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IRON NUTRITION, FLOODING, AND GROWTH OF POND APPLE TREES

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Abstract. **Pond apple (*Annona glabra* L.), a tree species native to wetlands of the Americas, including south Florida, may have potential as a flood-tolerant rootstock for commercial *Annona* species. In pond apple's native wetland soil, Fe^{3+} is reduced to Fe^{2+} which is readily absorbed by plants. In non-flooded, calcareous soils of south Florida, pond apple trees often exhibit symptoms of Fe deficiency. Therefore, the effects of Fe application on plant nutrition and growth of pond apple were studied under flooded and non-flooded conditions. Trees were grown in Krome very gravelly loam soil and fertilized with 0, 0.625, 1.25, 2.5, or 5.0 g/plant of chelated (Sequestrene-138, Fe-EDDHA) or non-chelated ($FeSO_4$) Fe under flooded or non-flooded conditions. Trees that survived 12 weeks of flooding exhibited morphological adaptations to flooding, such as development of adventitious roots and hypertrophied stem lenticels. Flooding decreased the concentration of N, P, K, Ca, Mg, Zn, and Cu, and increased the concentration of Fe and Mn in the leaves. For non-flooded trees, addition of chelated Fe to the soil resulted in a higher leaf chlorophyll index and more growth compared to trees fertilized with non-chelated Fe. The optimum amount of chelated Fe needed to achieve maximum growth in non-flooded trees was 2.5 to 5 g per plant. For flooded trees, the form of Fe did not affect leaf chlorophyll index and growth. To avoid Fe stress when the soil is not flooded, the use of pond apple as a flood-tolerant rootstock will require considerably higher rates of chelated Fe than the amount applied to traditional *Annona* rootstocks.**

Pond apple (*Annona glabra* L.) is a flood-tolerant (Zotz et al., 1997) perennial woody tree species native to tropical and subtropical Americas, including wetlands of south Florida (Morton, 1987). It is generally not considered a commercial species, although it has potential as an ornamental plant in

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Florida (Gettys and Sutton, 1999). The use of pond apple as a rootstock may allow commercial production of flood-sensitive *Annona* species (Núñez-Elisea et al., 1998, 1999) in flood prone areas. In some areas, including south Florida, *Annona* trees are commercially grown in calcareous soils, characterized by high pH (7.5 to 8.5), a high bicarbonate concentration, and a low organic matter content (Lucena, 2000). These soil conditions produce Fe deficiency in many fruit crops (Korcak, 1987), which is a major production expense to correct. Additionally, there are low-lying areas with this soil type that are prone to periodic flooding.

In alkaline soils, flooding decreases soil pH and nutrient elements such as Fe, Mg, and Mn become more soluble (Larson et al., 1991). Iron in the form of Fe^{3+} is reduced to Fe^{2+}, a form that is more available to plants (Larson et al., 1991; Ponnamperuma, 1984). Thus, periodic flooding in calcareous soils may result in increased Fe availability and improved plant nutritional status (Zude-Sasse and Lüdders, 2000). In calcareous soils under non-flooded conditions pond apple exhibits Fe deficiency symptoms, even when chelated Fe is added to the soil at rates commonly applied to commercial *Annona* species on traditional rootstocks. The most effective and common method to correct Fe deficiency in calcareous soils is a soil drench of chelated Fe (e.g., Sequestrene-138, Fe-EDDHA) since non-chelated Fe can precipitate in calcareous soils. Thus, under non-flooded soil conditions, pond apple trees must be fertilized with higher rates of chelated Fe than is typical for the traditional *Annona* rootstocks (B. Schaffer, pers. obs.). The objective of this study was to examine Fe nutrition of pond apple trees in flooded and non-flooded calcareous soils.

Materials and Methods

The study was conducted in a sunlit glasshouse at University of Florida in Gainesville. Average day/night air temperatures during the experimental period ranged from 36/27 to 25/20 °C, and relative humidity was 80 to 85%. Air temperature and relative humidity were monitored and recorded with a Hobo H8 Pro Series temperature logger (Onset Computer Corporation, Pocasset, Mass.).

Plant material. Seedling pond apple trees that were 1.5 years old were grown in 7.6-L containers in Krome very gravelly loam soil (loamy-skeletal, carbonatic, hyperthermic Lithic Rendoll), which is native to southern Florida (Noble et al., 1996). Trees were irrigated daily to container capacity, and fertilized (top dressing) at the beginning of the study with 15 g/plant of granulated fertilizer (10N-4.4P-8.3K, with ammonium and urea as N sources).

Treatments. Six weeks prior to treatment initiation, all trees were pruned to produce new growth flushes. After pruning, 100 uniform trees were selected and of these, 50 were flooded and 50 remained non-flooded. Treatments were arranged as a 2 (flooded and non-flooded) × 2 (Fe sources: chelated and non-chelated) × 5 (Fe rate) factorial. The Fe sources were Sequestrene-138 (Fe chelate containing 6% Fe, Fe-EDDHA), and $FeSO_4$ (containing 20% Fe). The five Fe rates used were 0, 0.625, 1.25, 2.5, or 5.0 g per plant. Iron rates and formulations were based on those applied to commercial atemoya (*A. squamosa* L. × *A. cherimola* Mill.) trees in south Florida (2.5 g Sequestrene-138 per tree, J. H. Crane, personal communication). Rates of $FeSO_4$ were also based on commercial recommendations of 2.5 g Fe per plant but were adjusted based on the concentration of Fe in $FeSO_4$. Iron was applied as a soil drench. There were five-single tree replications for each treatment combination. Trees were subjected to the treatments for 12 weeks using the onset of leaf chlorosis in the 0 g Fe per plant treatment as an indicator of when to terminate the experiment.

Soil measurements. For flooded trees, soil redox potential (Eh) was monitored with a platinum combination electrode (Ag^+/AgCl, Accumet, Fisher Scientific, Pittsburgh, Pa.) attached to a portable pH meter (Accumet AP62, Fisher Scientific, Pittsburgh, Pa.). Soil Eh was recorded 1, 3, 7, and 14 d after flooding treatments were initiated.

Sampling leaves for nutrient analysis. Nutrient element concentrations were determined by sampling leaves between the 4th and 6th nodes (young leaves) below the shoot apex at the beginning and the end of experiment (week 12). Six to seven leaves per tree in each treatment combination were sampled. Leaves were gently rinsed with deionized water for about 1 min. Samples were then oven-dried at 70 °C for 48 h. The dry samples were ground through a 40-mesh screen in a Wiley mill.

Total Kjeldahl nitrogen (TKN) concentration. Approximately 0.1 g of ground sample was transferred to a 50 mL digestion tube containing about 2.0 g of Kjeldahl mixture (10 g K_2SO_4: 3 g $CuSO_4$), and 2.5 mL of sulfuric acid. Tubes were covered with glass funnels and placed in an aluminum block digester at 380 °C for 8 to 10 h. After the tubes were allowed to cool overnight, the glass funnels covering the tubes were rinsed thoroughly into the tubes with 5 to 10 mL of deionized water. Each tube was shaken in a vortex mixer and the volume brought to 50 mL with deionized water. Subsamples were filtered through Whatman Q8 filter paper, and then transferred to 20 mL polyethylene scintillation vials for analysis. Samples were analyzed for Total Kjeldahl Nitrogen (TKN) concentration using a Flow Solution IV® automated continuous-flow analyzer (OI Analytical, College Station, Texas). Nitrogen concentration was expressed as % dry wt.

Leaf concentrations of other nutrients. The concentrations of P, K, Mg, Ca, Fe, Mn, Zn, and Cu were determined after extraction using a dry ash procedure (Hanlon et al., 1994). Approximately 0.5 g of ground plant tissue was transferred to a 10 mL beaker and placed in a muffle furnace at 500 °C for 10 to 12 h. After each sample was cooled, a few drops of 1N HCl were added to the ash, the samples were rinsed with 1 N HCl, transferred to a volumetric flask and brought to a volume of 50 mL. The samples were mixed thoroughly, and then filtered through Whatman Q8 filter paper. Subsamples were transferred to 20 mL polyethylene scintillation vials for analysis. For each treatment, concentrations of P, K, Mg, and Ca were expressed as % dry wt, and concentrations of Fe, Mn, Zn, and Cu were expressed as $mg \cdot kg^{-1}$ dry wt. Concentrations of P, K, Mg, Ca, Fe, Mn, Zn, and Cu were determined using an Inductively Coupled Argon Plasma Spectrometer (Spectro-CIROS CCD, FTCEA000, Germany) at the IFAS Analytical Research Laboratory, University of Florida, Gainesville, Fla.

Leaf chlorophyll and growth measurements. Leaf chlorophyll index was determined using a chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Japan). Measurements were made on six leaves located between the 4th and 6th nodes of the shoot apex (young leaves) from treatment initiation (day 1) and at weekly intervals thereafter. Data were expressed as SPAD readings (Schaper and Chacko, 1991).

Axillary shoot length was determined by measuring the length of one tagged shoot per tree from the leaf axil to the apical bud. Shoots were measured from the beginning (day 1) of the study and then at weekly intervals.

Statistical analysis. The treatments were arranged in a completely randomized design with five single-plant replications for each treatment combination. Data were analyzed by a combination of statistical tests, including analysis of variance and multiple comparison (LSMEANS), and repeated measures using SAS (SAS Institute, Cary, N.C.) statistical software. Regression analysis was done using SigmaPlot program (SPSS Science, Chicago, Ill).

Results and Discussion

Soil redox potential (Eh). There was no interaction between Fe rate or formulation (chelated vs. non-chelated) and flooding treatments for soil Eh ($P \leq 0.05$); therefore all flooding treatments were pooled for reporting Eh values. The soil became anaerobic (Eh ≤ 200 mV; Ponnamperuma, 1984) within 3 d after flooding (Fig. 1). Soil Eh decreased to -158 mV by the 7th d of flooding. Thereafter, soil Eh changed little reaching -163 mV on day 14. Thus, roots in the flooded treatments were under reduced soil conditions (low soil Eh), and therefore restricted availability of O_2 from day 3 through week 12. Núñez-Elisea et al. (1999) found similar results for *Annona* species in flooded Krome very gravelly loam soil. The rapid decrease of soil Eh within 1 week may be attributed to high air temperatures, which averaged 29.6 °C during the first week of the study. As temperatures increase, root respiration and microbial activity in the soil increase causing rapid O_2 depletion and reduction of soil Eh (Larson et al., 1992).

Morphological adaptations to flooding. Pond apple trees developed hypertrophied (swollen) trunk lenticels within 3 d of flooding. Trees also produced adventitious roots, upward root growth through the soil from preexisting roots, and basal trunk swelling. These morphological changes in response to flooding, reported previously in pond apple (Núñez-Elisea et al. 1998, 1999), were presumably adaptations to improve flood-tolerance since there was 100% plant survival after 12 weeks of flooding. These morphological adaptations may facilitate internal gas diffusion to flooded roots (Armstrong, 1968; Jackson and Attwood, 1996; Kozlowski, 1984) and/or function as excretory sites for potentially toxic metabolites formed in the roots during anaerobic respiration (Chirkova and Gutman, 1972).

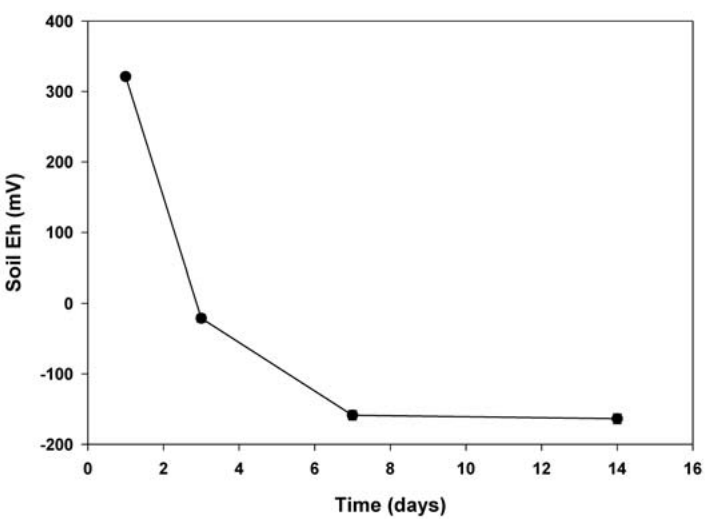

Fig. 1. Effect of flooding on soil redox potential (Eh) for container-grown pond apple trees. Symbols represent means ± SE of 5 Fe rates, 2 Fe sources and 5 single-tree replications. SE bars not visible are masked by the symbols.

Leaf nutrient concentrations. After flooding treatments were begun, significant interactions ($P \leq 0.05$) were observed between Fe fertilization and flooding treatment for leaf nutrient concentrations, leaf chlorophyll index, and shoot length. Therefore, the effect of Fe fertilization treatments on all variables is reported separately for each flooding treatment at the last measurement date (week 12).

At the beginning of the study, before treatments were imposed, means and standard errors (n = 100 plants) for N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu concentrations in young leaves were 1.28 ± 0.141%, 0.26 ± 0.014%, 2.06 ± 0.056%, 2.31 ± 0.205%, 0.50 ± 0.021%, 51.80 ± 5.83 $mg \cdot kg^{-1}$ dry wt, 50.44 ± 12.57 $mg \cdot kg^{-1}$ dry wt, 43.52 ± 6.62 $mg \cdot kg^{-1}$ dry wt, and 14.05 ± 2.76 $mg \cdot kg^{-1}$ dry wt, respectively.

Twelve weeks after treatments were initiated, there were significant differences for all macronutrients in young leaves among flooding and Fe treatments (data not shown). Leaf N concentration was generally lower in flooded than in non-flooded trees (Table 1). This response may be due to denitrification (Ponnamperuma, 1984; Olde et al., 2002), which occurs quickly because NO_3^- is the first electron acceptor to be reduced in anaerobic soil following O_2 depletion (Reddy and Patrick, 1983). The critical redox potential at which NO_3- is reduced to N_2 is 200 mV (Patrick and Jugsujinda, 1992), and in this study soil Eh was < 200 mV within 3 d of flooding. Similarly, Larson et al. (1991) reported a rapid decrease in NO_3^- concentration one week after Krome very gravelly loam soil was flooded.

Leaf N concentration in flooded trees receiving chelated Fe tended to be the highest at Fe rates of 0.625 to 1.25 g per plant. There was no effect of flooding on foliar N concentrations in the plants fertilized with non-chelated Fe regardless of Fe rate (Table 1).

Flooded plants generally had lower leaf P concentrations than non-flooded plants (Table 1), and P concentrations were lower than those reported for optimal growth of atemoya (George et al., 1987). In alkaline soils where P is not very soluble, flooding can increase P availability due to a reduction in insoluble P compounds (De Mello et al., 1998). However, Ca can also be dissolved and react with P to form insoluble complexes (Ponnamperuma, 1984), causing a decreased foliar concentration of P, as observed in flooded plants in this study.

Flooded trees receiving 5 g per plant of chelated Fe tended to exhibit a decrease in foliar P concentration (Table 1). However, applying non-chelated Fe to these trees did not affect foliar P concentration. Increased soluble Fe concentration in the soil, which is often a result of flooding, may interfere with P uptake causing P deficiency (Kozlowski and Pallardy, 1984). For non-flooded trees, application of chelated Fe did not affect leaf P concentrations. When non-flooded plants received non-chelated Fe, leaf P concentration generally decreased as Fe rates increased.

Flooding decreased foliar K concentration compared to that of non-flooded trees fertilized with either chelated or non-chelated Fe (Table 1). Leaf K concentrations in flooded trees were lower than the critical range for optimal growth of atemoya (George et al., 1987). Flooding generally inhibits K uptake and decreases leaf K concentration (Kozlowski and Pallardy, 1984).

For non-flooded trees receiving chelated Fe, the highest leaf K concentration occurred at 0 Fe per plant (Table 1). In non-flooded conditions, Fe deficiency generally increases leaf K concentration and decreases leaf Ca concentration, and consequently causes a marked increase in the K/Ca ratio (Abadía et al., 1985; Belkhodja et al., 1998). For flooded

Table 1. Effect of Fe fertilization and flooding treatments on young leaf macronutrient concentrations of pond apple 12 weeks after treatments were initiated.

Treatments		Fe rate	Nutrient (% dry wt)[z]				
Iron form	Flooding	(g/plant)	N	P	K	Ca	Mg
Chelated (Fe-EDDHA)	Flooded	0	1.39 b	0.13 a	0.70	2.08 a	0.35
		0.625	1.63 ab	0.12 ab	0.72	1.86 ab	0.30
		1.25	1.73 a	0.14 a	0.63	1.77 b	0.29
		2.5	1.36 b	0.14 a	0.83	1.66 b	0.31
		5.0	1.29 b	0.11 b	0.60	1.57 b	0.30
	Non-flooded	0	1.55	0.20	1.19 a	2.52 a	0.44 a
		0.625	1.45	0.18	0.69 b	2.79 a	0.43 a
		1.25	1.61	0.19	0.81 b	2.49 a	0.37 ab
		2.5	1.79	0.17	0.79 b	2.30 ab	0.35 b
		5.0	1.71	0.17	0.80 b	1.82 b	0.34 b
Non-chelated ($FeSO_4$)	Flooded	0	1.34	0.11	0.50 bc	2.11 a	0.34
		0.625	1.58	0.12	0.60 bc	1.73 bc	0.32
		1.25	1.53	0.14	0.65 abc	1.99 ab	0.33
		2.5	1.63	0.14	0.75 ab	1.65 c	0.29
		5.0	1.60	0.14	0.87 a	2.02 a	0.28
	Non-flooded	0	1.92	0.26 a	1.61	1.47	0.26 bc
		0.625	2.87	0.27 a	1.69	1.22	0.23 c
		1.25	2.38	0.18 ab	1.24	1.72	0.30 abc
		2.5	1.87	0.17 b	1.15	1.81	0.32 ab
		5.0	2.15	0.16 b	0.95	2.06	0.36 a

[z]Mean separation within columns by Fe form and flooding treatment by LSMEANS ($P \leq 0.05$). The absence of letters indicates no significant difference among means. Values represent 5 single-tree replications with 6 leaf samples per plant.

plants receiving non-chelated Fe, leaf K concentration increased as Fe rates increased. There was no effect of Fe rate on leaf K concentration of non-flooded trees fertilized with non-chelated Fe (Table 1).

Foliar Ca concentrations were higher in trees in all flooding and Fe treatments than those reported for optimum growth of atemoya (George et al., 1987). High leaf Ca concentrations may have been a result of the high Ca concentration in the soil solution rather than to increased uptake efficiency by the plant (Shuman, 1994) since Krome very gravelly loam soil is derived from limestone ($CaCO_3$) parent material (Noble et al., 1996).

In general, leaf Ca concentrations were higher in non-flooded trees receiving chelated Fe, than in flooded trees fertilized with chelated Fe (Table 1). Although flooding can increase Ca^{2+} concentration in alkaline soils, P can form insoluble complexes with Ca, making it less available to the plant (Ponnamperuma, 1984). The lowest leaf Ca concentrations were observed at Fe rates of 0.625 and 2.5 g per plant for flooded trees receiving non-chelated Fe. There was no effect of Fe rates on leaf Ca concentration of non-flooded trees fertilized with non-chelated Fe.

Trees fertilized with chelated Fe generally had a higher leaf Mg concentration than trees fertilized with non-chelated Fe (Table 1). There was no effect of flooding on leaf Mg concentrations among Fe rates in either the chelated or the non-chelated Fe treatments (Table 1). In flooded alkaline soils, $MgCO_3$ may dissolve and the increased concentration of reduced cations (Fe^{2+}, Mn^{2+}) in the soil solution can lead to displacement of Mg^{2+} from the exchange complex, thereby increasing its availability (Larson et al., 1991; Reddy and Patrick, 1983). However, flooded plants tended to have lower leaf Mg concentrations than non-flooded plants, especially for the plants receiving chelated Fe (Table 1). This response may be due to a decrease in Mg concentration in the soil solution (Ponnamperuma, 1984).

For flooded and non-flooded trees, application of non-chelated Fe tended to result in a leaf Mg concentration below the critical level for optimal growth of atemoya (George et al., 1987). Leaf Mg concentration decreased as the Fe rate increased for non-flooded trees receiving chelated Fe, with the lowest values occurring at 2.5 and 5 g Fe per plant. For non-flooded trees receiving non-chelated Fe, leaf Mg concentration generally increased as Fe rates increased. This response may be associated with the leaf Ca concentration (Table 1) because in soils with high pH, Mg uptake is more related to the ratio of Mg/Ca rather than to soil Mg concentration alone (Shuman, 1994).

At the end of study, regardless of flooding treatment, leaf Fe concentration generally was higher for plants receiving chelated Fe than those receiving non-chelated Fe (Fig. 2). In alkaline soils, Chelated Fe is more readily available for plant uptake than non-chelated Fe. Chelated Fe fertilizers, such as Sequestrene-138 (Fe-EDDHA), are synthetic compounds that bind or complex Fe and are highly water soluble. This protects Fe from the usual soil reactions avoiding formation of insoluble $Fe(OH)_3$ (Chen and Barak, 1982). Thus, more soluble Fe is available in the soil solution for plant uptake.

Twelve weeks after treatments were begun, there was a quadratic relationship between Fe rate and leaf Fe concentration for non-flooded trees fertilized with chelated Fe, (r^2 = 0.55), with the maximum value (65 mg kg^{-1} dry wt) at 2.5 g Fe per plant (Fig. 2A). Leaf Fe concentration increased from 0 to 5 g Fe per plant in non-flooded trees receiving chelated Fe, although the relationship between Fe rate and leaf Fe concentration was weaker (r^2 = 0.37) than for flooded plants fertilized with chelated Fe (Fig. 2A). The lower Fe rate required by the flooded trees (2.5 g per plant) than by non-flooded

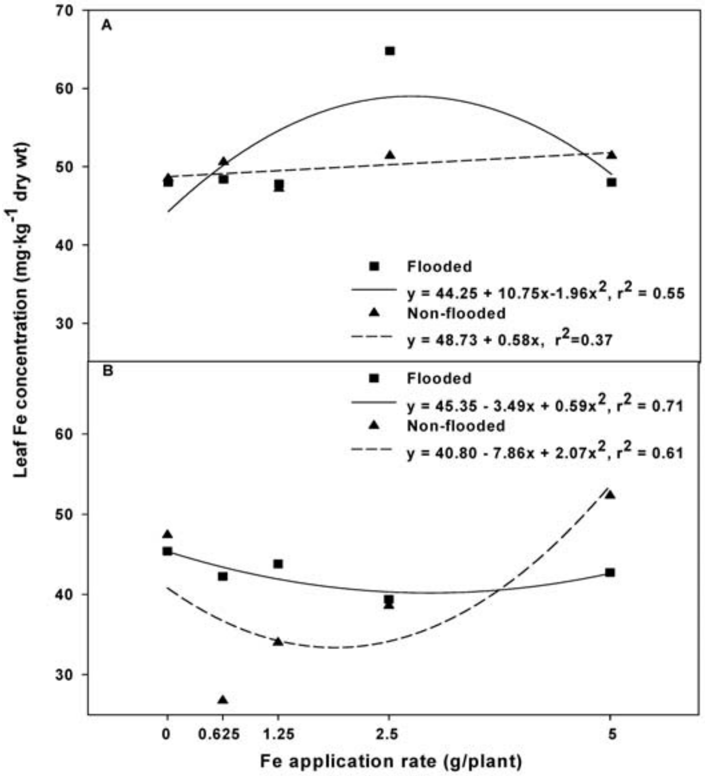

Fig. 2. Iron application rates and leaf Fe concentrations in flooded and non- flooded pond apple trees fertilized with (A) chelated Fe or (B) non-chelated Fe 12 weeks after treatments were initiated. Symbols represent the means of 5 single-tree replications with 6 leaf samples per tree.

trees (5 g per plant) may be due to an increased concentration of soluble Fe in the flooded soil (Larson et al., 1991). Under anaerobic conditions, Fe^{3+} is reduced to Fe^{2+} (Ponnamperuma, 1984) increasing solubility of Fe. The critical redox potential at which Fe^{3+} is reduced to Fe^{2+} is 100 mV (Patrick and Jugsujinda, 1992). In this study, soil Eh reached -21 mV within 3 d of flooding.

There were quadratic relationships between Fe rate and leaf Fe concentration for flooded ($r^2 = 0.71$) and non-flooded ($r^2 = 0.61$) trees fertilized with non-chelated Fe (Fig. 2B). For non-flooded trees receiving non-chelated Fe, the maximum leaf Fe concentration was at 5 g Fe/plant. The non-chelated Fe source was insoluble in calcareous soils, and only high Fe application rates increased leaf Fe concentration. For non-flooded plants fertilized with non-chelated Fe, Fe rates between 0.625 to 2.5 g per plant resulted in leaf Fe concentrations below the critical range for atemoya (George et al., 1987). For plants receiving non-chelated Fe at 0.625 and 1.25 g per plant, flooded trees had higher leaf Fe concentrations than non-flooded trees that received the same amount of Fe. This response may also have been due to flooding-induced increases in soil Fe^{2+} concentrations.

Twelve weeks after treatments were initiated, flooding increased Mn concentrations, and decreased Zn and Cu concentrations in young leaves regardless of Fe rate or form (data not shown).

Leaf chlorophyll index. Leaf chlorophyll index generally increased throughout the study (12 weeks) as Fe rates increased, regardless of Fe sources or flooding treatment (data not shown). Young leaves of trees fertilized with chelated Fe usually had a greater chlorophyll index than those of trees receiving non-chelated Fe (Fig. 3). Ferric-EDDHA (Sequestrene-138) increases availability of Fe in calcareous soils and consequently may increase leaf chlorophyll concentration because nearly all of a plant's Fe is in the chloroplast (Miller et al., 1995).

Twelve weeks after treatments were initiated, for flooded and non-flooded trees receiving chelated Fe, there were quadratic relationships between Fe rate and leaf chlorophyll index (Fig. 3A), although the relationship was weaker for non-flooded than flooded trees. For flooded trees fertilized with chelated Fe, the highest leaf chlorophyll index occurred at Fe rates between 1.25 and 2.5 g Fe per plant, and the lowest occurred at 5 g Fe per plant. The maximum leaf chlorophyll index occurred between 2.5 and 5 g Fe per plant for non-flooded plants receiving chelated Fe. Flooded trees receiving chelated Fe, generally had a lower leaf chlorophyll index than non-flooded trees. This response may be more associated with the lower leaf Mg concentrations observed for flooded trees receiving chelated Fe than for non-flooded trees receiving chelated Fe (Table 1), since Mg is one of the major constituents of the chlorophyll molecule.

There were quadratic relationships between the Fe rate and leaf chlorophyll index for flooded and non-flooded plants receiving non-chelated Fe, with the highest value at an Fe rate of 5 g Fe per plant for flooded trees and 2.5 g Fe per plant for non-flooded trees, both receiving non-chelated Fe (Fig. 3B). In general, flooded plants had higher leaf chlorophyll indices than non-flooded plants. This increase may be due to flood-induced increases in leaf Fe concentration observed for flooded trees receiving non-chelated Fe (Fig. 2B).

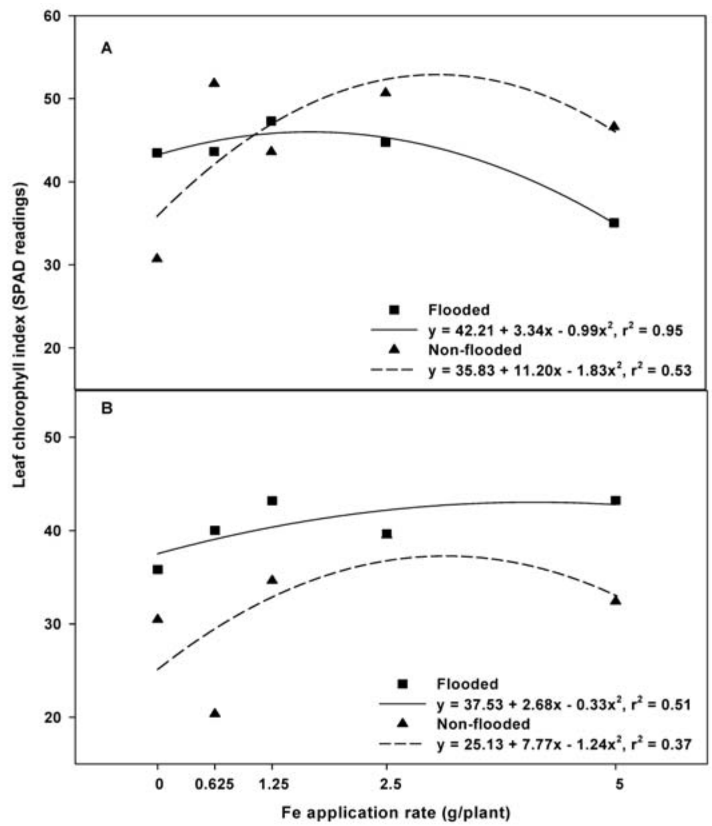

Fig. 3. Iron application rates and leaf chlorophyll index of flooded and non-flooded pond apple trees fertilized with (A) chelated Fe or (B) non-chelated Fe 12 weeks after treatments were initiated. Symbols represent the means of 5 single-tree replications with 6 leaf samples per tree.

Shoot length. In general, shoot length was greater for plants fertilized with chelated Fe than for plants receiving non-chelated Fe (Fig. 4). By week 12 for flooded and non-flooded plants that received chelated Fe, there were quadratic relationships between the Fe rate and shoot length (Fig. 4A). The greatest shoot growth occurred at 2.5 g Fe per plant for flooded plants receiving chelated Fe. In contrast, the greatest shoot length occurred between 2.5 and 5 g Fe per plant for non-flooded plants fertilized with chelated Fe.

For flooded trees, the longest shoots were produced at 5 g Fe per plant when non-chelated Fe per plant was applied (Fig. 4B), and at 2.5 and 5 g Fe per plant when chelated Fe was applied. Leaves of non-flooded trees receiving non-chelated Fe were smaller than those of trees in the other treatments. Increases in leaf nutrient concentrations concomitant with increased Fe chlorosis may be a result of reduced leaf expansion as a result of Fe stress leading to a relative increase in nutrient concentrations (Römheld, 2000).

In conclusion, under non-flooded soil conditions, addition of chelated Fe to calcareous soil resulted in a higher leaf chlorophyll index and more growth of pond apple trees compared with trees fertilized with non-chelated Fe. The optimum amount of chelated Fe needed to achieve maximum growth in non-flooded trees was 2.5 to 5 g per plant. For flooded trees, the form of Fe did not affect leaf chlorophyll index and growth. To avoid Fe stress when the soil is not flooded, the use of pond apple as a flood-tolerant rootstock will require the application of considerably higher rates of chelated Fe than the amount applied to traditional *Annona* rootstocks.

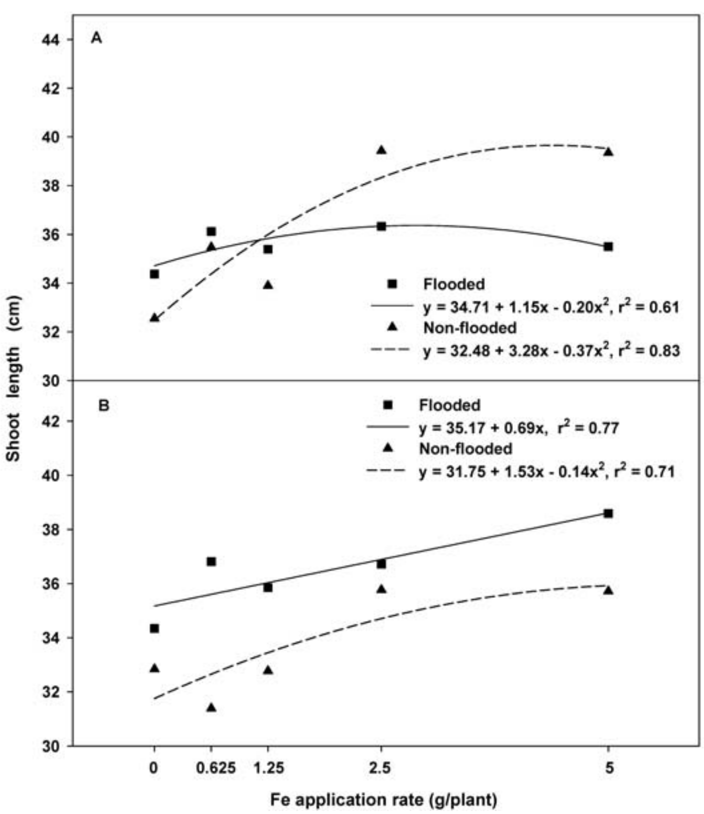

Fig. 4. Iron application rates and shoot length of flooded and non-flooded pond apple trees fertilized with (A) chelated Fe or (B) non-chelated Fe 12 weeks after treatments were initiated. Symbols represent the means of 5 single-tree replications.

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