SCIENTIFIC NOTE

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EFFECT OF INJECTING HYDROGEN PEROXIDE INTO HEAVY CLAY LOAM 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 located in poorly drained, low-oxygen soils, situation which limits fruit production and quality. The objective of this study was to evaluate the effect of injecting soil with hydrogen peroxide (H_2O_2) as a source of molecular oxygen, on plant water status, net CO_2 assimilation, biomass and anatomy of avocado trees set in 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 moisture content sustained at field capacity. Plants were divided into two treatments, (a) H_2O_2 injected into the soil through subsurface drip irrigation and (b) soil with no H_2O_2 added (control). Stem and root vascular anatomical characteristics were determined for plants in each treatment in addition to physical soil characteristics, net CO_2 assimilation (A), transpiration (T), stomatal conductance (gs), stem water potential (SWP), shoot and root biomass, water use efficiency (plant biomass per water applied [WUEb]). Injecting H_2O_2 into the soil significantly increased the biomass of the aerial portions of the plant and WUEb, but had no significant effect on measured A, T, gs, or SWP. Xylem vessel diameter and xylem/phloem ratio tended to be greater for trees in soil injected with H_2O_2 than for controls. The increased biomass of the aerial portions of plants in treated soil indicates that injecting H_2O_2 into heavy loam clay soils 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 trees are very sensitive to waterlogging (Schaffer *et al.*, 1992; Whiley and Schaffer 1994; Schaffer and Whiley, 2002). 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 low-oxygen soils. Thus, root asphyxiation is a growing concern for avocado growers when trees are planted on these marginal soils.

In heavy clay, compacted, 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 reduced stomatal conductance (gs), transpiration (T), net CO_2 assimilation (A), root and shoot growth, as well as inhibited leaf expansion, moderate to severe stem and leaf wilting, leaf abscission, and root necrosis (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 occurred 44 to 100% damage to roots of 'Mexicola' avocado trees. Ploetz and Schaffer (1987) observed a synergistic relationship between *Phytophthora* root rot and avocado root hypoxia, resulting in considerably more root damage than that caused by either stress alone.

Root anoxia or hypoxia often results in increased concentrations of 1-aminocyclopropane-1-carboxylic

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acid (ACC), ethylene and abscisic acid (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 involved as a stimulus for stomatal closure in flooded plants (Kramer and Boyer, 1995; Else *et al.,* 1995; Kozlowski, 1997). Finally, 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, adventitious roots or root and stem aerenchyma, and alterations in the relationship between xylem andphloem), premature senescence, and plant mortality (Schaffer *et al.,* 1992; Drew, 1997; Kozlowski, 1997).

At the present time, few methods exist to alleviate growth and production problems due to lack of oxygen in the root zone in avocado orchards. For other fruit tree species, the use of flood-tolerant rootstocks (Schaffer and Moon, 1991; Striegler *et al.*, 1993; Gettys and Sutton, 2001) has been tested, but there are currently no flood-tolerant rootstocks available for avocado trees. In Israel, a 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. 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 thus improving drainage of irrigation and rain water. However, the use of raised beds can result in significant soil erosion of steep hillside orchards and can cause water obstruction problems when heavy rains wash soil from raised beds into canals and streams.

Atmospheric enrichment of air surrounding the canopy with CO_2 or O_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 the oxygen content of the rhizosphere to improve root metabolism is a less-studied technique. In pepper plant production (*Capsicum annuum* L. var. *annuum*), injecting air into the water through subsurface drip irrigation (SDI) increased the oxygen concentration and partial pressure in the root zone, as well as N absorption fixation, and reduced plant stress leading to a 39% increase in fruit weight (Goorahoo *et al.*, 2001). Injecting air into the root zone also increased biomass and fruit production, as well as water use efficiency of zucchini (*Cucurbita pepo* L. subsp. *pepo*), soybean (*Glycine max* (L.) Merr.), and cotton (*Gossypium hirsutum* L.) in heavy clay soils (Bhattarai *et al.,* 2004).

An alternative technique to injecting 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 microorganisms and roots. H_2O_2 typically dissociates in the soil 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 catalase enzyme, which is ubiquitous in 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, which was saturated or at field capacity, increased biomass and yield of zucchini, soybean, and cotton after treatments of 1, 3, and 4 months, respectively. 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 are at risk of becoming deficient in oxygen.

The objective of this study was to evaluate the effect of injecting H_2O_2 into a low 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 3-year-old 'Hass' avocado trees (*Persea americana* Mill.) grafted onto 'Mexícola' avocado rootstock seedlings that were planted in a heavy clay loam soil in 200-L containers. These were constructed 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 typical soil characteristics 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 fosetyl-Al fungicides to avoid *Phytophthora* root damage. Plants were irrigated with a localized irrigation system consisting of 16 drippers (0.5 L h^{-1}) per plant. The irrigation frequency varied from 2 to 4 times per day (according to daily evapotranspiration) in order to maintain a relatively constant water content near field capacity (-0.33 kPa). Irrigation water and soil analyses indicated no salt or carbonate problems. Each tree was fertilized once a week from October to March with 145 g N, 10 g P, 63 g K, and 14 g Mg.

Climatic conditions

The outdoor study site was located at the INIA Regional Research Center in La Cruz (32º49' S; 71º13' W), Valparaíso Region, Chile. The region has a humid marine

Table 1. Physical characteristics of heavy loam clay soil.

Tmt	Soil texture	BD	FC ω	Porosity	Microporosity	Air capacity	Air content
		g cm^3	%				
T_0	Loam clay	1.4	20.0	46.0	28.6	17.5	17.4
T_1	Loam clay	1.5	18.3	43.8	27.3	16.5	15.6

Values are means of *in situ* and laboratory measurements; FC: field capacity; BD: bulk density.

Mediterranean climate with a mean annual temperature of 14.5 ºC, a minimum mean temperature of 5.2 ºC (July), and a maximum mean temperature of 29.3 ºC (January). The nine-month period from September to May is frost-free. The mean total annual precipitation in the region is 328.5 mm with over 80% of it occurring from May to August.

Experimental design

The experiment was carried out from November 2006 to March 2007 (spring-summer season). Plants were divided into two treatments, a control treatment (T_0) and an H_2O_2 injection treatment (T_1). The T_0 treatment consisted of frequently irrigated trees to maintain soil water content near field capacity and a seasonal mean of total soil aeration at 17%. The T_1 treatment consisted of frequently irrigated trees to maintain soil water content near field capacity and a seasonal mean of total soil aeration at 16%, plus a 1 mg kg^{-1} of H_2O_2 (50%) solution injected into the soil through the irrigation system at the end of the irrigation period. The injection time corresponded to 10% of the total irrigation period. The H_2O_2 concentration was chosen following the methodology described by Bhattarai *et al*. (2004). 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 water volume applied each day was calculated from estimated crop evapotranspiration, using a Class A evaporation pan with a coefficient (Kb) of 0.8 and crop coefficient (Kc) of 0.72 (Gardiazábal *et al*., 2003). 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 design was a completely randomized design with five replications per treatment.

Data collection

Soil physical properties. Soil bulk density (BD) was determined by using the cylinder method (Blake and Hartage, 1986). Final BD values were the mean of three *in situ* measurements and one laboratory measurement. Total soil porosity was calculated as described by Danielson and Sutherland (1986) using a real density soil 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 explained by Ball and Smith (1991). The *in situ* value was compared with a laboratory air capacity measurement obtained using the methodology illustrated by Carrasco (1997). The soil water content at field capacity (FC) was determined six times *in situ* during the experimental period by means of the Cassel and Nielsen (1986) method; the six *in situ* laboratory measurements were pooled to obtain a mean FC value. The FC was calculated by subtracting the percentage of macropores from the percentage of total porosity; the remaining percentage of pores corresponded to the total microporosity, which when filled with water equals the water content at field capacity (Danielson and Sutherland, 1986). The soil water content at field capacity was calculated by multiplying the gravimetric water content (ω) by the BD value as expressed by Cassel and Nielsen (1986).

Soil moisture. Soil water content was measured daily by means of frequency domain reflectometry (FDR) at a soil depth of 30 cm 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 water content was used to calibrate the FDR probe soil moisture data (mm) and FC determination in each soil replication. Calibration equation examples were ω = 1.2089 x mm - 14.795 (R^2 = 0.86) and ω = 0.9336 x mm - 7.6278 (R^2 = 0.86), for T_1 and T_0 soils respectively. The ω of the soil was determined by the formula: ω = [(wet soil weight - dry soil weight)/dry soil weight] ∗ 100. The soil θ was computed by multiplying ω with the BD value.

Soil air content. Volumetric air content of the soil was calculated as described by Benavides (1994). In brief, soil air content was calculated as the result of total porosity minus the mean of soil water content during the experimental period (% Soil air content = % Porosity - θ).

Soil oxygen diffusion rate (ODR), CO_2 and O_2 content. The soil oxygen diffusion rate (ODR) was measured

on three dates throughout the experimental period (12/27/07, 01/24/07, and 03/15/07) with a Pt-electrode (Oxygen Diffusion Meter, Eijkelkamp, Netherlands) as illustrated by Letey and Stolzy (1964). Measurements were made during the morning hours with two irrigation pulses applied during that period; the Pt-electrode was inserted at a depth of 15 cm into the soil. Soil air was sampled at a depth of 30-cm by using a "point-source soil atmospheric sampler" as found in Staley (1980). Samples were collected on two dates (22/02/07 and 14/03/07) during the morning before starting to irrigate. Samples were analyzed by injecting a 1 mL headspace sample into a gas chromatograph (Perkin-Elmer AutoSystem XL, Waltham, Massachusetts, USA) equipped with a thermal conductivity detector (TCD) 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, Lincoln, Nebraska, USA) as demonstrated in Prive and Janes (2003). Both gs and T were measured at 2-wk intervals during the morning (09:00-11:00) and in the afternoon (13:00-16:00). Three mature, sun-exposed leaves from each plant were measured. Stem (xylem) water potential (SWP) was calculated at the same frequency as for gs and T, whereas for SWP determinations, three sun-exposed leaves per tree were covered with plastic and aluminum foil and excised 30 min after covering (Meyer and Reicosky, 1985). The SWP of the excised leaves was immediately measured with a pressure chamber in accordance with Scholander *et al.* (1965). Leaves were excised and SWP was measured during the morning (09:00-11:00) and in the afternoon (13:00-16:00).

Net CO_2 assimilation. Net CO_2 assimilation (A) was measured once a month with an open system Li-6400 portable gas analyzer (LI-COR, Lincoln, Nebraska, USA). Measurements were made from 10:00 to 13:00 by taking three mature leaves from each plant, all of similar size and light exposure, located in the middle of a spring shoot. Measurements were made with 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 airflow rate into the cuvette was set at 200 µmol s^{-1}.

Plant water use efficiency (WUEb). 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 (Bhattarai *et al.*, 2004).

Biomass and leaf area. Plants were harvested at the end of the study period when aerial parts were separated from the roots and the fresh weights of leaves, shoots, and wood were determined using a digital scale (Shanghai SP-300, Shanghai Huade Weighing Apparatus, Shanghai, China). Shoot refers to the current season's branches whereas wood refers to the older trunk and branches. Tissues were then oven-dried at 70 ºC for 3 days when leaf shoot and wood dry weights were calculated with an electronic scale (Transcell ESW-5M, Transcell Technology, Buffalo Grove, Illinois, USA). Root density was found by subsampling roots with a 4.6-cm diameter, 1-m long tube sampler (Split tube sampler, Eijkelkamp, Netherlands) inserted into the soil (Ferreyra *et al.,* 1984; 1989). Depending on the depth of the soil in the sampled pot, the depth of the soil sampled for root density ranged between 35 and 45 cm. Root samples were washed and separated from the soil in order to determine fresh weights. Roots were then oven-dried at 70 ºC for 3 days and root dry weight and density (g cm^{-3}) were measured for each plant. Total root dry weight was estimated by multiplying the root density by the total soil volume in each pot.

After detaching and weighing 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 scale (Transcell ESW-5M, Transcell Technology, 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 three 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 water-soluble wax. Wax blocks with a thickness of 6-18 µm 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, New York, USA). Sections were stained with safranin and fast green.

Histological sections were observed at 100X for roots and 40X for shoots using a Leitz orthoplan optical microscope with an incorporated semiautomatic camera (Leitz, Wetzlar, Germany). Images were analyzed for mean 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 mean number of vessels per root in xylem tissue. To work out the xylem/phloem ratio in shoots and roots, both 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. Temperature and relative humidity were continuously monitored throughout the experiment with a Hobo datalogger (Onset Computer Corporation, Pocasset, Massachusetts, USA) and vapor pressure deficit was calculated using these variables.

Data Analysis

Data was expressed as means ± standard error (SE). Effects of treatment on ODR, CO_2 and O_2 soil concentrations, gs, T, SWP, A, leaf area, biomass, xylem/phloem ratio, and root xylem vessel diameter were analyzed utilizing ANOVA and the Bonferroni test using the SAS statistical software (SAS Institute, 1989).

RESULTS AND DISCUSSION

Water content and physical soil properties such as texture and structure are the factors that most affect soil aeration. The higher the soil water content, the lower the air volume, and greater are the limitations to the aerobic metabolism of the roots (Letey, 1961; Blokhina *et al.,* 2003). Fine textured soils have a greater water retention capacity 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). The physical soil characteristics measured in this experiment are summarized in Table 1. The mean air content in the soil was determined from this data as well as the mean gravimetric soil water content (θ) during the season (Table 1). Both volumetric soil water content (ω) and θ (data not shown) were similar for each treatment throughout the experimental period. Furthermore, laboratory analysis of *Phytophthora* in soil samples taken at the end of the experimental period did not indicate the presence of this soil pathogen.

Although plants in the present study were set in a loam clay-textured soil maintained near field capacity with average soil air content lower than 17% (Table 1), ODR never reached limit values for avocado (0.17 μg cm^{-2} min^{-1}, Stolzy *et al.,* 1967). The mean ODR throughout the experimental period is shown in Table 2. While ODR was higher in the T_1 treatment, differences between treatments were not statistically significant ($P > 0.1$). Moreover, no significant differences were found between treatments in CO_2 or O_2 concentrations in the soil (Table 2), although the O_2 soil concentration tended to be higher in soil treated with H_2O_2.

Table 2. Oxygen diffusion rate (ODR) and soil gas content.

Treatment	ODR	$\%O_2$	$\%CO_2$
	μg cm^{-2} m^{-1}		
Control	0.34 ± 0.03	4.97 ± 0.23	0.39 ± 0.09
H_2O_2 injection	0.39 ± 0.04	5.16 ± 0.12	0.40 ± 0.05
Significance	NS	NS	NS

Values represent means ± statistical error. ODR was the mean of three measurement dates, soil gas concentration was the mean of two measurement dates. NS: no significant difference (Bonferroni Test, $P > 0.1$).

Injecting H_2O_2 into heavy loam clay soil managed at near field capacity water content during 4 months resulted in an increase in biomass of the aerial portion of avocado trees as well as higher WUEb. 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 T_1 over the controls (Table 3). The T_1 treatment was also significantly higher in total plant dry weight than the controls ($P \leq 0.1$). However, no significant difference in root density (data not shown) or total root dry weight between treatments was noted (Table 3). Leaf area was significantly (43.1%) greater ($P \leq 0.05$) for plants in the T_1 treatment than in the controls (Table 3). WUEb calculated from the total biomass divided by the total water supply showed statistical differences between treatments ($P \leq 0.05$) (Table 3). Similar results have been reported for zucchini, soybean, and cotton (Bhattarai *et al.*, 2004). An increase in growth has also been observed in tomato (*Lycopersicon esculentum* Mill.) plants cultivated in flooded conditions when H_2O_2 was added to the flood solution (Bryce *et al*., 1982). Similar results were also identified for maize (*Zea mays* L.), where a significant gain in biomass was due to the application of H_2O_2 to soil exhibiting excellent structure and adequate irrigation rates (Melsted *et al.*, 1949).

Although injecting H_2O_2 into the soil significantly increased the biomass of avocado trees, no significant effect of H_2O_2 injection on gs, T, A or SWP was noted. No significant differences between treatments for gs T, A, or SWP ($P > 0.1$) were observed (Table 4). Although it is well-known that prolonged root hypoxia causes stomatal closure and lowers transpiration in avocado trees (Schaffer

Table 3. Final biomass, leaf area, and water use efficiency (WUEb) of avocado trees.

Tmt	Total biomass	Wood biomass	Leaf biomass	Root biomass	Leaf area	WUEb
	g dry weight				cm²	g L^{-1}
T_0	2 706.6 ± 149.8	877.32 ± 26	833.26 ± 75	996 ± 158	66 524 ± 8.1	2.41 ± 0.1
T_1	3 181.9 ± 147.1	1 111.50 ± 24	1 067.48 ± 13	1 003 ± 171	95 185 ± 11.8	2.83 ± 0.1
Sig.	*	**	**	NS	**	**

Values represent treatment means ± statistical error. * $P \leq 0.1$; ** $P \leq 0.05$.
NS indicates no significant difference between treatments according to Bonferroni test ($P > 0.1$); T_0: control treatment; T_1: H_2O_2 injection treatment.

et al., 1992; Schaffer, 1998; Schaffer and Whiley, 2002), it was not possible to observe any significant improvement of gs and T as a result of adding H_2O_2 to the soil oxygen supply in this study. The same phenomenon was found by Bhattarai *et al.* (2004) for zucchini, soybean, and cotton.

The greater biomass of plants in the H_2O_2 soil injection treatment as opposed to the plants in the control treatment should have resulted in a greater net CO_2 assimilation by plants in the treated soil. However, no significant difference was perceived in the measured net CO_2 assimilation rate between the H_2O_2 soil injection and control treatments (Table 4). Nevertheless, it must be pointed out that net CO_2 assimilation was measured on a leaf area basis in this study. Trees in the H_2O_2 soil injection treatment had a greater total leaf area than those in the control treatment (Table 3). Therefore, on a whole-plant basis, net CO_2 assimilation was significantly higher for plants in the H_2O_2 injection treatment than in the control treatment, presumably accounting for the greater biomass of plants in soil injected with H_2O_2. Moreover, an extra oxygen supply due injecting H_2O_2 to the soil might 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 on the H_2O_2 treatment, it must be pointed out that measurements were not continuous and were performed only three times during the measurement season. As a consequence, it was not possible to see changes in either O_2 and CO_2 composition or ODR, during a day with several irrigation pulses. It may therefore be presumed that changes would be momentary and that those specific changes in the concentration of O_2 in the soil could generate a better root oxygenation and thus ATP production.

The xylem/phloem ratio in roots and shoots, the number and mean area of root xylem vessels, and the total root xylem areas are shown in Table 5. An example of the anatomical features of xylem vessels in roots and spring shoots is shown in Figure 1. 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 ($P \leq 0.15$). Histological examination of avocado roots revealed larger xylem mean vessel diameters for plants in the H_2O_2 injection treatment than those in the control treatment, indicating that H_2O_2 soil injection leads to root anatomy improvement 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 in individual plants. Larger xylem vessels would increase the water conduction capacity 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

Table 4. Effect of injecting H_2O_2 into heavy clay loam soil maintained at field capacity on water relations and physiological variables of avocado plants.

	AM			PM		
	T_0	T_1	Significance	T_0	T_1	Significance
gs, cm s^{-1}	0.49 ± 0.03	0.51 ± 0.03	NS	0.29 ± 0.02	0.28 ± 0.02	NS
T, µg cm^{-2} s^{-1}	4.50 ± 0.18	4.80 ± 0.25	NS	5.30 ± 0.24	4.80 ± 0.22	NS
SWP, kPa	-0.60 ± -0.05	-0.63 ± -0.04	NS	-0.92 ± -0.04	-0.88 ± -0.04	NS
A, µmol s^{-1} m^{-2}	4.86 ± 0.48	5.38 ± 0.58	NS			

Values are means ± statistical error. T_0: control treatment; T_1: H_2O_2 injection soil treatment; gs: stomatal conductance; T: transpiration; SWP: soil water potential; A: net CO_2 assimilation; AM: 09:30 to 11:00; PM: 13:00 to 3:00. NS indicates no significant difference between treatments according to Bonferroni test ($P > 0.1$).

Table 5. Effect of injecting H_2O_2 to heavy clay loam soil maintained at field capacity on root and shoot vascular anatomy.

	T_0	T_1	Significance
Spring shoot xylem/phloem relationship	1.5	2.0	*
Root xylem/phloem relationship	1.5	1.4	NS
Number of root xylem vessels	59.8	63.6	NS
Mean xylem vessel area, µm²	2 392.5	2 418.4	NS
Xylem total area, µm²	146 630	154 202	NS

T_0: control treatment; T_1: soil H_2O_2 injection treatment. Values are means; NS: no significant difference according to Bonferroni test ($P > 0.15$).
* ($P > 0.15$).

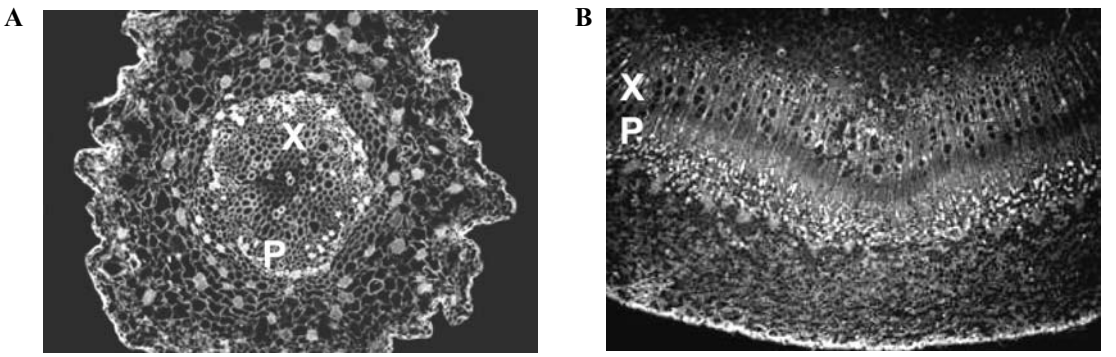

Figure 1. Anatomical features in avocado root and shoot vascular tissue. A) Root section from a plant in the H_2O_2 soil injection treatment (100X). B) Spring shoot section from a plant in the H_2O_2 soil injection treatment (40X). "X" indicates xylem tissue; "P" indicates phloem tissue.

in xylem vessel diameter could result in a significant increase in water conductivity through it. 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 higher xylem/phloem ratio was observed. According to several researchers (Kozlowski, 1997; Hsu *et al.,* 1999; Liao and Lin, 2001), plants in low-oxygen soils exhibit a reduction in the xylem/phloem ratio. Therefore, in the present study of avocado trees, the increased xylem/phloem ratio in plants in the H_2O_2 soil injection treatment might be an indirect indication that H_2O_2 increased the oxygen content of the root zone.

CONCLUSIONS

Injecting H_2O_2 through the irrigation system into a soil with low air content significantly increased the biomass of the aerial portions of the plant and water use efficiency (plant biomass per water applied [WUEb]), but had no significant effect on measured CO_2 assimilation, transpiration, stomatal conductance, or steam water potential. Furthermore, xylem vessel diameter and the xylem/phloem ratio tended to be greater for trees in soil injected with H_2O_2 than in the controls.

The increased biomass of the aerial portions of plants in treated soil indicates that H_20_2 soil injection might have potential as a method for improving soil oxygen content in a heavy clay loam soil. However, the present study was conducted with trees in containers, and before this method can be used to mitigate damage caused by low soil aeration in avocado orchards, further studies are needed to evaluate the practical and economic feasibility of using hydrogen peroxide on a larger scale in orchards.

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RESUMEN

Efecto de la inyección de peróxido de hidrógeno en suelo franco arcilloso pesado, sobre el estado hídrico, asimilación neta de CO_2, biomasa y anatomía vascular de paltos. En Chile, los huertos de palto (*Persea americana* Mill.) se ubican comúnmente en suelos pobremente drenados con bajo contenido de oxígeno, lo que limita producción y calidad de fruta. El objetivo de este estudio fue evaluar el efecto de la inyección de peróxido de hidrógeno (H_2O_2) al suelo como fuente de O_2, sobre el estado hídrico, asimilación de CO_2, biomasa y anatomía de paltos en suelo franco arcilloso con contenido de humedad cercano a capacidad de campo. Paltos cv. Hass de 3 años fueron establecidos en condiciones ambientales no forzadas, plantados en contenedores con suelo franco arcilloso pesado cuya humedad fue mantenida cercana a capacidad de campo. Las plantas se dividieron en dos tratamientos: inyección de H_2O_2 al suelo mediante riego por goteo subsuperficial y plantas sin inyección de H_2O_2 (control). Además de determinar las características físicas del suelo, se determinó la asimilación neta de CO_2 (A), transpiración (T), conductancia estomática (gs), potencial hídrico xilemático (SWP), biomasa aérea y radical, eficiencia de uso del agua (materia seca total producida en relación a la cantidad de agua aplicada [WUEb]) y características anatómicas vasculares en brotes y raíces. La inyección de H_2O_2 al suelo aumentó significativamente la biomasa aérea de las plantas y también el WUEb, pero no tuvo efectos significativos en A, T, gs, o SWP. El diámetro de los vasos xilemáticos y la relación xilema/floema tendieron a ser mayores en árboles situados en suelos tratados con H_2O_2 comparados con los controles. El aumento en la biomasa aérea de paltos en suelo tratado indica que la inyección de H_2O_2 en suelos franco arcillosos pesados puede ser una útil herramienta de manejo en suelos pobremente aireados.

Palabras clave: conductancia estomática, fotosíntesis neta, histología radical, inyección de oxígeno, hipoxia radical, riego por goteo subsuperficial.

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