

PHYSIOLOGICAL RESPONSES OF FLOODED AVOCADO TREES (*PERSEA
AMERICANA* MILL.) TO LEAF REMOVAL

By

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To my parents, Ale, and my best friend and favorite architect, Diana

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	7
LIST OF FIGURES.....	8
ABSTRACT	10
CHAPTER	
1 INTRODUCTION	12
2 LITERATURE REVIEW	15
Avocado.....	15
Flooding and Hypoxia stress.....	16
Effect of Low Oxygen Concentration on Avocado Physiology, Growth, and Yield ..	17
Effect of Low Oxygen Concentration on Leaf Gas Exchange.....	23
Interactions Between Above Ground Factors and Flooding Stress in Fruit Crops	24
Effect of Low Oxygen Concentration on Sap Flow	29
Summary	30
3 LEAF REMOVAL AND FLOODING EFFECTS ON LEAF GAS EXCHANGE, ROOT CARBOHYDRATE CONCENTRATION, GROWTH, AND SURVIVAL OF AVOCADO TREES.....	32
Background.....	32
Materials and Methods.....	35
Results	40
Discussion	44
4 PRE-AND POST-FLOODING LEAF REMOVAL EFFECTS ON LEAF GAS EXCHANGE, GROWTH, AND SURVIVAL OF AVOCADO TREES EXPOSED TO ROOT ZONE FLOODING.....	59
Background.....	59
Materials and Methods.....	63
Results	68
Discussion	70
5 CONCLUSIONS	88
LIST OF REFERENCES	93

BIOGRAPHICAL SKETCH..... 101

LIST OF TABLES

<u>Table</u>	<u>page</u>
3-1 Percentage of tree survival and mortality of 'Choquette' avocado trees on Waldin seedling rootstock in each canopy treatment at the end of Expt. 1 (day 49).	55
4-1 Number and percentage of tree mortality for flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock within each leaf removal treatment (Expt.1).Plants were harvested 52 days after plants were unflooded. Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed (2/3LR) before plants were flooded.	86
4-2 Number and percentage of tree mortality for flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each leaf removal treatment (Expt.2). Plants were harvested 52 days after flooding treatments were initiated (5 days after plants were unflooded). Leaf removal treatments were: one-third of the leaves removed (1/3LR), half of the leaves removed (1/2LR), and two thirds of the leaves removed (2/3LR) after plants were flooded.	87

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
3-1	Soil redox potential of flooded 2-year-old (Expt.1) and 1-year-old (Expt. 2) 'Choquette' avocado trees on Waldin seedling rootstock 49
3-2	Effect of flooding on net CO ₂ assimilation (A) for 2-year-old 'Choquette' avocado trees on Waldin seedling rootstock. 50
3-3	Effect of flooding on stomatal conductance to water vapor (g _s) for 2-year-old 'Choquette' avocado trees on Waldin seedling rootstock 51
3-4	Effect of flooding on net CO ₂ assimilation (A) for 1-year-old 'Choquette' avocado trees on Waldin seedling rootstock 52
3-5	Effect of flooding on stomatal conductance to water vapor (g _s) of 1-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 2). 53
3-6	Effect of flooding on root, leaf, stem, and total plant dry weight of 2-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of three canopy treatments (Expt. 1). 54
3-7	Root respiration of non-flooded (NF) and flooded (F) treatments within the leaf removal treatments and between the control and leaf removal treatment within each flooding treatment. 56
3-8	Root carbohydrate concentrations for control and leaf removal treatments for 1-year-old 'Choquette' avocado trees within flooding treatments (Expt. 2)..... 57
3-9	Root carbohydrate concentrations for non-flooded and flooded treatments for 1-year-old 'Choquette' avocado trees within leaf removal treatments (Expt. 2). 58
4-1	Soil redox potential of flooded 3-year-old 'Choquette' avocado trees (Expt. 1) and 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstocks (Expt. 2) 77
4-2	Net CO ₂ assimilation (A) of flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 1) 78
4-3	Stomatal conductance of water vapor (g _s) of flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 1) 79

4-4	Net CO ₂ assimilation (A) of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 2)	80
4-5	Stomatal conductance of water vapor (g _s) of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 2).....	81
4-6	Transpiration (E) of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 2)	82
4-7	Root, leaf, stem, and total plant dry weights of flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 1)	83
4-8	Root, leaf, stem, and total plant dry weight of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 2)	84
4-9	Daily sap flow of flooded and nonflooded trees of 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock (Expt. 2).....	85

Abstract of Thesis Presented to the Graduate School
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Effects of leaf removal before or after root zone flooding on the physiology and survival of avocado (*Persea americana*) trees was quantified. Plants had either no (control), one-third (1/3LR), one-half (1/2 LR), or two-thirds (2/3LR) of their leaves removed before or after flooding. Net CO₂ assimilation (A), stomatal conductance (g_s), transpiration (E), and plant growth measurements were made for flooded and non-flooded plants. Leaf removal or foliar application of Freeway[®] (a chemical photosynthetic inhibitor) prior to flooding, resulted in significantly lower A, g_s and dry weights of flooded compared to non-flooded plants. Survival of flooded plants sprayed with Freeway[®] was 66.6%, whereas survival of non-sprayed plants was 83.3%. 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. Flooded plants with no leaves removed tended to have higher root respiration than flooded plants with leaves removed. Leaf removal before flooding resulted in lower A and g_s compared to plants with no leaves removed. When leaves were removed before flooding, only 16.6% of flooded plants in the 2/3LR treatment survived compared to 50%

of flooded plants in the 1/3LR treatment. In contrast, removing the same amount of leaves after flooding resulted in 66.6% survival for the 2/3LR and 83.3% for the 1/3LR treatments. In all experiments, flooding reduced A and g_s . However, leaf removal after flooding resulted in A and g_s returning to levels similar to those of non-flooded trees several days after plants were unflooded. Leaf removal before flooding increased flooding stress, presumably by reducing carbohydrate reserves in the roots, whereas leaf removal after flooding reduced flooding stress presumably by reducing transpirational area.

CHAPTER 1 INTRODUCTION

Flooding in agricultural fields may develop due to anthropogenic (e.g., poor site selection, improper irrigation practices) or natural (e.g., flooding, soil compaction) factors leading to altered plant metabolism, growth, and/or yield and plant mortality. Furthermore, an anticipated increase in air temperature and resultant effects on other climatic variables, such as rainfall, as a result of global climate change, may increase the potential for flooding in many areas of the world (IPPC, 2001). Therefore, for agriculture to remain productive in flood-prone or potentially flood-prone areas, it is important to understand how flooding affects crop physiology, growth, yield, and survival. This information should assist with the selection and development flood-adapted crops and production systems for areas that experience periodic short-term flooding (Schaffer, 1998).

Avocado (*Persea americana* Mill.) is a subtropical to tropical evergreen tree in the Lauraceae family, which has been called “the most nutritious of all fruit crops” (Purseglove, 1968). This crop is grown internationally in Mediterranean, subtropical and tropical regions, and world production area and trade are rapidly increasing (Schaffer et al., 2012). In 2010, world production of avocado was estimated to be 3,840,905 tones/year (FAO, 2010). In the United States, the vast majority of avocado production is in California, where there are an estimated 21,108 hectares planted (FAO, 2010). Although there are only 2,995 hectares of avocado planted commercially in Florida, it is second only to citrus in state annual farm gate sales (Evans and Nalampang, 2010).

Rapidly increasing production of avocado in several regions of the world has resulted in production in marginal sites that are prone to flooding or poor soil drainage

(Schaffer, et al., 2012). In South Florida, avocado orchards are often planted in low-lying areas with a high water table (1.8-2.3 meters below the soil surface) (Barquin-Valle et al., 2009) and periodic flooding due to heavy rains from tropical storms or hurricanes (Crane et al., 1994). Thus, in Florida and other avocado production areas that are prone to waterlogging, there is a need to understand the negative impacts of soil flooding on avocado physiology to help devise adequate crop management strategies to avoid or ameliorate tree and crop losses due to periodic flooding.

The root system of avocado is relatively shallow and does not spread much beyond the tree canopy (Ferreyra et al., 2007). Roots are extensively suberized, with low hydraulic conductivity, few root hairs, poor water uptake and high sensitivity to low soil oxygen concentrations (Ferreyra et al., 2006). These characteristics make avocado trees one of the most susceptible fruit trees to soil flooding (Ferreyra et al., 2006). A few days of flooding avocado trees with a high shoot/root ratio can result in reductions in photosynthesis and yield, and a high degree of tree mortality (Schaffer, 2008). Part of the suggested pre-hurricane practices for avocado (and other subtropical fruit tree species) in southern Florida is to control tree size by removing part of the canopy to reduce wind damage (Crane et al., 1994). To mitigate the negative effects of flooding on stress and recovery avocado trees subjected to short-term flooding, removing a portion of the tree canopy after floodwaters subside has been recommended. This practice presumably compensates for roots damaged by flooding by bringing the shoot/ root ratio into balance; effectively reducing the transpirational demand of the canopy and helping to avoid plant desiccation (Crane et al., 1994). These pre- and post-flood pruning recommendations are based solely on observations and not on experimental evidence.

Thus, the effects of the proportion and timing of canopy removal relative to the time of flooding on avocado physiology has not been adequately quantified.

The objectives of the research described in this thesis were: 1) to determine if reducing the size of the canopy before or after flooding exacerbates stress and hinders recovery of avocado trees exposed to short-term flooding; and 2) to quantify fluctuations in carbohydrate concentrations in avocado trees exposed to short-term flooding and pruning.

The hypotheses tested were: 1) removing a portion of the canopy prior to the onset of flooding and the subsequent reduction in photosynthesis lowers the carbohydrate availability in the roots. Thus, there should be less substrate (carbohydrate) for normal root respiration during flooding and flooding stress is exacerbated; and 2). In contrast to pruning prior to flooding, pruning trees immediately after flooding increases survival of trees by bringing the shoot/root ratio into balance so that there is sufficient root volume to adequately provide the existing canopy with water and nutrients.

The results of this research should provide basic information about the physiological responses of avocado trees to root zone flooding and the interaction between leaf removal and flooding. Additionally, the study should enhance the understanding of the role of non-structural carbohydrates in responses of avocado trees to low soil oxygen. The practical application of the results should assist growers by providing quantitative information about how to rehabilitate trees damaged by flooding relative to the amount and timing of canopy removal (pruning).

CHAPTER 2 LITERATURE REVIEW

Avocado

Avocado (*Persea americana*) is a polymorphic tree species, belonging to the *Lauraceae* family (Judd, 1999), that evolved in the neotropical rainforest as an overstory canopy tree (Wolstenholme, 2002). It is native to a wide geographical zone including the highlands of central and eastern Mexico and Guatemala and the Pacific coast of Central America (Knight, 2002). *Persea americana* consists of several taxa, considered botanical varieties or subspecies that include varieties of commercial interest. The three subspecies, also referred to as “races” or “ecotypes” are *Persea americana* var. *americana* Mill. (Antillean or West Indian race), *Persea americana* var. *drymifolia* Blake (Mexican race), and *Persea americana* var. *guatemalensis* Williams (Guatemalan race) (Scora and Bergh, 1990). These three races originated wholly or partly within tropical latitudes in Central America and the tree is often considered a tropical species (Wolstenholme, 2002). Although the scientific names for the three races have become imbedded in the literature, it has recently been noted that they were not validly published according to standard procedures for taxonomic naming, and they will likely be renamed based on recent genetic analysis (Chanderbali et al., 2012).

Avocado trees evolved in andosol soils derived from volcanic ash which are considered the optimum soil type for tree growth due to their physical properties, mainly low bulk density (0.5-0.8 g/cm³), high macro-porosity (approx. 46%), high organic matter content, and a low pH between 5 and 6 (Ferreyra et al., 2007). The root system of avocado trees is relatively shallow and does not spread much beyond the tree canopy. Roots are extensively suberized having low hydraulic conductivity, few root hairs, poor

water uptake and sensitivity to low soil oxygen concentrations (Ferreyra et al., 2006; Wolstenholme, 2012). These characteristics may have evolved because of frequent rains that occur in the indigenous rainforest habitat and rapidly drained soils that are conducive to the high root oxygen requirement and sensitivity to poor soil drainage, and the presence of a rich surface organic mulch resulting in a tendency for healthy feeder roots to grow into any decomposing litter layer (Scora et al., 2002). Due to the high root oxygen requirement, avocado trees exposed to root hypoxia, even for short periods as a result of flooding, have decreased leaf expansion, reduced root and shoot growth, root necrosis, and moderate to severe leaf abscission (Ferreyra et al., 2006; Schaffer 2006).

Flooding and Hypoxia Stress

Abiotic plant stress is defined as any environmental condition that causes harmful biological effects (Salisbury and Ross, 1992). Plants are autotrophic and therefore any change that directly or indirectly reduces the accumulation of biomass should be considered biologically harmful even when it benefits parts of the plant (Salisbury and Ross, 1992). A major environmental factor limiting plant growth and yield worldwide, especially in high rainfall regions, is hypoxia (Bai et al., 2009). Hypoxia refers to the reduction of oxygen below optimal levels for the normal functioning of the plant, organ, or cell and generally occurs at soil concentrations less than $2 \text{ mg O}_2 \text{ L}^{-1} \text{ H}_2\text{O}$ (Gibbs and Greenway, 2003). Whereas, concentrations below $1 \text{ mg O}_2 \text{ L}^{-1} \text{ H}_2\text{O}$ or complete lack of oxygen in the soil or medium is referred to as anoxia (Drew, 1997). Low oxygen conditions occur in poorly drained soils or during periods of short-term flooding (Drew, 1997).

In many areas of the world, including southern Florida, tropical and subtropical fruit crops, including avocado trees, are grown in areas that often experience flooding from

rising water tables and/or heavy rain from tropical storms or hurricanes (Crane et al., 1994; Schaffer and Whiley, 2002). Although all higher plants require water, excess water in the root environment can be injurious or even lethal because it displaces oxygen and other gases in the soil (Drew, 1997; Geigenberger, 2003).

Redox potential is an indirect measure of soil oxygen content. Well-drained soils are characterized by redox potentials of +300mV or greater, whereas flooded soils have redox potentials of 200 mV or lower (Kozłowski, 1997). Soil flooding disrupts the metabolism of mesophytic plant species by displacing O₂ from soil pores and promoting O₂ depletion by roots and soil microbes (Nuñez-Elisea et al., 1999). These processes not only reduce the oxygen content, but also increase the concentration of toxic organic and inorganic compounds in the soil (Irfan et al., 2010). This production of toxic metabolites increases during high temperatures and at night, when root respiration increases, together with a simultaneous increase in microbial activity (Irfan et al., 2010). Since respiration increases exponentially with temperature, short-term increases or decreases in soil temperature will decrease or increase the overall requirements of energy associated with root maintenance (Atkin et al., 2000).

Effect of Low Oxygen Concentration on Avocado Physiology, Growth, and Yield

Reduction of root respiration is one the earliest responses of plants to anoxic soil conditions regardless of whether the plants are tolerant or intolerant to flooding (Liao and Lin, 2001). When the soil oxygen concentration is low, the activity of cytochrome oxidase is limited, thus reducing adenosine triphosphate (ATP) production during root respiration (Geigenberger, 2003). Reduced availability of oxygen as the final electron acceptor in the mitochondrial electron transport chain mediates a rapid reduction of the ATP/ADP ratio and adenylate energy charge (Serres and Voesenek, 2008). Cells adapt

to this energy reduction by relying on glycolysis and fermentation to generate ATP and regenerate ADP, respectively (Serres and Voesenek, 2008).

In plant cells, both aerobic and anaerobic respiration begins with glycolysis, where glucose is oxidized to pyruvic acid by a series of reactions. During the conversion of glucose to pyruvic acid, a net 2 ATP molecules are produced. After glycolysis, carbon metabolism will proceed to aerobic respiration if sufficient oxygen is available or otherwise, to anaerobic respiration (also called fermentation) (Geigenberger, 2003; Salisbury and Ross, 1992).

There are two types of fermentation defined by the product of the chemical reaction: lactic acid fermentation, where the end product is lactic acid; and alcoholic fermentation where the end products are CO₂ and ethanol (Taiz and Zieger, 2006). Both types of fermentation occur without producing ATP (Taiz and Zeiger 2006). In the second and third stages of aerobic respiration, respectively called the tricarboxylic acid (TCA) cycle and the electron transport chain, up to 36 ATP molecules can be produced (Salisbury and Ross, 1992). Therefore, aerobic respiration produces significantly more energy in the form of ATP than anaerobic respiration (Salisbury and Ross, 1992).

Root cell death due to rapid exposure to anoxic conditions has been associated with acidification of the cytoplasm, referred to as cytoplasmic acidosis (Drew, 1997). Sudden exposure to anaerobic conditions results in lactic acid fermentation, and protons leaking from the vacuole can significantly lower the cytoplasmic pH (Licausi and Perata, 2009; Schaffer, 2006). In anoxic conditions, cytoplasmic acidosis is believed to be the primary cause of plant cell death (Drew, 1997). The production of potentially toxic metabolites in flooded roots as a result of anaerobic respiration has been implicated in

plant cell death (Drew, 1997). Initially, ethanol produced in the roots during alcohol fermentation in anaerobic soils and transported through the xylem was thought to be toxic to the plant (Drew, 1997). However, the ethanol concentration required to damage plant tissue is extremely high and ethanol readily diffuses out of plant tissues to the surrounding solution where it is diluted or metabolized by microorganisms (Drew, 1997; Irfan et al., 2010). Atkinson et al. (2008) found that in *Forsythia*, the ethanol concentration in xylem sap and leaf tissue increased dramatically after four days of flooding; however, toxicity symptoms were not observed.

The immediate biochemical precursor to ethanol, acetaldehyde, is considerably more toxic to plant cells than ethanol and may be a factor in plant cell death during anaerobic root metabolism (Liao and Lin 2001; Schaffer, 2006). Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) are two enzymes whose activities increase during root submergence, leading to the production of ethanol and regeneration of nicotinamide adenine dinucleotide (NAD⁺) (Sarkar et al., 2006). The activity of PDC is usually many-fold lower than that of ADH, suggesting that the rate limiting enzyme for ethanol synthesis in flooded roots is more likely PDC than ADH (Liao and Lin, 2001). However, a high ADH/PDC ratio is required to prevent the accumulation of potentially toxic acetaldehyde (Liao and Lin, 2001; Su and Lin, 1996). In flooded luffa (*Luffa cylindrica*) roots, the acetaldehyde concentration did not rise in proportion to the induction of PDC activity, and it increased only slightly after five days of flooding indicating that a high ADH/PDC ratio is important to avoid accumulation of toxic levels of acetaldehyde (Su and Lin, 1996).

Alcohol dehydrogenase, synthesized under anaerobiosis, is essential for growth and/or survival of plants during hypoxia. This enzyme is a key regulator of glycolysis that supplements the depleting demand of NAD⁺ by catalyzing the conversion of acetaldehyde to ethanol (Keyhani et al., 2006). A significant amount of research with herbaceous plants has shown that increased ADH activity improves a plant's tolerance to anoxia. This effect may be due to a role for ADH in avoiding a build-up of acetaldehyde by enhancing its conversion to ethanol under anaerobic conditions (Schaffer, 2006). Wignarajah and Greenway (1976) found that ADH activity in the roots of *Zea mays* was highest with gas mixtures containing 8-13% oxygen in the root zone, intermediate with pure nitrogen, and lowest when the solution was flushed with gas containing 20% oxygen. The products of the fermentative pathway (acetaldehyde and ethanol) can be volatilized through lenticels (Kozlowski, 1997), or transported to the leaves via the transpiration stream (Atkinson et al., 2008). In flooded mango trees, survival during flooding periods was attributed, at least in part, to the formation of hypertrophic stem lenticels that enhances oxygen diffusion to the roots. These stem openings may serve as excretory sites for the elimination of potentially toxic compounds such as acetaldehyde that results from anaerobic metabolism in flooded roots (Larson et al., 1993). The development of hypertrophic stem lenticels enhances O₂ diffusion to the roots in flooded soils. Hypertrophic stem lenticels have also been observed in *Annona* species, soybean (*Glycine max*), and forest trees including *Erythrina speciosa* (Medina et al., 2009; Nuñez-Elisea et al., 1999; Shimamura 2010) exposed to flooding of the root zone.

Plant metabolic responses and adaptations to root zone hypoxia and anoxia have been thoroughly reviewed by Irfan (2010), Geigenberger (2003), and Kozlowski (1997). In a 1992 review of the effects of low soil oxygen levels on physiology and growth of fruit crops, including avocado (Schaffer et al., 1992), discussion of biochemical and cellular responses was based primarily on research conducted with herbaceous plants. To date, the majority of studies on the effects of low soil oxygen on plant biochemistry and cellular biology still have focused on herbaceous plants and published reports are limited for woody species, including avocado.

The phytohormone, ethylene, has been implicated in a wide range of plant responses and/or adaptations to hypoxia and anoxia in the root zone. These include development of aerenchyma and hypertrophic stem lenticels, development of adventitious roots, and leaf epinasty (Drew, 1997; Viser and Voesenek, 2004). Hypoxia generally increases ethylene production, stimulating anatomical or morphological adaptations to low soil oxygen (Yamamoto et al., 1995). In contrast, anoxia decreases ethylene formation due to the requirement of oxygen for conversion of aminocyclopropane-1-carboxylic acid (ACC) to ethylene, the final step in biosynthesis of ethylene (Yamamoto et al., 1995). In Japanese alder (*Alnus japonica*) seedlings, flooding increased ethylene production in the submerged parts of the stems, which was related to the formation of adventitious roots and hypertrophied stem lenticels (Yamamoto et al., 1995). Although ethylene has an important role in flooding symptomology for several herbaceous and some woody plants, the relationship between ethylene and plant responses to low soil oxygen content in fruit trees remains

unclear (Schaffer et al., 1992) and more work is needed to clarify the role of ethylene in these responses.

Root hypoxia inhibits root and shoot growth by affecting many plant physiological processes, including chlorophyll biosynthesis and reactive oxygen species (ROS) (Geigenberger, 2003). Exposure of plants to most adverse conditions causes oxidative stress, which affects plant growth by production of ROS, such as superoxide radicals, singlet oxygen, hydroxyl radicals and hydrogen peroxide (Bai et al., 2009). These ROS are all very reactive and can cause severe damage to membranes, deoxyribonucleic acid (DNA), and proteins. Thus, understanding the interaction between enzymatic and non-enzymatic ROS scavenging machinery is crucial for identifying key components involved in oxidative stress defense and manipulating hypoxia tolerance in plants. Bai et al. (2009) found that contents of superoxide radicals and hydrogen peroxide significantly increased in *Malus hupehensis* when roots were exposed to hypoxia stress. Elevated activities of the antioxidant enzymes superoxide dismutase, peroxidase, and ascorbate peroxidase were also observed. These activities increased after 12 days of hypoxia, in parallel with the accumulation of ascorbic acid and glutathione. These combined responses indicated that *M. hupehensis* may have a protective capacity against oxidative damage by maintaining higher induced activities of the antioxidant system. Additionally, Keyhani et al. (2006) found that in corms of *Crocus sativus*, the activities of superoxide dismutase and other ROS-scavenging enzymes were stimulated under hypoxic or anoxic conditions.

Avocado is considered a flood-sensitive species with physiological responses occurring shortly after soil becomes waterlogged (Schaffer et al., 1992). Short periods of

flooding often result in leaf abscission, and leaf size can also be reduced by inhibition of expansion (Ferreyra et al., 2006). In addition to inhibiting shoot growth, flooding of avocado trees generally leads to an inhibition of root growth (Schaffer, 1998), and often root necrosis (Schaffer and Ploetz, 1989). No anatomical, morphological, or physiological adaptations were observed in studies of avocado trees in response to low soil oxygen levels (Schaffer, 2006). However, hypertrophic stem lenticels have recently been observed in flooded avocado trees in California (Schaffer, 2012).

Effects of Low Soil Oxygen Concentration on Leaf Gas Exchange

In hypoxic or anoxic soils, one of the earliest measurable changes in plants is a decline in leaf gas exchange. Reductions of net CO₂ assimilation in leaves is generally accompanied by decreases of stomatal conductance, transpiration, and intercellular partial pressure of CO₂ (Irfan, 2010; Schaffer et al., 1992, 2006). Therefore, leaf gas exchange measurements are useful for determining the degree of flooding stress before any visible symptoms appear (Schaffer, 2006). In fruit crops, the time sequence for reductions of net CO₂ assimilation and stomatal conductance in response to flooding has not been clearly determined, and thus it is unclear which occurs first. Elucidating the temporal separation of these responses as a result of low soil oxygen content would be useful for determining if flood-induced reductions of photosynthesis are due to stomatal or non-stomatal factors (Schaffer et al., 1992). For avocado trees flooded in calcareous soils, net CO₂ assimilation and transpiration declined linearly after trees were flooded, with a mean net CO₂ assimilation of zero by 30 days after trees were flooded. These reductions were attributed to biochemical changes associated with photosynthetic reactions and reduced stomatal conductance rather than a hydraulic

effect, since flooding did not significantly decrease xylem water potential (Schaffer et al., 2006). Similar results have been reported for several other woody and herbaceous plant species when exposed to different periods of flooding. These include mamey sapote (*Pouteria sapota*) (Nickum et al., 2010), carambola (*Averrhoa carambola*) (Ismail and Noor, 1996), mango (*Mangifera indica*) (Larson et al., 1996), various *Annona* species (Nuñez-Elisea et al., 1999), Surinam cherry (*Eugenia uniflora*) (Martin et al., 2009, 2010; Mielke and Schaffer, 2010a,b, 2011), Japanese alder (*Alnus japonica*) (Iwanaga and Yamamoto, 2007), and corn (*Zea mays*) (Yordanova and Popova, 2007).

Interactions Between Above Ground Factors and Flooding Stress in Fruit Crops

During the growing season, root growth is assumed to be sustained by current photosynthate (Eissenstat and Duncan, 1992). In container-grown plants, a disruption of photosynthesis by defoliating or pruning shoots will decrease or stop root growth and enhance the relative growth rate of the shoots (Eissenstat and Duncan, 1992). Species vary in the response time before root growth is diminished and the length of time required for a plant to recover from defoliation so that its root to shoot ratio is in equilibrium. Eissenstat and Duncan (1992) found that above-ground factors in citrus trees (*Citrus sinensis*) modify carbohydrate partitioning. In normal (adequate oxygen content for normal plant functioning) soil conditions, the total amount of reducing and ketone sugars (including free fructose, sucrose, and fructans) in the fine roots of citrus trees were 24% lower in pruned than in non-pruned trees. They also found that 30 days after pruning, 20% of the roots in the pruned trees (at a soil depth of 9 to 35 cm) died, but after 63 days, root length of pruned trees was similar to that of the non-pruned trees.

However, at that time starch reserves remained 18% lower in the fine roots of pruned trees than in those of non-pruned trees.

Since root inundation reduces root growth of most woody plants more than it does stem growth, the root/shoot ratio is decreased as a result of flooding (Kozlowski, 1997). One result of a reduced root/shoot ratio is that when flood water drains away and better oxygenated root conditions return, previously flooded plants may be less drought tolerant than plants that were never flooded. This may result from less water absorption by diminished root systems that cannot adequately replenish transpirational losses (Kozlowski, 1997).

A high level of fermentative metabolism in roots is 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). A continuous supply of fermentable sugars in roots is critical for long-term survival of peas (*Pisum sativum*), pumpkins (*Cucurbita mixta*), and several herbaceous plants exposed to anoxia or flooding (Liao and Lin, 2001). In theory, if the assimilates in leaves are not transported to the roots, carbohydrate deficiency will occur. Photoassimilate transport to roots can decrease during flooding resulting in accumulation of starch in leaves (Carpenter et al., 2008). Vu and Yelenosky (2006) found that in citrus trees (*Citrus sinensis*), the total nonstructural carbohydrate concentrations were high in leaves but drastically reduced in roots as a result of flooding. Root inundation affects not only synthesis of carbohydrates, but also the transport of carbohydrates to meristematic sinks and carbohydrate utilization in metabolism and production of new tissues (Kozlowski, 1997). 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 drought, and to a lesser extent, periodic flooding on starch mobilization in resprouting of *S. nigra*. Implications of these findings extend to reduced survival in the field when plants are exposed to the combined stresses of pruning and flooding or drought (Carpenter et al., 2008).

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., 2002). These C₇ sugars often account for over 10% of the tissue dry weight of avocado, and can be found in substantial amounts in all tissues and organs, including the fruit peel and seeds (Liu et al., 2002). In comparison, the nonstructural carbohydrates that occur more commonly in other plant species are based on six-carbon hexose units, such as glucose and sucrose. These sugars are found in much lower concentrations in avocado tissues (Liu et al., 2002). In leaves of fruit-bearing and non-bearing 'Hass' avocado trees, the main carbohydrates were C₇ sugars, with mannoheptulose dominating over perseitol and C₆ sugars such as sucrose, glucose and fructose (Bertling and Bower, 2006). Furthermore, leaves of non-bearing trees generally have higher concentrations of these sugars than bearing trees (Bertling and Bower, 2006). This could be an indication that C₇ sugars play an important role in fruit growth and development because leaves cannot accumulate sugars when trees carry a heavy fruit load (Bertling and Bower, 2006). Thus, research with avocado that focused only on starch reserves overlooked the importance of the C₇ sugars in the carbon allocation

process (Liu et al., 1999). 'Fuerte' and 'Pinkerton' avocados have higher concentrations of C₇ sugars in the exocarp than in the mesocarp, while 'Hass' avocado has a higher concentration of sugar alcohols in the mesocarp (Bertling and Bower, 2005). However, such accumulation and/or active transport into the exocarp might be related to a need for stress resistance of these fruit parts, as sugar alcohols like perseitol have been suggested to act as active oxygen species scavengers (Dennison et al., 1999; Jennings et al., 1998).

In addition to their storage role, sugar alcohols are important for protection from oxidative damage as a result of salt and osmotic stress in celery (*Apium graveolens*), olive (*Olea europaea*), and wheat (*Triticum aestivum*) (Vitová et al., 2002; Rešjová et al., 2007; Abebe et al., 2003). In avocado fruit, Cowan (2004) proposed that C₇ sugars present in the fruit protect certain key enzymes essential for fruit growth and development from damage by reactive oxygen species (ROS). Bertling et al. (2007) found that avocado tissues have different predominant antioxidant systems and similar antioxidant activity except for the mesocarp where the antioxidant activity is lower than in leaves, seeds, and the exocarp. The main antioxidant in mesocarp tissue is the C₇ sugar, mannoheptulose (Bertling et al., 2007; Tesfay et al., 2010). Despite the importance of these seven carbon sugars, the exact function of heptose sugars is not completely understood and the number of related research papers published in the past is limited (Meyer and Terry 2008). Thus, there is a need to study how these sugars respond to environmental stress conditions including root hypoxia or anoxia.

Tree mortality can result from a few days of flooding avocado trees with a high shoot/root ratio due to extensive root damage (Schaffer, 1998). Tree mortality can

reportedly be offset by pruning trees after flooding, which is often recommended for avocado trees in Chile and Florida, to increase tree survival and recovery from flooding stress (Gil et al., 2008). Removing part or most of the canopy reduces the above-ground volume of the tree, making resetting and stabilizing the tree easier. This will also reduce transpirational water loss and prevent desiccation (Crane et al., 1994). However, this pruning recommendation for avocado trees exposed to flooding is based on observations and not experimental evidence (Gil et al., 2008). Additionally, as part of pre-hurricane practices in Florida, it is suggested that growers control tree size by removing part of the canopy to reduce potential wind damage (Crane et al., 1994). The effect of canopy removal on reducing tree damage as a result of low soil oxygen has not been quantified. To the author's knowledge, there is only one preliminary study that quantifies the effects of pruning or the timing of pruning in relation to the flooding period on reducing stress and increasing the growth and survival rate of avocado trees. Gil et al. (2008) found that pruning avocado trees immediately after floodwater drained from the root zone, resulted in a tendency for trees to recover faster than non-pruned trees, as indicated by net CO₂ assimilation of pruned trees returning to pre-flood levels, whereas those of non-pruned trees did not. Pruning the canopy immediately after flooding decreased stress, presumably by lowering the shoot/root ratio, thereby reducing transpirational surface area and transpirational demand, thus reducing potential water stress. In contrast, pruning avocado prior to flooding increased stress compared to flooded, non-pruned trees, presumably due to a reduction in leaf area and a subsequent reduction in photosynthesis and photoassimilate translocation to the roots (Gil et al., 2008). The reduced photosynthesis and production and transport of

assimilates likely limited the amount of substrate available for root respiration during flooding (Gil et al., 2008).

Effect of Low Soil Oxygen Concentration on Plant Sap Flow

Plant sap flow measurements provide direct and continuous measurements of whole plant water use with a high time resolution (Smith and Allen, 1996; Wullschlegel et al., 2000). There is little published information on the effects of flooding on continuous measures of plant water status such as stem (xylem) sap flow or fluctuations in trunk diameter in fruit trees (Nicolás et al., 2005).

Specific plant responses to flooding vary with many factors, including clones and species (Nicoll and Coutts, 1998). Thus, tolerance to flooding in fruit crops such as 'Fino' lemon (*Citrus limon*), apricot (*Prunus armeniaca*) and citrus (*Citrus sinensis*), may depend on the scion/rootstock interactions (Ruiz-Sanchez et al., 1996; Domingo et al., 2002, and Garcia-Sanchez et al., 2007). In 'Hass' avocado, the stem sap flow rate was significantly reduced when trees were grafted onto two different clonal rootstocks, Duke 7 and Toro Canyon, due to differences in the xylem anatomy of each cultivar that causes a discontinuity in the water conduction system that decreases water transport (Fassio et al., 2008). There are no reports of flood-tolerant rootstocks in avocado (Schaffer, 1998). However, information on differences in plant sap flow among avocado cultivars and the effects of flooding on stem sap flow may be useful for future development of flood-tolerant avocado rootstocks.

Plant age, the properties of the floodwater, and duration of flooding affect the response of trees to soil hypoxia caused by flooding (Kozlowski, 1984). In young apricot trees, three hours of flooding caused a decrease in sap flow and a significant reduction

in plant hydraulic conductance, decreasing water absorption by the roots (Nicolás et al., 2005). Similarly, lemon trees subjected to three days of soil flooding exhibited a progressive reduction in sap flow and stomatal closure (Ortuño et al., 2007). Flooding also reduced the mean sap flow of *Avicennia germinans* by 27%, in *Rhizophora mangle* by 17.3% and in *Laguncularia racemosa* by 16.7% (Krauss et al., 2007). Internal regulation of stomatal conductance and stem sap flow have generally been interpreted as a mechanism to restrict water uptake during drought or flooded conditions (Nicolás et al., 2005; Ruiz-Sanchez et al., 1996). Low concentrations of oxygen in the root zone reduce the permeability of roots to water (Smith et al., 1990; Zhang and Tyerman, 1991), increasing resistance to water uptake (Domingo et al., 2002). Under these conditions, water loss from the shoots exceeds the supply from the roots, leading to a reduction in leaf water potential and stomatal conductance (Domingo et al., 2002). Most studies of the effects of waterlogging of fruit trees have concentrated on the detrimental effect that root anoxia has on stomatal activity and growth (Savé and Serrano, 1986; Smith and Ager 1988). However, the ability of trees to recover from transient waterlogging of the root system is also important in assessing the effects of short-term flooding (Smith et al., 1990).

Summary

Flooding results in significant root damage to avocado, thereby reducing water uptake (Schaffer and Whiley, 2002). Removal of part of the canopy (pruning) may be a practical method to alleviate or prevent flooding stress to avocado trees. However, the underlying mechanisms that define differences in responses to leaf removal immediately after flooding compared to leaf removal from avocado trees shortly before

flooding remain unclear. Thus, there is a need to quantify the effect of the timing of leaf removal on whole plant physiology, growth, and survival of flooded avocado trees. Measuring the interaction between the time of leaf removal or pruning, with respect to flooding on net CO₂ assimilation, stomatal conductance, and transpiration may provide valuable clues to the mechanisms by which pruning influences response of avocado trees to flooding because these physiological variables are some of the earliest responses to flooding that can be easily and non-destructively measured (Schaffer et al., 2006). This research will provide basic information about the physiological response of avocado trees to root zone flooding and the interaction between pruning and flooding. Additionally, this work should enhance the understanding of the role of non-structural carbohydrates in responses of avocado trees to low soil oxygen. The practical application of this study should assist growers by providing quantitative information about how to rehabilitate trees damaged by flooding relative to the amount and timing of canopy removal.

CHAPTER 3
LEAF REMOVAL AND FLOODING EFFECTS ON LEAF GAS EXCHANGE, ROOT
CARBOHYDRATE CONCENTRATION, GROWTH AND SURVIVAL OF AVOCADO
TREES

Background

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 there are an estimated 21,108 hectares planted (FAO, 2010). Although there are only 2,995 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 et al., 2012). In southern Florida, avocado orchards can become saturated due to capillary water movement from a water table which is not far (1.8 – 2.3 m; Barquin-Valle, 2011) from the soil surface, and heavy rains from tropical storms and hurricanes (Schaffer and Whiley, 2002; Crane et al., 1994).

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). Responses of avocado trees to even short periods of root zone hypoxia include leaf abscission, root necrosis, and reductions

in net photosynthesis, shoot and root growth, and fruit production (Schaffer and Whiley, 2002). A few days of flooding can result in avocado tree death (Schaffer, 1998), which has been attributed to the 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 are reduced during flooding. 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, 2002, 2006, 2007).

The observation of a high shoot/root ratio as a result of flooded or poorly drained soils has led to the recommendation in southern Florida and 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 after flooding to mitigate flooding stress is based on observations rather than experimental evidence. Preliminary studies with avocado trees in containers showed that pruning the canopy immediately after the floodwater subsides reduced plant stress and increased tree survival (Gil et al., 2008).

In contrast to leaf removal after flooding, leaf removal before flooding was shown to increase flooding stress of avocado trees in containers (Gil et al., 2008). It was suggested that increased stress of flooded avocado trees due to canopy removal was the result of less carbohydrate produced and translocated to the roots as a result of reduced photosynthesis from the combined effects of less leaf area and root hypoxia.

Thus, it was hypothesized that reducing the leaf area resulted in less carbohydrate transported to the roots to act as a substrate for root respiration, and thus less ATP was 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, represent 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. 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 net CO₂ assimilation by 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.

Materials and Methods

Study site description

This study was conducted in an open-air (only the roof was covered.) greenhouse at the University of Florida, Tropical Research and Education Center in Homestead, Florida (25.5°N and 80.5°W). Temperature in the greenhouse was recorded with a StowAway, TidbiT sensor/data logger (Onset Computer Corp., Bourne, Massachusetts, USA) located 15 cm above the canopy. Photosynthetic photon flux (PPF) was measured with a quantum sensor (model Li 190) connected to a LI-1000 datalogger (Li-Cor Inc., Lincoln, Nebraska, USA).

There were two experiments. The first experiment (Expt. 1), conducted from June-August 2010, 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 second experiment (Expt. 2), conducted during September 2011, focused on determining the effects of reduced photosynthesis by leaf removal prior to flooding on root carbohydrate content, root respiration and plant stress from flooding.

Plant material

Two-year-old 'Choquette' avocado trees (*Persea americana* Mill) on Walden seedling rootstock growing in potting medium (40% Canadian peat, 10% coir, 40% pine bark and 10% perlite) in 3-L containers were obtained from a commercial nursery (Expt. 1).

One-year-old 'Choquette' avocado trees (Expt. 2) on Walden seedling rootstock in 1-L containers were obtained from a commercial nursery growing in the same type of

potting medium as trees in Expt. 1. Prior to initiating treatments, trees in each experiment were treated with the fungicide, Alliete® (Bayer Crop Science, Morganville, NC, USA), as a soil drench to help prevent Phytophthora root rot.

Experimental design

Expt.1 consisted of a randomized design with a 3 x 2 factorial arrangement of treatments. There were three canopy treatments: two-thirds of the leaves removed before flooding (leaf removal); use of Freeway® (alcohol ethoxylates, silicone-polyether copolymer, propylene glycol and dimethylpolysiloxane; Loveland Products Inc., Loveland, CO, USA), a chemical photosynthetic inhibitor sprayed on the canopy (C.P.); or no leaf removal or chemical spray (control); and two flooding treatments: flooded or non-flooded. Freeway® is a chemical surfactant that was previously found to inhibit photosynthesis of tropical fruit trees (Schaffer et al., 2010) including avocado (B. Schaffer, University of Florida, 2009, unpublished data. There were six single-tree replications for each treatment combination.

Expt. 2 consisted of a completely randomized design with a 2 x 2 factorial arrangement of treatments. There were two leaf removal treatments: no leaf removal (control) or two-thirds of the leaves removed (leaf removal); and two flooding treatments: flooded and non-flooded. There were 6 single-tree replications for each treatment combination.

Flooding treatments

Plants were flooded by submerging each entire plant container in a 19-L 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 unflooded after a statistically significant difference ($P \leq 0.05$) in net CO_2 assimilation or stomatal conductance to water vapor was observed between flooded and non-flooded trees in any of the canopy treatments (Expt. 1) or leaf removal treatments (Expt. 2). The flooding period was 4 days in each experiment. Trees were irrigated by overhead irrigation for 15 minutes twice each day, except during the flooding period, when non-flooded trees were manually irrigated twice each day. Tensiometers (Irrometer 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).

Leaf removal treatment

Two-thirds of the leaves were removed the day before the flooding treatments were initiated for plants in the leaf removal treatment in Expts. 1 and 2. The total leaf area was reduced to the desired level by counting the number of leaves per tree and then removing two-thirds of the total number of leaves in each tree starting from the base of the trunk upwards. Trees in the control treatment had no leaves removed.

Chemical photosynthetic inhibitor treatment

The foliage of plants in the C.P. treatment in Expt. 1 were sprayed with with 2 ml L^{-1} of Freeway[®] in distilled water to run-off with a hand sprayer, covering both the adaxial and abaxial surfaces. Applications started the day before initiating the flooding treatment. During the flooding period, flooded and non-flooded plants were sprayed

every other day until plants were unflooded. Plants in the control treatment (flooded or non-flooded) were not sprayed with Freeway[®].

Leaf gas exchange measurements

Net CO₂ assimilation and stomatal conductance to water vapor were measured with a Ciras-2 portable gas analyzer (PP Systems, Amesbury, MA, USA) at a light saturated PPF (1000 $\mu\text{m m}^{-2}\text{s}^{-1}$), a reference CO₂ concentration of 375 $\mu\text{m mol}^{-1}$ and an air flow rate into the leaf cuvette of 200 ml min⁻¹. Measurements were made between 900 HR and 1100 HR, starting 2 days prior of the initiation of flooding treatments. Leaf gas exchange was measured daily on the same two fully expanded, mature leaves of each plant. After the flooding period in Expt. 1, measurements were made at 1-2 day intervals, to monitor tree recovery, and were stopped 45 days after flooding when there were no significant differences in net CO₂ assimilation or stomatal conductance between flooding treatments. Measurements were stopped and plants were harvested in Expt. 2 immediately after the flooding period so that root respiration and carbohydrate concentrations could be measured.

Plant dry weights

Plants were harvested at the end of both experiments. 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.

Root respiration

Root respiration was measured in Expt. 2 in excised roots (~2 mm in diameter) using a method similar to that described by Burton and Pregitzer (2003). From each plant, 2 g fresh weight of roots were harvested and cleaned with a brush to remove soil particles. Root samples were placed in a polyvinyl chloride (PVC) cylinder sealed to a Plexiglas® bottom. The cylinder was covered with a soil respiration chamber attached to a Ciras-2 portable gas analyzer (PP Systems, Amesbury, MA, USA). Root respiration was determined using an airflow rate into the chamber of 100 ml min⁻¹. After measurements were made, root samples were oven dried at 70°C to a constant weight for dry weight determinations.

Root carbohydrate concentrations

Root samples from each plant in each treatment combination of Expt. 2 were collected immediately after plants 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 for extraction and analysis of C₇ sugars, sucrose, fructose and glucose. Carbohydrates were extracted 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 Landh usser, 2004).

Soil redox potential and soil temperature

Soil redox potential and pH were measured in each container with a metallic ORP indicating electrode for plants in the flooded treatment in both experiments (Accument Model 13-620-115, Fisher Scientific, Pittsburg, PA, USA) connected to a voltmeter. 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.

Soil temperature was recorded using a Hobo Tidbit v2 sensor/datalogger (Onset Computer Corp., Pocasset, MA, USA) placed 5 cm deep into the container of one randomly selected plant in each treatment.

Statistical analyses

Data were analyzed by a two-way analysis of variance (ANOVA) to assess interactions between flooding and canopy (leaf removal, Freeway[®] application, or control) treatments (Expt. 1), or between flooding and leaf removal treatments (Expt. 2). Differences between flooding treatments were compared by repeated measures ANOVA for leaf gas exchange variables, or a T-test for root respiration, carbohydrate concentration, and plant dry weights (SAS 9.1, SAS Institute, Cary, NC, USA).

Results

Soil and air temperature, soil redox potential and light intensity

Expt. 1. Mean daily air temperature in the greenhouse 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 redox potential for the flooded treatment was slightly

below 200 mV beginning one day after flooding and values continued to decrease to a mean of -18 mV by day 4 (Figure 3-1).

Expt. 2. Mean daily air temperature in the greenhouse ranged from 20 to 39°C with a mean of 28°C. Soil temperature in non-flooded soil ranged from 21 to 37°C with a mean of 30°C. Soil temperature in flooded soil ranged from 21 to 38°C with a mean of 28°C. Soil redox potential for the flooded treatment was slightly below 200 mV beginning the after 1 day of flooding and decreased to a mean of -18 mV by day 4 (Figure 3-1).

Leaf gas exchange

Expt. 1. There was a significant statistical interaction ($P \leq 0.05$) between the canopy and flooding treatments for net CO₂ assimilation and stomatal conductance on one or more measurement date(s). Therefore flooding treatments were compared separately within each canopy treatment.

There was no significant effect of flooding on net CO₂ assimilation in the untreated controls on any of the measured dates and stomatal conductance was significantly lower in flooded than in non-flooded plants ($P \leq 0.05$) on only one date during the recovery period (Figures 3-2, 3-3). Net CO₂ assimilation and stomatal conductance were significantly ($P \leq 0.05$) lower in flooded than in non-flooded plants in the leaf removal treatment after 4 days of flooding until about 28 days into the recovery period. In plants treated with Freeway[®], net CO₂ assimilation and stomatal conductance 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 plants were unflooded for net CO₂ assimilation and stomatal conductance, respectively (Figures. 3-2, 3-3).

Expt. 2. For plants in the control treatment after 3 and 4 days of flooding, net CO₂ assimilation and stomatal conductance, respectively, were significantly lower ($P \leq 0.05$) in flooded than in non-flooded plants (Figures 3-4, 3-5). Net CO₂ assimilation and stomatal conductance were significantly lower in flooded than in non-flooded plants for plants in the leaf removal treatment, ($P \leq 0.05$) after 3 and 4 days of flooding, respectively (Figures 3-4, 3-5).

Tissue dry weights and plant survival

Expt. 1. Although there was no significant interaction ($P > 0.05$) between flooding and Freeway[®] treatments for root, leaf or stem dry weights, there was significant statistical interaction ($P \leq 0.05$) for total plant dry weight. Therefore, for each plant tissue, differences between flooding treatments were compared separately within each Freeway[®] treatment.

Stem dry weight of control plants was significantly higher ($P \leq 0.05$) for plants in the flooded than those in the non-flooded treatment (Figure 3-6). For plants treated with Freeway[®], leaf dry weight was significantly lower ($P \leq 0.05$) for flooded than for non-flooded plants (Figures 3-6c). Total plant dry weight in the leaf removal treatment was significantly lower ($P \leq 0.05$) for flooded than for non-flooded plants (Figure 3-6b).

At the end of the experiment (day 49), 33% of the flooded plants treated with Freeway[®] died. In both the control and leaf removal treatments, 17% of the flooded plants died (Table 3-1).

Tissue dry weights and plant survival were not assessed in Expt. 2 because those plants were harvested immediately after the 4-day flooding period to determine root respiration and carbohydrate concentrations. Dry weight and survival differences

between flooded and non-flooded avocado trees can generally not be detected until several days after a short (3-5 day) flooding period (B. Schaffer, University of Florida, 2010, unpublished data).

Root respiration

There was no significant interaction ($P > 0.05$) between the flooding and the leaf removal treatments for root respiration in Expt. 2. Therefore, leaf removal treatments were pooled to compare flooding treatments and flooding treatments were pooled to compare leaf removal treatments.

There was no significant effect of flooding or leaf removal ($P > 0.05$) on root respiration. However, in the leaf removal treatment flooded plants had a higher mean root respiration than non-flooded plants. In addition, control plants in the flooded treatment tended to have higher root respiration than plants with leaves removed (Figure 3-7).

Root carbohydrate concentrations

There was a significant interaction in Expt. 2 between the flooding treatments and the leaf removal treatments only for sucrose and glucose concentrations ($P \leq 0.05$). 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 (Figure 3-8). 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., 2001).

D-mannoheptulose and sucrose concentrations were significantly higher in non-flooded than in flooded plants in the control treatment (Figure 3-8). For plants 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 plants (Figure 3-9).

For plants in the non-flooded treatment, perseitol was significantly lower in the control treatment than in the leaf removal treatment, whereas glucose was significantly higher in the control treatment ($P \leq 0.05$; Figure 3.8). Flooded control plants had higher perseitol concentrations than plants in the leaf removal treatment ($P \leq 0.05$; Figure 3.9).

Discussion

A decline in net CO₂ assimilation of flooded plants 2 days (Expt. 1) or 4 days (Expt. 2), respectively after flooding, coincided with reductions in stomatal conductance 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 (Schaffer et al., 1992), and that this response tends to occur within hours of exposure to root zone hypoxia (Schaffer and Ploetz 1989; Schaffer et al., 1992).

Leaf removal for flooded trees, or the use of Freeway[®] prior to flooding resulted in a greater reduction in net CO₂ assimilation, slower recovery from flooding stress, and more tree death compared to the control treatment. Thus, an inhibition of the photosynthesis before flooding negatively affected avocado tree recovery. Reduced

photosynthesis results in less carbohydrate available for glycolysis in the roots and thus less energy (ATP) production (Taiz and Zieger, 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. 2, D-mannoheptulose was found in considerably greater concentrations than any of the other carbohydrates. The reduction in stomatal conductance and net CO₂ assimilation of flooded plants coincided with lower total carbohydrate concentration, primarily D-mannoheptulose, in the roots. The C₇ 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, root hypoxia resulted in reductions in sucrose and the sugar alcohol, mannitol, in pigeon pea (*Cajanus cajan*; Kumuta et al., 2008). In the present study with avocado, the concentration of D-mannoheptulose was not only reduced by flooding, but also tended to be lower in plants with leaves removed prior to flooding. Additionally, the concentration of perseitol was significantly lower for plants with leaves removed before flooding than in the control plants that were flooded. This suggests that removing

leaves before flooding reduces carbohydrate production 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 (Kozlowski, 1997). 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 fermentable 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). Fermentable 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). However, in the present study with avocado, there was no significant statistical interaction between the flooding and the leaf removal treatment for root respiration. Presumably, the length of flooding was not long enough to observe a

decrease in root respiration as overall plant decay was observed days after flooding, even in plants in which the root carbohydrate concentration was reduced by flooding.

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 fruits (Tsfay et al., 2010). This may also explain why the D-mannoheptulose concentration of flooded plants was higher for those with leaves removed prior to flooding than in the control treatment. Plants in which photosynthesis was inhibited before flooding had less carbohydrate, thus 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*) (Vitová et al., 2002; Rešjová et al., 2007; Abebe et al., 2003) in response to environmental stresses. While in the present study with avocado, flooding reduced net CO₂ assimilation in the leaves and thus presumably carbohydrate mobilization to the roots, plants in the control treatment had more photoassimilate production than trees with leaves removed, as a result of reduced photosynthetic surface area, and thus a higher concentration of the D-mannoheptulose in the roots.

Based on leaf gas exchange, root respiration, carbohydrate concentration, growth, and plant survival, inhibition of photosynthesis by leaf removal or the application of Freeway® 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 plants with leaves removed prior to flooding, being more susceptible to flooding stress than plants with their canopies left intact. Therefore, this study provides evidence that reducing

photosynthesis by leaf removal before flooding exacerbates the flooding stress and decreases plant survival. Further studies are necessary to quantify the effect of prolonged flooding on root respiration and plant biomass of plants with photosynthesis inhibition before flooding.

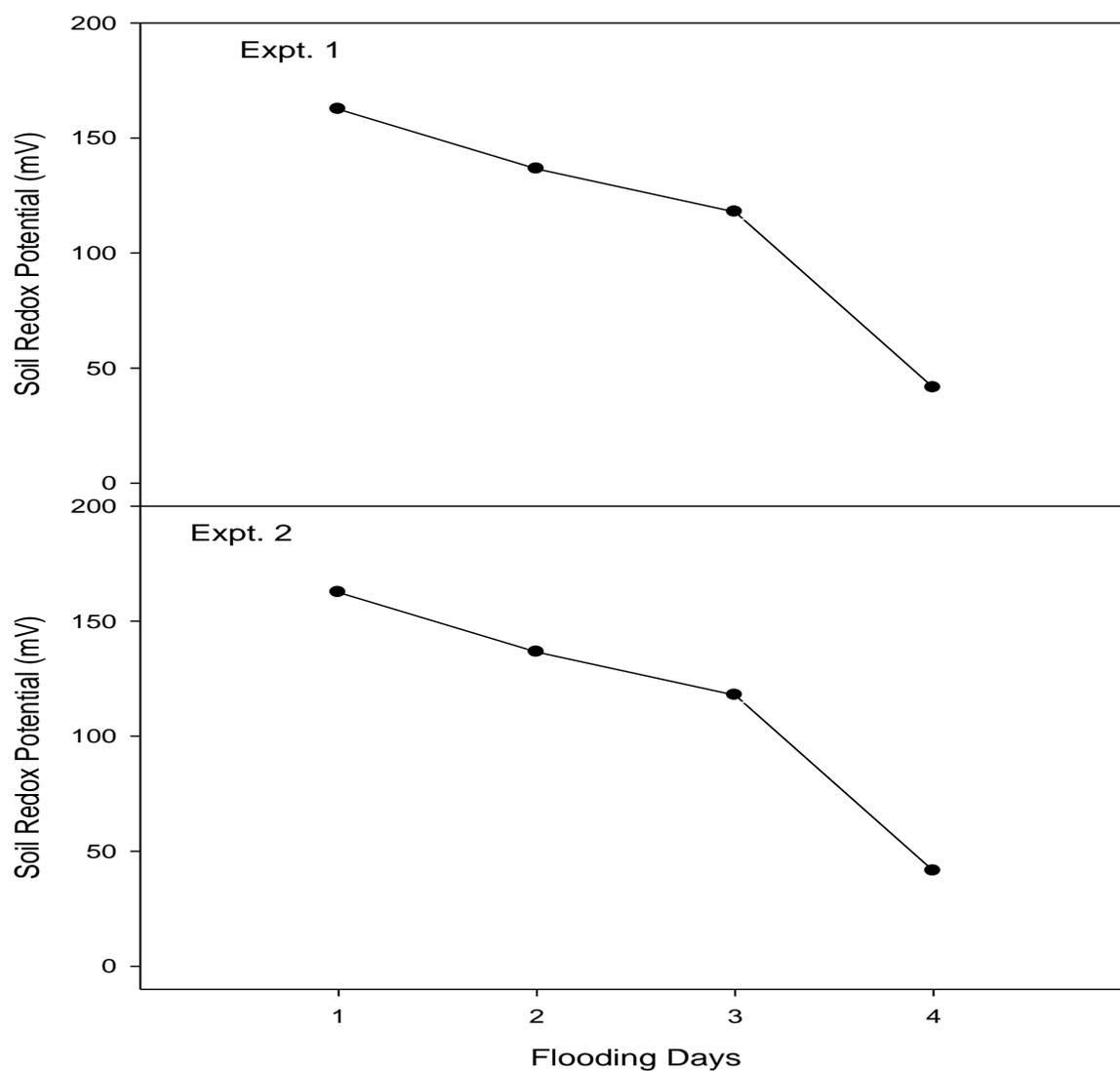


Figure 3-1. Soil redox potential of flooded 2-year-old (Expt. 1) and 1-year-old (Expt. 2) 'Choquette' avocado trees on Waldin seedling rootstock. Plants were flooded from 11 June to 14 June 2010(Expt. 1) and from 3 Sept. to 6 Sept. 2011(Expt. 2). Redox potentials below +200 mV indicate that soil conditions are anaerobic (Ponnamperuma, 1984), n=6.

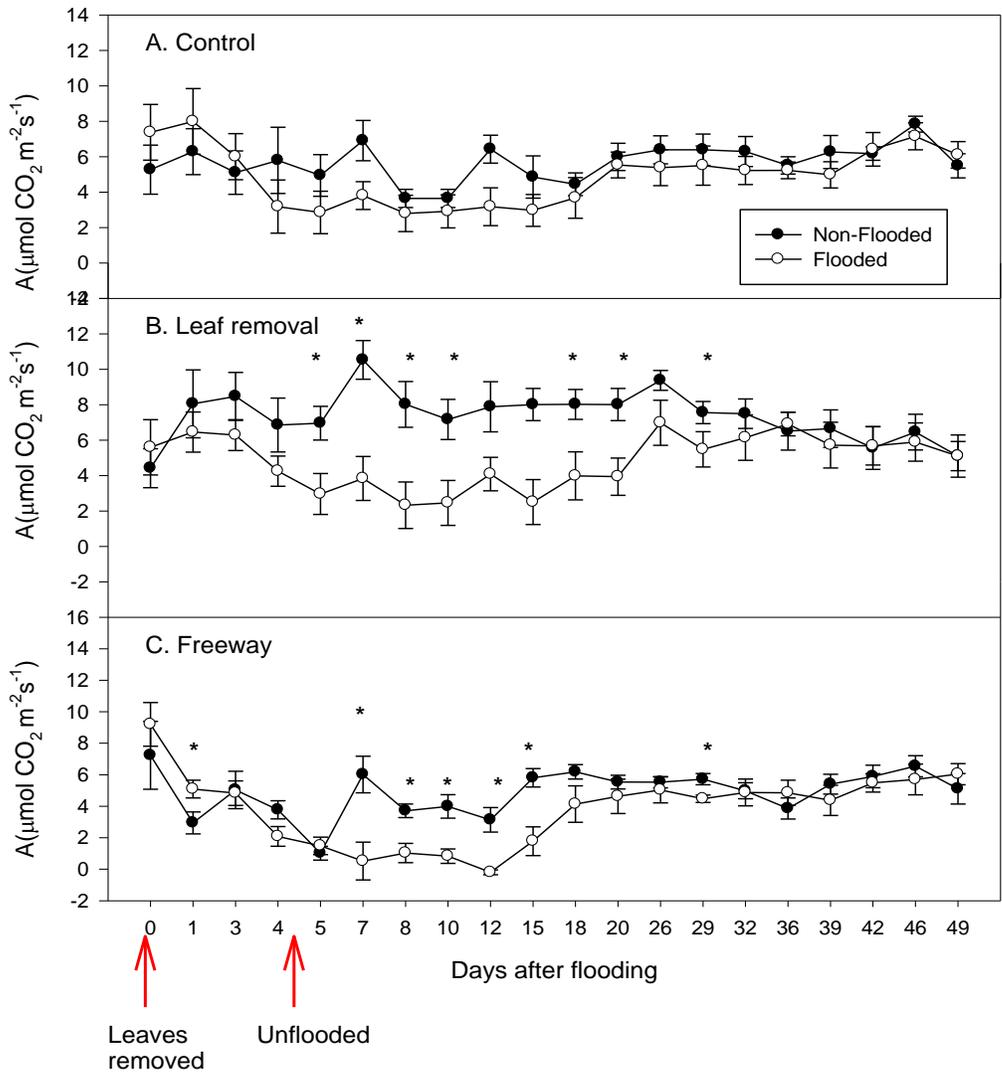


Figure 3-2. Effect of flooding on net CO₂ assimilation (A) for 2-year-old ‘Choquette’ avocado trees on Waldin seedling rootstock. Treatments included a control, leaf removal, and spraying with Freeway[®] from 10 Sept. to 29 July 2010 (Exp.1). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

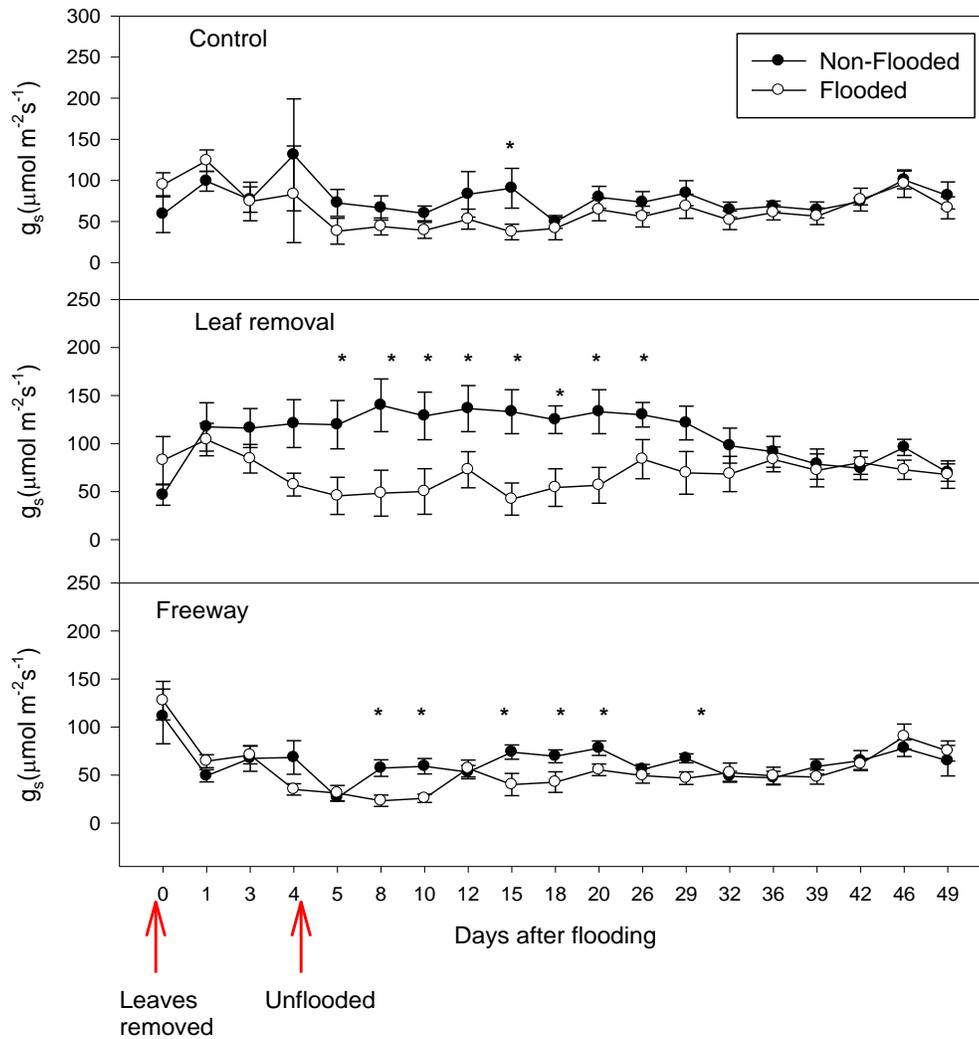


Figure 3-3. Effect of flooding on stomatal conductance to water vapor (g_s) for 2-year-old 'Choquette' on Waldin seedling rootstock avocado trees. Treatments included a control, leaf removal, and spraying with Freeway[®] from 10 Sept. to 29 July 2010 (Expt.1). An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

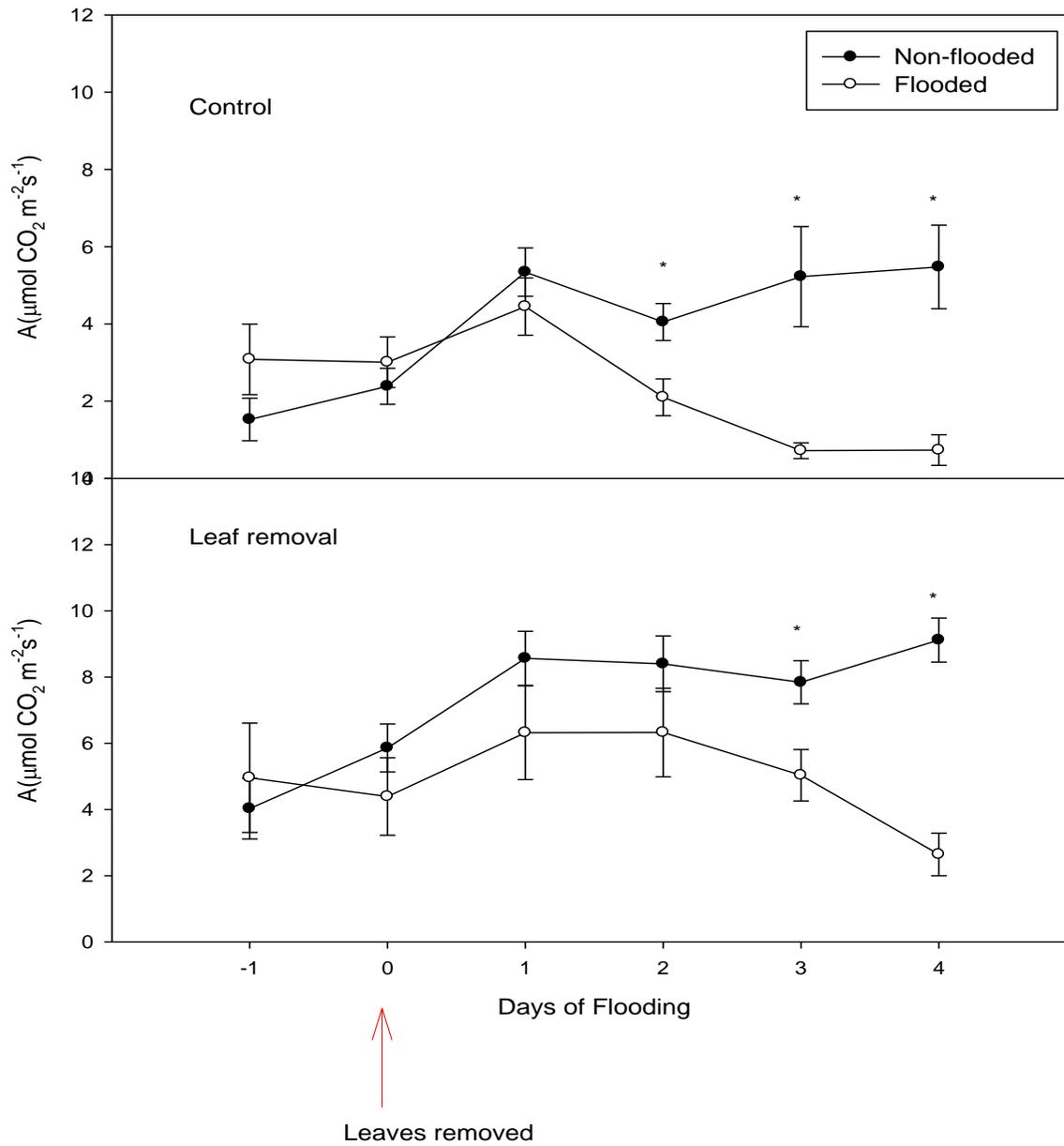


Figure 3-4. Effect of flooding on net CO₂ assimilation (A) for 1-year-old 'Choquette' avocado trees on Waldin seedling rootstock. Plants were flooded from 1 Sept. to 6 Sept. 2011. Treatments included a control and leaf removal. An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

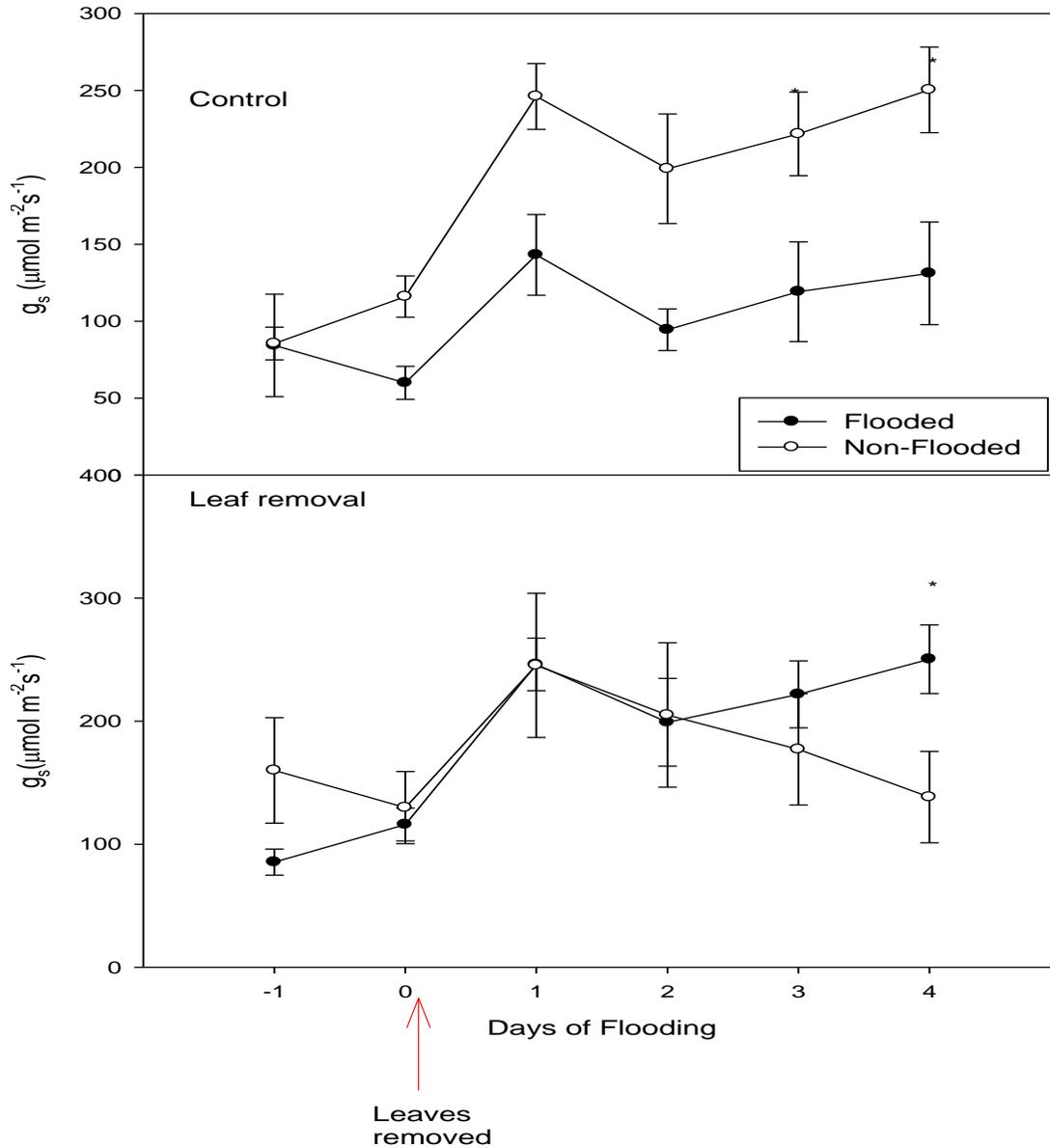


Figure 3-5. Effect of flooding on stomatal conductance to water vapor (g_s) of 1-year-old 'Choquette' avocado trees on Waldin seedling rootstock (Expt. 2). Plants were flooded from 1 Sept. to 6 Sept. 2011. Treatments included a control and leaf removal the day before flooding. An asterisk indicates a significant difference between treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

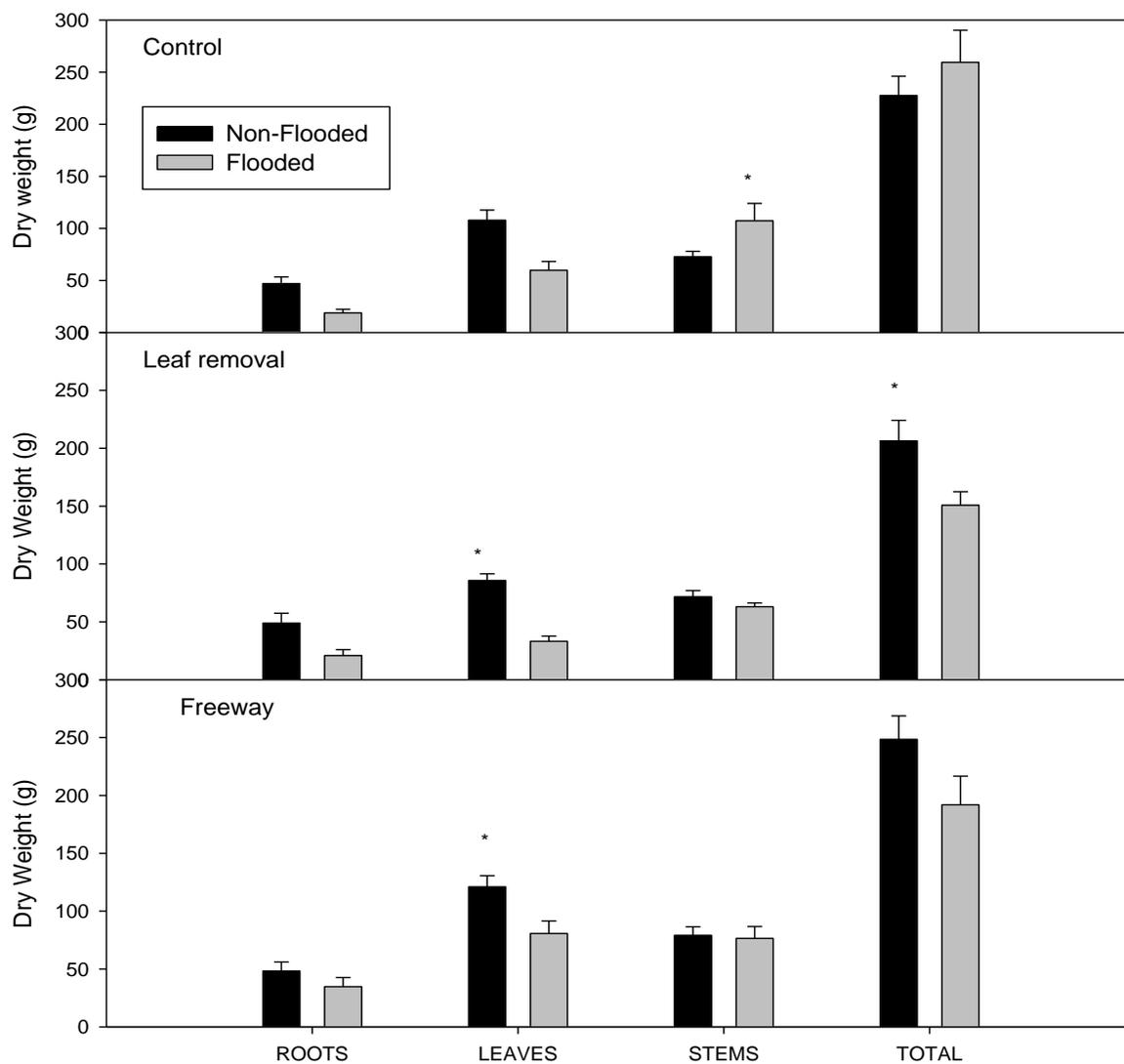


Figure 3-6. Effect of flooding on root, leaf, stem, and total plant dry weight of 2-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of three canopy treatments (Expt. 1). An asterisk indicates a significant difference between treatments according to a T-test ($P \leq 0.05$), $n = 6$.

Table 3-1. Percentage of tree survival and mortality of 'Choquette' avocado trees on Waldin seedling rootstock in each canopy treatment at the end of Expt. 1 (day 49).

Treatment		Control	Leaf removal	Freeway [®]
Flooded	No. of plants alive	5	5	4
	No. of dead plants	1	1	2
	Survival (%)	83.3	83.3	66.6
	Mortality (%)	16.6	16.6	33.3
Non-flooded	No. of plants alive	6	6	6
	No. of dead plants	0	0	0
	Survival (%)	100	100	100
	Mortality (%)	0	0	0

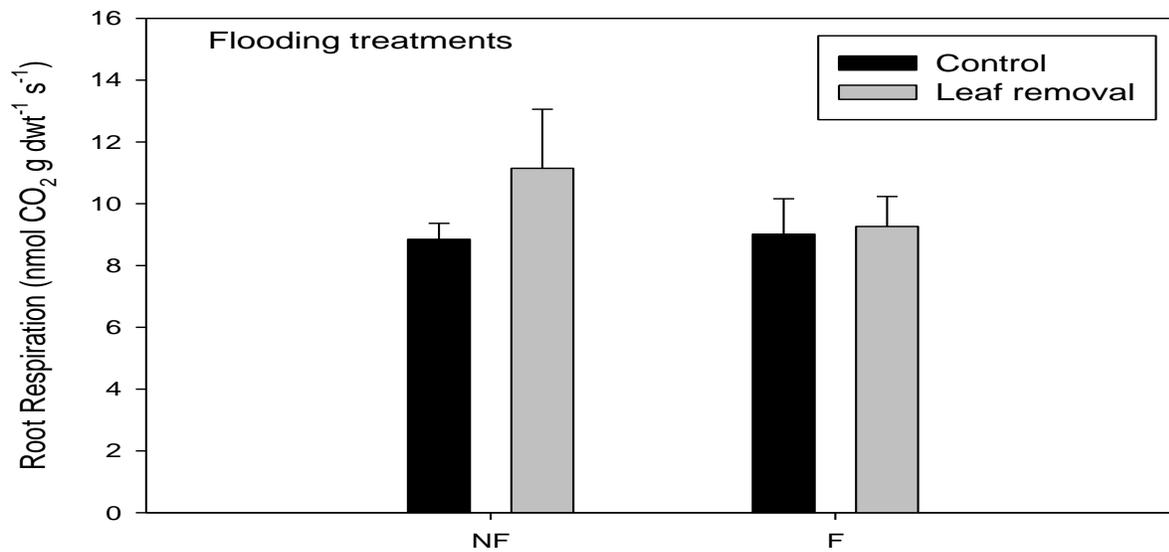
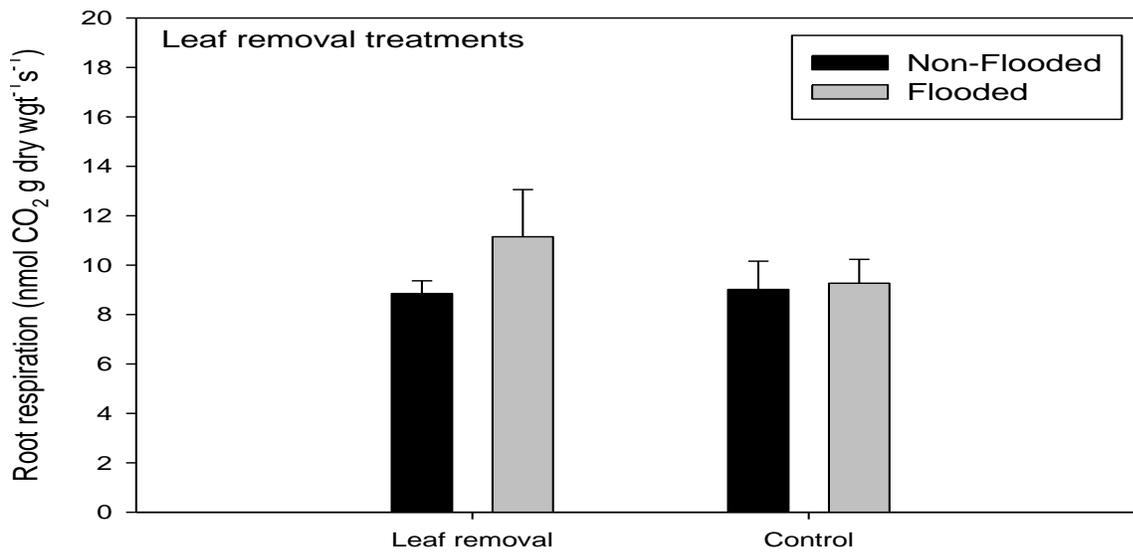


Figure 3-7. Root respiration of non-flooded (NF) and flooded (F) treatments within the leaf removal treatment and between the control and leaf removal treatment within each flooding treatment. There were no significant differences ($P \leq 0.05$) between flooding treatments according to a T-test ($P > 0.05$), $n = 6$.

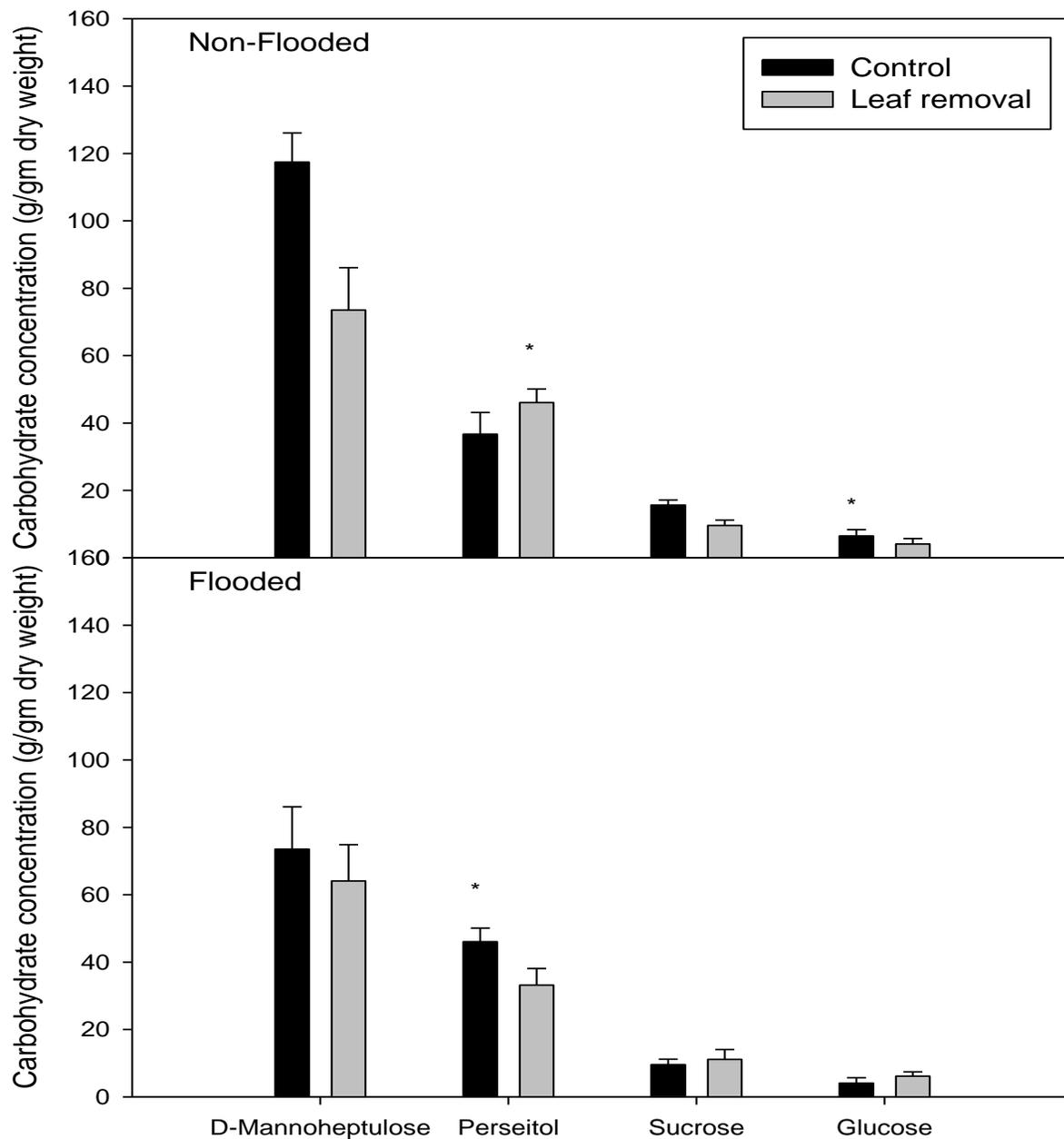


Figure 3-8. Root carbohydrate concentrations for control and leaf removal treatments for 1-year-old 'Choquette' avocado trees within flooding treatments (Expt. 2). An asterisk indicates a significant difference between treatments according to a T-test ($P \leq 0.05$), $n = 6$.

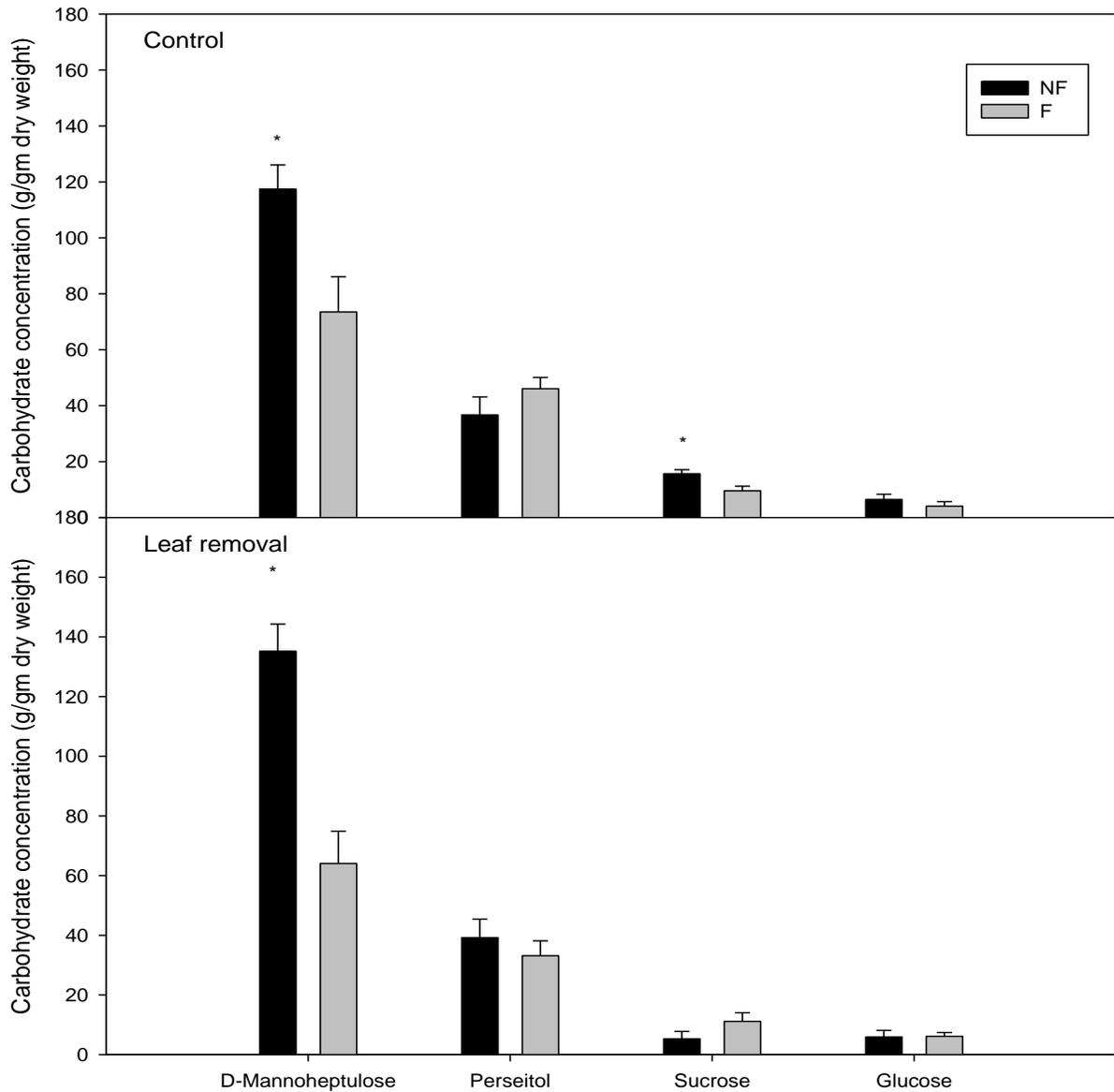


Figure 3-9. Root carbohydrate concentrations for control and leaf removal treatments for 1-year-old 'Choquette' avocado trees within flooding treatments (Expt. 2). An asterisk indicates a significant difference between treatments according to a T-test ($P \leq 0.05$), $n = 6$.

CHAPTER 4
PRE- AND POST-FLOODING LEAF REMOVAL EFFECTS ON LEAF GAS
EXCHANGE, GROWTH, AND SURVIVAL OF AVOCADO TREES EXPOSED TO
ROOT ZONE FLOODING

Background

In agricultural crop production areas, excess water produces hypoxic soil conditions (Gambrell and Patrick, 1978) by displacing oxygen and other gases in the soil which are necessary for normal root metabolism (Drew, 1997; Geigenberger, 2003). Although all plants require water, excess water in the root environment can be injurious or even lethal. Low oxygen in the soil due to flooding is one of the main environmental factors limiting plant growth and yield worldwide, especially in high rainfall regions (Bai et al., 2009). Therefore, for agriculture to remain viable in areas with poor soil drainage or that are prone to flooding from tropical storms or hurricanes, it is important to understand how flooding affects crop physiology, growth, and yield to help identify flood-adapted crops and production systems (Schaffer, 1998).

Avocado production is rapidly increasing worldwide. For example, from 2009 to 2010, avocado production increased by 13,610 ha in Colombia, by 3,380 ha in Peru, and by 2,000 ha in Mexico (FAO 2009, 2010). This rapid expansion of avocado production has resulted in orchard establishment on marginal sites that are prone to flooding or poor soil drainage (Schaffer et al., 2012). In addition, in many places avocado orchards are over-irrigated (du Plessis, 1991) which can result hypoxic conditions in the root zone. Flooding of avocado orchards occurs periodically in areas, such as southern Florida, where there are high water tables and heavy rains or tropical

storms and hurricanes during the “rainy” season (Schaffer and Whiley, 2002; Crane et al., 1994).

Avocado trees have a relatively shallow root system that does not spread much beyond the tree canopy (Wolstenholme, 2002). Roots are extensively suberized, with low hydraulic conductivity, few root hairs, poor water uptake and sensitivity to low soil oxygen concentrations (Ferreyra et al., 2006). These characteristics make avocado one of the most susceptible fruit trees to soil flooding (Ferreyra et al., 2006; Schaffer et al., 1992). Short periods (a few days) of standing water in the root zone of avocado trees has been shown to negatively impact physiological processes such as net carbon assimilation and transpiration, and cause tree mortality (Schaffer, 1998; Schaffer et al., 1992).

Tree mortality in flooded avocado orchards has been attributed to a reduction in root volume as a result of oxygen starvation of the roots (Schaffer and Ploetz, 1989; Kozlowski 1997). This reduction of root volume caused by flooding can be exacerbated if *Phytophthora cinnamomi* (the cause of Phytophthora root rot; the major disease of avocado worldwide) is present in the soil (Ploetz and Schaffer, 1989; Schaffer and Ploetz, 1989). When avocado trees are flooded, the reduction in root volume occurs before the canopy is affected (Schaffer et al., 1992). This results in a high canopy volume relative to root volume (Schaffer, 1998). The high shoot-to-root ratio of avocado trees as a result of orchard flooding has led to the recommendation in some areas, such as South Florida, of reducing the size of the canopy of trees exposed to flooding to bring the shoot to root ratio back into equilibrium (Crane et al., 1994). Removing a portion of the canopy decreases transpiration, which compensates for decreased water absorption

due to reduced total root volume as a result of death of some roots from flooding.

Therefore, sufficient water and nutrients can be supplied by the roots to the canopy, if the canopy volume is adequately reduced (Crane et al., 1994). Reducing canopy size in flooded orchards also decreases tree weight, thereby facilitating re-establishment of trees that may topple as a result of strong winds and flooded soils from tropical storms or hurricanes (Crane et al., 1994). In a preliminary study with avocado trees in containers, trees with two-thirds of the canopy removed (pruned) after flooding tended to recover faster from flooding stress than non-pruned trees (Gil et al., 2008). Pruned trees exhibited a more rapid recovery of net CO₂ assimilation and transpiration to pre-flood levels and had greater root dry weights than non-pruned trees (Gil et al., 2008). It was suggested that the reduction in leaf area and subsequent decreased transpiration due to pruning compensated for the reduction in viable root volume and the decreased capacity for water absorption by flood-damaged roots (Gil et al., 2008).

An alternative to removing a portion of the canopy after flooding to mitigate flooding effects on tree vigor and vitality is to remove a portion of the canopy prior to a predicted flooding event if there is sufficient time (Crane et al., 1994). In a study with young avocado trees in containers, Gil et al. (2008) removed a portion of the canopy prior to flooding. In contrast to results observed for post-flooding canopy reduction, removing a portion of the canopy immediately prior to flooding increased tree stress and mortality (Gil et al., 2008). It was hypothesized that pre-flooding leaf removal as a result of less carbohydrate production and translocation to the roots, as a result of reduced photosynthesis from the combined effects of decreased leaf area and stress caused by root hypoxia (Gil et al., 2008). Thus, there was less carbohydrate in the roots to serve

as a substrate for respiration during flooding, and consequently less ATP production for normal plant metabolism, resulting in increased tree stress (Chapter 3 of this thesis). Recent experiments have shown that the root concentrations of D-mannoheptulose and perseitol, the main photosynthetic products in avocado trees, were reduced under flooded conditions, and that concentrations of these carbohydrates tended to be lower in trees with leaves removed compared to those with no leaves removed before flooding (Chapter 3 of this thesis). Therefore, it appears that reducing leaf area before flooding exacerbates flooding stress, whereas leaf area removal after flooding can increase tree survival.

Recommendations related to pruning of avocado trees to reduce flooding stress are based mainly on observations (Gil et al., 2008). Thus, there is a need to quantify the effects of the amount and timing of leaf removal or pruning on physiology, growth and survival of flooded avocado trees. Additionally, the underlying basis for the different flooding responses between trees with leaves removed before or immediately after flooding remains unclear.

The objective of this study was to determine the relationship between the timing (pre- or post-flooding) and quantity of leaf removal on stress and survival of avocado trees exposed to flooding. The hypotheses tested were: 1) Leaf removal immediately prior to flooding exacerbates flooding stress due to decreased net CO₂ assimilation during flooding; and 2) Leaf removal immediately after flooding decreases water demand by reducing transpirational surface area.

Materials and Methods

Study site description

This study was conducted in an open-air (Only the roof was covered.) greenhouse at the University of Florida, Tropical Research and Education Center in Homestead, Florida (25.5°N and 80.5°W). Temperature in the greenhouse was recorded with a StowAway TidbiT sensor/data logger (Onset Computer Corp., Bourne, MA, USA) located 10 cm above the canopy. Photosynthetic photon flux (PPF) was measured with a quantum sensor (model LI?? 190) connected to a LI-1000 datalogger (Li-Cor Inc., Lincoln, Nebraska, USA).

There were two experiments: In the first experiment (Expt. 1), conducted from June-August 2011, the effect the percentage of leaves removed prior to flooding on leaf gas exchange and plant survival during and after flooding was determined. In the second experiment (Expt. 2), conducted during October-December 2011, the effect of the percentage of leaves removed after flooding on leaf gas exchange and plant survival during and after flooding was determined.

Plant material

Expt. 1. Three-year-old 'Choquette' avocado trees on Walden seedling rootstock in potting medium composed of 40% Canadian peat, 10% coir, 40% pine bark and 10% Perlite® in 3-L containers were obtained from a commercial nursery.

Expt. 2. Two-year-old 'Simmonds' avocado trees on Walden seedling rootstock in the same potting medium and the same size containers as described for plants in Expt. 1 were obtained from a commercial nursery .

Experimental design

Expt. 1. The experiment consisted of a completely randomized design with a 4 x 2 factorial arrangement of treatments. There were four leaf removal treatments: no leaf removal (control), one-third of the leaves removed (1/3LR), half of the leaves removed (1/2LR), and two-thirds of the leaves removed (2/3LR); and two flooding treatments: flooded (F) and non-flooded (NF). Leaves were removed 1 day before flooding. There were 6 single-plant replications for each treatment combination.

Expt. 2. The experiment consisted of a completely randomized design with a 4 x 2 factorial arrangement of treatments. Leaf removal treatments were similar to those in Expt. 1, except that instead of removing leaves before flooding, leaves were removed immediately after trees were unflooded. There were 6 single-plant replications for each treatment combination.

For each experiment, prior to initiating treatments, trees treated with the fungicide, Alliete® (Bayer Crop Science, Morganville, NC, USA), as a soil drench to help prevent Phytophthora root rot.

Leaf removal treatments

The desired percentage of leaves was removed by counting the number of leaves per tree and then removing a percentage of the total number of leaves from each tree starting from the base of the tree upward. No leaves were removed from trees in the control treatment.

Flooding treatments

Plants were flooded by submerging entire plant containers in 19-L plastic buckets 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 and to maintain a constant water level. Trees in the control (NF) treatment were not flooded. Flooded plants were unflooded after a statistically significant difference ($P \leq 0.05$) in net CO_2 assimilation or stomatal conductance of water vapor was observed between flooded and non-flooded trees in any of the leaf removal treatments. In Expt. 1, the flooding period was 3 days and in Expt. 2 the flooding period was 5 days. Trees were irrigated by overhead irrigation for 15 minutes twice each day except during the flooding period when non-flooded trees were manually irrigated twice daily. Tensiometers (Irrometer Company, Riverside, CA, USA) were installed in 5 randomly selected containers for plants in the non-flooded treatments and soil suction was maintained at 10-15 KPa to ensure that non-flooded trees were not drought stressed (Kiggundu et al., 2012).

Leaf gas exchange measurements

Expt. 1. Net CO_2 assimilation, stomatal conductance of water vapor, and transpiration were measured with a ADC3 portable gas analyzer and a PLC3 leaf cuvette with a halogen light source (Analytical Development Company, Bioscientific Ltd., Great Amwell, England) at a light saturated PPF of $1000 \mu\text{m m}^{-2}\text{s}^{-1}$, a reference CO_2 concentration of $375 \mu\text{m mol}^{-1}$ and an air flow rate into the leaf cuvette of 200 ml min^{-1} . Measurements were made between 900 HR and 1100 HR, starting 2 days prior to flooding. Leaf gas exchange was measured daily on the same two fully expanded, mature leaves of each plant. After the flooding period, measurements were made at 1-day intervals for 10 days after flooding and at 2-day intervals thereafter until 52 days after plants were unflooded, when there were no significant differences between flooded

and non-flooded plants in net CO₂ assimilation or stomatal conductance within any of the leaf removal treatments.

Expt. 2. Net CO₂ assimilation, stomatal conductance of water vapor, and transpiration were measured with a Ciras-2 portable gas analyzer with a broad-leaf cuvette and a halogen light source (PP Systems, Amesbury, MA, USA) at a light saturated PPF of 1000 $\mu\text{m m}^{-2}\text{s}^{-1}$, a reference CO₂ concentration of 375 $\mu\text{m mol}^{-1}$, and an air flow rate into the leaf cuvette of 200 ml min^{-1} . Measurements were made between 900 HR and 1100 HR, starting 2 days prior to flooding. During the flooding period, leaf gas exchange was measured daily on the same two fully expanded, mature leaves of each plant throughout the experiment. After the flooding period, measurements were made at 1-day intervals for 10 days after flooding and at 2-day intervals thereafter until 53 days after plants were unflooded when there were no significant differences between flooded and non-flooded plants for net CO₂ assimilation or stomatal conductance within any of the leaf removal treatments.

Plant biomass measurements

Plants were harvested and dry weights were determined at the end of each experiment. Roots were separated from the rooting medium and carefully washed with tap water. Tissue samples were then oven dried at 70°C to a constant weight, and leaf, stem, root and total plant dry weights were determined.

Soil redox potential and soil temperature

Soil redox potential was measured in each container with a metallic ORP indicating electrode for plants in the flooded treatment in both experiments (Accument Model 13-620-115, Fisher Scientific, Pittsburg, PA, USA) connected to a voltmeter.

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.

Soil temperature was recorded with a Hobo Tidbit v2 sensor/datalogger (Onset Computer Corp., Pocasset, MA, USA) placed 5 cm deep into the container of one randomly selected plant in each treatment combination.

Sap Flow

Expt. 2. Xylem sap flow was monitored daily in a subsample of 3 plants in the following treatment combinations: Control-NF, Control-F, 2/3P-NF, and 2/3P-F to determine the effects of treatments on transpirational demand. Xylem sap flow was monitored with a Dynagauge Flow 32-1KTM Sap Flow System (Dynamax Inc., Houston, TX, USA). This system is based on the heat balance technique which is a non-intrusive, non-destructive method to measure sap flow (Dynamax Inc. 2010). Measurements started 2 days before the initiation of the flooding treatments and stopped the day before plants were unflooded (day 4).

Statistical analysis

Data were first analyzed by a two-way analysis of variance (ANOVA) to test if there were significant interactions between flooding and leaf removal treatments. Differences between flooding treatments were determined by repeated measures ANOVA for leaf gas exchange variables and with a T-test for biomass variables (SAS Institute, Cary, NC, USA).

Results

Soil and air temperature, soil redox potential and light intensity

Expt. 1. Daily air temperature in the greenhouse ranged from 22 to 40°C with a mean of 28°C. Soil temperature ranged from 23 to 34°C with a mean of 28°C. Mean soil redox potential for the flooded treatment was slightly below 200 mV beginning on day 1 and continued to decrease to 94 mV by day 3 (Figure 4-1).

Expt. 2. Daily air temperature in the greenhouse ranged from 14 to 35°C with a mean of 23°C. Soil temperature ranged from 18 to 29°C with a mean of 23°C. Mean soil redox potential for the flooded treatment was 220 mV beginning on day 1 and continued to decrease to 48 mV by day 4 (Figure 4-1).

Leaf gas exchange

Expt. 1. There was a significant statistical interaction ($P \leq 0.05$) between the leaf removal and flooding treatments for net CO₂ assimilation and stomatal conductance on a few of the measurement dates. Therefore, flooding treatments were compared separately within each leaf removal treatment.

In each leaf removal treatment, net CO₂ assimilation, stomatal conductance and transpiration were significantly lower ($P \leq 0.05$) in flooded than in non-flooded trees after 4 days of flooding (Figures 4-2, 4-3, 4-4). Net CO₂ assimilation of flooded plants increased to values close to those of non-flooded plants 34 days after plants were unflooded only in the 1/3LR treatment. Net CO₂ assimilation of flooded plants did not recover to pre-flooding levels in the other leaf removal treatments (Figure 4-2).

Expt. 2. There was a significant statistical interaction ($P \leq 0.05$) between leaf removal and flooding treatments for stomatal conductance and transpiration on a few of

the measurement dates. Therefore, for all leaf gas exchange variables, flooding treatments were compared separately within each leaf removal treatment.

Net CO₂ assimilation was significantly lower ($P \leq 0.05$) in flooded than in non-flooded plants (Figure 4-4). Reductions in net CO₂ assimilation were first observed on the third and fourth day of flooding in the 1/3LR and 1/2LR treatments, respectively. In the control and 2/3LR treatments, reductions in net CO₂ assimilation were observed 1 and 8 days after plants were unflooded, respectively. The same pattern was observed for stomatal conductance (Figure 4-5) and transpiration (Figure 4-6) within each of the leaf removal treatment.

In control plants, net CO₂ assimilation and stomatal conductance values of flooded plants recovered to those of the non-flooded trees 35 days after plants were unflooded. In the 1/3LR and 1/2LR treatments, net CO₂ assimilation returned to levels similar to non-flooded plants 45 days after plants were unflooded. Net CO₂ assimilation of plants in the 2/3LR treatment returned to values similar to those of non-flooded plants 30 days after plants were unflooded (Figures 4-4, 4-6).

Plant growth and survival

Expt. 1. There was a significant statistical interaction ($P \leq 0.05$) between the flooding and the leaf removal treatments for root dry weight. Therefore, the effect of flooding on leaf, stem, root, and plant dry weights were determined separately within each leaf removal treatment. In the control and 1/2LR treatment, flooded plants had significantly lower ($P \leq 0.05$) leaf, stem, root, and total plant dry weights than non-flooded plants. In the 2/3LR treatment, flooded plants had significantly lower ($P \leq 0.05$) leaf and total plant dry weights than non-flooded plants. In the 1/3LR treatment, there

were no significant differences ($P > 0.05$) in dry weights between flooded and non-flooded plants (Figure 4-7).

At the end of the experiment (day 55), 83% of the flooded plants in the 2/3LR treatment died. In the control and 1/2LR treatments, 67% of the flooded plants died, whereas in the 1/3LR treatment, only 50% of the flooded plants died (Table 4-1). No non-flooded plants died.

Expt. 2. In the control treatment, flooded plants had significantly lower ($P \leq 0.05$) stem, root, and total plant dry weights than non-flooded plants. In the 1/3LR treatment, flooded plants had significantly lower ($P \leq 0.05$) root dry weights than non-flooded plants. In the 1/2LR and 2/3LR treatments, there were no significant differences ($P > 0.05$) between flooded and non-flooded plants for leaf, stem, root or plant dry weight (Figure 4-8).

At the end of the experiment (day 57), 33% of flooded plants in the control and 2/3LR treatments died. In the 1/3LR and 1/2LR treatments, 17% of the flooded plants died (Table 4-2).

Sap Flow

There was no significant interaction ($P > 0.05$) between leaf removal and flooding treatments for daily sap flow. Therefore leaf removal treatments were pooled to compare flooding treatments. There were no significant ($P > 0.05$) differences between flooded and non-flooded trees for daily xylem sap flow rate (Figure 4-9).

Discussion

Removal of avocado leaves prior to flooding (Expt. 1) resulted in significantly lower net CO_2 assimilation and stomatal conductance in flooded than in non-flooded plants.

Leaf removal prior to flooding also prevented plant recovery after plants were unflooded as indicated by significantly lower net CO₂ assimilation and stomatal conductance in flooded than in non-flooded plants with leaves removed until the end of the experiment (day 55). Tree mortality was higher and organ dry weights were lower in flooded plants with leaves removed prior to flooding than in plants with no leaves removed. In the present study, in order to leave the most photosynthetically active leaves intact, young leaves (Lambers et al., 2008) leaves were removed starting from the base of the trunk upwards until the desired percentage of leaves were removed. In a similar study, when avocado leaves were removed by pruning (from the top of the branches downward), the results were similar to this study.

When a portion of the leaves was removed from the base of avocado canopys immediately after flooding (Expt. 2), net CO₂ assimilation and stomatal conductance were lower in flooded plants than in non-flooded plants during the flooding period. However, in contrast to leaf removal before flooding, removing leaves after flooding facilitated recovery of flooded plants weeks after plants were unflooded as indicated by leaf gas exchange values that were similar to those of non-flooded plants. Leaf removal after flooding also resulted in higher organ dry weights and greater tree survival after flooding compared to plants with no leaves removed. Similarly, Gil et al. (2008) found that removal of two-thirds of the avocado canopy by pruning before flooding exacerbated stress, whereas removal of the same proportion of the canopy after flooding enhanced plant survival.

Increased stress of avocado trees as a result of leaf removal prior to flooding observed in the present study is consistent with previous research showing that

harvesting (shoot removal) forage legumes causes significant plant stress, especially when roots are in hypoxic conditions (Hatwig et al., 1987). Similarly, in alfalfa (*Medicago sativa*) root waterlogging following clipping, significantly increased injury of plants grown in greenhouse or the field (Barta and Schmitthener, 1986). Increased injury to plants with leaves removed prior to flooding may have been the result of a reduction in carbohydrate production due to a reduced amount of photosynthesizing tissue. Because a continuous supply of fermentable carbohydrates in root tissues is critical for long-term survival during flooding, the presence of leaves may be important as an assimilate source for root respiration (Barta, 1987; Liao and Lin, 2001). Root zone flooding also affects transport of carbohydrates to meristematic sinks (Kozlowski, 1997). Therefore, if a sufficient amount of assimilates in leaves is not produced and transported to the roots, carbohydrate deficiency will occur (Liao and Lin, 2001). In previous experiments with avocado trees, flooding resulted in a decrease in photosynthesis that coincided with a decrease in the primary photosynthetic products, D-mannoheptulose and perseitol in the roots (Chapter 3 of this thesis). Additionally, removal of two-thirds of avocado leaves prior to flooding resulted in an even greater reduction of these carbohydrates in roots compared to plants with no leaves removed prior to flooding. This may explain why in Expt.1 tree mortality was higher in plants with two-thirds of the leaves removed before flooding than in any other leaf removal treatment, including the control plants.

Reduction in root respiration is one the earliest responses of plants to root hypoxia (Liao and Lin, 2001). When oxygen is not available for energy production in the roots, plants undergo anaerobic respiration, which produces less energy (ATP) than aerobic

respiration for plant metabolism (Taiz and Zieger, 2010). Thus, the combined effect of reduced transport of photoassimilates and anaerobic respiration is exacerbated by the reduction of carbohydrate reserves when leaves are removed before flooding, resulting in severe stress and tree mortality. Flooding avocado trees reduced stomatal conductance, net CO₂ assimilation root and shoot growth, and caused root necrosis. These responses appeared to be greater in plants with two-thirds of the leaves removed than in plants with fewer leaves removed (i.e., one-third of the leaves removed), demonstrating that leaf removal before flooding increases flooding stress and reduces tree recovery.

Removing leaves immediately after flooding (Expt. 2) increased post-flooding survival of avocado trees. A reduction in net CO₂ assimilation and stomatal conductance were observed in flooded plants as seen in Expt. 1. Transpiration was also lower in flooded plants, presumably as a result of decreased stomatal conductance. This is consistent with previous research with young apricot trees (*Prunus armeniaca*) that showed a reduction of transpiration in flooded plants after 50 hours of flooding (Nicolás et al., 2005). In avocado trees, reductions in transpiration have been attributed to reductions in stomatal conductance rather than a hydraulic effect since flooding does not reduce xylem water potential (Schaffer et al., 1992; Schaffer et al., 2006). Similar results have been observed in other tropical fruit trees such as mamey sapote (*Pouteria sapota*) (Nickum et al., 2010) and carambola (*Averrhoa carambola*) (Ismail and Noor, 1996). The mechanisms for reductions in stomatal conductance in avocado trees as a result of flooding have not been elucidated (Gil et al., 2009). It has been hypothesized for other woody plants that an increase in

abscisic acid (ABA) concentration in leaves is the root-to-shoot signaling mechanism inducing stomatal closure in flooded plants (Kozłowski, 1997). In the present study, ABA concentration was not measured; however, Gil et al. (2009) could not relate stomatal closure to differences in ABA concentrations between flooded and non-flooded avocado trees. Therefore, the mechanism for increased stomatal closure of flooded avocado trees still needs to be elucidated.

Internal regulation of stomatal conductance and stem sap flow have generally been interpreted as mechanisms to restrict water movement during drought or flooding (Nicolás et al., 2005 and Ruiz-Sanchez et al., 1996). Low oxygen concentrations in the root zone have been shown to reduce the permeability of roots to water (Smith et al., 1990; Zhang and Tyerman, 1991), increasing the resistance to water uptake (Domingo et al., 2002). Under these conditions, water loss from the shoots exceeds the supply from the roots, leading to a reduction in leaf water potential and stomatal conductance (Domingo et al., 2002). In woody plants, the differences between water loss via transpiration and water uptake and short-term dynamics associated with changes in water status can be determined from measurements of trunk sap flow. Lemon (*Citrus limon*) trees subjected to 3 days of flooding exhibited a progressive reduction in sap flow and stomatal closure (Ortuño et al., 2007). Similarly, in young apricot trees (*Prunus armeniaca*), 3 hours of flooding caused a decrease in sap flow and a strong reduction in plant hydraulic conductance, decreasing water absorption by the roots (Nicolás et al., 2005). In the same experiment, a close relationship was observed between leaf water potential, leaf conductance, and plant hydraulic conductance, indicating that hydraulic signals are likely to play a dominant role in coordinating the observed responses of the

shoot. In the present study, there was a tendency for sap flow to decrease as the flooding days progressed. However, a relationship between plant water loss and sap flow was not observed because sap flow measurements were discontinued before the end of the flooding period. Sap flow sensors caused a slight browning of the stem a few days after flooding commenced. This may have been the result of removing part of the vascular tissue during stem preparation for the sap flow system. Measurements were halted after the symptoms were observed, to allow stems to heal and avoid further tree damage. However, this may have affected tree survival in control and 2/3LR treatments, since tree mortality in both treatments was 66.67%. Tree survival of flooded plants was 83.3% in both the 1/3LR and 1/2LR half leaf removal treatments when leaves were removed after the flooding period. Despite high tree mortality, possibly due to stem damage from the sap flow sensors, plants in the 2/3LR treatment that survived flooding recovered faster than plants with any other leaf removal level. This suggests that removing two-thirds of the leaves after flooding may be the optimum leaf removal level to ensure tree survival after flooding.

Based on leaf gas exchange, tree recovery, and plant biomass, leaf removal before flooding increased flooding stress, whereas leaf removal immediately after flooding increased plant survival. The results of this study support the observation that leaf removal from avocado trees immediately after flooding can reduce flooding stress of avocado trees (Crane et al., 1994; Gil et al., 2008). Removing two-thirds of the canopy immediately after flooding may be the optimum amount of leaf removal to ensure tree survival. On the other hand, leaf removal shortly before flooding can exacerbate flooding stress. Further studies are necessary to quantify the relationship

between the quantity of canopy removed and tree survival and recovery of trees pruned before flooding.

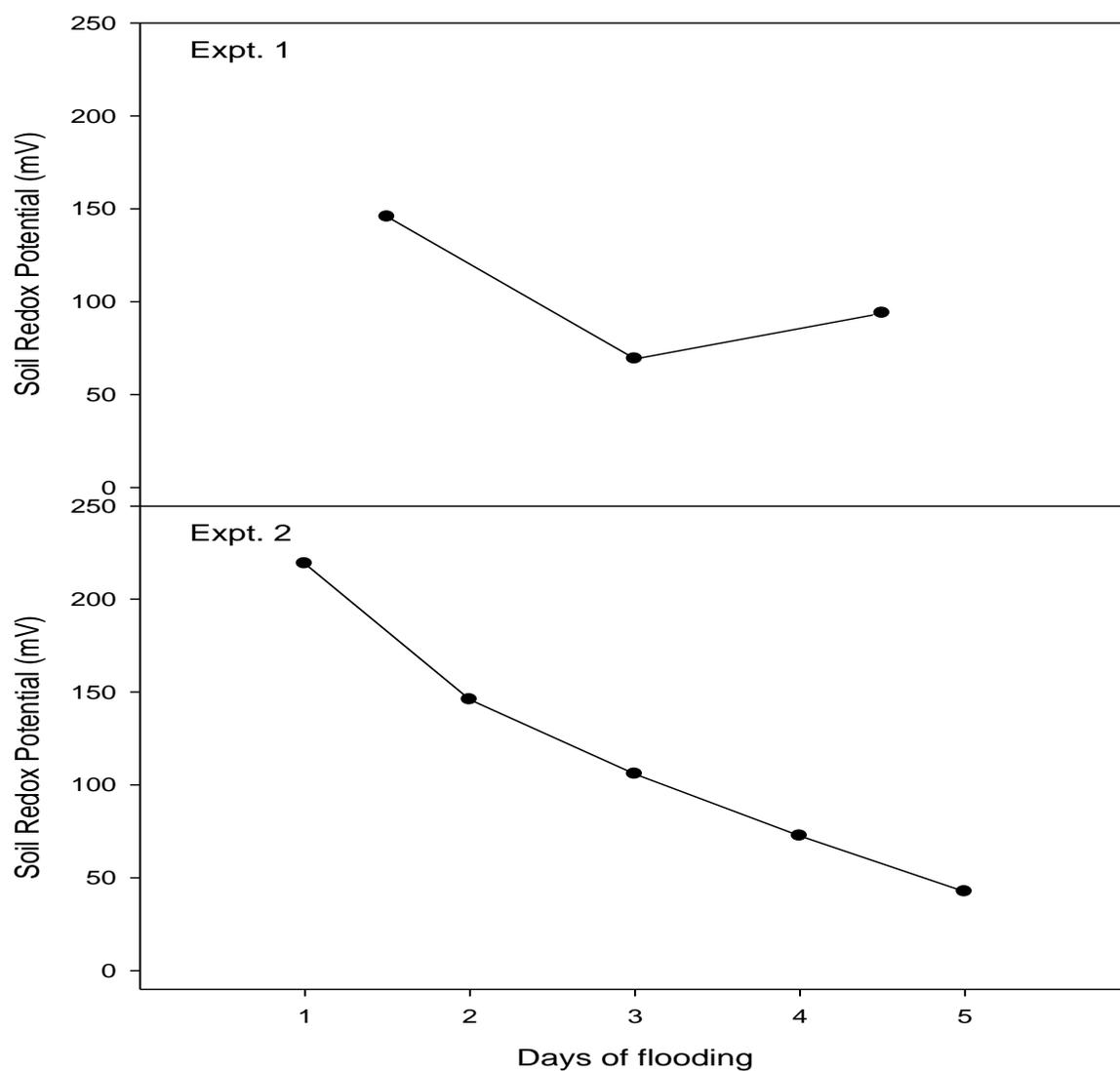


Figure 4-1. Mean soil redox potential of flooded 3-year-old 'Choquette' avocado trees (Expt. 1) and 2-year-old 'Simmonds' avocado trees (Expt. 2) on Waldin seedling rootstock. Plants were flooded from 11 June to 13 June 2011 (Expt. 1) and from 13 Oct. to 17 Oct. 2011 (Expt. 2). Redox potentials below +200 mV indicate that soil conditions are anaerobic (Ponnamperuma, 1984), n = 6.

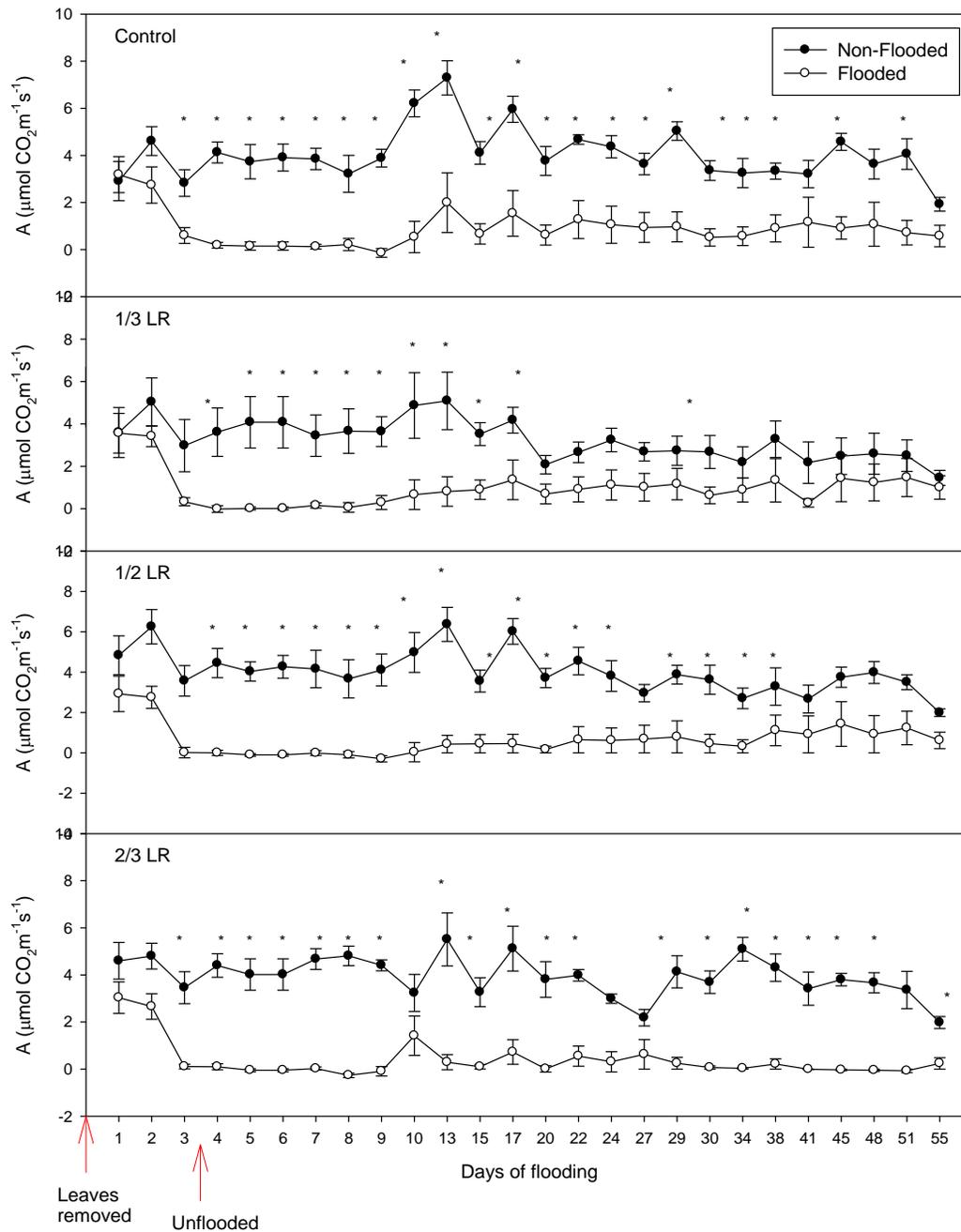


Figure 4-2. Net CO₂ assimilation (A) of flooded and non-flooded 3-year-old ‘Choquette’ avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 1). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed before flooding. An asterisk indicates a significant difference between flooding treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n=6$.

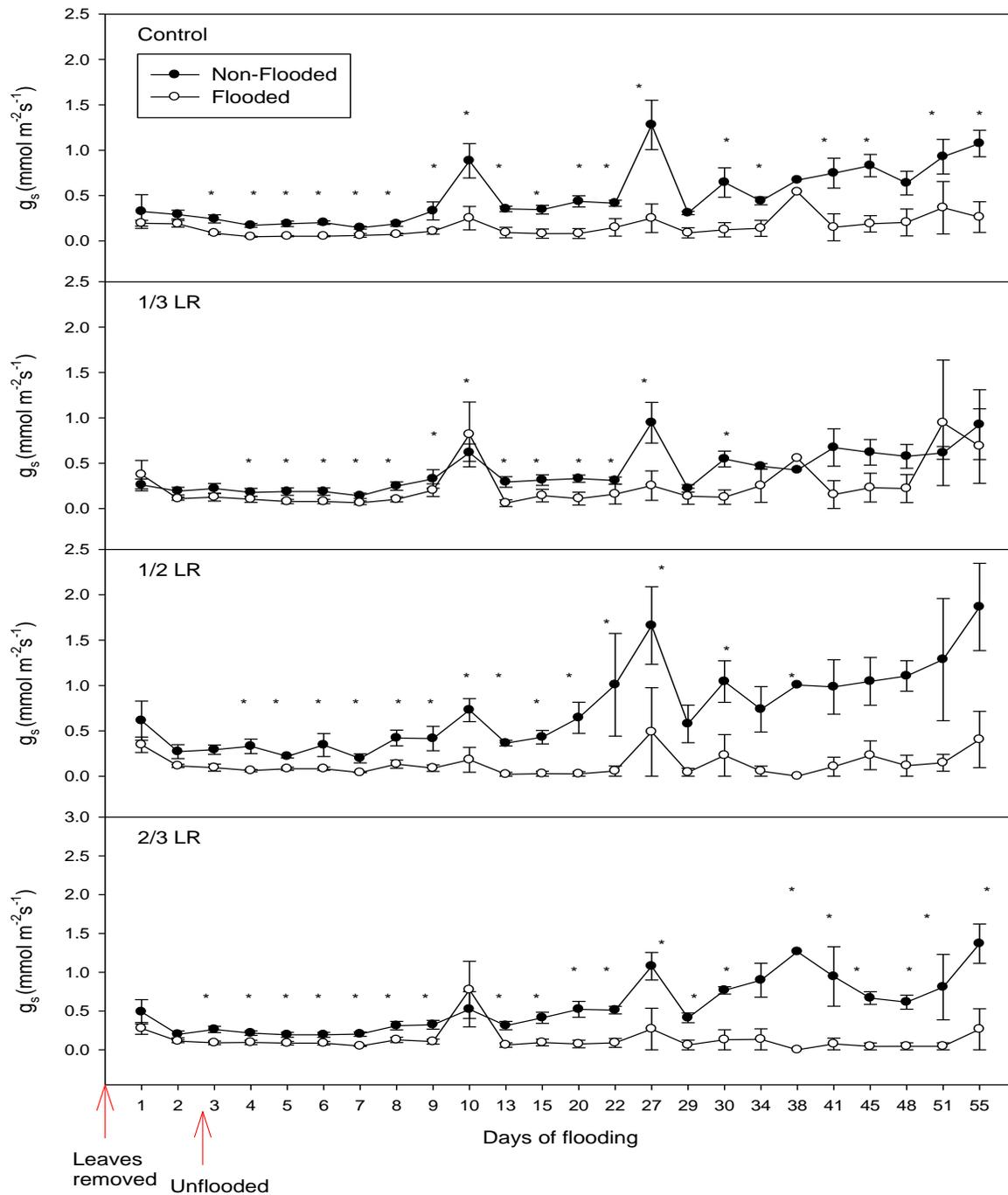


Figure 4-3. Stomatal conductance of water vapor (g_s) of flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 1). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed before flooding. An asterisk indicates a significant difference between flooding treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

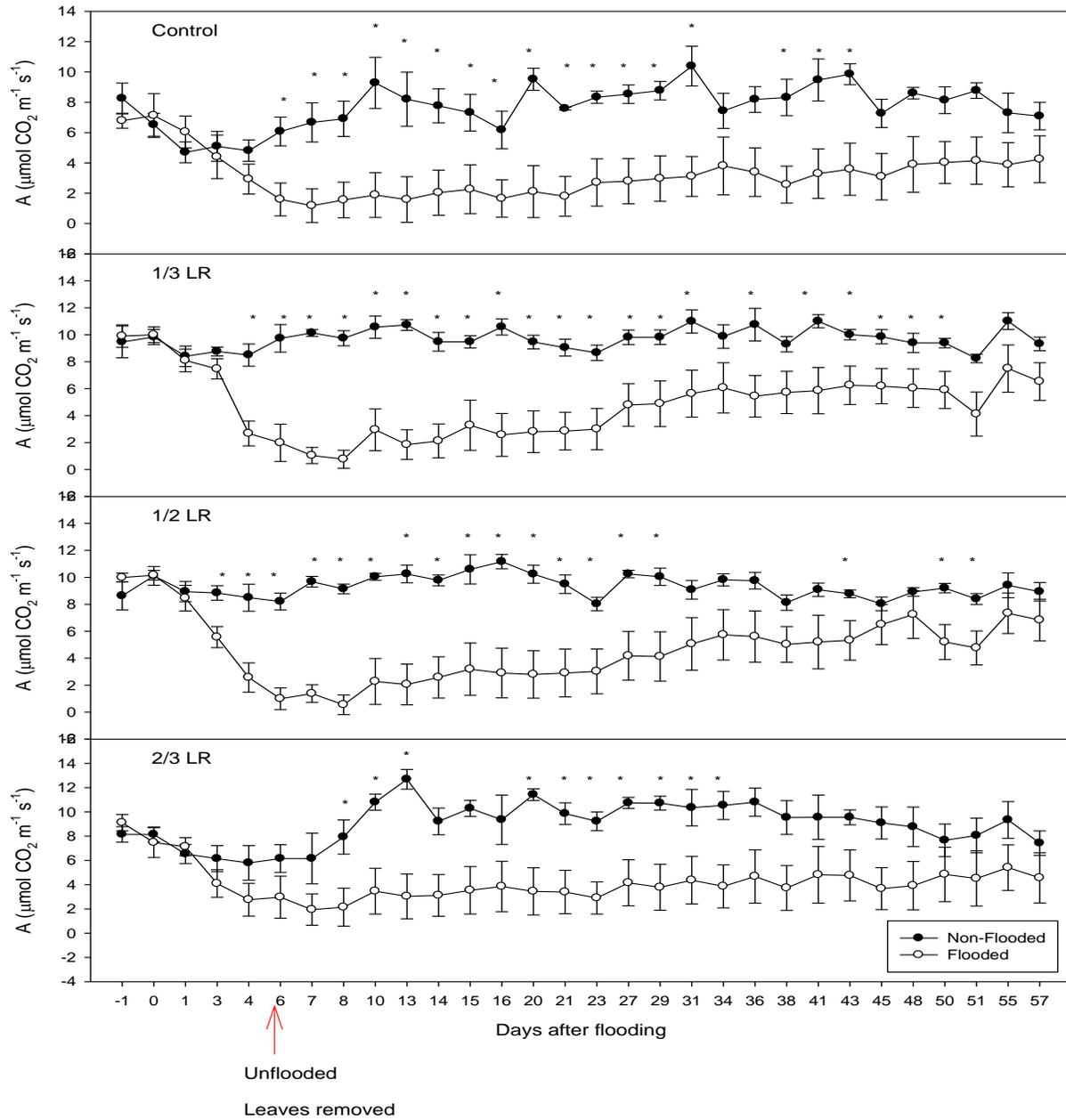


Figure 4-4. Net CO₂ assimilation (A) of flooded and non-flooded 2-year-old ‘Simmonds’ avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 2). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed after flooding. An asterisk indicates a significant difference between flooding treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

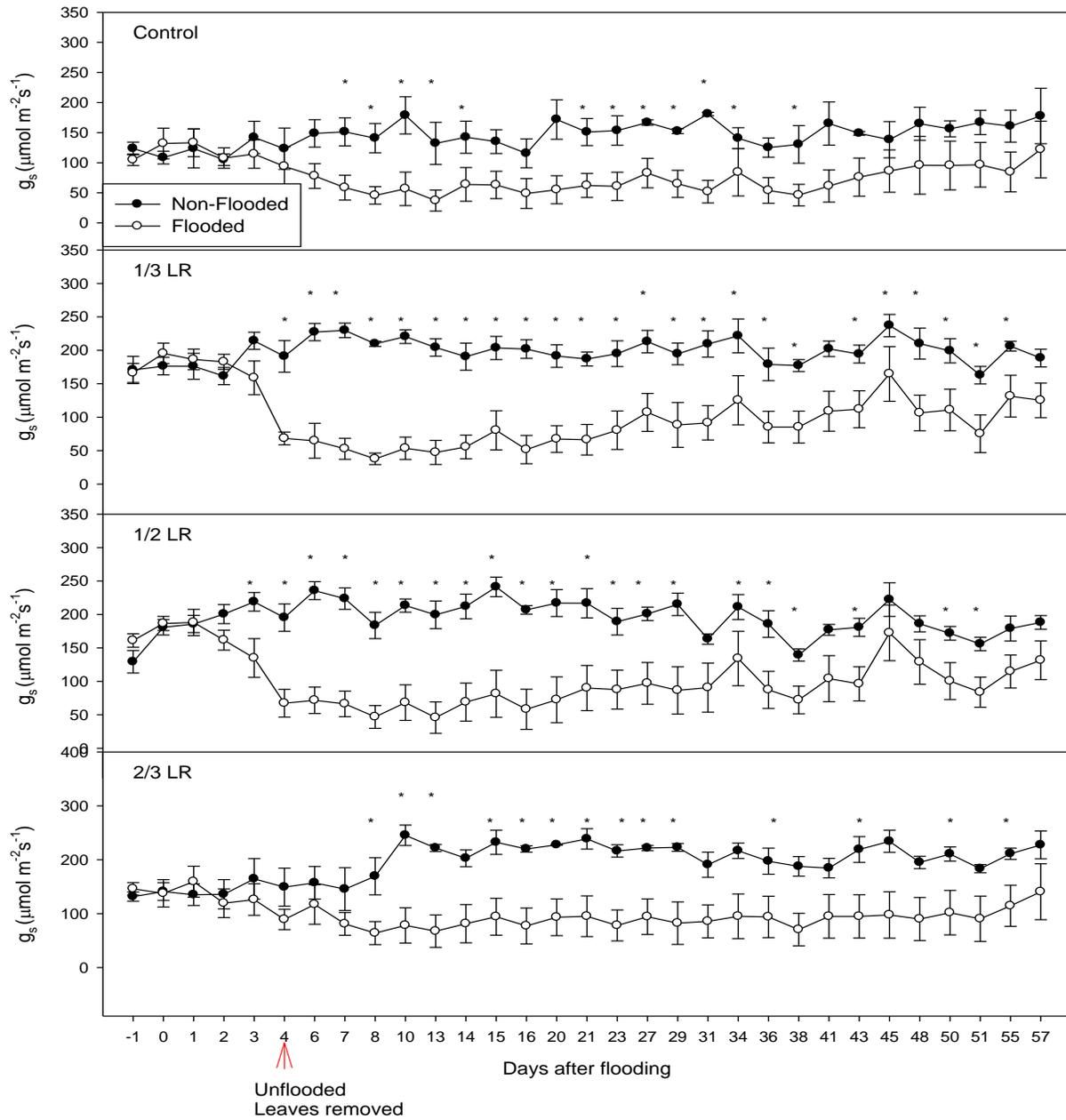


Figure 4-5. Stomatal conductance of water vapor (g_s) of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 2). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed after flooding. An asterisk indicates a significant difference between flooding treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

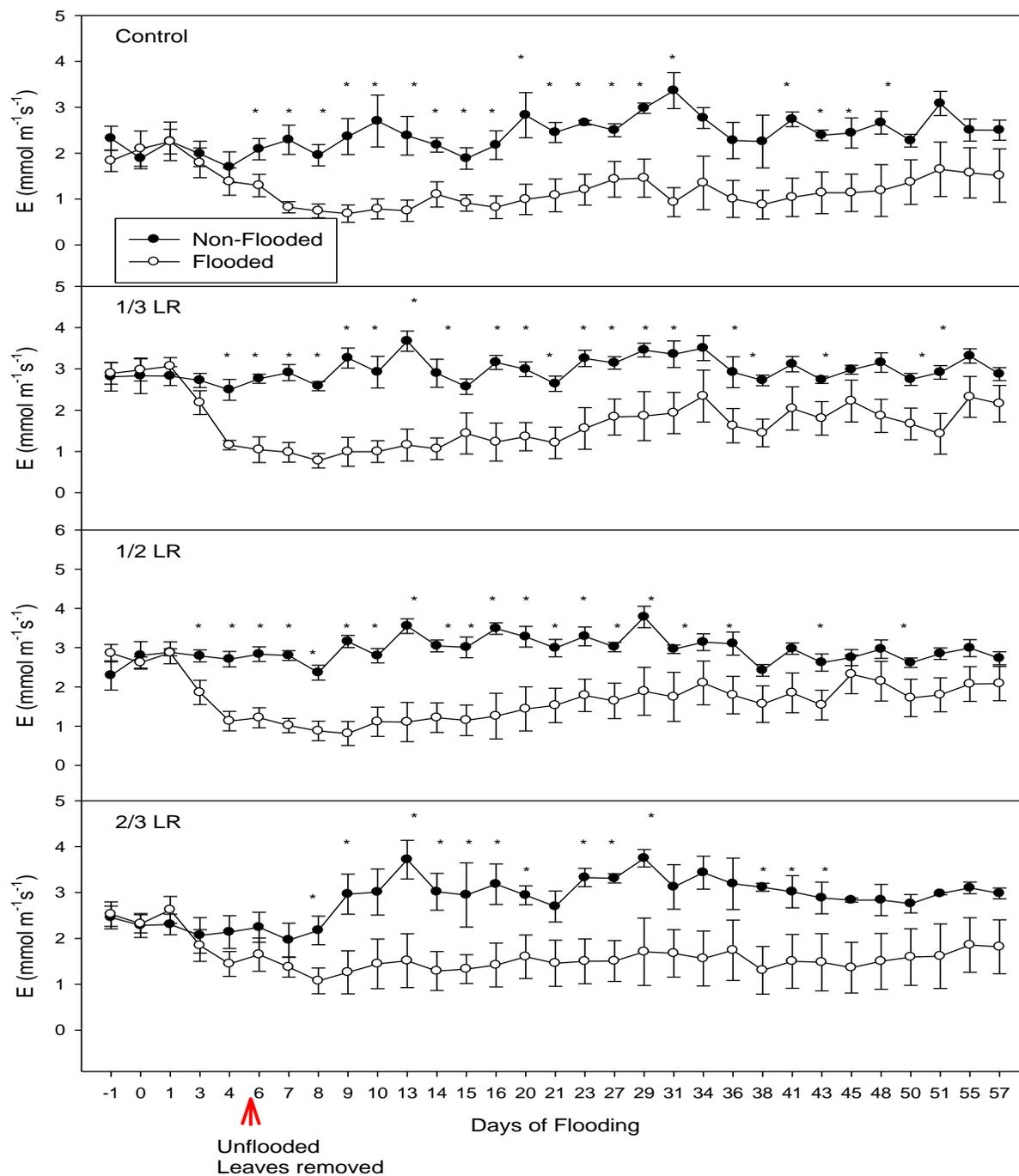


Figure 4-6. Transpiration (E) of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of 4 leaf removal treatments (Expt. 2). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed after flooding. An asterisk indicates a significant difference between flooding treatments according to a repeated measures ANOVA ($P \leq 0.05$), $n = 6$.

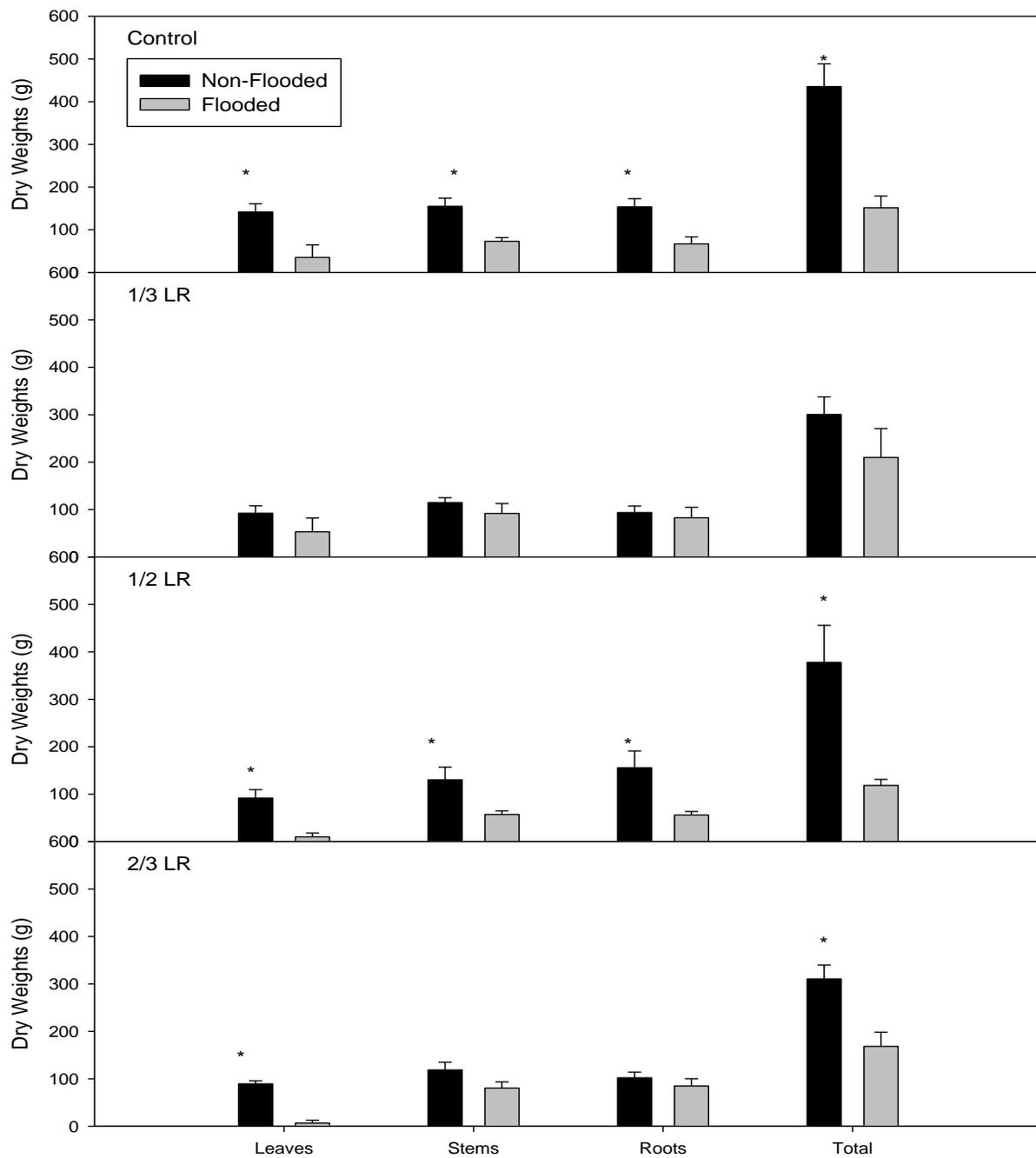


Figure 4-7. Root, leaf, stem, and total plant dry weights of flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 1). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3 LR) of the leaves removed prior to flooding. There were no significant differences between flooding treatments for any dry weight according to a T-test ($P > 0.05$), $n = 6$.

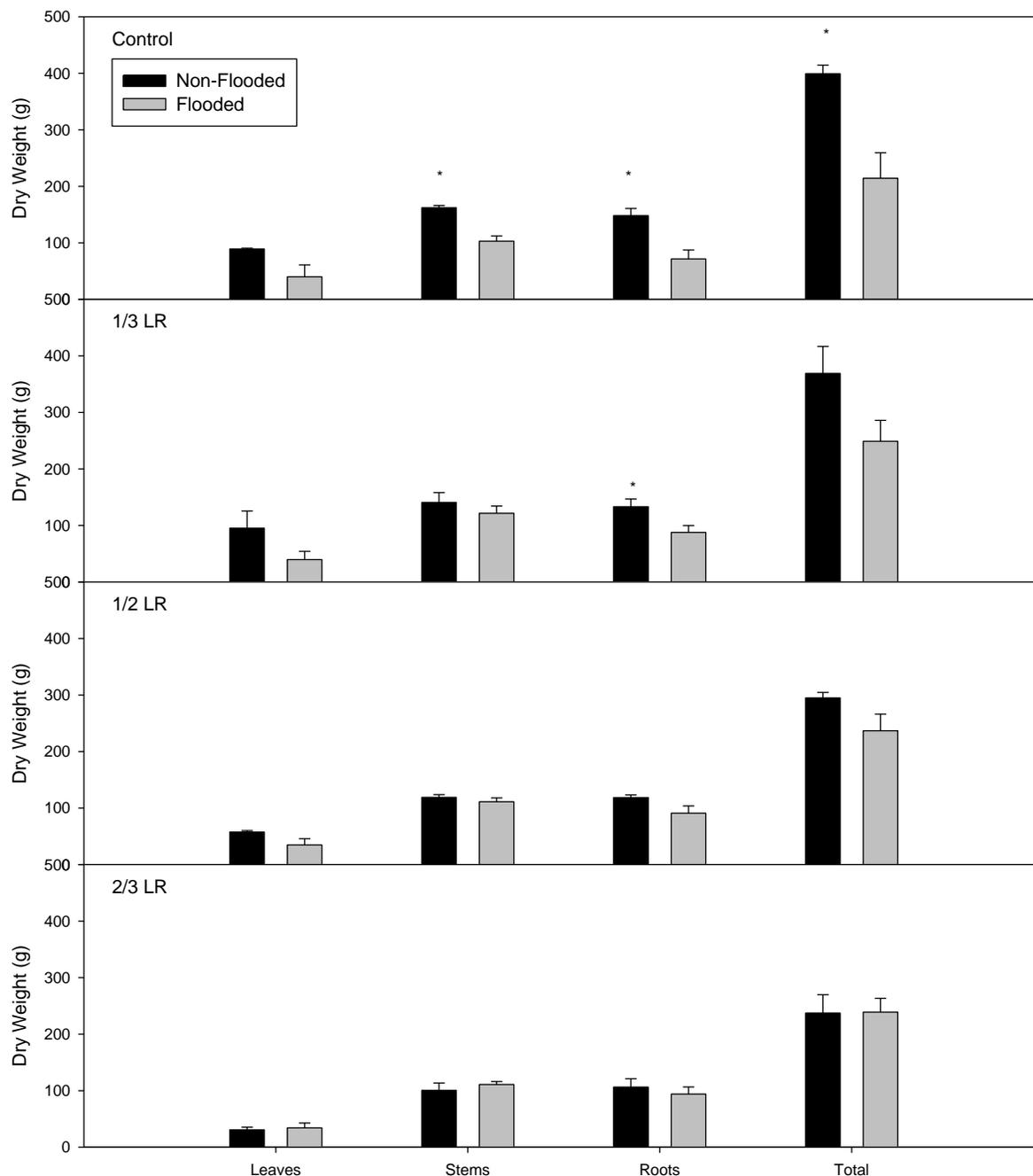


Figure 4-8. Root, leaf, stem, and total plant dry weight of flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each of four leaf removal treatments (Expt. 2). Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed after flooding. An asterisk indicates a significant difference between flooding treatments according to a T-test ($P \leq 0.05$), $n = 6$.

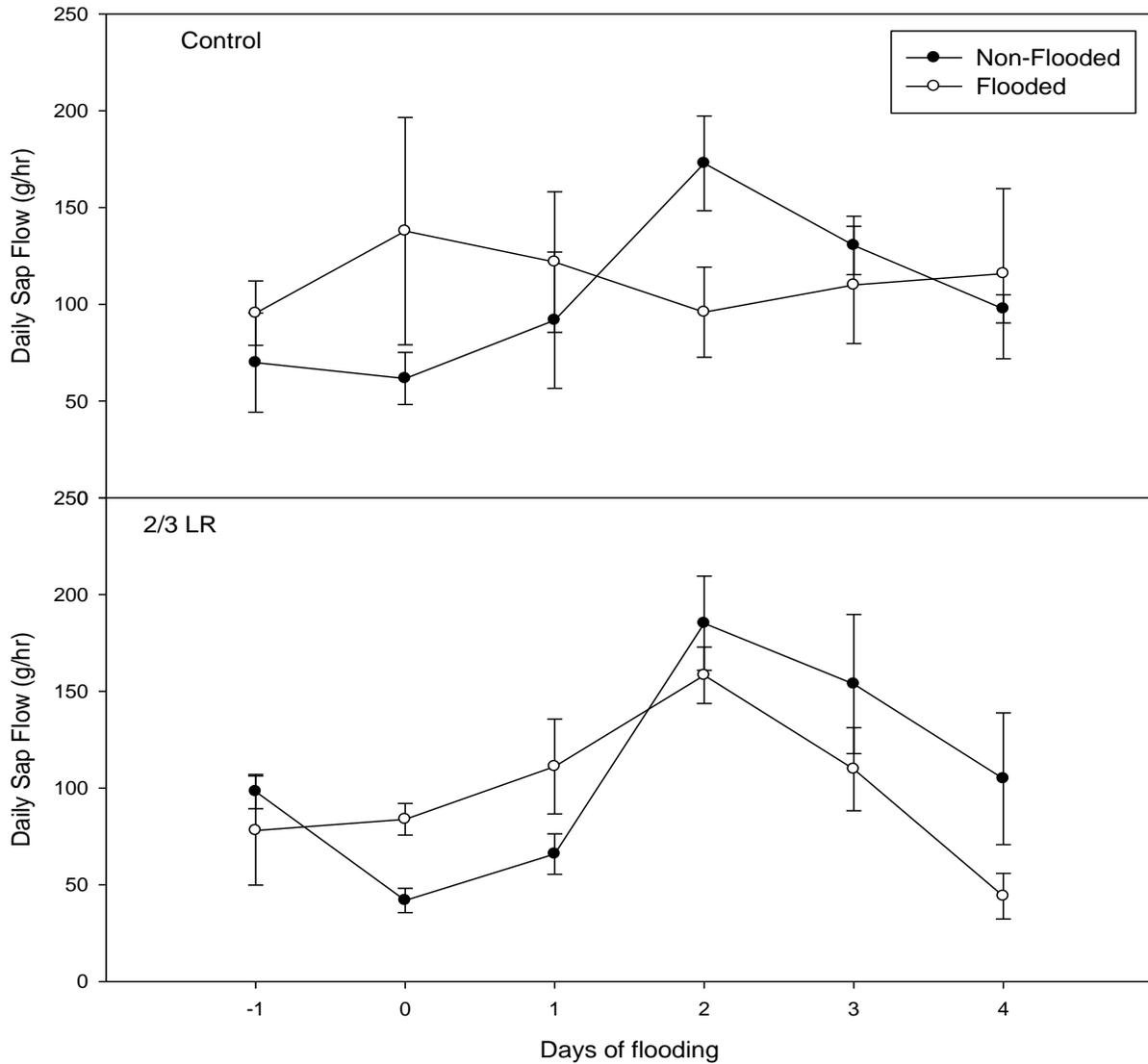


Figure 4-9. Daily sap flow of flooded and non-flooded trees of 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock (Expt. 2). Leaf removal treatments were: no leaves removed (control) and two thirds of the leaves removed (2/3LR) after flooding. There were no significant differences between flooding treatments on any measurement date ($P > 0.05$), $n = 3$.

Table 4-1. Number and percentage of tree mortality for flooded and non-flooded 3-year-old 'Choquette' avocado trees on Waldin seedling rootstock within each leaf removal treatment (Expt.1). Plants were harvested 52 days after plants were unflooded. Leaf removal treatments were: no leaves removed (control), one-third (1/3LR), one-half (1/2LR) or two-thirds (2/3LR) of the leaves removed (2/3LR) before plants were flooded.

Treatment		Control	1/3LR	1/2LR	2/3LR
Flooded	No. of plants alive	2	3	2	1
	No. of dead plants	4	3	4	5
	Mortality (%)	66.6	50	66.6	83.3
Non-flooded	No. of plants alive	6	6	6	6
	No. of dead plants	0	0	0	0
	Mortality (%)	0	0	0	0

Table 4-2. Number and percentage of tree mortality for flooded and non-flooded 2-year-old 'Simmonds' avocado trees on Waldin seedling rootstock in each leaf removal treatment (Expt.2). Plants were harvested 52 days after flooding treatments were initiated (5 days after plants were unflooded). Leaf removal treatments were: one-third of the leaves removed (1/3LR), half of the leaves removed (1/2LR), and two thirds of the leaves removed (2/3LR) after plants were flooded.

Treatment		Control	1/3LR	1/2LR	2/3LR
Flooded	No. of plants alive	4	5	5	4
	No. of dead plants	2	1	1	2
	Mortality (%)	33.3	16.6	16.6	33.3
Non-flooded	No. of plants alive	6	6	6	6
	No. of dead plants	0	0	0	0
	Mortality (%)	0	0	0	0

CHAPTER 5 CONCLUSIONS

In several areas of the world where avocados are commercially produced, including southern Florida, trees can experience periodic flooding of the root zone due to poor soil drainage, or heavy rains from tropical storms or hurricanes. The rapid depletion of oxygen in the root zone as a result of flooding can be injurious or even lethal to plants. Pruning the canopy has been recommended as a method to rehabilitate injured avocado trees after flooding. Since roots are damaged by flooding, reducing transpiration by canopy pruning presumably offsets the reduced water uptake by damaged roots and thus alleviates water stress. Pruning trees prior to flooding (in anticipation of a predicted storm or hurricane or after harvest during hurricane season) may actually exacerbate flooding stress by reducing carbohydrate concentration from reduced photosynthesis (as a result of less leaf area), thereby reducing the substrate for root respiration. Consequently, reduced respiration in the roots of pruned trees during flooding may result in increased flooding stress compared to non-pruned, flooded trees.

The objectives of this research were: 1. to determine if limiting net CO₂ assimilation by leaf removal or foliar application of a chemical photosynthetic inhibitor prior to flooding exacerbates plant stress and delays or prevents recovery of avocado trees from short-term (a few days) flooding; 2. to determine the combined effects of leaf removal prior to flooding and root zone hypoxia on root carbohydrate concentrations, particularly C₇ sugars; and 3. to determine the relationship between the timing (pre- or post-flooding) and quantity of leaf removal on stress and survival of avocado trees exposed to short-term flooding.

After a few days of flooding, in all experiments, soil redox potential measurements indicated that the rhizosphere of flooded plants was hypoxic. Decreased net CO₂ assimilation and stomatal conductance were observed for flooded plants in all experiments. This is consistent with previous studies showing that avocado is a flood-sensitive species which exhibits reductions in leaf gas exchange and shoot and root growth shortly after plants are flooded (Schaffer et al., 1992). Leaf removal immediately (1-day) or foliar application of the chemical photosynthetic inhibitor, Freeway[®], immediately (1-day) before and when roots were flooded, increased the negative impact of flooding on net CO₂ assimilation, stomatal conductance, organ and whole plant dry weights and tree survival. This was presumably due to a reduction of carbohydrate reserves for root respiration during the flooding period.

Unlike other fruit crops, D-mannoheptulose and a related C₇ sugar alcohol, perseitol, represent major forms of nonstructural carbohydrates in avocado (Liu et al., 1999). The nonstructural carbohydrates found in other tree fruit species are based on a six-carbon hexose skeleton, such as glucose, sucrose and even starch. While these six-carbon sugars are found also found in avocado trees, they are present in much lower concentrations than the C₇ sugars in avocado tissues (Liu et al., 1999). Flooded plants with no leaves removed prior to flooding had higher D-mannoheptulose and perseitol concentrations than plants with leaves removed prior to flooding. Reduction of carbohydrate reserves by leaf removal resulted in carbohydrate deficiency in root tissues and thus less carbohydrate in the roots for use as a substrate for respiration. These C₇ sugars have also been shown to have antioxidant properties in avocado fruit (Bertling et al., 2007). Although studies have shown that root respiration is reduced in

flooded plants (Liao and Lin 2001; Geigenberger, 2003; Serres and Voeselek, 2008), significant differences in root respiration of avocado trees between flooding or leaf removal treatments could not be detected in the present study. However, flooded plants with no leaves removed prior to flooding tended to have higher root respiration than flooded plants with leaves removed.

Based on leaf gas exchange, root respiration, carbohydrate concentration, plant growth, and plant survival, inhibition of photosynthesis by leaf removal or the application of Freeway[®] 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 (although antioxidant activity was not measured in this study) apparently resulted in plants with leaves removed prior to flooding, being more susceptible to flooding stress than plants with their canopies left intact. These observations provide evidence that reducing photosynthesis by leaf removal before flooding can increase flooding stress and decrease plant survival. However, additional studies are necessary to quantify the effect of prolonged flooding on root respiration and plant biomass of plants with photosynthesis inhibited, either by leaf removal or with a chemical photosynthetic inhibitor, before flooding.

In separate experiments, plants were subjected to varying amounts of leaf removal [0 (control), one-third (1/3LR), half (1/2LR), and two-thirds (2/3LR)] immediately before or after flooding. Similarl to previous experiments, removal of leaves immediately before flooding resulted in severe stress and significantly lower net CO₂ assimilation and stomatal conductance in flooded than in non-flooded trees. Tree mortality of plants in the 2/3LR treatment was 83.3%, whereas tree mortality in 1/3LR treatment was 50%

and in the control and 1/2LR treatments was 66.6%. Plants in the 1/3LR treatment were the only ones that recovered well after flooding, as indicated by higher survival and higher organ dry weights than in any other leaf removal treatment. The reasons for high tree mortality in the control treatment remain unclear. Although no leaves were removed from control plants and sufficient quantities of carbohydrates may have been available for respiration, these plants had higher transpirational surface area than plants with leaves removed. Roots damaged by flooding may have been unable to supply enough water to a large canopy, thus preventing recovery and increasing tree mortality.

In contrast to leaf removal before flooding, leaf removal after flooding significantly reduced net CO₂ assimilation and transpiration of flooded plants. However, plants with leaves removed after flooding recovered faster than control plants as indicated by greater organ dry weights and tree survival than for plants with leaves removed prior to flooding. Tree survival in both the 1/3LR and 1/2LR treatments was 83% and for both the control and 2/3LR treatments was 66.6%. Plants in the 2/3LR treatment recovered faster than any other leaf removal level presumably due to equilibrium between the shoot and root volume. Similar results were observed by Gil et al. (2008), who observed that 1-year-old 'Choquette' avocado trees in containers recovered faster if trees were pruned after flooding compared to non-pruned trees. This was presumably due to reduced transpirational demand as a result of a reduction in canopy volume of pruned trees that had the root system partially damaged from flooding.

This research provides evidence that reducing photosynthesis and carbohydrate reserves by removing leaves or the use of a chemical photosynthetic inhibitor before flooding, increases the flooding stress and decreases survival of avocado trees. In

contrast to removing leaves prior to flooding, removing leaves from avocado trees immediately after a flooding event appears to reduce flooding stress and increases tree survival, presumably by reducing transpiration demand on a root system that has been damaged by flooding. Of the percentages of leaf removal tested, removing two-thirds of the canopy immediately after flooding was the was the most effective amount of leaf removal to reduce tree stress and help trees recover from and survive flooding. Further studies are necessary to quantify the effect of prolonged flooding on root respiration and biomass of plants with leaves removed prior to flooding.

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BIOGRAPHICAL SKETCH

Maria Angélica Sanclemente Galindo was born in Cali, Colombia in 1983. After graduating from high school in 1999, she attended the Universidad del Valle to study biology. After taking her first botany class during the second year of her undergraduate program, Angélica became interested in plants. In her second year as an undergraduate, she worked as a student assistant for the basic botany and plant physiology classes offered in her department. Additionally, she worked in the herbarium of the university for one year.

Upon graduation in 2005, she moved to St. Petersburg, Florida with her parents. After taking English classes for six months, she started working at the University of South Florida in St. Petersburg. There, she worked as a research assistant in the Department of Internal Medicine, doing research in diabetes, aging, and obesity. During her time there, she enjoyed working with non-human primates.

In January 2010, she decided to go back to plants and started her Master of Science degree in horticultural science at the University of Florida under the mentorship of Dr. Bruce Schaffer. Her master's degree thesis focused on the physiological responses of flooded avocado trees to leaf removal. Upon completing her MS degree, Angélica will pursue a PhD degree focusing on plant physiology. After her graduate studies, she plans to pursue a career in academia.