



Leaf removal before flooding influences recovery of avocado (*Persea americana* Mill.) trees from flooding stress

Maria Angelica Sanclemente^{a,b}, Bruce Schaffer^{a,*}, Pilar M. Gil^c, Frederick S. Davies^b, Jonathan H. Crane^a

^a University of Florida, Tropical Research and Education Center, 18905 S.W. 280 Street, Homestead, FL 33031, USA

^b Department of Horticultural Sciences, University of Florida, P.O. Box 110690, Gainesville, FL 32611, USA

^c Departamento de Fruticultura y Enología, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306-22, Santiago, Chile

ARTICLE INFO

Article history:

Received 17 August 2012

Received in revised form 1 November 2012

Accepted 3 November 2012

Keywords:

Persea americana

Avocado

Flooding

Pruning

Net CO₂ assimilation

Stomatal conductance

D-mannoheptulose

Perseitol

ABSTRACT

Three experiments (Expts. 1, 2 and 3) were conducted to test the effects of removing a portion of the leaf canopy of avocado trees (*Persea americana* Mill.) prior to flooding on plant stress, survival and recovery from short-term flooding. Trees in each experiment were divided into two flooding treatments: (1) flooded, or (2) non-flooded. Trees in each flooding treatment were divided into two pruning treatments in Expt. 1: (1) approximately two-thirds of the canopy removed by pruning, or (2) non-pruned (control). In Expt. 2, trees in each flooding treatment were divided into three “canopy” treatments: (1) removal of two-thirds of the total number of leaves from the bottom of the canopy upward (2/3 LR), (2) foliar application of a chemical photosynthetic inhibitor and no leaf removal (CP), or (3) no leaf removal or photosynthetic inhibitor applied (control). Trees in each flooding treatment in Expt. 3 were divided into two treatments: (1) two-thirds of the total number of leaves removed from the bottom of the canopy upward, or (2) no leaves removed (control). In each experiment, net CO₂ assimilation (*A*) and stomatal conductance of water vapor (*g_s*) were determined daily during the flooding period and periodically after the flooding period until trees were harvested. Plant survival and tissue dry weights were determined several weeks after plants were removed from flooding (unflooded) in Expts. 1 and 2. Root carbohydrate concentrations were determined immediately after the flooding period in Expt. 3. Pruning, leaf removal or foliar application of a chemical photosynthetic inhibitor immediately prior to flooding resulted in significantly lower *A* of flooded compared to non-flooded trees. Concentration of the C₇ sugar, D-mannoheptulose, was higher in non-flooded than in flooded plants. In flooded plants, concentration of the C₇ sugar alcohol, perseitol, was higher in plants with no leaves removed than those in the 2/3 LR treatment. Reducing photosynthesis of avocado trees prior to flooding, either by pruning, leaf removal, or the application of a chemical photosynthetic inhibitor, makes trees more susceptible to flooding stress. Reducing *A* prior to flooding limits carbohydrate transport to the roots, thereby limiting the amount of substrate for respiration of flooded roots, making the plants more susceptible to flooding stress.

Published by Elsevier B.V.

1. Introduction

Avocado (*Persea americana* Mill.), a subtropical evergreen tree native to Central American and Mexico, is grown commercially in Mediterranean, subtropical and tropical climates worldwide (Whiley and Schaffer, 1994). In the United States, the vast majority of avocado production is in California, where in 2010 there was an estimated 21,108 ha planted (FAOSTAT, 2010). Although there are only 2995 ha of avocado trees planted commercially in Florida, it is second only to citrus among tree fruit crops in annual farm gate sales (Evans and Nalampang, 2010).

Rapidly increasing avocado production in several regions of the world has resulted in orchard establishment on marginal sites that are prone to flooding or poor soil drainage (Schaffer and Whiley, 2002; Gil et al., in press). In southern Florida, soils in avocado orchards can become saturated due to capillary water movement from a water table which is not far (1.8–2.3 m) below the soil surface (Barquin-Valle et al., 2011) and heavy rains from tropical storms or hurricanes (Crane et al., 1994; Schaffer and Whiley, 2002). Flooding displaces oxygen and other gases and increases the concentrations of toxic organic and inorganic compounds in agricultural soils (Drew, 1997; Geigenberger, 2003; Irfan et al., 2010). Thus, flooding can negatively impact crop physiology, growth, production and even survival of fruit trees, including avocado (Schaffer et al., 1992).

Avocado is a flood-sensitive species with physiological responses occurring shortly after soil becomes waterlogged (Schaffer et al., 1992; Whiley and Schaffer, 1994; Schaffer and

* Corresponding author. Tel.: +1 305 246 7000; fax: +1 305 246 7003.

E-mail address: bas56@ufl.edu (B. Schaffer).

Whiley, 2002). Responses of avocado trees to even short periods of root zone hypoxia include leaf abscission, root necrosis, and reductions in Net CO₂ assimilation (*A*), stomatal conductance of water vapor (*g_s*), shoot and root growth, and fruit production (Schaffer and Whiley, 2002; Gil et al., in press). A few days of flooding can result in avocado tree death (Schaffer, 1998), which has been attributed to a high canopy to root ratio as a result of damage to roots which occurs prior to canopy damage when trees are flooded (Schaffer, 1998). Root water absorption and nutrient uptake by avocado trees are often reduced during flooding (Gil et al., in press). As a result, the resistance to water movement across the root cortex is increased. This eventually leads to leaf dehydration, stomatal closure, and tree death (Schaffer et al., 1992, 2007; Schaffer and Whiley, 2002).

The observation of a high canopy to root ratio as a result of flooded or poorly drained soils has led to the recommendation in some avocado growing regions (e.g. southern Florida, Chile) to prune or remove part of the canopy after flooding to mitigate stress from root hypoxia (Gil et al., 2008). Due to extensive root damage of partially uprooted trees after a tropical storm or hurricane, removing part or most of the canopy reduces the weight of the tree, making resetting and stabilizing the tree easier, and also reduces transpiration and prevents desiccation (Crane et al., 1994). Nevertheless, canopy pruning to mitigate flooding stress is based on observations rather than experimental evidence. Preliminary studies with flooded avocado trees in containers showed that pruning the canopy immediately after flooded trees were unflooded reduced plant stress and increased tree survival (Gil et al., 2008).

In contrast to leaf removal after flooding, leaf removal before flooding increased flooding stress of avocado trees in containers (Gil et al., 2008). It was speculated that increased stress of flooded avocado trees due to canopy removal prior to flooding was the result of less carbohydrate produced and translocated to the roots as a result of reduced photosynthesis from the combined effects of less photosynthesizing surface (leaf) area and root hypoxia. Thus, it was hypothesized that limiting photosynthesis by reducing leaf area resulted in less carbohydrate transported to the roots to act as a substrate for root respiration, and thus less ATP produced to help maintain normal plant metabolism under hypoxic root conditions (Gil et al., 2008). If this hypothesis is correct, reducing carbohydrate production and transport to the roots by means other than leaf removal, i.e. the use of a chemical photosynthetic inhibitor, should also increase flooding stress and delay tree recovery after flooding.

The seven-carbon (C₇) sugar, mannoheptulose, and a related C₇ sugar alcohol, perseitol, are the major forms of nonstructural carbohydrates in avocado trees (Liu et al., 1999). These C₇ sugars often account for more than 10% of the tissue dry weight and can be found in substantial quantities in all tissues and organs. The concentrations of primary nonstructural carbohydrates based on a six-carbon hexose skeleton (i.e. glucose and sucrose) common in most other fruit trees, are in much lower concentrations than the C₇ sugars in avocado tissues (Liu et al., 1999). Research with avocado has often focused only on starch reserves and thus overlooked the importance of the C₇ sugars in the carbon allocation process (Liu et al., 1999). Little is known about how these sugars mediate root/shoot relations or respond to environmental stress conditions, particularly root hypoxia or anoxia.

The main objective of this study was to determine if limiting photosynthesis by pruning, leaf removal, or foliar application of a chemical photosynthetic inhibitor prior to flooding, exacerbates stress and delays or prevents recovery of avocado trees exposed to short-term flooding. Additionally, the combined effects of leaf removal prior to flooding and root zone hypoxia on root carbohydrate concentrations, particularly C₇ sugars, were determined.

2. Materials and methods

2.1. Study site description

This study was conducted at the University of Florida, Tropical Research and Education Center in Homestead, Florida (25.5°N and 80.5°W) in a shade-house with a clear polyethylene roof and shade cloth (30% light exclusion) on all four sides.

Temperature in the shade-house was monitored with a StowAway, TidbiT sensor/data logger (Onset Computer Corp., Bourne, Massachusetts, USA) located ~15 cm above the plant canopies.

There were three experiments conducted with avocado trees in plastic containers. The first experiment (Expt. 1) assessed the effects of pruning a portion of the canopy prior to flooding on plant stress and recovery from short-term flooding. The second experiment (Expt. 2), determined the effects of reduced photosynthesis, by either leaf removal or the use of a foliar applied chemical photosynthetic inhibitor prior to flooding, on plant stress and recovery from flooding. The third experiment (Expt. 3) focused on determining the effects of reduced photosynthesis by leaf removal prior to flooding on plant stress and root carbohydrate content as a result of flooding.

2.2. Plant material

Two-year-old 'Choquette' avocado trees on Waldin seedling rootstock growing in potting medium (40% Canadian peat, 10% coir, 40% pine bark and 10% perlite) in 11.3 L containers were obtained from a commercial nursery (Expts. 1 and 2). One-year-old 'Choquette' avocado trees (Expt. 3) on Waldin seedling rootstock, growing in the same type of potting medium as trees in Expts. 1 and 2, in 3.8 L containers were obtained from a commercial nursery.

The potting medium used in all experiments was steam sterilized prior to planting trees and initiating treatments. Prior to initiating treatments, trees in each experiment were treated with fungicide; Ridomyl® (Syngenta International AG, Basel, Switzerland) in Expt. 1 or Alliete® (Bayer Crop Science, Morganville, NC, USA) in Expts. 2 and 3 as a soil drench, to help prevent Phytophthora root rot.

2.3. Experimental design

Expt. 1 was set up as a completely randomized design with a 2 × 2 factorial arrangement of treatments. There were two pruning treatments: (1) approximately two-thirds of the canopy pruned away before flooding (pruned), or (2) non-pruned; and two flooding treatments (flooded or non-flooded). There were five single-tree replications for each treatment combination.

Expt. 2 was set up as a completely randomized design with a 3 × 2 factorial arrangement of treatments. There were three "canopy" treatments: (1) two-thirds of the leaves removed before flooding (2/3 LR), (2) use of a chemical photosynthetic inhibitor sprayed on the foliage (CP), or (3) no leaf removal or chemical spray (control), and two flooding treatments (flooded or non-flooded). There were six single-tree replications for each treatment combination.

Expt. 3 was set up as a completely randomized design with a 2 × 2 factorial arrangement of treatments. There were two leaf removal treatments: (1) two-thirds of the leaves removed (LR), or (2) no leaf removal (control), and two flooding treatments (flooded or non-flooded). There were 6 single-tree replications for each treatment combination.

2.4. Flooding treatments

Plants were flooded by submerging each plant container in a 19L plastic bucket filled with tap water to 5 cm above the soil surface. Buckets were refilled each day with stagnant water collected on the same day that the flooding treatment was initiated to avoid re-oxygenation of the medium and maintain a constant water level. Trees in the control treatment were not flooded. Plants were removed from flooding (unflooded) after a statistically significant difference ($P \leq 0.05$) in net A or g_s between flooded and non-flooded trees was observed in any of the leaf removal (Expts. 1 and 3) or canopy treatments (Expt. 2). This occurred on the fourth day of flooding for each experiment so the flooding period was 4 days in all experiments. Trees were irrigated by overhead irrigation for 15 min twice each day, except during the flooding period, when non-flooded trees were manually irrigated twice each day. Tensiometers (Irrrometer Company, Riverside, CA, USA) were installed in five randomly selected containers for plants in the non-flooded treatments and soil suction was maintained at 10–15 KPa to ensure that trees were not drought stressed (Kiggundu et al., 2012).

2.5. Pruning or leaf removal treatments

In each experiment, leaves were removed one day prior to initiation of the flooding treatments. Trees in the control treatments had no leaves removed.

In Expt. 1, approximately two-thirds of the canopy was removed by counting the leaves and pruning the upper branches until only about the lower one-third of the initial canopy remained by cutting the top of the central leader and trimming some of the remaining lateral shoots. This resulted in the older leaves remaining on the pruned trees.

In Expts. 2 and 3, in order to maintain the most actively photosynthesizing leaves on trees in each treatment for A and g_s measurements (Schaffer et al., 1991), two-thirds of the leaves were removed starting from the base of the canopy upward to allow the most recently developed leaves to remain on trees in both the leaf removal and no leaf removal (control) treatments.

2.6. Chemical photosynthetic inhibitor treatment

The foliage of trees in the CP treatment in Expt. 2 was sprayed to run-off with 2 mL L^{-1} of a photosynthetic inhibitor (Freeway[®]; alcohol ethoxylates, silicone-polyether copolymer, propylene glycol and dimethylpolysiloxane; Loveland Products Inc., Loveland, CO, USA) with a hand sprayer, covering both the adaxial and abaxial surfaces. The photosynthetic inhibitor used is an organosilicone surfactant that was previously found to inhibit photosynthesis of lychee (*Litchi chinensis*) (Schaffer et al., 2011) and avocado trees (B. Schaffer, University of Florida, 2009, unpublished data). Applications started the day before initiating the flooding treatment. During the flooding period, flooded and non-flooded trees were sprayed every other day until plants were unflooded. Trees in the control (no photosynthetic inhibitor application or leaves removed) treatment (flooded or non-flooded) were not sprayed.

2.7. Net CO_2 assimilation (A) and stomatal conductance (g_s) measurements

As physiological indicators of plant stress in the absence of visible symptoms, A and g_s were measured for all trees in each experiment with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, MA, USA) at a light saturated photosynthetic photon flux (PPF) of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, a reference CO_2 concentration of $375 \mu\text{mol mol}^{-1}$, and an air flow rate into the leaf cuvette of 200 mL min^{-1} . In Expt. 1, measurements were made on the two

most recently mature (youngest) leaves that remained on pruned trees after pruning. Similar age leaves of non-pruned (control) trees were used for A and g_s measurements. In Expts. 2 and 3, measurements were made on the two most recently matured leaves of each plant in each treatment. In all experiments measurements were made between 900 HR and 1100 HR. In Expts 1 and 2, measurements were made two days prior of the initiation of flooding, daily during the flooding period, and periodically after trees were unflooded to monitor tree recovery from flooding. Measurements of A and g_s were stopped after there were no significant differences in A or g_s between flooding treatments. Measurements were stopped immediately after the flooding period in Expt. 2 because plants were harvested so that root carbohydrate concentrations could be measured.

2.8. Plant dry weights

Plants were harvested at the end of each experiment. In Expts. 1 and 2, plant organs (leaves, stems, and roots) were collected for dry weight determinations. Roots were separated from the rooting medium by carefully washing them in tap water. Tissue samples were oven dried at 70°C to a constant weight, and leaf, stem root and whole plant dry weights were determined. Tissue dry weights were not determined in Expt. 3 because fresh root tissue was collected and immediately freeze dried for carbohydrate determination.

2.9. Root carbohydrate concentrations

Root samples from each tree in each treatment combination in Expt. 3 were collected immediately after trees in the flooded treatment were unflooded. Samples were freeze-dried at -50°C for 50 h in a Freezone 4.5 freeze dryer (Labconco, Kansas City, MO, USA). Freeze-dried samples were ground using a Proctor Silex[®] coffee grinder (Hamilton Beach, Southern Pines, NC, USA) to obtain 0.1 g per sample. The samples were sent to the Department of Botany at the University of California, Riverside, USA for extraction and analysis of C_7 sugars, sucrose, fructose and glucose using the ethanol-based method described by Liu et al. (2002). This method is sufficiently sensitive to detect sugar or starch concentrations as low as 0.04% (Chow and Landhäusser, 2004).

2.10. Soil redox potential and soil temperature

In each experiment, soil redox potential was measured with a metallic ORP indicating electrode (Accument Model 13-620-115, Fisher Scientific, Pittsburg, PA, USA) connected to a voltmeter for plants in the flooded treatment. Measurements were made daily in each container during the flooding period by placing the electrode into a polyvinyl chloride (PVC) pipe inserted 10 cm deep into the media of each container.

In experiments 2 and 3, soil temperature was recorded using a Hobo Tidbit v2 sensor/datalogger (Onset Computer Corp., Pocasset, MA, USA) placed 5 cm below the soil surface into the container of one randomly selected plant in each treatment.

2.11. Statistical analyses

Data were analyzed by a two-way analysis of variance (ANOVA) to assess interactions between flooding treatments and pruning (Expt. 1), canopy (Expt. 2), or leaf removal treatments (Expt. 3). Differences between flooding treatments were compared by repeated measures ANOVA for A and g_s , or a T -test for carbohydrate concentration and plant dry weights using the SAS statistical software package (SAS 9.1, SAS Institute, Cary, NC, USA).

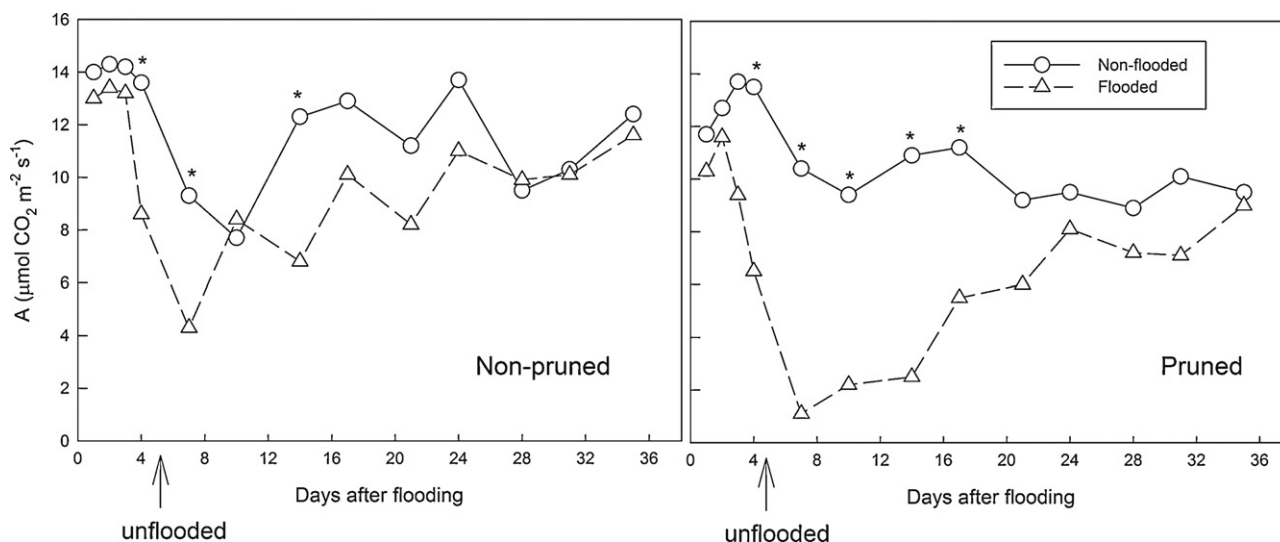


Fig. 1. Effect of flooding on net CO_2 assimilation (A) of pruned and non-pruned two-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 1). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 5$.

3. Results

3.1. Air and soil temperature, and soil redox potential

In Expt. 1, daily air temperature in the shade-house ranged from 15.1 to 25.2 °C with a mean of 22.1 °C. Mean soil redox potential for the flooded treatment was 129.1 mV one day after flooding and values decreased to 82.2 mV by day 4. Soil redox potential below 200 mV indicates anaerobic soil conditions (Ponnamperuma, 1972).

In Expt. 2, daily air temperature in the shade-house ranged from 23 to 41 °C with a mean of 29 °C. Soil temperature in the non-flooded treatment ranged from 24 to 39 °C with a mean of 30 °C. Soil temperature in flooded treatment ranged from 21 to 38 °C with a mean of 28 °C. Mean soil redox potential for the flooded treatment was 162.4 mV beginning one day after flooding and continued to decrease to a mean of 41.4 mV by day 4.

In Expt. 3, daily air temperature in the shade-house ranged from 20 to 39 °C with a mean of 28 °C. Soil temperature in non-flooded

treatment ranged from 21 to 37 °C with a mean of 30 °C. Soil temperature in flooded treatment ranged from 21 to 38 °C with a mean of 28 °C. Mean soil redox potential for the flooded treatment was 219.0 mV beginning after 1 day of flooding and decreased to a mean of 42.5 by day 4.

3.2. Leaf gas exchange

There was a significant statistical interaction ($P \leq 0.05$) between pruning and flooding treatments for A and g_s on several measurement dates in Expt. 1. While flooding resulted in a significant reduction in A (Fig. 1) and g_s (Fig. 2) of both non-pruned and pruned trees on several measurement dates, the reductions in both A and g_s due to flooding were greater and more frequent for trees that were pruned before flooding than for non-pruned trees (Figs. 1 and 2).

In Expt. 2, there was a significant statistical interaction ($P \leq 0.05$) between the canopy and flooding treatments for A and g_s on several measurement dates. There was no significant effect of flooding on A (Fig. 3) in the control treatment on any of the measurement

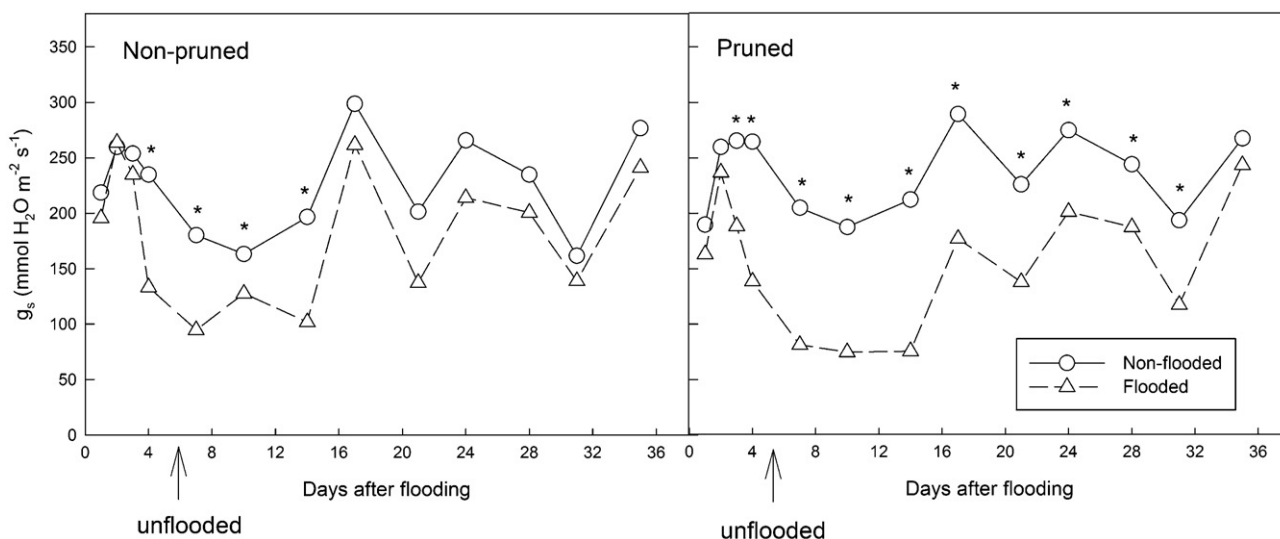


Fig. 2. Effect of flooding on stomatal conductance of water vapor (g_s) of pruned and non-pruned two-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 1). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 5$.

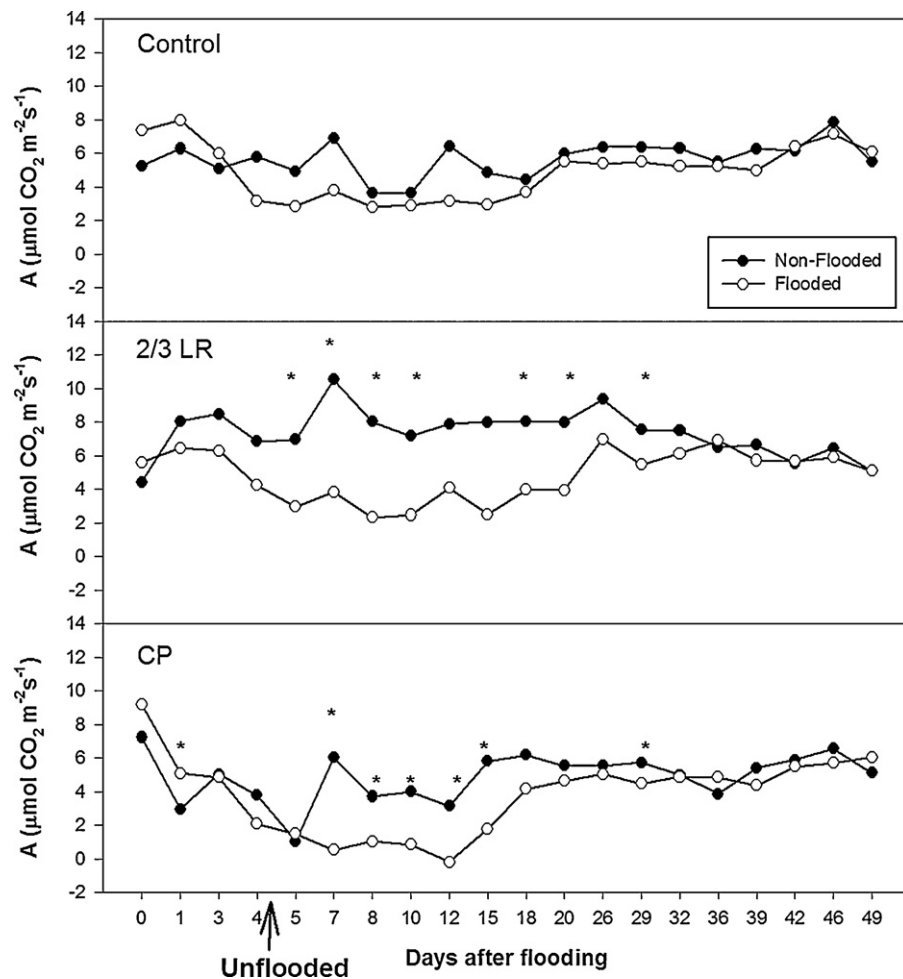


Fig. 3. Effect of flooding on net CO₂ assimilation (*A*) of two-year-old 'Choquette' avocado trees on Waldin seedling rootstock. Treatments included a control, two-thirds of the leaves removed one day before flooding (2/3 LR), and a photosynthetic inhibitor applied to the foliage (CP) (Expt. 2). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

dates and g_s was significantly lower in flooded than in non-flooded trees ($P \leq 0.05$) on only one date during the recovery period (Fig. 4). Net CO₂ assimilation and g_s were significantly lower ($P \leq 0.05$) in flooded than in non-flooded trees in the leaf removal treatment after 4 days of flooding until about 28 days after trees were unflooded. In plants treated with the photosynthetic inhibitor, *A* and g_s were significantly lower ($P \leq 0.05$) in flooded than in non-flooded trees on several dates after the flooding period, beginning 1 and 3 days after trees were unflooded for *A* and g_s , respectively (Figs. 3 and 4).

In Expt. 3, there was a significant statistical interaction ($P \leq 0.05$) between leaf removal and flooding treatments. For trees in the control treatment after 3 and 4 days of flooding, *A* (Fig. 5) and g_s (Fig. 6), respectively, were significantly lower ($P \leq 0.05$) in flooded than in non-flooded trees (Fig. 5). Net CO₂ assimilation (Fig. 5) was significantly lower in flooded than in non-flooded trees in the leaf removal treatment ($P \leq 0.05$) after 3 days of flooding, whereas as g_s was higher in the flooded trees after 4 days of flooding (Fig. 6), indicating that reduced *A* as a result of flooding plants in the leaf removal treatment was not due to stomatal limitation to CO₂ uptake.

3.3. Tissue dry weights and plant survival

There was a significant interaction ($P \leq 0.05$) between flooding and pruning treatments in Expt. 1. There was no significant

effect of flooding on leaf, root, stem, or whole plant dry weight of non-pruned trees. However, for trees pruned immediately prior to flooding, flooding significantly ($P \leq 0.05$) reduced leaf, root, stem, and whole plant dry weights (Table 1). By the end of the experiment (day 36), 20% of the non-pruned, flooded plants died, whereas 40% of the pruned, flooded plants died.

In Expt. 2, due to a significant interaction ($P \leq 0.05$) between flooding and canopy treatments, dry weight differences between flooding treatments were compared separately within each canopy treatment. Stem dry weight of control plants was significantly higher ($P \leq 0.05$) for trees in the flooded than those in the non-flooded treatment (Table 2). For trees treated with the photosynthetic inhibitor, leaf dry weight was significantly lower ($P \leq 0.05$) for flooded than for non-flooded trees (Table 2). Total plant dry weight in the leaf removal treatment was significantly lower ($P \leq 0.05$) for flooded than for non-flooded trees (Table 2). By the end of the experiment (day 49), 33% of the flooded trees treated with the chemical photosynthetic inhibitor died. In both the control and leaf removal treatments, 17% of the flooded plants died.

Tissue dry weights and plant survival were not assessed in Expt. 3 because those plants were harvested immediately after the 4-day flooding period to determine root carbohydrate concentrations. Dry weight and survival differences between flooded and non-flooded avocado trees can generally not be detected until several days to

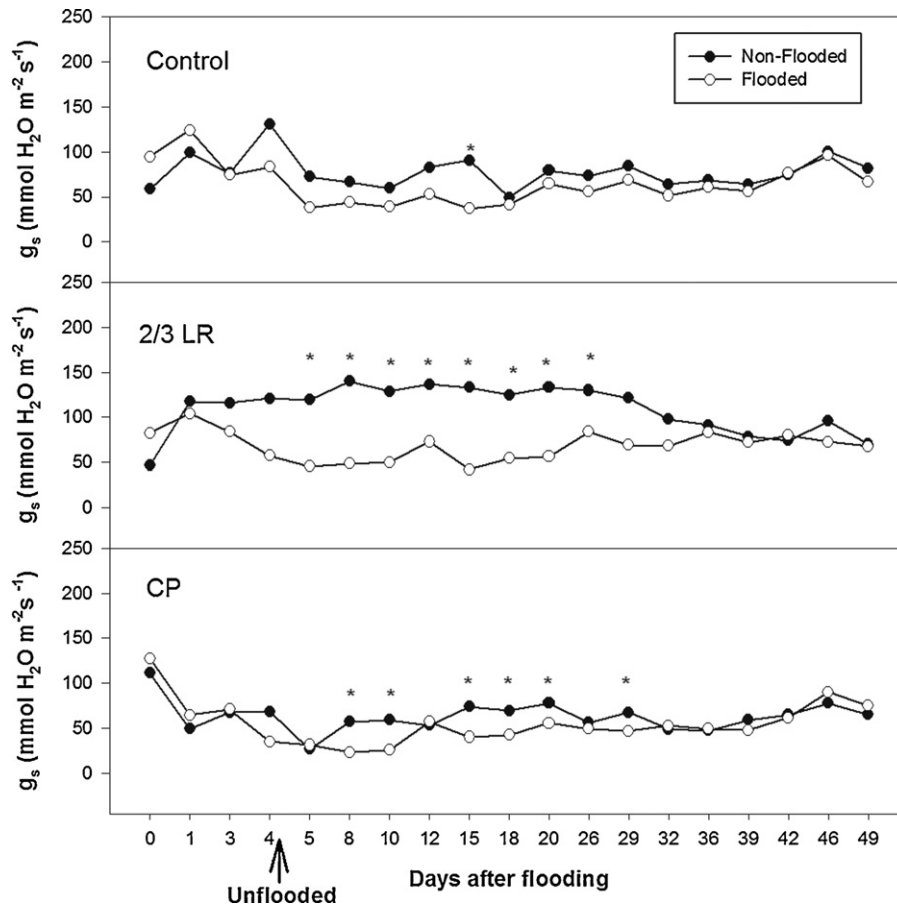


Fig. 4. Effect of flooding on stomatal conductance to water vapor (g_s) of two-year-old ‘Choquette’ avocado trees on Waldin seedling rootstock. Treatments included a control, two-thirds of the leaves removed one day before flooding (2/3 LR), and a photosynthetic inhibitor applied to the foliage (CP) (Expt. 2). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

Table 1

Effect of flooding on root, leaf, stem, and total plant dry weight of pruned (approximately two-thirds of the canopy removed by pruning) and non-pruned two-year-old ‘Choquette’ avocado trees on Waldin seedling rootstock (Expt. 1). An asterisk indicates a significant difference and ns indicates no significant difference between flooding treatments within each pruning treatment according to a *T*-test ($P \leq 0.05$), $n = 5$.

Flooding treatment		Leaf dry wt. (g)	Root dry wt. (g)	Stem dry wt. (g)	Total plant dry wt. (g)
Non-pruned	Non-flooded	29.1	31.4	32.5	93.0
	Flooded	23.0	24.7	26.6	74.2
	<i>P</i>	ns	ns	ns	ns
Pruned	Non-flooded	12.5	24.0	23.8	60.2
	Flooded	4.1	14.7	19.6	38.4
	<i>P</i>	*	*	*	*

Table 2

Effect of flooding on root, leaf, stem, and total plant dry weights of two-year-old ‘Choquette’ avocado trees on Waldin seedling rootstock (Expt. 2) with two-thirds of the leaves removed prior to flooding (2/3 LR), treated with a foliar-applied photosynthetic inhibitor (CP), or no leaves removed (Control). An asterisk indicates a significant difference and ns indicates no significant difference between flooding treatments within each canopy treatment according to a *T*-test ($P \leq 0.05$), $n = 6$.

Flooding treatment		Leaf dry wt. (g)	Root dry wt. (g)	Stem dry wt. (g)	Total plant dry wt. (g)
Control	Non-flooded	107.8	47.0	72.8	227.6
	Flooded	59.8	18.8	107.3	185.9
	<i>P</i>	ns	ns	*	ns
2/3 LR	Non-flooded	85.7	48.9	71.7	206.3
	Flooded	33.2	20.8	63.1	117.1
	<i>P</i>	*	ns	ns	*
CP	Non-flooded	121.0	48.2	79.1	247.3
	Flooded	80.7	34.7	76.5	191.9
	<i>P</i>	*	ns	ns	ns

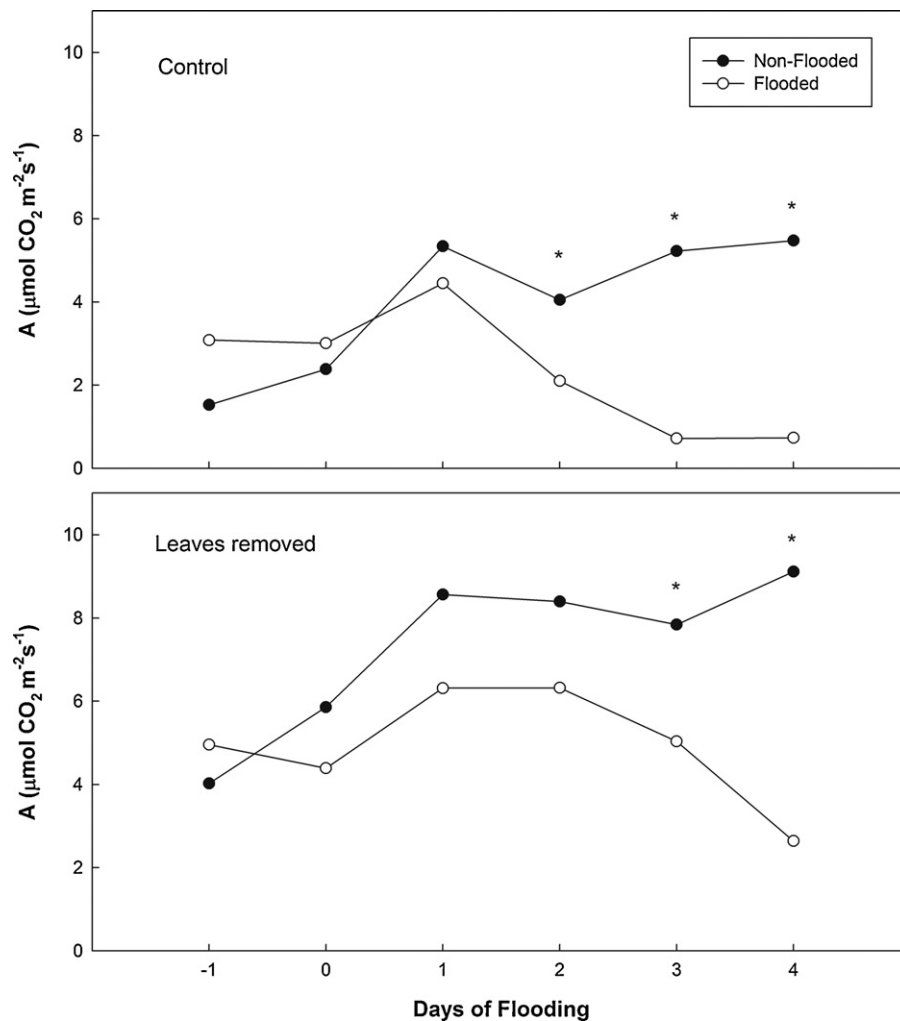


Fig. 5. Effect of flooding on net CO₂ assimilation (*A*) of one-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 3). Treatments included no leaf removal (Control) or two-thirds of the leaves removed one day before flooding (Leaves removed). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

several weeks after plants are unflooded when exposed to a short (3–5 day) flooding period (B. Schaffer, personal observations).

3.4. Root carbohydrate concentrations

There was a significant statistical interaction between flooding and leaf removal treatments only for sucrose and glucose concentrations in Expt. 3 ($P \leq 0.05$). Therefore, the effects of flooding treatment on root concentrations of each carbohydrate were analyzed separately within each leaf removal treatment and the effects of leaf removal were analyzed separately within each flooding treatment.

The carbohydrate found in the greatest concentration in the roots was D-mannoheptulose, followed by perseitol. Sucrose and glucose were detected in the roots, but in much lower concentrations than the C₇ sugars (Fig. 7). Fructose was not detected, either because it was not present or its levels were below the detection limit of 0.04% of the tissue dry weight (Liu et al., 2002).

D-mannoheptulose and sucrose concentrations were significantly higher in non-flooded than in flooded plants in the control treatment (Fig. 7). For trees in the leaf removal treatment, only the D-mannoheptulose concentration was significantly higher in non-flooded than flooded plants ($P \leq 0.5$). In both the leaf removal

and control treatments, the D-mannoheptulose concentration was nearly twice as high in the non-flooded as in the flooded trees (Fig. 7).

For trees in the non-flooded treatment, the concentration of perseitol was significantly lower in the control treatment than in the leaf removal treatment, whereas glucose was significantly higher in the control treatment ($P \leq 0.05$; Fig. 7). Flooded control trees had higher perseitol concentrations than trees in the leaf removal treatment ($P \leq 0.05$; Fig. 7).

4. Discussion

A decline in *A* of flooded control plants, 2 days (Expt. 2) or 4 days (Expts. 1 and 3) after flooding, coincided with reductions in *g*_s and soil redox potential. This is consistent with previous research that showed that a reduction in leaf gas exchange is the earliest symptom of flooding stress of avocado (Ploetz and Schaffer, 1989; Schaffer et al., 1992), and that this response tends to occur within a few days after exposure to root zone hypoxia (Whiley and Schaffer, 1994; Schaffer and Whiley, 2002).

Pruning, leaf removal, or the use of a chemical photosynthetic inhibitor prior to flooding resulted in a greater reduction in *A*, slower recovery from flooding stress, and a higher incidence of tree mortality compared to the control treatment. Thus, inhibition

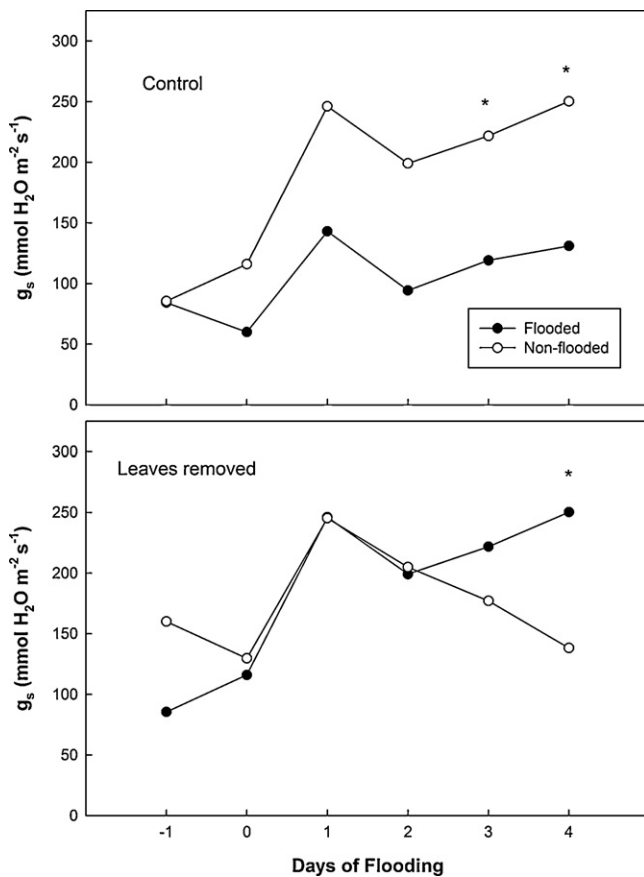


Fig. 6. Effect of flooding on stomatal conductance to water vapor (g_s) of one-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 3). Treatments included no leaf removal (Control) or 2/3 of the leaves removed one day before flooding (Leaves removed). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

of photosynthesis before flooding negatively affected avocado tree recovery after roots were unflooded. Reduced photosynthesis results in less carbohydrate available for glycolysis in the roots and thus less energy (ATP) production (Taiz and Zeiger, 2010). Based on similar findings to those of the present study, Gil et al. (2008) postulated that pruning avocado trees prior to flooding increases stress and delays recovery of flooded trees due to reduced ATP production as a result of less carbohydrate being produced and transported to the root. Thus, there is presumably less substrate (carbohydrate) available for root respiration as a result of less photosynthetic surface area.

In Expt. 3, D-mannoheptulose was found in considerably greater concentrations than any of the other carbohydrates. The reduction in A and g_s of flooded plants coincided with lower total carbohydrate concentration, primarily D-mannoheptulose, in the roots. The C_7 sugar, D-mannoheptulose, is the primary photosynthetic product in avocado. This is catalyzed by aldoses in the Calvin cycle to form the storage product, perseitol (Liu et al., 1999). In experiments with tomato (*Solanum lycopersicum*), root zone hypoxia resulted in reductions in fructose and glucose concentrations, the primary non-structural carbohydrates metabolized in tomato (Horchani et al., 2009). Similarly, in pigeon pea (*Cajanus cajan*), root hypoxia resulted in reductions in sucrose and the sugar alcohol, mannitol (Kumutha et al., 2008). In the present study, the concentration of D-mannoheptulose in avocado roots was not only reduced by flooding, but also tended to be lower in trees with leaves removed prior to flooding. Additionally, the concentration of perseitol was significantly lower in roots of trees with leaves removed before flooding

than in flooded plants with no leaves removed prior to flooding. This suggests that removing leaves before flooding reduces carbohydrate production thereby making plants more susceptible to flooding damage.

Root inundation affects not only carbohydrate synthesis, but also photoassimilate transport to meristematic sinks and their utilization in metabolism and production of new tissues (Kozłowski, 1997). Gimeno et al. (2012) recently observed that flooding inhibited carbohydrate transport from roots to shoots of 'Verna' lemon (*Citrus limon*) trees on sour orange (*Citrus aurantium*) rootstock with a 'Valencia' orange (*Citrus sinensis*) interstock. Carpenter et al. (2008) found that in cuttings of *Salix nigra*, the total nonstructural carbohydrate pool was reduced in response to complete shoot removal, but increased in response to periodic flooding and water stress. These results were attributed to changes in carbon partitioning as indicated by increased soluble carbohydrates in roots and shoots. These results also illustrated the negative effects of periodic flooding on starch mobilization and resprouting of *S. nigra*. Implications of these findings extend to reduced survival in the field when plants are exposed to the combined stresses of reduced canopy size and flooding (Carpenter et al., 2008). In theory, if the translocation pathway is blocked, which typically occurs with flooding (Carpenter et al., 2008), assimilates in leaves will not be translocated to the roots, thus resulting in carbohydrate deficiency in the roots. A continuous supply of sugars in roots was found to be critical for long-term survival of pea (*Pisum sativum*), pumpkin (*Cucurbita maxima*), and several herbaceous plants exposed to anoxia or flooding (Liao and Lin, 2001). Sugars are important for plant survival when soil is flooded because this process can potentially supply a high enough energy charge to sustain root metabolism (Liao and Lin, 2001).

Although, antioxidant activity was not measured in this study, it has been suggested that D-mannoheptulose acts as a major antioxidant in mesocarp tissue of avocado fruit (Tsfay et al., 2012). This may also explain why the D-mannoheptulose concentration was higher for in flooded plants with leaves removed prior to flooding than in the control treatment. Plants in which photosynthesis was inhibited before flooding had less carbohydrate, thus presumably less antioxidant concentration during and after the flooding stress. Sugar alcohols that serve as antioxidants have also been observed in celery (*Apium graveolens*), olive (*Olea europaea*), and wheat (*Triticum aestivum*) (Rešjová et al., 2002; Vítová et al., 2002; Abebe et al., 2003) in response to environmental stresses. While in the present study of avocado, flooding reduced A in the leaves and thus presumably carbohydrate mobilization to the roots, trees in the control treatment had more photoassimilate production than trees with leaves removed, and thus a higher concentration of D-mannoheptulose in the roots.

Based on leaf gas exchange, carbohydrate concentration, growth, and plant survival, inhibition of photosynthesis by leaf removal or the application of a chemical photosynthetic inhibitor prior to flooding exacerbates flooding stress. Reduction of the main photosynthetic products, D-mannoheptulose and perseitol, in the roots and possibly the role of the former as an antioxidant appeared to result in flooded trees with leaves removed prior to flooding, being more susceptible to flooding stress than trees with their canopies left intact.

This study provides evidence that reducing photosynthesis by leaf removal before flooding exacerbates flooding stress and decreases plant survival. In Expts. 2 and 3, leaves were removed starting from the bottom upward to keep the most active leaves on all trees for the purpose of monitoring leaf gas exchange. Pruning the exterior of the canopy, as in Expt. 1, had a greater effect on exacerbating flooding stress with respect to reduced A and tissue dry weights than removing leaves from the bottom of the canopy. Of courses, in a practical orchard situation, leaves are removed from

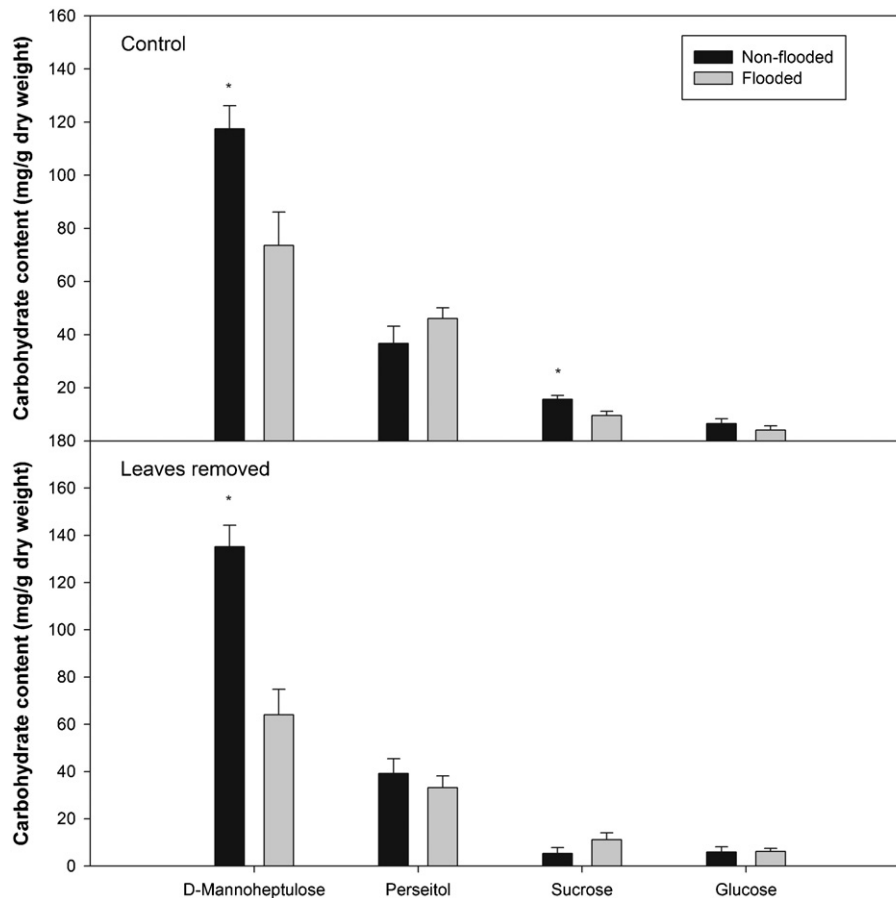


Fig. 7. Root carbohydrate concentrations for control and leaf removal treatments of non-flooded and flooded one-year-old 'Choquette' avocado trees on Waldin seedling rootstock within flooding treatments (Expt. 3). Treatments consisted of no leaf removal (Control) or two-thirds of the leaves removed one day before flooding (Leaves removed). An asterisk indicates a significant difference between treatments according to a T-test ($P \leq 0.05$), $n = 6$.

the exterior of the canopy by pruning. Thus, the data obtained in this study suggests that reducing the size of avocado canopies by pruning, in anticipation of flooding from a predicted impending storm or hurricane, should not be recommended for mitigating flooding stress. However, further studies are needed with larger, mature, crop yielding trees in an orchard to quantify the effects of pruning before flooding on avocado tree survival and recovery.

Acknowledgements

We thank Robert Dowell, Letty Almanza, Manuel Sacramento and Chunfang Li for assistance with data collection. We also thank Fayek Negm and Mary Lu Arpaia for root carbohydrate analyses.

References

- Abebe, T., Guenzi, A.C., Martin, B., Cushman, J.C., 2003. Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol.* 131, 1748–1755.
- Barquin-Valle, L.P., Migliaccio, K.W., Schaffer, B., Muñoz-Carpena, R., Crane, J.H., Li, Y.C., 2011. Predicting soil water content using the 'drained to equilibrium' concept. *Vadose Zone J.* 10, 675–682.
- Carpenter, L.T., Pezeshki, S.R., Shields Jr., F.D., 2008. Responses of nonstructural carbohydrates to shoot removal and soil moisture treatments in *Salix nigra*. *Trees-Struct. Funct.* 22, 737–748.
- Chow, P.S., Landhäusser, S.M., 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiol.* 24, 1129–1136.
- Crane, J., Balerdi, C., Campbell, C., Goldweber, S., 1994. Managing fruit orchards to minimize hurricane damage. *HortTechnology* 4, 21–27.
- Drew, M.C., 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 223–250.
- Evans, E.A., Nalampang, S., 2010. Sample avocado production costs and profitability analysis for Florida. In: Electronic Data Information Source (EDIS) FE837. Department of Food and Resource Economics, University of Florida, Gainesville, Florida, USA. (<http://edis.ifas.ufl.edu/fe837>).
- FAOSTAT. 2010. Food and Agriculture Organization of the United Nations. (<http://faostat.fao.org/site/339/default.aspx>).
- Geigenberger, P., 2003. Response of plant metabolism to too little oxygen. *Curr. Opin. Plant Biol.* 6, 247–256.
- Gil, P.M., Schaffer, B., Gutierrez, S.M., Li, C., 2008. Interacting effects of waterlogging and pruning on leaf gas exchange and biomass of avocado (*Persea americana* Mill.). *HortScience* 43, 1276 (Abstr.).
- Gil, P.M., Bonomelli, C., Schaffer, B., Ferreyra, R., Gentina, C. Effect of soil water-to-air ratio on biomass and mineral nutrition of avocado. *J. Soil Sci. Plant Nutr.*; in press.
- Gimeno, V., Syvertsen, J.P., Simon, I., Martinez, V., Camara-Zapata, J.M., Garcia-Sanchez, F., 2012. Interstock of 'Valencia' orange affects the flooding tolerance in 'Verna' lemon trees. *HortScience* 47, 403–409.
- Horchani, F., Aloui, A., Brouquisse, R., Ashi-Smiti, S., 2009. Physiological responses of flooded tomato (*Solanum lycopersum*) as affected by root hypoxia. *J. Agron. Crop Sci.* 194, 297–303.
- Irfan, M., Hayat, S., Hayat, Q., Afroz, S., Ahmad, A., 2010. Physiological and biochemical changes in plants under waterlogging. *Protoplasma* 241, 3–17.
- Kiggundu, N., Migliaccio, K.W., Schaffer, B., Li, Y.C., Crane, J.H., 2012. Water savings nutrient leaching, and fruit yield in a young avocado orchard as affected by irrigation and nutrient management. *Irrigation Sci.* 30, 275–286.
- Kozłowski, T.T., 1997. Responses of woody plants to flooding and salinity. In: *Tree Physiology Monograph No.1*. Heron Publishing, Victoria, Canada. (<http://www.heronpublishing.com/tp/monograph/kozłowski.pdf>).
- Kumutha, D., Sairam, R.K., Ezhilmathi, K., Chinnusamy, V., Menna, R.C., 2008. Effect of waterlogging on carbohydrate metabolism of pigeon pea (*Cajanus cajan* L.): upregulation of sucrose synthase and alcohol dehydrogenase. *Plant Sci.* 175, 706–716.
- Liao, C.T., Lin, C.H., 2001. Physiological adaptations of crop plants to flooding stress. *Proc. Natl. Sci. Council, Rep. Chin.* 25, 148–157.
- Liu, X., Sievert, J., Arpaia, M.L., Madore, M.A., 2002. Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *J. Am. Soc. Hortic. Sci.* 127, 108–114.

- Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L., 1999. 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. *J. Am. Soc. Hortic. Sci.* 124, 671–675.
- Ploetz, P.C., Schaffer, B., 1989. Effects of flooding and Phytophthora root rot on net gas exchange and growth of avocado. *Phytopathology* 79, 204–208.
- Ponnamperuma, F.N., 1972. The chemistry of submerged soils. *Adv. Agron.* 24, 29–96.
- Rešjová, A., Patkova, L., Stodulkova, E., Livpaska, L., 2002. The effect of abiotic stress on carbohydrate status of olive shoots (*Olea europea* L.) under in vitro conditions. *J. Plant Physiol.* 16, 174–184.
- Schaffer, B., Whiley, A.W., Kohli, R.R., 1991. Effects of age on gas exchange characteristics of avocado (*Persea americana* Mill.) leaves. *Sci. Hortic.* 48, 21–28.
- Schaffer, B., Andersen, P.C., Ploetz, R.C., 1992. Responses of fruit trees to flooding. *Hortic. Rev.* 13, 257–313.
- Schaffer, B., 1998. Flooding responses and water-use efficiency of subtropical and tropical fruit trees in an environmentally-sensitive wetland. *Ann. Bot.* 81, 475–481.
- Schaffer, B., Whiley, A.W., 2002. Environmental physiology. In: Whiley, A.W., Schaffer, B., Wolstenholme, B.N. (Eds.), *The Avocado: Botany, Production and Uses*. CABI Publishing, Wallingford, UK, pp. 133–154.
- Schaffer, B., Davies, F.S., Crane, J.H., 2007. Responses of subtropical and tropical fruit trees to flooding in calcareous soil. *HortScience* 41, 549–555.
- Schaffer, B., Crane, J.H., Li, C., Li, Y.C., Evans, E.A., 2011. Re-greening of lychee (*Litchi chinensis* Sonn) leaves with foliar applications of iron sulfate and weak acids. *J. Plant Nutr.* 34, 1341–1359.
- Taiz, L., Zeiger, E., 2010. *Plant Physiology*, fifth ed. Sinauer Associates, Sunderland, Massachusetts, USA.
- Tesfay, S.Z., Bertling, I., Bower, J.P., 2012. D-mannoheptulose and perseitol in 'Hass' avocado: metabolism in seed and mesocarp tissue. *S. Afr. J. Bot.* 79, 159–165.
- Vitová, L., Ikova, E.S., Bartoni, A., Livpaska, H., 2002. Mannitol utilization by celery (*Apium graveolens*) plants grown under different in vitro conditions. *Plant Sci.* 163, 907–916.
- Whiley, A.W., Schaffer, B., 1994. Avocado. In: Schaffer, B., Andersen, P.C. (Eds.), *Environmental Physiology of Fruit Crops. Subtropical and Tropical Crops*, vol. 2. CRC Press, Boca Raton, Florida, pp. 3–35.