PHYSIOLOGICAL AND GROWTH RESPONSES OF MAMEY SAPOTE (*POUTERIA SAPOTA*) TO FLOODING

By

MARK THOMAS NICKUM

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

© 2009 Mark Thomas Nickum

To all of my family: Those who have passed, those who are here,
and those who are still to be

ACKNOWLEDGMENTS

I would like to thank the Tropical Research and Education Center (TREC) for its contribution to my studies. In particular, my committee chair, advisor, and mentor, Jonathan Crane, has offered me invaluable encouragement and research assistance, and has given me a superior view of the tropical fruit industry in all its facets. This time and experience in South Florida will bear greatly wherever my future endeavors take me. Great thanks to Bruce Schaffer for his research and editorial experience throughout this dissertation. My work is certainly all the better due to his input and academic rigor. The University of Florida Alumni Fellowship was the primary source of support, funding, and tuition for my graduate studies at the University of Florida, without which this dissertation would not have been possible. Thank you to Yuncong Li and his laboratory technicians for their support, aid, and guidance for much of the laboratory work contained in this dissertation. Their cheerful accommodation in the midst of their own busy work days made all the difference. Also thank you to Fred Davies and Pete Andersen for their reviews and guidance throughout the writing of this work.

On a personal note, I would like to honor the memory of my mother and father, who throughout my life always wanted me to follow my dreams and become the best person I could be. Integrity was always the utmost value in each of their lives, and I hope I am living up to the example they both set for me. I would also like to thank my Grandma Dolly (Marguerite Eipers). Her unconditional love is a continued source of inspiration and motivation for me in everything I do, everywhere I go.

TABLE OF CONTENTS

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

PHYSIOLOGICAL AND GROWTH RESPONSES
OF MAMEY SAPOTE (*POUTERIA SAPOTA*) TO FLOODING

By

Mark Thomas Nickum

May 2009

Chair: Jonathan H. Crane
Cochair: Bruce A. Schaffer
Major: Horticultural Sciences

Physiology and growth responses of mamey sapote (*Pouteria sapota*) trees to low oxygen in the root zone were examined. For trees in containers, stomatal conductance and net CO_2 assimilation decreased within 3 d of flooding, leaf epinasty occurred between days 5 to 10, leaf senescence and abscission occurred between days 15 to 30, branch dieback and tree mortality occurred between days 30 to 60. Three cycles of 3-d flooding and 3-d recovery in containers had little effect on leaf gas exchange of 'Magaña' trees. 'Pantin' trees tolerated 3 cycles of 6-d flooding interspersed with 3 to 6 d of recovery despite consistent declines in stomatal conductance and net CO_2 assimilation during flooding. In the field, non-root rot infested mamey sapote trees exhibited good tolerance to flooding during fall-winter and less tolerance during the warmer spring-summer period in which tree decline and death occurred, if coupled with root rot. Physiological responses and survival of *Pouteria sapota* trees were assessed in response to three different oxygen concentrations in the root zone, including an aerated hydroponic treatment (7-8 mg $O_2 \cdot L^{-1}$ H_2O), an O_2-purged hydroponic treatment (0-1 mg $O_2 \cdot L^{-1}$ H_2O), and an aeroponic treatment (~150 mg $O_2 \cdot L^{-1}$ air). Roots in the O_2-purged hydroponic treatment evolved

significantly higher levels of CO_2, developed a glycolysis rate 5 to 10 times higher, and produced levels of ATP similar to those in the aerated hydroponic treatment. Although root alcohol dehydrogenase (ADH) activity was detected in all treatments, there were no observable trends of ADH up-regulation or down-regulation common to all trials or treatments. Development of hypertrophic stem lenticels appeared to be a response to high moisture levels rather than lack of oxygen in the root zone because they developed on all of trees in the aeroponic treatment, some trees in the aerated hydroponic treatment and fewer trees in the O_2-purged hydroponic treatment. Alcohol dehydrogenase activity alone was not sufficient to ensure *P. sapota* survival when oxygen concentrations in the root zone were low, but other leaf responses and morphological developments may be necessary for long term survival in flooded soil.

CHAPTER 1
INTRODUCTION

Flooded conditions may occur in many areas where subtropical and tropical fruit trees are grown (Kozlowski, 1997; Schaffer, 1998; Schaffer and Andersen, 1994; Schaffer et al., 2006) and can lead to large economic losses (Crane et al., 1997). In Miami-Dade County, examples of agricultural losses due to flooding include $77 million and nearly 7,700 hectares of vegetable crops in October 1999 due to hurricane Irene, and $13 million in December 2000 after 35 cm of rainfall (Schaffer and Muñz-Carpena, 2002). Mamey sapote is reported to be intolerant of flooded conditions and has been observed to decline or die under excessive soil moisture or flooded conditions (Morton, 1987). However, these observations were made after tropical storms which were usually accompanied by strong winds confounding the effects of flooding and wind stress on mamey sapote survival. There have been no investigations to quantify this or to determine the mechanisms of intolerance and if cultivars differ in flood tolerance. For the tropical fruit tree species mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn], environmental stress may be of particular concern because of the long period before fruit production begins, (i.e., 3 to 4 years for grafted trees and 10 or more year for seedlings) and because fruit development takes anywhere from 10 to 24 months from flowering to maturity (Balerdi et al., 2005). Knowledge of responses of *P. sapota* to low soil oxygen conditions and flooding may determine the potential inherent flood tolerance in the species that could be used in rootstock selection and breeding new cultivars. Furthermore, understanding the effect of flooding on mamey sapote may suggest cultural practices to ameliorate the negative impacts of flooding on this tree species.

There has been a considerable amount of research on the effects of flooding on subtropical and tropical fruit trees (Schaffer and Andersen, 1994; Schaffer et al., 2006). These studies have

focused on banana (*Musa* spp. L.) (Turner, 1994), avocado (*Persea americana* Miller) (Ploetz and Schaffer, 1987; 1989; Whiley and Schaffer, 1994), *Annona* spp. (Nuñez-Elisea et al., 1998), mango (*Mangifera indica* L.) (Larson et al., 1991a; 1991c), and carambola (*Averrhoa carambola* L.) (Joyner and Schaffer, 1989). In the calcareous Krome soil of local agricultural areas in Miami-Dade County, avocado trees can survive up to 30 d of flooding when not infested with root-rot (*Phytophthora cinnamomi* Rands) (Ploetz and Schaffer, 1987; 1989). Also in the same calcareous Krome soils, seedlings of the flood sensitive *Annona* species bullock's heart (*A. reticulata* L.) and sugar apple (*A. squamosa* L.) survived between 30 to 50 d of flooding (Nuñez-Elisea et al., 1998), mango survived over 110 d of continuous flooding (Larson, 1991; Schaffer et al., 2006), and carambola survived over 126 d of continuous flooding (Joyner and Schaffer, 1989). To the author's knowledge, there are no published reports on the effects of flooding on the physiology and growth of mamey sapote trees.

Research examining flood-stress physiology of mamey sapote is warranted due to the potential for flooding in many areas where the crop is grown and the lack of information about physiological and growth responses of this species to low soil oxygen conditions. Flooding responses of crops can vary by species, cultivar, and soil type; therefore, experiments were conducted with the two main south Florida cultivars, Pantin and Magaña. The growth habit for 'Pantin' is upright and vigorous, and the growth habit for 'Magaña' is more spreading and slower growing than 'Pantin' (Campbell and Lara, 1982).

Container-grown plants in an open-air screenhouse were used to examine continuous and cyclic flooding. Field planting experiments were conducted to examine continuous flooding in the field. The goals of these experiments were to determine mamey sapote's basic physiological responses to flooded conditions, the time-line of physiological responses, and how long mamey

sapote trees survive under anoxic soil conditions. Leaf gas exchange, leaf and stem water potential, and leaf chlorophyll index were monitored. Plant decline and visible responses such as leaf epinasty, chlorosis, and abscission, hypertrophied stem lenticel development, and plant death were recorded. These experiments were conducted using crushed Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) (Burns et al., 1965; Colburn and Goldweber, 1961; Leighty and Henderson, 1958; Nobel et al., 1996) which is the major agricultural soil type in the flood-prone tropical fruit production area of southern Florida.

Further experiments were conducted to examine root physiology during flooding. These experiments examined the relationships among leaf gas exchange, root electrolyte leakage, root respiration and glycolysis rates, and root alcohol dehydrogenase enzyme activity. Plants were maintained in soil media until the time of the experiments, when excess soil media was removed and the root zones were placed under hydroponic and aeroponic conditions to approximate normoxic, hypoxic, and anoxic soil conditions. The goals of these experiments were to determine: 1) the physiological response of mamey sapote roots when exposed to hypoxic and anoxic conditions; 2) if mamey sapote roots respond to flooding by upregulating alcohol dehydrogenase enzyme activity; 3) the levels of root tissue alcohol dehydrogenase activity in mamey sapote; and 4) the glycolysis rates of roots under aerobic and anaerobic conditions.

CHAPTER 2
LITERATURE REVIEW

Botany and Production of Mamey Sapote

Mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn] is a commercially grown tropical fruit crop popular throughout Latin America and the Caribbean (J.H. Crane and C.F. Balerdi, University of Florida, personal communication). Mexico is the largest producer with about 1,416 ha, worth an estimated $4 million with most production in the tropical states of Chiapas, Guerrero, Tabasco, Oaxaca and Yucatán (Otero-Sánchez et al., 2008; SAGARPA, 2008). Commercial mamey sapote orchards also exist in Guatemala, Nicaragua, Costa Rica, Cuba (at least before 1959), Ecuador, Puerto Rico, the Dominican Republic, and Florida (Balerdi and Shaw, 1998). Mamey sapote was introduced into Florida during the mid-1800s (Reasoner, 1887), and by the 1980's was grown on a commercial scale (Degner et al., 2002). As of 2009, mamey sapote is estimated to be grown commercially in southern Florida on 233 ha (575 acres) and is annually worth an estimated $7.5 million at the farm level, and about $18.5 million at the wholesale level (E. Evans, University of Florida, personal communication). The tree produces 45 to 113 kg (100 to 250 pounds) of fruit per tree with 173 to 271 trees per ha (70 to 110 trees per acre). The average yield per acre in south Florida is between 11.2 MT to 30.3 MT per ha (10,000 to 27,000 pounds per acre) with harvest predominantly from May to August, with some year-round production (Balerdi et al., 2005).

The center of origin for mamey sapote is the humid lowlands of southern Mexico extending south through portions of Central America to northern Nicaragua, where it was originally cultivated by the Mayan civilization (Balerdi and Shaw, 1998; Verheij and Coronel, 1992). Ecologically, mamey sapote does best in hot, humid climates with a relatively even rainfall distribution. The species is seldom planted above 1500 m (Balerdi and Shaw, 1998;

Bayuelo-Jimenez and Ochoa, 2006). Mature trees can survive light frost and may abscise leaves during cold periods. Mamey sapote is reported to be intolerant of flooded soil conditions (FAO-AGL, 2007; Verheij and Coronel, 1992).

Related tropical fruit species include the canistel or egg fruit (*P. campechiana* Baehni), green sapote (*P. viridis* Crong.), abiu (*P. caimito* Radlk.), lucumo (*P. lucuma* O. Ktze.), caimito (*Chrysophyllum cainito* L.), and sapodilla (*Manilkara sapota* L.). These crops are all in the Sapotaceae and are native to and most well known in Mexico and Central America. Worldwide distribution of these Sapotaceous fruit has been relatively slow due to the short storage life of their seeds. However spread of these crops has reached the Caribbean, South America, Florida, Puerto Rico, Dominican Republic, and as far away as Hawaii, India, Australia, the Philippines, Vietnam, China, Taiwan, Japan, Spain, and Israel. In some of these regions, only a few trees are represented (Balerdi and Shaw, 1998).

Climate and Soils of South Florida

South Florida has a warm subtropical climate, high humidity, and a rainy season from May to as late as November in which 70% of the annual rainfall occurs, amounting to at least 1,270 mm of rain annually (Black, 1993). The rainy season also coincides with a hurricane season which lasts from June 1 to November 30 (City of Homestead, 2008; NOAA-AOML, 2008).

Flooding in Miami-Dade County is a significant problem for agriculture. Over nearly 150 years from 1859 to 2006 there were approximately 108 significant storms including tropical depressions, tropical storms, and hurricanes within 120 km (65 nautical miles) of Miami-Dade County (NOAA, 2007). Flood damage to agricultural crops in Miami-Dade County as a result of these storms can be quite extensive. For example, Tropical Storm Gordon struck Miami-Dade County on 12-17 November, 1994. Flooding from that storm was estimated by the USDA Farm Services Agency to cover 526 ha (1300 acres) out of 5,308 ha (13,116 acres) of tropical fruit

orchards in production (~10%) (Crane et al., 1997). Of the 132 ha (325 acres) of mamey sapote, 54 ha (136 acres) were flooded, and this was approximately 40% of the mamey sapote produced in Miami-Dade County at the time of the storm. Other flood related storms have included Hurricane Dennis in August 1981, Hurricane George in September 1998, Hurricane Irene in October 1999 and the 'No Name Storm' in October 2000 (NWS-NHC, 2008).

Nearly 12% of the world's agricultural soils are calcareous (FAO-AGL, 2007) and in southern Florida, the major soil type for subtropical and tropical fruit crops is a calcareous soil with a high pH due to large amounts of calcium carbonate. This soil is classified as a Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) consisting of only a few centimeters of loose soil above a hard, water permeable, oolitic limestone bedrock (Burns et al., 1965; Leighty and Henderson, 1958; Nobel et al., 1996). In order to use this soil for fruit production the bedrock must be prepared by crushing. This is accomplished by the use of a bulldozer with a scarifying plow which crushes the limestone bedrock to a plow layer depth of 15 to 20 cm. For tree crops, the orchard floor is then often trenched to 45 to 60 cm below the plow layer in parallel lines corresponding to the row and tree spacing. Each tree is planted at the intersection points of the trenches (Burns et al., 1965; Colburn and Goldweber, 1961; Li, 2001). The deeper tree roots are thus able to develop within the trenches, as well as have surface roots form within the plow layer.

The agricultural area of south Florida is highly susceptible to flooding because of the relatively low elevations, i.e., <7.6 m above sea level, and high water table (Li, 2001). Flood conditions may exist during the rainy summer and fall months when periods of heavy rainfall and/or hurricanes occur. Flooding of mamey sapote orchards in this area has generally resulted in tree decline and death (Crane et al., 1997; Degner et al,. 2002).

Oxygen Content in the Rhizosphere

Waterlogging nearly eliminates gas filled pore spaces in soils creating hypoxic or anaerobic soil conditions. Molecular diffusion rates for oxygen and carbon dioxide is 10,000 times slower in water than in air and oxygen levels in the soil are depleted by microorganisms and roots within a few hours (Grable, 1966; Ponnamperuma, 1984; Slowik et al., 1979; Stolzy et al., 1967). Oxygen diffusion rates (ODR) of 0.19 $\mu g \cdot cm^{-2} \cdot min^{-1}$ or lower frequently result in root decay for avocado (Stolzy et al., 1967) and low soil oxygen levels in the roots can lead to significant declines in root dry weight in young avocado plants (Slowik et al., 1979). Low soil oxygen levels can also lead to significantly reduced leaf concentrations of N, P, K, Ca, Mg, Zn, Mn, and Cu and significantly increased Fe concentrations (Slowik et al., 1979).

Soil redox potential (Eh) is a method of indirectly quantifying the oxygen content in the soil. This is important since soils that are only periodically flooded may have a wider range of Eh (-300 mV to +700 mV) than aerated (Eh > +400 mV) or permanently saturated soils (Eh < +350) (Kozlowski, 1997; Pezeshki, 2001). A lower Eh indicates a more hypoxic condition. Larson et al. (1991b) examined the flood-induced chemical transformation of two common soils in south Miami-Dade County, Krome and Chekika very gravelly loam soils. Their results showed a drop from normoxic conditions before waterlogging, to between -100 to -300 mv within 3 d after waterlogging, followed by a stable redox potential of -165 mv achieved after 21 d of saturated conditions for both soils. Low Eh levels such as these indicate a reduced soil respiration rate and depletion of oxidizable organic matter and electron acceptors (Larson et al., 1991b).

Root Responses to Flooding

Basic root morphology and physiology have been studied for many temperate tree species under flood stress (Kozlowski, 1997) and a few tropical tree species (De Simone et al., 2002)

including mango (Larson et al., 1991a) and *Annona* species (Nuñez-Elisea et al., 1998). Root responses to flooding may vary. The predominant challenges to roots include hypoxia, anoxia, and the build up of toxins produced by soil microbes and/or plants (Kozlowski, 1997). Low oxygen conditions cause changes in root metabolism (Drew, 1997), membrane permeability (Crane and Davies, 1987; Islam et al., 2003; Kolb et al., 2002; Kozlowski and Pallardy, 2002; Ojeda et al., 2004), root aquaporin activity (Javot and Maurel, 2002; Luu and Maurel, 2005), and halts the growth of the main root system (Poot and Lambers, 2003). Root mortality also occurs (Kozlowski and Pallardy, 2002). These changes may reduce the amount of water uptake for physiological processes and transpiration.

Shift from aerobic to anaerobic respiration. Under normal oxygen conditions, cells respire aerobically beginning with sucrose (a 12 carbon sugar), following through glycolysis in the cytoplasm to pyruvate (3 carbon) (Fig. 2-1). From the cytoplasm, pyruvate moves into the mitochondria, where the citric acid cycle and oxidative phosphorylation produce adenosine triphosphate (ATP) for energy, and regenerate nicotinamide dinucleotide (NAD^+) by oxidizing NADH via the electron transport chain (Equation 2-1) (Bailey-Serres and Voesenek, 2008; Brand, 1994; Gibbs and Greenway, 2003; Taiz and Zeiger, 2002).

$$NADH + H^+ + \tfrac{1}{2} O_2 \rightarrow NAD^+ + H_2O \qquad (2\text{-}1)$$

Without oxygen, the NAD^+ cannot be regenerated via the electron transport chain and without NAD^+ regeneration, glycolysis is severely reduced or stopped. Under these conditions, plants shift from aerobic respiration to either lactic acid fermentation or ethanol fermentation which regenerates NAD^+ (Fig. 2-1) (Bailey-Serres and Voesenek, 2008; Hole et al., 1992; Taiz and Zeiger, 2002).

This shift from aerobic to anaerobic respiration may take place within a few hours. An anoxic cell generally first undergoes lactic acid fermentation via lactate dehydrogenase (LDH), and then ethanol fermentation via acetaldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH). As lactic acid fermentation occurs, there is a build up of lactic acid which lowers the pH of the cell generally from 7.5 to 6.8 (in maize roots for example) (Roberts et al., 1989). Alcohol dehydrogenase (ADH) functions at a relatively lower pH optimum than LDH, thus ADH is promoted. In ADH-deficient roots, LDH will continue to produce lactic acid, causing cytosolic acidification and cell death, often referred to as cytoplasmic acidosis (Bailey-Serres and Voesenek, 2008).

Anaerobic stress may result in adverse effects on the root cell membrane such as disruption of solute and water movement. Root electrolyte leakage is a measure commonly made to determine the extent of damage to roots under anaerobic stress (Kozlowski, 1984). Increased electrolyte leakage may indicate an increased permeability of the root cell membrane due to anaerobic stress and cytoplasmic acidosis (Crane and Davies, 1987; Islam et al., 2003; Kolb et al., 2002; Kozlowski, 1984; Kozlowski and Pallardy, 2002; Ojeda et al., 2004). Root membrane lipids of flood-sensitive species can be hydrolyzed when roots are under anoxic conditions (Kolb et al., 2002).

Rates of glycolysis and ATP generation. This shift from the aerobic respiration processes of the citric acid cycle and oxidative phosphorylation which take place in the mitochondria to the anaerobic fermentation pathways which take place in the cytoplasm significantly reduces the level of adenosine triphosphate (ATP) generated for cell metabolism (Bailey-Serres and Voesenek, 2008; Gibbs and Greenway, 2003; Taiz and Zeiger, 2002). In response to this loss in ATP generation, the rate of glycolysis may significantly increase, a

condition which is termed the Pasteur effect (Gibbs and Greenway, 2003; Schaffer et al., 1992; Taiz and Zeiger, 2002). In aerobic respiration, one sucrose molecule (12 carbon sugar) will yield 10 ATP during the process of glycolysis, and the resulting four pyruvates will produce 50 ATP when fully processed through the citric acid cycle and oxidative phosphorylation in the mitochondria. Thus, aerobic respiration yields 60 ATP from one sucrose molecule, whereas anaerobic respiration only yields the initial 10 ATP from glycolysis (Fig. 2-1) (Brand, 1994; Taiz and Zeiger, 2002). Therefore energy yielded by anaerobic respiration is one-sixth that of aerobic respiration.

Other estimates of actual ATP energy produced from glycolysis via ethanolic fermentation begin with 1 mole of hexose (6 carbon sugar) and estimate 2-3 mol of ATP per mole hexose (Gibbs and Greenway, 2003; Hole et al., 1992). Whereas the estimate for aerobic respiration via oxidative phosphorylation yields 24-36 ATP. Thus, under anaerobic conditions, the anoxic cell would need to increase its rate of glycolysis 10 to 18 times in order to reach the same levels of energy as aerobic cells (Gibbs and Greenway, 2003; Hole et al., 1992).

A number of plant species and plant tissues which have been documented to exhibit high rates of glycolysis indicating a Pasteur effect during anoxia include carrot storage tissue, beetroot storage tissue, excised maize root tips, excised rice shoots, and excised rice coleoptiles (Gibbs and Greenway, 2003). Little information has been cited about the Pasteur effect for woody trees.

Studies of alcohol dehydrogenase. As previously mentioned, the ADH enzyme may be upregulated during periods of anaerobic respiration. Depending on the plant species, the *Adh* gene family is made up of one to four members (Preiszner et al., 2001). Many environmental stresses such as anoxia, heat, dehydration, and cold, as well as the hormone abscisic acid (ABA) are known to upregulate ADH activity (Preiszner et al., 2001). With most species, the

meristematic tissue in growing root tips is in a state of high metabolic activity, and it is normal for them to be somewhat oxygen deficient (Bailey-Serres and Voesenek, 2008; Gibbs and Greenway, 2003). Consequently, root tips are often a preferred tissue targeted for harvest when anaerobic enzymes such as ADH are sought.

Experiments commonly found in the literature tended to examine root physiology and the development of ADH upregulation utilizing *Arabidopsis thaliana* L., maize (*Zea mays* L.), rice (*Oryza sativa* L.), soybean (*Glycine max* L.), *Lepidium latifolium* L. and *Echinochloa* Pal. (Chen and Qualls, 2003; Chung and Ferl, 1999; Gibbs et al., 2000; Kato-Noguchi, 2000; Kimmerer, 1987; Morimoto and Yamasue, 2007; Preiszner et al. 2001; Rumpho and Kennedy, 1981; Russell et al., 1990), although mesic-adapted woody trees such as swamp tupelo [*Nyssa sylvatica* (Walt.) Sarg.] and *Melaleuca cajuputi* Powell have been investigated (Angelov et al., 1996; Yamanoshita et al., 2005). Most often in these investigations, seed is germinated in agar, Petri dishes, beakers, or other controlled conditions, with controlled oxygen and temperature levels, followed by examining the respiration and anaerobic peptide (enzyme) upregulation from seed to seedling during the course of seed germination and early plant development. This is an effective technique for studying the developmental responses and upregulation of anaerobic peptides such as lactate dehydrogenase (LDH), pyruvate decarboxylase (PDC), and alcohol dehydrogenase (ADH), for young plants. While neither the alcohol nor lactate fermentation pathways of anaerobic respiration produce ATP, they do regenerate NAD^+ which is necessary for the glycolytic pathway to continue when cells are deficient in oxygen.

Levels of root ADH activity have been determined for herbaceous plants and mesic-adapted tree species. Maize had a range of ADH activity between about 60 to 360 nmol $\cdot$ NADH $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$ (Kato-Noguchi, 2000), *Lepidium latifolium* with ADH activity from 150 to

500 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Chen and Qualls, 2003) and *Arabidopsis thaliana* with ADH activity levels between about 50 to 480 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Chung and Ferl, 1999).

Mesic-tolerant trees such as swamp tupelo (*Nyssa sylvatica* var. biflora) exhibited root ADH activity in nonflooded seedlings of about 100 to 125 nmol · NADH · min^{-1} · mg $protein^{-1}$, and seedlings flooded for up to 30 d exhibited activity of about 200 to 300 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Angelov et al., 1996). The flood-tolerant *Melaleuca cajuputi* seedlings exhibited nonflooded levels of about 500 to 900 nmol · NADH · min^{-1} · mg $protein^{-1}$, and flooded levels after 2 d up to 1700 nmol · NADH · min^{-1} · mg $protein^{-1}$, followed by a decline in activity to about 500 nmol · NADH · min^{-1} · mg $protein^{-1}$ by day 14 of flooding (Yamanoshita et al., 2005).

Leaf Responses to Flooding

Leaf responses to flooding include the closing of stomata, epinasty, senescence, abscission, ability or inability to maintain leaf canopy, reduction of stomatal conductance (g_s) and net CO_2 assimilation (A), reduction in leaf and stem water potential (ψ_l and ψ_s), nutrient deficiencies, and buildup of leaf carbohydrate concentrations (Kozlowski, 1997). Some of these responses are due to a decrease in water uptake from the roots, and a reduced ability to translocate photosynthate from source (leaf) to sink (roots or other storage structures). Electrolyte leakage in the needles of black spruce [*Picea mariana* (Mill.) BSP] and tamarack [*Larix laricina* (Du Roi) K. Koch] when exposed to flooding indicated that flood stress can damage cell membrane function in the leaves (Islam et al., 2003).

Stomatal closure appears to be a common early response involved in the reduction of A as a result of flooding. For mango, avocado, banana, citrus, and *Annona* spp. trees, A, g_s, and transpiration (E) significantly decreased as early as 1 to 3 d after onset of flooding, and sometimes internal leaf CO_2 concentration (Ci) values increase (Larson et al., 1991a; 1991c;

Nuñez-Elisea et al., 1998; Ploetz and Schaffer, 1987, 1989; Syvertsen and Lloyd, 1994; Turner, 1994). This varies widely, so 1-3 d is not definitive, as it also depends on environmental conditions, cultivar, soil type, and plant age (B. Schaffer, personal communication).

Plant signaling for stomatal closure. One of the first physiological responses a plant has to flooding is often stomatal closure, which leads to a decrease in g_s resulting in a decrease in E. When roots are submerged, the synthesis and translocation of cytokinin (CK) from roots to leaves is reduced (Reid and Railton, 1974). Cytokinin is known to help keep stomata open. Abscisic acid (ABA), which is synthesized in the roots and leaves is known to close stomata and the ratio of ABA:CK provides a critical signal to the leaf stomata (Else et al., 1996). However, studies have shown that it may not be an increase in root production of ABA which affects the stomatal closure, but instead the ABA present in the apoplast adjacent to the guard cells (Else et al., 1996). In flooded avocado trees, however, stomatal closure could not be related to changes in root or leaf ABA activity (Gil et al., 2009).

The pH of the xylem sap may increase within 24 h of flooding, and in some plants this may be the signal that has a critical impact on ABA, and how it impacts stomatal closure (Else et al., 1996). When a leaf has sufficient water status, its apoplast is relatively more acidic, while the apoplast of a leaf under water stress is relatively more alkaline. Under more acidic conditions in the leaf apoplast, ABA exists in an undissociated form (ABAH) which passes through cell membranes more easily and thus favors mesophyll cell uptake of ABA. Under more alkaline conditions in the apoplast, ABA exists as a dissociated form (ABA-) (Taiz and Zeiger, 2002) which does not pass into the mesophyll cells easily, and thus more ABA reaches the guard cells by way of the apoplast and transpiration stream (Taiz and Zeiger, 2002). Both drought stress

(Wilkinson and Davies, 1997) and flooding stress (Else et al., 1995; Jackson et al., 1996) have been found to increase the pH of the xylem sap.

The leaf mespophyll cells are also capable of producing ABA (Loveys, 1977) under water stress (Farquhar and Sharkey, 1982; Walton, 1980). In the light, chloroplasts accumulate ABA, as the light causes H^+ uptake into the grana, which makes the stroma more alkaline. The ABAH in the stroma is dissociated into ABA- and H^+ and the H^+ continue to pass into the grana. If the chloroplast continues to maintain its alkalinity in the stroma, the passive diffusion of ABAH from the cytosol across the chloroplast membrane into the chloroplast's stroma is facilitated, and the total combined concentration of ABAH and ABA- is thus much greater in the stroma of the chloroplast than the cytosol of the mesophyll cell (Farquhar and Sharkey, 1982). This mode of action of transmission of ABAH across membranes from regions of lower pH to regions of higher pH also illustrates how an increase in pH of the leaf apoplast resulting from an increase in pH from the xylem sap might make for a fast signal to the leaf, resulting in a rush of ABAH into the leaf apoplast, and stomatal closure (Farquahar and Sharkey, 1982; Raschke, 1975a). Also, when the leaf is under water stress, the chloroplast envelope may become leaky, contributing to the ABA release (Farquahar and Sharkey, 1982; Kaiser and Heber, 1981).

Another hypothesis for signals promoting stomatal closure was tested by looking at potential ionic and pH signals translocated from roots to shoots of flooded tomato plants. Xylem sap was sampled during approximately 30 h of flooding. After the first 2.5 h of flooding, total osmolites and PO_4^{3-}, SO_4^{2-}, Ca^{2+}, K^+, NO_3^- and H^+ decreased compared to the control, and Na^+ continued to be excluded. After about 10 h the roots' ability to function became damaged, which led to an increase in PO_4^{3-}, SO_4^{2-}, Ca^{2+}, and Na^+ in the xylem sap above control values, while K^+ and H^+ were still maintained at lower levels than control (Jackson et al., 2003). Follow up

experiments with detached leaves tested if K^+ or H^+ were signals for stomatal closure. Low concentrations of K^+ or no K^+ were delivered in solution to the detached leaves, as well as low or no H^+ solutions. Stomatal closure was not cued. The conclusion from these experiments was that ionic and pH signals from the roots do not play a role in leaf stomatal closure in flooded tomato plants (Jackson et al., 2003).

Stomatal limitation on photosynthesis. Stomatal closure is one obvious cause for the observed declines in A due to assimilation reducing the level of CO_2 present in the air spaces of the leaf mesophyll and closed stomata limiting the replacement of that CO_2 from the ambient air. This has been documented as an early plant response to flooding in numerous fruit crops such as mango, avocado, banana, citrus, and *Annona* spp. (Larson et al., 1991a; 1991c; Núñez-Elisea et al., 1998; Ploetz and Schaffer, 1987, 1989; Syvertsen and Lloyd, 1994; Turner, 1994). However, this does not explain all observed leaf gas exchange responses, particularly over extended periods of flooding.

Relative stomatal limitation (L_s) can be calculated directly by measuring A, first at ambient CO_2 concentrations (C_a = 350 μmol mol-1), and then at equal internal leaf CO_2 concentrations (Ci = 350 μmol mol-1). This allows for the calculation of a percent level of stomatal limitation on A (Farquhar and Sharkey, 1982; Fernández, 2006) which is summarized by the equation $L_s = 100\ (P_{NO} - P_N)/P_{NO}$, where P_N is the assimilation rate at ambient CO_2 concentrations and P_{NO} is the assimilation rate at equivalent internal leaf CO_2 concentrations. This work has been done with a mamey sapote relative, *Pouteria orinocoensis* which is considered flood tolerant (Fernández, 2006). Flooded seedlings with non-submerged leaves had L_s of 36% one day prior to flooding (day 0) which increased to 50% after 3 d of flooding, and 71% after 7 d of flooding, where it remained relatively constant at least through day 20 (Fernández, 2006).

Non-stomatal limitations on photosynthesis. Photosynthesis is made up of thylakoid reactions and carbon fixation reactions. The thylakoid reactions involve such components as carotenoids, chlorophylls, light harvesting complexes, photosystems (PS) I and II, and ATP synthase. The thylakoid reactions are the source of ATP and nicotinamide adenine dinucleotide (NADPH) production which are required by the carbon fixation reactions which take place in the stroma. The carbon fixation reactions are also known as the photosynthetic carbon reduction (PCR) cycle or the Calvin Cycle.

Plants under environmental stress may experience a decrease in PSII efficiency (Cai and Xu, 2002; Farquhar and Sharkey, 1982; Laisk et al., 1997; Li et al., 2007; Mauchamp and Méthy, 2004). Photosystem II is responsible for the oxidation of water and the buildup of a proton gradient between the lumen side of the thylakoid membrane (high H+), and the stroma side (low H+) (Taiz and Zeiger, 2002). It is the diffusion of protons from the lumen side to the stroma side which powers photophosphorylation, which regenerates ATP by ATP synthase. The oxidation of water by PSII is the source of electrons which are carried from PSII to PSI and then are used by PSI to reduce NADP+ to NADPH. Thus, the decrease in intrinsic PSII efficiency can lead to a decrease in production of ATP and NADPH in the chloroplast and ATP and NADPH are required for the carbon fixation reactions (Taiz and Zeiger, 2002).

Carbon fixation is made up of the three main stages, carboxylation, reduction, and regeneration. Two important components of carbon fixation include ribulose-1,5-bisphosphate (RuBP) which is the CO_2 acceptor, and the enzyme ribulose bisphosphate carboxylase / oxygenase (Rubisco) which catalyses the reaction. In the carboxylation reaction, CO_2 and H_2O are fixed with RuBP to form 3-phosphoglyceric acid (3-PGA). In the reduction reaction, 3-PGA, ATP and NADPH yield 3-phosphoglyceraldehyde (3-PGAld), ADP, NADP+, and Pi. Some of

the 3-PGAld is then exported and used to make sucrose, but most of it enters the regeneration reaction where it is converted back into RuBP. The regeneration reaction also requires ATP.

Many points in the above processes can be interrupted for various reasons, and there are many ways to measure and test the health, efficiency, and productivity of the photosynthetic apparatus itself. The status of relative mesophyll limitation (L_m) can be measured as a whole. The health of PSII can be assessed through measurements of chlorophyll fluorescence and quantum yield. Photochemical and nonphotochemical quenching can be assessed. On the carbon fixation side, carboxylation efficiency (CE) and net CO_2 assimilation can be determined. These variables offer insight into the health and condition of different components of the photosynthetic apparatus and where limiting factors lie.

Relative mesophyll limitation (L_m) can be calculated by measuring saturation assimilation rates (A_{sat}) at saturated internal CO_2 concentrations (e.g. Ci = 1600 $\pm$ 100 μmol · mol^{-1}) for both flooded and control plants. Therefore there is no limitation placed on assimilation by either stomata or insufficient Ci (Farquhar and Sharkey, 1982; Fernández, 2006). Thus, $L_m = 100 (A_C - A_F) / A_C$, where A_C is the assimilation rate of the control leaves at saturated Ci and A_F is the assimilation rate of the flooded plants at saturated Ci (Farquhar and Sharkey, 1982; Fernández, 2006). Therefore nonstomatal or mesophyll limitation is detected as a decrease in A_{sat} by the flooded plants relative to the nonflooded plants (Herrera et al., 2008; Jacob and Lawlor, 1991). In studying the effect of flooding on A of *Pouteria orinocoensis*, a species in the same genus as mamey sapote, Fernandez *et al.* (2006) calculated L_m limitations by saturating the air spaces in the leaf mesophyll with CO_2 to eliminate the limitation on A imposed by stomata (Farquhar and Sharkey, 1982). Fernández found flooded seedlings with non-submerged leaves had L_m beginning at 0% on day 0 and steadily increasing throughout the flooding period to 7% on day 3,

16% day 7, 48% day 12, and 61% day 20. On each measurement day, L_m of flooded trees was significantly greater than the previous measurement day (Fernández, 2006). However, even with significant increases in L_s and L_m, A still remained between 3.5 to 3.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ until at least day 20 (Fernández, 2006).

In citrus it was found that flood-stressed plants had higher Ci levels than control plants, which indicated that non-stomatal factors such as chlorophyll degradation were more important to the limitation of A than stomatal limitations (García-Sánchez et al., 2007). Flooded mango trees in containers also had an increase in Ci after only 3 d of flooding which suggested nonstomatal limitations were greater than stomatal limitations (Larson, 1991).

Photoinhibition and photochemical and non-photochemical quenching. Another possibility for what causes a leaf's photosynthetic ability to decline during flooding stress is photoinhibition (Fernández, 2006; Mauchamp and Méthy, 2004). Inhibition of photosynthesis can take place by either a reduction in photosynthetic activity due to protective mechanisms, or excess light causing damage to the photosynthetic system. If the amount of excess light energy is moderate, then the xanthophyll cycle may quench the excess energy and keep it from the antenna complexes which lead the light's energy to the reaction center complex PSII. The xanthophyll cycle converts that excess light energy to heat and prevents formation of superoxide, singlet oxygen, and peroxides which can damage cellular components, especially lipids and the D1 protein of PSII (Cai and Xu, 2002; Taiz and Zeiger, 2002). Also there are light harvesting complexes (LHCII) associated with PSII which can dissociate from PSII and reduce damage to PSII (Cai and Xu, 2002; Hong and Xu, 1999).

Another description of nonphotochemical quenching (NPQ) is that it is related to the thermal de-excitation of PSII (Li et al., 2007). Violaxanthin is converted via the intermediate

antheraxanthin to zeaxanthin by the enzyme de-epoxidase through a process called de-epoxidation. This conversion is aided by an ascorbate cofactor and is favored under excess light and a low pH optimum of 5.1. The reversal of zeaxanthin back to violaxanthin is called epoxidation and takes place when the light intensity is reduced. Epoxidation is favored under a higher pH optimum of 7.5 with NADPH as a cofactor (Pallet and Young, 1993).

As these protective mechanisms operate, quantum efficiency may be reduced, and a condition known as dynamic photoinhibition may occur. This is only a temporary reduction in quantum efficiency, which may return to normal when the photon flux again drops below saturated levels.

If the amount of excess light is too great to be dissipated by these kinds of defensive mechanisms, then the D1 protein in the PSII reaction center may be damaged (Melis, 1999). When the D1 protein is damaged, it is removed from the membrane, and it must be resynthesized in order for the damage to the photosynthetic apparatus to be repaired. This condition is known as chronic photoinhibition and it may take weeks or months for the damage to be repaired (Melis, 1999).

Carboxylation efficiency, photorespiration, and RuBP regeneration. Non-stomatal limitations of photosynthesis can include reduced carboxylation efficiency and reduced RuBP regeneration. As discussed above, ATP and NADPH is required for the reduction reaction, and the regeneration of RuBP. Thus, if any part of the thylakoid reactions broke down, ATP regeneration would be limited due to the reduced proton gradient across the thylakoid membrane and reduced ATP synthesis. NADPH generation would also be reduced as the electron flow from PSII to PSI to the reduction of NADP+ to NADPH would be reduced. Predominantly due to reduced ATP levels, RuBP regeneration would thus be reduced (Lawlor, 2002). Reduced

relative water content in the leaves can also have important consequences leading to the reduction of ATP synthesis, due to increased relative concentrations of Mg^{2+} in the chloroplast as relative water content decreases (Lawlor, 2002; Younis et al., 1979).

Effects of Flooding on Water Potential, Leaf Epinasty, and Leaf Senescence

Water potential is the measure of free energy found in a given volume of water, generally measured in pascals, which is a pressure unit (Taiz and Zeiger, 2002). Its components are solute potential, pressure potential, and matric potential. Understanding water potential can be a useful tool for understanding the soil-plant-atmosphere continuum, and water status / health of the plant. For example, as water evaporates into the mesophyll air spaces of the leaf leading to transpiration via the stomata, more water is drawn by cohesion through the leaf xylem, thus drawing more water up through the petiole, stem, and trunk xylem. Besides bulk flow, roots are also capable of absorbing ions from the soil solution, building up solute or osmotic potential inside the root xylem, and causing the movement of water into the roots. The path of water into the roots involves either the symplastic path through the root hairs, through cells, and finally through the cells of the Casparian strip into the root xylem, or the apoplastic pathway, between the root cells, until the water is forced into and through the cells of the Casparian strip by its suberized walls.

Thus, measurements of stem water potential may indicate the ability of the plant to take up water. If the stem water potential remains relatively high, then the roots are probably still functioning sufficiently to allow water to be drawn up by the plant. If the stem water potential drops, then it is likely that root function has been negatively impacted by hypoxic or anoxic conditions, compromising the root cells' ability to function properly. If stem water potential declines very rapidly, then it is likely that the leaves will undergo necrosis, wilt, turn brown and dry, as opposed to undergoing the physiological processes involved with senescence, chlorosis,

and abscission. If the stem water potential does not drop too rapidly, the plant may have sufficient time to respond to flooding conditions via hormonal and other signaling mechanisms. Stem water potential after 9 d of flooding in citrus was found not to decline and remained similar to control plants (García-Sánchez et al., 2007). Flooding of mango in containers for 14 d was found to not affect leaf water potential of flooded plants compared to nonflooded plants, although A did decrease within 7 d (Larson, 1991). In flooded avocado trees, leaf water potential did not decline after 4 d of flooding, however, if the plants were infested with *Phytophthora cinnamomi* Rands, then their leaf water potential did significantly decline after 4 d of flooding (Schaffer et al., 1992).

Signaling mechanisms such as ABA, CK, and ethylene, initiate leaf responses making it possible for the leaves to senesce and translocate nitrogen from the leaves back into the plant. In addition to their impacts on stomatal opening and closure, both ABA and CK also impact leaf senescence. Abscisic acid is known to promote leaf senescence, while CK is known to inhibit leaf senescence. Significant increases in ABA content were observed in continuously flooded citrus after 14, 20, and 32 d of flooding, depending on the citrus genotype (Arbona and Gómez-Cadenas, 2008). There was also a transient increase in jasmonic acid (JA) in the leaf prior to the increase in ABA, which might indicate JA involvement as well (Arbona and Gómez-Cadenas, 2008). In oat and wheat, ABA and ethylene result in growth inhibition during flooding, and auxins, CK, and GB result in repair processes (Bakhtenko et al., 2007). In drought stress it was found that ABA and sugar signaling in the leaf impact the induction of senescence (Wingler and Roitsch, 2008).

During the process of leaf senescence, chlorophyll is broken down and the leaves turn yellow. This clearly would cause a reduction in the leaves' photosynthetic ability. Chlorosis is

measurable as a reduced leaf chlorophyll index or leaf greenness by a SPAD meter. The leaf chlorophyll index is typically correlated with SPAD values and used to provide an indication of the extent of leaf chlorophyll loss due to an environmental stress (Ojeda et al., 2004; Schaper and Chacko, 1991).

The hormone with perhaps the greatest impact on leaf senescence is ethylene. In citrus, there is a late increase in 1-amino-cyclopropane-1-carboxylate (ACC) concomitant with severe leaf injury, which indicates ethylene promotes leaf senescence (Arbona and Gómez-Cadenas, 2008). In tomato, ethylene levels can be found to increase in the leaf as early as 1 h after flooding (Shiu et al., 1998). Waterlogging roots can cause synthesis of the precursor to ethylene, ACC, and this can be transported to other plant parts within 6-12 h (Shiu et al., 1998). ACC is exported from the roots via the xylem sap in tomato to the shoots and leaves. Early flood induced ACC arrives from the root to the shoot of tomato within 6 h after flooding (English, 1995). In the shoots and leaves, ACC may be converted to ethylene in a process which requires oxygen.

Epinasty is an induced response to ethylene and causes the cells on the upper (adaxial) surface of the leaf petiole to expand more rapidly. This process induces leaf epinasty, which can reduce the light incidence on the leaf by bending the leaf down out of the direct angle of the sunlight (Reid and Bradford, 1984). In the case of apricot, epinasty was observed and was associated with a decrease in leaf water potential to -6.0 MPa and death of the plants (Domingo et al., 2002).

Morphological Adaptations to Flooding

Common morphological adaptations to flooding include development of root and trunk aerenchyma, adventitious root development, the root hypodermal tissue may suberize, and lenticels on the trunk may hypertrophy (Kozlowski, 1997). Aerenchyma may be produced in the

cortex of extant roots of herbaceous (e.g., corn) and woody plants (e.g., pond apple) upon the introduction of hypoxic conditions (Drew et al., 2000; Nuñez-Elisea et al., 1998). Hypoxia results in the formation of ethylene via ACC synthase and ACC oxidase. Ethylene receptors then induce a signaling cascade which leads to programmed cell death of cortical cells which creates air spaces in the cortex called aerenchyma (Drew et al., 2000). These spaces permit oxygen flow from the stomata (or lenticels in woody species) to the roots. The formation of aerenchyma also reduces the number of respiring cells in the roots requiring oxygen.

Prevention of oxygen escape from the root to the rhizosphere may also be an important adaptation to flooding. Rice and other wetland species produce a suberized layer of hypodermal cells, and the cells just inside of the hypodermis become lignified. These layers produce a barrier of low gas permeability (Drew et al., 2000). The mamey sapote relative, *P. glomerata* appears to be flood tolerant due to the formation of aerenchyma in the root cortex and a thick suberized layer in the tangential and radial walls of the root hypodermis (De Simone et al., 2002). Non-suberized cells in this layer were not often observed, while the epidermis showed little sign of suberin. The suberized hypodermal layer extended up to, but did not include the root tip. This layer functions to keep oxygen in the root and toxins from the reduced environment out of the root (De Simone et al., 2002).

Hypertrophic lenticels may form on the trunk of flooded trees below or above the waterline. Ethylene plays a roll in the formation of lenticels and underlying bark tissue. Some lenticels are formed under water and permit the exchange of dissolved gases, as well as the possible release of toxic byproducts of root anaerobic respiration such as acetaldehyde and ethanol (Chirkova and Gutman, 1972; Kozlowski and Pallardy, 2002). Ethylene may also increase cellulase activity which weakens the cell walls of targeted cells and dehydration follows

due to competition for water from healthier cells. This kills the weakened cells, forming intercellular spaces (Kozlowski and Pallardy, 2002). Other hypertrophic lenticels are formed above the waterline, as in the case of mango (Larson et al., 1991a; Schaffer, 1998) and pond apple (Nuñez-Elisea et al., 1998; Ojeda et al., 2004). When mango is flooded, some trees will form hypertrophic lenticels on the stem just above the waterline. The swollen lenticels are accompanied by changes in the phellem tissue leading to intercellular spaces in the phellem and cortex (Larson et al., 1991a).

Adventitious root formation occurs in some tree species as a response to flooding including *Rumex* spp. and *Fraxinus mandshurica* (Blom et al., 1994; Visser, et al.,1996; Yamamoto et al., 1995). Ethylene accumulation under waterlogged conditions can promote adventitious root formation (Visser et al., 1996). These roots can increase water uptake to compensate for the loss of original rooting structures (Tsukahara and Kozlowski, 1985). Aerenchyma can develop in the outer bark of adventitious roots permitting flow of oxygen to the roots (Yamamoto et al., 1995). Gibberellins and cytokinins can be supplied to the rest of the plant from adventitious roots, making up for a decline in production elsewhere (Reid and Bradford, 1984).

The quantity of adventitious roots formed in response to flooding can differ within a genus. *Hakea* is a woody Proteaceae with species from wetland and non-wetland environments. The wetland species formed twice the number of adventitious roots as a non-wetland *Hakea* species (Poot and Lambers, 2003). Development of adventitious root primordia may occur from ray parenchyma in the secondary phloem or from xylem parenchyma, depending on the species (Kozlowski, 1997). In some species, stem morphology or anatomy is affected by flooding. Flooded *Fraxinus mandshurica* seedlings had a cumulative stem diameter of about four times that of nonflooded control seedlings after 70 d of flooding. Aerenchyma tissues also formed in

the bark tissue, as well as numerous hypertrophic lenticels and adventitious roots (Yamamoto et al., 1995). Ethylene is known to promote programmed cell death, thus forming lysigenous aerenchyma which promotes oxygen transport in roots of waterlogged plants (Shiono et al., 2008). However, it was found that flooded *Annona glabra* did not develop aerenchyma tissue in response to flooding, although they did develop an outgrowth of new roots into the flood water as well as hypertrophic lenticels on submerged roots which could allow increased diffusion of gases (Núñez-Elisea et al., 1999).

Flooding can significantly reduce the root:shoot ratio and reduce root depth. These reductions due to hypoxic conditions and abundant water can be detrimental to trees in climates subject to both flooding and drought. Trees with a shallower root system and reduced root:shoot ratio could be more susceptible to drought stress than trees with normal root development (Lopez and Kursar, 1999).

Conclusions

As discussed above, a significant body of research exists about the physiological responses of forest trees, agricultural crops, and tropical fruit trees to waterlogged conditions in the root zone. Leaf gas exchange factors such as stomatal conductance and net CO_2 assimilation frequently decline after a few days. Leaves may senesce. Roots and stems may undergo morphological changes such as the development of hypertrophic lenticels, aerenchyma tissue, and adventitious roots to help cope with the hypoxic or anoxic environment. Root cells may deteriorate, membranes may become more permeable, and roots may loose functionality and deteriorate. Plant water potential may decrease. Most of these responses have been documented already for tropical fruit trees such as mango, *Annona* spp., avocado, and carambola. Previous research with other tree species has demonstrated root cells may shift from aerobic to anaerobic respiration, glycolysis rates increase, and the activity of anaerobic enzymes such as alcohol

dehydrogenase may be upregulated. These flood responses have not been documented in tropical fruit trees to the author's knowledge.

Based on the above literature review, the following experiments with *Pouteria sapota* were conducted to gauge the plants' responses to flooded soil conditions. Leaf gas exchange, root respiration, root anaerobic enzymes, plant morphological changes, and time-line of plant responses were all measured and assessed in a variety of flooding treatments from field plantings, to potted plants in a screenhouse, to hydroponic treatments in a greenhouse. Chapter 3 investigates the response of two common grafted cultivars of mamey sapote, 'Magaña' and 'Pantin' to continuous flooding in containers filled with the calcareous soil native to the production area of south Florida. Responses such as leaf gas exchange, stem water potential, leaf chlorophyll index, soil redox potential, and other visible symptoms such as leaf epinasty and leaf senescence were all documented for up to 60+ d until the plants died. Chapter 4 investigates mamey sapote responses to repeated cycles of short flooding in containers for 3 d or 6 d with short periods of recovery in between. Flooding time periods were based on declines in leaf gas exchange in response to flooding during continuous flooding experiments (Chapter 3), and were intended to mimic a more likely flooding scenario that might be experienced by the crop in the orchard based on the weather patterns and water table conditions of both south Florida and other areas of the world. Chapter 5 continues to investigate further what mamey sapote's response to flooding is in the orchard by planting 60 grafted 3-yr-old 'Magaña' in the field in mounds of native calcareous soil placed on top of a water resistant barrier. Flooding treatments were initiated by raising the sides of the barrier and filling each one with water to form a pool up to the soil-line. The final set of experiments in Chapter 6 takes a deeper look at the root physiology of mamey sapote under flooded conditions. Hydroponic and aeroponic treatments were designed

in order to subject mamey sapote roots to the desired normoxic and anoxic conditions, while still permitting access to the roots for harvest and data collection. Root respiration, glycolysis rates, root electrolyte leakage, and alcohol dehydrogenase enzyme activity were all determined. While this kind of work has been done with other tree species, it has not been well documented in tropical fruit trees (alcohol dehydrogenase activity in particular) including mamey sapote. Collectively, these chapters explore the physiological and growth responses of mamey sapote to a wide variety of flooding or low oxygen conditions in the rhizosphere.

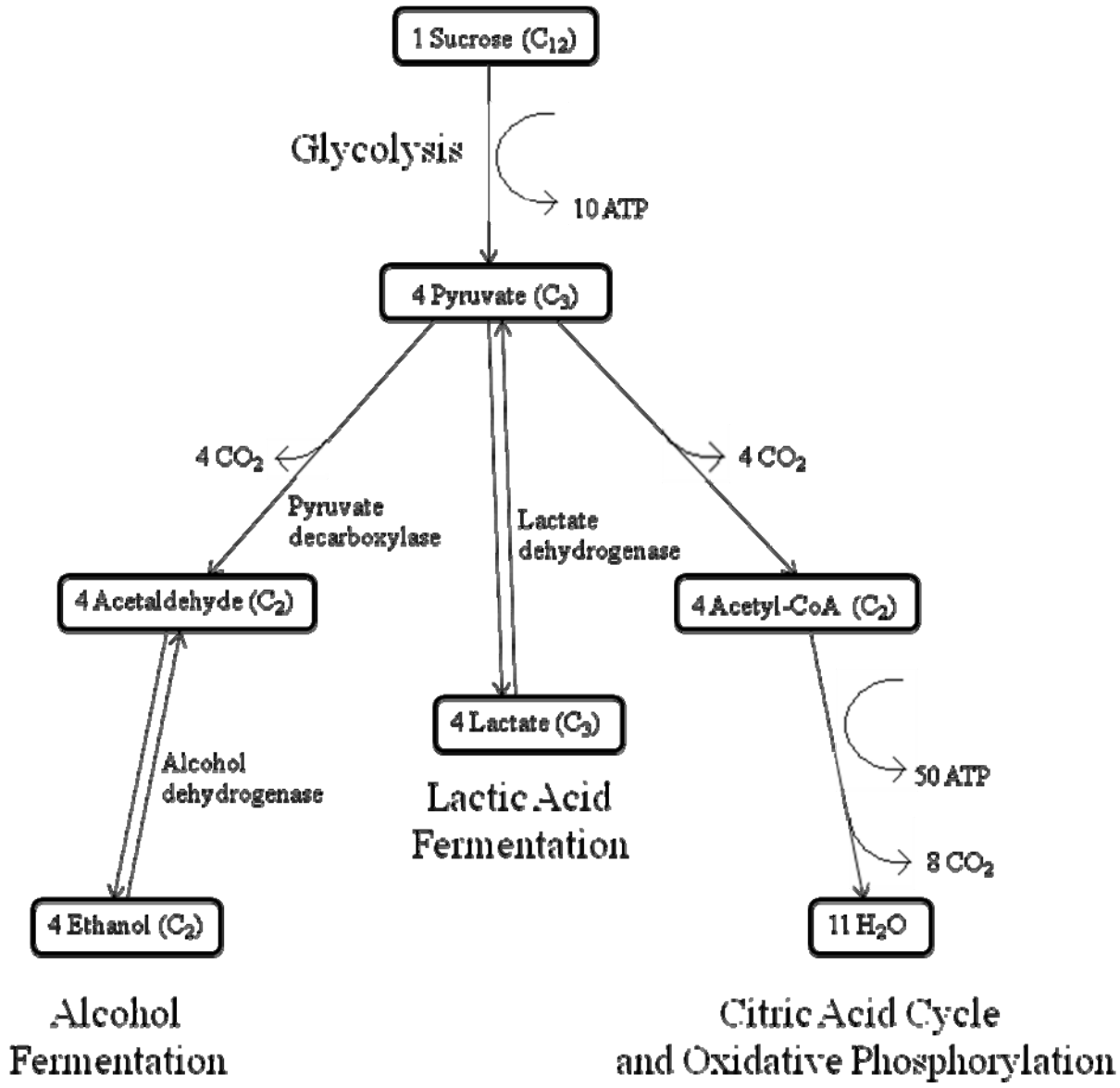

Figure 2-1. Respiration pathways. Simplified diagram of respiration pathways tracing ATP generation and CO_2 evolution via glycolysis, citric acid cycle and oxidative phosphorylation, lactic acid fermentation, and alcohol fermentation. Number of carbon atoms present in each molecule noted in parentheses. Diagram information based on Brand (1994) and Taiz and Zeiger (2002).

CHAPTER 3
RESPONSE OF MAMEY SAPOTE (*POUTERIA SAPOTA*) TREES TO FLOODING IN A CALCAREOUS SOIL IN CONTAINERS

Introduction

Mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn] is a tropical tree native to the humid lowlands of southern Mexico to as far south as northern Nicaragua (Balerdi and Shaw, 1998; Verheij and Coronel, 1992). It is grown as a fruit crop in the subtropics and tropics including Mexico, Central America and in the Caribbean Basin (Balerdi and Shaw, 1998; SAGARPA, 2008). As of 2009, mamey sapote is estimated to be grown commercially in southern Florida on 233 ha (575 acres) and is annually worth an estimated $7.5 million at the farm level, and about $18.5 million at the wholesale level (E. Evans, University of Florida, personal communication). The calcareous agricultural soil in south Florida on which fruit crops are grown is classified as Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) (Burns et al. 1965; Leighty and Henderson, 1958; Nobel et al., 1996). In southern Florida, mamey sapote orchards are subjected to periodic flooding during high water table conditions which coincide with periods of heavy rainfall and/or tropical storms. Flooding of mamey sapote orchards in this area has generally resulted in tree decline and death (Crane et al., 1997; Degner et al,. 2002).

One of the earliest detectable physiological responses of trees to flooding is a decrease in stomatal conductance (g_s) due to stomatal closure that results in decreased transpiration (E) and maintenance of high leaf water potential (Kozlowski, 1997; Kozlowski and Pallardy, 1984; Schaffer et al., 1992). A decline in net CO_2 assimilation (A) is generally concomitant with reductions in g_s as a result of flooding of fruit trees (Kozlowski and Pallardy, 1984; Kozlowski, 1997; Schaffer et al., 1992). Calculations of internal partial pressure of CO_2 (Ci) in leaves may provide a clue to determining if reductions in A are due to stomatal or non-stomatal factors. A

decline in Ci concurrent with declines in A and g_s may indicate stomatal limitation (L_s) to a sufficient quantity of CO_2 entering the leaf for maintenance of of an optimum rate of CO_2 fixation. However, an increase in Ci accompanied by decreased A and g_s in flooded trees may indicate a non-stomatal or mesophyll (L_m) limitation to A (Farquhar and Sharkey, 1982) which can result from increased CO_2 in the intercellular space of the leaf which has been associated with stomatal closure (Mansfield et al., 1990; Raschke, 1975a; 1975b)

There has been a considerable amount of research on the effects of flooding on subtropical and tropical fruit crops grown in the calcareous soil of southern Florida (Schaffer et al., 2006). These studies have focused on avocado (*Persea americana* Mill.) (Ploetz and Schaffer, 1987, 1989), *Annona* spp. (Nuñez-Elisea et al., 1998), mango (*Mangifera indica* L.) (Larson et al., 1991a; 1991c), and carambola (*Averrhoa carambola* L.) (Joyner and Schaffer, 1989). In calcareous soil, avocado trees survived up to 30 d of flooding when not infested with root-rot (*Phytophthora cinnamomi* Rands) (Ploetz and Schaffer, 1987, 1989). Seedlings of the flood sensitive bullock's heart (*Annona reticulata* L.) and sugar apple (*Annona squamosa* L.) survived between 30 to 50 d of flooding (Nuñez-Elisea et al., 1998), and mango can survive up to 110 d of continuous flooding (Larson, 1991; Schaffer et al., 2006) in these calcareous soils. To the author's knowledge, there are no published reports on the effects of flooding on the physiology and growth of mamey sapote trees. The purpose of this study was to determine physiological and growth responses of young mamey sapote trees to continuous flooding in a calcareous soil.

Materials and Methods

Plant material. In March 2004, two-year-old 'Pantin' and 'Magaña' mamey sapote trees grafted onto seedling rootstocks were obtained from a commercial nursery and repotted into 19-L containers filled with Krome very gravelly loam soil. After about one year of acclimation in Krome soil, plants were treated with metalyxl (Ridomil™; Syngenta Crop Protection, Inc.,

Greensboro, NC) and Fosetyl-Al (Aliette™; Bayer CropScience. Research Triangle Park, NC) to prevent phytophthora (*Phytophthora cinnamomi* Rands) or pythium (*Pythium splendens* Braun) root rots.

Experimental design. The experiment was conducted in an open-air structure consisting of screen cloth on all sides and an arch-shaped roof composed of two sheets of clear polyethylene. Two flooding trials were conducted. In trial 1, 'Pantin' trees were continuously flooded for 66 d from 12 Apr. to 17 June 2005, and in trial 2, 'Magaña' trees were continuously flooded for 45 d from 31 May to 15 July 2005. Thus, each trial consisted of a flooded treatment and a nonflooded control treatment. Plants were flooded by placing the 19-L containers inside 38-L containers, and filling the larger containers with well water until the water level was 5 cm above the soil surface.

Trees in both treatments were arranged in a completely random design. In trial 1, there were ten single-tree replications per treatment, and in trial 2 there were seven single-tree replications per treatment. All nonflooded plants were drip irrigated for 10 min daily, receiving about 3.8 L of water per plant per day. When all trees in the flooded treatment were dead, the experiment was terminated. Trees were considered dead when scratching the bark on the lower trunk no longer revealed green tissue beneath.

Pre-treatment and post-treatment measurements. In both trials, plant height was measured from the soil surface to the top of the apical bud one day prior to initiating treatments (Day 0) and at the end of the experiment. Trunk diameter was measured at 5 cm above the soil surface on Day 0 and at the end of the experiment. In trial 2, 'Magaña' tree height and the number of leaves on the trees varied. Trees were selected and grouped into treatments so that each treatment had similar means and variances of the number of leaves per tree.

Temperature and soil redox potential. In both trials, soil temperatures were monitored with a HOBO Water Temp Pro sensor and datalogger (Onset Computer Co., Bourne, MA) and canopy temperatures were monitored with a StowAway TidbiT sensor and datalogger (Onset Computer Co., Bourne, MA). Soil redox potential was measured in the flooded treatment with a metallic ORP indicating electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) connected to a volt meter. Soil redox potential was measured in trial 1 on days 0, 1, 3, 7, and 10 and in trial 2 on days 0, 1, 3, 5, and 8.

Leaf gas exchange. Leaf gas exchange measurements of A, gs, Ci, and E, were made with a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA). Measurements were made at a photosynthetic photon flux of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a reference CO_2 concentration of 330 $\mu mol \cdot mol^{-1}$ and an air flow rate into the leaf cuvette of 200 $mL \cdot min^{-1}$. Measurements were made every 1 to 4 d for 7 to 14 d until leaves of the flooded trees wilted or abscised. The fifth or sixth most recently matured leaf from the apical meristem of each tree was repeatedly sampled over time.

Leaf chlorophyll index. A chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Ramsey, NJ) was used to measure leaf greenness (leaf chlorophyll index). Measurements with the SPAD meter were made during trial 1 on days 0, 7, 9, and 10 and during trial 2 on days 0, 5, 7, and 9. In trial 1, both the fifth and sixth most recently matured leaves were measured on each plant. In trial 2, either the fifth or sixth most recently matured leaf was repeatedly measured per plant.

Stem water potential. During trial 1, stem water potential (Ψs) was measured on days 0 and 8 and during trial 2 on days 0, 3, 5, and 8. Leaves were selected from the middle of the canopy and enclosed for about 1 hr prior to measurements in a zip lock bag covered with

reflective aluminum foil (Shackel et al., 1997). Stem water potential was measured immediately after leaf harvest with a pressure chamber (Plant Water Status Console 3000 Series, Soilmoisture Equipment Corporation, Santa Barbara, CA). In trial 1, three leaves were sampled per tree at each measurement time, and in trial 2 one leaf was sampled per tree.

Data analysis. All data were analyzed by repeated measures ANOVA and standard T-test at the 5% significance level (unless otherwise noted), using the SAS statistical software package (Version 9.1, SAS Institute, Cary, North Carolina)

Results

Soil and Air Temperatures and Soil Redox Potential

Trial 1. Air temperatures ranged from 12 to 39°C. Nonflooded soil temperatures ranged from 15 to 38°C and flooded soil temperatures ranged from 17 to 35°C. Soil redox potential for the flooded treatment was slightly below 200 mV beginning on day 0, and values continued to decrease to a mean of -18 mV by day 10 (Fig. 3-1a).

Trial 2. Air temperatures ranged between 22 to 44°C. Nonflooded soil temperatures ranged from 23 to 40°C and flooded soil temperatures ranged from 22 to 38°C. Mean soil redox potential for the flooded treatment was 273 mV on day 0 and was 166 mV by day 8 (Fig 3-1b).

Leaf Gas Exchange

Trial 1. Net CO_2 assimilation for the nonflooded 'Pantin' plants remained consistently near 6 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ throughout the first 14 d (Fig. 3-2). Net CO_2 assimilation of flooded plants became significantly lower than that of the nonflooded plants by day 3, decreased to very low values by day 7 and then to 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ by day 10. By day 3, g_s of flooded plants was significantly lower than that of nonflooded plants and continued to decline further on subsequent days (Fig. 3-2). Transpiration of flooded plants became significantly lower than that of nonflooded plants by day 3 (data not shown). Internal CO_2 concentration was significantly

higher for the leaves of flooded plants than for nonflooded plants after 7 d of flooding (Fig. 3-2). By days 10 and 14, Ci for flooded plants was more than twice that of the nonflooded plants.

Trial 2. Net CO_2 assimilation of the nonflooded 'Magaña' trees remained consistently near 8 or 9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ throughout the first 7 d (Fig. 3-3). On day 1, Ci of the flooded plants was significantly greater than that of the nonflooded plants (Fig. 3-3). Beginning day 3, A for the flooded plants was only about one third that of the nonflooded plants, and E (data not shown), and g_s of flooded plants were significantly lower than nonflooded plants (Fig. 3-3).

Leaf Temperature and Chlorophyll Index (SPAD Values)

Leaf temperatures became significantly higher (by up to 1°C) for the flooded trees compared to nonflooded trees 7 and 14 d after flooding in trial 1 (Fig. 3-4a). In contrast, leaf temperatures were similar for flooded and nonflooded trees in trial 2, though there was a detectible increase in leaf temperature for the flooded treatment vs. nonflooded on day 7 at the $P \leq 0.1$ level (Fig. 3-4b). In trial 1, the leaf chlorophyll index was similar between treatments until day 10 when the chlorophyll index of the flooded treatment declined by about 20% to become significantly lower than that of the control (Fig. 3-5a). In trial 2, the leaf chlorophyll index was significantly lower for the flooded treatment than the nonflooded control on days 5, 7 and 9, steadily declining to about 25% lower values (Fig. 3-5b).

Stem Water Potential

Trial 1. Stem water potential was similar on day 0 for both treatments with means for nonflooded plants = -0.18 MPa and for flooded plants = -0.19 MPa (Fig. 3-6a). On day 8, Ψs of plants in the flooded treatment was significantly lower than that of the nonflooded plants with means for nonflooded = -0.21 MPa and flooded = -0.54 MPa.

Trial 2. Stem water potential was not significantly different between treatments on day 0 with means for nonflooded = -0.18 MPa and flooded = -0.17 MPa (Fig. 3-6b). By day 5, Ψs was lower for the flooded trees than the nonflooded trees, with a mean for nonflooded = -0.12 MPa and flooded = -0.20 MPa, though the differences were not statistically significant. However, by day 8, Ψs was significantly lower for flooded trees with a mean for nonflooded = -0.18 MPa and for flooded = -2.1 MPa.

Visible Stress Symptoms

Trial 1. Leaf chlorosis, epinasty, wilting, and leaf abscission were observed for flooded plants. In 'Pantin' trees, the flooding symptoms were often observed in the lower canopy before the upper canopy. Many of the flooded 'Pantin' trees showed epinasty in the lower canopy by day 8 and by day 10 epinasty occurred throughout the canopy. Most of the epinastic leaves became wilted with the leaf margins drying and becoming curled. By day 12, all epinastic leaves were desiccated and many lower canopy leaves began to abscise.

A few of the flooded trees did not show epinasty on day 8, and finally began to show slight wilting by day 13. These trees began to defoliate in the lower canopy by day 22, while leaves in the upper canopy wilted but did not undergo chlorosis or abscision. Some trees eventually became completely defoliated, whereas others had no leaf abscission at all, even though the entire canopy showed epinasty and desiccation.

Many of the 'Pantin' trees had a young apical flush with about 12 to 15 juvenile leaves, each about 7 cm long, about 2 cm wide, and somewhat pubescent. Of the flooded trees, by day 22 this apical flush was sometimes wilted. Lenticels on the trunk above the soil surface of flooded trees did not hypertrophy; however, woody roots of the nonflooded plants had some

hypertrophic lenticels. Stem dieback in flooded plants began occurring in most plants by day 30 until all plants were dead by day 66.

Trial 2. Flooded trees displayed leaf chlorosis, epinasty, wilting, and leaf abscission. 'Magaña' trees did not display a lower and upper canopy division in visible symptoms as a result of flooding. Between days 5 and 7, epinasty occurred on six out of seven of the flooded trees, with mild leaf chlorosis on two trees and more severe chlorosis on one tree. Epinasty occurred throughout the plant, and did not appear to occur at different times based on an upper and lower division of canopy. By day 9, flooded plants exhibited marginal leaf curling on either the upper leaves or all leaves, and the leaves were either wilted or desiccated. By day 14, all flooded plants exhibited epinasty and the leaves were completely desiccated. Between days 14 and 22, most of the leaves in the canopy had abscised. No young apical flush was present or developed on the 'Magaña' trees. Lenticels on the trunk above the soil line did not become hypertrophied, although woody roots of the nonflooded plants had some hypertrophic lenticels. Branches began to dieback in most trees by day 30 until all trees were dead by day 44.

Tree Growth

Trial 1. There were no significant differences in tree height or trunk diameter between treatments at the beginning of the experiment on day 0, however, at the end of the experiment the height and trunk diameter of the nonflooded trees was significantly greater than those of the flooded trees (Figs. 3-7a and 3-7b).

Trial 2. Tree height was significantly different between treatments on day 0 (Fig. 3-7c), however the mean number of leaves per plant was not significantly different between treatments with the nonflooded plants averaging 91 leaves per tree, and the flooded plants averaging 82 leaves per tree. Height of nonflooded trees increased slightly by day 44 (Fig. 3-7c). Trunk

diameter was not significantly different between nonflooded and flooded plants on day 0, however, by day 44 the trunk diameter of the nonflooded plants was significantly greater than that of the flooded plants (Fig. 3-7d).

Harvest

At the end of trial 1, mean fresh weights and mean dry weights for roots, stems, and leaves were significantly lower for plants in the flooded treatment compared to those in the nonflooded treatment (Fig. 3-8). At the end of trial 2, mean fresh weights and mean dry weights for leaves and stems of the flooded plants were significantly lower than those of the nonflooded plants (Fig. 3-9). However, root fresh and dry weights of the flooded plants were lower, but not significantly lower than those of the nonflooded plants (Fig. 3-9).

Discussion

In mamey sapote, a decline in A after 3 d of flooding appeared to occur simultaneously with a decline in g_s. These early declines in A were likely due to L_s, as the Ci levels at this stage were still below the ambient CO_2 level. If Ci levels were equal or above the ambient CO_2 level, that could indicate reduced A due to L_m. It is unlikely that the root to shoot signal for stomatal closure in flooded plants was due to altered water balance because reduced Ψs did not occur at the same time as, or prior to, the reduced g_s. While Ψs did decline over time, this did not occur until the 8th day of flooding. Thus, it is unlikely that the signal for the initial reduction in g_s was related to water or xylem potential. Thus, early reduction in g_s may have been due to other plant signals such as an increase in guard cell abscisic acid (ABA) content, although ABA content was not measured in this study. Anaerobic flooding stress has been found to increase the pH of the xylem sap and leaf apoplast and lead to increased stomatal guard cell ABA content, thus closing the stomata in as little as 24 h (Else et al., 1995; 1996; Jackson et al. 1996).

In studying the effect of flooding on A of *Pouteria orinocoensis* (Aubr.) Penn. Ined., a species in the same genus as mamey sapote, Fernandez *et al.* (2006) calculated L_s and L_m limitations. It was found that flooded seedlings with non-submerged leaves had L_s of 36% one day prior to flooding (day 0) which increased to 50% after 3 d of flooding, and 71% after 7 d of flooding, where it remained relatively constant at least through day 20. Corresponding measures of L_m began at 0% on day 0 and steadily increased throughout the flooding period to 7% on day 3, 16% day 7, 48% day 12, and 61% day 20. However, even with these significant increases in L_s and L_m, A still remained between 3.5 to 3.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in flooded *P. orinocoensis* until at least day 20 of flooding. Comparatively, in our study, mamey sapote A reached 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ within 7 to 10 d of flooding. *P. orinocoensis* is considered flood tolerant and found in a seasonally flooded forest in Venezuela, while data from our study indicates mamey sapote is not as flood tolerant. Thus, for mamey sapote, initial reductions in A may have been due to L_s. However, a large L_m may also be responsible for reductions of A to 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in flooded mamey sapote, also leading to the significant increase in Ci of the flooded plants over the nonflooded plants (Figs. 3-2 and 3-3). Mesophyll limitations to A may be due to changes in carboxylation enzymes, reduced chlorophyll content, a reduction in ATP and/or RuBP synthesis (Fernández, 2006; Herrera et al., 2008; Kozlowski, 1982; Kozlowski and Pallardy, 1984; Lawlor, 2002). In the present study leaf chlorophyll index dropped 20 to 25% in the flooded plants, indicating reduced chlorophyll content may also have been a factor contributing to mesophyll limitations for mamey sapote.

Declines in A as a result of flooding may have been due to photoinhibition. Decline in photochemical quenching (photosynthesis reactions) can take place in leaves that are photoinhibited (excess light energy used up in nonphotochemical quenching reactions) (Laisk et

al., 1997). Thus, if heat is given off during nonphotochemical quenching as part of the protective mechanism for PSII, this might be observed as an increase in leaf temperature. In some instances later in the flooding period for mamey sapote, leaf temperature was significantly greater for the flooded plants than the nonflooded plants (Fig. 3-4). In experiments with mamey sapote trees planted in an orchard, leaf temperatures of flooded plants became significantly greater than nonflooded plants by 1°C to 2°C (Chapter 5).

Net CO_2 assimilation in *P. orinocoensis* declined 37% from day 0 to day 3, but did not decline beyond that through day 20, remaining at between 3.5 to 3.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Fernández, 2006). In mamey sapote, A rates were near 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ by day 7 and later in both trials. While it was determined that *P. orinocoensis* exhibited dynamic (temporary) photoinhibition, but not chronic (permanent) photoinhibition (Fernández, 2006), perhaps in contrast, mamey sapote experienced chronic photoinhibition because A rates of 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ suggested significant enough deterioration of the photosynthetic apparatus took place.

In mamey sapote, Ci levels generally rose in the days following the initial decline in A, g_s, (Figs. 3-2 and 3-3) and E (data not shown). In trial 1, the Ci in 'Pantin' increased to above the ambient C_a level in the leaf cuvette of 330 μmol·mol^{-1} CO_2 to about 400 μmol·mol^{-1} CO_2 by day 10, and to just above 500 μmol·mol^{-1} CO_2 by day 14. Thus, it does not appear that the decline in A in later stages are due to a lack of internal CO_2 but rather due to some other inhibition or damage. In later stages of flood stress the cessation of carbon fixation may be due to a drop in carboxylation efficiency due to a lack of ATP being generated by photochemistry in the leaf mesophyll (Farquhar and Sharkey, 1982; Herrera et al. 2008; Lawlor, 2002). This indicates the possibility of damage to the photosynthetic apparatus and possible chronic photoinhibition.

The observed declines in A in mamey sapote may also be a response to ethylene, which can increase in response to flooding (Schaffer et al., 1992). Responses to a build-up of ethylene in leaves include leaf epinasty, yellowing and/or senescence (Kozlowski and Pallardy, 1984; English et al., 1995). In mamey sapote, leaf epinasty was visible by day 5 in both trials, followed by leaf yellowing and leaf senescence. Kozlowski and Pallardy (1984) attributed reduction of photosynthesis in flooded plants to be in part due to reduced chlorophyll content of leaves, early leaf senescence, and abscission. Flooded, 'Pantin' mamey sapote exhibited epinasty and chlorosis in the lower canopy while still maintaining a more healthy upper canopy. This epinasty and leaf senescence of the lower half of the canopy likely reduced the plants' overall transpiration.

Annona reticulata and *A. squamosa* seedlings are tropical fruit trees that are considered flood-sensitive and mango is considered relatively flood tolerant (Nuñez et al., 1999; Schaffer et al., 2006). *A. reticulata* and *A. squamosa* may respond within 1 d of flooding with reduced A and g_s. Vegetative growth such as budbreak, shoot growth, and leaf expansion are also reduced, and finally leaf wilting and necrosis followed by defoliation occurred between 15 to 30 d of flooding. Eventually branch dieback and tree death occurred if flooding continued for 30 to 50 d (Nuñez-Elisea et al., 1998; 1999). Mamey sapote's response timeline is very similar, with reductions in A, g_s, and E within 3 d, leaf epinasty between days 5 to 10, and leaf senescence and abscission between days 15 to 30. Branch dieback and tree death occurred in mamey sapote between days 30 to 60. In contrast, short term flooding of mango has shown A, g_s and E to drop by day 3. However, mango has also shown some long-term adaptability to flooding. While some trees may die soon after flooding, those that survive have shown ability to survive up to

110 days of flooding (Larson, 1991; Schaffer et al., 2006). Mamey sapote did not demonstrate ability to survive such long periods under the conditions tested.

It appears that 'Pantin' was able to tolerate flooded conditions for a longer period of time than 'Magaña' before there were significant reductions in A, g_s or E , leaf greenness, increased leaf epinasty, or chlorosis. Further studies would be needed to determine a more refined timeline on photosynthesis decline and to pinpoint damage to the photosynthetic apparatus, and stomatal and mesophyll limitations to photosynthesis. Mamey sapote appears to be relatively flood-sensitive because of the rapid decline in A, leaf epinasty and abscission, leaf chlorophyll index, and stem dieback.

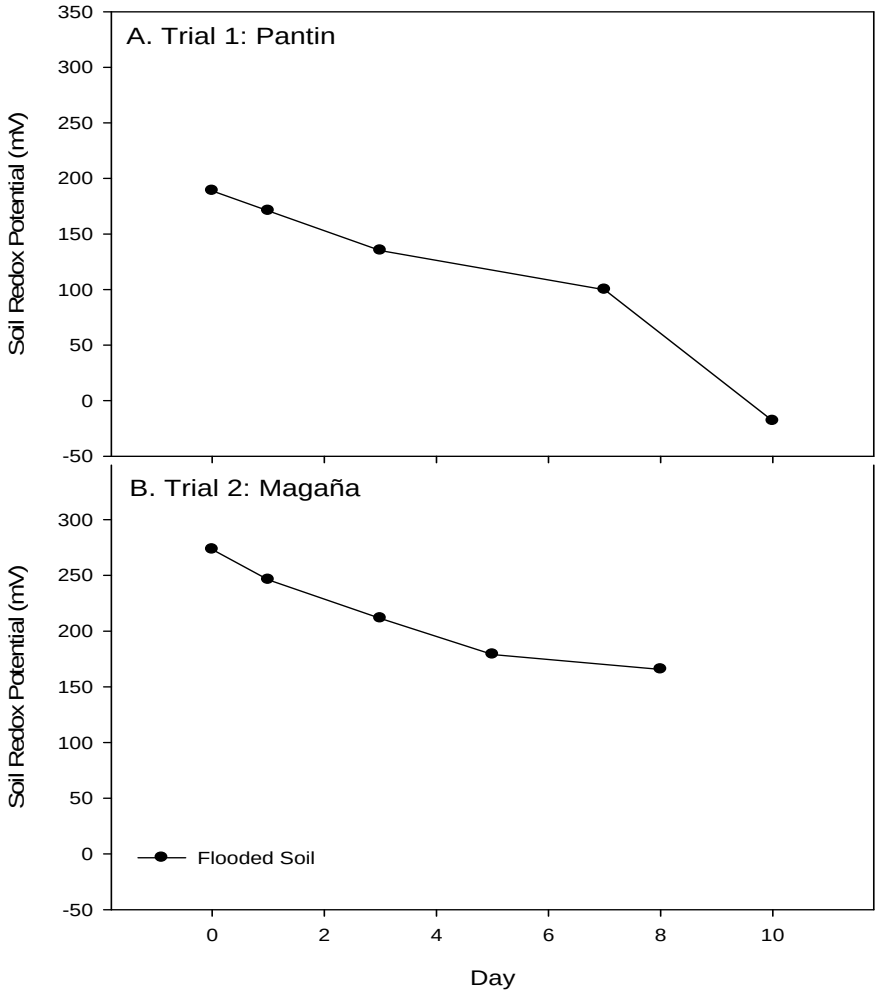

Figure 3-1. Soil redox potential of A) flooded 'Pantin' mamey sapote trees from 12 Apr. to 22 Apr. 2005 (Trial 1) and B) flooded 'Magaña' mamey sapote trees from 31 May to 8 June 2005 (Trial 2). Redox potentials below +200 mV indicate that soil conditions are anaerobic (Ponnamperuma, 1984).

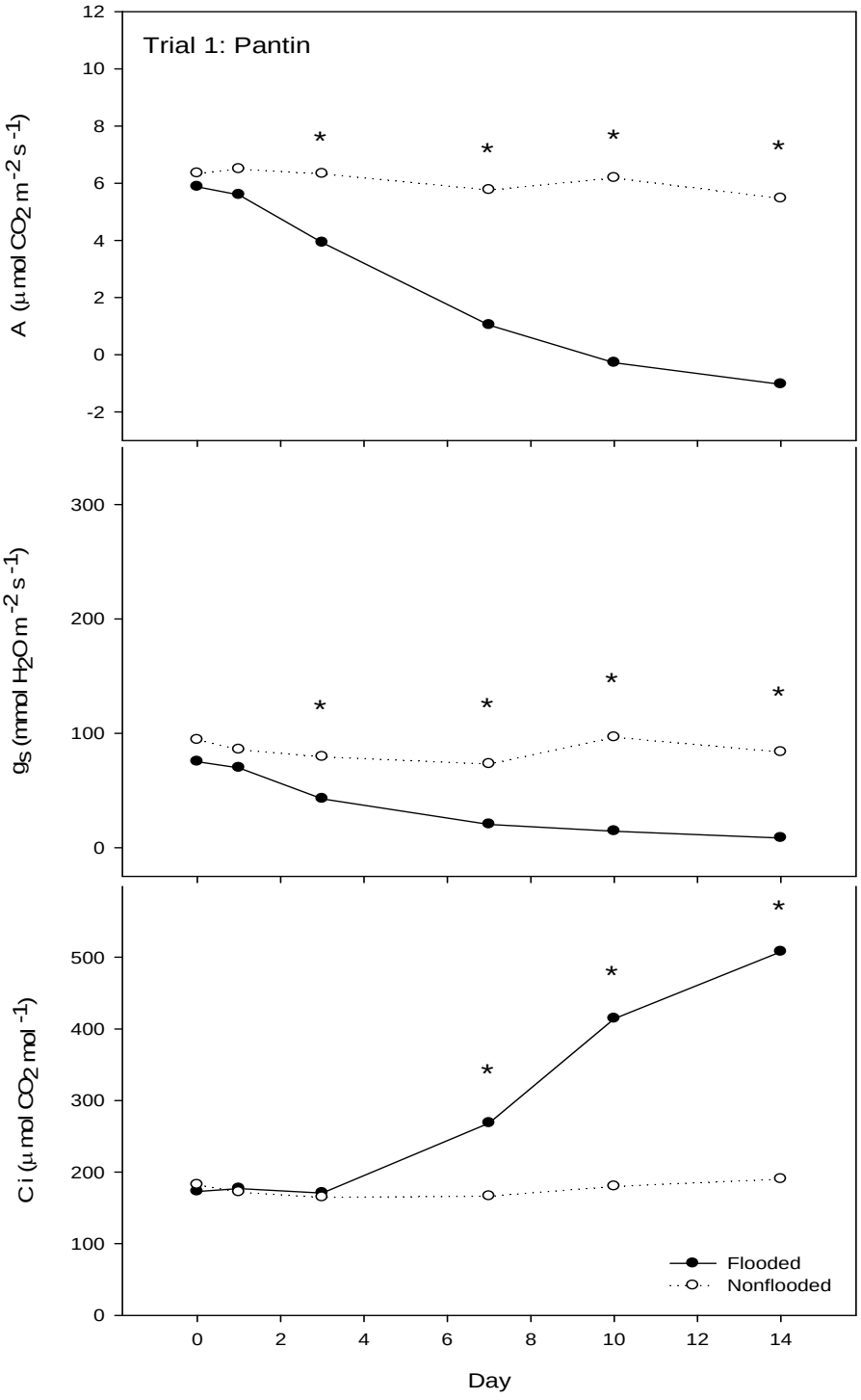

Figure 3-2. Effects of flooding on net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), and internal CO_2 concentration (Ci) in leaves of 'Pantin' mamey sapote trees from 12 Apr. to 26 Apr. 2005 (Trial 1). Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=10.

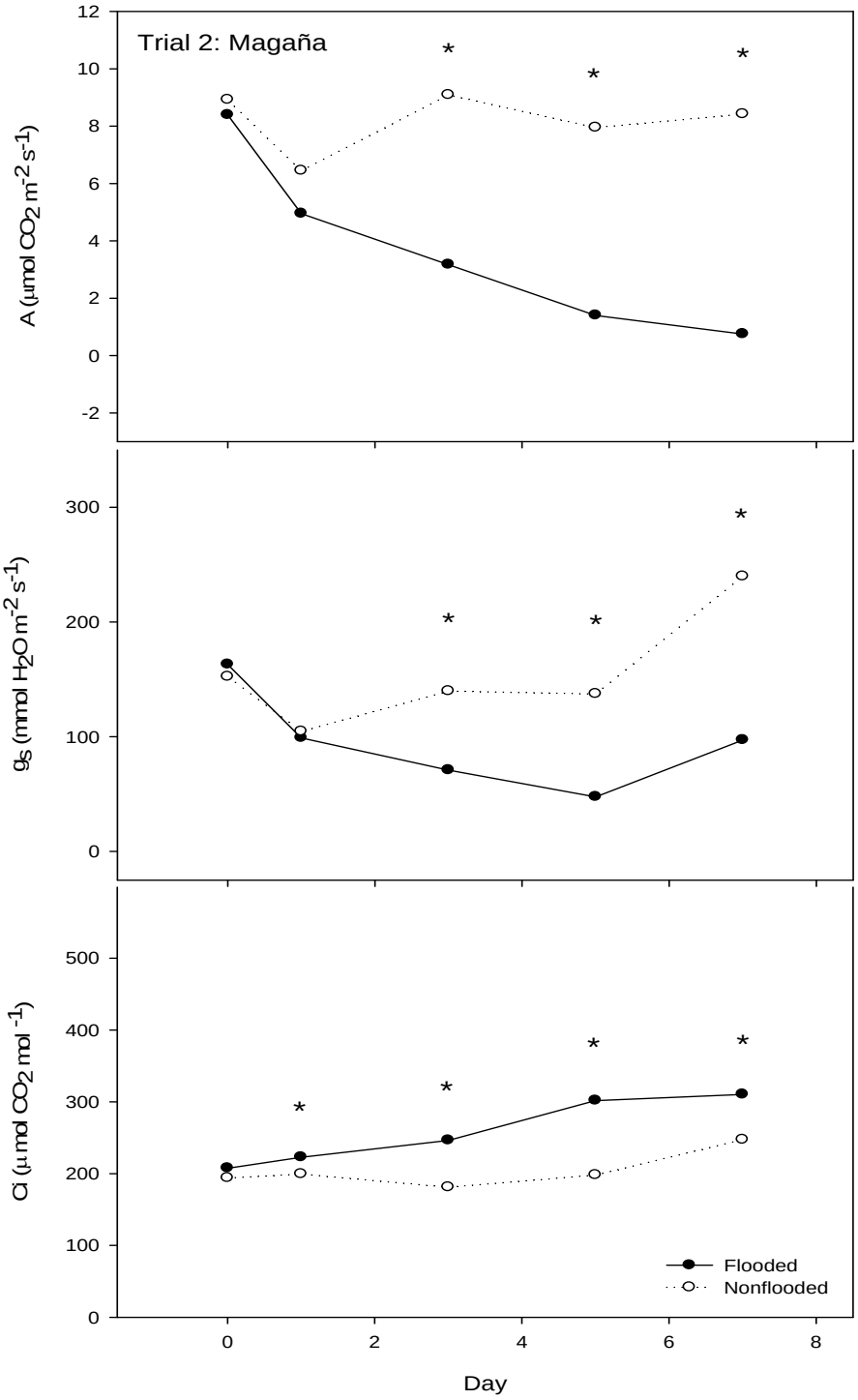

Figure 3-3. Effects of flooding on net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), and internal CO_2 concentrations (Ci) in leaves of 'Magaña' mamey sapote trees from 31 May to 7 June 2005 (Trial 2). Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=7.

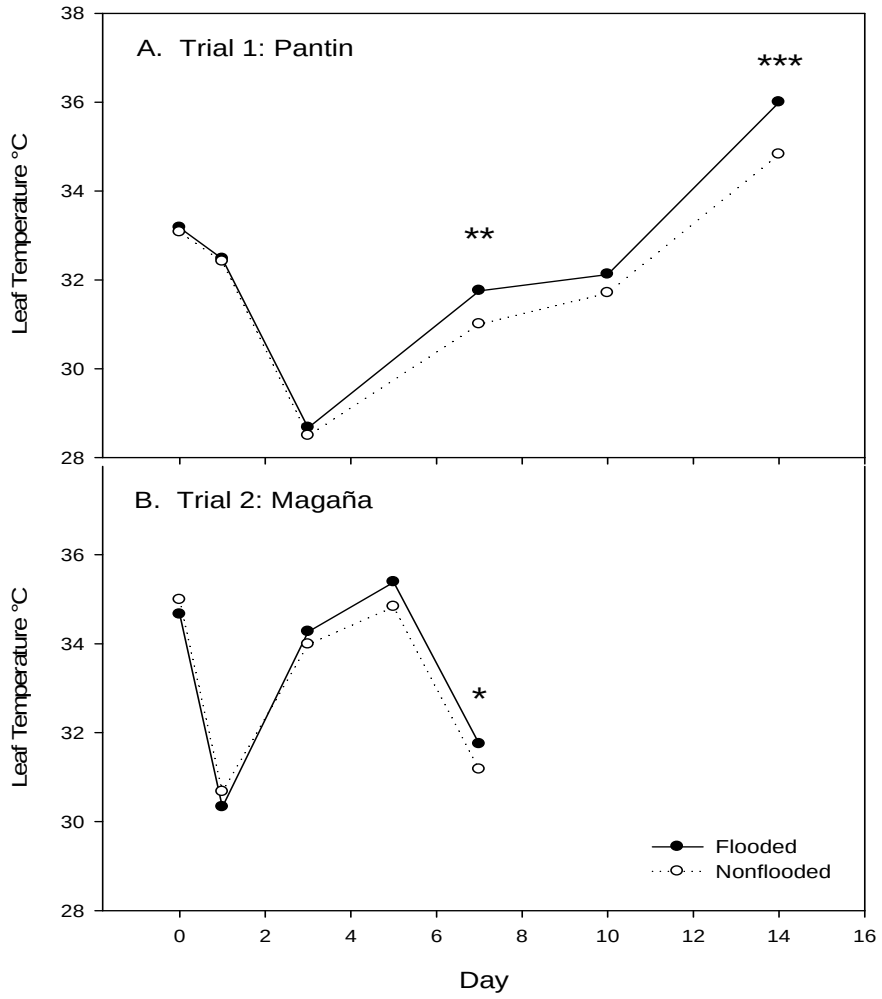

Figure 3-4. Effects of flooding on leaf temperature of A) 'Pantin' mamey sapote trees from 12 Apr. to 20 Apr. 2005 (Trial 1) and B) 'Magaña' mamey sapote trees from 31 May to 8 June 2005 (Trial 2). Asterisks *, **, and *** indicate significant differences according to a T-test, $P \leq 0.1$, 0.05, and 0.01, respectively.

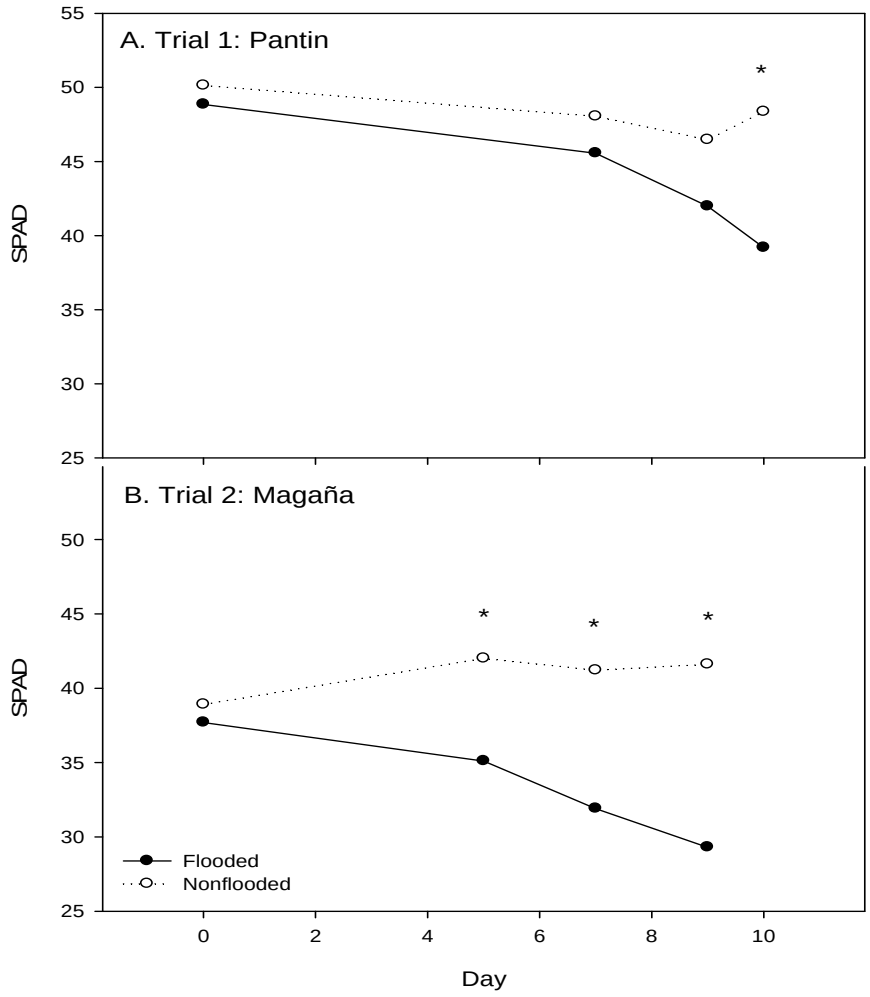

Figure 3-5. Effects of flooding on leaf chlorophyll index (SPAD values) of A) 'Pantin' mamey sapote trees from 12 Apr. to 22 Apr. 2005 (Trial 1) and B) 'Magaña' mamey sapote trees from 31 May to 9 June 2005 (Trial 2). Asterisks indicate significant differences according to a T-test ($P \leq 0.05$).

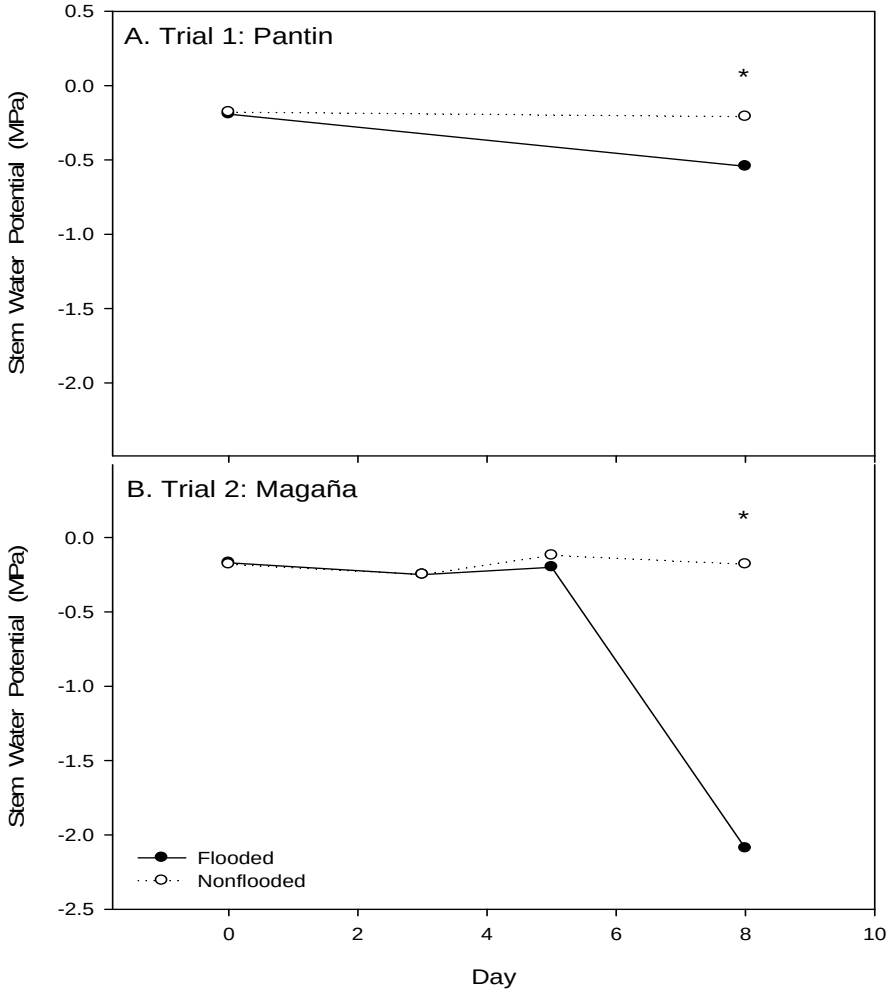

Figure 3-6. Effects of flooding on stem water potential of A) 'Pantin' mamey sapote trees from 12 Apr. to 20 Apr. 2005 (Trial 1) and B) 'Magaña' mamey sapote trees from 31 May to 8 June 2005 (Trial 2). Asterisks indicate significant differences according to a T-test ($P \leq 0.05$).

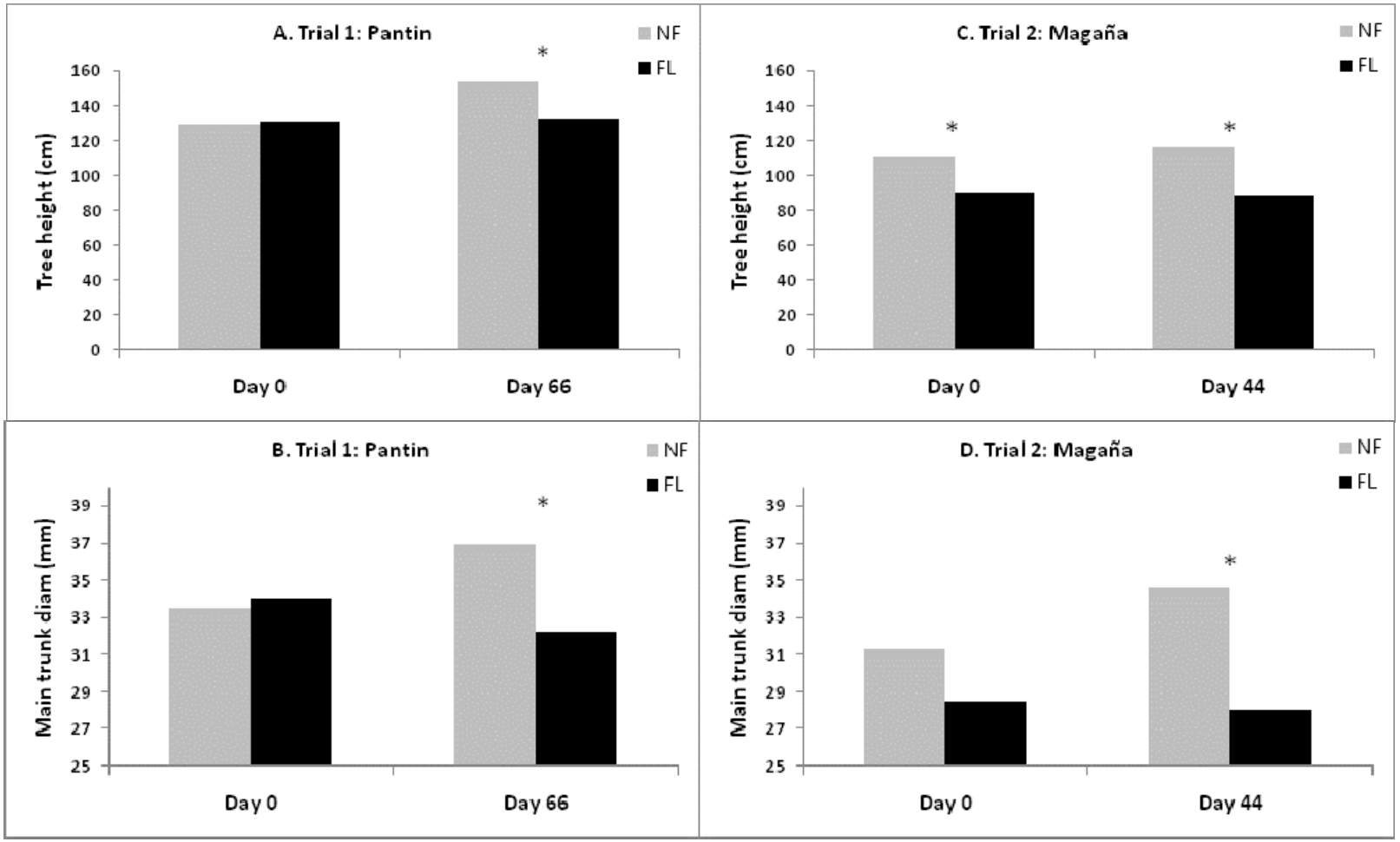

Figure 3-7. Tree height and trunk diameter. A) Mean tree height, and B) mean trunk diameter for nonflooded and flooded 'Pantin' trees in trial 1. C) Mean tree height, and D) mean trunk diameter for nonflooded and flooded 'Magaña' trees in trial 2. Asterisks indicate significant differences according to a T-test ($P \leq 0.01$).

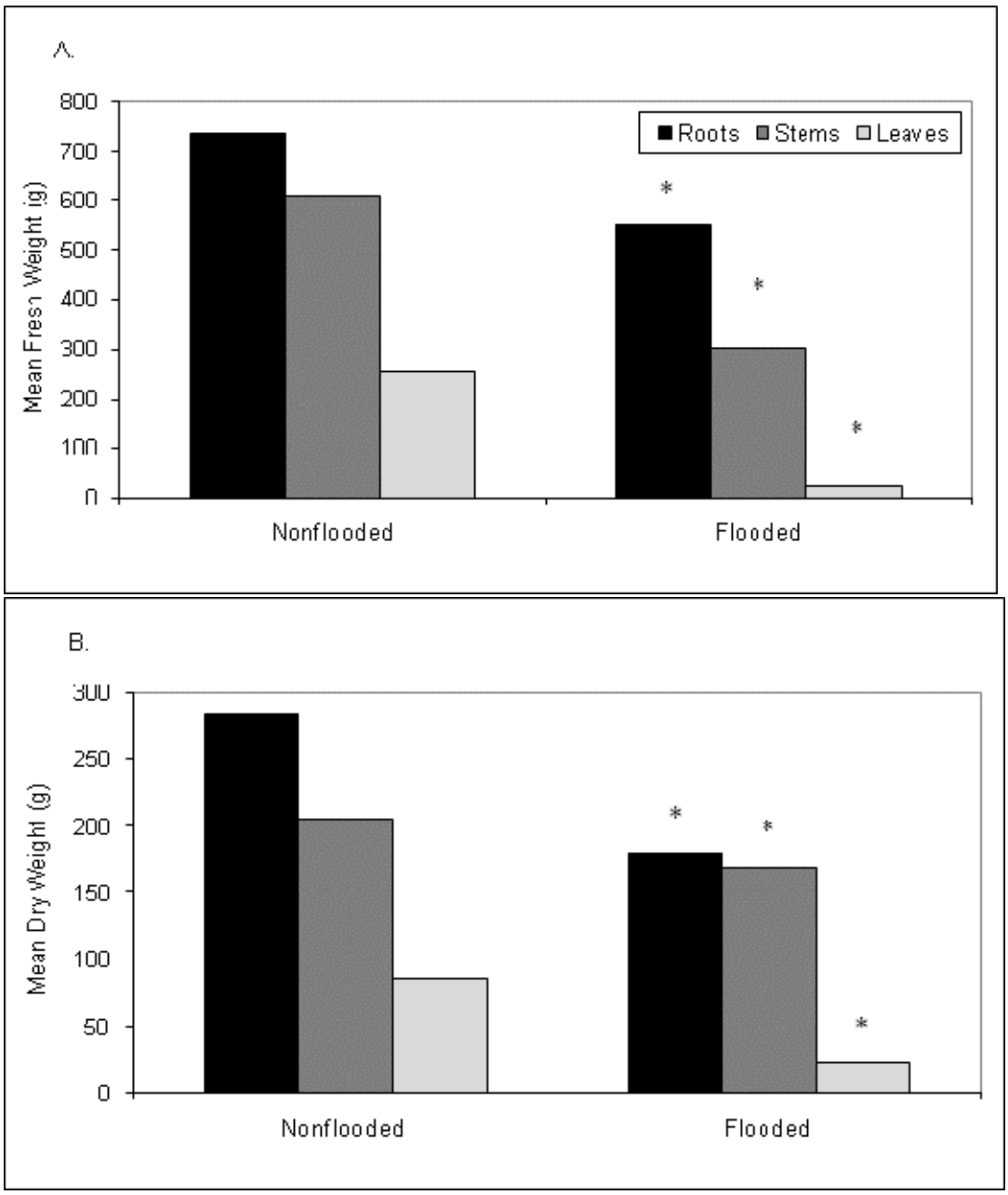

Figure 3-8. Mean harvest weights for Trial 1. A) fresh weights, and B) dry weights for roots, stems, and leaves of nonflooded and flooded 'Pantin' mamey sapote trees after 66 days of flooding. Asterisks indicate significant differences according to a T-test ($P \leq 0.01$), where roots, stems, and leaves of flooded plants weigh significantly less than nonflooded plants.

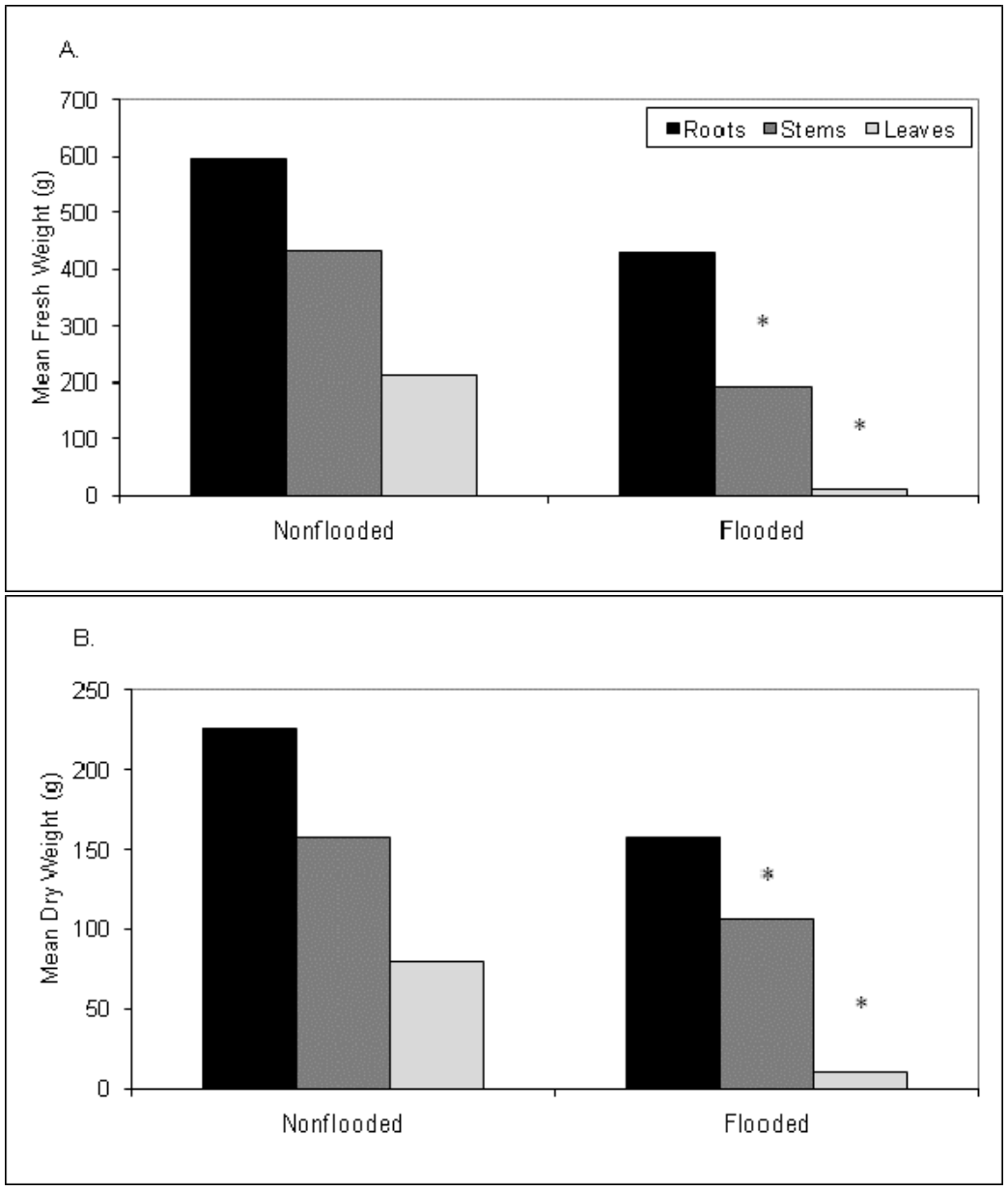

Figure 3-9. Mean harvest weights for Trial 2. A) fresh weights, and B) dry weights for roots, stems, and leaves of nonflooded and flooded 'Magaña' mamey sapote trees after 45 days of flooding. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), where stems and leaves of flooded plants weigh significantly less than nonflooded plants.

CHAPTER 4
RESPONSE OF MAMEY SAPOTE (*POUTERIA SAPOTA*) TREES TO CYCLICAL FLOODING IN CALCAREOUS SOIL IN CONTAINERS

Introduction

Mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn] is a tropical tree native to the humid lowlands of southern Mexico to northern Nicaragua in Central America (Balerdi and Shaw, 1998; Verheij and Coronel, 1992). The species is grown commercially as a tropical fruit crop in several parts of the world including Mexico, Central America and the Caribbean Basin (Balerdi and Shaw, 1998; SAGARPA, 2008). In the United States commercial production is concentrated primarily in Miami-Dade County, Florida, where as of 2009, it is estimated to be grown on 233 ha (575 acres) and is annually worth an estimated $7.5 million at the farm level, and about $18.5 million at the wholesale level (E. Evans, University of Florida, personal communication). The calcareous agricultural soil on which fruit crops are grown in this area is classified as Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) (Burns et al. 1965; Leighty and Henderson, 1958; Nobel et al., 1996).

Periodic flooding is a problem in some lowland tropical fruit production areas. Typically flood-prone areas are flooded repeatedly due to recurrent wet-dry seasons and climate patterns such as monsoon climates where one or more wet seasons may be interspersed with one or more dry seasons per year (Jackson, 1989; Schaffer and Andersen, 1994). In southern Florida, mamey sapote orchards on calcareous soils may be subjected to periodic flooding during high water table conditions which coincide with periods of heavy rainfall and/or tropical storms (J.H. Crane, personal communication). Depending upon water management and storm activity in south Florida multiple flooding events which may last 2 to 10 d each may occur within any given year

(NWS-NHC, 2008). Flooding of mamey sapote orchards in this area has generally resulted in tree decline and death (Crane et al., 1997; Degner et al., 2002).

One of the first physiological responses of trees to flooding is a decrease in stomatal conductance (g_s) due to stomatal closure, which results in decreased transpiration (E) and maintenance of high leaf water potential (Kozlowski, 1997; Kozlowski and Pallardy, 1984; Schaffer et al., 1992). Concomitant with this decrease in g_s is a decline in net CO_2 assimilation (A) (Kozlowski, 1997; Kozlowski and Pallardy, 1984; Schaffer et al., 1992). The temporal separation between reduced A and g_s in flooded fruit trees, if there is one, has not been determined, and thus, it is not clear if reductions in A as a result of flooding are due to stomatal or non-stomatal factors (Schaffer et al., 1992). Measuring internal partial pressure of CO_2 (Ci) in leaves may provide a clue to determining if reductions in A are due to stomatal or non-stomatal factors. Concurrent decline in Ci with decreased A and g_s may indicate stomatal limitation to a sufficient quantity of CO_2 entering the leaf for maintaining A at an optimum level. However, an increase in Ci accompanied by decreased A and gs in flooded trees may indicate a non-stomatal or mesophyll limitation to A (Farquhar and Sharkey, 1982) which can result from increased CO_2 in the intercellular space of the leaf which has been associated with stomatal closure (Mansfield et al. 1990; Raschke, 1975a; 1975b). Previous research has determined that continuous flooding of mamey sapote for 45 to 66 d, resulted in decreased A, g_s, and transpiration (E) after 3 d, leaf epinasty between days 5 to 10, and leaf senescence and abscission between days 15 to 30 after trees were flooded. Branch dieback and tree death occurred within 30 to 60 d of continuous flooding (Chapter 3).

Relatively little has been published about the tolerance of fruit crops to repeated short-term flooding (Crane and Davies, 1988; Gur et al., 1998; Joyner and Schaffer, 1989; Nuñez-Elisea et

al., 1999). Peach trees (*Prunus persica* L.) appear to be intolerant of repeated flooding of 4 to 5 d per wk over a 9 to 10 wk period (Gur et al., 1998). In contrast, 'Golden Star' carambola (*Averrhoa carambola* L.) tolerated intermittent flooding periods of 3 wks followed by 3-wk periods of nonflooded recovery, repeated over 18 wks, with leaf gas exchange returning to near normal levels post-flooding (Joyner and Schaffer, 1989). The effect of repeated short-term flooding of rabbiteye blueberry plants (*Vaccinium ashei* Reade) was also found to decrease A; however, seasonally high air and soil temperatures also contributed to reduced A (Crane and Davies, 1988). The purpose of this study was to determine the effects of repeated cycles of short-term flooding on leaf gas exchange, leaf and stem water potential, and overall vigor of mamey sapote trees.

Materials and Methods

Plant material. In March 2004, two-year-old 'Pantin' and 'Magaña' mamey sapote (*Pouteria. sapota*) grafted onto seedling rootstocks were obtained from a commercial nursery and repotted into 19 L plastic containers filled with Krome very gravelly loam soil. Trees were acclimated in the soil for about 1 year. To preclude phytophthora (*Phytophthora cinnamomi* Rands) or pythium (*Pythium splendens* Braun) root rots, trees were treated with soil applications of the fungicides, metylaxyl (Ridomil™; Syngenta Crop Protection, Inc., Greensboro, NC) on 28 Jan. 2005, and fosetyl-Al (Aliette™, Bayer CropScience. Research Triangle Park, NC) on 4 Apr. 2005. They were also treated with metylaxyl one to two weeks prior to initiating treatments. All trees were housed in an open-air structure in which all sides were screened and an arch shape roof was covered with two sheets of clear plastic (screenhouse).

Experimental design. Three cyclical flooding trials were conducted with each period of flooding (F) followed by a period of nonflooded recovery (R). In trial 1, the flooding period of 3 d was based on data from a previous study flooding mamey sapote in which significant decreases

in leaf gas exchange occurred after 3 d of continuous flooding (Chapter 3). Flooding periods were doubled to 6 d for trials 2 and 3 after examination of data from trial 1. Thus, trial 1 comprised 3 d of flooding followed by 3 d of recovery (F3-R3), trial 2 comprised 6 d flooding and 6 d recovery (F6-R6) and trial 3 comprised 6 d flooding and 3 d recovery (F6-R3). The flooding and recovery cycles in each trial were repeated three times total.

Trial 1 was conducted from 7 Oct. to 22 Oct. 2005 with the cultivar Magaña and trials 2 and 3 were conducted concurrently with the cultivar Pantin from 24 Oct. to 29 Nov. 2006. For a nonflooded control treatment, the same group of plants was used for trials 2 and 3. In all trials there were 7 single-plant replications per treatment arranged in a completely randomized design.

Plants were flooded by submerging the 19-L containers containing the plants and soil inside 38-L containers filled with water from a well. Plants were submerged to a water level of 5 cm above the soil surface. The nonflooded plants were drip irrigated for 10 min daily, which amounted to about 3.8 L of water per day. During the "recovery periods" the plant containers were removed from the flooding containers and the soil was allowed to drain. During the recovery period, plants were drip irrigated at the same rate and schedule as the nonflooded control plants. After the recovery periods, trees were returned to their flooding containers for the next flooding period.

Data collection. For each single-plant replication, leaf gas exchange and stem water potential were determined periodically. Plants in trial 1 were not harvested due to damage from a hurricane at the end of the experimental period.

At the completion of trials 2 and 3 on 29 Nov. 2006, plants were left for long-term recovery. Leaf gas exchange was measured a final time in trials 2 and 3 on day 84, which was 54 and 60 d respectively, after final removal of plants from flooding. Plants were harvested on

day 167 (9 Apr. 2007) for fresh and dry weights, 137 and 143 d after final removal from flooding in trials 2 and 3, respectively.

Leaf gas exchange, including A, g_s, internal CO_2 concentration (Ci), and E, was monitored with a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA) for plants in each treatment. Leaf gas exchange measurements were made at a photosynthetic photon flux (maintained with a halogen light affixed to the leave cuvette) of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an air flow rate of 200 $mL \cdot min^{-1}$ into the leaf cuvette. The reference CO_2 concentration for leaf gas exchange measurements was 350 $\mu mol \cdot mol^{-1}$ in trial 1, and 375 $\mu mol \cdot mol^{-1}$ in trials 2 and 3. Leaf gas exchange was measured every 3 d beginning on day 0 and ending the last day of the last flooding-recovery cycle for all trials. One recently mature leaf (the 5th or 6th leaf below the apical meristem) was measured on each plant.

Leaf or stem water potential (Ψ_l or Ψs) was measured every 3 d beginning on day 0 and ending the last day of the trial for all trials. Water potential was determined with a pressure chamber (Plant Water Status Console 3000 Series, Soilmoisture Equipment Corporation, Santa Barbara, CA). Non-bagged leaves were used to measure leaf water potential, and bagged leaves were used to measure stem water potential. Although it has been suggested that leaves should be enclosed in plastic bags prior to water potential measurements (Shackel et al., 1997), this was not done in trial 1 because a preliminary study showed no significant difference in water potential between bagged and nonbagged leaves of plants not under water stress (M. Nickum, unpublished data), and bagged leaves of trees under long-term flooding stress were observed to abscise. However, in trial 1 cyclical flooding did not result in leaf abscission as observed in the preliminary, long-term flooding study. Therefore for trials 2 and 3 all leaves were covered with plastic bags surrounded by aluminum foil for at least 1 hr prior to water potential measurements.

In trial 1, Ψ_l was measured in the field whereas in trials 2 and 3 bagged leaves were detached and placed into a styrofoam cooler and immediately taken to a laboratory for Ψs measurements.

In all trials, soil temperatures were monitored with a HOBO Water Temp Pro (Onset Computer Co., Bourne, MA) and canopy temperatures were monitored with a StowAway TidbiT (Onset Computer Co., Bourne, MA). In trials 2 and 3, relative humidity (RH) was measured with a HOBO RH/Temp (Onset Computer Co., Bourne, MA). During the final recovery period for trials 2 and 3, ambient temperatures were monitored with an automated weather station located within a few hundred meters of the experimental site at 60 cm above soil line (University of Florida Automated Weather Network, http://fawn.ifas.ufl.edu).

Soil redox potential was measured in the flooded treatments with a metallic ORP indicating electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) connected to a volt meter. In all trials soil redox potential was measured after the first day of flooding (Day 1) and again on day 15 to confirm anoxic soil conditions. Sleeves made from irrigation tubing with several holes drilled in the sides were permanently installed into soil in each container to permit insertion of the electrode into the soil water for measurement.

Data analysis. All data were analyzed by ANOVA and standard T-test using the SAS statistical software package (Version 9.1, SAS Institute, Cary, North Carolina)

Results

Soil redox potentials declined rapidly after flooding, with the soil in each flooded container reaching a redox potential below 200 mV, with a mean near 160 mV during all three cyclic flooding trials (data not shown). No symptoms of leaf chlorosis, epinasty, wilting, or abscission were observed in any trial.

Trial 1

Temperatures. Ambient air temperatures ranged between 22-39°C, and flooded and non-flooded soil temperatures were slightly lower than air temperatures (Fig. 4-1).

Leaf gas exchange. Net CO_2 assimilation remained relatively constant for the nonflooded plants throughout the experiment with a mean for the nonflooded plants between 6 to 8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Fig. 4-2), whereas A for the flooded plants decreased to about 4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ during each of the first two flooding periods, and recovered to control levels during the nonflooded period. However, there was no significant difference between treatments during any flooding period. During the last 3 d flooding period from days 12 to 15, A of both flooded and nonflooded treatments was slightly above 8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. There were no significant effects of flooding on g_s, Ci (Fig. 4-2), or E (data not shown) and the general trends appeared similar for both nonflooded and flooded treatments.

Water potential. Leaf water potential of the nonflooded treatment remained between -0.1 to -0.2 MPa over a 15-d period (Fig. 4-3). After the first 3-d flood period, Ψ_l of flooded plants decreased significantly to about -0.8 MPa; however, after 3 d of recovery (on day 6), Ψ_l of the flooded plants returned to that of the non-flooded plants. During the second 3-d flood period to day 9, there was also a decrease in the Ψ_l in the flooded treatment to about -0.6 MPa, although this was not significantly different from that of the nonflooded treatment. The 3-d recovery period from days 9 to 12 again showed recovery of the flooded treatment to nonflooded Ψ_l levels. During the final 3-d flood period Ψ_l of the flooded plants again decreased to levels lower than those of plants in the nonflooded treatment. Thus Ψ_l decreased during each flooding period, and recovered to levels similar to those of the non-flooded plants after each recovery period.

Trials 2 and 3

Temperatures and relative humidity. Ambient temperatures during the 54-d treatment period ranged from 10 to 41°C with a mean of about 27°C (Fig. 4-4). Relative humidity ranged from about 22 to 88% with a mean of about 35% (Fig. 4-4). Soil temperatures in the flooded and nonflooded treatments soils were slightly lower than air temperatures (Fig. 4-5). Soil and air temperatures reached lows close to 10°C over the course of about 5 d, the lowest night reaching about 6°C during the third flooding period for trial 2 and after the third flooding period for trial 3 (Fig. 4-5). Over the post-experiment recovery period from day 54 to 168, ambient air temperatures ranged from 0 to 30°C (Fig. 4-6).

Leaf gas exchange. Net CO_2 assimilation of plants in the nonflooded treatment remained between 8 to 10 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for the first two flood-recovery cycles (24 d) in both trials (Figs. 4-7 and 4-8).

Trial 2. After the first 6-d flood period, A declined to about 2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ and Ci levels became significantly higher for the flooded treatment than the nonflooded treatment (Fig. 4-7). Stomatal conductance was significantly lower on days 3 and 6 for the flooded treatment than the nonflooded treatment (Fig. 4-7). During the first recovery period, stomatal conductance returned to pre-flood levels. Net CO_2 assimilation of the flooded plants returned to pre-flood levels after the first 6-d recovery period (to ~ 9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ by day 12), although A was still significantly lower for plants in the flooded treatment than those in the nonflooded treatment due to an increase in the A of the nonflooded plants.

Plant responses during the second cycle of flood and recovery were similar to responses during the first cycle. On the third day of the flooding period gs and A declined in plants in the flooded treatment. Net CO_2 assimilation declined to about 3 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in the flooded

treatment by the sixth day of the flood period, and Ci significantly increased. However, 3 d into the second recovery period A of plants in the flooded treatment was only 2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, which was significantly lower than that of the nonflooded treatment which was 10 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. Internal CO_2 concentration remained higher and gs remained lower in plants in the flooded treatment compared to those in the nonflooded treatment. Six days into the second recovery period, A of plants in the flooded treatment increased slightly to 5 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, but this was still significantly lower than that of the nonflooded plants. The Ci was still slightly, but not significantly higher and g_s was still significantly lower in plants in the flooded treatment compared to those in the nonflooded treatment.

During the third flooding period, plants in both flooded and nonflooded treatments exhibited reduced A; however, A of plants in the flooded treatment was significantly lower than that of plants in the nonflooded treatment.

For plants in the flooded treatment, Ci was significantly higher by the end of the third flooding period (same trend as the previous two flooding periods) compared to that of plants in the nonflooded treatment. Stomatal conductance was not significantly different between treatments during this flooding cycle. By day 3 of the recovery period, temperatures returned to previous levels of about 20°C or above. Net CO_2 assimilation for the flooded treatment slowly increased to nonflooded levels, until day 41 (11 d recovery after the third flooding period) when there was no significant difference in A between the nonflooded and flooded treatments. Stomatal conductance and Ci also returned to nonflooded levels over time. On day 84, (54 d after final flooding period) there were no significant differences in A, g_s, Ci, or E between treatments (data not shown).

Trial 3. Leaf gas exchange results for trial 3 were similar to those observed in trial 2. During the first flooding period, A and g_s declined significantly by day 3 (Fig. 4-8). By day 6, A of plants in the flooded treatment decreased significantly to 3 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, and Ci increased slightly for the flooded plants, but differences in Ci were not significant between treatments. There were no significant differences in A, g_s, and Ci between plants in the flooded and nonflooded treatments after the first 3-d recovery period (day 9).

During the second flood period there were significant reductions in A and g_s by day 3, and significant increase in Ci for plants in the flooded treatment by day 6 of flooding. At the end of the second 3-d recovery period A and g_s were still significantly different between treatments.

During the third flood period, A of plants in the flooded treatment were significantly lower than that of plants in the nonflooded treatment and remained at about 5 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ without a noticeable decline (Fig. 4-8). Stomatal conductance continued to be significantly lower in plants in the flooded treatment than those in the nonflooded treatment, but Ci of plants in the flooded treatment did not increase significantly during the third flood period. However, 5 d into recovery after the third flood period, Ci of flooded plants increased significantly compared to that of plants in the nonflooded treatment. Air and soil temperatures were low (6-10°C) during this period (Figs. 4-5 and 4-6). On days 33 and 36 (9 and 12 d after the last flood period) Ci levels of plants in the flooded treatment returned to those of plants in the nonflooded treatment, while A was significantly lower for flooded than nonflooded plants until day 50. On day 50 there were still significant differences in gs and E (data not shown) between flooded and nonflooded treatments. On day 84 there was no significant difference between nonflooded and flooded plants for all gas exchange variables measured (data not shown).

Water potential. For both trials 2 and 3 there was no significant difference in Ψs during the 6-d flooding periods or after the 6- or 3-d recovery periods between plants in the nonflooded and flooded treatments. Mean Ψs levels were in the range of -0.2 to -0.6 MPa (data not shown).

Plant fresh and dry weights. There were no significant differences between treatments for mean fresh or dry wt of roots, shoots, or leaves in either trial 2 or trial 3 (Fig. 4-9). However, root fresh and dry wt tended to be greater for repeatedly flooded plants compared to nonflooded plants. The dry wt root:shoot ratio of plants in the nonflooded treatment was 1.0, and for the cyclically flooded plants of trials 2 and 3 was 1.20 and 1.16, respectively.

Discussion

Three cycles of 3-d flooding and 3-d recovery had little effect on leaf gas exchange (i.e., A, g_s, and Ci) of 'Magaña' mamey sapote trees. Leaf water potential temporarily declined the third day of flooding during each cycle. This suggests that young mamey sapote trees under orchard conditions may tolerate brief periods of soil saturation or flooding which may occur during the rainy season. Similarly, rabbiteye blueberry plants tolerated 2 cycles of 2 to 7 days of flooding, although the recovery period was much longer (Crane and Davies, 1988).

'Pantin' mamey sapote trees tolerated 3 cycles of 6-d flooding interspersed with 3 to 6 d of recovery despite a consistent decline in A and g_s during flooding. The temporary decrease in A during the flooding period did not appear to be due to stomatal closure as the Ci increased during, or immediately, after each flooding period and then declined to nonflooded levels. Similarly, Ci was higher for the leaves of continuously flooded 'Pantin' and 'Magaña' mamey sapote plants than for nonflooded plants, and g_s and A decreased for the flooded plants (Chapter 3). Again, this suggests negative non-stomatal effects on A during flooding.

The decrease in A for all treatments on day 25 was probably due to cool temperatures which dropped to about 11°C (Adams III et al., 1994; Zhou et al., 2007). In both trials 2 and 3 it

appears that the low temperatures coupled with any moderate existing damage to the photosynthetic apparatus of the flooded treatment accentuated the observed raise in Ci on day 29.

There was a tendency for Ci levels to become higher in plants in the flooded treatment than those in the nonflooded treatment during the latter part of the flooding period and the early part of the recovery period (Figs. 4-7 and 4-8). This may indicate some form of damage or limitation to the photosynthetic system as a contributing cause to lower A levels, because if normal carbon fixation was occurring, A would have been positive, and the Ci in the leaf would have been reduced as CO_2 was fixed. However, in both trials 2 and 3, flooded Ci returned to levels similar to those of nonflooded plants within 6 to 10 d after the final flooding period, and Ci never reached levels above the reference CO_2 level of 375 $\mu mol \cdot mol^{-1}$. Leaf water potential was reduced after cyclic flooding periods, and reduced leaf water content can result in photoinhibition (Lawlor, 2002). When the relative water content of the leaf is reduced, the relative ionic concentration of Mg^{2+} is increased which results in the inactivation or loss of function of the ATP synthase coupling factor. The loss of ATP production limits RuBP synthesis, thus reducing carboxylation efficiency and decreasing overall A potential (Lawlor, 2002). In *Pouteria orinocoensis* (Aubr.) Penn. Ined., which is considered flood tolerant, carboxylation efficiency was reduced to 70% of its pre-flooded level after 7 d of flooding, and reduced to 30% of its pre-flooded level after 20 d of flooding (Fernández, 2006). The level of damage which may have occurred to the photosystems of mamey sapote during these cyclic flooding experiments is not known. Recovery of leaf gas exchange to near nonflooded levels took place within 10 to 25 d after the final flooding period ended. Chronic photoinhibition may take a long time to be reversed, sometimes months, as it is necessary for the photosystems,

particularly the D1 proteins, to be rebuilt (Melis, 1999). Another option plants may have to overcome leaf damage is the formation of new leaves.

In the shorter 3-d flooding periods of trial 1, Ψ_l became lower for the flooded treatment after each 3-d flooding period and recovered to normal levels after each 3-d recovery period (Fig. 4-3), whereas in the longer 6-d flooding periods in trials 2 and 3, there were no significant differences in Ψs between treatments. From the differences in Ψ_l and Ψs between treatments, it appears that the petiole may play a role in control of water flow into the leaf of mamey sapote. Petioles may exert control on xylem sap flow rate by the use of aquaporins (Secchi et al., 2007; Ye et al., 2008), and also by the diameter and number of xylem vessels per vascular bundle, which can be different between varieties or different phenotypes of the same species (Dodd et al., 2008).

Mamey sapote appears much more flood-sensitive than some tropical fruit trees in Krome very gravelly loam soil, including carambola and mango, but similar to sugar apple (*Annona squamosa*) and custard apple (*Annona reticulata*) (Joyner and Schaffer, 1989; Larson et al., 1991a; 1991c; Nuñez-Elisea et al., 1998; 1999; Schaffer et al., 2006), For example, much longer periods of flooding were utilized in cyclic flooding experiments with carambola trees, with flooding periods lasting 3 wks and recovery periods lasting 3 to 6 wks (Joyner and Schaffer, 1989). Despite these relatively long flooding cycles, carambola leaves were able to recover to relatively normal gas exchange levels when unflooded (Joyner and Schaffer, 1989).

Evidence from these three cyclic flooding trials indicate that mamey sapote is capable of withstanding repeated periods of flooding for 3 to 6 d followed by at least an equal period of recovery. However, periods of flooding longer than 7 d may lead to both physiological decline (e.g., stomatal and nonstomatal decreases in A) and physical decline (e.g., leaf epinasty,

desiccation, and senescence) (Chapter 3). Thus under natural flooding cycles which in monsoon climates or rainy seasons can lead to multiple floods of 2 to 10 d per event (Jackson, 1989; NWS-NHC, 2008; Schaffer and Andersen, 1994), mamey sapote trees may be able to survive.

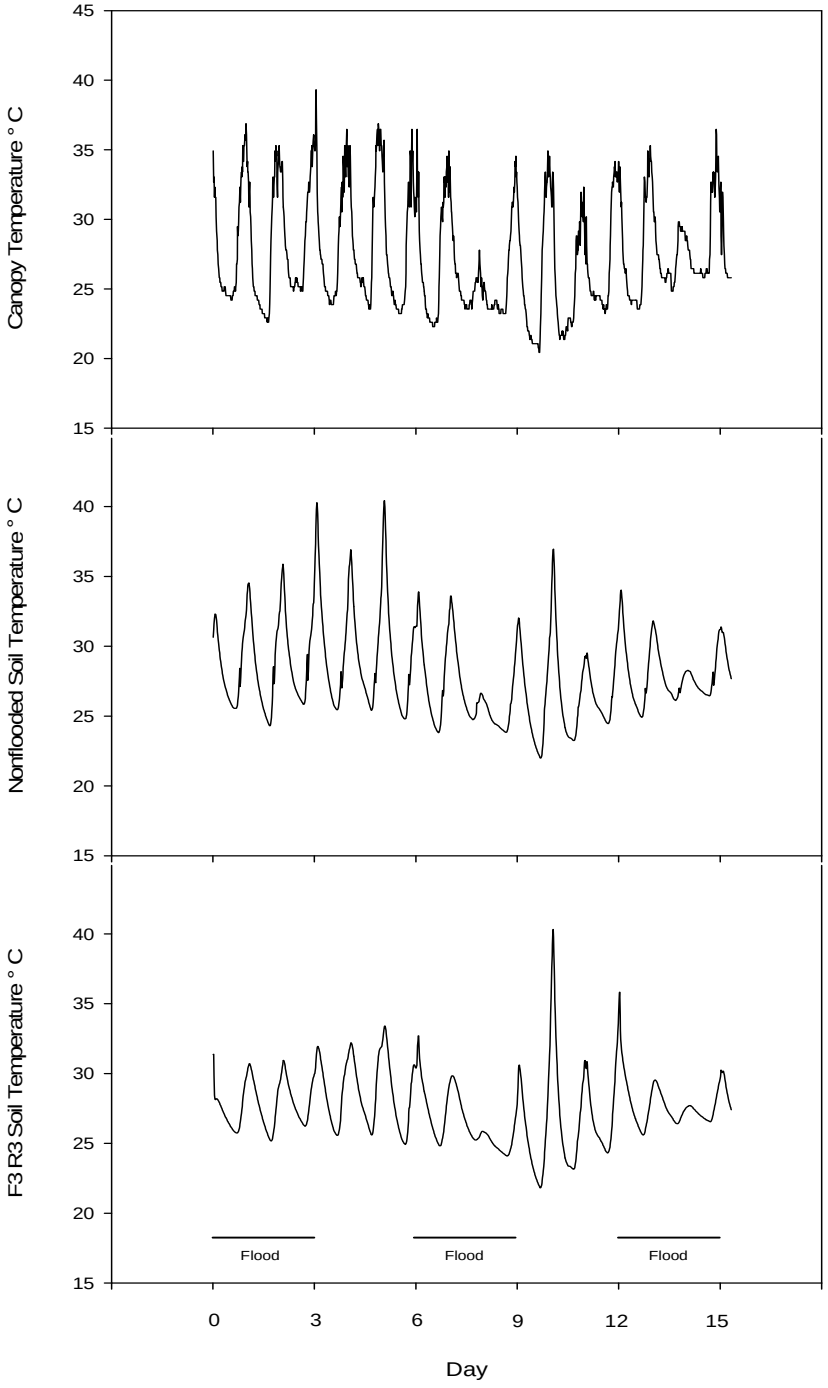

Figure 4-1. Canopy air temperature and nonflooded and flooded soil temperature from 7 Oct. to 22 Oct. 2005 for trial 1 – F3R3 (flooded 3 days/unflooded 3 days).

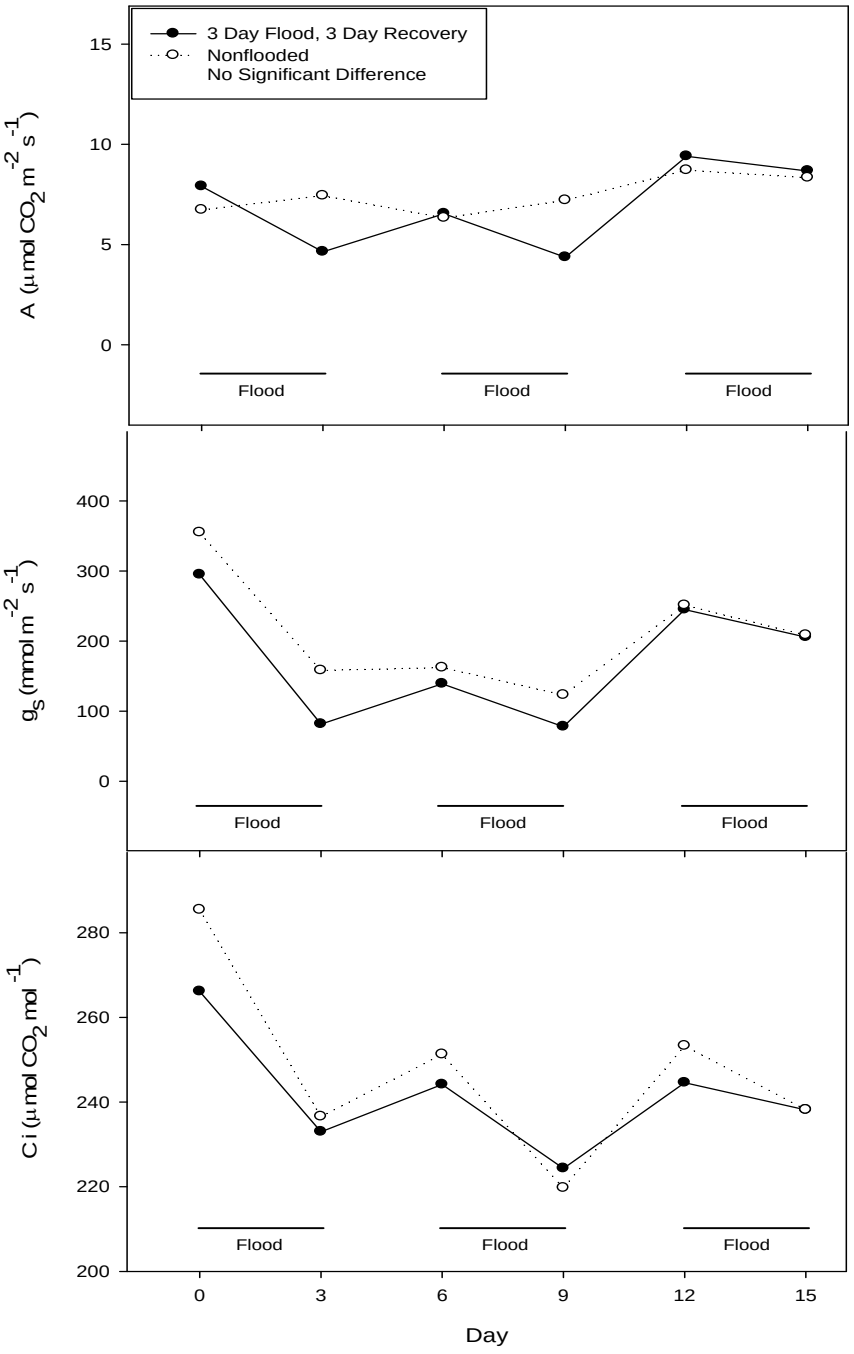

Figure 4-2. Effect of flooding on net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), and internal CO_2 concentrations (Ci) in leaves of 'Magaña' mamey sapote trees from 7 Oct. to 22 Oct. 2005 for trial 1 – F3R3. No significant differences were found according to a T-test ($P \leq 0.05$), n=7. Reference (ambient) CO_2 level = 350 µmol·mol^{-1}.

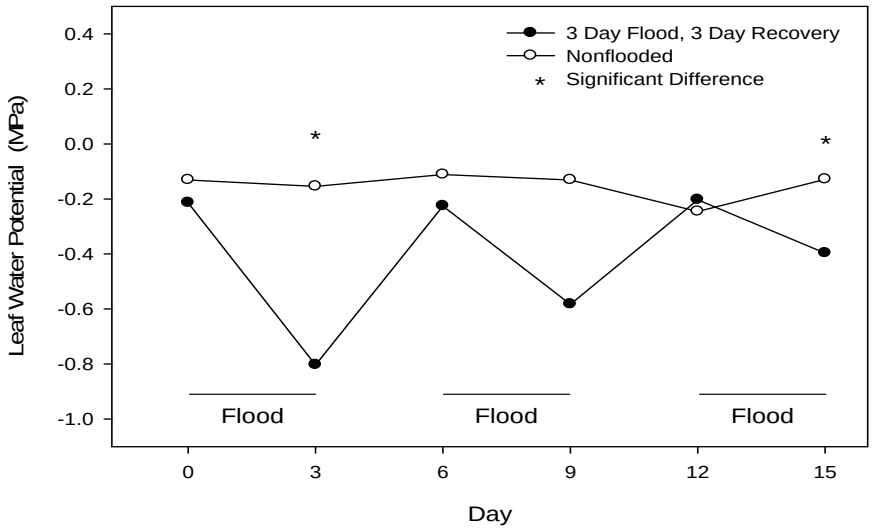

Figure 4-3. Effect of flooding on leaf water potential (Ψ_l) in leaves of 'Magaña' mamey sapote trees from 7 Oct. to 22 Oct. 2005 for trial 1 – F3R3. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=7.

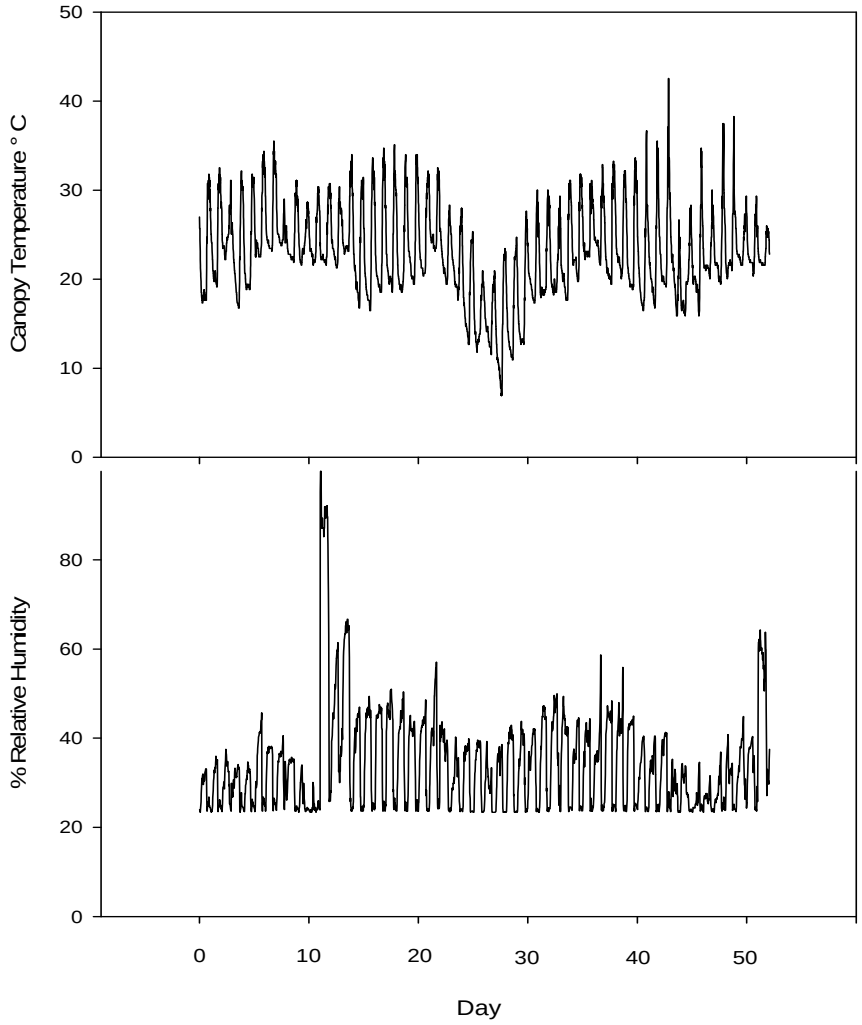

Figure 4-4. Air temperature and percent relative humidity within the tree canopy from 24 Oct. to 16 Dec. 2006 for trial 2 – F6-R6 and trial 3 – F6-R3.

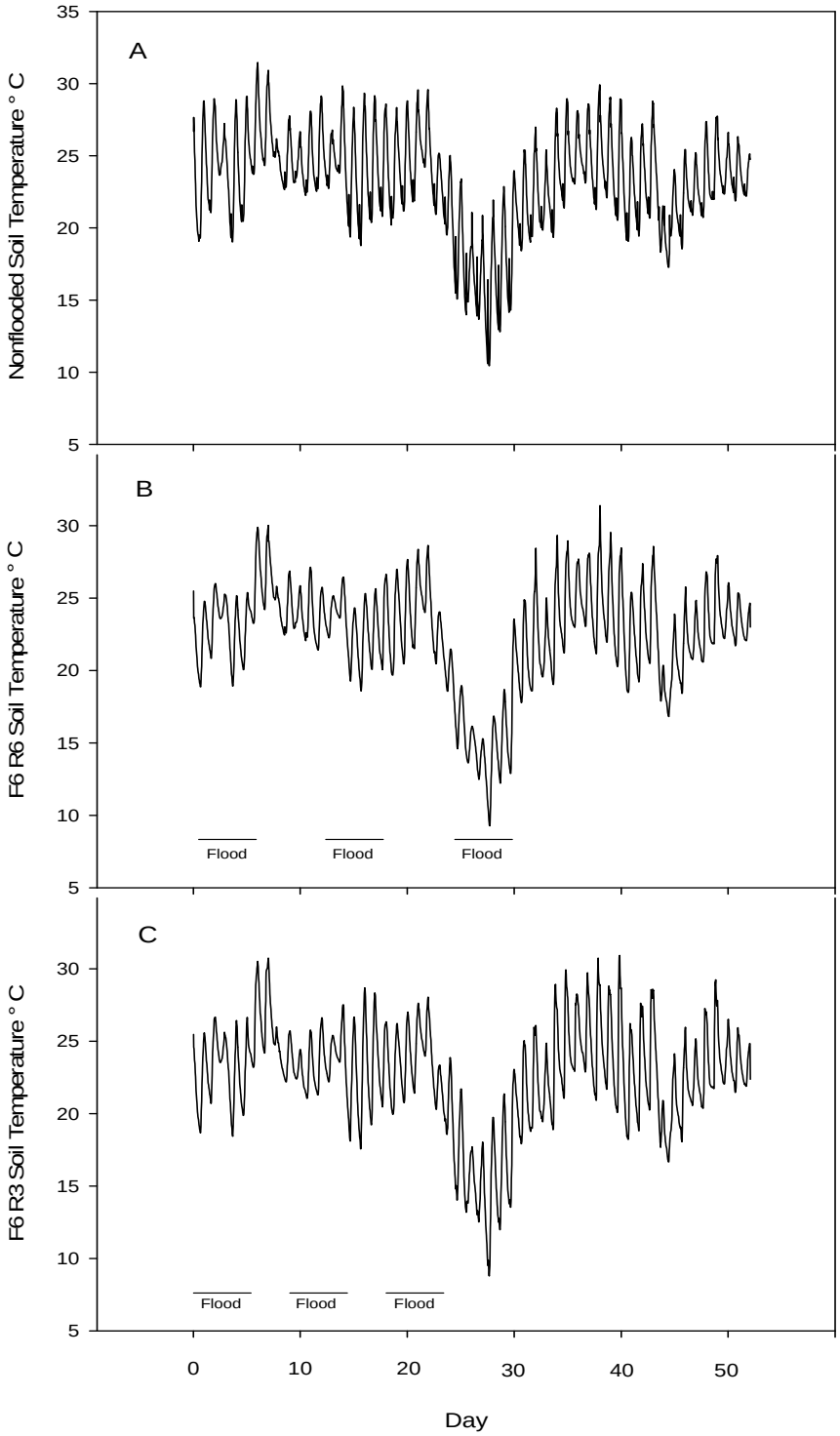

Figure 4-5. Soil temperatures for nonflooded (A) and flooded soil in trial 2 (B) and trial 3 (C) from 24 Oct. to 16 Dec. 2006.

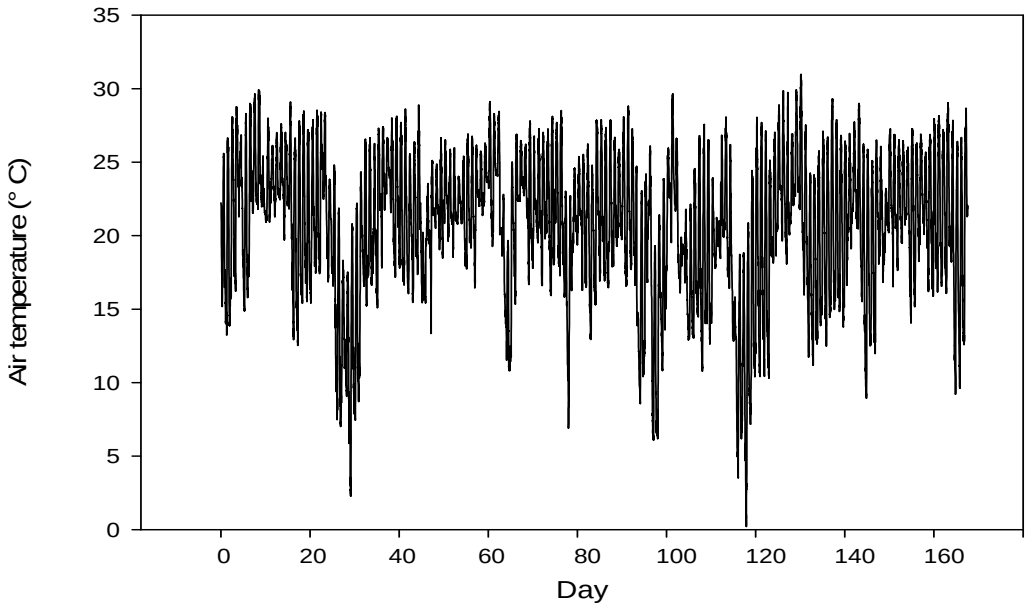

Figure 4-6. Air temperature at 60 cm above soil line as recorded by the FAWN field station 33 m north of the screenhouse for 168 days from 24 Oct. 2006 to 9 Apr. 2007 for trial 2 – F6R6 and trial 3 – F6R3.

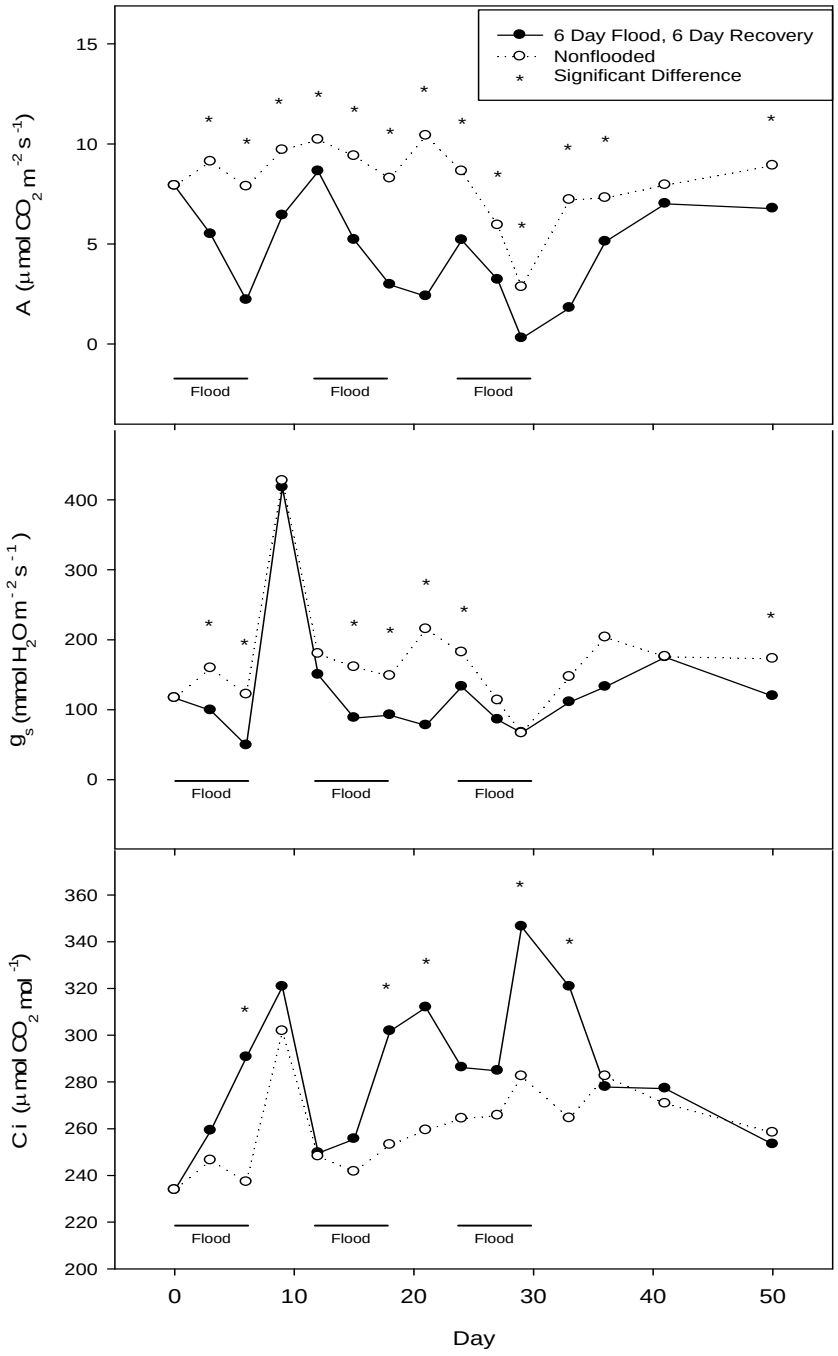

Figure 4-7. Effect of flooding on net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), and internal CO_2 concentrations (Ci) in leaves of 'Pantin' mamey sapote trees from 24 Oct. to 29 Nov. 2006 for trial 2 - F6R6. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=7. Reference (ambient) CO_2 level = 375 µmol·mol^{-1}.

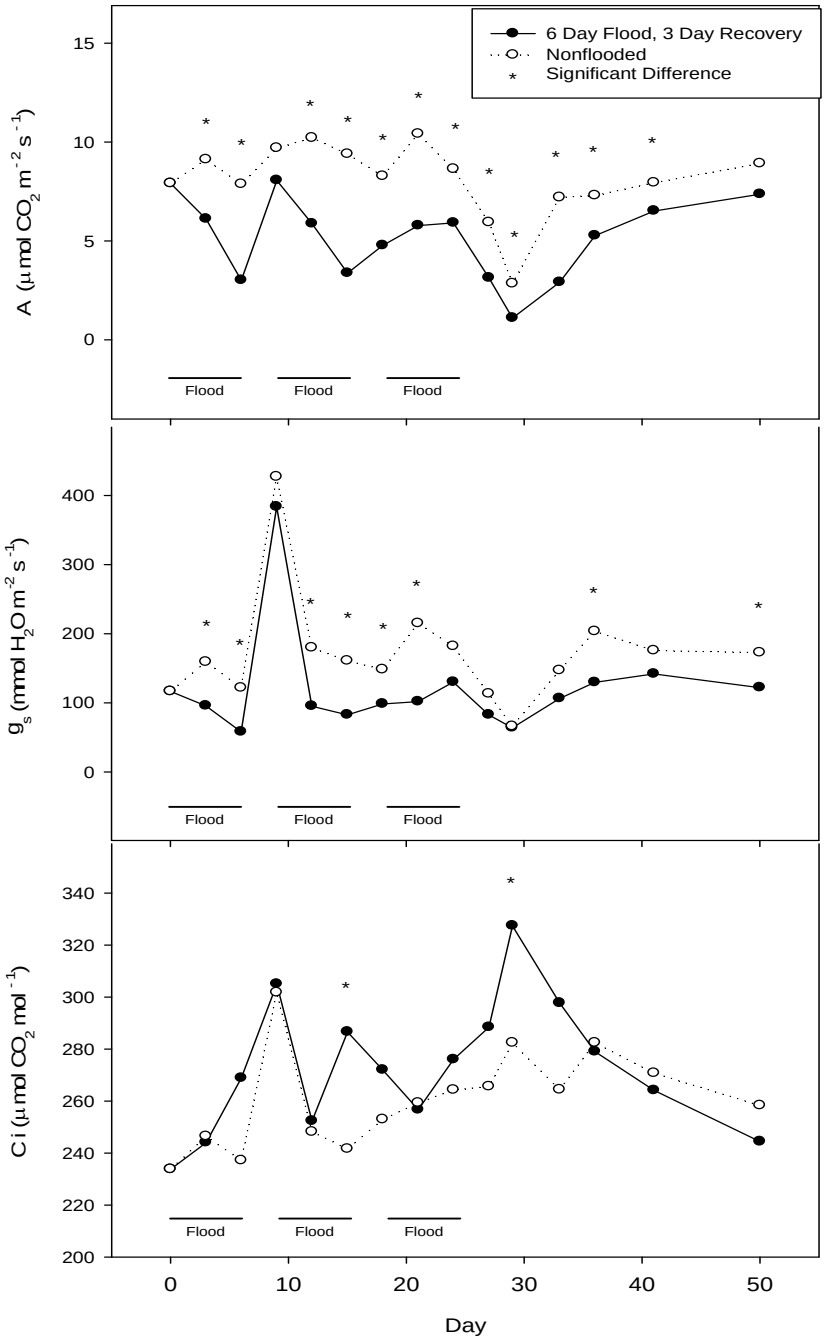

Figure 4-8. Effect of flooding on net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), and internal CO_2 concentrations (Ci) in leaves of 'Pantin' mamey sapote trees from 24 Oct. to 29 Nov. 2006 for trial 3 - F6R3. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=7. Reference (ambient) CO_2 level = 375 µmol·mol^{-1}.

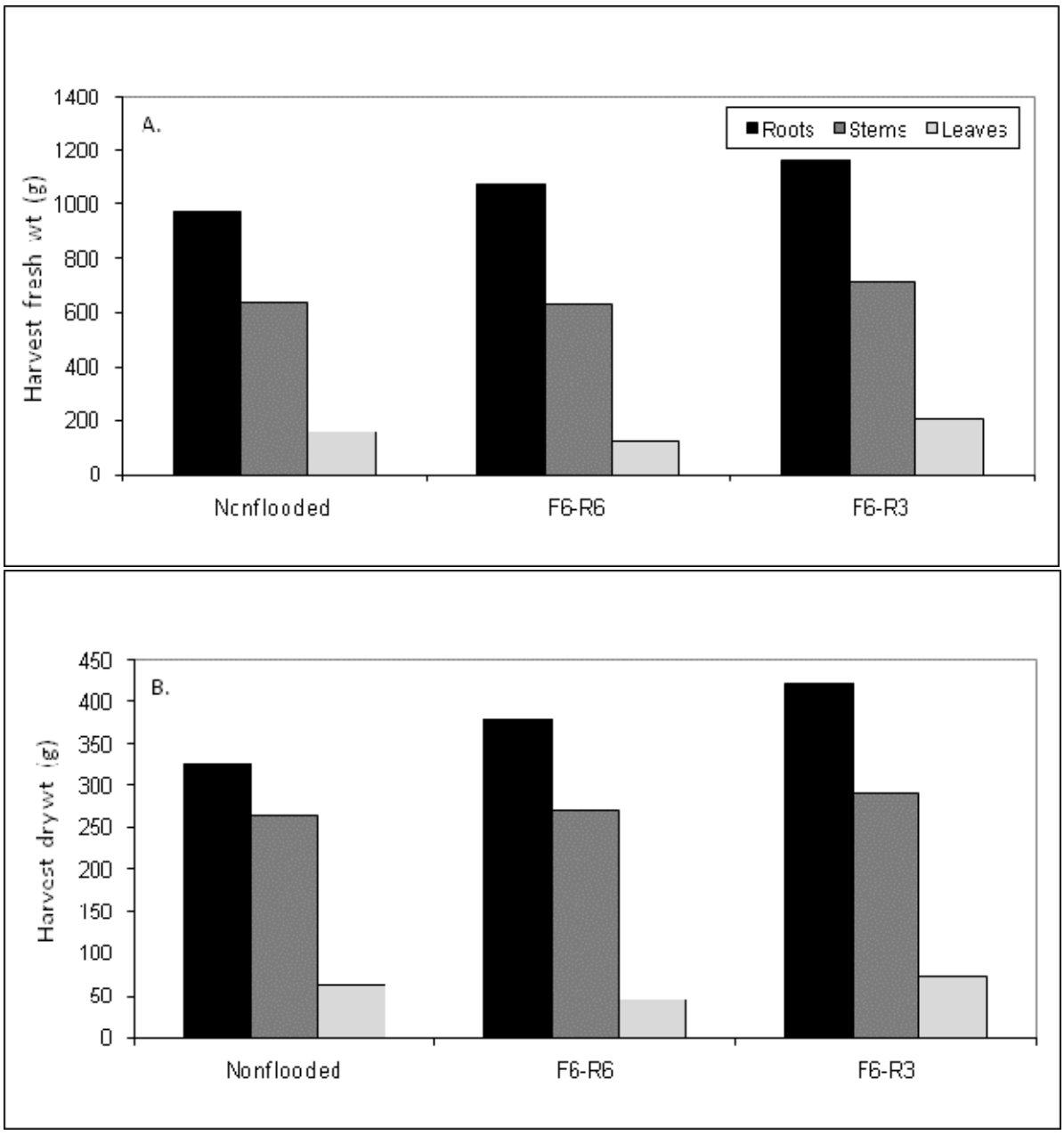

Figure 4-9. Mean harvest fresh weights (A), and mean harvest dry weights (B), of roots, stems, and leaves from nonflooded and cyclic-flooded 'Pantin' trees in trial 2 – F6R6 and trial 3 – F6R3 harvested on day 167, 9 Apr. 2007. No significant differences between nonflooded and F6R6 treatments for respective plant parts according to a T-test ($P > 0.05$). No significant differences between nonflooded and F6R3 treatments for respective plants parts according to a T-test ($P > 0.05$), n=7.

CHAPTER 5
RESPONSE OF MAMEY SAPOTE (*POUTERIA SAPOTA*) TREES TO FLOODING IN A CALCAREOUS SOIL IN THE FIELD

Introduction

Mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn] is a commercially grown tropical fruit crop which is popular among the Latin community. The center of origin for mamey sapote is the humid lowlands of southern Mexico and Central America (Verheij and Coronel, 1992), and extends south to northern Nicaragua (Balerdi and Shaw, 1998). Mamey sapote requires a hot climate with a relatively even rainfall distribution and grows best in the lowland humid tropics. Mamey sapote is seldom planted above 1000 m elevation (Verheij and Coronel, 1992). As of 2009, mamey sapote is estimated to be grown commercially in southern Florida on 233 ha (575 acres) and is annually worth an estimated $7.5 million at the farm level, and about $18.5 million at the wholesale level (E. Evans, University of Florida, personal communication). The Miami-Dade County agricultural area is subjected to periodic flooding during high water table conditions which coincide with periods of heavy rainfall and/or hurricanes. Flooding in mamey sapote orchards observed in this area has generally resulted in tree decline and death (Crane et al., 1997; Degner et al. 2002). The objective of this study was to investigate the physiological responses and survival of mamey sapote subjected to flooding under field conditions in very gravelly loam soil.

Materials and Methods

Experimental design. A single row of 60 grafted 3-yr-old mamey sapote (*Pouteria sapota*) cv. Magaña trees was planted on 11 May 2006 at the University of Florida, Tropical Research and Education Center in Homestead, FL. Trees were obtained from Lara Farm Nursery, Homestead, FL. The row was prepared by scraping the Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) (Burns et al. 1965; Leighty and Henderson,

1958; Nobel et al., 1996) down to the bedrock with a front end loader. Then 3.2 x 3.2 m (10 x 10 ft) water resistant tarps were sandwiched between two black ground cloths of the same size to form a water resistant barrier. The ground cloth layers were used to protect the plastic tarps from puncturing. A mound of soil approximately 1.5 x 1.5 m (4 x 4 ft) and 1 m (3 ft) high was placed on top of each barrier. Trees were planted in the mounds of native soil and allowed to establish in the field for 6 months before the first flooding trial. During this establishment period the trees were irrigated using microsprinklers according to the irrigation schedule used for the rest of the orchard that was planted with avocado and carambola trees. Two separate trials were conducted from 6 Nov. 2006 to 9 Jan. 2007 (Fall-Winter) and from 23 Apr. to 11 June 2007 (Spring-Summer). The Fall-Winter trial was flooded for 70 d and the Spring-Summer trial was flooded for 50 d. A fungicide was applied to the soil around each plant one to two weeks prior to each trial (Ridomil Gold EC®, Syngenta Crop Protection, Inc., Greensboro, NC) for the purposes of controlling Phytophthora (*Phytophthora cinnamomi* Rands) and Pythium (*Pythium splendens* Braun) root rots.

Sixteen healthy trees were selected for each trial and divided into two treatments: flooded (FL) and nonflooded (NF), with eight single-tree replications per treatment. The trees were randomly assigned to each treatment. For the trees to be flooded, the edges of the tarp and plastic were raised above the soil surface and soil was backfilled behind the raised barrier using a backhoe and shovels. Water was applied using a water wagon to form a pool.

Data collection. Leaf gas exchange [net CO_2 assimilation (A), stomatal conductance of water vapor (g_s), internal CO_2 concentration (Ci), and transpiration (E)], stem water potential (Ψs), soil redox potential, and soil and canopy temperature data were collected during the Fall-Winter trial. Leaf gas exchange, leaf chlorophyll index, soil redox potential, and soil and canopy

temperature data were collected during the Spring-Summer trial. Leaf gas exchange was monitored using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA). Leaf gas exchange measurements were made at a photosynthetic photon flux (PPF) of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a reference CO_2 of 375 $\mu mol \cdot mol^{-1}$ and a flow rate of 200 $mL \cdot min^{-1}$ into the leaf cuvette. Leaf gas exchange monitoring took place every 2 to 4 d during the first 3 wks and at varying intervals thereafter. One fully mature, sun exposed leaf on the west side of the plant was measured between 1100 and 1400 hr (EST). If any measurement was suspect as being low due to windy conditions, leaf damage, or other factors, another leaf was selected to either confirm or replace that particular reading. During the Fall-Winter trial measurements were made on day 0, 2, 5, 7, 9, 11, 14, 16, 20, 23, 28, 34, 39, 53, and 71 and during the Spring-Summer trial on day 0, 4, 8, 10, 14, 17, 21, 25, and 30.

During the Fall-Winter trial, Ψs was measured on days 1 and 7. One leaf per plant from about the middle of the canopy was covered with a zip lock bag which was covered with reflective aluminum foil for 1 hr. Subsequently, each leaf petiole was cut with a razor blade and placed into a styrofoam cooler, with the bag still surrounding the leaf. Stem water potential was measured immediately after harvest with a pressure chamber (Plant Water Status Console 3000 Series, Soilmoisture Equipment Corporation, Santa Barbara, CA) in a laboratory.

During the Spring-Summer trial, leaf chlorophyll index was measured using a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Ramsey, NJ), to assess the effect of flooding on leaf greenness as a general indication of plant vigor. Three leaves in the upper canopy and three leaves in the lower canopy of each tree were tagged and monitored to make comparisons between treatments, plants and canopy levels. Leaf chlorophyll index measurements were made on day 0, 4, 8, 10, 14, 17, 21, 25, and 30.

In the Spring-Summer trial, root samples were taken from all flooded trees to determine if root rot fungal pathogens were involved in the observed plant symptoms. The samples were analyzed at the Plant Diagnostic Clinic at the University of Florida, Tropical Research and Education Center in Homestead.

Soil redox potential was measured in the flooded plots using a metallic ORP indicating electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) connected to a volt meter. Redox potential readings below +200 mv indicate that soil conditions are anaerobic (Ponnamperuma, 1984). In Fall-Winter, redox potential was measured on days 2 and 39 and in Spring-Summer, redox potential was measured on days 4 and 24. A large spike was driven into the soil of each flooded tree to form a hole which immediately backfilled with soil water solution when the spike was removed. The redox potential probe was then inserted into the hole and gently moved up and down until the redox potential remained constant. In both experiments, soil temperature was monitored with a HOBO Water Temp Pro (Onset Computer Co., Bourne, MA), at approximately 10 to 15 cm deep, and canopy temperature was monitored with a StowAway TidbiT (Onset Computer Co., Bourne, MA). The University of Florida, IFAS FAWN weather station at TREC (http://fawn.ifas.ufl.edu) was also used to collect temperature data. Observations of the overall plant condition including leaf epinasty, wilting, leaf drop, and mortality were recorded.

Data were analyzed by two-way ANOVA to test for interactions between treatment and measurement date, and a standard T-test ($P \leq 0.05$) was used to compare gas exchange data between treatments on each measurement date.

Results

Fall-Winter trial. Tree canopy temperatures ranged from about 10 to 30°C, and soil temperatures from 15 to 25°C from 6 Nov. 2006 to 9 Jan. 2007 (Fig. 5-1). The mean soil redox

potential for the flooded treatment was 128 mV by day 2 and by day 29 was -107 mV (data not shown). Net CO_2 assimilation, g_s (Figs. 5-2 and 5-3), and E (data not shown) decreased for both flooded and nonflooded trees beginning on day 2. By day 5, g_s of flooded plants was significantly lower than that of nonflooded plants ($P \leq 0.01$). By day 8, epinasty was visible on two flooded trees. On day 16, A of both flooded and nonflooded trees approached zero. The reduced A may have been due to over a week of relatively low temperatures which ended on day 16 with 13°C soil temperatures and 6°C canopy temperatures. After that cool period, A and g_s of nonflooded trees increased and was significantly greater than in flooded plants. Trees in the nonflooded treatment reached an A of 9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ by day 39 and this level was maintained or slightly increased throughout the remaining 32 d of flooding. Net CO_2 assimilation of flooded trees decreased by more than half to 4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ at the end of 39 d, and that level of A was maintained or slightly increased throughout the last 32 d of monitoring (Fig. 5-2). After the low temperature period, Ci was nearly the same in both flooded and nonflooded trees for at least 1 week, even though the other gas exchange variables were significantly different between treatments. Then on day 34, plants in the flooded treatment had a significantly higher Ci than those in the nonflooded treatment ($P \leq 0.01$).

Measurements of A, g_s, and Ci combine to provide a physiological indication of plant vigor (Schaffer et al., 1992). Stomatal conductance changed in response to flooding prior to A or Ci. Net CO_2 assimilation showed the next observable change related to treatments. Finally, Ci increased in the flooded tree leaf compared to the nonflooded tree leaf. The increase in Ci suggests that the photosynthetic apparatus may have been damaged or its capacity compromised. If the photosynthetic apparatus was not damaged and A was reduced due to low g_s, then Ci

would be expected to drop, since the available CO_2 inside the leaf mesophyll would be assimilated (Schaffer et al., 1992).

There was no significant difference in Ψs among treatments (data not shown). Mean Ψs for flooded and nonflooded trees was -0.32 and -0.41 MPa, respectively on day 1, and -0.67 and -0.52 MPa, respectively on day 7. No leaves on nonflooded trees exhibited chlorosis or epinasty. On day 8, the first signs of slight epinasty were observed on two flooded trees, and only those two trees had epinasty by day 39. By day 70, one flooded plant died (12% of all flooded plants); all other plants survived and maintained their canopy.

Spring-Summer trial. Tree canopy temperatures ranged from about 17 to 40°C and soil temperatures from 22 to 27°C from 23 Apr. 2007 to 11 June 2007 (Fig. 5-4). The mean soil redox potential for the flooded soil was 141 mV on day 4 and -12 mV by day 24 indicating that the soil in the flooded treatment was anaerobic. In general, A, g_s, (Figs. 5-5 and 5-6) and E (data not shown) were significantly greater for trees in the nonflooded treatment than the flooded treatment. After day 17, leaf wilting and abscission on flooded trees precluded further gas exchange measurements. On day 17, leaves on two of the flooded trees were dead. Further leaf desiccation and plant mortality occurred, one tree at a time every 4 d, on days 21, 25, and 29 for a total of five dead trees. Of the eight flooded trees, 5 (63%) exhibited this rapid 1 to 2 day decline noted on each day listed above. In contrast, all nonflooded trees survived. *Pythium splendens* Braun was found infesting six of the eight flooded trees, and may have been the cause of, or contributed to, the rapid decline and death of these trees.

There was no significant difference between leaf chlorophyll index for upper and lower canopy leaves, therefore the data were combined. In general, there was a significant difference in SPAD values between the flooded and nonflooded treatments. This condition existed prior to

the beginning of the experiment. During the first 30 days of the Spring-Summer trial leaf chlorophyll index fluctuated only slightly and remained consistent within each treatment (Fig. 5-7).

Discussion

Anecdotal evidence from growers suggested that mamey sapote trees were intolerant of flooded conditions (J.H. Crane, University of Florida, personal communication). However, young trees (~3 year-old) not infested with Pythium or Phytophthora root rot appear to possess moderate to good flood tolerance under orchard conditions during the fall and winter months having survived about 70 d of continuous flooding until the water was removed. One possible explanation for this tolerance is the type of rootstock. During the past 15 to 30 years seed from various locally grown cultivars (mostly 'Pantin' and 'Magaña') was used to produce rootstocks. More recently, a large amount of seed for rootstock has been imported from the Dominican Republic (J.H. Crane, University of Florida, personal communication). The variability in rootstocks and potential shift in the source of rootstocks used in mamey sapote grafting may explain in part the relative tolerance to flooding observed in these field-flooding experiments compared to lower flooding tolerance of older orchards in the area.

Compared to other tropical and subtropical fruit crops grown in south Florida, mamey sapote's flood tolerance may lie somewhere between that of the moderately flood-tolerant carambola (Schaffer et al., 1992; 2006), and the flood-sensitive avocado (Schaffer et al., 1992; 2006). Carambola has been capable of withstanding continuous flooding for periods of up to 18 weeks, in container-grown plant studies in alkaline soil, although there were reductions in gas exchange and dry weights compared to nonflooded trees (Joyner and Schaffer, 1989). Soil type was found to strongly influence avocado flooding survival. Healthy container-grown avocado trees in organic soil with high water-holding capacity showed a reduction in A after 5 d of

flooding (Ploetz and Schaffer, 1989), while there was no effect on A after 28 d for trees with healthy roots grown in a porous, limestone soil (Schaffer and Whiley, 2002). Consequently, even flood-sensitive species are capable of surviving extended flooding in calcareous soils if roots are disease free. If, however, the roots are not disease free or are infected by a root rot pathogen, rapid decline and death may occur within one to two weeks. For example, it was demonstrated in containerized studies with calcareous soil, that when avocado trees were inoculated with *Phytophthora cinnamomi* Rands prior to flooding, a reduction in A within 3 d and reached nondetectible levels in 7 to 9 d, followed by tree death within 2 weeks (Ploetz and Schaffer, 1989).

Other experiments with flooded mamey sapote trees in containers in Krome very gravelly loam soil (conducted Apr. to June 2005) also showed A reduced to near nondetectible levels in 7 to 10 d after flooding, and typical flooding symptoms of leaf epinasty, leaf chlorosis, and leaf abscission began at the end of the first week of flooding (Chapter 3). By contrast, in the Fall-Winter field trial, only about 25% of flooded trees showed some level of leaf epinasty after 2 months, thus practically no visible tree decline occurred. In the Spring-Summer field period, flooded trees did show a reduced A level by day 10. However, flooded trees did not exhibit the visible progression of decline (i.e., leaf epinasty, chlorosis, desiccation, abscission), nor did they decline rapidly during the first one to two weeks of flooding which can indicate a pre-existing infestation of root rot. Instead, it was not until day 17 when two trees died (25%) followed by one more tree every 4 d, until five total trees died (63%). This may have been due to the pre-flooding soil application of a systemic fungicide which provided some measure of root protection. However, it may be speculated that as new root growth occurred and/or the efficacy of the systemic fungicide decreased or was possibly leached from the soil throughout the course

of flooding, Pythium root rot infestation increased and eventually caused the observed rapid decline in tree health.

Based on the above results and comparisons, non-root rot infested mamey sapote trees appear to exhibit good tolerance to flooding conditions during the fall-winter period, and less tolerance during the spring-summer period in the field. Young trees or recently planted orchards on currently available rootstocks and/or treated with systemic fungicides may be able to survive 1 week of sustained flooding with minimal effect on tree health beyond reduced A. However, higher temperatures during the summer and/or root rot infestation may reduce the length of this time frame dramatically. Also, if trees are not treated regularly with soil fungicides during the season when flooding is more likely (i.e., hurricane season) then flooding will likely lead to rapid and irrecoverable tree decline and death due to root rot, as has been found in other tropical fruit species in Miami-Dade County, such as avocado (Ploetz and Schaffer, 1989). In summary, the results of this study suggest that non-root rot infested mamey sapote trees are moderately tolerant to flooding in a Krome very gravelly loam soil. However, more work is needed to separate tree decline due to flooding from that due to *Pythium splendens* infection in this soil.

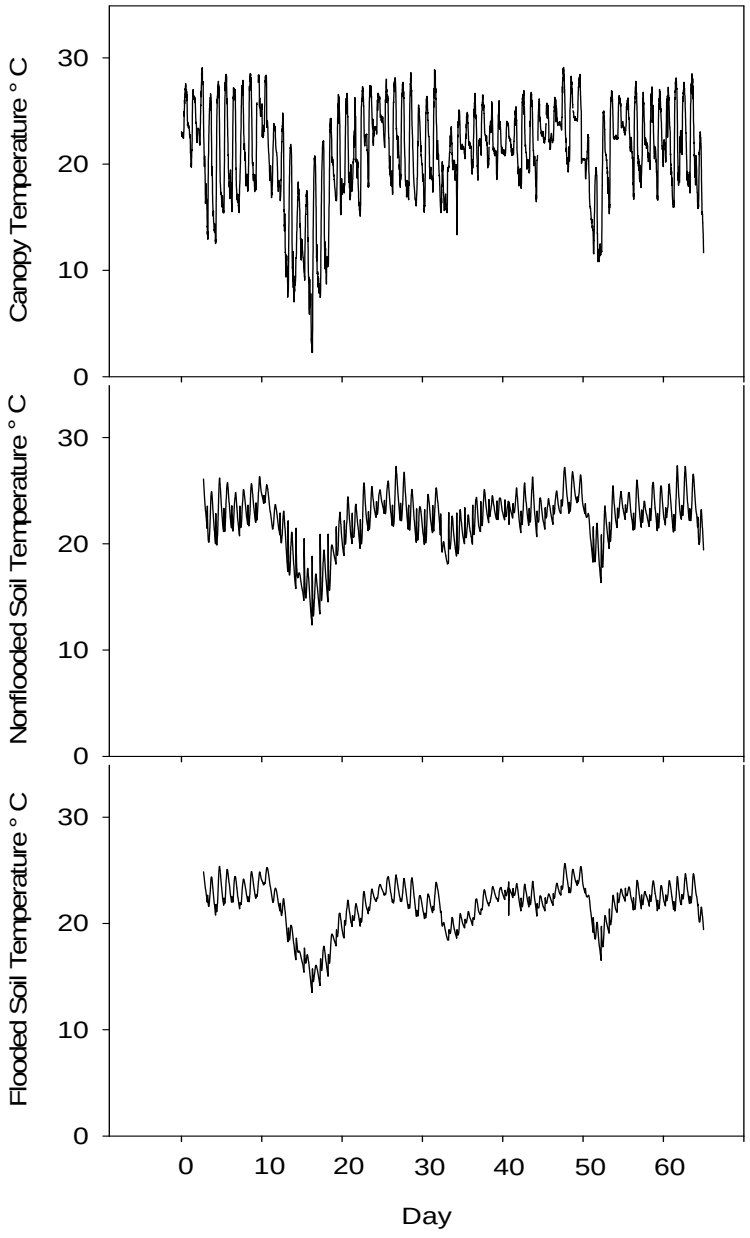

Figure 5-1. Air temperature of tree canopy and temperature of nonflooded and flooded soil at 10 cm soil depth. [Tree canopy temperature at 60 cm above ground downloaded from Univ. of Fla., IFAS, FAWN weather site at TREC. http://fawn.ifas.ufl.edu (accessed May 15, 2008)]. Fall-Winter trial, 6 Nov. 2006 to 9 Jan. 2007.

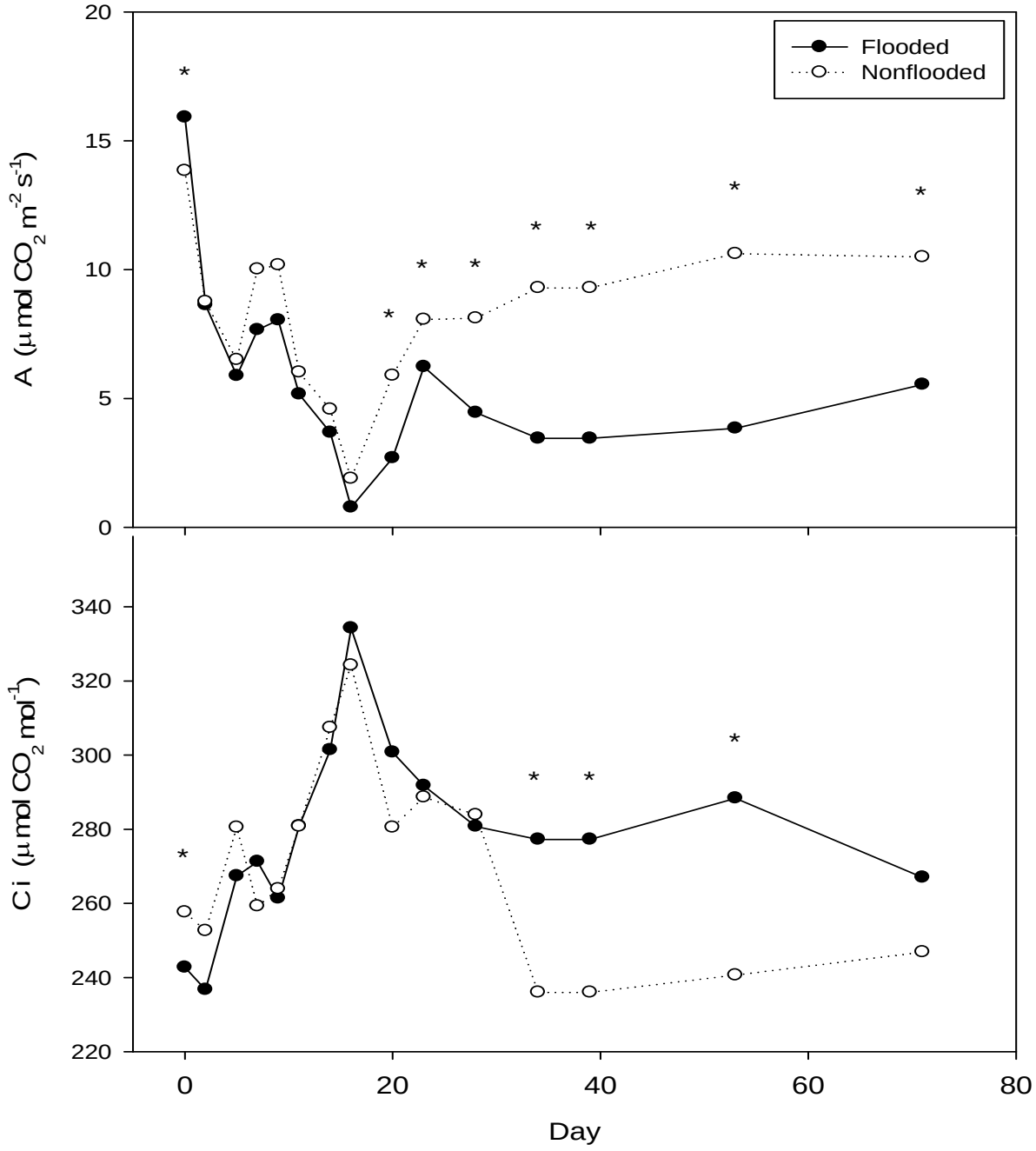

Figure 5-2. Effects of flooding on net CO_2 assimilation (A) and internal CO_2 concentrations (Ci) in leaves of mamey sapote trees. Fall-Winter trial, 6 Nov. 2006 to 9 Jan. 2007. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=8.

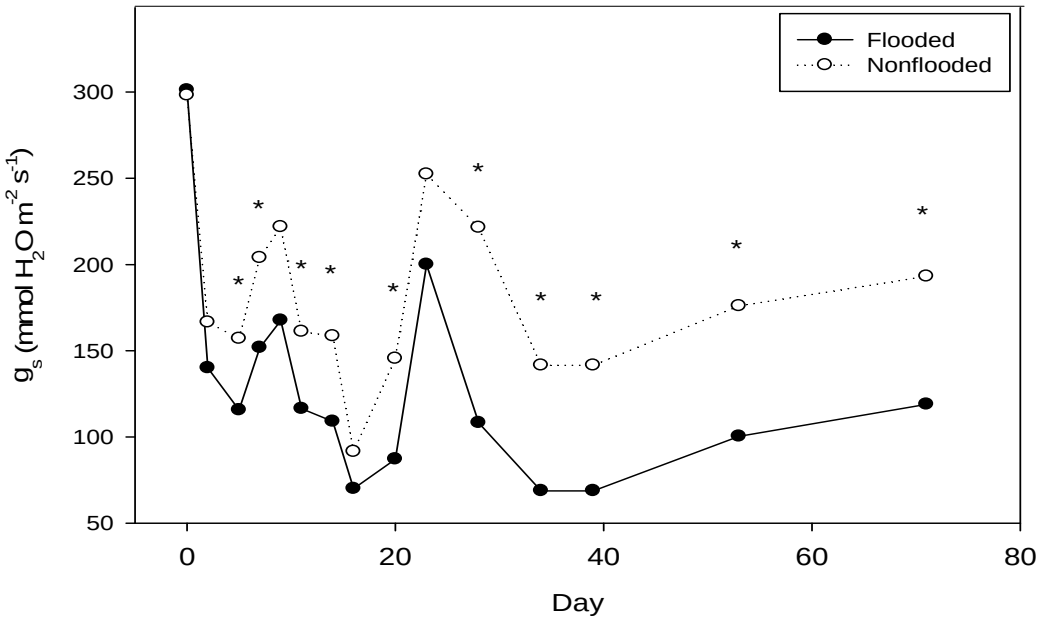

Figure 5-3. Effects of flooding on stomatal conductance of water vapor (g_s) in leaves of mamey sapote trees. Fall-Winter trial, 6 Nov. 2006 to 9 Jan. 2007. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=8.

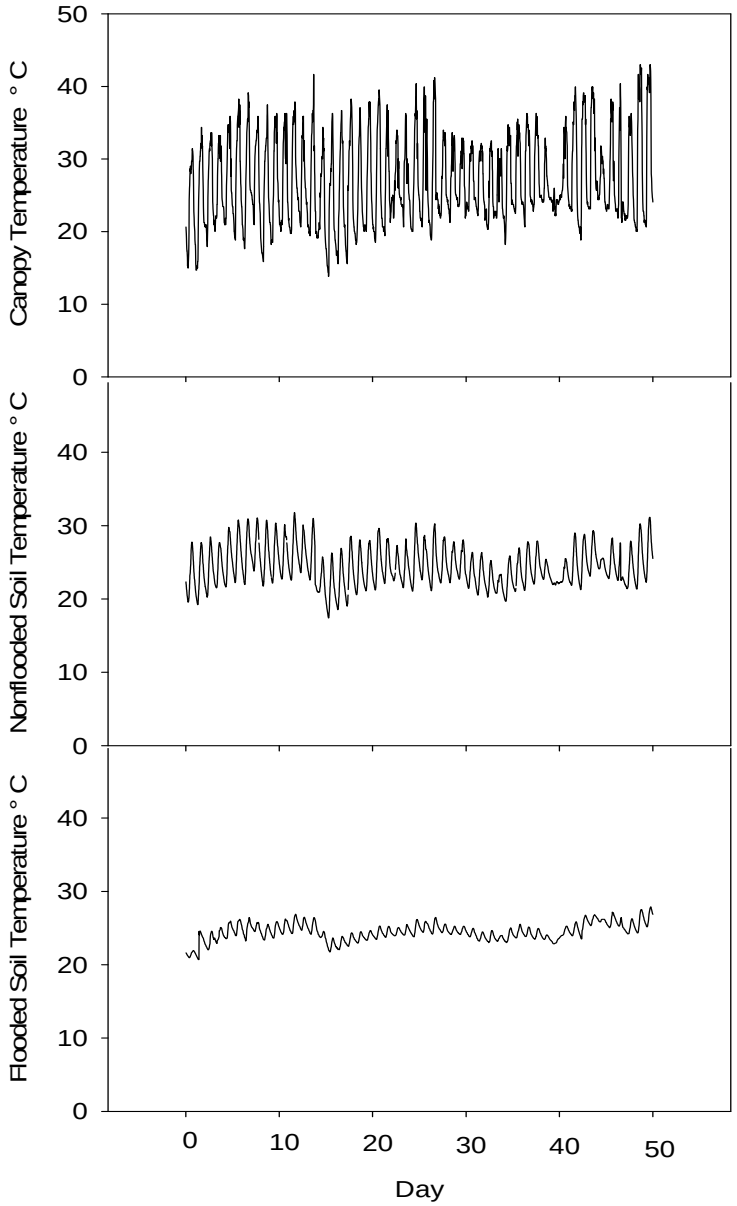

Figure 5-4. Air temperature within the tree canopy and temperature of nonflooded and flooded soil. [Nonflooded soil temperature at 10 cm soil depth downloaded from Univ. of Fla., IFAS, FAWN weather site at TREC. http://fawn.ifas.ufl.edu (accessed May 15, 2008)]. Spring-Summer trial, 23 Apr. to 11 June 2007.

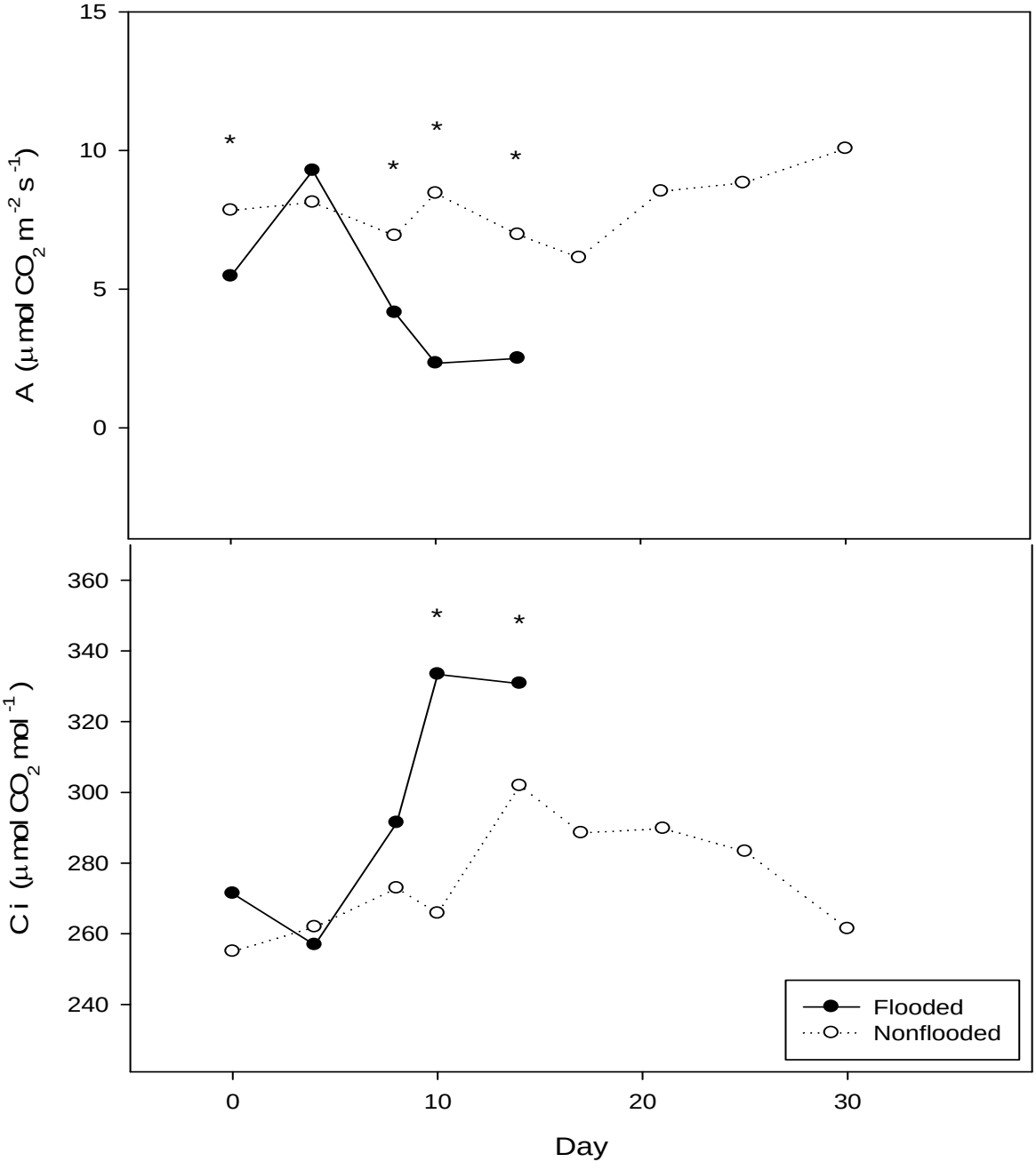

Figure 5-5. Effects of flooding on net CO_2 assimilation (A) and internal CO_2 concentration (Ci) in leaves of mamey sapote trees. Spring-Summer trial, 23 Apr. to June 11 2007. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=8.

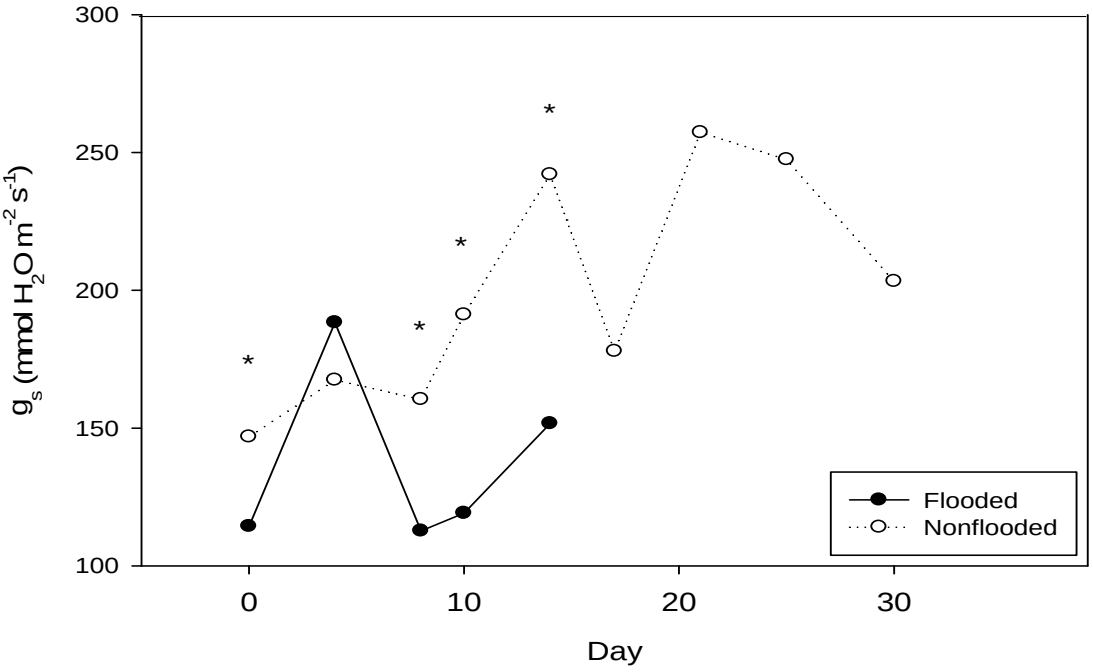

Figure 5-6. Effects of flooding on stomatal conductance of water vapor (g_s) in leaves of mamey sapote trees. Spring-Summer trial, 23 Apr. to 11 June 2007. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=8.

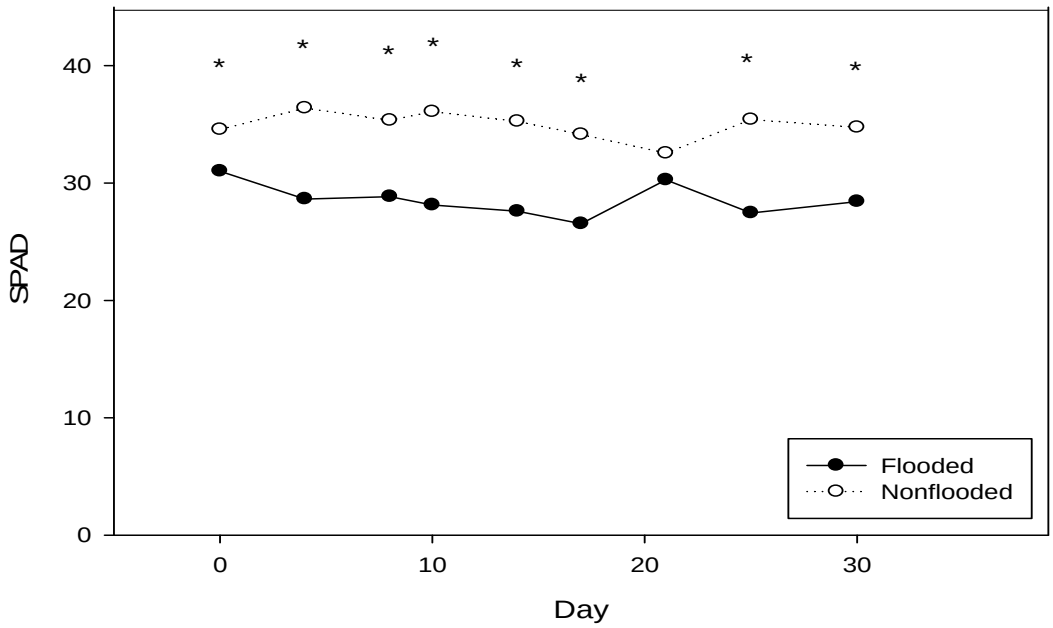

Figure 5-7. Effects of flooding on leaf chlorophyll index (SPAD) values of leaves of mamey sapote trees. Spring-Summer trial, 23 Apr. to June 11 2007. Asterisks indicate significant differences according to a T-test ($P \leq 0.05$), n=8.

CHAPTER 6
ROOT ZONE OXYGEN CONTENT, LEAF GAS EXCHANGE, ROOT RESPIRATION, AND ALCOHOL DEHYDROGENASE ACTIVITY IN *POUTERIA SAPOTA*

Introduction

Pouteria sapota (Jacq.) H.E. Moore and Stearn is a tropical tree native to the humid lowlands of southern Mexico, and south through parts of Central America to northern Nicaragua (Balerdi and Shaw, 1998; Verheij and Coronel, 1992). In southern Florida, *P. sapota*, commonly called mamey sapote, is grown commercially as a fruit crop in calcareous limestone soil (loamy-skeletal carbonatic, hyperthermic Lithic Udorthents) (Nobel et al., 1996). These soils are subjected to periodic flooding during high water table conditions which coincide with periods of heavy rainfall and/or tropical storms. Flooding of mamey sapote orchards in this area has often resulted in tree decline and death (Crane et al., 1997).

In flooded or poorly drained soils, oxygen concentration may become low enough to inhibit normal aerobic root respiration, a condition known as hypoxia. Hypoxia generally occurs at soil concentration less than 2 mg O_2 L^{-1} H_2O, although the O_2 content at which plants become hypoxic may differ for different species (Gibbs and Greenway, 2003). Curtis (1949) found that roots of avocado (*Persea americana* L.) in a hydroponic medium were able to an withstand oxygen content of 1 mg O_2 L^{-1} H_2O for 10 d without experiencing root damage, whereas concentration below 1 mg O_2 L^{-1} H_2O or complete lack of O_2 in the medium, a condition referred to as anoxia, caused root damage. Most plant species are able to survive only brief periods of anoxia (Drew, 1997). Under natural conditions, plant roots rarely are exposed to sudden anoxia. Rather there is a gradual transition from normoxia (an adequate supply of oxygen in the root zone) to hypoxia and then to anoxia, and thus an opportunity for plants to acclimate to low soil oxygen levels before conditions become potentially lethal to the plant (Drew, 1997).

Adaptive mechanisms to increase plant survival under conditions of low soil oxygen have been documented for some subtropical and tropical fruit tree species. These include the development of adventitious roots for increased oxygen absorption (e.g., mango or *Mangifera indica* L.; Larson et al., 1993, Schaffer et al., 1994), development of aerenchyma tissue in the stem for increased internal oxygen transport (e.g., *Annona* spp.; Nuñez-Elisea et al., 1999) and the development of hypertrophic (swollen) stem lenticels which function to increase oxygen absorption and for excretion of potentially toxic metabolites resulting from anaerobic metabolism in the roots (Chirkova and Gutman, 1972; Hook, 1984; Larson et al., 1991a; 1991c; 1993). However, these anatomical or morphological adaptations have not been reported for mamey sapote trees in response to low soil oxygen levels or soil flooding.

There has been a considerable amount of work over the last few decades on physiological responses of plants to hypoxia or anoxia in the root zone caused by flooding or poor soil drainage (for reviews see Bailey-Serres and Voesenek, 2008; Drew, 1997; Gibbs and Greenway, 2003; Givan, 1999; Kozlowski, 1997; Schaffer et al, 1992; 2006). For woody, perennial species including fruit trees, many studies have focused on assessing leaf gas exchange or plant water relations (Kozlowski, 1997; Schaffer et al., 1992). One of the earliest responses of woody perennial species including tropical fruit trees to low soil oxygen content is a reduction in net CO_2 assimilation (A) (Kozlowski, 1997; Schaffer et al., 1992). Significant reductions in A as a result of low soil oxygen content occur before visible stress symptoms. In woody plants, reductions in leaf gas exchange as a result of low soil oxygen are often concomitant with reductions in stomatal conductance (g_s). As a result of reduced g_s, transpiration (E) also often declines (Kozlowski, 1997; Schaffer et al., 1992). Thus, leaf gas exchange measurements have been useful for quantifying stress in tree species in response to soil hypoxia or anoxia.

Another early effect of low soil oxygen content on plants is an alteration of root metabolism. When the oxygen concentration in soil is low, there is a reduction in energy (adenosine triphosphate or ATP) production during root respiration. As a result of root exposure to hypoxic conditions, there is a shift from the citric acid cycle and oxidative phosphorylation (aerobic respiration) in the mitochondria to the fermentative pathways (anaerobic respiration) in the cytoplasm, which significantly reduces the level of ATP generated for the cell (Brand, 1994; Taiz and Zeiger, 2002). To make up for this loss in ATP, the rate of glycolysis may significantly increase, a condition which is termed the Pasteur effect (Gibbs and Greenway, 2003). While neither the alcohol or lactate fermentation pathways will produce ATP, they regenerate nicotinamide adenine dinucleotide (NAD^+) which is necessary for the glycolysis pathway to continue.

The production of potentially toxic metabolites in the roots as a result of anaerobic respiration has been implicated in plant cell death as a result of soil anoxia (Drew, 1997; Vartapetian and Jackson, 1997). When soil becomes hypoxic, the shift from aerobic to anaerobic respiration may take place in the roots within a few hours. In response to low soil oxygen content, root cell death due to a rapid exposure to anoxic conditions has been associated with acidification of the cytoplasm, referred to as cytoplasmic acidosis (Drew, 1997; Felle, 2005; Vartapetian and Jackson, 1997). Sudden exposure to anaerobic conditions results in lactic acid fermentation and the build-up of lactic acid which reduces the pH of the cell. After a short time, the pH in the cell stabilizes due to a shift from lactic acid fermentation to alcohol fermentation. Thereafter, a second phase of cytoplasmic acidosis occurs due to the loss of protons from the cell vacuole into the cytoplasm (Bailey-Serres and Voesenek, 2008; Drew, 1997).

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 in response to anaerobic root metabolism (Drew, 1997; Vartapetian and Jackson, 1997) . The conversion of acetaldehyde to ethanol is catalyzed by the enzyme, alcohol dehydrogenase (ADH). A significant amount of research with herbaceous plants has shown that increased ADH activity can improve a plant's tolerance to anoxia. Most studies of ADH upregulation in response to root anoxia have focused on *Arabidopsis thaliana* L., maize (*Zea mays* L.), rice (*Oryza sativa* L.), soybean (*Glycine max* L.), *Lepidium latifolium* L. and *Echinochloa* Pal. (Chen and Qualls, 2003; Chung and Ferl, 1999; Gibbs et al., 2000; Kato-Noguchi, 2000; Kimmerer, 1987; Morimoto and Yamasue, 2007; Preiszner et al., 2001; Rumpho and Kennedy, 1981; Russell et al., 1990). Levels of root ADH activity have also been determined for mesic trees such as swamp tupelo [*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.] (Angelov et al., 1996), and *Melaleuca cajuputi* Powell (Yamanoshita et al., 2005). However, ADH activity in response to root anoxia or hypoxia has not been reported for tropical fruit tree species, including *P. sapota*.

The purpose of this study was to determine the effects of root hypoxia and anoxia on leaf gas exchange, root ADH activity, root respiration, rates of glycolysis, and development of hypertrophic stem lenticels in *P. sapota* trees. Tree survival was also assessed.

Materials and Methods

Plant material. Four trials were conducted in a greenhouse to evaluate the effects of root hypoxia or anoxia on root alcohol dehydrogenase (ADH) enzyme activity, root respiration, and root glycolysis levels in two-year-old mamey sapote trees. All trees were grafted on mamey sapote seedling rootstocks, which is the common nursery propagation practice. The cultivar Pantin was used as the scion in trials 1 through 3, and the cultivar Magaña was used as the scion in trial 4.

Greenhouse conditions. Temperatures in the greenhouse were monitored in the hydroponic root medium with a HOBO® Water Temp Pro (Onset Computer Co., Bourne, MA, USA). Temperatures in the hydroponic medium ranged from 19°C to 31°C. One StowAway® TidBit® temperature logger (Onset Computer Corporation, Bourne, MA, USA) was placed in the plant canopy at a height of 1.5 m. Air temperature in the canopy ranged from 19°C to 43°C during the experiments.

Treatments. The first two trials consisted of two treatments, both with roots placed in a hydroponic medium. In one treatment an oxygen concentration of 7-8 mg O_2 L^{-1} H_2O was maintained in the root zone by bubbling air into the hydroponic medium (aerated hydroponic treatment). In the other treatment the oxygen concentration in the root zone was maintained at 0-1 mg O_2 L^{-1} H_2O by purging O_2 from the medium with N_2 gas (O_2-purged hydroponic treatment). In trials 3 and 4 a third treatment was added in which trees roots were grown aeroponically with roots exposed to a high oxygen concentration of ~150 mg O_2 L^{-1} air (aeroponic treatment). In all treatments roots were in 19-L polyethylene containers. Dissolved oxygen (DO) content in hydroponic medium was monitored using an Oakton DO 100 handheld dissolved oxygen meter (Oakton Instruments, Vernon Hills, Illinois, USA).

The O_2-purged hydroponic treatments were initiated by purging tap water with N_2 gas until a DO concentration of 0 mg O_2 L^{-1} H_2O was achieved prior to submerging roots into the water. The DO concentration was maintained at 0-1 mg O_2 L^{-1} H_2O.

For the aerated hydroponic treatment, the medium was aerated with ambient air at a rate of 0.16 L air · min^{-1} · L^{-1} water, with an air pump (Whisper® Model 100, Tetra®, Blacksburg, VA). A bubbling stone was added to the end of each air tube inserted into the hydroponic medium to increase air dispersal.

For the aeroponic treatment, plants were placed in a medium-less 19-L container with roots misted at regular intervals. Each container had two misting emitters affixed opposite to each other on the inside of each container and placed about 5 cm from the container lid so that the mist would be sprayed on the top of the root ball and drip downward to cover the entire root system. Four holes (3-cm diameter) were drilled into the bottom of each container to allow for drainage. A solenoid controlled the misting at a frequency of 6 sec every 2 min. The roots were in ambient air and therefore the oxygen levels of the root zone were presumed to be ~150 mg O_2 L^{-1} air, which is the oxygen concentration in the ambient atmosphere by volume.

Treatments in all trials were arranged in a completely randomized design. Trial 1 had 6 single-plant replicates per treatment and treatments were imposed for 10 d. Trial 2 had 8 single-plant replicates per treatment and treatments were imposed for 54 d. Trial 3 had 7 single-plant replicates per treatment and treatments were imposed for 48 d. Trial 4 had 7 single plant replicates per treatment and treatments were imposed for 50 d.

Leaf gas exchange. Net CO_2 assimilation, g_s and E were measured with a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA). Leaf gas exchange measurements were made on one recently matured leaf per plant at a photosynthetic photon flux of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a reference CO_2 of 375 $\mu mol \cdot mol^{-1}$ and a flow rate of 200 $mL \cdot min^{-1}$ into the leaf cuvette. Measurements were made on all plants in each treatment sampling one leaf per tree on the day of or day prior to each root sampling.

Root electrolyte leakage. In trial 2, electrolyte leakage from roots was determined by harvesting 2 g fresh weight of terminal sections of the healthiest appearing roots. Roots sections were rinsed with deionized water (DI), blotted dry, and placed into a 60-mL tube with 30 mL of DI water. The tubes were capped and placed on a shaker at 200 rpm for 24 h. The electrical

conductivity was determined with a Fisher Scientific Accumet® AR50 pH/ION/conductivity meter (Fisher Scientific, Waltham, MA, USA) for each solution after the roots were removed. The roots were then frozen for 24 h at -80°C to lyse the cells. Roots were then removed from the freezer, the corresponding solution was returned to each tube, and the samples were shaken at 200 rpm for 24 h. The roots were removed from the solution and electrical conductivity of the solution was determined. Root electrolyte leakage was calculated as a percentage by dividing the electrical conductivity prior to freezing roots by the electrical conductivity after freezing roots (Crane and Davies, 1987; Stergios and Howell, 1973).

In addition to electrolyte leakage, the total amount of electrolytes present in the root cells per unit dry weight was determined to more accurately assess root health. While electrolyte leakage assesses permeability of the root cell membrane, total electrolyte assesses the total amount of electrolyte maintained in the root cell. Roots were frozen at -80°C and then shaken for 24 hrs in 30 mL of DI water as previously described. The resulting electrical conductivity was measured, and then multiplied by 30 to mathematically concentrate the electrical conductivity units ($S \cdot m^{-1}$) to 1 mL DI water. The roots were then oven dried and weighed.

Alcohol dehydrogenase activity. Root ADH activity was determined in trials 1, 2, 3, and 4 on the following days after the treatments were imposed: Trial 1: days 0, 2, 4, 6, 8, and 10; Trial 2: days 9, 13, 17, 21, 26, 33, 40, 47, and 54; Trial 3: days 0, 2, 4, 6, 8, 10, 21, 44; and Trial 4: days 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, and 50.

Root tips, the preferred target tissue for analyzing ADH (Bailey-Serres and Voesenek, 2008; Gibbs and Greenway, 2003), were harvested from each plant, rinsed with DI water, placed into a 1.5-mL microcentrifuge tube and immediately placed on ice. Samples were then stored at -80°C for 1 to 2 d until enzyme extraction. Root samples were processed and ADH activity was

measured according to modification of protocols described by Chung and Ferl (1999) and Russell et al. (1990). Roots were ground on ice with sea sand in 1.5 to 2 mL of an extraction solution containing 50 mM Tris-HCl and 15 mM DTT at a pH 8.8. Samples were centrifuged at 12,000 g for 12 min at 4°C. The enzyme reaction was initiated by placing 500 µL of reaction solution (50 mM Tris-HCl, 1.7 mg NAD^+ mL^{-1}, pH 8.5), 100 µL 50% ethanol, and 100 µL extract in disposable cuvettes which were inverted seven times. The cuvettes were allowed to sit for 1 to 2 min and then placed in a Beckman DU-640 Spectrophotometer (Beckman Instruments, Fullerton, California, USA). Absorbance was zeroed and recorded at A340 every 15 sec. for 2 min. Protein content of the extract was determined with a protein assay kit (Bio-Rad Laboratories, Hercules, California, USA) and the ADH enzyme activity was calculated as nmol NADH min^{-1} mg $protein^{-1}$.

Evolution of CO_2 from roots. Evolution of CO_2 from excised roots was determined in trial 2 using a similar method as that described by Burton and Pregitzer (2003). From each plant, 2 g fresh weight of roots was harvested for CO_2 evolution measurements. Root samples were placed in a cuvette made from a 60-mL vial with air holes in the lid. One hole served as the air inlet. A rubber tube was inserted through the other hole into the vial to 1 cm from the bottom with the opposite end of the tube connected to a portable gas analyzer (CIRAS-2, PP Systems, Amesbury, Massachusetts, USA). The gas analyzer was set in an open configuration with a flow rate of 300 mL · min^{-1}. At this rate, air was exchanged through the cuvette 5 times per min. After determining CO_2 evolution from roots, each root sample was dried in an oven at 70°C for 2 d and dry weights were used to make the final calculation of root CO_2 evolution rate in nmol $CO_2 \cdot s^{-1} \cdot g\ dwt^{-1}$.

Calculation of glycolysis rates and ATP energy production. In trial 2, it was determined if the plant roots were undergoing aerobic or anaerobic respiration as evidenced by visual symptoms (leaf chlorosis, leaf abscission), root ADH activity, root electrolyte leakage, and increased root CO_2 evolution. Glycolysis rates were calculated from the mean evolution of CO_2 into units of nmol sucrose · g dwt^{-1} · s^{-1} and nmol pyruvate · g dwt^{-1} · s^{-1}. Glycolysis rates for aerobic respiration were calculated as 12 mol CO_2 released per mol sucrose processed by glycolysis and the citric acid cycle. Glycolysis rates for anaerobic respiration were calculated as 4 mol CO_2 released per mol sucrose processed by glycolysis and the alcohol fermentation pathway. No other anaerobic respiration pathways release CO_2. To compare the rate of carbohydrate consumption between the anaerobic and aerobic treatments, a glycolysis rate ratio was determined by dividing the glycolysis rate of the anaerobic treatment by the glycolysis rate of the aerobic treatment (Gibbs and Greenway, 2003; Hole et al., 1992).

The quantity of ATP energy produced was calculated to determine if the resultant increased rate of glycolysis via anaerobic respiration produced equivalent ATP levels to that of aerobic respiration. Based on previous studies, anaerobic roots were assumed to have produced 10 mol ATP per mol sucrose processed during glycolysis, and aerobic roots were assumed to have produced 60 mol ATP per mol sucrose processed by glycolysis and the citric acid cycle (Brand, 1994; Taiz and Zeiger, 2002).

Hypertrophic lenticel development and tree survival. At the end of trials 3 and 4, the development of hypertrophic lenticels on the main trunks of each plant, and the visible appearance of the canopy and trunk tissue beneath the bark was assessed. Trees in trial 4 were retained in their respective treatments for 14 d past the 50-d treatment period before assessing plant survival and hypertrophic lenticel development. Hypertrophic lenticel development was

rated as present or absent for each individual tree and ranked as percent of individuals with hypertrophic lenticels per treatment. For each tree, survival of the main trunk was determined by nicking the bark to expose if the underlying tissue was green (alive) or brown to black (dead). Tree survival was ranked as percent of individuals alive in each treatment.

Data analyses. In trials 1 and 2 on each measurement date, means of the O_2-purged and aerated hydroponic treatments were compared using a standard T-test. In trials 3 and 4 on each measurement date, means among of the O_2-purged hydroponic, aerated hydroponic, and aeroponic treatments were compared by a one-way analysis of variance (ANOVA) and a Waller-Duncan K-ratio test. All data were analyzed with SAS Version 9.1 Statistical Software (SAS, Inc., Cary, North Carolina, USA).

Results

Leaf gas exchange. In each trial, A in the O_2-purged hydroponic treatment became lower than the aerated hydroponic or aeroponic treatments by day 5, and declined to near 0 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in less than 10 d (Fig. 6-1). Trees in the aerated hydroponic treatment were able to maintain A levels between 2-4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for between 20 to 30 d. There were no significant differences in g_s or E between aerated and O_2-purged hydroponic treatments in trials 1, 2, and 3 until gas exchange for plants in the O_2-purged hydroponic treatment could no longer be measured due to defoliation of trees in the O_2-purged hydroponic treatment (Figs. 6-2 and 6-3). From about 10 to 14 d after treatments were initiated, all trees in the O_2-purged hydroponic were completely defoliated, precluding gas exchange measurements for that treatment. In trials 3 and 4, g_s and E decreased more for plants in the O_2-purged and aerated hydroponic treatments than in the aeroponic treatment (Figs. 6-2 and 6-3).

Root electrolyte leakage and total electrolyte. In trial 2, root electrolyte leakage was significantly greater from trees in the O_2-purged hydroponic treatment than trees in the aerated

hydroponic treatment beginning by day 4 and continuing for nearly 50 d (Fig. 6-4). By day 13 root electrolyte leakage was just over 70% for trees in the O_2-purged hydroponic treatment, and this level was maintained for the rest of the trial.

The greatest loss in total root electrolyte for trees in the O_2-purged hydroponic treatment occurred between days 4 and 9, during which time more than half of the roots' electrolyte was lost (Fig. 6-5). By day 17, trees in the O_2-purged treatment reached the lowest levels of total electrolyte, which were maintained until the end of the treatment period. Roots of trees in the aerated hydroponic treatment slowly and steadily lost their electrolytes linearly over time, until by day 40 trees in the aerated hydroponic treatment had a low level of total electrolyte which was similar to what was observed for trees in the O_2-purged treatment (Fig. 6-5).

Alcohol dehydrogenase (ADH) activity. Alcohol dehydrogenase enzyme activity was detected in root tips of plants in all treatments (Figs. 6-6 and 6-7). The normally observed range of mean ADH activity in roots in both the O_2-purged and aerated hydroponic treatments was between 5 to 125 nmol NADH $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$. Mean ADH activity in the aeroponic treatments ranged from near 0 to 50 nmol NADH $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$ in trial 3, and as high as 250 nmol NADH $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$ in trial 4. Overall, there were no observable trends of ADH up-regulation or down-regulation common to all trials or treatments.

During the first 10 d of flooding in trials 1 and 4 there were no significant differences in ADH activity among treatments (Fig. 6-6). In trial 3 some differences in ADH activity among treatments were observed on days 4 and 10; when roots in the O_2-purged hydroponic treatment had the highest ADH activity levels, followed by those in the aerated hydroponic treatment, and then the aeroponic treatment. In trial 3, roots in the aeroponic treatment generally had the lowest

levels of ADH activity. Conversely, in trial 4 roots in the aeroponic treatment exhibited some of the highest ADH activity of all treatments and all trials (Figs. 6-6 and 6-7).

During long-term treatment (more than 50 d) in trial 2, ADH activity in roots in the O_2-purged hydroponic treatment remained in the range of 50 to 100 nmol NADH · min^{-1} · mg $protein^{-1}$ from about day 10 to day 40 until it finally declined past day 50. ADH activity in roots in the aerated hydroponic treatment remained low during that period until day 54, when there appeared to be an upregulation in ADH activity (Fig. 6-7).

Root CO_2 evolution, root respiration, and glycolysis rates. By day 9 the roots in the O_2-purged hydroponic treatment evolved significantly higher levels of CO_2 than roots in the aerated hydroponic treatment, and roots in the aerated hydroponic treatment evolved a lower level of CO_2 than on day 0 (Fig. 6-8).

For plants in trial 2 between days 4 to 21, roots in the O_2-purged hydroponic treatment were undergoing glycolysis at between 12 and 18 nmol sucrose · g dwt^{-1} · s^{-1}, and roots in the aerated hydroponic treatment were undergoing glycolysis at between 1 and 4 nmol sucrose · g dwt^{-1} · s^{-1} (Fig. 6-9A).

The glycolysis rate was typically 5 to 10 times higher for trees in the O_2-purged hydroponic treatment (anaerobic respiration) than those in the aerated hydroponic treatment (aerobic respiration) with a peak on day 17 in which the glycolysis rate of trees in the O_2-purged hydroponic treatment was more than 20 times higher than that of trees in the aerated hydroponic treatment (Fig. 6-9B). Trees in the O_2-purged hydroponic treatment produced levels of ATP in a similar range to those of trees in the aerated hydroponic treatment (Fig. 6-9C).

Hypertrophic lenticel development. In trials 3 and 4, hypertrophic lenticels developed on the main trunk of 100% of the trees in the aeroponic treatment. Trees in the aerated

hydroponic treatment developed hypertrophic lenticels in only 14% and 57% of individuals in trials 3 and 4, respectively, compared to even fewer plants in the O_2-purged treatment with 0% in trial 3 and 29% in trial 4.

Tree survival. In trials 3 and 4, 100% of the trees in the aeroponic treatment survived with no observable damage to the leaf canopy, stems, or main trunk by the end of the treatment period. For trees in the aerated hydroponic treatment, the main trunks survived for 100% and 86% of individuals in trials 3 and 4, respectively. The main trunks of plants in the O_2-purged hydroponic treatment had living tissue in 57% and 86% of individuals in trials 3 and 4, respectively and the canopy was completely defoliated on each tree in that treatment.

Discussion

Previous studies of leaf gas exchange responses of *P. sapota* to low oxygen concentrations in flooded soil in containers showed that A, g_s, and E declined significantly after 3 d of flooding and continued to decline to their lowest points after 7 to 10 d (Chapter 3). After 5 to 10 d, epinasty and chlorosis of the leaves developed, and between 14 to 22 d of flooding, leaf abscission occurred. Branch dieback and tree death occurred between 30 to 60 d of flooding. In the present study, leaf gas exchange of plants in the aerated and O_2-purged hydroponic treatments declined similarly to those of flooded plants in the previous study. Leaf gas exchange of plants in the aeroponic treatments in the present study was similar to that of *P. sapota* in nonflooded soil conditions in the previous study. Thus, in the present study, plant vigor declined as a result of reduced oxygen availability to the roots as evidenced by a similar reduction in A, g_s and E observed due to soil flooding in previous studies of the same tree species.

The levels of root ADH activity in mamey sapote were similar to those reported for herbaceous plants such as maize (*Zea mays* L.) which had a range of ADH activity of 60 to 360 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Kato-Noguchi, 2000), *Lepidium latifolium* with ADH

activity of 150 to 500 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Chen and Qualls, 2003) and *Arabidopsis thaliana* with ADH activity levels of about 50 to 480 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Chung and Ferl, 1999). Mesic trees such as swamp tupelo [*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.] exhibited root ADH activity in nonflooded seedlings of about 100 to 125 nmol · NADH · min^{-1} · mg $protein^{-1}$, and seedlings flooded for up to 30 d exhibited activity of about 200 to 300 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Angelov et al., 1996). Flood tolerant *Melaleuca cajuputi* Powell seedlings exhibited nonflooded levels of about 500 to 900 nmol · NADH · min^{-1} · mg $protein^{-1}$, and flooded levels after 2 d up to 1700 nmol · NADH · min^{-1} · mg $protein^{-1}$, followed by a decline in activity to about 500 nmol · NADH · min^{-1} · mg $protein^{-1}$ by day 14 of flooding (Yamanoshita et al., 2005). The ADH enzyme activity observed in roots of *Pouteria sapota* in this study lies well within the ranges of these species. The highest individual extremes of ADH activity were found in trial 4 in some of the aeroponic plants. One particular plant repeatedly had very high ADH levels on days 2, 4, 6, and 8 of 1344, 770, 1127, and 1448 nmol NADH · min^{-1} · mg $protein^{-1}$, respectively. Another plant in that same treatment had extremely high levels on days 4 and 15 of 636 and 711 nmol NADH · min^{-1} · mg $protein^{-1}$, respectively. Plants in trial 3 also exhibited extreme levels: on day 0, two individual plants read 805 and 518 nmol NADH · min^{-1} · mg $protein^{-1}$, and on day 2 a different individual read 500 nmol · NADH · min^{-1} · mg $protein^{-1}$. Thus, *P. sapota* has the genetic capacity for ADH activity equivalent to flood-adapted species such as *Melaleuca cajuputi*; however, there was a high degree of plant to plant variation. This variation may be attributable to possible genetic variation found in the seedling rootstock. Perhaps more importantly, while the genetic capacity for ADH activity sufficient for flood tolerance may be present in *P. sapota*, the level of ADH activity alone may not be the limiting factor inhibiting greater survival of this species to flooded conditions.

Roots of *P. sapota* in the aerated hydroponic treatment lost total electrolytes more slowly than those of the O_2-purged hydroponic treatment, but by day 40 total root electrolyte content was similar between the two treatments. Measuring the total root electrolyte content allows for treatment comparisons over time as well as between studies, which may be useful particularly for hydroponic circumstances where even a "slow membrane leak" of root electrolyte (revealed in the standard electrolyte leakage measurement technique as only a small percentage of electrolyte leakage) could heavily impact roots sitting directly in water over time. There has been some published work with regards to root electrolyte leakage and flooding stress. Crane and Davies (1987) found an increase in root electrolyte leakage from flooded rabbiteye blueberry plants after 6 d of flooding. Ojeda et al. (2004) found that flooded *Annona glabra* L. (pond apple) and *Annona muricata* L. (soursop) experienced more electrolyte leakage from roots of flooded than to nonflooded trees. Chang et al. (1983) found that one flood-susceptible cultivar and one flood-tolerant cultivar of *Ipomoea batatas* (L.) Lam. (sweet potato) with roots treated in an anaerobic, CO_2 enriched environment for 2 d experienced an increase in root electrolyte leakage. Chang *et al.* (1983) also found that anaerobiosis alone resulted in increased electrolyte leakage, and that applications of ethanol had no effect on electrolyte leakage in roots of the sweet potato cultivars tested. As ADH activity and the fermentative pathways of anaerobic respiration take place in the cytosol of the cell, the cells' total electrolyte content could have an impact on cellular health and respiration levels, although it was difficult from this study with mamey sapote to correlate the two.

In *P. sapota*, development of hypertrophic stem lenticels requires oxygen in the root zone as evidenced by the fact that stem lenticles hypertrophied in 100% of the trees in the aeroponic treatment, whereas only some trees in the aerated hydroponic treatment and even fewer trees in

the O_2-purged hydroponic treatment developed hypertrophic lenticels. Thus, in contrast to observations with mango which developed a significantly higher incidence of hypertrophic lenticels in lower oxygen water (1-7 mg L^{-1}) than higher oxygen water (15 mg L^{-1}) (Larson et al., 1993), lenticel hypertrophy in mamey sapote appears to be more related to moisture level in the root zone and around the main stem with a concomitant presence of oxygen. The requirement of both moisture and oxygen to induce hypertrophic lenticel development may be demonstrated by a low number of hypertrophic lenticels in the aerated and O_2-purged hydroponic treatments potentially due to altered metabolism resulting in lower ethylene production, while the higher oxygen level and constant misting of the root system in the aeroponic treatment may have facilitated ethylene production and acted as a barrier to ethylene loss by reduced gas diffusion (Larson et al., 1993).

In preliminary hydroponic experiments, smaller mamey sapote trees with roots placed in an aerated hydroponic medium where they developed hypertrophic stem lenticels prior to being transferred to an O_2-purged hydroponic root environment, maintained aerobic root respiration in the O_2-purged environment (M.T. Nickum, unpublished data). According to Bailey-Serres and Voesenek (2008), oxygen deprivation can become progressively more severe as root tissue distance from the oxygen source increases, and porosity of the tissue decreases. Thus, small trees with porous aerenchyma and hypertrophic lenticels may have been able to maintain aerobic root respiration and tolerate an O_2-purged root environment, whereas much larger plants in trial 2 of this experiment which did not develop hypertrophic lenticels shifted to anaerobic respiration in an O_2-purged root environment and many plants did not survive the very low O_2 concentrations in the root zone. Trees exposed to very low oxygen content (O_2-purged hydroponic treatment) developed some hypertrophic lenticels, but considerably fewer than trees

exposed to higher root oxygen concentrations (aerated hydroponic and aeroponic treatments). In flooded soils, plants are rarely exposed to sudden anoxia, but generally there is a gradual change in the root zone from normoxia to hypoxia and then to anoxia (Drew, 1997). Thus, trees may acclimate to very low soil oxygen concentrations in response to very high soil moisture with sufficient O_2 concentrations (in the aerated hydroponic treatment and from constant misting in the aeroponic treatment) by developing hypertrophic stem lenticels prior to anoxic shock. Development of hypertrophic stem lenticels may enable the maintenance of sufficient cellular oxygen levels in the root tissues for maintaining aerobic respiration under anoxic conditions.

The normally observed glycolysis rate for anaerobic respiration was 5-10 times higher than that of aerobic respiration, with a peak glycolysis rate over 20 times higher. Aerobic respiration was maintained in roots of trees in the aerated treatment, although the rate of glycolysis declined as a result of oxygen limitations. The Pasteur effect has been reported previously as inducing a glycolysis rate anywhere from 6 to 18 times higher in anaerobic tissues than in aerobic tissues (Gibbs and Greenway, 2003; Hole et al., 1992; Summers et al., 2000), so it is evident that the roots of *P. sapota* are capable of inducing a Pasteur effect in order to maintain ATP production. It is questionable if the maintained ATP production in the O_2-purged trees improved plant survival as it did not seem to be able to maintain the integrity of their cell membranes or total root electrolyte content.

In conclusion, *P. sapota* trees grafted onto seedling rootstock appear to have some adaptability to hypoxic conditions by reducing water loss, as evidenced by reduced g_s and E, and increasing their glycolysis rate (Pasteur effect). However, there is little long lasting adaptability to anoxic shock (sudden exposure to very low or anoxic soil oxygen concentrations in the O_2-purged hydroponic treatment). Although variable among individual trees, the genetic capacity

for ADH in *P. sapota* is comparable to many other plant species, including mesic adapted forest species. However, alcohol dehydrogenase activity alone is not sufficient to ensure plant survival in conditions of very low soil oxygen content.

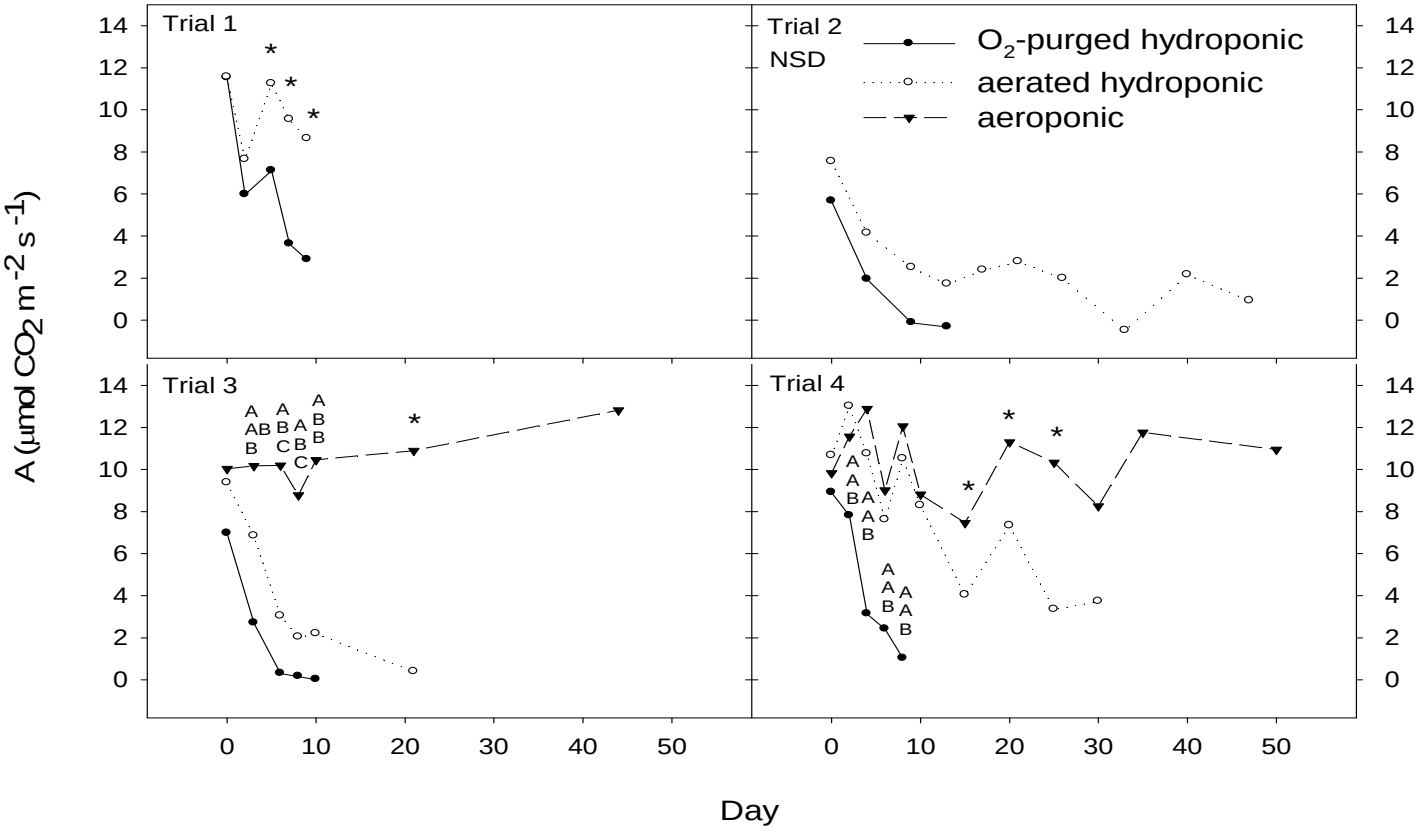

Figure 6-1. Effects of root zone oxygen level on net CO_2 assimilation (A) in trials 1-4. Asterisks represent significant difference at $P \leq 0.05$ level according to a standard T-test. Letters indicate significant differences at $P \leq 0.05$ according to a Waller-Duncan K-ratio Test. NSD indicates no significant differences ($P > 0.05$) for the entire trial. Means are based on measurement of a single mature leaf from each plant. Trial 1: n = 6, Trial 2: n = 8, Trials 3 and 4: n = 7.

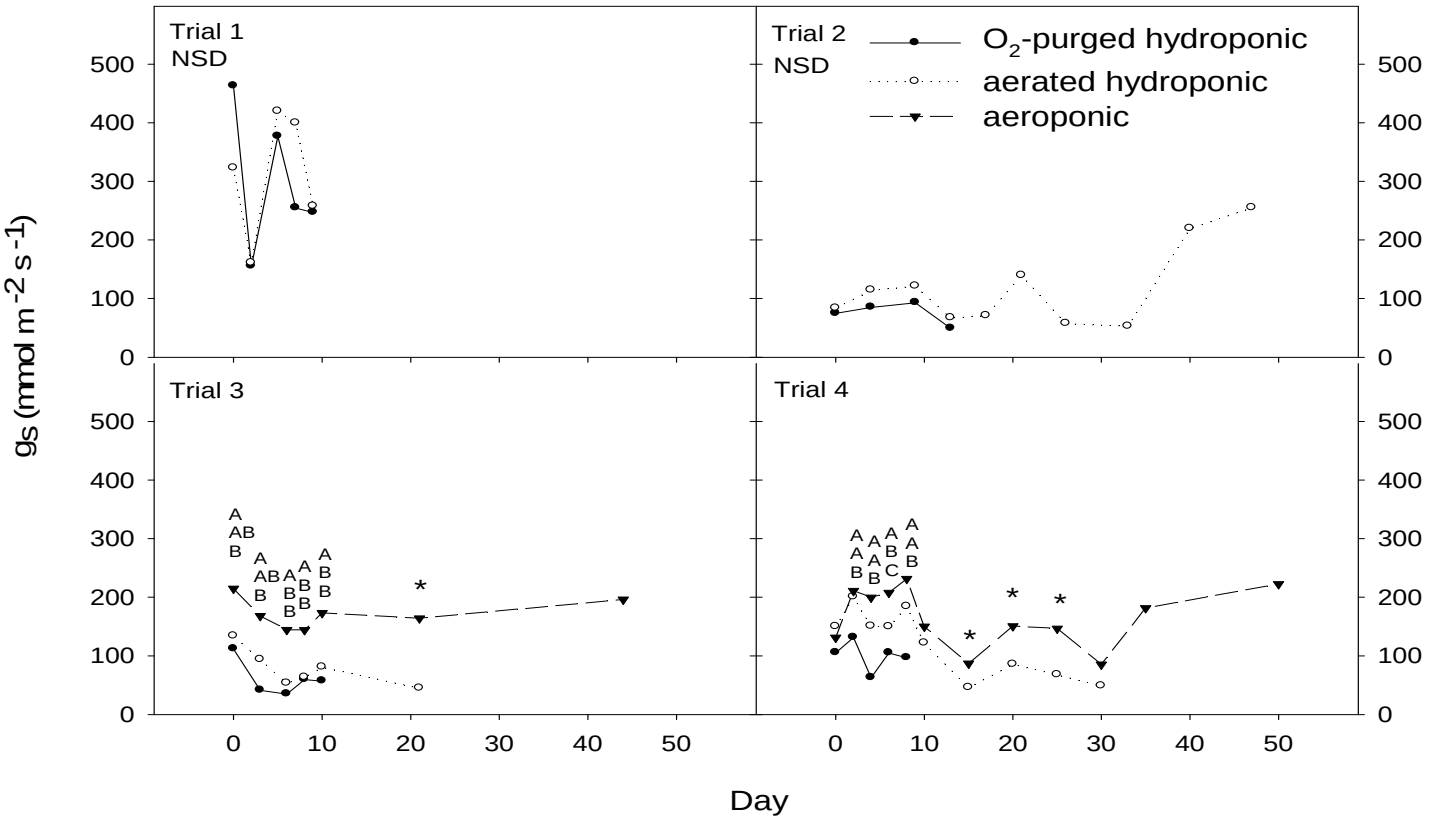

Figure 6-2. Effects of root zone oxygen level on stomatal conductance of water vapor (g_s) in trials 1-4. Asterisks represent significant difference at $P \leq 0.05$ level according to a standard T-test. Letters indicate significant differences at $P \leq 0.05$ according to a Waller-Duncan K-ratio Test. NSD indicates no significant differences ($P > 0.05$) for the entire trial. Means are based on measurement of a single mature leaf from each plant. Trial 1: n = 6, Trial 2: n = 8, Trials 3 and 4: n = 7.

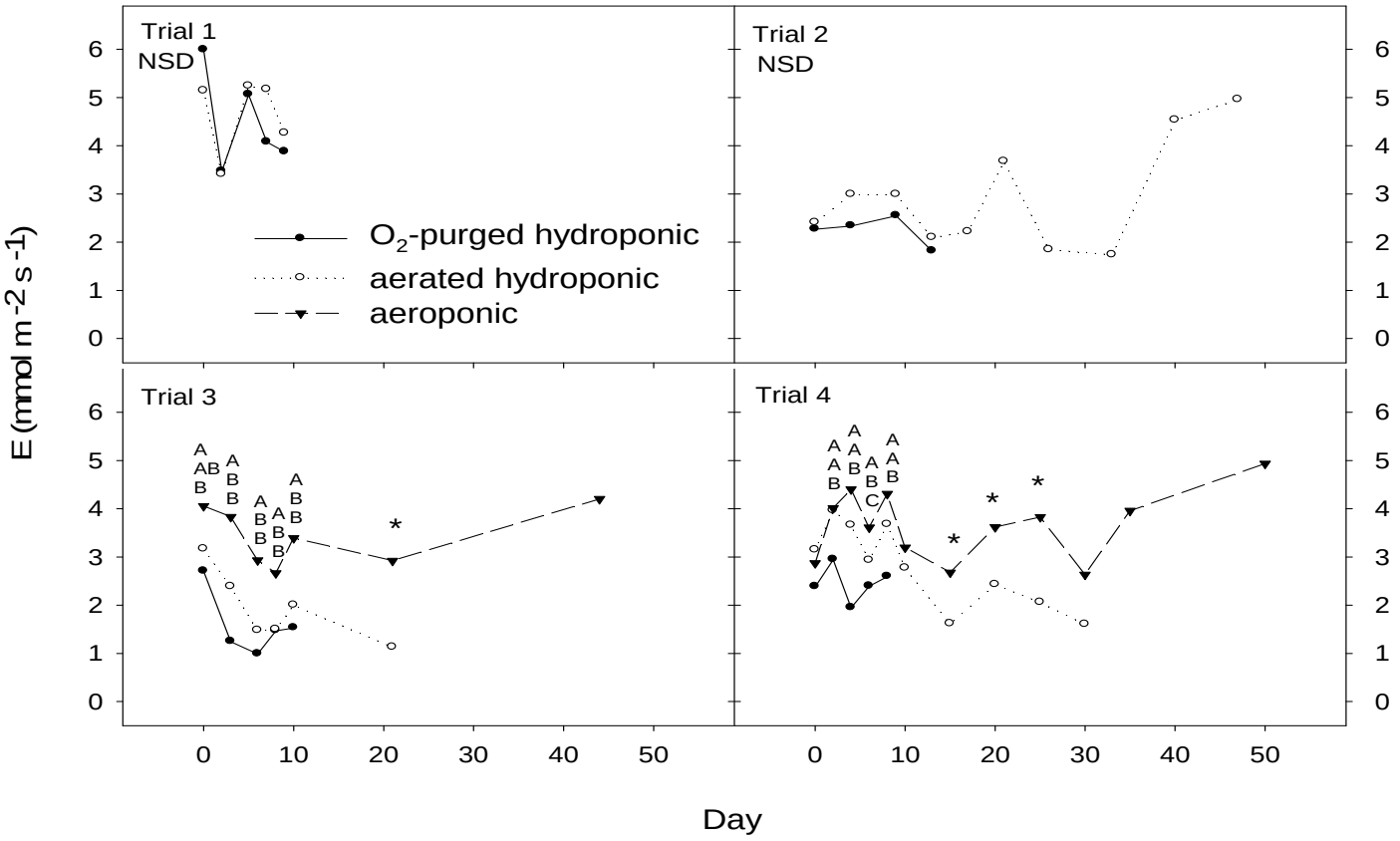

Figure 6-3. Effects of root zone oxygen level on transpiration (E) in trials 1-4. Asterisks represent significant difference at $P \leq 0.05$ level according to a standard T-test. Letters indicate significant differences at $P \leq 0.05$ according to a Waller-Duncan K-ratio Test. NSD indicates no significant differences ($P > 0.05$) for the entire trial. Means are based on measurement of a single mature leaf from each plant. Trial 1: n = 6, Trial 2: n = 8, Trials 3 and 4: n = 7.

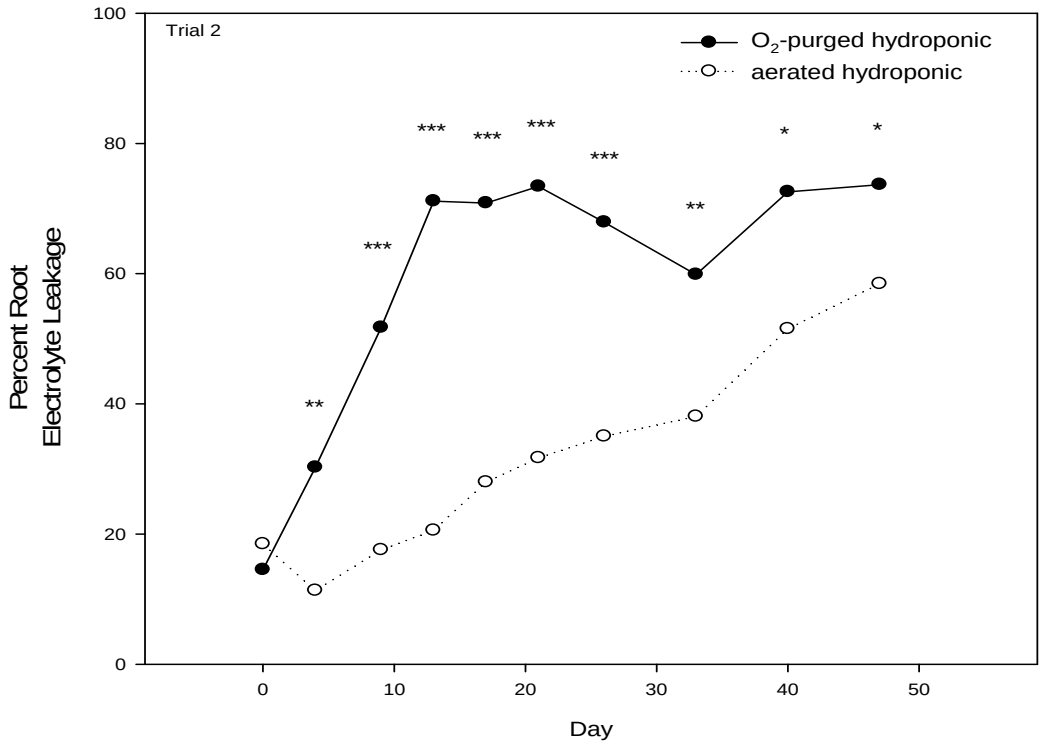

Figure 6-4. Percent root electrolyte leakage for O_2-purged hydroponic and aerated hydroponic treatments in trial 2. Single, double and triple asterisks indicate significant differences at $P \leq 0.1$, 0.05, and 0.01, respectively according to a standard T-test.

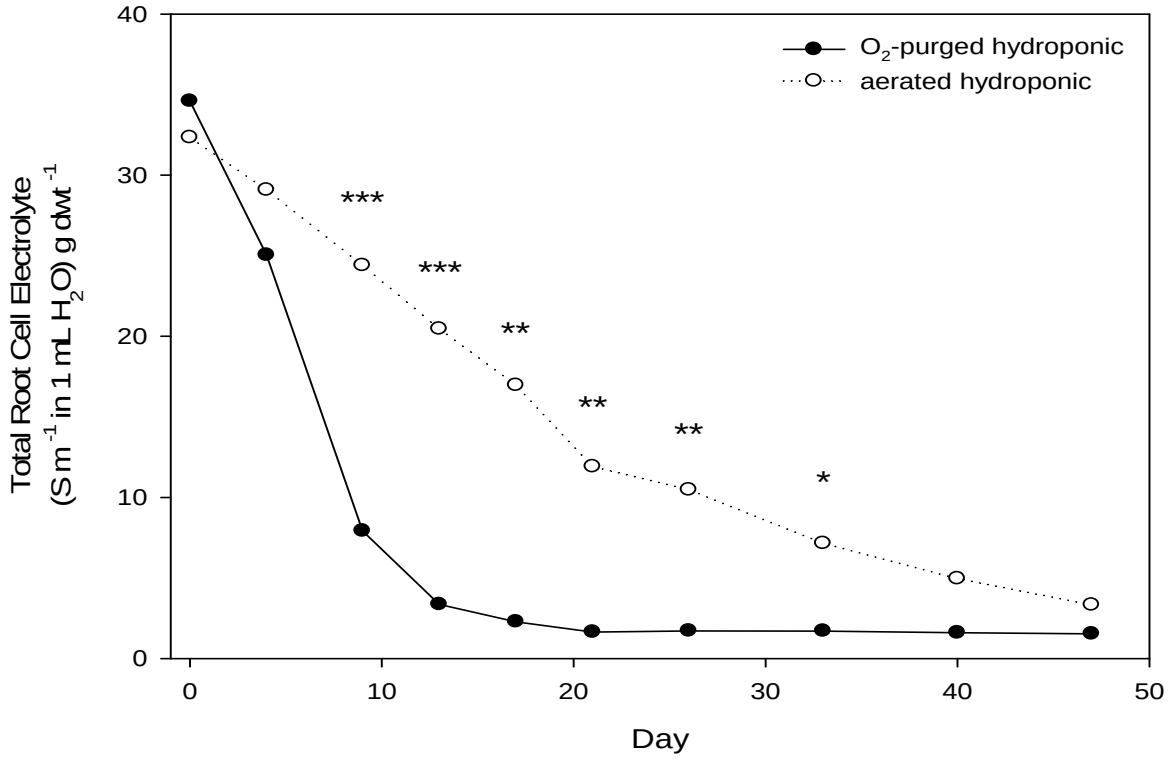

Figure 6-5. Total electrolyte present in roots for O_2-purged hydroponic and aerated hydroponic treatments in trial 2. Units measured as amount of electrical conductivity ($S \cdot m^{-1}$) if concentrated into 1 mL of DI water per gram root dry weight. Single, double and triple asterisks indicate significant differences at $P \leq 0.1$, 0.05, and 0.01, respectively according to a standard T-test.

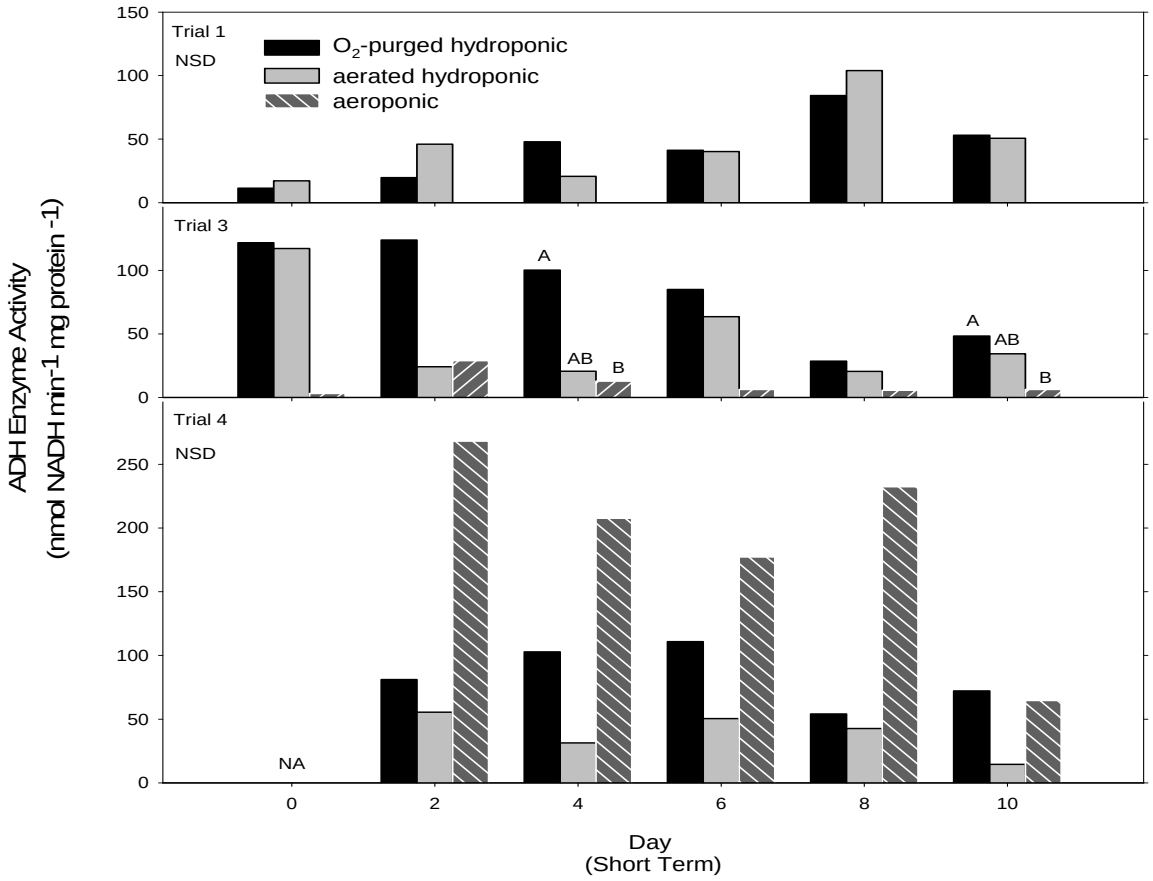

Figure 6-6. Alcohol dehydrogenase enzyme activity for O_2-purged hydroponic, aerated hydroponic, and aeroponic treatments during 0 to 10 d of treatment for trials 1, 3, and 4. Different letters indicate significant differences among treatments at $P \leq 0.05$) according to a Waller-Duncan K-ratio Test.

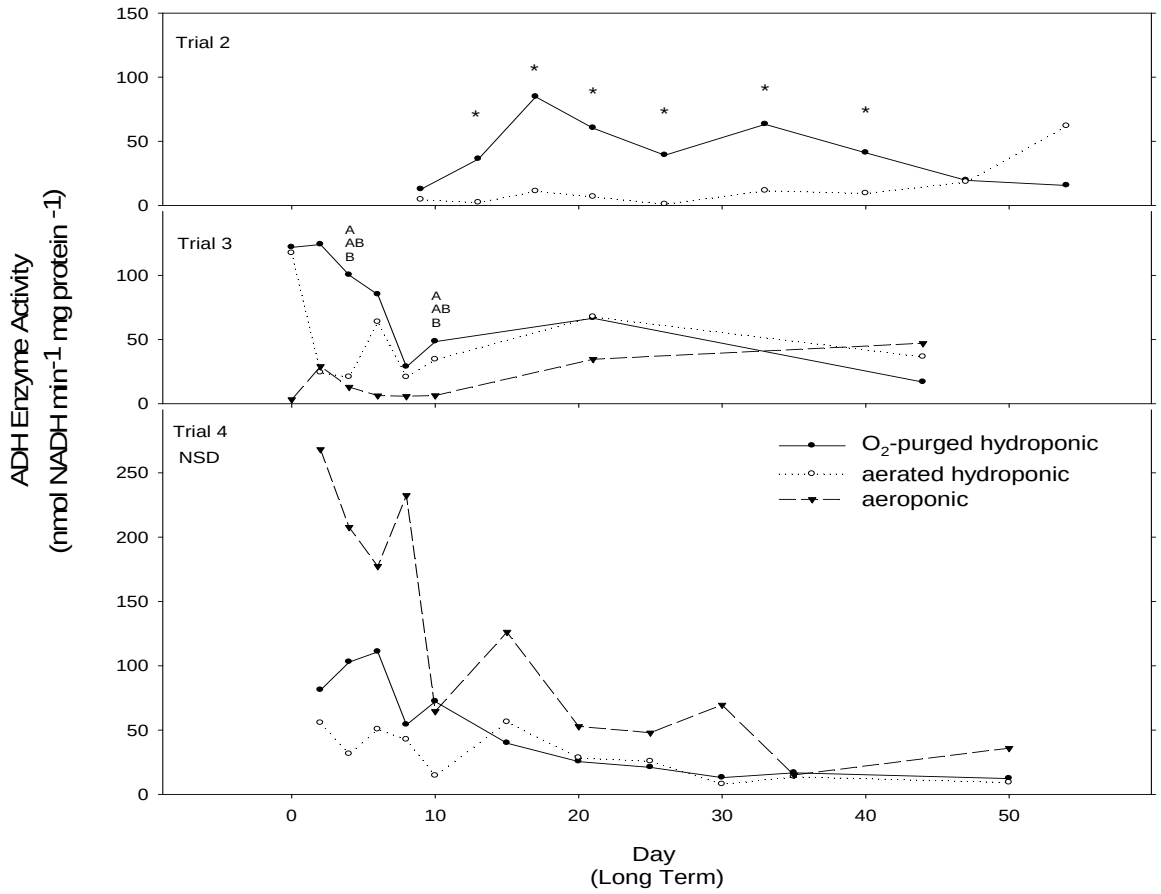

Figure 6-7. Alcohol dehydrogenase (ADH) enzyme activity for O_2-purged hydroponic, aerated hydroponic, and aeroponic treatments during 0 to 50+ d of flooding in trials 2, 3, and 4. For trial 2, asterisks represent significant difference at $P \leq 0.05$ level according to a standard T-test. For trial 3, different letters indicate significant differences at $P \leq 0.05$ according to a Waller-Duncan K-ratio test. For trial 4, no significant differences found according to a Waller-Duncan K-ratio Test.

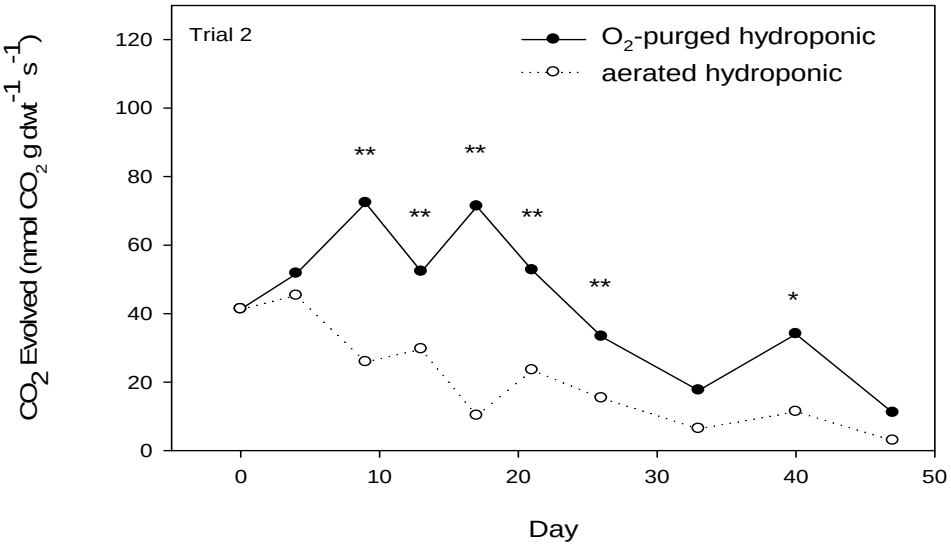

Figure 6-8. Root CO_2 evolution for O_2-purged and aerated hydroponic treatments in trial 2. Single and double asterisks indicate significant difference at $P \leq 0.1$ or 0.05, respectively according to a standard T-test.

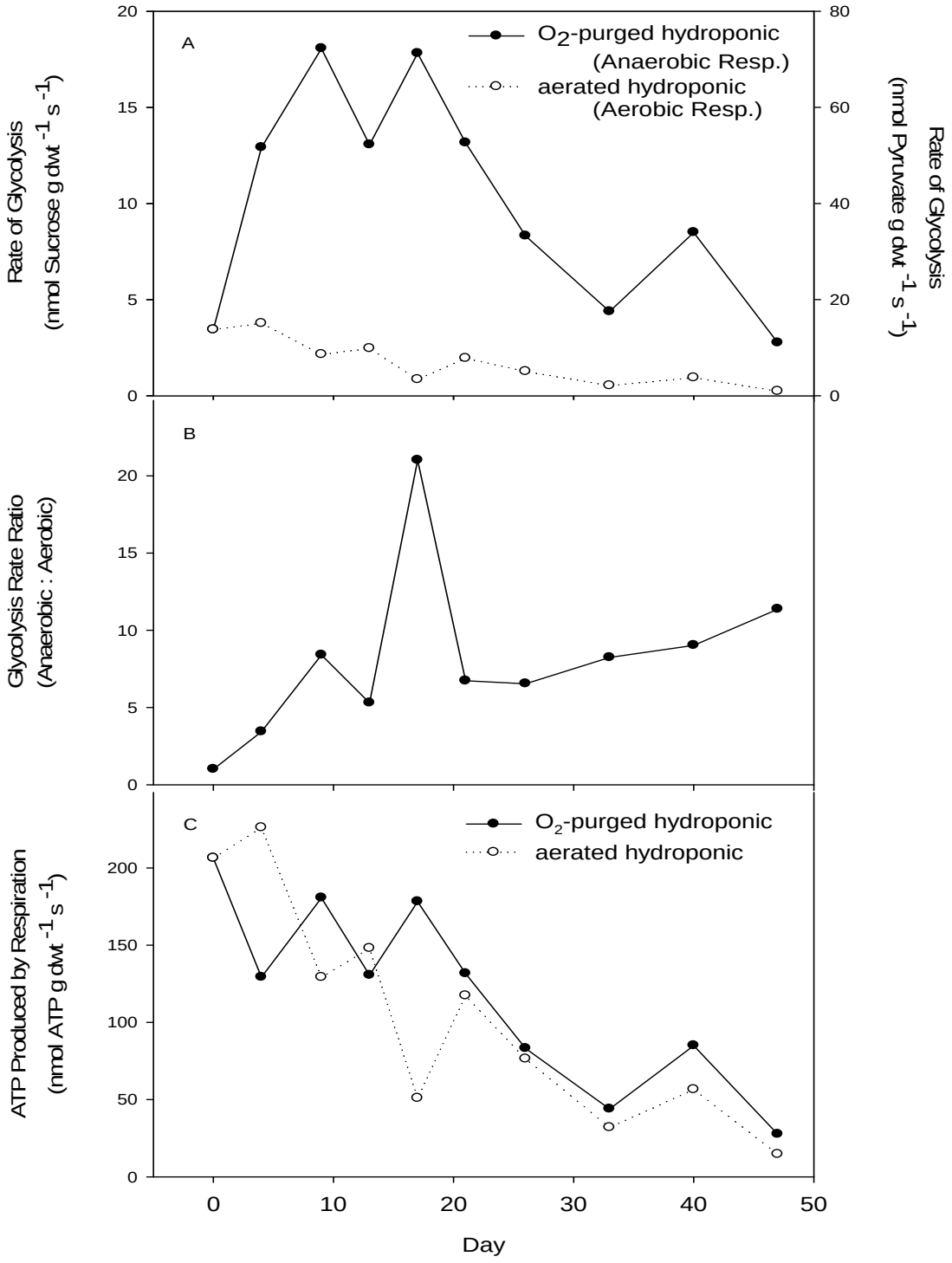

Figure 6-9. A) Root glycolysis rate for O_2-purged hydroponic (anaerobic respiration) and aerated hydroponic (aerobic respiration) treatments, B) Ratio of anaerobic to aerobic glycolysis, and C) amount of ATP produced by respiration, all for trial 2.

CHAPTER 7
CONCLUSION

Mamey sapote [*Pouteria sapota* (Jacq.) H.E. Moore and Stearn] appears to possess only limited flood tolerance. Young grafted trees grown in containers filled with calcareous soil (Krome very gravelly loam) and continuously flooded, showed a rapid decline in stomatal conductance (g_s) and net CO_2 assimilation (A), leaf epinasty and abscission, reduced leaf chlorophyll index, and stem dieback. Stomatal conductance and A declined within 3 d of flooding, leaf epinasty occurred between days 5 to 10, and leaf senescence and abscission occurred between days 15 to 30. Branch dieback and tree mortality occurred in mamey sapote between days 30 to 60. Mamey sapote did not demonstrate ability to survive long periods of flooding under the conditions tested. Some differences between cultivars were observed. In flooded 'Pantin' trees, the lower canopy experienced epinasty, leaf senescence, and leaf abscission prior to the same symptoms in the upper canopy. This "self-pruning" may have reduced the plants' overall water stress. 'Magaña' did not demonstrate this separation in lower and upper canopy response.

Three cycles of 3-d flooding and 3-d recovery in containers filled with native soil had little effect on A, g_s, or internal concentration of CO_2 in the leaf (Ci) of 'Magaña' trees. Leaf water potential temporarily declined by the third day of flooding during each cycle. This may suggest that in orchard conditions young mamey sapote trees can tolerate brief periods of soil saturation or flooding which may occur during the rainy season. 'Pantin' trees tolerated 3 cycles of 6-d flooding interspersed with 3 to 6 d of recovery, despite a consistent decline in A and g_s during flooding. The temporary decrease in A during the flooding period did not appear to be due to stomatal limitation as the Ci increased during, or immediately, after each flooding period and then declined to nonflooded levels. Similarly, Ci was higher for the leaves of continuously

flooded ‘Pantin’ and ‘Magaña’ trees than for nonflooded plants, and g_s and A decreased in the flooded plants. This suggests non-stomatal limitations on A during continuous and short duration cyclic flooding conditions.

In the field, non-root rot infested mamey sapote trees appeared to exhibit good tolerance to flooding during the fall-winter period, and less tolerance during the spring-summer period. Young trees or recently planted orchards on seedling rootstocks and/or treated with systemic fungicides may be able to survive 1 week of sustained flooding with minimal effect on tree health beyond reduced A. However, higher temperatures during the summer and/or root rot infestation may reduce the length of this time frame dramatically. Flooding in the field can lead to rapid and irrecoverable tree decline and death due to root rot such as that caused by as *Pythium splendens* Braun. Thus in the field, results indicate that mamey sapote is moderately tolerant to flooding in a very gravelly loam soil under fall-winter temperatures and without root rot infestation. However, more work is needed to separate tree decline due to flooding from that due to *Pythium splendens* root infection in this soil.

Root physiological responses and survival of *Pouteria sapota* trees were assessed in response to three different oxygen concentrations in the root zone: aerated hydroponic treatment (7-8 mg $O_2 \cdot L^{-1}$ H_2O), O_2-purged hydroponic (0-1 mg $O_2 \cdot L^{-1}$ H_2O), and aeroponic (~150 mg $O_2 \cdot L^{-1}$ air). Electrolyte leakage was significantly greater from roots in the O_2-purged hydroponic treatment than roots in the aerated hydroponic treatment. Roots in the O_2-purged hydroponic treatment evolved significantly higher levels of CO_2 than those in the aerated hydroponic treatment. The glycolysis rate was typically 5 to 10 times higher in roots in the O_2-purged hydroponic treatment than in the aerated hydroponic treatment, thus indicating a strong Pasteur effect. Roots of trees in the O_2-purged hydroponic treatment produced levels of adenosine

triphosphate (ATP) in a similar range to those of trees in the aerated hydroponic treatment. Although root alcohol dehydrogenase (ADH) activity was detected in all treatments, there were no consistent differences in ADH activity among treatments. The normally observed range of mean ADH activity in roots in both the O_2-purged and aerated hydroponic treatments was between 5 to 125 nmol NADH · min^{-1} · mg $protein^{-1}$. Mean ADH activity in the aeroponic treatments ranged from near 0 to 50 nmol NADH · min^{-1} · mg $protein^{-1}$ to as high as 250 nmol NADH · min^{-1} · mg $protein^{-1}$. Overall, there were no observable trends of ADH up-regulation or down-regulation common to all trials or treatments. Mesic trees such as swamp tupelo [*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.] exhibited root ADH activity in nonflooded seedlings of about 100 to 125 nmol · NADH · min^{-1} · mg $protein^{-1}$, and seedlings flooded for up to 30 d exhibited activity of about 200 to 300 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Angelov et al., 1996). Flood tolerant *Melaleuca cajuputi* Powell seedlings exhibited nonflooded levels of about 500 to 900 nmol · NADH · min^{-1} · mg $protein^{-1}$, and flooded levels after 2 d up to 1700 nmol · NADH · min^{-1} · mg $protein^{-1}$ (Yamanoshita et al., 2005). The ADH enzyme activity observed in roots of *Pouteria sapota* in this study lies well within the ranges of these species. The highest individual extremes of ADH activity were found in some of the aeroponic treatment plants of up to 1448 nmol NADH · min^{-1} · mg $protein^{-1}$.

Development of hypertrophic stem lenticels appeared to be a response to high moisture levels rather than lack of oxygen in the root zone because they developed on all of trees in the aeroponic treatment, some trees in the aerated hydroponic treatment and fewer trees in the O_2-purged hydroponic treatment. All trees survived in the aeroponic treatment, whereas 86-100% survived in the aerated hydroponic treatment and 57-87% of the trees survived in the O_2-purged hydroponic treatment (Chapter 6: in trials 3 and 4). The results indicate that while *Pouteria*

sapota is sensitive to very low oxygen concentrations in the root zone (O_2-purged hydroponic treatment), trees may adapt to fairly low oxygen concentration in the root zone (aerated hydroponic treatment) by reducing water loss, as evidenced by reduced g_s and E, and maintaining aerobic respiration in the roots. Alcohol dehydrogenase activity alone was not sufficient to ensure *P. sapota* survival under flooding conditions; however, the development of hypertrophic stem lenticels and/or leaf epinasty may increase the survival potential of mamey sapote if sufficient time and favorable conditions occur for the development of these adaptations under flooded conditions.

Unlike *Pouteria orinocoensis* (Aubr.) Penn. Ined. which appears to be extremely flood tolerant (Fernández, 2006), *P. sapota* appears to have limited flood tolerance under the conditions investigated. Further investigations into the mechanisms of flood tolerance among these *Pouteria* species and whether *P. orinocoensis* could be utilized as a flood-tolerant rootstock for *P. sapota* warrant further investigation.

REFERENCE LIST

Adams III, W.W., B. Demmig-Adams, A.S. Verhoeven, and D.H. Barker. 1994. 'Photoinhibition' during winter stress: Involvement of sustained xanthophylls cycle-dependent energy dissipation. Aust. J. Plant Physiol. 22:261-276.

Angelov, M.N., S.-J.S. Sung, R.L. Doong, W.R. Harms, P.P. Kormanik, and C.C. Black, Jr. 1996. Long- and short-term flooding effects on survival and sink-source relationships of swamp-adapted tree species. Tree Physiol. 16:477-484.

Arbona, V. and A. Gómez-Cadenas. 2008. Hormonal modulation of citrus responses to flooding. J. Plant Growth Regul. 27:241-250.

Bailey-Serres, J. and L.A.C.J. Voesenek. 2008. Flooding stress: Acclimations and genetic diversity. Annu. Rev. Plant Biol. 59:313-339.

Bakhtenko, E. Yu., I.V. Skorobogatova, and N.P. Karsunkina. 2007. The role of hormonal balance in plant adaptation to flooding. Plant Physiol. (Biol. Bul.) 34(6):569-576.

Balerdi, C.F., J.H. Crane, and I. Maguire. 2005. Mamey sapote growing in the Florida home landscape. FC30. Horticultural Sciences Dept., Fla. Coop. Extn. Service, IFAS, Univ. of Fla., Gainesville, Fla. p. 1-11. http://edis.ifas.ufl.edu/pdffiles/MG/MG33100.pdf

Balerdi, C.F. and P.E. Shaw. 1998. Sapodilla, sapote and related fruit, p. 78-136. In: P.E. Shaw, H.T. Chan Jr., and S. Nagy (eds.). Tropical and subtropical fruits. AgScience, Inc. Auburndale, Fla.

Bayuelo-Jimenez, J.S.B. and I. Ochoa. 2006. Caracterization morfologica de sapote mamey [*Pouteria sapota* (Jacquin) H.E. Moore&Stern] del centro occidente de Michoacan, Mexico. Revista Fitotecnia Mexicana 29(1):9-17.

Black, R.J. 1993. Florida climate data. IFAS Extension. Univ. of Fla. http://edis.ifas.ufl.edu/EH105

Blom, C.W.P.M., L.A.C.J. Voesenek, M. Banga, W.M.H.G. Engelaar, J.H.G.M. Rijnders, H.M. Van de Steeg, and A.J.W. Visser. 1994. Physiological ecology of riverside species: adaptive responses of plants to submergence. Ann. of Bot. 74:253-263.

Brand, M.D. 1994. The stoichiometry of proton pumping and ATP synthesis in mitochondria. Biochemist. 16(4):20-24.

Burns, R. M., D. A. Roberts, S. Goldweber, and B. E. Colburn. 1965. Avocado soil and root rot survey of Dade County, Florida. Proc. Fla. State Hort. Soc. 78:345-349.

Burton, A.J. and K.S. Pregitzer. 2003. Field measurements of root respiration indicate little to no seasonal temperature acclimation for sugar maple and red pine. Tree Physiol. 23:273-280.

Cai. S.-Q. and D.-Q. Xu. 2002. Light intensity-dependent reversible down-regulation and irreversible damage of PSII in soybean leaves. Plant Sci. 163:847-853.

Campbell, C.W. and S.P. Lara. 1982. Mamey sapote cultivars in Florida. Proc. Fla. State Hort. Soc. 95:114-115.

Chang, L.A., L.K. Hammett, and D.M. Pharr. 1983. Internal gas atmospheres, ethanol, and leakage of electrolytes from a flood-tolerant and a flood-susceptible sweet potato cultivar as influenced by anaerobiosis. Can. J. Bot. 61:3399-3404.

Chen, H. and R.G. Qualls. 2003. Anaerobic metabolism in the roots of seedlings of the invasive exotic *Lepidium latifolium*. Env. and Expt. Bot. 50:29-40.

Chirkova, T.V. and T.S. Gutman. 1972. Physiological role of branch lenticels in willow and poplar under conditions of root anaerobiosis. Soviet Plant Physiol. 19:289-295.

Chung, H.-J. and R.J. Ferl. 1999. Arabidopsis alcohol dehydrogenase expression in both shoots and roots is conditioned by root growth environment. Plant Physiol. 121:429-436.

City of Homestead. 2008. Emergency preparedness guide 2008. http://www.cityofhomestead.com/pdf/HurricaneGuide08.pdf

Colburn, B. and S. Goldweber. 1961. Preparation of oolitic limestone soil for agricultural use. Proc. Fla. State Hort. Soc. 74:343-344.

Crane, J.H. 2009. Personal communication. Univ. of Fla.

Crane, J.H. and C. Balerdi. 2005. Personal communication. Univ. of Fla.

Crane, J.H., C.F. Balerdi, M. Lamberts, D. Hull, and T. Olczyk. 1997. Flood damage assessment of agricultural crops in South Dade County as a result of Tropical Storm Gordon. Proc. Fla. State Hort. Soc. 110:152-155.

Crane, J.H. and F.S. Davies. 1987. Flooding, hydraulic conductivity, and root electrolyte leakage of rabbiteye blueberry plants. HortScience 22(6):1249-1252.

Crane, J.H. and F.S. Davies. 1988. Periodic and seasonal flooding effects on survival, growth, and stomatal conductance of young rabbiteye blueberry plants. J. Amer. Soc. Hort. Sci. 113:488-493.

Curtis, D.S. 1949. Further investigations on avocado decline: Effect of oxygen supply in nutrient solution on avocado and citrus seedlings studied in greenhouse tests. California Agr. 3(12):8-9.

De Simone, O., E. Müller, W.J. Junk, and W. Schmidt. 2002. Adaptations of central Amazon tree species to prolonged flooding: Root morphology and leaf longevity. Plant Biol. 4:515-522.

Degner, R.L., T.J. Stevens, and K.L. Morgan. 2002. Miami-Dade county agricultural land retention study. App. B. Vol. 3 of 6. Fla Agr. Market Res. Center. Institute of Food and Agr. Sci.. Univ. of Florida. p. 60-62. http://www.agmarketing.ifas.ufl.edu/dlfiles/DadeAgLandRetentionAppendixVolumeB.pdf

Dodd, I.C., B.J. Ferguson, and C.A. Beveridge. 2008. Apical wilting and petiole xylem vessel diameter of the *rms2* branching mutant of pea are shoot controlled and independent of a long-distance signal regulating branching. Plant Cell Physiol. 49(5):791-800.

Domingo, R., A. Pérez-Pastor, and M.C. Ruiz-Sánchez. 2002. Physiological responses of apricot plants grafted on two different rootstocks to flooding conditions. J. Plant Physiol. 159:725-732.

Drew, M.C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annu. Rev. of Plant Physiol. and Plant Mol. Biol. 48: 223-250.

Drew, M.C., Chuan-Jiu He, and P.W. Morgan. 2000. Programmed cell death and aerenchyma formation in roots. Trends in Plant Sci. 5(3): 123-127.

Else, M., K. Hall, G. Arnold, W. Davies, and M. Jackson, 1995. Export of abscisic acid, 1-aminocyclopropane-1-carboxylic acid, phosphate, and nitrate from roots to shoots of flooded tomato plants. Accounting for effects of xylem sap flow rate on concentration and delivery. Plant Physiol. 107:377-384.

Else, M.A., A.E. Tiekstra, S.J. Croker, W.J. Davies, and M.B. Jackson. 1996. Stomatal closure in flooded tomato plants involves abscisic acid and a chemically unidentified anti-transpirant in xylem sap. Plant Physiol. 112:239-247.

English, P.J., G.W. Lycett, J.A. Roberts, and M.B. Jackson. 1995. Increased 1-amino-cyclopropane-1-carboxylate oxidase activity in shoots of flooded tomato plants raises ethylene production to physiologically active levels. Plant Physiol. 109:1435-1440.

Evans, E.A. 2009. Personal communication. Univ. of Fla.

FAO-AGL. 2007. Calcareous soils. In: FAO-AGL ProSoil – Problem Soils Database, land and plant nutrition management service. [http://www.fao.org/ag/agl/agll/prosoil/calc.htm] (accessed 30 Oct., 2007).

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33:317-345.

Felle, H.H. 2005. pH regulation in anoxic plants. Ann. Bot. 96:513-532.

Fernández, M.D. 2006. Changes in photosynthesis and fluorescence in response to flooding in emerged and submerged leaves of *Pouteria orinocoensis*. Photosynthetica 44(1):32-38.

García-Sánchez, F., J.P. Syvertsen, V. Gimeno, P. Botía, and J.G. Perez-Perez. 2007. Responses to flooding and drought stress by two citrus rootstock seedlings with different water-use efficiency. Physiol. Plant. 130:532-542.

Gibbs, J. and H. Greenway. 2003. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. Funct. Plant Biol. 30:1-47.

Gibbs, J. S. Morrell, A. Valdez, T.L. Setter, and H. Greenway. 2000. Regulation of alcoholic fermentation in coleoptiles of two rice cultivars differing in tolerance to anoxia. J. Expt. Bot. 51(345):785-796.

Gil, P.M., L. Gurovich, B. Schaffer, N. García, and R. Iturriaga. 2009. Electrical signaling, stomatal conductance, ABA, and ethylene content in avocado trees in response to root hypoxia. Plant Signaling and Behavior 4(2):100-108.

Givan, C.V. 1999. Evolving concepts in plant glycolysis: two centuries of progress. Biol. Reviews 74:277-309.

Grable, A.H. 1966. Soil aeration and plant growth. Adv. in Agron. 18:57-106.

Gur, A., A. Gabai, and J. Mouyal. 1998. Combatting flooding damage in peach trees. Acta Hort. 465:609-614.

Herrera, A., W. Tezara, O. Marin, and E. Rengifo. 2008. Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. Physiol. Plant. 134:41-48.

Hole, D.J.,B.G. Cobb, P.S. Hole, and M.C. Drew. 1992. Enhancement of anaerobic respiration in root tips of *Zea mays* following low-oxygen (hypoxic) acclimation. Plant Physiol. 99:213-218.

Hong, S.S. and D.-Q.Xu. 1999. Reversible inactivation of PSII reaction centers and the dissociation of LHCII from PSII complex in soybean leaves. Plant Sci. 147:111-118.

Hook, D.D. 1984. Adaptations to flooding with fresh water. p. 265-294. In: T.T. Kozlowski (ed.). Flooding and plant growth. Acad. Press, Inc. Orlando, Florida.

Islam, M.A., S.E. MacDonald, and J.J. Zwiazek. 2003. Responses of black spruce (Picea *mariana*) and tamarack (*Larix laricina*) to flooding and ethylene. Tree Physiol. 23:545-552.

Jackson, I.J. 1989. Climate, water and agriculture in the tropics. Longman Press, N.Y. p.1-377.

Jackson, M.B. 2002. Long-distance signaling from roots to shoots assessed: the flooding story. J. Expt. Bot. 53(367):175-181.

Jackson, M.B., W.J. Davies, and M.A. Else. 1996. Pressure-flow relationships, xylem solutes and root hydraulic conductance in flooded tomato plants. Ann. Bot. 77:17-24.

Jackson, M.B., L.R. Saker, C.M. Crisp, M.A. Else, and F. Janowiak. 2003. Ionic and pH signaling from roots to shoots of flooded tomato plants in relation to stomatal closure. Plant and Soil 253:103-113.

Jacob, J. and D.W. Lawlor. 1991. Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. J. Expt. Bot. 42:1003-1011.

Javot, H. and C. Maurel. 2002. The role of aquaporins in root water uptake. Ann. Bot. 90:301-313.

Joyner, M.E.B, and B. Schaffer. 1989. Flooding tolerance of 'golden star' carambola trees. Proc. Fla. State Hort. Soc. 102:236-239.

Kaiser, W.M. and U. Heber. 1981. Photosynthesis under osmotic stress. Effect of high solute concentration on the permeability properties of the chloroplast envelope and on activity of stroma enzymes. Planta 153(5)423-429.

Kato-Noguchi, H. 2000. Osmotic stress increases alcohol dehydrogenase activity in maize seedlings. Biologia Plantarum 43(4):621-624.

Kimmerer, T.W. 1987. Alcohol dehydrogenase and pyruvate decarboxylase activity in leaves and roots of eastern cottonwood (*Populus deltoides* Bartr.) and soybean (*Glycine max* L.). Plant Physiol. 84:1210-1213.

Kolb, R.M., A. Rawyler, and R. Braendle. 2002. Parameters affecting the early seedling development of four neotropical trees under oxygen deprivation stress. Ann. Bot. 89:551-558.

Kozlowski, T.T. 1982. Water supply and tree growth. II. Flooding. For. Abstr. 43:145-161.

Kozlowski, T.T. 1984. Flooding and plant growth. Acad. Press, Inc. New York.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. Tree Physiology Monograph No. 1. Heron Publishing – Victoria, Canada. http://www.heronpublishing.com/tp/monograph/kozlowski.pdf

Kozlowski, T.T. and S.G. Pallardy. 1984. Effect of flooding on water, carbohydrate, and mineral relations. p. 165-193. In: T.T. Kozlowski (ed.). Flooding and plant growth. Acad. Press, Inc. New York.

Kozlowski, T.T. and S.G. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. The Bot. Rev. 68(2): 270-334.

Laisk, A., V. Oja,, B. Rasulov, H. Eischelmann, and A. Sumberg. 1997. Quantum yields and rate constants of photochemical and nonphotochemical excitation quenching. Plant Physiol. 115:803-815.

Larson, K.D. 1991. Flooding, leaf gas exchange, and growth of mango trees in containers. p. 57-68. In: Physiological, anatomical and growth responses of mango trees to flooding. Ph.D. Diss., Univ. of Fla., Gainesville.

Larson, K.D., F.S. Davies, and B. Schaffer. 1991a. Floodwater, temperature, and stem lenticel hypertrophy in *Mangifera indica* (Anacardiaceae). Amer. J. Bot. 78:1397-1403.

Larson, K.D., D.A. Graetz, and B. Schaffer. 1991b. Flood-induced chemical transformations in calcareous agricultural soils of South Florida. Soil Sci. 152(1):33-40.

Larson, K.D., B. Schaffer, and F.S. Davies. 1991c. Mango responses to flooding in limestone soil. Proc. Fla. State Hort. Soc. 104:33-39.

Larson, K.D., B. Schaffer, and F.S. Davies. 1993. Floodwater oxygen content, lenticels hypertrophy, and ethylene evolution from mango trees. J. Expt. Bot. 44:665-671.

Lawlor, D. 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann. Bot. 89:871-885.

Leighty, R.G. and J.R. Henderson. 1958. Soil survey (detailed reconnaissance) of Dade County, Florida. Series 1947, no. 4. U.S. Dept. of Agr. and Fla. Agr.. Expt. Sta.

Li, Y. 2001. Calcareous soils in Miami-Dade County, SL183. Soil and Water Sci. Dept., Fla. Coop. Extn. Service, IFAS, Univ. of Fla., Gainesville, Fla. p.1-3.

Li, M., D. Yang, and W. Li. 2007. Leaf gas exchange characteristics and chlorophyll fluorescence of three wetland plants in response to long-term soil flooding. Photosynthetica 45(2):222-228.

Lopez, O.R. and T.A. Kursar. 1999. Flood tolerance of four tropical tree species. Tree Physiol. 19: 925-932.

Loveys, B.R. 1977. The intracellular location of abscisic acid in stressed and non-stressed leaf tissue. Physiol. Plant. 40:6-10.

Luu, D.T. and C. Maurel. 2005. Aquaporins in a challenging environment: molecular gears for adjusting plant water status. Plant, Cell and Env. 28:85-96.

Mansfield, T.A., A.M. Hetherington, and C.J. Atkinson. 1990. Some current aspects of stomatal physiology. Annu. Rev. Plant Physiol. Mol. Biol. 41:55-75.

Mauchamp, A. and M. Méthy. 2004. Submergence-induced damage of photosynthetic apparatus in *Phragmites australis*. Env. and Expt. Bot. 51:227-235.

Melis, A. 1999. Photosystem-II damage and repair cycle in chloroplasts: What modulates the rate of photodamage in vivi? Trends Plant Sci. 4:130-135.

Morimoto, K. and Y. Yamasue. 2007. Differential ability of alcohol fermentation between the seeds of flooding-tolerant and flooding-susceptible varieties of *Echinochloa crus-galli*. Weed Biol. and Mngt. 7:62-69.

Morton, J.F. 1987. Sapote, p. 398-401. In: Fruits of warm climates. J.F. Morton Publisher, Miami, Fla.

NOAA. 2007. http://maps.csc.noaa.gov/hurricanes/viewer.html (Accessed 21 Aug. 2007).

NOAA-AOML. 2008. Frequently asked questions. National Oceanic and Atmospheric Administration – Atlantic Oceanographic and Meteorological Laboratory. Hurricane Research Division. [http://www.aoml.noaa.gov/hrd/tcfaq/G1.html] (Accessed 22 Dec. 2008).

NWS-NHC. 2008. National Hurricane Center Archive of Hurricane Seasons. National Weather Service – National Hurricane Center. [http://www.nhc.noaa.gov/pastall.shtml] (Accessed 22 Dec. 2008).

Nobel, C.V., R.W. Drew, and J.D. Slabaugh. 1996. Soil survey of Dade county area Florida. USDA, Nat. Res. Cons. Serv., Washington, D.C.

Núñez-Elisea, R., B. Schaffer, J.H. Crane, and A.M. Colls. 1998. Impact of flooding on *Annona* species. Proc. Fla. State Hort. Soc. 111:317-319.

Núñez-Elisea, R., B. Schaffer, J.B. Fisher, A.M. Colls, and J.H. Crane. 1999. Influence of flooding on net CO2 assimilation, growth and stem anatomy of *Annona* species. Ann. Bot. 84:771-780.

Ojeda, M., B.Schaffer, and F.S. Davies. 2004. Flooding, root temperature, physiology and growth of two *Annona* species. Tree Physiol. 24:1019-1025.

Otero-Sánchez, M.A., A.C. Michel-Aceves, R. Ariza-Flores, A. Barrios-Ayala, A. Rebolledo-Martinez, and D. Segura-Torres. 2008. Producción y commercialización de mamey en Guerrero, p. 69-78. In: I.A. Tejacal, A.V. Monter, V.L. Martínez, MA. Rodríguez, C.M.A. Durán, O.G.V. Torres, and D.G. Sánchez (eds.). El zapote mamey en México: advances de investigación. Universidad Autónoma del Estado Morelos. Cuernavaca, Morelos, México.

Pallet, K.E. and A.J. Young. 1993. Carotenoids. In: R.G. Alscher and J.L. Hess (eds.). Antioxidants in plants. CRC Press. Boca Raton, FL.

Pezeshki, S.R. 2001. Wetland plant responses to soil flooding. Env. and Expt. Bot. 46: 299-312.

Ploetz, R.C. and B. Schaffer. 1987. Effects of flooding and phytophthora root rot on photosynthetic characteristics of avocado. Proc. Fla. State Hort. Soc. 100:290-294.

Ploetz, R.C. and B. Schaffer. 1989. Effects of flooding and phytophthora root rot on net gas exchange and growth of avocado. Phytopathology 79: 204-208.

Ponnamperuma, F.N. 1984. Effects of flooding on soil. p. 9-45. In: Flooding and plant growth. T.T. Kozlowski (ed.). Acad. Press, Inc., Orlando, FL.

Poot, P. and H. Lamberts. 2003. Growth responses to waterlogging and drainage of woody Hake (Proteaceae) seedlings, originating from contrasting habitats in south-western Australia. Plant and Soil. 253: 57-70.

Preiszner, J., T.T. VanToai, L. Huynh, R.I. Bolla, and H.H. Yen. 2001. Structure and activity of a soybean *Adh* promoter in transgenic hairy roots. Plant Cell Rep. 20:763-769.

Raschke, K. 1975a. Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. Planta 125:243-259.

Raschke, K., 1975b. Stomatal action. Annu. Rev. Plant Physiol. 26:309-340.

Reasoner, P.W. 1887. Report on the condition of tropical and semitropical fruits in the United States in 1887. U.S. Dept. Agr., Divn. Pomol. Bul. No. 1:14.

Reid, D.M. and K.J. Bradford. 1984. Effects of flooding on hormone relations, p. 195-219. In: T.T. Kozlowski (ed.). Flooding and Plant Growth. Academic Press, Orlando, FL.

Reid, D.M. and I.D. Railton. 1974. Effect of flooding on the growth of tomato plants: Involvement of cytokinins and gibberellins, p. 789-792. In R.L. Bieleski, A.R. Ferguson, and M.M. Cresswell (eds.). Mechanisms of regulation of plant growth. Bul. 12. The Royal Society of New Zealand, Wellington.

Roberts, J.K.M, K.Chang, C. Webster, J. Callis, and V. Walbot. 1989. Dependence of ethanolic fermentation, cytoplasmic pH regulation, and viability on the activity of alcohol dehydrogenase in hypoxic maize root tips. Plant Physiol. 89:1275-1278.

Rumpho, M.E. and R.A. Kennedy. 1981. Anaerobic metabolism in germinating seeds of *Echinochloa crus-galli* (Barnyard Grass). Plant Physiol. 68:165-168.

Russell, D.A., D.M.-L.Wong, and M.M. Sachs. 1990. The anaerobic response of soybean. Plant Physiol. 92:401-407.

SAGARPA. 2008. Mamey. In: Produccion Agricola: ciclocos y perennes 2007. Anuario estadístico de la producción agrícola. Servicio de information agroalimenatria y pesquera. [http://www.siap.sagarpa.gob.mx/ventana.php?idLiga=1043&tipo=1] (Accessed 22 Dec. 2008).

Schaffer, B. 1998. Flooding responses and water-use efficiency of subtropical and tropical fruit trees in an environmentally-sensitive wetland. Ann. Bot. 81:475-481.

Schaffer, B. and P.C. Andersen (eds.). 1994. Handbook of environmental physiology of fruit crops, Vol. II. Sub-tropical and tropical Crops. CRC Press, Inc., Boca Raton, Fla. 310 pp.

Schaffer, B., P.C. Andersen, and R.C. Ploetz. 1992. Responses of fruit crops to flooding, p. 257-313. In: J.Janick (ed.). Hort. Rev. Vol. 13. Wiley, New York.

Schaffer, B., F.S. Davies, and J.H. Crane. 2006. Responses of subtropical and tropical fruit trees to flooding in calcareous soil. HortScience 41:549-555.

Schaffer, B. and R. Muñoz-Carpena. 2002. Flooding in agricultural fields in south Florida, p. 1-4. In: Degner, R.L., T.J. Stevens, and K.L. Morgan (eds.). Miami-Dade county agricultural land retention study. App. E. Vol. 6 of 6. Fla Agr. Market Res. Center. Institute of Food and Agr. Sci.. Univ. of Florida. http://www.agmarketing.ifas.ufl.edu/dlfiles/AppE_Flooding.pdf

Schaffer, B. and A.W. Whiley. 2002. Environmental physiology of avocado. p. 135-160. In: A.W. Whiley, B. Schaffer, and B.N. Wolstenholme (eds.). Avocado: botany, production and uses. CAB Intl. Press. Wallingford, U.K.

Schaffer. B, A.W. Whiley, and J.H. Crane. 1994. Mango, p. 165-197. In: Schaffer, B. and P.C. Andersen (eds.). Handbook of environmental physiology of fruit crops. Vol. II: Sub-tropical and tropical Crops. CRC Press, Inc. Boca Raton, Fla.

Schaper, H. and E.K. Chacko. 1991. Relation between extractable chlorophyll and portable chlorophyll meter readings in leaves of eight tropical and subtropical fruit-tree species. J. Plant Physiol. 138:674-677.

Secchi, F., C. Lovisolo, and A. Schubert. 2007. Expression of *OePIP2.1* aquaporin gene and water relations of *Olea europaea* twigs during drought stress and recovery. Ann. Appl. Biol. 150:163-167.

Shackel, K.A., H. Ahmadi, W. Biasi, R. Buchner, D. Goldhammer, S. Gurusinghe, J. Hasey, D. Kester, B. Krueger, B. Lampinen, G. McGourty, W. Micke, E. Mitcham, B. Olsen, K. Pelletrau, H. Philips, D. Ramos, L. Schwankl, S. Sibbett, R. Snyder, S. Southwick, M. Stevenson, M. Thorpe, S. Weinbaum, and J. Yeager. 1997. Plant water status as an index of irrigation need in deciduous fruit trees. HortTechnology 7:23-29.

Shiono, K., H. Takahashi, T.D. Colmer, and M. Nakazono. 2008. Role of ethylene in acclimations to promote oxygen transport in roots of plants in waterlogged soils. Plant Sci. 175:52-58.

Shiu, O.Y., J.H. Oetiker, W.K. Yip, and S.F. Yang. 1998. The promoter of *LE-ACS7*, an early flooding-induced 1-amino-cyclopropane-1-carboxylate synthase gene of the tomato, is tagged by a *Sol3* transposon. Proc. Natl. Acad. Sci. USA. 95:10334-10339.

Slowik, K., C.K. Labanauskas, L.H. Stolzy, and G.A. Zentmyer. 1979. Influence of root stocks, soil oxygen, and soil-moisture on the uptake and translocation of nutrients in young avocado plants. J. Amer. Soc. Hort. Sci. 104(2):172-175.

Stergios, B.G. and G.S. Howell, Jr. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98:325-330.

Stolzy, L.H., G.A. Gentmyer, L.J. Klotz, and C.K. Labanaus. 1967. Oxygen diffusion, water, and *Phytophthora cinnamomi* in root decay and nutrition of avocados. Proc. Amer. Soc. Hort. Sci. 90:67-76

Summers, J.E., R.G. Ratcliffe, and M.B. Jackson. 2000. Anoxia tolerance in the aquatic monocot *Potamogeton pectinatus*: absence of oxygen stimulates elongation in association with an unusually large Pasteur effect. J. Expt. Bot. 51(349):1413-1422.

Syvertsen, J.P. and J.J. Lloyd. 1994. Citrus, p. 65-100. In: Schaffer, B. and P.C. Andersen (eds.). Handbook of environmental physiology of fruit crops, Vol. II. Sub-tropical and tropical crops. CRC Press, Inc., Boca Raton, Fla.

Taiz, L. and E. Zeiger. 2002. Plant Physiology. Third Edition. Sinauer Associates, Inc., Publishers. Sunderland, Mass.

Tsukahara, H. and T.T. Kozlowski. 1985. Importance of adventitious roots to growth of flooded *Platanus occidentalis* seedlings. Plant and Soil 88: 123-132.

Turner, D.W. 1994. Banana, p. 37-64. In: Schaffer, B. and P.C. Andersen (eds.). Handbook of environmental physiology of fruit crops, Vol. II. Sub-tropical and tropical crops. CRC Press, Inc., Boca Raton, Fla.

Vartapetian, B.B. and M.B. Jackson. 1997. Plant adaptations to anaerobic stress. Ann. Bot. 79(Suppl. A):3-20.

Verheij, E.W.M. and R.E. Coronel (eds.). 1992. Plant Resources of South-East Asia. No. 2. Edible fruits and nuts. Prosea. Bogor, Indonesia.

Visser, E.J.W., G.M. Bögemann, C.W.P.M. Blom, and L.A.C.J. Voesenek. 1996. Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. J. Expt. Bot. 47(296):403-410.

Walton, D.C. 1980. Biochemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol. 31:453-489.

Whiley, A.W. and B. Schaffer. 1994. Avocado, p. 3-35. In: B. Schaffer and P.C. Andersen (eds.). Handbook of environmental physiology of fruit crops. Vol. II: Sub-tropical and tropical crops. CRC Press, Inc. Boca Raton, Fla.

Wilkinson, S., and W.J. Davies. 1997. Xylem sap pH increase: A drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol. 113:559-573.

Wingler, A. and T. Roitsch. 2008. Metabolic regulation of leaf senescence: interactions of sugar signaling with biotic and abiotic stress responses. Plant Biol. 10(Suppl. 1):50-62.

Yamanoshita, T., M. Masumori, H. Yagi, and K. Kojima. 2005. Effects of flooding on downstream processes of glycolysis and fermentation in roots of *Melaleuca cajuputi* seedlings. J. For. Res. 10:199-204.

Yamamoto, F., T. Sakata, and K. Terazawa. 1995. Physiological, morphological and anatomical responses of *Fraxinus mandshurica* seedlings to flooding. Tree Physiol. 15: 713-719.

Ye, Q., N.M. Holbrook, and M.A. Zwieniecki. 2008. Cell-to-cell pathway dominates xylem-epidermis hydraulic connection in *Tradescantia fluminensis* (Vell. Conc.) leaves. Planta 227:1311-1319.

Younis, H.M., J.S. Boyer, and Govindjee. 1979. Conformation and activity of chloroplast coupling factor exposed to low chemical potential of water in cells. Biochimica et Biophysica Acta 548:328-340.

Zhou, Y., L. Huang, Y. Zhang, K. Shi, J. Yu, and S. Nogués. 2007. Chill-induced decrease in capacity of RuBP carboxylation and associated H2O2 accumulation in cucumber leaves are alleviated by grafting onto figleaf gourd. Ann. Bot. 100:839-848.

BIOGRAPHICAL SKETCH

Mark Thomas Nickum was born in 1975 in Bloomington, Illinois, to Gary John Nickum and Lynnette Evelyn Eipers. His early grade school days took place in Lenexa, Kansas, after which his family moved back to Bloomington, where Mark attended Bloomington Junior High School and University High School, graduating in 1993. In high school, Mark read the journals of Captain James Cook, who circumnaviagated the earth three times and discovered much of Polynesia, including Tonga and Hawaii.

Mark attended a small liberal arts college named Knox College in Galesburg, Illinois, and studied biology and ecology, earning his B.A. in biology in 1997. Knox was famous for being a site for the Lincoln-Douglas debates. While at Knox, Mark studied abroad in Costa Rica, in 1995, with the School for Field Studies on their program in sustainable development. Finally learning Spanish, which was his worst subject in school, and breaking the language barrier paved the way for future international travels and research. Toward the end of his college career, Mark worked at Oak Ridge National Laboratory, in Tennessee, for a semester internship conducting an environmental impact assessment for railroad tracks comparing wooden crosstie systems with concrete crosstie systems. At the end of college, Mark remembered the journals of Captain James Cook and applied for the Watson Fellowship with a proposed project to travel the Pacific Islands, learning how to build Polynesian voyaging canoes; however, this fellowship was not to be. After college, Mark worked at Missouri Botanical Garden, in St. Louis, Missouri, for about a year as an herbarium assistant. It was here that he began learning about botany and how to identify the flowering plant families. It was also here where he first learned about a discipline called ethnobotany, and decided to apply for graduate school.

The desire to travel the world compelled Mark to attend the University of Hawai`i at Manoa, in order to study ethnobotany with Dr. Will McClatchey. While in Hawaii, Mark chose

to study the construction and ethnobotany of a 108 foot long, two hulled, Tongan voyaging canoe. During the course of this project, he travelled to Tonga three times to learn how the expert canoe builder, Tuione Pulotu, could build such a mammoth creation. Particularly fascinating was watching how five gigantic logs, 6 to 8 feet wide at the base, and 20 to 30 feet long, could be joined end to end to form the basis for each canoe hull, and how in the end, community ties were strengthened. Along the way, Mark learned the importance of international travel, appreciated the beauty in diverse cultures, and even learned how to play Hawaiian contemporary music on the guitar with good friends. While living on the island of Oahu, Mark travelled to the windward side many times to Waiahole, where there was a small community taro patch (*lo`i*) tucked back into the valley, away from roads, and requiring hiking through the woods and crossing streams to get to. While collecting fiddlehead ferns from the stream, wild growing passionfruit (*lilikoi*) fallen to the ground, and fresh taro (*kalo*) from the *lo`i*, Mark was overwhelmed by the community of local growers who lived and farmed in the valley. After completing his M.S. in botany in 2002, Mark decided to move back to the mainland and attend the University of Florida in the Horticultural Science Department, ultimately studying tropical fruit tree physiology at UF's Tropical Research and Education Center (TREC) in Homestead, Florida with Drs. Jonathan Crane and Bruce Schaffer. While at TREC, Mark has studied the physiology of flooding stress on a tropical fruit tree called mamey sapote (*Pouteria sapota*). He has enjoyed living in South Florida, particularly for the mango season, the avocado orchards, and exploring Big Cypress Nature Preserve and Everglades National Park, where he has slogged through cypress domes and swamps to see orchids, bromeliads, alligators, and snakes. Mark received his Ph.D. in horticultural science in May 2009.