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EFFECT OF FOLIARLY-APPLIED ACIDS AND FERROUS SULFATE ON IRON NUTRITION OF AVOCADO TREES

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Iron deficiency is a major problem of avocado grown in calcareous soils. Applying chelated iron to calcareous soil is efficacious but very expensive. The effects of foliarly applied organic acids and organic acids plus ferrous sulphate (FS) on iron nutrition of 10-year-old 'Donnie' avocado trees in calcareous soil was investigated in southern Florida. At 13-14 day intervals, trees received the following foliar treatments: ascorbic acid (A), A plus FS (AFS), citric acid plus FS (CFS), and sulphuric acid plus FS (SFS). Additional treatments were chelated iron (EDDHA-Fe) applied to the soil 3 times at 27-28 day intervals and a control receiving no iron (CNT). An organosilicone adjuvant was added to all foliar sprays. On 4 of 7 measurement dates, trees in the EDDHA-Fe treatment had higher leaf chlorophyll indexes determined with a SPAD meter (SPAD values) than trees in all other treatments. On 2 measurement dates, trees in the SFS and AFS treatments had higher SPAD values than trees in the CNT and A treatments. Total leaf iron content was higher for the SFS and CFS treatments than the AFS, EDDHA-Fe, CNT, and A treatments. There was no difference in total leaf iron content between the CFS and AFS treatments. Leaf ferrous iron (Fe^{2+}) content was higher for the SFS, CFS, AFS treatments than the EDDHA-Fe, A, and CNT treatments after 6 foliar acid applications and 3 EDDHA-Fe applications to the soil. Economic analysis indicated that foliar acid-iron treatments were 75 to 88% less costly than soil applications of EDDHA-Fe.

EFFECTO DE ÁCIDOS Y SULFATO FERROSO APLICADOS FOLIARMENTE EN LA NUTRICIÓN DE HIERRO EN AGUACATES

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La deficiencia de hierro es un serio problema en suelos calcáreos. La aplicación de quelato de hierro a suelos calcáreos es eficaz pero muy costosa. Se investigó los efectos de aplicaciones foliares de ácidos orgánicos y de ácidos orgánicos más sulfato ferroso (SF) en la nutrición de hierro de aguacate "Donnie" de 10 años, en suelos calcáreos del sur de Florida. Los árboles recibieron a intervalos de 13-14 días los siguientes tratamientos foliares: acido ascórbico (A), A más SF (ASF), ácido cítrico más SF (CSF) y ácido sulfúrico más sulfato ferroso (SSF). Otros tratamientos adicionales fueron: quelato de hierro (EDDHA-Fe) aplicado al suelo 3 veces cada 27 ó 28 días y un testigo sin recibir hierro (CNT). Se añadió un adyuvante de silicona orgánica a todos los tratamientos foliares. En cuatro de las siete fechas en que se hicieron mediciones, los árboles en el tratamiento con EDDHA-Fe tuvieron índices de clorofila más altos

determinados con un equipo SPAD (valores SPAD) que aquellos bajo otros tratamientos. En dos de las fechas en que se hicieron mediciones, los árboles bajo tratamientos SSF y ASF tuvieron valores SPAD más altos que aquellos bajo tratamientos CNT y A. El contenido total de hierro en las hojas fue más alto en los tratamientos SSF y CSF que en los tratamientos ASF, EDDHA-Fe, CNT y A. No hubo diferencia en el total de hierro en las hojas entre los tratamientos CSF y ASF. El contenido de hierro ferroso fue más alto en los tratamientos SSF, CSF, ASF que en aquellos con EDDHA-Fe, A y CNT después de 6 aplicaciones foliares con ácido y 3 de EDDHA-Fe al suelo. El análisis económico indicó que los tratamientos foliares con ácido-hierro fueron entre un 75% y un 88% menos costosos que las aplicaciones de EDDHA-Fe al suelo.

Introduction

Iron is an essential element for several plant metabolic functions, including chlorophyll synthesis and the electron transport system of respiration (Taiz and Zeiger, 1998). All plants need a continuous supply of iron during growth because it is not translocated from the mature to developing leaves and is classified as an immobile nutrient element (Mengel and Kirkby, 1982).

Root iron uptake involves a reduction of iron from Fe^{3+} to Fe^{2+} at the cell membrane of epidermal root cells; this reduction is catalyzed by the enzyme, ferric chelate reductase (Mengel, 1994). After iron is reduced in the roots, it is re-oxidized back to Fe^{3+} in the apoplast where Fe^{3+} then binds with citric acid (Schmidt, 1999). Iron is then transported in the xylem from the roots to the leaves as ferric-citrate and re-reduced in the leaf apoplast to the Fe^{2+} form and is actively transported across the plasma membrane into the symplast where it is metabolized by the plant (De la Guardia and Alcántara, 1996; Kosegarten et al., 2001; Mengel, 1994; Taiz and Zeiger, 1998). Often, in calcareous soils, a sufficient quantity of iron is translocated from the roots to the leaves, but the reduction of Fe^{3+} to Fe^{2+} by ferric chelate reductase in the leaves is hampered by the high pH environment of the apoplast (Brüggemann et al., 1993; Gonzáles-Vallejo, 2000; Mengel et al., 1994; Mengel and Geurtzen, 1986).

Nearly 12% of the world’s agricultural soils are calcareous (FAO-AGL, 2007) and in the western hemisphere, avocado production areas with calcareous soils include south Florida, the Caribbean Region (e.g., Virgin Islands and Puerto Rico) (Anonymous, 2004; Martinez et al., 1998) and Chile (Britannica, 2007; Vera, 2003). Calcareous soils are characterized by a high pH due to large amounts of calcium carbonate (limestone). Most of the iron in these soils is unavailable for plant uptake because it is tightly bound to soil particles or precipitated as water-insoluble iron oxides (Mengel and Kirkby, 1982). The apoplastic bicarbonate concentration and the pH of plants growing in calcareous soils is also high (Mengel and Geurtzen, 1988; Mengel 1994)

Avocados trees growing in calcareous soils may exhibit typical signs of iron deficiency including yellowing of the tissue between veins of young leaves which is called iron chlorosis or lime-induced chlorosis (Lucena, 2000; Mengel and Guertzen, 1986). Iron deficient avocado trees may produce light green to yellow fruit and leaves that are severely Fe deficient become completely pale yellow. In calcareous soils of south Florida, avocado trees showing signs of iron deficiency often have higher total (extractable) leaf iron concentrations than trees growing in other types of soils such as sands or clays (Chen et al., 2000; Li et al., 2003). In these types of soil, plants exhibiting iron deficiency symptoms can have the same or higher leaf iron concentrations than asymptomatic plants (Mengel, 1994; Mengel et al., 1994).

Soil applications of non-chelated iron fertilizers do not prevent or alleviate iron deficiency in avocado trees grown in calcareous soils (Malo, 1965). An effective method to prevent iron deficiency in avocado grown on calcareous soil is by soil application of chelated iron (Li et al., 2002; Malcolm, 1953; Malo, 1965; Malo, 1966; Harkness and Malcolm, 1957; Lahav and Whiley, 2002). Of the many chelated materials tested, EDDHA-Fe applied as a soil drench has consistently outperformed all other materials on avocado and has become standard practice for growing avocado in the calcareous soils of south Florida (Malcolm, 1953; Harkness and Malcolm, 1957; Malo, 1965; Malo, 1966). However, chelated iron is very expensive and is by far the single highest fertilizer material cost for the production of avocado trees on calcareous soils.

The cost of chelated iron ranges from US$13.00 to $22.00 per kg and tropical fruit growers in southern Florida generally need to apply 25 to 80 kg per ha per year, depending on the crop and tree nutrient status (Crane et al., 2005). Therefore, chelated iron can represent up to 80% of the total fertilizer cost and up to 50% of the total agricultural chemical costs of subtropical and tropical fruit production (Evans, 2005). There is obviously a need for a cheaper alternative to chelated iron for preventing or alleviating iron deficiency in tropical fruit crops grown in calcareous soils.

Foliar applications of standard iron fertilizers are not very effective on subtropical and tropical fruit crops (Davenport, 1983; Malo, 1965). For example, foliar iron sprays had no effect on re-greening and in some cases burned leaves of iron-deficient avocado trees on calcareous soil (Embleton and Jones, 1966; Harkness and Malcolm, 1957; Kadman and Lahav, 1971-72; Malo, 1965).

Reducing the apoplastic pH of iron-chlorotic leaves with foliar applications of dilute acids such as sulfuric, citric, or ascorbic acid, resulted in a decrease in the leaf apoplast pH and “re-greening” of leaves of some fruit crops including kiwifruit (Tagliavini et al., 1995), orange (Pestana et al., 2001), pear (Garcia-Lavina et al., 2002) and peach (Tagliavini et al., 2000) growing in calcareous soil. Preliminary research on lychee, carambola, pond apple, and avocado using weak acids with ferrous iron resulted in higher leaf ferrous iron content than leaves treated with acid only (B. Schaffer, unpublished data), or non-treated control plants. Thus, the potential exists for the use of foliar applications of weak acids as a low-cost alternative to expensive chelated iron for preventing iron deficiency in avocado trees growing on calcareous soils. The purpose of this study was to test the effects of folair applications of weak acids or weak acids plus iron sulfate on re-greening and ferrous and total iron content of leaves of avocado trees showing signs of iron deficiency.

Materials and Methods

An 11-year-old commercial 'Donnie' avocado (grafted onto 'Waldin' rootstock) orchard was used to compare the efficacy of foliarly-applied sulfuric (SA+Fe), citric (CA+Fe), or ascorbic (AA+Fe) acid, each with ferrous sulfate and adjuvant (Freeway®, Loveland Products, Inc., Greeley, Colorado, USA), foliarly applied ascorbic acid plus adjuvant (AA alone), soil applied EDDHA-Fe (chelated soil drench), and no acid or iron control (Table 1). Foliar acid-iron applications were made every 13 to 14 days from 15 September to 22 November 2006 and chelated soil drenches were made at about a 28 day interval (Table 1). There were 5 to 6 trees per treatment arranged in a completely randomized design.

The leaf chlorophyll index which is a measure of leaf "greenness" was measured prior to each foliar acid-iron application from 5 randomly-selected mature (hardened off) leaves on the east and west side of the canopy from 5 to 6 (but repeatedly measured) trees in each treatment with a SPAD chlorophyll meter (Minolta, Inc., Japan). SPAD values were recorded 1 to 2 days prior to each acid-iron foliar application and about 3 months after the last foliar treatment. There is a high correlation between SPAD readings and avocado leaf chlorophyll content ($r^2 = 0.91$) (B. Schaffer, unpublished data).

On 2 Dec. 2006 total iron and ferrous iron content was determined from 3 mature leaves per replication. To determine total leaf iron, samples were washed with detergent and diluted acid (1% HCL), and rinsed with deionized water. Washed samples were dried in an oven at 70°C and then ground. Samples (0.5 g) were then ashed at 500°C for 5 hours and then dissolved in 20 ml HCl and injected into an atomic absorption spectrophotometer (Shimadzu AA-6300 with GFA-EX7i, Columbia, Maryland, USA). Ferrous iron was determined from 0.5 g of fresh leaf tissue (taken from the 3 sampled leaves per replication) using a modification of the technique described by Katyal and Sharma (1980, 1984). After the tissue was washed (as described for total iron extraction), it was ground in a miniature grinding mill (Mini-Mate Plus Chopper/Grinder, Cuisinart, East Windsor, New Jersey, USA) and 5 ml 76 mMol 1,10-phenanthroline was added to the tissue. The solution was shaken on an automatic shaker for 17 hours and then filtered through a 0.45μl syringe filter (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA). The absorbance of the filtrate was determined at 510 nm with a spectrophotometer (Model No. Du 640, Beckman Coulter Co., Fullerton, California, USA). The absorbance values were calibrated with ferrous sulfate standards, and leaf ferrous iron concentration was calculated as described by Katyl and Sharma (1980).

Throughout the experiment, trees in each treatment were observed for signs of phytotoxicity and any obvious differences in tree phenology. Data were analyzed by ANOVA and Duncan-Waller multiple range test using SAS statistical software (SAS Institute, Cary, North Carolina).

Results and Discussion

Total and ferrous leaf iron content was significantly higher for the SA-Fe treatment than all other treatments (Table 2). However, all acid plus iron sulfate foliar treatments tended to have higher total and ferrous iron concentrations than the chelated soil drench, AA alone, or the control treatments. Avocado root activity decreases at soil temperatures below 15℃ which may have decreased EDDHA-Fe uptake (Whiley and Schaffer, 1994). Total and ferrous leaf iron content was similar among soil drench, AA-alone, and control treatments.

There was no significant difference in SPAD values among leaves sampled from the east and west sides of the canopy and therefore data from both sides of the tree were combined (data not shown). There was no significant difference in SPAD values prior to beginning the treatments, however, significant differences were found among treatments from mid-Sept. through 2006 and again in Feb. 2007 (Fig. 1). Although not always statistically significant, trees in the chelated soil drench and AA+Fe treatments consistently had significantly higher SPAD values than AA-alone and the control treatment from late Sept. through October. SPAD values were generally intermediate for trees in the CA+Fe and SA+Fe treatments compared to chelated soil drench, AA+Fe and AA alone, and control treatments during the Fall. There were no significant difference in SPAD values among treatments during early and late November although there was a trend for the EDDHA-Fe and AA+Fe treatments to have higher SPAD values than other treatments. Three months after the last treatment application, SPAD values for the chelated soil drench treatment were higher than for all other treatments and trees in the AA+Fe, and SA+Fe treatments were higher than AA alone and control treatments.

There was a great difference in the cost per hectare among treatments (Table 3). As was expected, applying EDDHA-Fe as a soil drench was the most expensive treatment with a total cost of $954 on a per hectare basis. The SA+Fe was the cheapest treatment costing only $178 per hectare, however as pointed out above, this treatment was not as affective as the chelated soil drench and AA+Fe treatments. All foliar application treatments would result in considerable savings compared to the soil drench treatment, ranging from $604 per hectare to $776 per hectare or savings of 63% to 81% of the cost of applied EDDHA-Fe to the soil.

SPAD values for the AA+Fe treatment were consistently similar to those of the chelated soil drench treatment. Furthermore, the AA+Fe treatment had a potential cost savings of 63% compared to the chelated soil drench treatment. These results suggest that further investigation utilizing weak acids plus iron is warranted. Foliar applications of ascorbic acid plus iron was more effective during the spring and summer for 'Arkin' carambola trees than during the late fall and winter (Crane et al., 2007). This was attributed to a cessation of carambola tree growth at ambient and soil temperatures below 20^{o}C (George et al., 2002a; 2002b, and 2000). This may also be the case for avocado. Therefore, further tests are underway to determine if there are temporal changes in the effectiveness of applying weak acids and iron for alleviating iron deficiency of avocado trees in calcareous soils in Florida.

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Table 1. Foliar acid plus iron and soil applied chelated-iron treatments applied to 'Donnie' avocado trees.

Treatment[z]	Materials applied	Method of application	Dates treatments were applied
SA+Fe	Sulfuric acid (0.9 ml/l) + ferrous sulfate (2.5 g/l) + Freeway® (1.9 ml/l)	Foliar	Sept. 15, 2006 Sept. 28, 2006 Oct. 12, 2006 Oct. 26, 2006 Nov. 11, 2006 Nov. 22, 2006
CA+Fe	Citric acid (5.1 g) + ferrous sulfate (2.5 g/l) + Freeway® (1.9 ml/l)	Foliar	
AA+Fe	Ascorbic acid (8.6 g/l) + ferrous sulfate (2.5 g/l) + Freeway® (1.9 ml/l)	Foliar	
AA alone	Ascorbic acid (8.6 g/l) + Freeway® (1.9 g/l)	Foliar	
Chelated soil drench (EDDHA-Fe)	[(technical sodium ferric ethylenediamine di-(o-hydroxyphenylacetate)] iron (92 g/tree on 6 Feb., all other dates 56.7 g/tree)	Chelated soil drench	Feb. 6, 2006 Sept. 15, 2006 Oct. 12, 2006 Nov. 9, 2006
Control (no acid or iron applied)	None	None	Not applicable

[z]The adjuvant Freeway® (Loveland Products, Inc., Greeley, Colorado, USA) was added to all foliar treatments.

Table 2. Effect of foliar application of acid plus iron or acid alone or soil application of chelated iron on total and ferrous iron content in leaves of 'Donnie' avocado trees.

	Iron content (mg/kg dry wt.)	
Treatment[z]	Total	Ferrous[y]
SA+Fe	287.3a	27.0a
CA+Fe	188.8ab	22.0ab
AA+Fe	140.6bc	22.5ab
AA alone	58.2c	19.1bc
Chelated soil drench (EDDHA-Fe)	69.8c	12.43c
Control (no acid or Fe applied)	63.5c	11.6c

[z]SA+Fe=sulfuric acid+iron sulfate; CA+Fe=citric acid+iron sulfate; AA+Fe= ascorbic acid+iron sulfate; AA-alone= ascorbic acid alone and; EDDHA-Fe=sodium ferric ethylenediamine di-(o-hydroxyphenylacetate) iron. [y] Ferrous iron was calculated by the difference between total leaf iron content – ferric iron content.

Table 3. Cost Comparison for foliar applications of acid plus iron with cost of applying chelated iron (EDDHA-Fe) as a soil drench.

Treatment	No. of applications	Material[z] cost per application ($)	Other[y] costs per application ($)	Total[x] cost ($/ha)	Potential[w] savings ($/ha)	Potential[v] savings (%)
SA + Fe	6	17.65	11.93	178	776	81
CA + Fe	6	20.49	11.93	195	759	80
AA + Fe	6	46.45	11.93	350	604	63
AA alone	6	45.99	11.93	348	606	64
Chelated soil drench (EDDHA-Fe)	4	214.60	23.87	954	0	0

[z]Include only the cost of chemicals, assuming there are 215 trees per ha.
[y]Include the costs of labor and fuel to apply treatments, assuming 215 per ha.
[x]Total cost represents the sum of the material and other costs times the number of applications.
[w]Potential savings ($) calculated relative to the cost of applying EDDHA-Fe.
[v]Potential savings (%) represents the dollar savings expressed as percentage of the cost of applying EDDHA-Fe.

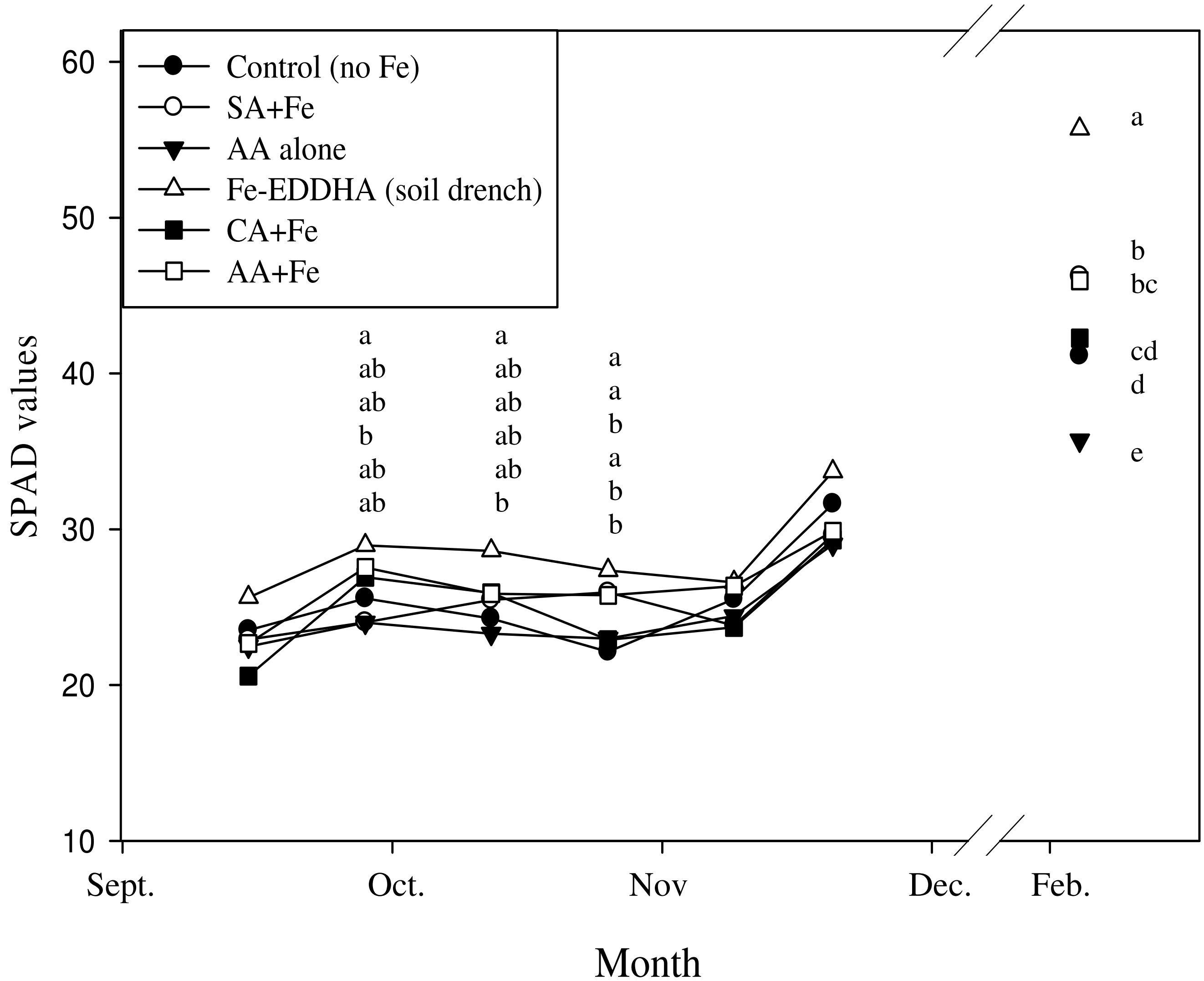

Fig. 1. SPAD values recorded at 2-week intervals (from 27 October 2006 to 20 November 2006) and on 20 February 2007 for mature leaves of 'Donnie' avocado trees. Different letters indicate significant differences among treatment means according to a Duncan-Waller multiple range test (P< 0.05 on 27 Sept. and 25 Oct. 2006 and 20 Feb. 2007; P≤0.10 on 11 Oct. 2006).