Flooding, root temperature, physiology and growth of two Annona species

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Summary The effects of root zone temperature (RZT) and flooding on physiology and growth of Annona glabra L. (pond apple) and A. muricata L. (soursop) were investigated. Trees planted in containers were exposed to RZTs of 5, 10, 20, 25 or 35 °C in controlled root temperature chambers. Trees at each RZT were either non-flooded (control) or continuously flooded. There were four replications over time for each treatment combination. Pond apple was more flood-tolerant than soursop. A combination of flooding and RZTs of 5 and 10 °C resulted in tree mortality of both species by Week 4. Only trees that appeared to develop morphological adaptations survived continuous flooding. In both species, net CO2 assimilation (A) decreased to nearly zero within 1 week following exposure to RZTs of 5 or 10 °C and became consistently negative over the remaining experimental period. Flooding reduced leaf chlorophyll index (measured with a SPAD meter), A and plant growth, and increased root electrolyte leakage from soursop. Optimum growth occurred at RZTs of 25 to 35 °C for non-flooded pond apple trees and at 20 to 25 °C for flooded trees. Soursop exhibited maximum growth at RZTs of 35 °C under non-flooded conditions and at 25 °C under flooded conditions.

Keywords: Annona muricata, Annona glabra, chlorophyll, net CO2 assimilation, pond apple, root electrolyte leakage, soil redox potential, soursop.

Introduction

The genus Annona includes several economically important subtropical and tropical fruit tree species (Morton 1987, Nakasone and Paull 1998), including cherimoya (A. cherimola Mill.), soursop (A. muricata L.), sugar apple (A. squamosa L.), and atemoya (A. squamosa L. × A. cherimola Mill.). Pond apple (A. glabra L.) is native to tropical and subtropical wetlands of the Americas and is generally not considered a commercial crop (Nakasone and Paull 1998).

Most Annonaceae species cultivated for their fruit, such as sugar apple and atemoya, are susceptible to flooding damage (Núñez-Elisea et al. 1999). In contrast, soursop and pond apple are relatively tolerant to flooding and have the potential for use as flood-tolerant Annona rootstocks (Núñez-Elisea et al. 1999). The most notable effect of flooding on trees is a reduction in root and shoot growth because of decreased soil oxygen content (Schaffer et al. 1992). Various physiological and metabolic processes are affected by flooding, including decreases in net CO2 assimilation (A) (Larson et al. 1991a, Núñez-Elisea et al. 1999), stomatal conductance to CO2 (gs) (Davies and Flore 1986, Crane and Davies 1987), transpiration (E) (Davies and Flore 1986, Crane and Davies 1989) and root hydraulic conductivity (Syvertsen et al. 1983, Crane and Davies 1987). Flooding of commercial Annona species, even for short periods, reduces growth, may cause defoliation and severely reduces flowering and fruit set (Marler et al. 1994).

Some areas where Annonas are grown commercially are periodically subjected to low winter temperatures (< 10 °C). The effect of low air temperature is more pronounced on soursop than on atemoya, sugar apple or pond apple: branches of juvenile soursop trees are killed when air temperatures are less than 0 °C (Campbell et al. 1977). There is potential for using flood-tolerant soursop as a rootstock for less cold-sensitive commercial Annona species in flood-prone areas. Although soursop is the most sensitive of the commercial Annona species to low temperatures, no reports distinguish between air temperature effects on the canopy and soil temperature effects on the roots of this species. Characterizing the effects of root temperature on soursop in flooded and non-flooded conditions would help define the suboptimal temperature range where soursop could be used as a rootstock in flood-prone areas.

We evaluated the effects of root zone temperatures (RZTs) on physiology and growth of flooded and non-flooded pond apple and soursop trees. The hypotheses tested were: (1) physiological and growth responses to flooding differ between pond apple and soursop; and (2) the relative flood tolerance of pond apple and soursop is influenced by medium (or soil) temperature.

Materials and methods

Plant material

Trees between 6 and 18 months of age were studied. Trees of
Two uniform trees of each species were selected and placed in each of five root temperature chambers, and subjected to one of five RZTs (5, 10, 20, 25 or 35 ± 2 °C) in a greenhouse in Gainesville, Florida. Trees at each RZT were either non-flooded (control) or continuously flooded. Because of the limited number of temperature chambers, treatments were replicated and blocked over time for a total of four replications over a 1-year period. The treatments were replicated from August–September, October–November, April–May and May–June. A similar experimental design was used previously in a soil temperature study with Averrhoa carambola L. trees (George et al. 2002). The treatment period was 6 weeks (Ojeda et al. 2004). Day/night ambient air temperatures in the greenhouse throughout the experiment ranged from 40/25 to 25/15 °C and relative humidity ranged from 80 to 95%.

Root temperature chambers

The chambers were thermostatically controlled freezers, modified by replacing the lids with styrofoam lids. The potted trees were inserted through holes in the styrofoam lids and positioned with roots in the controlled-temperature chamber and canopy exposed to the ambient air temperature. Styrofoam was also placed on the top of the containers to reduce evaporation from the potting medium and moderate temperature changes.

An oscillating fan was placed inside each growth chamber to help maintain uniform temperatures throughout the chamber and facilitate air circulation. Five 20-l plastic buckets filled with water were placed inside the 5, 10 and 20 °C chambers to reduce temperature fluctuations. The 25 and 35 °C treatment chambers were half-filled with water and an aquarium water heater was installed below the water level to help maintain RZTs. Ambient air temperatures in the greenhouse and RZTs in each chamber were continuously monitored and recorded with a calibrated Hobo H8 Pro Series temperature logger (Onset Computer, Pocasset, MA) and a mercury thermometer. The thermometers monitoring RZTs were positioned at the center of the potting medium in one container per chamber. Ambient air temperatures and relative humidity in the greenhouse were measured at 1.82 m above ground level.

Medium redox potential (Eh) was monitored with a platinum combination electrode (Ag+/AgCl, Accumet, Fisher Scientific). Medium Eh was recorded for each flooded tree for the first 3 days of each replication and then 7 days later, because Eh tends to increase before reaching a stable potential (Larson et al. 1991b).

Physiological measurements

Electrolyte leakage was measured as described by Crane and Davies (1987) with 0.5 g of non-woody roots (fresh mass) at the end of each replication. Roots were excised, rinsed in deionized water, and placed in test tubes containing 15 ml of deionized water. Test tubes were shaken for 1 h at room temperature (25 °C), and conductivity of the effusate (the liquid resulting from the mix of roots and deionized water after the soaking period) was measured with a conductivity bridge (Model 31A, YSI, Yellow Springs, OH) and an electrode (Model 3403, YSI). Roots were then frozen at −20 °C for a minimum of 12 h, boiled for 30 min, cooled to room temperature, and the conductivity of the effusate remeasured as an estimate of the total electrolyte content of the roots. Electrolyte leakage was calculated as the quantity of water-soluble electrolytes normalized to total electrolyte content.

Leaf chlorophyll index was determined with a chlorophyll meter (SPAD-502, Minolta, Japan) on six of the most recently matured, fully exposed leaves located at the 4th or 5th node below the shoot apex.

The leaves used for the SPAD meter measurements were also used for gas exchange measurements. Net CO₂ assimilation, g, and E were measured weekly with a portable infrared gas analyzer (LCA-2, Analytical Development, U.K.). Measurements were made in sunlight between 1200 and 1500 h at a photosynthetic photon flux (PPF) > 700 µmol m⁻² s⁻¹, which is above light saturation for Annona photosynthesis (Marler and Zozor 1996, Utsunomiya and Higuchi 1996).

Growth measurements

Tree height, trunk diameter, total number of leaves and fresh and dry mass were determined for all trees. All growth measurements, except trunk diameter and fresh and dry mass, were made at the beginning (Day 1) of each replication and repeated weekly for 6 weeks. Trunk diameter was measured every 2 weeks.

Tree height was determined on the main stem by measuring from the soil surface to the apical bud. Trunk diameter was measured 10 cm above the soil surface with a micrometer caliper. Fresh and dry masses were recorded at the end of each replication (Week 6). Leaves, stems (including axillary branches and shoots) and roots were weighed. Roots were separated from the rooting medium by carefully washing them in tap water. Tissue samples were oven-dried at 70 °C to a constant weight prior to dry mass determinations.

Statistical analysis

Treatments were arranged as a 2 (species) × 5 (RZTs) × 2 (flooding treatments) factorial design, with species, RZTs and flooding treatments as main effects. Treatments were replicated and blocked over time in a randomized complete block design with four replications. Data were subjected to analysis of variance (ANOVA) using SAS (SAS Institute, Cary, NC) statistical software to test for significant interactions among main effects. Soil Eh was analyzed by ANOVA and LSMEANS multiple comparison test to determine signifi-
cant treatment differences. Physiological and growth responses were analyzed by regression with SigmaPlot software (Systat Software, Richmond, CA).

Results

Medium redox potential (Eh)

There were no significant differences in medium Eh between species ($P > 0.05$, data not shown); therefore, data from both species were pooled for all RZTs. Redox potential was about 360 mV on Day 1, when treatments were initiated (Figure 1). Thereafter, an inverse relationship was found between RZTs and Eh for the flooded treatments. After 2 days of flooding, Eh at RZTs of 10, 20, 25 and 35 °C was below 200 mV, indicating anaerobic conditions (Ponnampерuma 1984). At an RZT of 5 °C, Eh decreased more slowly than at the other RZTs and this trend was consistent until the last measurement on Day 7.

Morphological adaptations to flooding and tree mortality

Pond apple trees developed hypertrophied (swollen) trunk lenticels at RZTs of 20, 25 and 35 °C within 1 week of flooding. Although not quantified, most lenticels appeared to develop at 25 °C. In addition, flooded pond apple trees exposed to RZTs of 5 and 10 °C often exhibited leaf epinasty 2 weeks after flooding treatments were initiated. Epinasty occurred earlier at 10 °C than at 5 °C. Additionally, pond apple developed adventitious roots, upward root growth through the medium from preexisting roots, and basal trunk swelling in response to flooding. Soursop trees also developed some hypertrophied trunk lenticels at 20 and 25 °C, but lenticel development appeared to be most pronounced at 35 °C. Fewer lenticels were observed in soursop than in pond apple. Soursop trees produced some vegetative shoots at the base of the trunk and some trees developed adventitious roots at 20 and 25 °C. In both species, only trees with visible morphological adaptations survived extended flooding. All pond apple and soursop trees at RZTs of 5 and 10 °C showed symptoms of flooding damage, such as leaf wilting and necrosis, followed by defoliation after 3 weeks of flooding and death after 4 weeks of flooding.

Root electrolyte leakage

There were significant species × flooding, species × RZT and flooding × RZT interactions for root electrolyte leakage (Table 1). There were linear and quadratic relationships between root electrolyte leakage and RZT for non-flooded pond apple and soursop trees, respectively (Figure 2A). For flooded and non-flooded trees combined, root electrolyte leakage appeared to be less sensitive to low RZTs (5 and 10 °C) in pond apple than in soursop (Figure 2A). Flooded trees had more overall root electrolyte leakage than non-flooded trees (Figures 2A and 2B), and soursop had more overall root electrolyte leakage than pond apple.

Leaf chlorophyll index

There was a significant species × flooding × RZT interaction for leaf chlorophyll index (Table 1). As RZT decreased, leaf chlorophyll index generally decreased linearly for non-flooded trees of both species by Week 4 (Figure 3A). Extractable leaf chlorophyll concentration is highly correlated ($r^2 = 0.96$) with leaf SPAD readings from Annona leaves (Schaper and Chacko 1991). Although we did not measure leaf chlorophyll concentration, it presumably increased linearly in non-flooded pond apple and soursop, because RZT increased in parallel with leaf chlorophyll index. In flooded conditions, leaf chlorophyll index of pond apple was unaffected by RZT until it dropped to 10 °C, whereas there was a steady decrease in leaf chlorophyll index of soursop as RZT decreased (Figure 3B).

Leaf gas exchange

There were no effects of species, flooding or RZT on $E$ or $g_s$ (Table 1). There was a significant interaction among species, flooding and RZT for $A$ (Table 1). Non-flooded and flooded pond apple and soursop trees exposed to RZTs of 5 or 10 °C had negative $A$, indicating a net respiratory loss of carbon within 1 week of initiating treatments (Figure 4) and $A$ remained negative for the next 3 weeks. Net CO$_2$ assimilation was not measured after Week 3 because flooded trees in the 5 and 10 °C treatments died by Week 4.

For non-flooded soursop trees, $A$ increased linearly as RZT increased, whereas $A$ of non-flooded pond apple trees did not decrease until RZTs reached 10 °C (Figure 4A). At RZTs of 20, 25 or 35 °C, flooding caused a reduction in $A$ of soursop (Figure 4B) compared with nonflooded trees, whereas there was no significant effect of flooding on $A$ of pond apple trees at these RZTs (Figures 4A and 4B).

Growth measurements

Height of non-flooded pond apple and soursop trees decreased linearly as RZTs decreased 4 weeks after treatment initiation (Figure 5A). Flooding did not significantly reduce tree height in pond apple and the tallest trees were observed at RZTs greater than 20 °C. In contrast, height of soursop trees was less
for flooded trees than for non-flooded trees by Week 4 (Figure 3B). There was little change in tree height from Week 4 to Week 6 for non-flooded and flooded trees of each species exposed to RZTs of 20, 25 or 35 °C (data not shown).

Table 1. Summary of analysis of variance (F values) of the effects of species (Annona glabra and Annona muricata), flooding (flooded and non-flooded), and root zone temperature (RZT) (5, 10, 20, 25 and 35 °C) on electrolyte leakage, leaf chlorophyll index, net CO₂ assimilation (A), stomatal conductance (gₛ), transpiration (E), trunk diameter, tree height, total number of leaves, root dry mass (DM), stem DM and leaf DM. Significant effects are denoted as: * = P ≤ 0.10, ** = P ≤ 0.05; and *** = P ≤ 0.01.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Electrolyte leakage</th>
<th>Leaf chlorophyll</th>
<th>A</th>
<th>gₛ</th>
<th>E</th>
<th>Trunk diameter</th>
<th>Tree height</th>
<th>Total leaf no.</th>
<th>Root DM</th>
<th>Stem DM</th>
<th>Leaf DM</th>
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</thead>
<tbody>
<tr>
<td>Species</td>
<td>22.33 ***</td>
<td>4.92 ***</td>
<td>1.76</td>
<td>0.07</td>
<td>0.01</td>
<td>16.93 ***</td>
<td>51.46 ***</td>
<td>11.49 ***</td>
<td>0.13</td>
<td>20.70 ***</td>
<td>0.08</td>
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<td>Flooding</td>
<td>39.79 ***</td>
<td>13.69 ***</td>
<td>2.02</td>
<td>0.30</td>
<td>0.07</td>
<td>16.00 ***</td>
<td>0.02 ***</td>
<td>3.93 **</td>
<td>1.05</td>
<td>0.10</td>
<td>1.30</td>
</tr>
<tr>
<td>RZT</td>
<td>42.18 ***</td>
<td>25.53 ***</td>
<td>4.17 ***</td>
<td>1.26</td>
<td>0.17</td>
<td>7.60 ***</td>
<td>25.67 ***</td>
<td>8.95 ***</td>
<td>1.46</td>
<td>1.88</td>
<td>12.54 ***</td>
</tr>
<tr>
<td>Species × flooding</td>
<td>2.96 *</td>
<td>2.25</td>
<td>0.96</td>
<td>0.12</td>
<td>0.04</td>
<td>17.27 ***</td>
<td>8.66 ***</td>
<td>0.10</td>
<td>0.07</td>
<td>0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>Species × RZT</td>
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<td>1.04</td>
<td>0.40</td>
<td>0.05</td>
<td>0.03</td>
<td>3.98 ***</td>
<td>4.77 ***</td>
<td>0.62</td>
<td>0.41</td>
<td>0.03</td>
<td>0.42</td>
</tr>
<tr>
<td>Flooding × RZT</td>
<td>3.77 ***</td>
<td>1.31</td>
<td>0.88</td>
<td>0.03</td>
<td>0.08</td>
<td>2.52 **</td>
<td>3.84 ***</td>
<td>0.67</td>
<td>0.14</td>
<td>0.34</td>
<td>1.29</td>
</tr>
<tr>
<td>Species × flooding × RZT</td>
<td>0.33</td>
<td>2.09 *</td>
<td>2.29 *</td>
<td>0.27</td>
<td>0.07</td>
<td>2.45 *</td>
<td>4.48 ***</td>
<td>0.64</td>
<td>0.09</td>
<td>0.41</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Figure 3. Root zone temperatures (RZT) and leaf chlorophyll index (SPAD readings) of (A) non-flooded and (B) flooded pond apple and soursop trees 4 weeks after flooding and RZT treatments were initiated. Symbols represent the means of four replications with six leaf samples per tree.
Although there were significant linear (pond apple) and quadratic (soursop) relationships between RZT and trunk diameter for non-flooded trees of each species, the response was small (Figure 5C). However, flooding caused the trunk of pond apple to thicken (Figures 5C and 5D), with the most thickening occurring at the higher RZTs (25 and 35 °C). In contrast, the trunk of soursop trees did not thicken in response to flooding (Figures 5C and 5D).

For non-flooded trees of each species and flooded pond apple trees the total number of leaves decreased linearly by Week 4 as RZTs decreased (data not shown). However, total leaf number of flooded soursop trees was unaffected by RZT (data not shown).

There was no significant relationship between RZT and stem or root dry mass of non-flooded or flooded soursop trees and stem dry mass of non-flooded pond apple trees (data not shown). Stem dry mass of flooded pond apple (Figure 6A) decreased linearly as RZT decreased. There were no significant effects of flooding on root dry mass of pond apple over the experimental period (Figure 6B).

For non-flooded trees of each species (Figure 7A), and flooded soursop trees (Figure 7B), leaf dry mass decreased linearly as RZT decreased (Figure 7A). However, for flooded pond apple trees, leaf dry mass did not significantly decrease until RZT decreased to 10 °C (Figure 7B). Leaf, stem and root fresh mass of each species showed similar patterns to dry mass (data not shown).

Discussion

Temperature effects on Eh of flooded media were similar to those observed in flooded calcareous soil by Larson et al. (1991c), who found a more rapid decrease in Eh at soil temperatures of 22.5 and 30 °C than at 15 °C. This is a typical response to increased soil temperature, and results in increased root respiration and microbial activity and rapid oxygen depletion at high soil temperatures, leading to more rapid reduction in soil Eh (Ponnamperuma 1972). After 7 days of flooding, Eh of the media in all RZT treatments was less than 100 mV, indicating low oxygen availability.

The greater root electrolyte leakage at relatively low RZTs (5 and 10 °C) is consistent with the putative subtropical and tropical origins of pond apple and soursop (Morton 1987, Nakasone and Paull 1998), respectively. Subtropical and tropi-
Calx species are susceptible to chilling damage (< 10 °C), which causes a loss of semi-permeability of cell membranes (Björkman et al. 1980). In addition, flooded pond apple, which is native to wetland areas, had less root electrolyte leakage than flooded soursop over the higher range (20 to 35 °C) of RZTs, attesting to its greater flood tolerance compared with soursop. The comparable A values in non-flooded and flooded pond apple trees at RZTs of 20 to 35 °C after 3 weeks of flooding were similar to the observations of Núñez-Elisea et al. (1999). However, at RZTs of 20–35 °C, A was lower in flooded soursop than in non-flooded soursop, confirming the greater flood tolerance of pond apple compared with soursop at these RZTs. There were no significant effects of flooding or RZT on g (Table 1), suggesting that A of soursop was mainly decreased by non-stomatal limitations.

Development of adventitious roots and hypertrophied lenticels observed in flooded pond apple and soursop appeared to be a temperature-dependent process, because no morphological adaptations were observed in trees at RZTs of 5 or 10 °C. The development of adventitious roots and hypertrophied lenticels in flooded pond apple and soursop, also reported previously (Núñez-Elisea et al. 1998), were presumably adaptations to flooding because there was 100% plant survival at RZTs of 20, 25 and 35 °C after 6 weeks of flooding. Other tree species adapt to flooding stress by either avoiding oxygen deficits by developing aerenchyma, adventitious roots or hypertrophied trunk lenticels for improved root gas exchange, or adapting physiologically to oxygen deficits (Schaffer et al. 1992, Armstrong et al. 1994, Crawford and Brändle 1996). The basal trunk swelling and development of hypertrophied trunk lenticels may partly facilitate oxygen diffusion to flooded roots of pond apple (Kozlowski 1984) and produce energy to maintain ion uptake. The finding that flooded pond apple appeared to produce more hypertrophied lenticels than flooded soursop may partly explain the greater flood tolerance of pond apple. Pond apple has an efficient hydraulic system that may help maintain nutrient uptake and transport under flooded conditions (Zotz et al. 1997).

Flooding inhibited the growth of soursop trees, but did not inhibit growth of pond apple trees, probably because pond apple trees have adapted to their native wetland habitat where they often thrive and grow despite prolonged waterlogging of the root system. Flooded pond apple trees had slightly lower root dry mass than non-flooded trees, which may have been associated with altered partitioning patterns, because greater allocation of carbon to aboveground tissue is characteristic of trees subjected to flooding (Tang and Kozlowski 1982, Megenigal and Day 1992). Low root dry mass as a result of flooding has also been reported in other flood-tolerant species such as Fraxinus mandshurica Rupr. (Yamamoto et al. 1995) and Taxodium distichum L. (Pezeshki et al. 1996).

In contrast to pond apple, flooded soursop trees generally grew less than non-flooded trees, especially at RZTs of 5 and 10 °C. Flooded pond apple and soursop trees grown at RZTs of...
5 and 10 °C exhibited negative A within the first week of the study, indicating a net carbon loss.

Growth of non-flooded trees of both species generally decreased as RZT decreased. This response reflects the subtropical and tropical origin, respectively, of pond apple and sour-sop, where soil temperatures during the summer are quite high. Similarly, George and Nissen (1987) found significantly more dry matter accumulation in sugar apple (Annona squamosa L.) trees grown at a soil temperature of 28 °C compared with 15 °C.

In summary, both pond apple and sour-sop trees tolerated flooding at RZTs of 20–35 °C, which are similar to root temperatures occurring in subtropical and tropical areas where Annona trees are commercially cultivated. Pond apple was more flood-tolerant than sour-sop at RZTs of 20–35 °C, most likely because of its adaptation to a native wetland habitat where it often thrives under prolonged waterlogging. In both species, net gas exchange and growth were inhibited at RZTs at or below 10 °C in both non-flooded and flooded conditions.

**Note**

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**References**


