

Responses of mamey sapote (*Pouteria sapota*) trees to continuous and cyclical flooding in calcareous soil

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ABSTRACT

Physiological and growth responses of 'Pantin' and 'Magana' mamey sapote (*Pouteria sapota*) trees to continuous and cyclical flooding were studied in a series of experiments. Trees were grown in containers in a very gravelly loam soil and were subjected to continuous flooding of the root zone for 30–66 days (Experiments 1 and 2) or alternating flooding–unflooding cycles for 50 days (Experiments 3–5). For all experiments, the control treatment consisted of nonflooded trees. Net CO₂ assimilation (*A*) and stomatal conductance (*g_s*) decreased within 3 days of continuous flooding and internal CO₂ concentration was significantly higher in leaves of flooded than nonflooded plants. In the cyclic flooding experiments, trees were flooded in 3- to 6-day cycles and then unflooded for the same time periods. Stomatal conductance and *A* decreased within 3 days of flooding, leaf epinasty occurred between days 5 and 10, leaf senescence and abscission occurred between days 15 and 30, and branch dieback and tree death occurred between days 30 and 60. Three cycles of 3-day flooding and 3-day recovery of trees had little effect on leaf gas exchange of 'Magaña' trees. Similarly, 'Pantin' trees survived 3 cycles of 6 days of flooding interspersed with 3–6 days of recovery despite consistent decreases in *g_s* and *A* during flooding. Stomatal conductance and *A* of both mamey sapote cultivars decreased within a few days of flooding and this species appears to have intermediate flooding tolerance compared with other tropical fruit crops based on tree survival.

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1. 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 (Verheij and Coronel, 1992; Balerdi and Shaw, 1998). 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 southern Florida, where it is grown on about 233 ha and is annually worth an estimated \$US 7.5 million at the farm level, and about \$US 18.5 million at the wholesale level (E.A. Evans, personal communication). In southern Florida, tropical fruit crops are grown in a calcareous soil classified as Krome very gravelly loam soil (loamy-skeletal carbonatic, hyperthermic lithic Rendoll, pH, 7.2–8.4) (Leighty and Henderson, 1958; Burns et al., 1965; Noble et al., 1996).

Flooding of the root zone 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 monsoons 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 either continuous and/or cyclic (periodic) flooding during the rainy season when the water table is high (J.H. Crane, personal communication). Depending upon water management and storm activity in south Florida, multiple flooding events which may last 2–10 days 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).

An early physiological response of trees to flooding is a decrease in stomatal conductance (*g_s*), which results in decreased transpiration (*E*) (Kozłowski and Pallardy, 1984; Schaffer et al., 1992; Kozłowski, 1997). Studies of several trees species showed that although flooding reduced *g_s*, flooded plants had similar or higher leaf water potentials than nonflooded plants. Therefore, flooding did not cause leaf water deficits in several tree species tested (Kozłowski, 1997). Concomitant with a decrease in *g_s* is a decline in net CO₂ assimilation (*A*) in flooded plants (Kozłowski and Pallardy, 1984; Schaffer et al., 1992; Kozłowski, 1997). A temporal separation, if it exists, between declines in *A* and *g_s* in flooded

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mamey trees, 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). A concurrent decline in internal partial pressure of CO_2 in the leaf (C_i) with decreased A and g_s may indicate a stomatal limitation to a sufficient quantity of CO_2 entering the leaf for maintaining A at an optimum level. However, an increase in C_i accompanied by decreased A and g_s in flooded trees may indicate a stomatal limitation (L_s) or mesophyll (internal) conductance limitation (L_m) to A (Farquhar and Sharkey, 1982). This can result from increased CO_2 in the intercellular space of the leaf which has been associated with stomatal closure (Raschke, 1975a,b; Mansfield et al., 1990). Previous field research showed that continuous flooding of mamey sapote for 45–66 days resulted in decreased A , g_s , and E after 3 days, leaf epinasty between days 5 and 10, and leaf senescence and abscission between days 15 and 30 after trees were flooded. Branch dieback and tree death occurred within 30–60 days of continuous flooding (Nickum et al., 2008).

The hypothesis was that mamey sapote trees have evolved several adaptations to flooding including regulation of transpiration and net carbon assimilation related to stomatal and non-stomatal factors. These adaptations affect growth and survival of this subtropical–tropical species. Consequently, the objectives of this study were to determine the effects of continuous and repeated cycles of short-term, cyclical flooding on leaf gas exchange, leaf and stem water potential, and overall growth and survival of container-grown mamey sapote trees growing in a very gravelly soil.

2. Materials and methods

2.1. Continuous flooding (Experiments 1 and 2)

2.1.1. Plant material and experimental design

In March 2004, 2-year-old 'Pantin' and 'Magaña' mamey sapote trees grafted onto seedling rootstocks were obtained from a commercial nursery and repotted into 19-L containers (26 cm in height) filled with Krome very gravelly loam soil. After about 1-year of acclimation in Krome soil, plants were treated with metalyxl (Ridomil™; Syngenta Crop Protection, Inc., Greensboro, NC, USA) and fosetyl-Al (Aliette™; Bayer CropScience, Research Triangle Park, NC, USA) to prevent phytophthora (*Phytophthora cinnamomi* Rands) or pythium (*Pythium splendens* Braun) root rots from developing.

Two experiments were 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 (screenhouse). In Experiment 1, 'Pantin' trees were continuously flooded for 66 days from 12 April to 17 June 2005, and in Experiment 2, 'Magaña' trees were continuously flooded for 45 days from 31 May to 15 July 2005. Each experiment consisted of a flooded and a nonflooded (control) treatment. Plants were flooded by placing the 19-L containers inside 38-L plastic 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 Experiment 1, there were ten single-tree replications per treatment, and in Experiment 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. The experiment was terminated when all trees in the flooded treatment were dead. Trees were considered dead when the lower trunk no longer had green tissue and trees would not regrow when placed in favorable growing conditions.

2.1.2. Temperature and soil redox potential measurements

In both experiments, soil temperatures were monitored with a HOBO Water Temp Pro sensor and datalogger (Onset Computer Co.,

Bourne, MA, USA) and canopy air temperatures were monitored with a StowAway TidbiT sensor situated in a shaded location in the canopy and attached to a datalogger (Onset Computer Co., Bourne, MA, USA). Soil redox potential was measured at a 4-cm depth in the flooded containers with a metallic ORP indicating electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA, USA) connected to a volt meter. Soil redox potential was measured in Experiment 1 on days 0, 1, 3, 7, and 10 and in Experiment 2 on days 0, 1, 3, 5, and 8.

2.1.3. Leaf gas exchange measurements

In both experiments, leaf gas exchange measurements of A , g_s , C_i , and E , were made with a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA). Measurements were made at a light-saturated photosynthetic photon flux (PPF) of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, a reference CO_2 concentration of $375 \mu\text{mol mol}^{-1}$ and an air flow rate into the leaf cuvette of 200 mL min^{-1} . Measurements were made from 9:00 to 12:00 h every 1–4 days for 7–14 days 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.

2.1.4. Stem water potential measurements

Stem water potential (Ψ_s) was measured on days 0 and 8 in Experiment 1 and on days 0, 3, 5, and 8 in Experiment 2. Leaves were selected from the middle of the canopy and enclosed for about 1 h prior to measurements in a plastic 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, USA). In Experiment 1, three leaves were sampled per tree at each measurement time, and in Experiment 2 one leaf was sampled per tree to reduce the overall number of leaves that were removed.

2.1.5. Leaf chlorophyll index measurements

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

2.1.6. Plant growth

Plant height was measured in both experiments from the soil surface to the top of the apical bud 1-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 Experiment 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 for the number of leaves per tree.

Immediately after the flooding period for both continuous flooding experiments, all plants were harvested, oven dried to a constant weight at 70°C and dry weights were determined.

2.1.7. Statistical analysis

All data were analyzed by 2-way (repeated measures) analysis of variance (ANOVA) and standard T -tests within dates using the SAS statistical software package (Version 9.1, SAS Institute, Cary, NC, USA).

2.2. Cyclical flooding (Experiments 3–5)

2.2.1. Plant material and experimental design

Two-year-old 'Pantin' and 'Magaña' mamey sapote (*P. sapota*) trees grafted onto seedling rootstocks were obtained from a commercial nursery in March 2004, and repotted into 19-L plastic containers filled with crushed Krome very gravelly loam soil. Trees were acclimated in the soil for about 1-year. To eliminate the influence of phytophthora (*P. cinnamomi* Rands) or pythium (*P. splendens* Braun) root rots, trees were treated with soil applications of the fungicides/algacides, metylaxyl (Ridomil™; Syngenta Crop Protection, Inc., Greensboro, NC, USA) on 28 January 2005, and fosetyl-AI (Aliette™, Bayer CropScience, Research Triangle Park, NC, USA) on 4 April 2005. Trees were also treated with metylaxyl 1–2 weeks prior to initiating treatments. All trees were housed in the same screenhouse that was used for the continuous flooding Experiments 1 and 2.

Three cyclical flooding experiments were conducted with each flooding period (*F*) followed by an unflooded recovery period (*R*) to determine the effects of cyclic flooding on tree growth and physiology. In the first cyclical flooding experiment (Experiment 3), the flooding period of 3 days was based on data from a previous mamey sapote flooding study in which significant decreases in leaf gas exchange occurred after 3 days of continuous flooding (Nickum et al., 2008). Flooding periods were doubled to 6 days for Experiments 4 and 5 after examining data from Experiment 1. Thus, Experiment 3 consisted of 3 days of flooding followed by 3 days of recovery (*F3–R3*); Experiment 4 consisted of 6 days of flooding and 6 days of recovery (*F6–R6*); and Experiment 5 consisted of 6 days of flooding and 3 days of recovery (*F6–R3*). The flooding and recovery cycles in each experiment were repeated three times over the course of each study.

Experiment 3 was conducted from 7 October to 22 October 2005 with 'Magaña' and Experiments 4 and 5 were conducted concurrently with 'Pantin' from 24 October to 29 November 2006. The same group of nonflooded plants was used for Experiments 4 and 5. In all 3 experiments there were 7 single-plant replications per treatment arranged in a completely randomized design.

Plants roots were flooded as described previously for the continuous flooding experiments. 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 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.

2.2.2. Temperature and soil redox potential measurements

In all 3 experiments, soil temperatures were monitored with a HOBO Water Temp Pro (Onset Computer Co., Bourne, MA USA) and canopy air temperatures were monitored with a StowAway TidbiT situated in a shaded location in the canopy (Onset Computer Co., Bourne, MA, USA). In Experiments 4 and 5, relative humidity (RH) was measured with a HOBO RH/Temp (Onset Computer Co., Bourne, MA, USA). During the final recovery period for Experiments 4 and 5, ambient temperatures were monitored at 60 cm above the soil surface with an automated weather station located within a few hundred meters of the experimental site (University of Florida Automated Weather Network, <http://fawn.ifas.ufl.edu>).

In all three cyclical flooding experiments, soil redox potential was measured in the flooded treatments as previously described for the continuous flooding experiments. Soil redox potential was

measured after the first day of flooding (day 1) and again on day 15 in all experiments to confirm that the soil was anoxic. Sleeves made from irrigation tubing with several holes drilled in the sides were permanently installed in the soil in each container to permit insertion of the electrode into the soil.

2.2.3. Leaf gas exchange measurements

For all experiments, leaf gas exchange was measured periodically for each single-plant replication as described in Section 2.1.3. Leaf gas exchange was measured from 9:00 to 12:00 h every 3 days beginning on day 0 and ending the last day of the last flooding-recovery cycle for all experiments. One recently matured leaf (the 5th or 6th leaf below the apical meristem) was measured on each plant using the method previously described for the continuous flooding experiments.

2.2.4. Leaf and stem water potential measurements

Leaf or stem water potential (Ψ_l or Ψ_s) was measured every 3 days beginning on day 0 and ending the last day of the experiment. Water potential was determined with a pressure chamber (Plant Water Status Console 3000 Series, Soilmoisture Equipment Corporation, Santa Barbara, CA, USA). Non-bagged leaves were used to measure Ψ_l and bagged leaves were used to measure Ψ_s (Shackel et al., 1997). Leaf bagging was not done in Experiment 3 because a preliminary study showed no significant difference in water potential between bagged and non-bagged leaves of non-stressed plants (M. Nickum, unpublished data). In addition, bagged leaves of trees under long-term flooding abscised. However, in Experiment 3 cyclical flooding did not result in leaf abscission as observed in a preliminary, long-term flooding study. Therefore for Experiments 4 and 5 all leaves were covered with plastic bags surrounded by aluminum foil for at least 1 h prior to water potential measurements. In Experiment 3, Ψ_l was measured in the field, whereas in Experiments 4 and 5 bagged leaves were detached and placed into a styrofoam cooler and immediately taken to a laboratory for Ψ_s measurements.

2.2.5. Plant growth

Plants were harvested 137 and 143 days after flooding was complete in Experiments 4 and 5, respectively for fresh tissue weight determinations. Plants were then oven dried at 70 °C to a constant weight and dry weights were determined.

2.2.6. Statistical analysis

All data were analyzed by ANOVA and treatment means were compared within each date with standard *T*-tests using SAS statistical software (Version 9.1, SAS Institute, Cary, NC, USA).

3. Results

3.1. Continuous flooding (Experiments 1 and 2)

3.1.1. Soil and air temperatures and soil redox potential

3.1.1.1. Experiment 1. Air temperatures ranged from 12 to 39 °C in Experiment 1. Nonflooded soil temperatures ranged from 15 to 38 °C and flooded soil temperatures ranged from 17 to 35 °C. Mean soil redox potential for the flooded treatment was 188.9 ± 17.3 mV beginning on day 0 and values decreased to a mean of -18 ± 57.2 mV (anoxic) by day 10 (data not shown).

3.1.1.2. Experiment 2. Air temperatures ranged from 22 to 44 °C in Experiment 2. 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 273.3 ± 11.3 mV on day 0 and 11.3 ± 32.7 mV by day 8 (data not shown).

3.1.2. Leaf gas exchange

3.1.2.1. Experiment 1. Net CO₂ assimilation for the nonflooded 'Pantin' plants remained consistently near 6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ throughout the first 14 days of the study (Fig. 1). Net CO₂ assimilation of flooded plants was significantly lower than that of the nonflooded plants by day 3, decreased to very low values by day 7 and then to 0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ 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. 1). Transpiration of flooded plants became significantly lower than that of nonflooded plants by day 3 (data not shown). Internal CO₂ concentration was significantly higher for the leaves of flooded than for nonflooded plants after 7 days of flooding (Fig. 1). By days 10 and 14, C_i for flooded plants was more than twice that of the nonflooded plants.

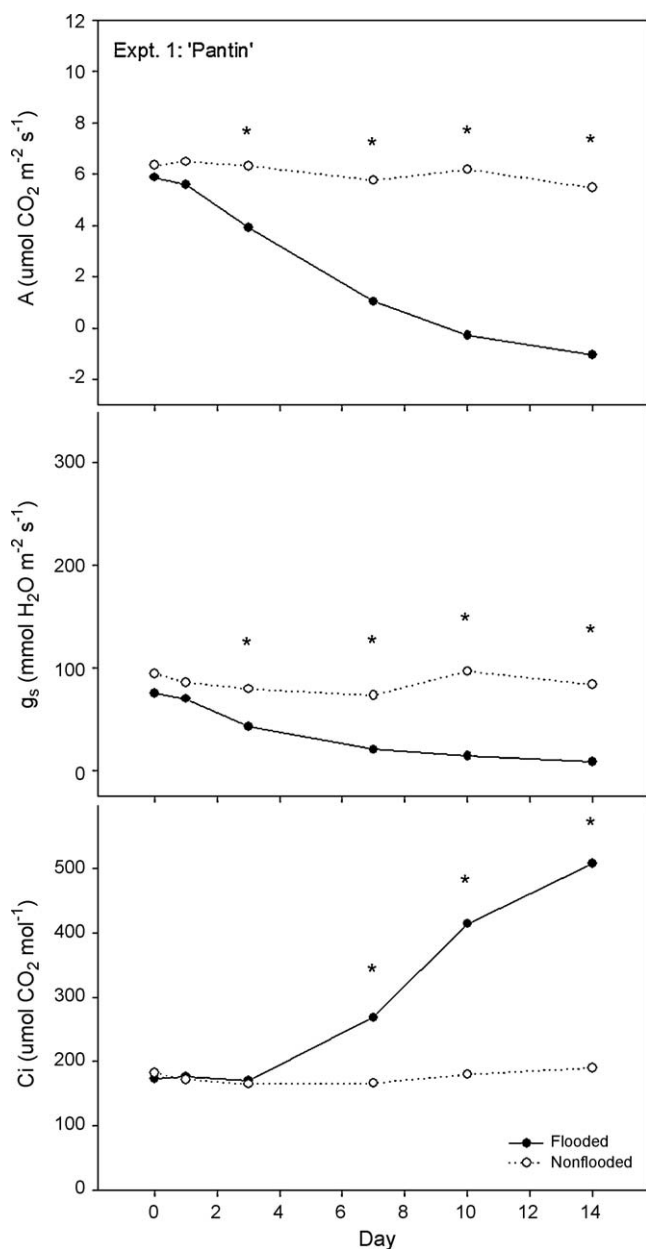


Fig. 1. Effects of flooding on net CO₂ assimilation (A), stomatal conductance of water vapor (g_s), and internal CO₂ concentration (C_i) in leaves of 'Pantin' mamey sapote trees from 12 April to 26 April 2005 (Experiment 1). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

3.1.2.2. Experiment 2. Net CO₂ assimilation of the nonflooded 'Magaña' trees remained consistently near 8 or 9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ throughout the first 7 days of the study (Fig. 2). On day 1, C_i of the flooded plants was significantly greater than that of the nonflooded plants (Fig. 2). Beginning on 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 those of nonflooded plants (Fig. 2).

3.1.3. Stem water potential

3.1.3.1. Experiment 1. Initial stem water potential was similar for both treatments with means for nonflooded and flooded plants of -0.18 and -0.19 MPa, respectively (data not shown). On day 8, Ψ_s of flooded plants was significantly lower than that of the nonflooded plants with means of -0.54 and -0.21 MPa, respectively.

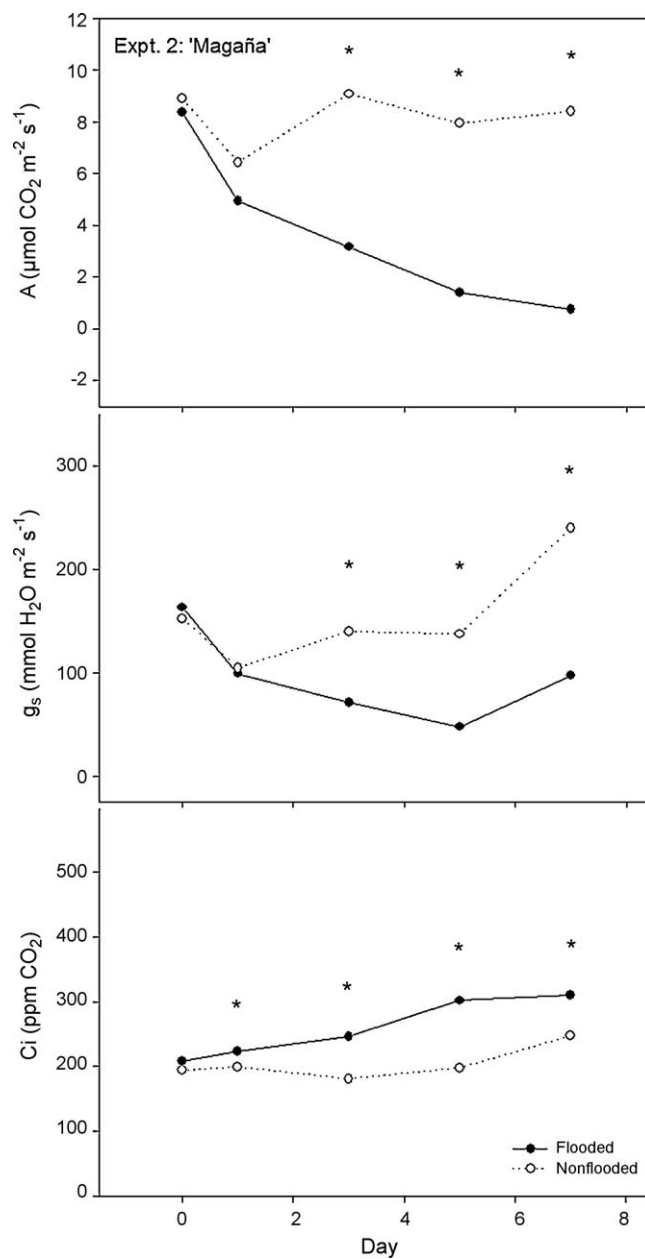


Fig. 2. Effects of flooding on net CO₂ assimilation (A), stomatal conductance of water vapor (g_s), and internal CO₂ concentrations (C_i) in leaves of 'Magaña' mamey sapote trees from 31 May to 7 June 2005 (Experiment 2). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

3.1.3.2. Experiment 2. Initial stem water potential was not significantly different between treatments with means for non-flooded and flooded plants of -0.18 and -0.17 MPa, respectively (data not shown). By day 5, Ψ_s was lower for the flooded than the nonflooded trees with means of -0.20 and -0.12 MPa, respectively, although the differences were not statistically significant. However, by day 8, Ψ_s was significantly lower for flooded trees, -2.1 MPa, compared with -0.18 MPa for nonflooded trees.

3.1.4. Leaf temperature and chlorophyll index (SPAD values)

3.1.4.1. Experiments 1 and 2. Leaf temperatures became significantly higher (by up to 1°C) for the flooded trees compared to nonflooded trees 7 and 14 days after flooding in Experiment 1 (Fig. 3A). In contrast, leaf temperatures were similar for flooded and nonflooded trees in Experiment 2, although there was an increase in leaf temperature for the flooded compared with the nonflooded leaves on day 7 (Fig. 3B). In Experiment 1, the leaf chlorophyll index (SPAD value) was similar between treatments until day 10 when the SPAD value of the flooded treatment declined by about 20% becoming significantly lower than that of the nonflooded plants (Fig. 4A). In Experiment 2, SPAD values were significantly lower for the flooded than the nonflooded treatment on days 5, 7 and 9, steadily declining to about 25% of nonflooded values (Fig. 4B).

3.1.5. Visible stress symptoms

3.1.5.1. Experiment 1. Leaf chlorosis, epinasty, wilting, and abscission were observed for flooded plants. Flooding symptoms of 'Pantin' trees were often observed in the lower canopy prior to

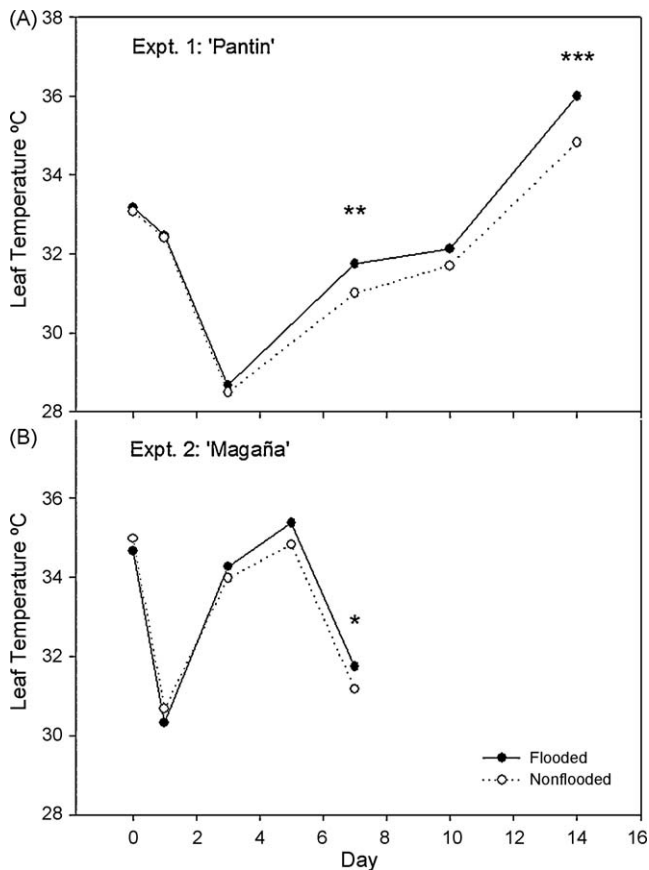


Fig. 3. Effects of flooding on leaf temperature of (A) 'Pantin' mamey sapote trees from 12 April to 20 April 2005 (Experiment 1) and (B) 'Magaña' mamey sapote trees from 31 May to 8 June 2005 (Experiment 2). Asterisks *, **, and *** indicate significant differences using a *T*-test ($P \leq 0.1$, 0.05 , and 0.01), respectively; $n = 7$.

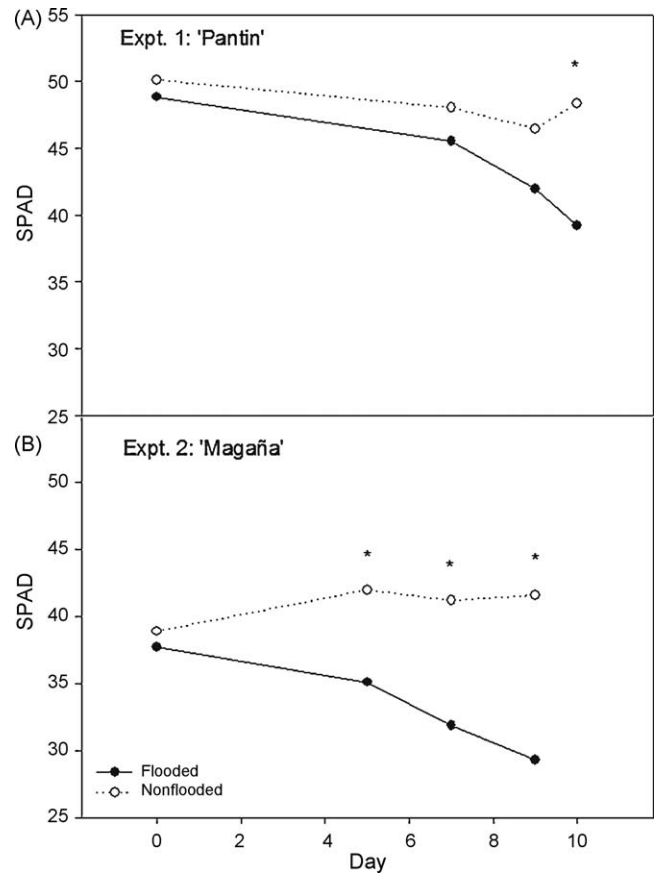


Fig. 4. Effects of flooding on leaf chlorophyll index (SPAD values) of (A) 'Pantin' mamey sapote trees from 12 April to 22 April 2005 (Experiment 1) and (B) 'Magaña' mamey sapote trees from 31 May to 9 June 2005 (Experiment 2). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

observing them in the upper canopy. Many of the flooded 'Pantin' trees showed epinasty in the lower canopy after 8 days of flooding and by day 10 epinasty occurred throughout the canopy. Most of the epinastic leaves became wilted with the leaf margins, drying and becoming curled. All epinastic leaves had become desiccated by day 12 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 become chlorotic or abscise. Some trees eventually became completely defoliated, whereas others had no leaf abscission at all, even though leaves in the entire canopy displayed leaf epinasty and desiccation.

Many of the 'Pantin' trees had a young apical flush with about 12–15 juvenile, pubescent leaves. This apical flush sometimes wilted after 22 days of flooding. 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. In the flooded treatment, stem dieback began occurring in most plants by day 30 until all plants were dead by day 66.

3.1.5.2. Experiment 2. Flooded 'Magaña' trees also displayed leaf chlorosis, epinasty, wilting, and abscission. Epinasty occurred on six out of seven flooded trees between days 5 and 7, with mild leaf chlorosis on two trees and more severe chlorosis on one tree. Epinasty occurred throughout the plant, and did not occur at different times based on upper and lower canopy positions. Flooded plants exhibited marginal leaf curling by day 9 on either the upper leaves or all leaves, and the leaves were either wilted or

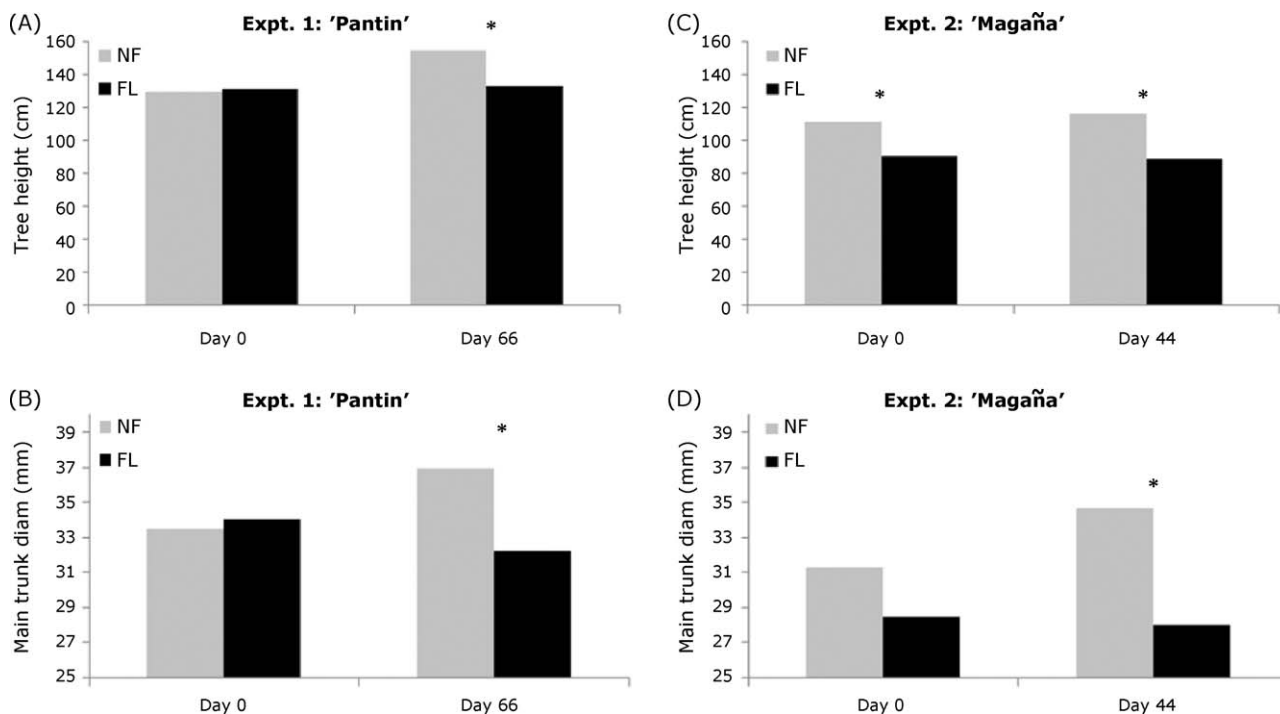


Fig. 5. Effects of flooding on (A) mean tree height, and (B) mean trunk diameter of nonflooded and flooded 'Pantín' trees (Experiment 1). (C) Mean tree height, and (D) mean trunk diameter of nonflooded and flooded 'Magaña' trees (Experiment 2). Asterisks indicate significant differences using a *T*-test ($P \leq 0.01$); $n = 7$.

desiccated. Leaves on all flooded plants exhibited epinasty and became completely desiccated by day 14. Most of the leaves in the canopy had abscised after 14–22 days of flooding. 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.

3.1.6. Tree height and trunk diameter

3.1.6.1. Experiment 1. There were no significant differences in tree height or trunk diameter between treatments at the beginning of the experiment; however, at the end of the experiment the heights and trunk diameters of the nonflooded trees were significantly greater than those of the flooded trees (Fig. 5A and B).

3.1.6.2. Experiment 2. Initial tree height was significantly different between treatments (Fig. 5C); however, the mean number of leaves per plant was not significantly different between treatments, with the nonflooded plants averaging 91.4 ± 27.8 leaves per tree, and the flooded plants averaging 81.9 ± 23.5 leaves per tree. Height of nonflooded trees increased slightly by day 44 (Fig. 5C). Initial trunk diameter was not significantly different between nonflooded and flooded plants; however, by day 44 the trunk diameter of the nonflooded plants was significantly greater than that of the flooded plants (Fig. 5D).

3.1.7. Fresh and dry weights

3.1.7.1. Experiments 1 and 2. Mean fresh and dry weights for roots, stems, and leaves were significantly lower for plants in the flooded compared to those in the nonflooded treatment at the end of Experiment 1 (Fig. 6). Similarly, at the end of Experiment 2, mean fresh and dry weights for leaves and stems of the flooded plants were significantly lower than those of the nonflooded plants. However, root fresh and dry weights of the flooded plants were

not significantly different from those of the nonflooded plants (Fig. 7).

3.2. Cyclical flooding (Experiments 3– 5)

3.2.1. Temperature, soil redox potential, and tree symptoms

Soil redox potentials declined rapidly after flooding in all 3 experiments, with the soil in each flooded container reaching a

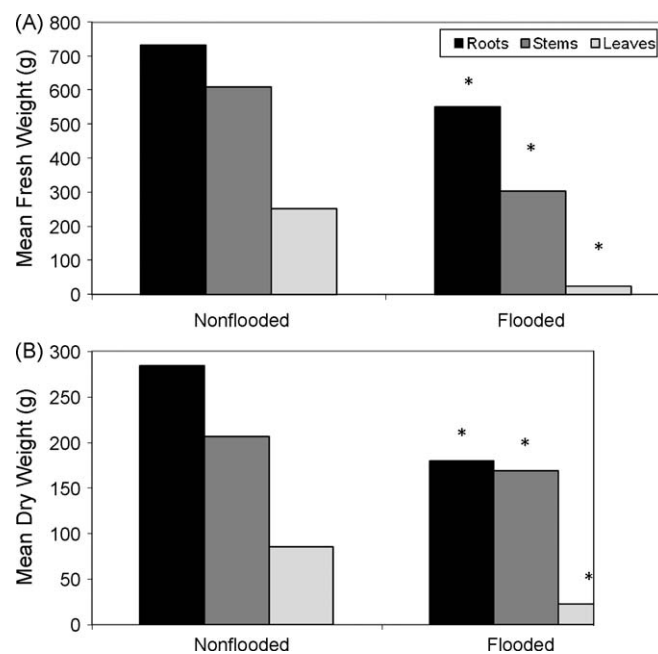


Fig. 6. Effects of flooding on (A) mean fresh weights, and (B) dry weights of roots, stems, and leaves of nonflooded and flooded 'Pantín' mamey sapote trees after 66 days of flooding (Experiment 1). Asterisks indicate significant differences using a *T*-test ($P \leq 0.01$); $n = 7$.

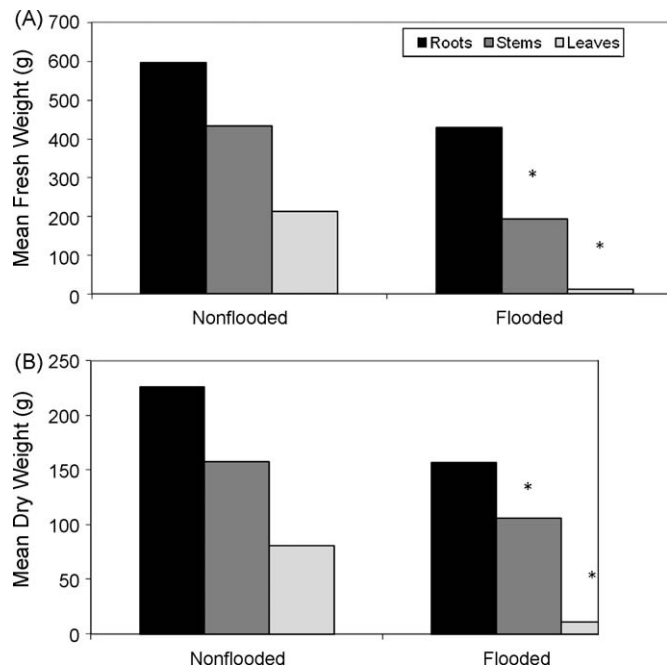


Fig. 7. Effects of flooding on (A) fresh weights, and (B) dry weights of roots, stems, and leaves of nonflooded and flooded 'Magaña' mamey sapote trees after 45 days of flooding (Experiment 2). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

redox potential of 160.6 ± 20.4 mV on day 1 and decreasing to 109.3 ± 59.3 by day 15 of flooding.

3.2.1.1. Experiment 3. Ambient air temperatures ranged from 22 to 39 °C, and flooded and nonflooded soil temperatures were slightly lower than air temperatures. No symptoms of leaf chlorosis, epinasty, wilting, or abscission were observed in any experiment.

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

3.2.2. Leaf gas exchange

3.2.2.1. Experiment 3. Net CO₂ assimilation remained relatively constant for the nonflooded plants throughout the experiment with a mean between 6 and 8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 8), whereas *A* for the flooded plants decreased to about 4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ during each of the first two flooding periods, and recovered to control levels during the unflooded period. However, there was no significant difference between treatments during any flooding period. During the last 3 days of flooding, *A* of both flooded and nonflooded treatments was slightly above 8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. There were no significant effects of flooding on *g_s*, *C_i* (Fig. 8), or *E* (data not shown) and the general trends appeared similar for both nonflooded and flooded treatments.

3.2.2.2. Experiment 4. Net CO₂ assimilation of nonflooded plants remained between 8 and 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the first two flood-recovery cycles (24 days; Fig. 9). After the first 6-day flooding

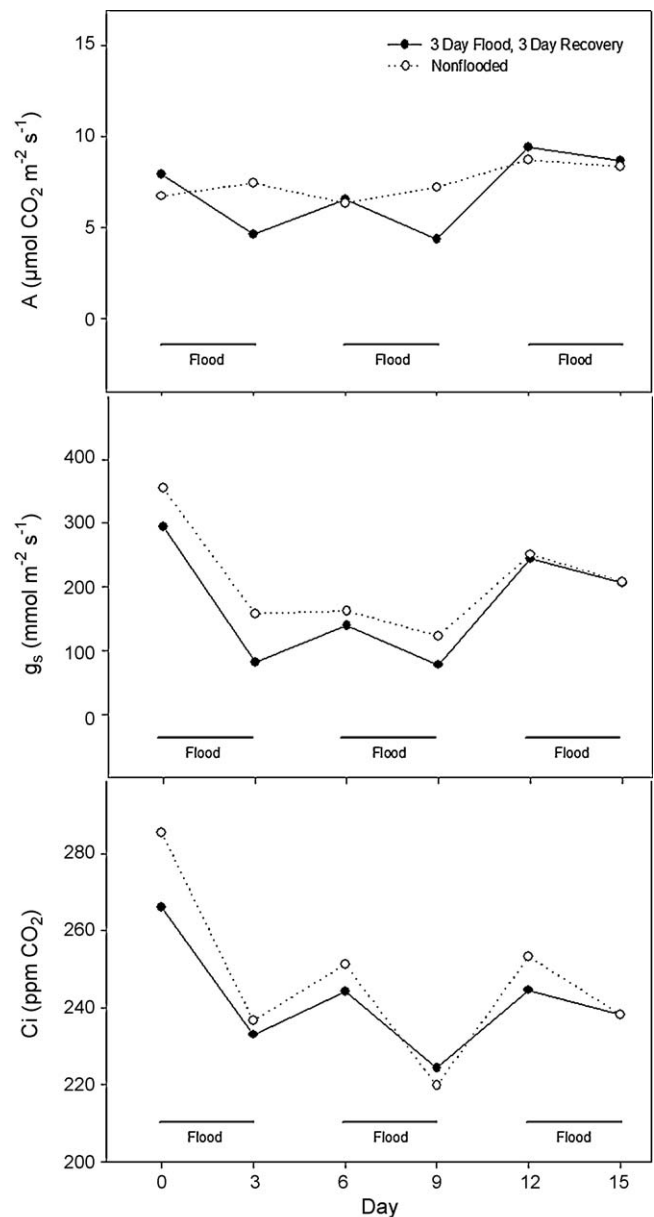


Fig. 8. Effects of cyclical flooding (3 days flooding followed by 3 days of recovery) on net CO₂ assimilation (*A*), stomatal conductance of water vapor (*g_s*), and internal CO₂ concentrations (*C_i*) in leaves of 'Magaña' mamey sapote trees from 7 October to 22 October 2005 (Experiment 3). No significant differences were found using a *T*-test ($P \leq 0.05$); $n = 7$.

period, *A* decreased to about 2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and *C_i* levels became significantly higher for the flooded than the nonflooded plants (Fig. 9). Stomatal conductance was significantly lower on days 3 and 6 for the flooded than the nonflooded plants. During the first recovery period, *g_s* returned to pre-flood levels. Net CO₂ assimilation of the flooded plants returned to pre-flood levels after the first 6-day recovery period (to $\sim 9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ by day 12), although *A* was still significantly lower for plants in the flooded than those in the nonflooded treatment due to an increase in the *A* of the nonflooded plants.

Plant responses during the second cycle of flooding and recovery were similar to responses during the first cycle. On the third day of the flooding period, *g_s* and *A* decreased in flooded plants. Net CO₂ assimilation decreased to about 3 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the flooded treatment by the sixth day of flooding, and *C_i* significantly increased. However, 3 days into the second recovery period *A* of flooded plants

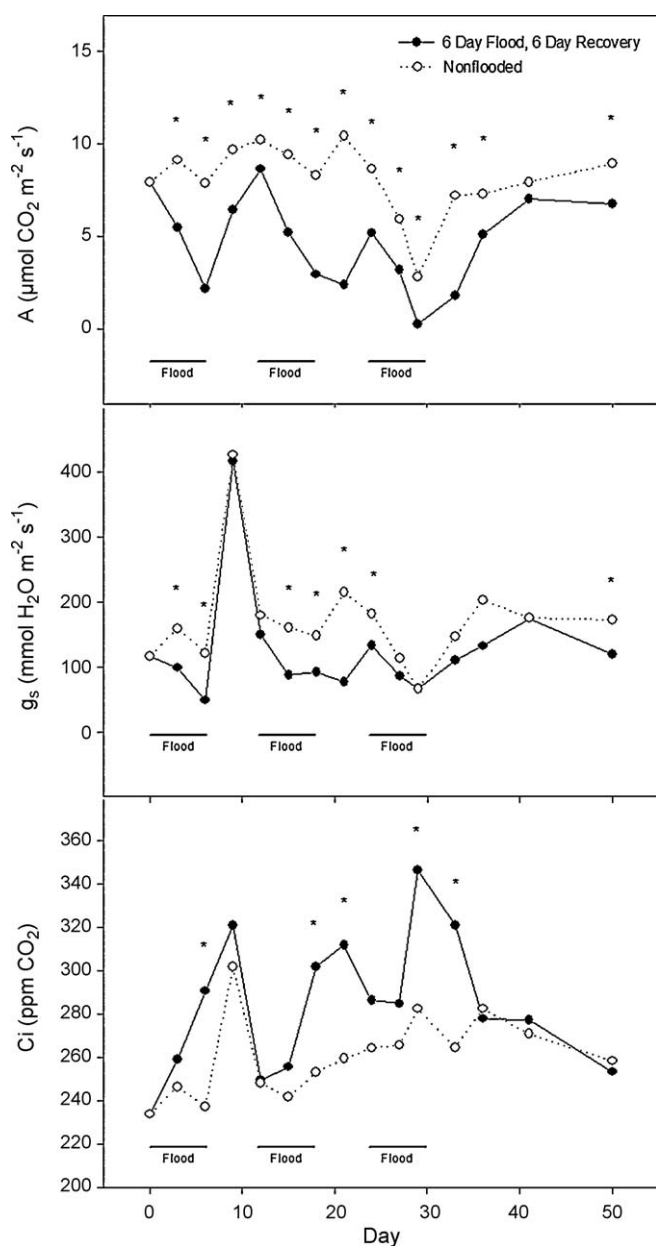


Fig. 9. Effects of cyclical flooding (6 days of flooding followed by 6 days of recovery) on net CO₂ assimilation (*A*), stomatal conductance of water vapor (*g_s*), and internal CO₂ concentrations (*C_i*) in leaves of 'Pantin' mamey sapote trees from 24 October to 29 November 2006 (Experiment 4). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

was only $2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, which was significantly lower than that of the nonflooded plants which averaged $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Internal CO₂ concentration remained higher and *g_s* remained lower in plants in the flooded compared to those in the nonflooded treatment. Six days into the second recovery period, *A* of flooded plants increased slightly to $5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, but this was still significantly lower than that of the nonflooded plants. The *C_i* was not significantly higher and *g_s* was still significantly lower in plants in the flooded compared to those in the nonflooded treatment.

Plants in both treatments had low *A* during the third flooding period; however, *A* of flooded plants was significantly lower than that of nonflooded plants. For flooded plants, *C_i* became 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

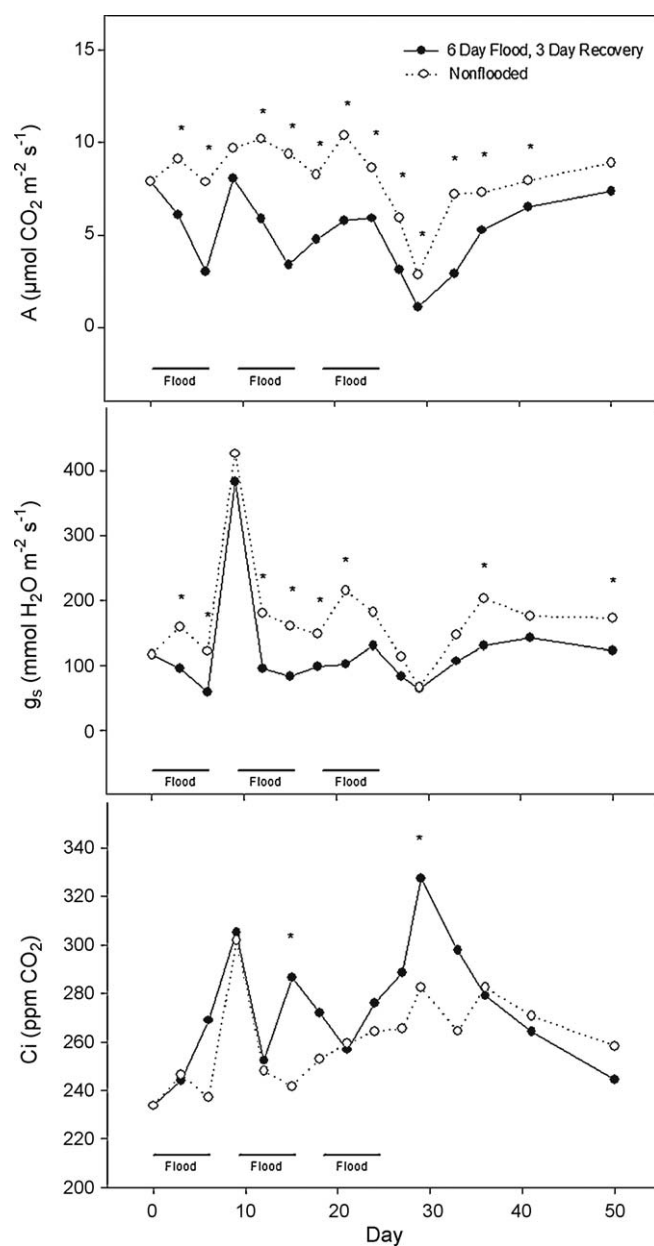


Fig. 10. Effect of cyclical flooding (6 days of flooding followed by 3 days of recovery) on net CO₂ assimilation (*A*), stomatal conductance of water vapor (*g_s*), and internal CO₂ concentrations (*C_i*) in leaves of 'Pantin' mamey sapote trees from 24 October to 29 November 2006 (Experiment 5). Asterisks indicate significant differences using a *T*-test ($P \leq 0.05$); $n = 7$.

significantly different between treatments during this flooding cycle. Temperatures had returned to their previous levels by day 3 of the recovery period. Net CO₂ assimilation for the flooded plants slowly increased to nonflooded levels until day 41 (11 days recovery after the third flooding period) when there was no significant difference in *A* between the treatments. Stomatal conductance and *C_i* also returned to nonflooded levels over time. There were no significant differences in *A*, *g_s*, *C_i*, or *E* between treatments on day 84 (54 days after the final flooding cycle) (data not shown).

3.2.2.3. Experiment 5. Results of Experiment 5 were similar to those in Experiment 4. During the first flooding period, *A* and *g_s* declined significantly by day 3 (Fig. 10). By day 6, *A* of flooded plants fell significantly to $3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and *C_i* increased

slightly, but differences in C_i were not significant between treatments. There were no significant differences in A , g_s , and C_i between plants in both treatments after the first 3-day recovery period (day 9).

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

During the third flooding period, A of flooded plants was significantly lower than that of plants in the nonflooded treatment and remained at about $5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, without decreasing further (Fig. 10). Stomatal conductance continued to be significantly lower for flooded compared with nonflooded plants, but C_i of plants in the flooded treatment did not increase significantly during the third flooding period. However, 5 days into recovery after the third flooding period, C_i of flooded plants increased significantly compared to that of nonflooded plants. Air and soil temperatures were low (6–10 °C) during this period. On days 33 and 36 (9 and 12 days after the last flooding period) C_i levels of flooded plants returned to those of plants in the nonflooded treatment, while A was significantly lower for flooded than nonflooded plants until day 50. There were still significant differences in g_s and E (data not shown) on day 50 between treatments. There were no significant differences in gas exchange variables for both treatments by day 84 (data not shown).

3.2.3. Leaf water potential

3.2.3.1. Experiment 3. Leaf water potential of trees in the nonflooded treatment remained between -0.1 and -0.2 MPa over a 15-day period (Fig. 11). After the first 3-day flooding period, Ψ_l of flooded plants decreased significantly to about -0.8 MPa; however, after 3 days of recovery (on day 6), Ψ_l of the flooded plants returned to that of the nonflooded plants. During the second 3-day flooding period to day 9, there was also a decrease in the Ψ_l in the flooded treatment to about -0.6 MPa, although this value was not significantly different from that of the nonflooded treatment. The 3-day recovery period from days 9 to 12 again showed recovery of the flooded trees to nonflooded Ψ_l levels. During the final 3-day flooding 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

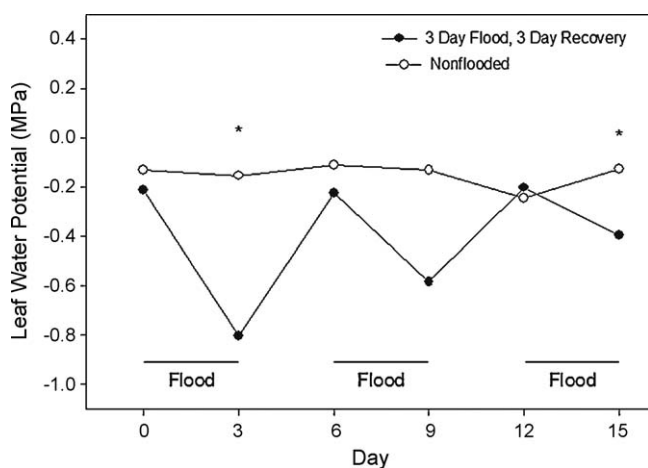


Fig. 11. Effects of cyclical flooding (3 days of flooding followed by 3 days of recovery) on leaf water potential (Ψ_l) in leaves of 'Magaña' mamey sapote trees from 7 October to 22 October 2005 (Experiment 3) – F3R3. Asterisks indicate significant differences using a T -test ($P \leq 0.05$); $n = 7$.

similar to those of the nonflooded plants after each recovery period.

3.2.3.2. Experiments 4 and 5. There was no significant difference in Ψ_s during the 6-day flooding or after the 6- or 3-day recovery periods between plants in both treatments. Mean Ψ_s levels were in the range of -0.2 to -0.6 MPa (data not shown).

3.2.4. Fresh and dry weights

3.2.4.1. Experiments 4 and 5. There were no significant differences between treatments for mean fresh or dry weights of roots, shoots, or leaves in either experiment (data not shown). However, root fresh and dry weight tended to be greater for cyclically flooded plants compared to nonflooded plants. The dry weight root:shoot ratio of plants in the nonflooded treatment was 1.0, and for the cyclically flooded plants was 1.20 and 1.16, respectively.

4. Discussion

4.1. Continuous flooding

The decrease in A after 3 days of flooding appeared to occur simultaneously with a decrease in g_s for mamey sapote trees in this study. These early decreases in A were likely due to L_s , as the C_i levels at this stage were still below the ambient CO_2 concentration. If the C_i levels were equal or above the ambient CO_2 level, that could indicate that reduced A was 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 Ψ_s did not occur at the same time as, or prior to, reductions in g_s . While Ψ_s did decrease over time, this did not occur until the 8th day of flooding.

Fernández (2006) calculated L_s and L_m limitations for *Pouteria orinocoensis* (Aubr.) Penn., a species related to mamey sapote. They found that flooded seedlings with non-submerged leaves had L_s of 36% 1-day prior to flooding, which increased to 50% after 3 days of flooding, and 71% after 7 days of flooding, where it remained relatively constant at least through day 20. Corresponding measures of L_m increased from 0% initially to 61% after 20 days of flooding. However, even with these significant increases in L_s and L_m , A still remained between 3.5 and $3.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ until day 20 of flooding. In our study, A of mamey sapote reached $0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ within 7–10 days of flooding. *P. orinocoensis* is considered flood tolerant and is found in seasonally flooded forests in Venezuela. Our data suggests that mamey sapote is less 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 $0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in flooded mamey sapote, also leading to the significant increase in C_i of the flooded over the nonflooded plants (Figs. 1 and 2). In addition, the leaf chlorophyll index dropped 20–25% in flooded plants, indicating that reduced chlorophyll content may also have been a factor contributing to mesophyll (internal) limitations as well as a reduction in photosynthetic capacity of mamey sapote. Similarly, Kozłowski and Pallardy (1984) attributed reductions of photosynthesis in flooded plants to be in part due to reduced chlorophyll content of leaves, early leaf senescence, and abscission.

4.2. Cyclical flooding

Three cycles of 3-day flooding and 3-day recovery had little effect on leaf gas exchange (i.e., A , g_s , and C_i) of 'Magaña' mamey sapote trees. Leaf water potential temporarily decreased the third day of flooding during each cycle. This suggests that young mamey sapote trees under orchard conditions may tolerate brief periods of flooding which may occur during the rainy season.

In contrast, 'Pantin' mamey sapote trees tolerated 3 cycles of 6-day flooding interspersed with 3–6 days of recovery despite a consistent decrease in A and g_s during flooding. The temporary decrease in A during flooding presumably was not due to L_s , as the C_i in flooded trees increased during or immediately after each flooding period and then declined to nonflooded levels.

The decrease in A for all treatments on day 25 was probably due to low temperatures which dropped to about 11 °C (Adams et al., 1994; Zhou et al., 2007). In both Experiments 4 and 5 it appeared that the low temperatures coupled with any moderate existing damage to the photosynthetic apparatus of the flooded treatment accentuated the increase in C_i on day 29.

In the short 3-day flooding periods of Experiment 3, Ψ_1 decreased for the flooded treatment after each 3-day flooding period and recovered to normal levels after each 3-day recovery period (Fig. 11), whereas in the longer 6-day flooding periods in Experiments 4 and 5, there were no significant differences in Ψ_s between treatments. It appears that the petiole may play a role in control of water flow into the leaf of mamey sapote based on differences in Ψ_1 and Ψ_s between treatments.

Mamey sapote appears much more flood-sensitive to cyclic flooding than some other tropical fruit trees growing in Krome very gravelly loam soil, including carambola (Joyner and Schaffer, 1989; Schaffer et al., 2006) and mango (Larson et al., 1991a,b). However, its flood tolerance is similar to that of sugar apple (*Annona squamosa*) and custard apple (*Annona reticulata*) (Núñez-Elisea et al., 1998, 1999; Schaffer et al., 2006). For example, flooding periods lasted 3 weeks and recovery periods lasted 3–6 weeks for carambola trees. Despite these relatively long flooding cycles, carambola leaves were able to recover to relatively normal gas exchange levels when unflooded (Joyner and Schaffer, 1989).

The three cyclic flooding experiments suggest that mamey sapote is capable of withstanding repeated periods of flooding for 3–6 days followed by at least an equal period of recovery. However, flooding periods longer than 7 days may lead to both physiological decline (e.g., stomatal and non-stomatal decreases in A) and physical decline (e.g., leaf epinasty, desiccation, and senescence) (Nickum et al., 2008). Thus, under natural flooding cycles, which in monsoon climates or rainy seasons can lead to multiple floods of 2–10 days per event (Jackson, 1989; Schaffer and Andersen, 1994; NWS-NHC, 2008), mamey sapote trees are capable of surviving without long-term damage.

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