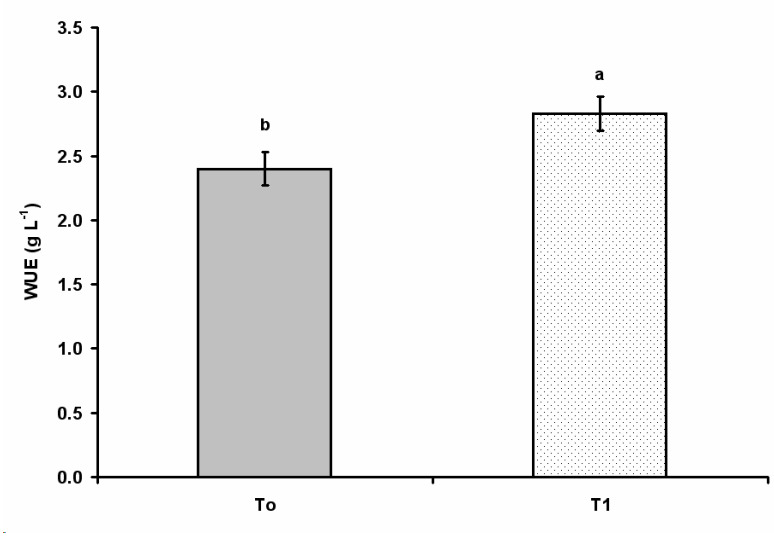

Figure 6. Water use efficiency (WUE) obtained from total biomass. Bars indicate means ± SE. Different letters indicate significant differences between treatments (Bonferroni test, $P \leq 0.05$).

Control treatment (To) and soil H_2O_2 injection treatment (T1).

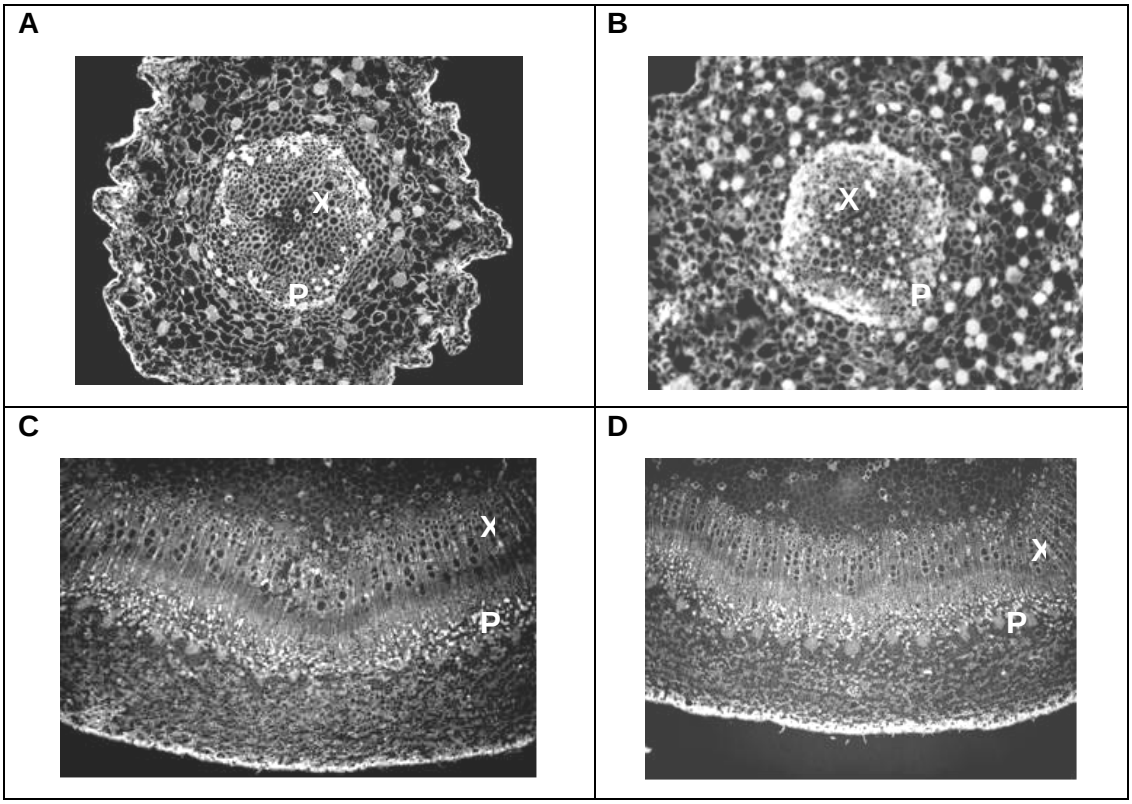

Figure 7. Anatomical differences in root and shoot vascular tissue. A) Root section from a plant in the soil H_2O_2 injection treatment. B) Root section from a plant in the control treatment. C) Spring shoot section from a plant in the soil H_2O_2 injection treatment. D) Spring shoot section from a plant in the control treatment. “X” indicates xylem tissue; “P” indicates phloem tissue. Magnification for root sections was 100 X and for shoot sections was 40 X.