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ORIGINAL ARTICLE

Overcoming Fe deficiency in guava (*Psidium guajava* L.) by *co-situs* application of controlled release fertilizers

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Abstract

Among micronutrient deficiencies, Fe deficiency is the most difficult nutritional disorder to prevent in the fruits of trees growing on calcareous soils. In this study, a pot experiment was carried out to evaluate the potential of *co-situs* application of controlled release fertilizers (CRF) in alleviating Fe deficiency and improving the growth of fruit trees growing on calcareous soil (pH 9.3). Guava (*Psidium guajava* L.) seedlings were used as test plants because of their sensitivity to Fe deficiency. Treatments consisted of the following: (1) broadcast application of readily soluble Fe, Zn, Cu, B and Mn fertilizers (Control) or (2) *co-situs* application of CRF containing N, P, K, Mg, Fe, Zn, B, Cu and Mn (*Co-situs*). For the Control treatment, CRF containing only N, P and K was used. Both treatments received the same amount of all nutrients. Plants were more chlorotic in young leaves under the Control treatment and the Fe content of young leaves was significantly (least significant difference [$LSD_{0.05}$]) higher under the *Co-situs* treatment. Dry matter production of shoots under the *Co-situs* treatment was 5.2-fold higher than under the Control treatment, and the total accumulations of macro and micronutrients were much higher under the *Co-situs* treatment than the Control treatment. Total accumulations of N, P, K, Ca and Mg were 5.0, 4.1, 9.6, 3.2 and 2.2-fold higher, respectively, under the *Co-situs* treatment compared with the Control treatment, and Fe, Zn, Cu and Mn accumulations were 3.2, 4.1, 6.0 and 3.7-fold higher, respectively. Iron deficiency in guava seedlings was successfully alleviated by the *co-situs* application of controlled fertilizer, proving the high potential of this method in alleviating Fe deficiency in fruit trees growing on calcareous soils.

Key words: calcareous soils, controlled release fertilizers, Fe deficiency, guava.

INTRODUCTION

Iron (Fe) chlorosis is a major limiting factor for fruit trees grown on calcareous soils. Iron deficiency in fruit trees is characterized by chlorotic young leaves, resulting from decreased leaf chlorophyll concentration because of inadequate Fe absorption and/or utilization, and is responsible for significant decreases in yield, fruit size and fruit quality. When plants cannot acquire enough Fe to sustain growth, Fe chlorosis appears. This disorder has been reported in several fruit tree species such as pea, kiwifruit, vineyard and guava (Abadia *et al.* 1989; Kamal *et al.* 2000; Rombolà *et al.* 2000; Tagliavini and Rombolà 2001)

Ferrous sulfate, applied to either the soil or the tree (leaf sprays, trunk injection), has been a major therapy against Fe chlorosis since the first description of this nutritional disorder, and is still widely used by fruit growers in developing countries because of its low cost. If supplied alone, however, soil-applied Fe sulfate is of little or no agronomic value in calcareous soils where the Fe^{2+} is subject to rapid oxidation and insolubilization as hydroxide. In calcareous soils, the treatment of Fe chlorosis in trees is normally achieved by the application of Fe(III)-chelates such as Fe-EDDHA to the soil (Legaz *et al.* 1992; Papastylianou 1990). However, this practice has to be repeated annually because Fe is rapidly immobilized in the soil. Moreover, these chelating agents might also affect the absorption of other metals

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Table 1 Selected soil chemical characteristics

pH (H_2O)	9.3
OM (g kg^{-1})	0.1
$CaCO_3$(g kg^{-1})	383.5
EC (dS m^{-1})	0.05
P_2O_5 (µg g^{-1})[†]	1
AB-DTPA[‡]	(µg g^{-1})
Fe	3.2
Cu	Trace
Zn	0.2
Mn	2.4
Ammonium acetate – pH7[§]	(g kg^{-1})
Ca	107.1
Mg	0.53
K	1.72
Na	0.59
Water (1:20)[¶]	(g kg^{-1})
Ca	2.86
Mg	0.45
K	0.08
Na	0.58

[†]Phosphorus was analyzed using the Olsen method (Olsen *et al.* 1954). [‡]Ammonium bicarbonate diethylene triamine pentaacetic acid (AB-DTPA). extractable Fe, Zn, Mn and Cu were analyzed using the method of Soltanpour and Schwab (1977). [§]Ammonium-acetate extractable Ca, Mg, K and Na were determined according to Schollenberger and Simon (1945). [¶]Water-extractable Ca, Mg, K and Na were determined to be in a 1:20 ratio. EC, electrical conductivity; OM, organic matter.

such as manganese (Mn), copper (Cu) and nickel (Ni). It has also been reported that injection of Fe salts (mainly ferrous sulfate and Fe ammonium citrate) in liquid form into xylem vessels alleviates Fe chlorosis symptoms in several woody plants, such as apple, pear, peach, kiwifruit and olive (Fernandez-Escobar *et al.* 1993; Wallace 1991; Wallace and Wallace 1986).

Recently, the use of controlled release fertilizers (CRFs) in agriculture has been increasing, bringing advantages including labor-saving production, increased nutrient efficiency, improved yields and reduced negative environmental effects. Moreover, we recently succeeded in alleviating Fe chlorosis in paddy rice growing in calcareous soil by *co-situs* application of CRFs containing Fe (Morikawa *et al.* 2004, 2005). However, the situation is different under aerobic upland conditions, where Fe deficiency is more difficult to prevent. Therefore, the use of this method with upland crops is a challenge. The objective of the present study was to evaluate the potential of *co-situs* application of CRF on alleviating Fe deficiency and improving the growth of guava growing on a calcareous soil.

MATERIAL AND METHODS

A pot experiment was carried out for 1 year in a greenhouse of the Field Science Center of Tohoku University, Miyagi, Japan. Calcareous subsoil (shell fossil soil) of pH 9.3 was collected in Ishikawa Prefecture and used as the test soil. One-year-old guava (*Psidium guajava* L.) seedlings were grown in individual pots with 10 kg of calcareous soil. Guava was chosen because of its sensitivity to micronutrient deficiency, particularly Fe deficiency, and its nutritional importance in developing countries as a vitamin C source. The main chemical characteristics of the calcareous soil are described in Table 1. The soil had a low amount of organic matter and low amounts of oxalate, dithionite and pyrophosphate-extractable Fe, giving it a low capacity to supply Fe for crop growth.

The experimental design was a completely randomized block with three repetitions. Treatments consisted of: (1) broadcast application of readily soluble Fe, Zn, Cu, B and Mn fertilizers (Control) or (2) *co-situs* application of CRF containing N, P, K, Mg, Fe, Zn, B, Cu and Mn (*Co-situs*). For the Control treatment, CRF containing only N, P and K was used. As shown in Table 2, fertilizer containing a total of 4 g N per plant was applied twice for each treatment. Both CRFs used in this experiment require 180 days to release 80% of the nitrogen in water at 25°C.

Soil Fe was extracted with acid ammonium oxalate (McKeague 1976) using sodium pyrophosphate solution (0.1 mol L^{-1}; pH 10) (McKeague 1967) and citrate–bicarbonate–dithionite (Holmgrenn 1967), and Fe

Table 2 Total amounts of nutrients applied in the treatments

Treatment	Time	N (g $plant^{-1}$)	P (g $plant^{-1}$)	K (g $plant^{-1}$)	Mg (g $plant^{-1}$)	Fe (mg $plant^{-1}$)	Zn (mg $plant^{-1}$)	B (mg $plant^{-1}$)	Cu (mg $plant^{-1}$)	Mn (mg $plant^{-1}$)
Control	July 2003	2	1.6	2	0.3[†]	81.6[‡]	4.6[§]	9.2[¶]	4.6[††]	15.4[‡‡]
	Jan. 2004	2	1.5	2	0.3[†]	81.6[‡]	4.6[§]	9.2[¶]	4.6[††]	15.4[‡‡]
Co-situs	July 2003	2	1.6	2	0.3	81.6	4.6	9.2	4.6	15.4
	Jan. 2004	2	1.5	2	0.3	81.6	4.6	9.2	4.6	15.4

Source: [†]$MgSO_4{\cdot}7H_2O$; [‡]$FeSO_4{\cdot}7H_2O$; [§]$ZnSO_4{\cdot}7H_2O$; [¶]H_2BO_3; [††]$CuSO_4{\cdot}5H_2O$; [‡‡]$MnSO_4{\cdot}7H_2O$.

concentrations of the extracts were measured using atomic absorption (Hitachi Z-6100 Hitach, Tokyo, Japan). The carbon content of the soil samples was measured with a Sumigraph NC-80S analyzer, and the organic matter content was calculated by multiplying the amount of organic carbon (%) by 1.724.

The soil pH was determined for a 1:2.5 (w/w) soil : water suspension using a glass electrode. Ammonium bicarbonate-diethylene triamine pentaacetic acid (AB-DTPA) extractable Fe, Zn, Mn and Cu were analyzed according to the method of Soltanpour and Schwab (1977).

Before harvest, the net growth length of the branches was measured by subtracting the plant length at transplant time from the total length of the branches at harvest time. After harvest, shoots were washed with distilled water and then dried at 70°C for 48 h in an oven before the dry weights were measured. The dried material was ground and ashed in a muffle furnace at 500°C. The ash was then treated with acid solution on a hot plate according to the method of Howitz (1980). The Ca, Mg, K, Fe, Mn, Zn and Cu contents of the extracts were analyzed using atomic absorption spectrometry and P was measured using the colorimetric method.

All data were analyzed using nested ANOVA and means were compared using least significant difference ($LSD_{0.05}$).

RESULTS AND DISCUSSION

As shown in Fig. 1, plants under the Control treatment were more chlorotic than those under the *Co-situs* treatment. Interveinal chlorosis and characteristics of Fe deficiency appeared mainly on the young leaves of plants under the Control treatment. Leaves under the Control treatment were reduced in size compared with those under the *Co-situs* treatment.

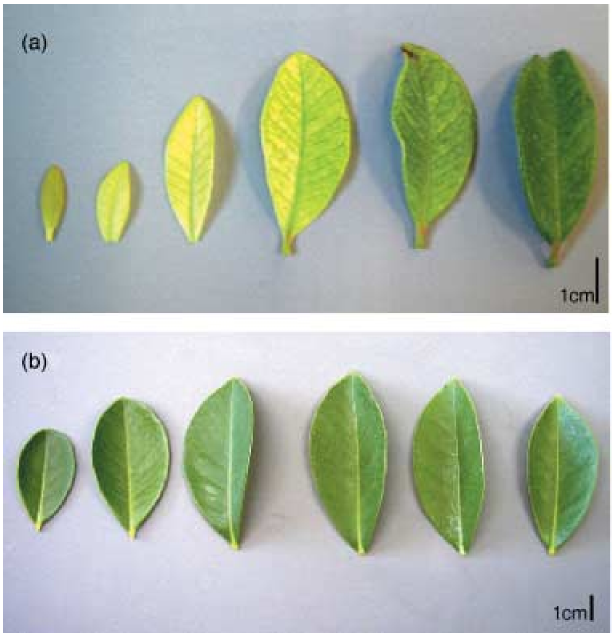

Figure 1 View of leaves of (a) Control and (b) Co-*situs* treatments. The order of leaf position indicates from young to older leaf (from left to right).

Trees develop Fe deficiency because of the continuous need for this element for growth and physiological processes. To treat Fe deficiency, synthetic chelates are widely used by farmers in soil applications. Common recommended doses are approximately 30–50 g of product per tree, which is equivalent to Fe supplementation of approximately 1.8–3.0 g per tree (considering a 6% Fe content). These rates are much higher than the 81.6 mg Fe used in our experiment.

Table 3 shows the effect of the fertilization method on the total dry matter production. The total dry matter production of stems, leaves and fruits under the *Co-situs* treatment was much higher than production under the Control treatment. Moreover, the total dry matter production of shoots under the *Co-situs* treatment was 5.2-fold higher than production under the Control treatment.

Table 3 Effect of the treatments on dry matter production (g $plant^{-1}$)

	Treatment		
Plant part	Control	*Co-situs*	$LSD_{0.05}$
Young leaves	7.7	19.3	7.9
Old leaves	16.3	40.9	13.7
Young stems	20.3	38.3	10.8
Old stems	11.8	27.9	7.1
Fruits	0.0	164.2	25.1
Total shoot	56.1	290.6	33.2

LSD, least significant difference.

Table 4 shows the nutrient contents of the leaves, stems and fruits as affected by the fertilization method. No significant differences ($LSD_{0.05}$) were observed between treatments in the macronutrient contents of N, P and K in older leaves. The Ca contents of older leaves under *Co-situs* treatment were much higher than those under Control treatment, and the opposite was found for Mg content. The Mg contents of older leaves under the Control treatment were statistically ($LSD_{0.05}$) higher than those under the *Co-situs* treatment. For young leaves, the contents of N, K and Mg under the Control treatment were statistically higher than those under the *Co-situs* treatment. There were no significant differences between treatments in the P and Ca contents of young leaves.

Except for the Fe content of older leaves, drastic differences between treatments were found with regard

Table 4 Content of nutrients in leaves, stems and fruits

			N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
Plant part		Treatment	Contents (g kg^{-1})								
Leaf	Young	Control	19.5	3.8	44.0	64.6	15.3	107.3	17.4	9.6	96.5
		Co-situs	16.0	3.9	24.2	64.2	10.4	137.4	22.6	25.8	148.4
	Old	Control	13.2	2.6	10.3	62.1	18.9	202.0	12.7	15.7	170.7
		Co-situs	12.3	3.6	11.0	103.8	12.2	205.0	25.0	31.4	209.7
Stem	Young	Control	10.1	2.0	16.9	47.5	7.9	50.4	13.7	2.9	48.5
		Co-situs	7.6	2.0	11.6	52.7	6.6	69.8	23.9	16.1	77.7
	Old	Control	7.9	2.1	6.2	37.2	4.0	58.9	13.9	8.0	26.6
		Co-situs	5.7	1.8	5.5	36.7	5.7	60.6	13.7	7.5	33.1
Fruits		Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Co-situs	12.0	1.4	29.5	3.2	2.2	24.7	4.7	3.9	8.6
	$LSD_{(0.05)}$		1.1	0.7	3.6	20.6	2.3	15.2	5.6	5.8	23.1

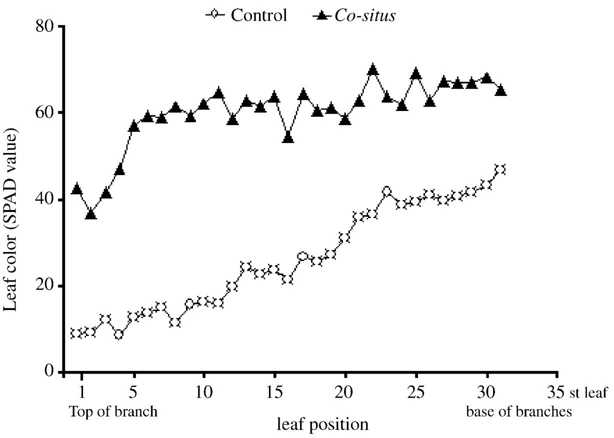

Figure 2 Effect of treatments on leaf color according to leaf position at harvest time. In the x axe, lower values represent young leaves (top of branches) and higher values represent older leaves (base of branches). The y axe represents the SPAD values. The longest branches of each repetition were used for measurements. Each SPAD value represents the mean value of leaves at same position in the branches.

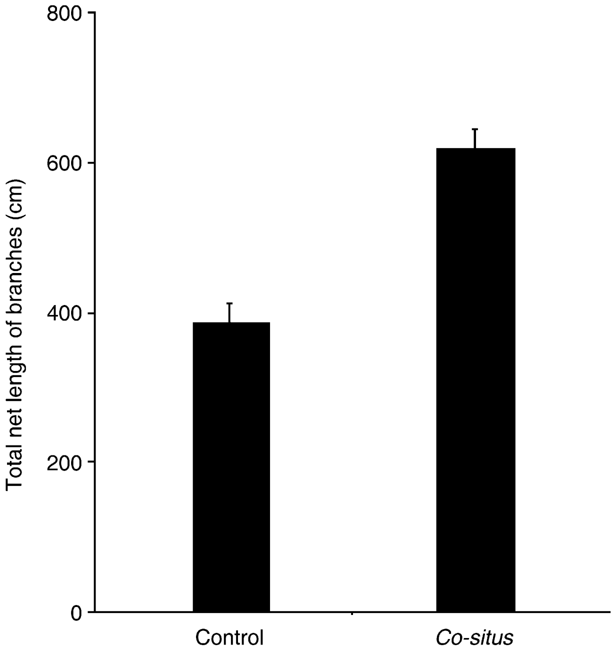

Figure 3 Effect of treatments on the total net length of brances ($LSD_{0.05} = 25.9$).

to the contents of micronutrients. The Zn, Cu and Mn contents of young and older leaves under the Control treatment were significantly ($LSD_{0.05}$) lower than those under the *Co-situs* treatment. No significant differences between treatments were found in the Fe contents of older leaves; however, the Fe contents of young leaves under the *Co-situs* treatment were much higher than those under the Control treatment.

Except for N content, no significant differences between treatments were found for nutrient contents of older stems. In contrast, as with young leaves, the N and K contents of young stems under the Control treatment were higher than those under the *Co-situs* treatment. No differences between treatments were found for P, Ca and Mg contents of young stems. The Fe, Zn, Cu and Mn contents of young stems under the *Co-situs* treatment were much higher than those under the Control treatment.

As shown in Fig. 2, the SPAD values of leaves under the Control treatment were lower than the values recorded under the *Co-situs* treatment. Moreover, the color differences increased from the base to the top of the branches. The lowest SPAD value of 6 was found for young leaves under the Control treatment, and the highest value of 72 was found for older leaves under the *Co-situs* treatment.

Figure 3 shows the total net length of branches according to the fertilization method. The total net length under the Control treatment was significantly higher than under the *Co-situs* treatment ($P < 0.05$).

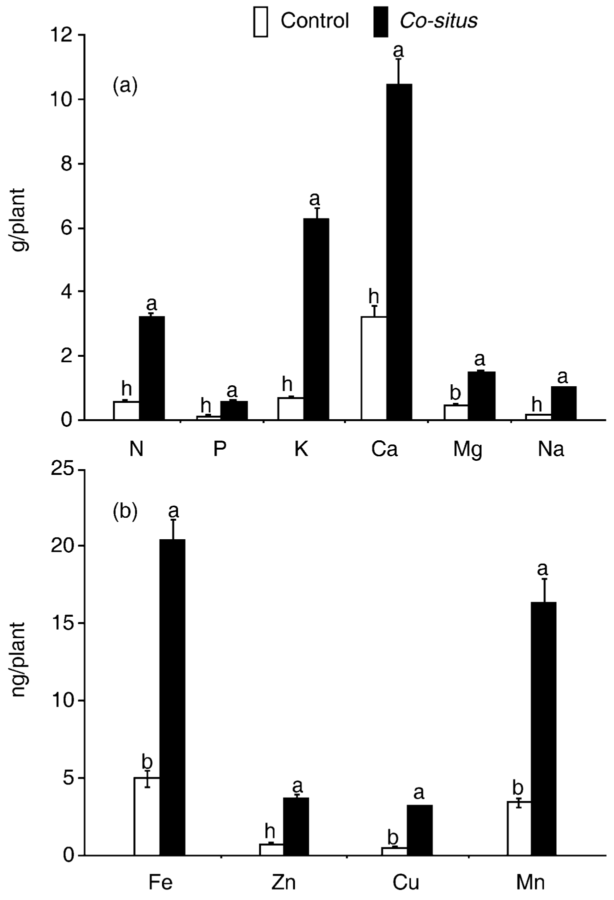

Figure 4 Total of (a) macronutrients and (b) micronutrients accumulated by guava seedlings. Bars are means of three replicates ± SE. For same element, bars with same letters are not significantly different at $LSD_{0.05}$.

Reflecting dry matter production rather than nutrient concentration, drastic differences between treatments were found for the total nutrients accumulated by the plants during the growth period (Fig. 4). For both macro and micronutrients, the total nutrients accumulated by plants under the *Co-situs* treatment were much higher than those accumulated under the Control treatment. The total N, P, K, Ca and Mg accumulated by plants under the *Co-situs* treatment were 5.0, 4.1, 9.6, 3.2 and 2.2-fold higher, respectively, than those under the Control treatment. The Fe, Zn, Cu and Mn accumulations under the *Co-situs* treatment increased by 3.2, 4.1, 6.0 and 3.7-fold, respectively, compared with the Control treatment. These findings clearly show that the *co-situs* application of CRF increased fertilizer use efficiency to a great extent.

Thus, the *co-situs* application of micronutrients significantly ($LSD_{0.05}$) increased the content and accumulation of these elements in guava tissue. Similar results were found by Morikawa *et al.* (2004) for paddy rice. The drastic differences between treatments with regard to the content and accumulation of micronutrients suggest that micronutrient deficiency was the main constraint affecting guava seedling growth on calcareous soil in the present study. An increased accumulation of Fe was found under the *Co-situs* treatment compared with the Control treatment. These results can be explained by the low effectiveness of soil applications of readily soluble Fe sources in ameliorating Fe deficiency and, in contrast, the high effectiveness of the *co-situs* method in supplying Fe to guava seedlings. Soil applications of rapidly soluble Fe sources are usually not effective unless high rates are applied because they are rapidly converted to forms not available to plants. With the *co-situs* method, the fertilizer granules come in direct contact with the guava roots, directly supplying ferrous Fe to the guava seedlings.

In our study, the broadcast application of 81.6 mg Fe per plant was likely to be insufficient to sustain normal plant growth, as shown by the progressive development of chlorosis associated with the reduction of Fe content and accumulation (Table 4, Fig. 4). Despite large differences in the leaf color of young leaves under the Control treatment compared with the *Co-situs* treatment, a considerable concentration of Fe was observed in young leaves under the Control treatment. This probably resulted from the following: (1) an overestimation of the amount of Fe in the chlorotic leaves as a consequence of a reduction in leaf size as previously reported by Toselli *et al.* (2000) and/or (2) existence of Fe pools that are somehow inactivated in chlorotic leaves (Mengel 1994; Tagliavini *et al.* 2000). However, more investigation of the mechanism for the inactivation of Fe in tissues is required. According to Römheld (2000), Fe inactivation is a secondary effect occurring in a leaf after the occurrence of Fe chlorosis: a high HCO_3^- concentration in the soil would lead to a decrease in the uptake and availability of Fe for canopy growth, so the higher Fe concentration in chlorotic leaves would be the final consequence of the leaf growth inhibiting effect of bicarbonate. However, Mengel (1994) considered Fe inactivation to be of major importance for the development of Fe chlorosis, suggesting that the poor efficiency of Fe in leaf tissues is primary related to the high pH of the leaf apoplast under alkaline conditions, which would impair Fe^{3+} reduction by mesophyll cells and consequently depress Fe transport across the plasmmalema.

In fruit trees, Fe deficiency results in considerable loss of yield (Pestana *et al.* 2003), delayed fruit ripening and impaired fruit quality, as reported for peach (Sanz *et al.* 1997) and orange (Pestana *et al.* 2001). In our experiment, despite the same amounts of nutrients being applied in both treatments, no fruits were found in plants under the Control treatment, while plants under the *Co-stius* treatment had a mean of 11 fruits per plant.

The central concept of *co-situs* application is to apply a large amount of CRF to the intensive rooting zone with release patterns that synchronize with plant demand throughout the growing season. In our experiment, Fe deficiency of guava seedlings was successfully prevented by *co-situs* application of CRFs, suggesting the potential of this method in alleviating Fe deficiency and improving the growth of trees in calcareous soils.

We conclude that the *co-situs* application of CRFs can easily be adopted if the release rates of the nutrients from the used CRF match the plant demand for the same nutrients. A specific nutrient can be supplied to the plants using this method. In our case, Fe was successfully supplied to guava trees grown in an extremely alkaline soil (pH 9.2). The application cost is reduced and innovative farming systems can be developed using the *co-situs* method. However, future studies under field conditions are required.

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