DOI: 10.1007/s11099-016-0679-7 PHOTOSYNTHETICA 55 (4): 655-663, 2017

Growth, photosynthetic performance and antioxidative response of ‘Hass’ and ‘Fuerte’ avocado (*Persea americana* Mill.) plants grown under high soil moisture

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Abstract

‘Hass’ and ‘Fuerte’ avocado plants were grown under well-watered or waterlogged conditions. Results indicated significant effects on the majority of the allometric parameters in waterlogged plants, with ‘Fuerte’ displaying a more pronounced growth inhibition. Waterlogged conditions caused a progressive and simultaneous decline in net photosynthetic rate and stomatal conductance, earlier in ‘Fuerte’ than in ‘Hass’. Maximal potential quantum yield of PSII was unaffected by the soil water regime and/or variety and leaf water potential values in waterlogged plants were not more negative compared with control plants. ‘Fuerte’ waterlogged plants exhibited increased contents of thiobarbituric acid reactive substances, whereas oxidative injury was not detected in ‘Hass’. Finally, none of the two cultivars displayed valuable antioxidant potential, as evidenced by the decreased activities of the antioxidant enzymes superoxide dismutase, guaiacol peroxidase, glutathione peroxidase, and ascorbate peroxidase.

Additional key words: cell membrane stability; chlorophyll fluorescence; gas exchange; oxidative stress; waterlogging.

Introduction

Soil is considered waterlogged whenever water content of the surface layer is at least 20% higher than the field capacity, a condition describing the water saturation at the root zone (Aggarwal *et al.* 2006). Waterlogging and/or flooding (*i.e.* shoots are also either partially or even fully submerged) of agricultural fields may develop due to anthropogenic (*e.g.* excessive irrigation, growing plants in low-lying areas with a high water table or in poorly drained soils, management practices that exacerbate soil degradation) or natural (*e.g.* highly intense rainfall, overflow of rivers, snowmelting) factors leading to altered plant metabolism and growth, increasing susceptibility to diseases, reduced crop yield or even plant mortality (Schaffer 1998, Colmer and Voesenek 2009). Furthermore, according to the “Special Report on Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation” of the Intergovernmental Panel on Climate Change (IPCC), the frequency of heavy precipitation events is likely to increase in the 21st century, enhancing the potential for soil waterlogging/flooding in many areas of the world, and thus addressing a new concept of flood risk management projects for the agricultural sector (Kundzewicz *et al.* 2014).

Although all higher plants require access to free water, when water saturates the root environment, plants are challenged by a significant depletion of free oxygen in the rhizosphere because of the increased microbial activity, the slow diffusion rate, and the limited solubility of oxygen in water (Irfan *et al.* 2010). Hypoxia refers to the reduction of oxygen below optimal concentration for the normal functioning of the plant, while anoxia describes the complete lack of oxygen in the soil. Both terms label

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Received 26 July 2016, accepted 27 October 2016, *published as online-first* 8 December 2016.

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Abbreviations: APX – ascorbate peroxidase; Chl – chlorophyll; C_i/C_a – ratio of intracellular CO_2 concentration to atmospheric CO_2 concentration; DAE – days of experiment; DM – dry mass; *E* – transpiration rate; FC – control Fuerte; F_0 – minimal fluorescence yield of the dark-adapted state; F_m − maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; FW – waterlogged Fuerte; GPX – glutathione peroxidase; g_s – stomatal conductance; HC – control Hass; HW – waterlogged Hass; P_N – net photosynthetic rate; POD – guaiacol peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances; ψ_m – soil matric potential; ψ_w – water potential.

the transfer from aerobic to anaerobic metabolism in roots, with dramatic restrictions to ATP synthesis and carbohydrate economy (Voesenek and Sasidharan 2013). Anaerobic lactic acid and ethanol fermentation (Bailey-Serres and Voesenek 2008), cytoplasmic acidification (Colmer and Voesenek 2009), accumulation of reduced soil components, such as Mn^{2+}, Fe^{2+}, and S^{2-}, and carboxylic acids to toxic concentrations (Irfan *et al.* 2010), modification of plant hormone biosynthesis (Kramer and Boyer 1995), stomatal closure and reductions in photosynthetic rate (Pimentel *et al.* 2014), excessive formation of reactive oxygen species (ROS) (Blokhina *et al.* 2003), and a decrease in the hydraulic conductivity of roots (Tournaire-Roux *et al.* 2003) are the main features caused by lack of soil oxygen. The severity of waterlogging effects depends on plant species, the stage of plant development, the duration of the waterlogging event, and the depth of the water level, but if air contact cannot be re-established, the exhaustion of sugars and the energy deficit lead to severe tissue damage, reduced root and shoot growth and reproductive disorders and, eventually, to mortality (Drew 1997).

Avocado (*Persea americana* Mill.) is a profitable tree crop with a total world production more than 4.5 million tons (Bost *et al.* 2013). Several economically important cultivars, such as cv. 'Hass' and cv. 'Fuerte', are grown under Mediterranean climate conditions, with main commercial avocado orchards located in California, Chile, south-western Australia, and the northern parts of the Mediterranean basin (Carr 2013, González-Sánchez *et al.* 2013). Moreover, avocado has recently become a dynamic crop at the island of Crete, Greece, where it is grown on more than 800 ha. In avocado, the root system does not spread much beyond the tree canopy and the majority of roots, which function for water and nutrient uptake, are mainly restricted to the upper 60 cm of the soil profile (Lahav *et al.* 2013). Roots are extremely suberized, with low hydraulic conductivity, few or no root hairs, high oxygen demand and poor water-uptake efficiency (Ferreyra *et al.* 2007). Furthermore, mechanisms of waterlogging tolerance through anatomical and morphological adaptations (aerenchyma formation, development of hypertrophic stem lenticels and/or adventitious roots) have not been observed in avocado trees (Schaffer *et al.* 2013). These characteristics classify avocado among the tree species most sensitive to root hypoxia with physiological and growth responses reported shortly after soil becomes waterlogged (Schaffer 1998). Low soil oxygen content due to poor soil drainage is often accompanied by wilting, reduced leaf size, and leaf abscission (Schaffer *et al.* 2006), restriction of vegetative and root growth (Gil *et al.* 2007), and root necrosis (Schaffer *et al.* 2013). Schaffer (1998) reported that a few days of flooding can result in avocado tree death as a result of extensive root system damage. Root decay caused by waterlogging can be exacerbated by *Phytophthora* root rot (caused by the soil-borne fungal pathogen *Phytophthora cinnamomi* Rands), the major disease of avocado worldwide (Reeksting *et al.* 2014). Physiological responses of avocado tree to oxygen deficiency in the rooting zone include reductions in leaf gas exchange parameters, such as net CO_2 assimilation rate (P_N), stomatal conductance (g_s), transpiration (E), and ratio of internal to atmospheric CO_2 concentrations (C_i/C_α) (Schaffer *et al.* 2013, Sanclemente *et al.* 2014), reduced PSII efficiency (Reeksting *et al.* 2014), and water and nutrient uptake restrictions (Gil *et al.* 2012).

Although the generation of ROS is a well-known effect of excessive water in the soil (Blokhina *et al.* 2003), to our best knowledge, there is no study regarding the consequences of low soil oxygen content-induced oxidative stress and elucidating the ability to induce the antioxidant defense mechanism in avocado tree. This study: (*1*) addressed this lack by investigating potential antioxidative responses under excessive soil moisture conditions and (*2*) conducted a comprehensive evaluation of the growth and physiological responses of the avocado cultivars 'Hass' and 'Fuerte' to soil waterlogging.

Materials and methods

Plant material and treatments: Forty-eight, two-year-old avocado plants (*Persea americana* Mill.), cv. 'Hass' and 'Fuerte', grafted onto Zutano seedling rootstock were used in this experiment, which was conducted under nethouse conditions at the facilities of the Institute of Olive Tree, Subtropical Plants and Viticulture in Chania, Crete, Greece (35°32'00"N, 24°04'09"E). The plants were grown in 35-L pots filled with a slightly alkaline sandy loam soil.

Two soil water regimes were applied during the experimental period (October–July): (*1*) a control, well-watered treatment (for 'Fuerte' – FC and for 'Hass' – HC), where the plants were irrigated manually when soil matric potential (ψ_m) value reached −0.03 MPa [average value from tensiometers (*Irrometer Company*, Riverside, CA, USA), which were installed at 20 cm depth in five randomly selected pots] and (*2*) a waterlogging treatment (for 'Fuerte' – FW and 'Hass' – HW) where each 35-L pot was submerged in an about half-sized (20-L) plastic bucket filled with tap water. To maintain a constant water level, the buckets were refilled as needed. Each treatment was applied to twelve uniform plants of each avocado variety.

Measurements of gas-exchange characteristics (P_N, g_s) were carried out after 1, 6, 17, 29, 72, 155, and 274 days of the experiment (DAE). Leaf water potential (ψ_w) and maximal potential quantum yield of PSII (F_v/F_m) were also recorded on 274 DAE. One day later (275 DAE), plants were harvested for the determination of allometric parameters, and leaf and root samples were taken for the determination of lipid peroxidation level (TBARS), hydrogen peroxide concentration, and antioxidant enzyme activities.

Allometric parameters: At the end of the experiment (275 DAE), 12 plants (replicates) of each treatment were removed and the diameter of the trunk was measured 10 cm above (scion's diameter) and 10 cm below (rootstock's diameter) the grafting line. Shoots formed during the experiment were separated into stems and leaves. Total stem length per plant was calculated as the sum of the lengths of all new stems. Total leaf area per plant was measured using a *LI-3100C* area meter (*LI-COR Biosciences Inc.*, Lincoln, Nebraska, USA). Subsequently, stems and leaves were weighted for the determination of fresh mass (FM), while stem and leaf total dry mass (DM) was determined after the material was dried in an oven at 70°C to a constant mass.

Leaf gas exchange, chlorophyll (Chl) fluorescence and leaf water potential: Net CO_2 assimilation rate (P_N) and stomatal conductance (g_s) were recorded with a *Li-6400*, portable photosynthesis system (*LiCor Bioscience Inc.*, Lincoln, Nebraska, USA) in the morning (between 09:30 and 11:00 h) at a light-saturated photosynthetic photon flux (PPFD greater than 1,000 μmol m^{-2} s^{-1}) and a reference CO_2 concentration of 380 μmol mol^{-1}. The measurements were taken on one healthy and fully expanded leaf from five plants (n = 5) per treatment. Chl fluorescence was measured with a portable fluorometer (*Mini PAM*, *Walz*, Effeltrich, Germany), on the same leaves used for gas-exchange determinations. Prior the measurement of Chl fluorescence, the leaves were dark-adapted for 20−30 min, to ensure oxidation of the plastoquinone pool. Measurements of the minimal (F_0) and maximal (F_m) fluorescence yield were performed, thus allowing the determination of variable to maximal fluorescence ratio (F_v/F_m), which represents a measure of the maximum efficiency of PSII in darkness. Finally, leaf water potential (ψ_w) was measured on mature leaves from the middle of the last season shoots using a pressure chamber (*PMS Instrument Company*, Corvallis, Oregon, USA) and following the recommendations of Scholander *et al.* (1965).

Enzymatic antioxidant activity, lipid peroxidation and hydrogen peroxide: Superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (POD, EC 1.11.1.7), glutathione peroxidase (GPX, EC 1.11.1.9), and ascorbate peroxidase (APX, EC 1.11.1.11) activities were determined in four samples (n = 4) of leaves and roots per treatment and variety. Plant material was collected at the end of the experiment and was immediately frozen at −80°C. The extraction medium consisted of 0.1 M potassium phosphate buffer (pH 7.6), containing 1 mM ethylenediaminetetraacetic acid disodium salt, 0.5 mM ascorbate (in the case of the APX assay), and 1% polyvinylpolypyrrolidone. Plant tissue (0.3 g) was homogenized in 1.5 ml of the extraction buffer and the homogenate was centrifuged at 13,000 × *g* for 30 min. The supernatant was used for activity assays. The absorbance of the crude enzyme extracts was measured with a *Hitachi U-1100* spectrophotometer (*Hitachi Ltd.*, Tokyo, Japan). Total SOD activity was assayed according to the methodology of Becana *et al.* (1986). One unit of SOD activity (U) was defined as the amount of enzyme that produced a 50% inhibition of nitroblue tetrazolium (NBT) reduction. Guaiacol POD assay was performed using the method described by Zhang (1992). POD activity was measured by the increase in absorbance following the oxidation of guaiacol at 470 nm. One unit of POD was defined as 1 μmol of guaiacol oxidized min^{-1}. The determination of GPX activity was based on the method of Drotar *et al.* (1985). Activity was measured by the decrease in absorbance at 340 nm for 10 min. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 nmol of NADPH min^{-1}. APX activity was determined, according to Nakano and Asada (1981), by measuring the oxidation rate of ascorbate at 290 nm. One unit of APX was defined as the amount of enzyme that oxidized 1 μmol ascorbate min^{-1}. All enzyme activities were calculated on fresh mass basis.

Leaf tissue (0.5 g) obtained from the same leaves that were used for the antioxidant enzyme assays, was homogenized with 5 ml trichloroacetic acid (TCA) 0.1% (w/v) in an ice bath. The homogenate was centrifuged at 10,000 × *g* for 30 min and the supernatant was used for the determination of thiobarbituric acid reactive substances (TBARS) and H_2O_2 content. Lipid peroxidation was determined by quantifying the TBARS content as described by Hodges *et al.* (1999) with minor modifications. In particular, 1 ml of the supernatant was added to an equal volume of either (*1*) 20% (w/v) TCA solution containing 0.01% butylated hydroxytoluene or (*2*) 20% (w/v) TCA solution containing 0.65% (w/v) TBA and 0.01% butylated hydroxytoluene. The mixture was incubated at 95°C for 30 min, quickly cooled and centrifuged at 10,000 × *g*. The absorbance was read at 532 nm with the values for nonspecific absorption at 600 nm and 440 nm subtracted. TBARS content was expressed as as nmol g^{-1}(FM). H_2O_2 content was determined according to the methodology of Alexieva *et al.* (2001). Absorbance measurements were carried out by above spectrophotometer at 390 nm using a solution consisting of TCA 0.1% and pure catalase reagent as a blank, to ensure zero interference. The H_2O_2 content was calculated by comparing the reads with a standard curve obtained from different concentrations of H_2O_2. Final concentration was expressed as μmol g^{-1}(FM).

Data analysis: The experimental design was a 2 × 2 factorial, with two avocado varieties and two soil water regimes. Totally, 48 plants were used, *i.e.* 12 control plants and 12 high soil moisture-treated ones per variety ('Hass' and 'Fuerte'). Data were analyzed by a two-way analysis of variance (*ANOVA*) to assess interactions between soil moisture (SM) treatments and varieties (V), using the *SPSS 15.0* statistical package (*SPSS Inc.*, Chicago, USA). For the comparison of the means, the *Student*'s *t*-test as well as the *Duncan*'s multiple range test were employed (P<0.05).

For gas-exchange characteristics, treatment means were further compared using the 'repeated measures' feature of *SPSS 15.0*. Calculation of correlation coefficients (R^2) and figures preparation were performed using *GraphPad Prism 5.0* software (*GraphPad Software Inc*., La Jolla, California, USA).

Results

In our study, scion's diameter, total stem length, total leaf and stem DM, and total leaf area were significantly higher in control plants than that of waterlogged plants for both varieties. Rootstock's diameter was also negatively affected by the waterlogging conditions but only in 'Fuerte' (Fig. 1). Furthermore, a significant soil moisture × variety interaction effect was detected in total leaf DM, because of the greater percentage reduction observed in stressed 'Fuerte' plants (76%) compared to those of 'Hass' (45%) (Fig. 1*C*).

High soil moisture affected both P_N and g_s values in a similar way, as shown by the parallel decline in both parameters, as the experiment progressed (Fig. 2*A*,*B*). P_N significantly decreased in waterlogged plants, which exhibited an average reduction in P_N values, throughout the experimental period, of about 21% compared with control treatments. The largest reduction in P_N values was recorded in the FW plants, being 28% of FC values. The average g_s values of waterlogged avocado plants of both cultivars were 22% lower than those of control plants, with FW showing in average 26% higher stomatal closure. According to *Student*'s *t*-test (statistical analysis not shown) a significant decline in P_N and g_s values was observed earlier in 'Fuerte' (from 72 DAE and thereafter) than that in 'Hass' (from 155 DAE and thereafter) (Fig. 2*A*,*B)*

At the end of the experimental period (274 DAE), persistently high F_v/F_m values, between 0.83 and 0.87, were unexpectedly detected in leaves, irrespective of the variety and/or the soil moisture regime. However, a minor but significant decrease of the maximal quantum yield of PSII (F_v/F_m) was observed only in FW compared with FC (Fig. 3*A*). At the same time, leaf ψ_w values of the FW plants were less negative compared to all other treatments (Fig. 3*B*). With regard to 'Hass' plants, similar leaf ψ_w values were measured in both treatments (HC, HW).

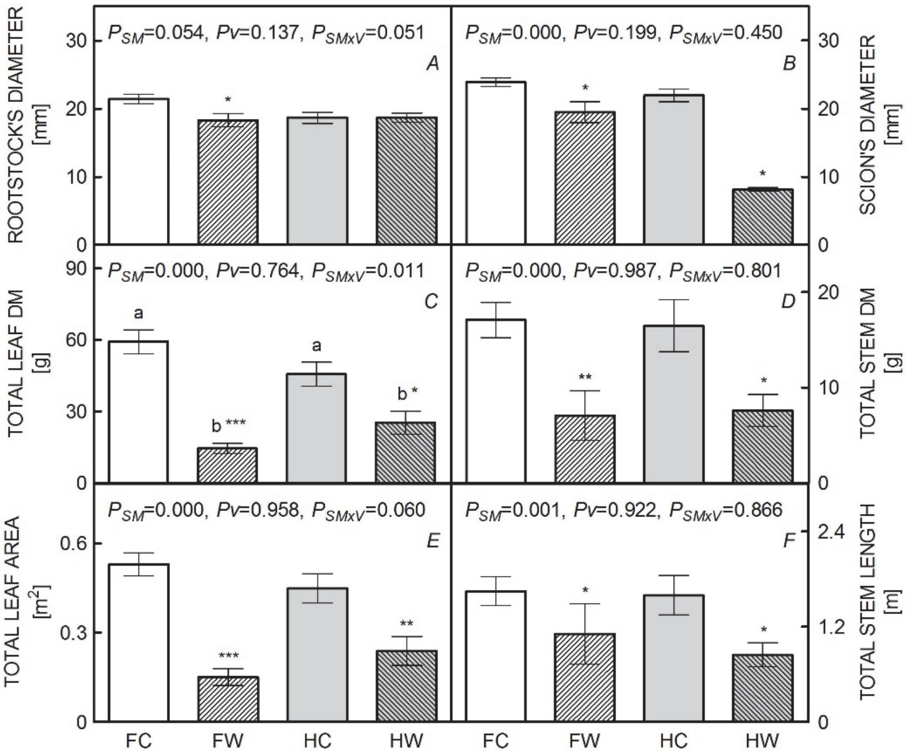

Fig. 1. The effect of soil moisture, variety, and their combination on various allometric parameters of 'Hass' and 'Fuerte' avocado plants. Data represent average values ± SE (n = 12). For each parameter, *different letters* above bars indicate significant differences at $P<0.05$ (*Duncan*'s multiple range test). Asterisks indicate significant differences between control and waterlogged plants of the same variety, at $P<0.05$ (*), $P<0.010$ (**) or $P<0.001$ (***); (*Student*'s *t*-test). P_{SM} – significance of the soil moisture level; P_V – significance of the different varieties; P_{SMxV} – significance of the soil moisture × variety interaction. FC – Fuerte control; FW – Fuerte waterlogged; HC – Hass control; HW – Hass waterlogged.

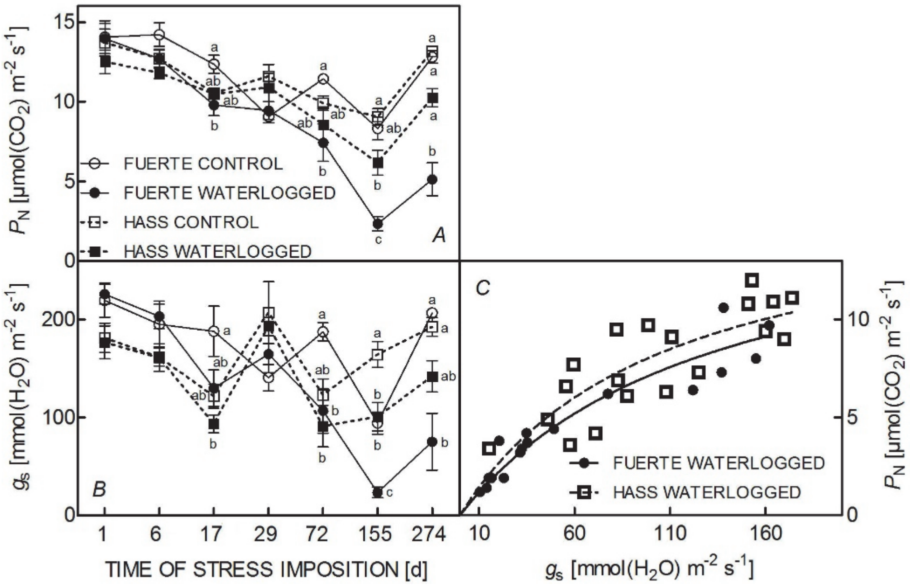

Fig. 2. Variation of net photosynthetic rate (P_N, *A*) and stomatal conductance (g_s, *B*) in leaves of 'Hass' and 'Fuerte' avocado plants during the experimental period (each value represents the average of five replicates ± SE) and the relationship between g_s and P_N for the high soil moisture treatment at the end of the stress imposition (*C*). For each parameter and day after the stress imposition, means of P_N and g_s followed by *the same letter* were not significantly different at $P<0.05$ (*Duncan*'s multiple range test). Nonlinear regressions of P_N with g_s: 'Fuerte' Waterlogged: y = 2.93 ln(x) − 6.31, $R^2 = 0.90$ and 'Hass' Waterlogged: y = 3.45 ln(x) – 7.73, $R^2 = 0.72$. Fitted lines were statistically significant at $P<0.001$.

A downregulation of APX, GPX, and POD activity was revealed in leaves of waterlogged trees in both cultivars during the last day of the stress imposition, whereas the activation of SOD isoforms under excessive soil moisture was not so prominent (Fig. 4). Of the four enzymes studied, a significant increase was detected only in APX activity in roots of FW, whereas GPX, POD, and SOD activity in roots of waterlogged plants did not differ significantly compared with control ones (Table 1, Fig. 4).

The extent of oxidative membrane damage was significantly higher in FW than that in HW (Fig. 5). TBARS and H_2O_2 content in leaves of FW were four times and three times higher, respectively, than that in HW plants.

Discussion

At soil redox values below +350 mV, oxygen begins to disappear from the soil, roots of flooded and/or waterlogged plants are exposed to anaerobic conditions, and various changes in the overall plant metabolism are induced in order to preserve survival under low energy availability (Irfan *et al.* 2010). Avocado is considered a waterlogging-sensitive tree species with tree growth and metabolism negatively impacted by low soil oxygen content (Schaffer *et al.* 2006). In our study, excessive soil moisture for nine months significantly affected the majority of the allometric parameters examined, except for rootstock's diameter in 'Hass' variety (Fig. 1). Stressed plants were shorter, with smaller total leaf area and decreased biomass production compared with controls. However, we noted that, although no significant differences were found between the two cultivars in terms of vegetative growth ($P_v>0.05$), 'Fuerte' plants imposed to reduced soil oxygen (FW treatment) exhibited a higher percentage decrease in the majority of the allometric traits examined in relation to HW treatment. This relative maintenance of plant growth was reported in 'Hass' avocado, a variety of the Guatemalan race that is replacing the traditional variety 'Fuerte' in most places with a Mediterranean type of climate (Carr 2013); it might imply that 'Hass' could perform slightly better during a prolonged period with high soil moisture.

In contrast to some other subtropical species (*e.g.* mango tree), there are no reports on morphological and anatomical adaptations of avocado under waterlogging conditions, and hence physiological modifications could probably relieve the stress of insufficient oxygen supply and sustain metabolic activity and growth. As seen in different woody and herbaceous species (Malik *et al.* 2001, Pang *et al.* 2004, Gimeno *et al.* 2012, Reeksting *et al.* 2014) "wet soil"-induced reductions of photosynthesis occur due to stomatal or nonstomatal limitations, which are related to the biochemical reactions of photosynthesis. In addition, Castonguay *et al.* (1993) suggested that reduced respiratory activity in roots implies a feedback mechanism, when a production of photosynthates is limited,

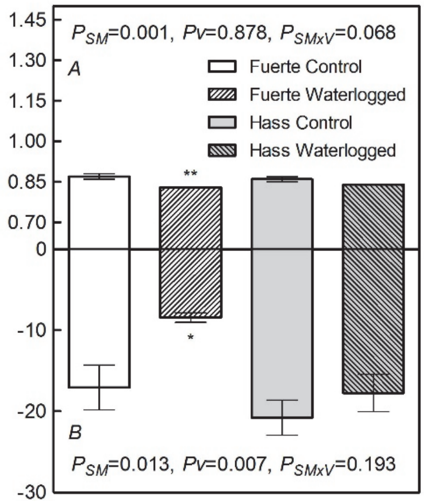

Fig. 3. Effect of soil moisture on maximal potential quantum yield of PSII (F_v/F_m) and leaf water potential (ψ_w) during the last day of the experimental period. Values are the average of five replicates ± SE. For each parameter *different letters* denote significant differences at $P<0.05$ (*Duncan*'s multiple range test). Asterisks indicate significant differences between control and waterlogged plants of the same variety, at $P<0.05$ (*), $P<0.010$ (**) or $P<0.001$ (***); (*Student*'s *t*-test). P_{SM} – significance of the soil moisture level; P_V – significance of the different varieties; P_{SMxV} – significance of the soil moisture × variety interaction.

exacerbating P_N decline in plants subjected to hypoxia. In the present study, avocado tree induced stomatal closure with a subsequent downregulation of the photosynthetic machinery (Fig. 2*A*,*B*). Closing the stomata limits the ability of the plant to capture CO_2, thus reducing photosynthetic enzyme substrate and lowering the P_N. The simultaneous and parallel decline of P_N and g_s in flooded plants, under limited rhizosphere oxygen concentrations, indicates that the decline in net photosynthesis is mainly a consequence of stomatal limitation (Farquhar and Sharkey 1982), and that the inhibition of key metabolic processes is not detectable even when g_s dropped to minimum values during the latter stages of the experiment (Fig. 2*C*, 155 and 274 DAE). In addition, at the end of the experiment (274 DAE), F_v/F_m values were around 0.85 and very similar in the waterlogged as well as in the control plants of both varieties (Fig. 3*A*), supporting the absence of photochemical and biochemical irreversible impairments of the photosynthetic apparatus. Our results do not agree with Schaffer *et al.* (1992) and Reeksting *et al.* (2014) who attributed changes in P_N in flooded avocado plants to non-stomatal limitations associated with metabolic impairment of photosynthetic processes. However, in any case, (*1*) different scion-rootstock combinations, (*2*) the extent and duration of anaerobic conditions, (*3*) the temporal separation between these physiological modifications, and (*4*) the presence of *Phytophthora* root rot in the substrate should be considered when determining if waterlogging-induced reductions in photosynthesis occur due to stomatal or nonstomatal factors in avocado.

Table 1. Significance of the soil moisture level (P_{SM}), significance of the different varieties (P_V); significance of the soil moisture × variety interaction effect on antioxidant enzymes (P_{SMxV}) activity in leaves and roots of 'Hass' and 'Fuerte' avocado plants. Values lower than 0.05 indicate significant effects. SOD – superoxide dismutase; APX – acsorbate peroxidase; GPX – glutathione peroxidase; POD – guaiacol peroxidase.

Source of variance	Leaves				Roots			
	SOD	APX	GPX	POD	SOD	APX	GPX	POD
P_{SM}	0.225	0.000	0.000	0.000	0.695	0.944	0.357	0.603
P_V	0.288	0.090	0.469	0.131	0.493	0.006	0.023	0.386
P_{SMxV}	0.963	0.129	0.122	0.820	0.264	0.004	0.619	0.803

It is also worth noting that the stomatal closure of both waterlogged treatments occurred without a decrease in leaf water potential (Fig. 3*B*). Furthermore, FW plants, which exhibited greater sensitivity during vegetative growth, also displayed the higher overall ψ_w values at the end of the waterlogging period. Our findings indicate that, in avocado, stomatal response to high soil moisture was independent of leaf water status and in addition, reduced transpiratory losses following stomatal closure could be considered as an adaptive mechanism to prevent leaf dehydration (Irfan *et al.* 2010). Similar behavior has been observed in other plant species, such as kiwi fruit (Savé and Serrano 1986), mango tree (Larson *et al.* 1991), oak species (Dreyer *et al.* 1991), and citrus seedlings (Rodríguez-Gamir *et al.* 2011), and it was attributed to abscisic acid accretion in leaves, losses of root hydraulic conductance, increase in CO_2 concentration caused by import of gas from the soil, and the involvement of growth regulators, such as ethylene, auxin, and gibberellins. In the current study, maintaining a stable leaf hydration did not improve the assimilation potential, and the underlying growth potential of the stressed avocado trees; additionally the hyperbolic equation characterizing the relationship between P_N and g_s (Fig. 2*C*) revealed insignificant, but consistent larger P_N reduction than that of g_s (and thus a lower water-use efficiency) in FW treatment, suggesting that a lower degree of stress was affecting 'Hass' avocado.

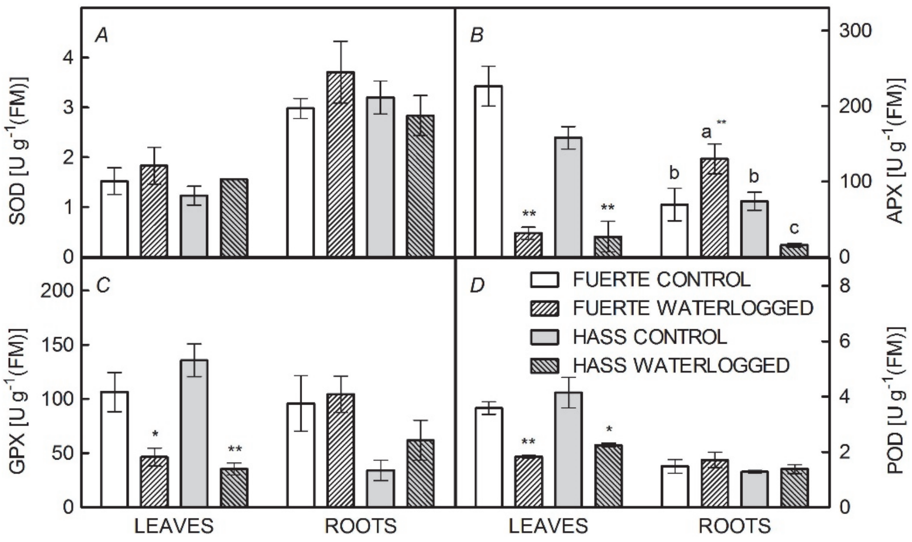

Fig. 4. Effects of soil moisture, variety and their combination on superoxide dismutase (SOD), APX, GPX, and POD at the end of the experimental period. Data represent average values ± SE (*n* = 4). For each enzyme and plant tissue (leaves, roots), means followed by *the same letter* were not significantly different at *P*<0.05 (*Duncan*'s multiple range test). Asterisks indicate significant differences between control and waterlogged plants of the same variety, at *P*<0.05 (*), *P*<0.010 (**) or *P*<0.001 (***); (*Student*'s *t*-test).

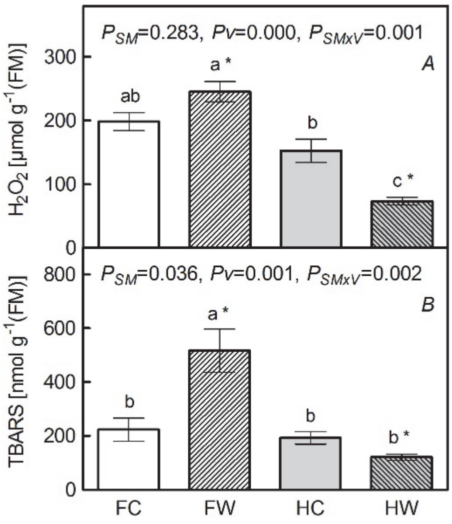

Fig. 5. The effects of soil moisture, variety and their combination on thiobarbituric acid reactive substances (TBARS) and H_2O_2 content in leaves of 'Hass' and 'Fuerte' avocado plants, at the end of the experimental period. Data represent average values ± SE (*n* = 4). For each parameter, *different letters* indicate significant differences at *P*<0.05 (*Duncan*'s multiple range test). Asterisks indicate significant differences between control and waterlogged plants of the same variety, at *P*<0.05 (*), *P*<0.010 (**) or *P*<0.001 (***); n.s. – insignificant differences (*P*>0.05) (*Student*'s *t*-test). P_{SM} – significance of the soil moisture level; P_V – significance of the different varieties; P_{SMxV} – significance of the soil moisture x variety interaction. FC – Fuerte control; FW – Fuerte waterlogged; HC – Hass control; HW – Hass waterlogged.

The better photosynthetic performance exhibited by 'Hass' variety under high soil moisture was also validated by the progressive decline of P_N values in HW treatment, compared to the prolonged inhibition of carbon assimilation in FW treatment as the experimental period progressed.

Several authors have reported increased accumulation of ROS under hypoxic/anoxic conditions (for a review, *see* Blokhina *et al.* 2003). Waterlogging-induced limitation of CO_2 fixation and lack of soil oxygen provoke the over-reduction of the photosynthetic electron transport chain forming superoxide radicals ($O_2^{\bullet-}$) and singlet oxygen species ($^1O_2$) in chloroplasts and H_2O_2 in mitochondria. Moreover, possible sources for hypoxia-stimulated enhanced ROS production involve acetaldehyde formation and lipoxygenase reaction. Disturbances in the equilibrium between ROS production and scavenging under stress conditions induce both protective responses and cellular damage (Gill and Tuteja 2010). A variety of enzymatic, such as SOD, CAT, and peroxidases, and nonenzymatic scavengers, such as ascorbate, glutathione, carotenoids, phenolic compounds, and tocopherols, play an active role in controlling the concentration of ROS, while the excessive accumulation of ROS and molecules due to the limited or ineffective antioxidant response results in oxidized damage to lipids, proteins, and nucleic acids (Halliwell and Gutteridge 1989). In our study, the content of TBARS produced during peroxidation of membrane lipids – a general marker of oxidative stress – at the end of the experimental period was significantly increased in FW, indicating excess soil moisture-induced oxidative injury (Fig. 5*B*). In contrary, 'Hass' avocado responded to waterlogging with a significantly lower extent of lipid peroxidation, with TBARS values relatively depressed in HW plants with respect to controls, revealing a considerable adaptability of 'Hass' to ROS pressure under high soil moisture conditions. Increasing oxidative stress is also supported by increasing concentration of leaf H_2O_2 content in FW plants, while the absence of oxidative stress in HW plants is coupled with a reduction in H_2O_2 generation (Fig. 5*A*). Our results were in agreement with reports of Bansal and Srivastava (2012) and Alam *et al.* (2010) who concluded that there is a direct correlation between H_2O_2 accumulation and lipid peroxidation, leading to oxidative injury during waterlogging in pigeon pea and soybean, respectively.

While in other tree species the activation of the antioxidant defense system as indicative of tolerance or sensitivity under soil waterlogging conditions has been studied, showing a direct relationship between increased antioxidant enzymes activity and stress tolerance (Blokhina *et al.* 2003, Arbona *et al.* 2008, Hossain *et al.* 2009, Porto *et al.* 2013), no information exists concerning avocado tree antioxidant response under low soil oxygen concentrations. In this work, antioxidant enzyme activities remained unchanged in roots and decreased significantly in leaves of waterlogged plants (Fig. 4). Our results suggest that: (*1*) the increased contents of ROS did not stimulate the cellular protective mechanism to mitigate damages in FW, (*2*) 'Hass' avocados were not subjected to oxidative stress after a prolonged period under high soil moisture, and (*3*) the activation of the major enzymes involved in the antioxidant system under waterlogging conditions did not constitute a generic response, but might depend on plant species, cultivar, stress intensity, and duration. Plants under hypoxic conditions typically use a complex antioxidative defense system consisting of several nonenzymatic and enzymatic components responsible for the scavenging and/or detoxification of the toxic oxygen radicals (Blokhina *et al.* 2003). According to Chugh *et al.* (2011), the major cause of oxidative stress is the disability of the scavenging system to metabolize the toxic oxygen radicals due to either increased ROS formation or decreased activity of the antioxidant enzymes. Our results revealed a differential response to oxidative stress in the two avocado cultivars, with 'Hass' cultivar exhibiting an increased protection, which did not correlate positively with the activation of the enzymes studied and thus, suggesting that different mechanisms might be involved in the enhancement of the antioxidant defense.

In conclusion, conditions of high water soil moisture significantly affected vegetative growth and photosynthetic capacity of both avocado cultivars. No evidence was found that the alteration of leaf gas exchange following a prolonged period of excessive soil moisture was associated with biochemical limitations to photosynthesis and/or cell membrane damage, as a result of H_2O_2 accumulation, and similarly, no evidence was found that stomatal closure was initiated by a loss of leaf hydration. We also suggest that the four antioxidant enzymes studied (SOD, POD, GPX, and APX) had no major antioxidative function in avocado trees imposed to low soil oxygen supply. In addition, the results of our study allowed us to speculate that 'Hass' cultivar possesses a certain level of waterlogging tolerance as indicated by: (*1*) the less pronounced effects on allometric parameters in HW, (*2*) the maintenance of a better photosynthetic performance in HW, and (*3*) the ROS damage avoidance in HW. The aforementioned 'Hass' cultivar traits might be of large interest for avocado management programs in waterlogging-prone agricultural areas and, thus, should be in the agenda of further research as they could provide a better insight into avocado's adaptability to specific regions.

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