EFFECT OF WATERLOGGING ON PLANT WATER STATUS, LEAF GAS EXCHANGE AND BIOMASS OF AVOCADO (*Persea americana* Mill)

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Avocado trees are highly sensitive to soil waterlogging. An early physiological response of avocado trees to soil waterlogging is reduced stomatal conductance which limits transpiration. The objective of this study was to determine the effect of waterlogging on plant water relations, leaf gas exchange, and biomass of avocado and to relate stomatal conductance and stem water potential to stomatal closure in response to soil waterloging. In August, 2006 in Homestead, Florida, USA, one-year-old 'Beta' and 'Hass' avocado trees, grafted onto Waldin seedling rootstocks were either continuously flooded for 11 days or not flooded (control plants). Net CO_2 assimilation (A), stomatal conductance (gs), transpiration (T) and stem water potential (SWP) were measured every two days during the waterlogging period and periodically for 21 days after such period. During that period, stomatal impressions were made for plants in each treatment, while stomatal closure was related to leaf gas exchange and SWP. There was a significant reduction in A, gs and T after 9 days of waterlogging, whereas SWP was significantly reduced 14 days after waterlogging was initiated. 'Beta' plants were more susceptible to waterlogging and had less leaf and root biomass at the end of the study period than 'Hass'. Stomatal closure as a result of waterlogging occurred when gs values decreased to 50 mmol m^{-2} s^{-1}. However, there was no correlation between SWP and the percentage of stomatal closure under waterlogging conditions.

Key words: root asphyxiation, flooding, stomatal closure, net photosynthesis, biomass.

EFECTO DEL ANEGAMIENTO EN EL ESTATUS HÍDRICO E INTERCAMBIO GASEOSO Y BIOMASA DEL PALTO (*Persea americana* Mill.).

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El palto es una especie muy sensible al anegamiento. Una respuesta fisiológica temprana ante suelos inundados es la reducción de la conductancia estomática, lo que limita la transpiración. El objetivo de este estudio es determinar el efecto del anegamiento sobre la respuesta hídrica, intercambio gaseoso y biomasa del palto, además de relacionar la conductancia estomática y potencial hídrico xilemático al momento de cierre estomático en respuesta al anegamiento. El ensayo se realizó en Homestead, Florida, Estados Unidos, durante agosto de 2006. Se evaluaron paltos de 1 año cv. Beta y Hass, injertados sobre patrón Waldin de semilla, inundados continuamente por 11 días o bien mantenidos bajo condiciones normales de riego (control). La asimilación de CO_2 (A), conductancia estomática (gs), transpiración (T) y potencial hídrico xilemático (PHX) fueron medidos cada 2 días durante el periodo de anegamiento y periódicamente luego de terminado el tratamiento de inundación. Durante este periodo se realizaron impresiones estomáticas, relacionándose la proporción de estomas cerrados con gs y PHX. Se determinó una significativa reducción de A, gs y T, luego de 9 días de anegamiento, mientras que el PHX se redujo significativamente luego de 14 días de comenzado el tratamiento. Los paltos Beta mostraron mayor susceptibilidad al anegamiento y tuvieron mayor pérdida de biomasa foliar y radical comparado con Hass. Por su parte se observó que el cierre estomático como resultado del anegamiento ocurre cuando gs es menor de 50 mmol m^{-2} s^{-1}. No se observó correlación ente PHX y porcentaje de estomas cerrados bajo condiciones de asfixia.

Palabras claves: asfixia radical, inundación, cierre estomático, fotosíntesis, biomasa.

1. Introduction.

In Chile, avocado production is primarily in the IV and VI regions with a large proportion of orchards planted on hill slopes to reduce the risk of frost. In these regions, avocado production has expanded to areas with soils that drain slowly. This combined with poor irrigation design and management has increased the potential for flood-induced root asphyxiation leading to root decay which is often severe within four years after orchards are planted.

Oxygen diffuses about 10,000 times more slowly in water than in air. Therefore, in poorly drained or flooded soils, the main factor causing root damage is a lack of sufficient O_2 for normal root respiration (Drew, 1997; Kramer and Boyer, 1995). Lack of O_2 in the soil is termed anoxia whereas low soil O_2 concentration is called hypoxia (Drew, 1997). As a result of soil hypoxia or anoxia, a change from aerobic to anaerobic respiration takes place in the root. This causes a reduction in ATP production resulting in reduced energy for normal plant metabolic processes. During anaerobic root respiration, potentially toxic metabolites such as ethanol, lactic acid, acetaldehyde and cyanogenic compounds can accumulate in the plant. Finally, cytosolic acidosis can occur in the cells, due to lactic acid accumulation in the cytoplasm, which results in cell death. (Koslowski, 1997, Liao and Lin, 2001, Jackson, 2002). Other physiological changes in plants as a result of root asphyxiation include an increase in the production of the ethylene precursor, ACC (carboxilic 1-Aminociclopropano-acid) (Bradford and Yang, 1980), a reduction in cytokinin and gibberellin synthesis in the roots (Kramer and Boyer, 1995) and an increase in abscisic acid concentration in leaves which has been implicated in causing stomatal closure (Kramer and Boyer, 1995, Else et al., 1995). Ultimately, hypoxia or anoxia of the soil can result in root tissue damage, inhibition of the vegetative and reproductive growth, changes in plant anatomy, premature senescence and plant mortality (Drew, 1997; Kozlowski, 1997). Avocado is among the most susceptible of fruit tree species to poor soil aeration (Schaffer et al., 1992).

In avocado trees, root asphyxiation has resulted in a delay of vegetative Spring shoot growth, a reduction in fruit yield (40%), reduced biomass accumulation, loss of foliage and tree death. When root asphyxia occurs during critical periods of fruit development, reproductive disorders such as abortion of organs (flowers and fruits), peduncle rings, elongated fruit and a reduction of the post harvest fruit life can develop (Whiley et al., 1996; Whiley and Schaffer, 1994; Zamet, 1997; Schaffer, 1998; Hofman et al. 2002). Valoras *et al.* (1964) observed that that avocado roots cease growing when the oxygen diffusion rate in the soil is lower than 0.20 µg cm^{-2} min^{-1}. Stolzy *et al.* (1967) found that 'Mexicola' avocado trees cultivated in soils with an oxygen diffusion rate lower than to 0.17 µg cm^{-2} min^{-1} had between 44 to 100% of their roots damaged.

Among the early physiological responses of avocado trees to flooding stress (or low soil O_2 content) are reductions in leaf gas exchange variables including stomatal conductance, transpiration and net CO_2 assimilation (often called net photosynthesis) (Ploetz and Schaffer, 1987, 1992; Schaffer and Ploetz, 1989;

Schaffer et al., 1992; Schaffer, 1998). Studies of the effects of poor soil aeration on 'Hass' avocado trees have focused primarily on tree growth and leaf and root mineral nutrition (Labanauskas et al,., 1968; Stolzy et al., 1967) and there is little information on the effects of flooding or poor soil aeration on leaf gas exchange of 'Hass' avocado trees.

There has been a considerable amount of research on the effects flooding on leaf gas exchange of West Indian avocado cultivars, mainly in porous limestone soils native to southern Florida. In these soils, the presence of Phytophthora root rot greatly exacerbated reductions of net photosynthesis, stomatal conductance, and transpiration caused by flooding (Ploetz and Schaffer, 1987, 1992; Schaffer and Ploetz, 1989; Schaffer et al., 1992; Schaffer, 1998). It was postulated that the reduction in net photosynthesis and transpiration of avocado due to flooding is most likely due to reduced stomatal closure rather than a hydraulic effect because there was no difference in stem (xylem) water potential (SWP) between flooded and non-flooded plants (Schaffer et al., 1992). Therefore, it is likely that stomata close as an early response to flooding to reduce transpirational water loss resulting in reduced stomatal conductance and net photosynthesis. However, the effect of flooding on stomatal closure and the relationship among stomatal closure, xylem water potential, and stomatal conductance in response to flooding have not been reported.

The purpose of this study was to determine the effect of flooding on leaf gas exchange, xylem water potential, stomatal closure, growth and survival of 'Beta', a West Indian cultivar, and 'Hass' avocado trees and to relate leaf gas exchange and plant water potential to stomatal closure under flooded conditions.

2. Materials and Methods.

Date and location of the experiment: The experiment was conducted at the Tropical Research and Educational Center (TREC), University of Florida, Homestead, Florida, USA, in August and September 2006, in a screened enclosure with a clear polyethylene roof.

Plant material: One-year-old 'Beta' and 'Hass' avocado trees grafted on seedling 'Waldin' rootstock were evaluated. Plants were in 11.4 liter plastic pots in a potting medium composed of 60% Canadian peat, 40% pine bark by volume, and 10% sand, which filled in the pore space in the media and didn't add additional volume.

Treatments: Plants in the control treatment (To) were irrigated twice daily and allowed to drain between irrigations. Plants in the flooded treatment (T1) were flooded for 11 days, by submerging the pots in plastic tubs with the water level 5 cm above the soil surface. There were 3 pots per tub (to maintain similar environments for each treatment, plants in the To treatment were also placed in tubs with no water added). After 11 days, plants in the T1 treatment were

removed from flooding (unflooded). Plants were harvested 11 days after unflooding for 'Beta' plants and 15 days after the unflooding for 'Hass' plants.

Experimental design: The experiment was arranged in a randomized complete block design. Treatments were blocked by size and cultivar (Beta: medium and small size, Hass: small size). An experimental unit consisted of 3 plants per tub (each plant with more than 30 fully expanded leaves). This provided a sufficient number of leaves per experimental unit to periodically excise leaves for SWP measurements without removing too much leaf area per plant to create additional stress. There were 4 tubs (12 'Beta' plants - 2 tubs with large 'Beta' and 2 tubs with small 'Beta' plants) and 2 tubs (6 'Hass' plants - 2 tubs) in each treatment.

Measurements:

Leaf gas exchange: Net CO_2 assimilation (A), stomatal conductance (gs) and transpiration (T) were measured with a CIRAS-2 infrared gas analyzer (IRGA) (PP Systems, Amesbury, Massachusetts, USA) on 3 recently matured leaves per plant from 9:00 to 11:00 hr, every two days during the flooding period and periodically from the time plants were unflooded until they were harvested.

Stem water potential: Stem (xylem) water potential (SWP) was measured with the same frequency as A, gs and T using the technique described by Meyer and Reickosky (1985). For SWP determinations, 3 leaves per experimental unit were covered with plastic and aluminum foil and then excised between 11:30 and 12:30 hr. Excised leaves were immediately placed in a cooler with ice and brought to a laboratory where SWP was determined with pressure chamber (Scholander *et al.*, 1965)

Stomatal impressions: Immediately after measuring gs, A and T, tooth silicone (Oranwash L ®, Zhermack) was applied to a 10 cm^2 area of the abaxial surface of same the leaves used for gas exchange measurements. After the material hardened, it was painted with clear nail polish to obtain an impression of the surface of the leaf which was then observed with an optical microscope (at 400 x) to determine if the stomata were open or closed (Weyers and Johanson, 1985). The relative proportion of open and closed stomata was related to gs.

Chlorophyll fluorescence: Prior to initiating the flooding treatment and 15 days after plants were initially flooded, the variable to maximal chlorophyll fluorescence ratio (Fv/Fm) was determined with a portable fluorometer (Opti-Sciences, Inc., Tyngsboro, Massachusetts, USA). The Fv/Fm ratio is an indicator of plant stress resulting from damage to photosystem II (Björkman, 1987) and indicates if reductions of A in stressed plants are the result of photoinhibitory damage to the photosynthetic apparatus.

Leaf area: Throughout the study period, leaf area of the absised leaves was determined with a leaf area meter (model LI-3000, Li-Cor, Inc., Lincoln, Nebraska, USA)

Biomass: at the end of the experiment the plants were harvested and roots and aerial part were separated and dried in an oven at 90ºC for 3 days. Root and shoot dry weights were then determined.

Soil redox potential: Four and 10 days after flooding was initiated, soil redox potential was measured with an $Ag^+/AgCl$ platinum combination electrode (Accumet, Fisher Scientific, Pittsburgh, Pennsylvania, USA) attached to a portable pH/voltmeter (Accumet model AP62, Fisher Scientific, Pittsburgh, Pennsylvania, USA) to determine changes in the oxygen content of the soil. Soil redox potentials lower than -200 mV are considered to be anaerobic (Ponnamperuma, 1984).

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

3. Results

Leaf gas exchange and plant water status. Responses of 'Beta' plants to flooding were different from responses of 'Hass' plants to flooding for all variables measured, even though both cultivars were on the same ('Waldin' seedling) rootstock. There was a significant statistical interaction ($P \leq 0.05$) between cultivar and treatment for all of the variables measured. Therefore, each cultivar was analyzed separately. For 'Beta', there was no significant statistical interaction between plant size (large or small) and treatment and no significant plant size effect ($P > 0.05$) on any of the variables measured. Therefore, data from large and small 'Beta' plants were pooled for statistical analysis.

Net CO_2 assimiliation, gs and T of flooded 'Beta' plants were significantly lower than those of control plants beginning 8 days after flooding was initiated (Figure 1). Differences in SWP between flooded and control 'Beta' plants were first significant 15 days after flooding was initiated, with flooding plants having higher (less negative) SWP than control plants (Figure 1). After 'Beta' plants were unflooded they did not recover from flooding stress and exhibited progressive leaf abscission with complete defoliation 20 days after flooding was initiated. All but two of the flooded 'Beta' plants eventually died prior to the end of the experiment.

'Hass' exhibited greater tolerance to flooding than 'Beta'. For 'Hass', flooded plants had significantly lower gs and T 10 days after flooding was initiated (Figure 2). For flooded 'Hass' plants, there was a recovery of gs 7 days after plants were unflooded. Flooding of 'Hass' resulted in a decrease in A 12 to 15 days after the flooding treatment was initiated (Figure 2). After that time, A of flooded 'Hass' plants increased to values near those of the control treatment. There were no effects of treatment on SWP of 'Hass' (Figure 2).

Soil redox potential was not significantly different between treatments at the beginning of the experiment (day 4) with values of 218 and 210.5 mV for the

control and flooded treatments, respectively. On day 10, significant differences in soil redox potential were observed between flooded and control plants with values of 194.4 mV and 88.4 mV for the control and flooded treatments, respectively. Although this indicated lower oxygen content in the flooded treatment, the flooded soil was still not severely low in oxygen. During the experiment the maximum VPD was 3.2 KPa (Figures 1 and 2).

Chlorophyll fluorescence. There were no significant differences in Fv/Fm between the control and flooded plants. The Fv/Fm ratios were 0.78 and 0.81 for the control and flooded treatments, respectively at the beginning of the experiment and 0.62 and 0.7 for the control and flooded treatment, respectively 12 days after flooding was initiated (Data not shown).

Leaf area. Flooded 'Beta' plants exhibited severe wilting beginning 4 days after flooding was initiated. Wilting was followed by leaf abscission beginning on day 5. After unflooding, flooded 'Beta' plants did not recover from wilting and more leaf abscission occurred until plants were completely defoliated. In contrast, flooded 'Hass' plants generally did not exhibit wilting or significant leaf abscission throughout the experimental period (Data not shown).

Biomass
Flooding of 'Beta' plants resulted in 77.4% less leaf dry weight and 10.6% less root dry weight than plants in the control treatment (Figures 3 and 4). Flooded 'Hass' plants also had lower leaf and root dry weights than control plants (Figures 3 and 4), but the difference in leaf and root dry weight between flooded and control plants were not a great for 'Hass' as for 'Beta' plants.

Relationship between stomatal conductance and the percentage of open stomata. Data from a sample of 30 combined leaves (`Hass´ and `Beta´ avocado, control and treatments) were taken to do the correlation. There was a moderate positive linear relationship (R = 0.6) between gs and the percentage of open stomatas. Only a 20% of stomata were open when gs values were near 30 mmol m^{-2} s^{-1} (Figure 5).

Relationship between stem water potential and stomatal conductance. Linear regression analysis of pooled data from both treatments and cultivars showed that there was no significant linear relationship between SWP and gs (Figure 6).

4. Conclusions

• For young 'Beta' avocado plants grafted onto Waldin rootstock, T, gs and A were significantly reduced after 8 days of continuous flooding and SWP was reduced after 15 days of flooding.

• 'Hass' was less sensitive to flooding stress than 'Beta' on the same ('Waldin' seedling) rootstock as evidenced by the longer period of flooding required to reduce A, gs and T of 'Hass' compared to 'Beta'. Also, 'Hass' plants on 'Waldin'

rootstock were able to survive 11 days of continuous flooding, whereas 'Beta' plants on the same rootstock succumbed to 11 days of continuous flooding. Additionally, the reduction in leaf and root biomass caused by flooding was greater for 'Beta' than for 'Hass' plants.

• The reduction of A as a response to flooding is presumably due to a reduction of gs and not photoinhibition (damage to PSII) because flooding had no effect on Fv/Fm.

 • The reduction in transpiration resulting from flooding appeared to be due to a reduction in gs and not to a hydraulic change. It was not possible to correlate SWP with stomatal closure resulting from flooding.

 • There was a moderate positive linear relationship between gs (mmol m^{-2} s^{-1}) and the percentage of opened stomata. Thus, it appears that in response to flooding, stomata close to reduce transpirational water loss resulting in reduced gs. For flooded plants reduced gs and A lead to a reduction in biomass. Significant stomatal closure in avocado happens with gs lower than 50 mmol m^{-2} s^{-1}.

• If a cultivar is susceptible to flooding (i.e., 'Beta') severe defoliation and plant death can occur several days or weeks after plants are unflooded.
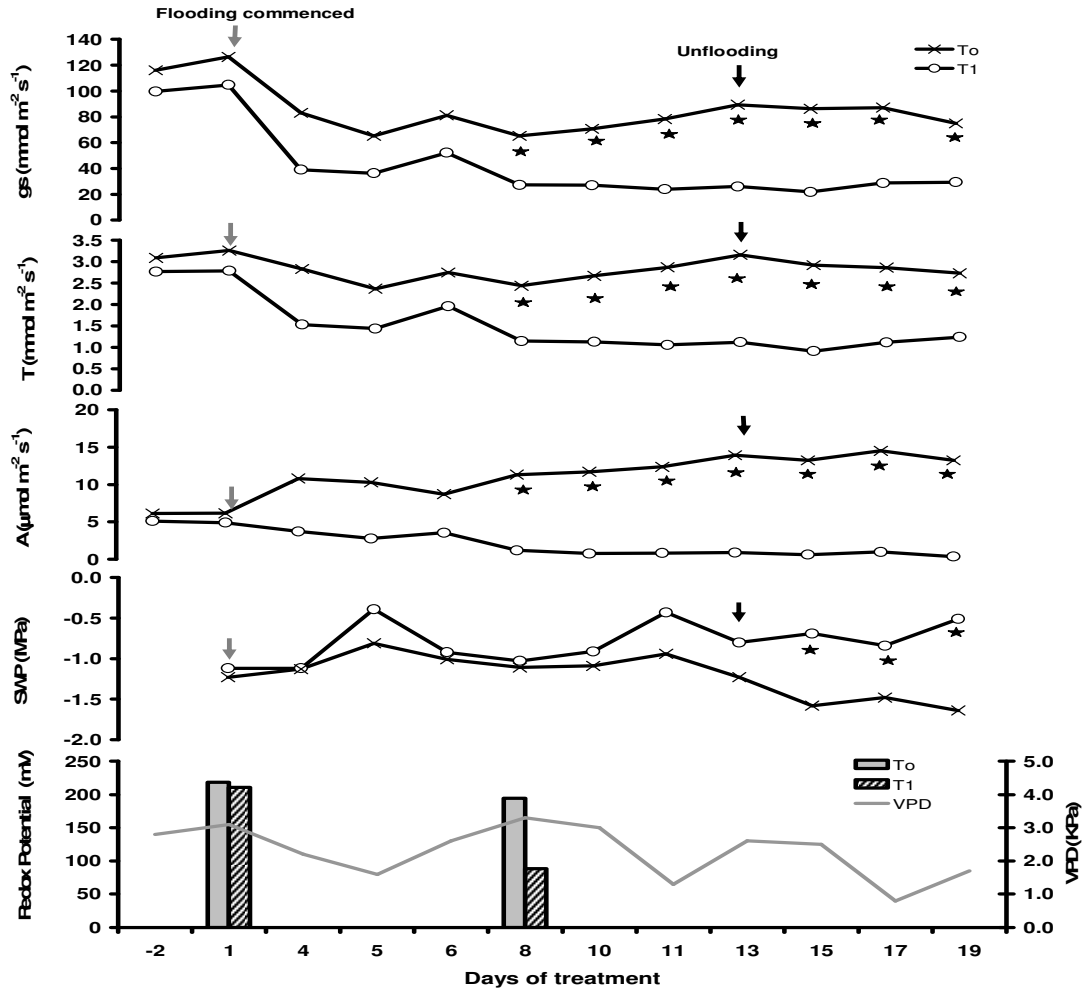
5. Figures

Figure 1: Effect of flooding in stomatal conductance (gs), transpiration (T), net CO_2 assimilation (A), stem water potential (SWP) and soil redox potential of 'Beta' avocado trees and vapor pressure deficit (VPD) during the experiment. An asterisk indicates a significant difference ($P \leq 0.05$) according to a standard T-Test.
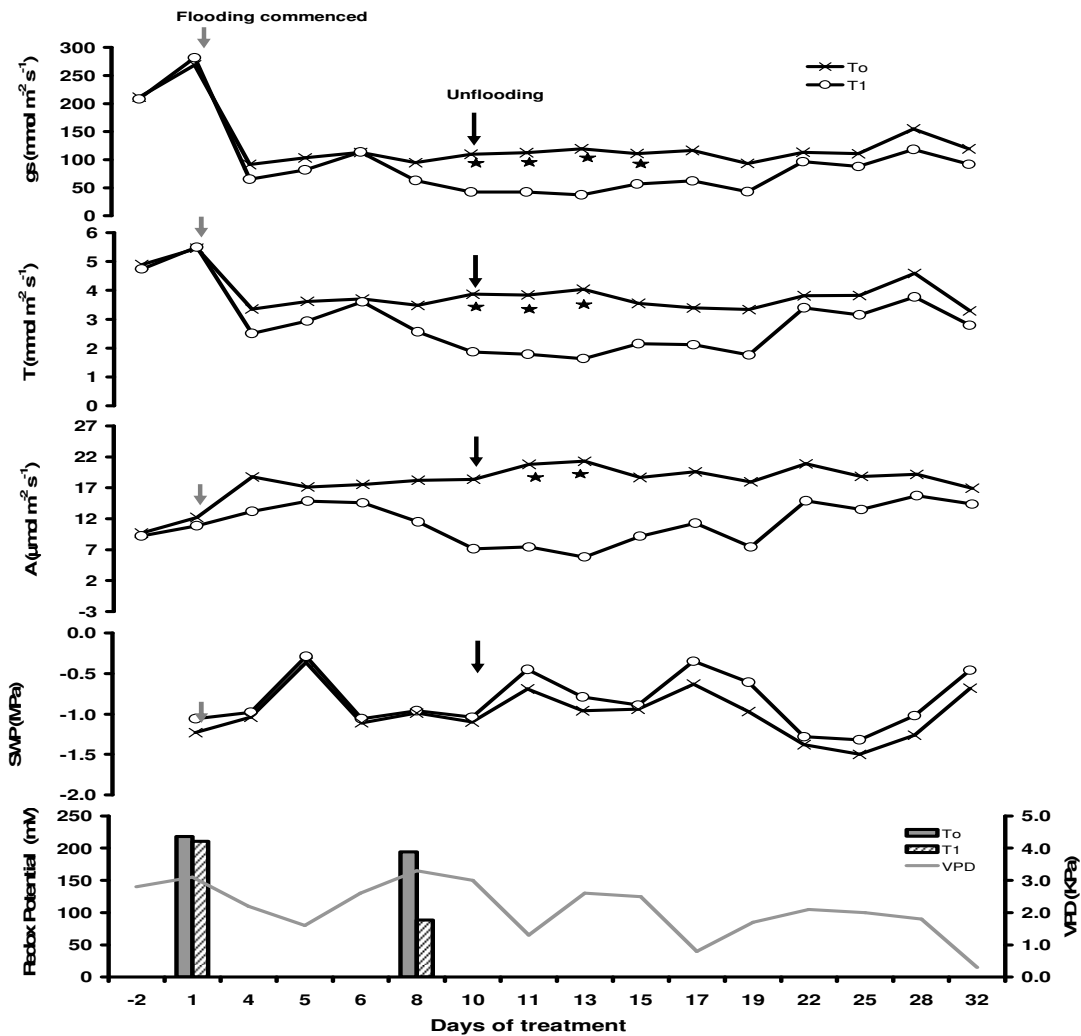
Figure 2: Effect of flooding in stomatal gs, T, A, SWP of 'Hass' avocado trees and soil redox potential and vapor pressure deficit (VPD) during the experiment. An asterisk indicates a significant difference (P < 0.05) according to a standard T-Test.
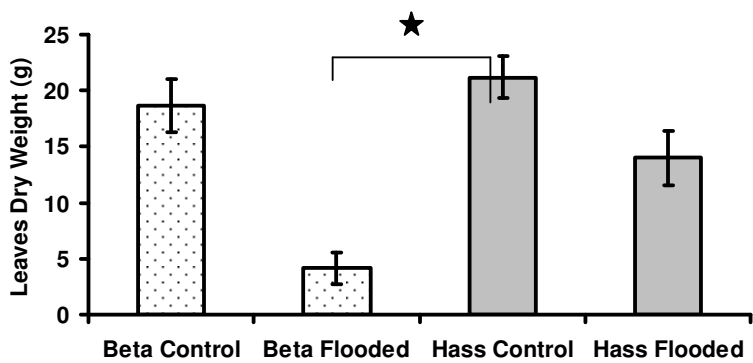

Figure 3: The effect of flooding on leaf dry weight of 'Beta' and 'Hass' avocado. Asterisks indicate significant different between the 2 treatments within each cultivar according to a standard T-test (P ≤ 0.05)"
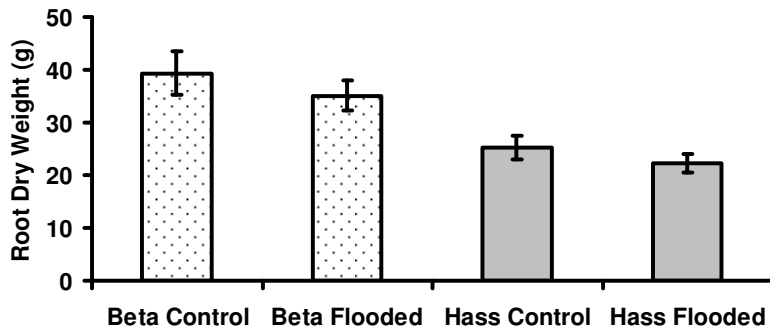
Figure 4: The effect of flooding on root dry weight of 'Beta' and 'Hass' avocado.

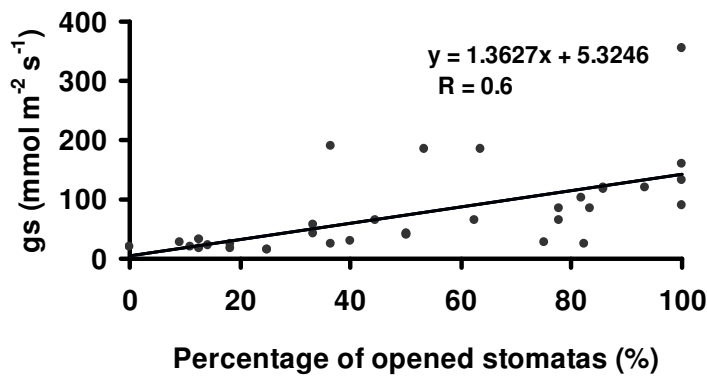

$y = 1.3627x + 5.3246$
$R = 0.6$

Figure 5. Relationship between stomatal conductance (gs) and the percentage of open stomata. Plants of both cultivars and in the flooded and control treatments were used in this analysis.
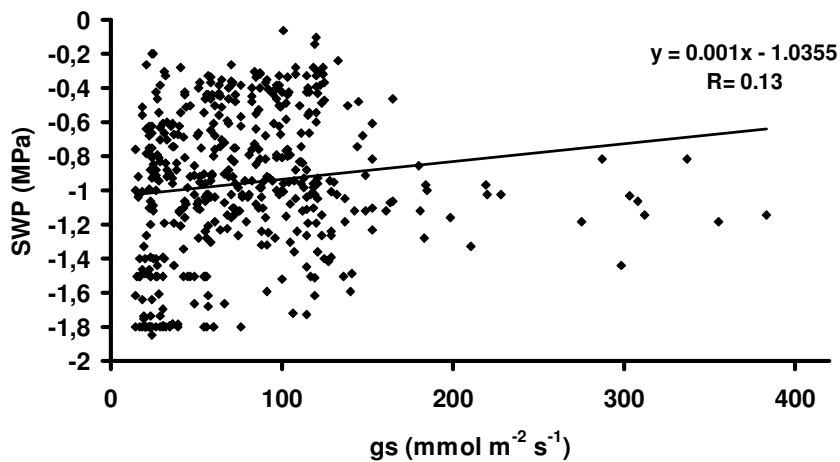

$y = 0.001x - 1.0355$
$R = 0.13$

Figure 6. Relationship between SWP and gs of flooded plants.

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