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Rates of Photosynthesis and the Hill Reaction in Citrus Seedlings Affected by Fe, Mn, and Zn Nutrition[1]

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Additional index words. Citrus sinensis, orange seedlings, plant nutrition

Abstract. **Foliar application of Fe and Mn to Fe-deficient 'Pineapple' orange seedlings (*Citrus sinensis* (L.) Osbeck) substantially increased the rate of photosynthesis and the Hill activity over Fe alone; Zn plus Fe and Mn had little effect. The Hill reaction was impaired more by a deficiency of Mn than of Fe. The disappearance of chlorotic symptoms or increased Fe and chlorophyll contents in the leaves may not indicate complete recovery of the leaves from photosynthetic impairment. Photosynthetic rate and the Hill reaction could be used as additional indicators for diagnosing mineral element deficiencies in citrus plants.**

Foliar application of mineral elements has been used both experimentally and commercially over the past 4 decades on many crop plants in attempts to control mineral deficiencies. Supplying Fe to Fe-chlorotic plants was a problem until Fe chelates were introduced in 1950 (13). However, correction of chlorosis by Fe sprays has through the years been only moderately successful. Fixation of absorbed Fe in sprayed leaves from certain Fe compounds, with little or no translocation to growing regions, still remains a problem. In almost all the previous work with Fe nutrition, much attention has been focused on the effects of sprayed Fe with respect to the degree of leaf regreening or increases in leaf Fe content as indicators for plant recovery. There is a quantitative relationship between absorbed Fe and the Fe content of leaves, but a precise correlation between Fe content of the leaf and the degree or the intensity of chlorotic symptoms of the same leaf has not yet been established. It is possible to have plants lacking distinctive symptoms of Fe chlorosis over a wide range in Fe content. Lack of symptoms of mineral deficiencies, including those of Fe, does not indicate the existence of optimum nutritional status in the plant. Symptoms of chlorosis can serve only as approximate indicators of the severity of Fe deficiency and only in the more advanced stages (33). The cation: anion ratio within the leaf is a constant. Based on the concept of nutrient-element balance outlined by Shear, Crane and Myers (25, 26), it is evident that variation in the accumulation of cations or anions influences not only the accumulation of other ions of the same charge but also may either increase or decrease the oppositely charged ions. It has long been known that absorption and accumulation of each nutrient ion is dependent on absorption and accumulation of every other available ion (26). The absolute concentration of an element in the leaf is not a logical measure of its functional concentration. For maximum efficiency and normal function, each nutrient element must occur in the leaf in the proper proportion to every other essential element (25).

The purpose of this study was to determine if application of Fe alone or Fe in combination with other elements to Fe-

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Table 1. Composition of Fe formulations used for dipping chlorotic 'Pineapple' orange seedlings.

Formulation[z]		
#1	#2	#3
50 mM FeEDTA 800 mM urea 1% PEG 1000MO[y]	50 mM FeEDTA 130 mM $MnSO_4$ 800 mM urea 1% PEG 1000MO	50 mM FeEDTA 130 mM $MnSO_4$ 120 mM $ZnSO_4$ 800 mM urea 1% PEG 1000MO

[z]Quantities per 3.75 liters. pH 5.5 to 6.0.

[y]Polyethylene Glycol 1000 mono-oleate (Armour Industrial Chemical, Co.)

Table 2. Effects of different nutritional formulations on Fe, Mn, Zn and chlorophyll contents of 'Pineapple' orange leaves 96 hr after treatment.[z]

Formulation	Fe (ppm)	Mn (ppm)	Zn (ppm)	Total chlorophyll (mg/g fresh wt)
# 1	65.0 b	14.6 b	22.3 b	1.178 b
# 2	47.0 c	17.8 c	21.6 a	1.303 c
# 3	79.0 c	17.5 c	24.4 c	1.577 d
Green[y]	93.6 d	18.0 c	25.0 d	1.944 e
Chlorotic[x]	48.3 a	13.1 a	20.1 a	0.877 a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

[y]4-month-old 'Pineapple' orange leaves before induction of Fe chlorosis.

[x]4-month-old 'Pineapple' orange leaves after induction of Fe chlorosis.

deficient citrus leaves would bring about high rates of photosynthesis and Hill reaction activity.

Materials and Methods

Four-month-old 'Pineapple' orange seedlings (*Citrus sinensis* (L.) Osbeck) were grown in vermiculite (Zonolite Company, Chicago, Ill.) in small plastic pots in the greenhouse. Fe-deficiency symptoms were induced by preparing nutrient solution as described by Hoagland and Arnon (12) but without the addition of Fe during the experiment. When Fe deficiency became evident (after 7 weeks), the seedlings were dipped thoroughly for 1 min in the formulations (Table 1). Care was taken not to allow contamination of the culture media.

Photosynthesis measurements. Photosynthetic activities of intact leaves were determined at 24, 48, 72, and 96 hr after treatment. Measurements were made on mature leaves with a Beckman Infra-Red Analyzer 315B equipped with automatic recorder. Each leaf tested was enclosed in a transparent, double-walled chamber that was maintained at 30 ± 1°C. CO_2 assimilation rates were determined per unit of leaf area by comparing the CO_2 concentration of air at the entrance and exit ports of the chamber, and expressing the difference as uptake of CO_2 in mg dm^{-2} hr^{-1} (36).

Hill reaction activity measurements. The procedure adopted for the chloroplast suspension was essentially the same as the one described by Jagendorf (15) with slight modification. Two g fresh leaf tissue, with midribs removed, were macerated in 10 ml ice-cold solution containing 0.05 M tris (pH 8.0), 0.35 M sucrose, 0.002 M EDTA, and 0.001 M $MgC1_2$. The homogenate was filtered through 8 layers of cheesecloth, and then filtered through Nitex nylon (20 μ pores). The homogenate was centrifuged at 1000 g for 5 min The pellets were then resuspended in 1 ml of the original solution immediately before measuring the Hill reaction activity.

Assay of the Hill reaction was basically a modification of the procedures of Jagendorf (15), Jagendorf and Evans (16), and Spencer and Possingham (29). Chloroplast suspension and 2, 6-dichloroindophenol (DCIP) dye (Eastman Kodak) were mixed in a Beckman cuvette in 1 : 1 proportion to give a total volume of 3 ml. Changes in optical density (ΔOD) were recorded at 620 nm before and after illumination of the reaction mixture for 45 sec. Absorbance measurements were made in a Beckman DB-G spectrophotometer; μg/μl chlorophyll in the chloroplast suspension was determined by the method described by Walker (32). Hill reaction activity was expressed as ΔOD at 620 nm/mg chl. hr.

Chlorophyll content and mineral analyses. Chlorophyll pigments were extracted with 80% acetone. Total chlorophyll was calculated by Arnon's modification (2) of MacKinney's method (21). Mineral analyses were carried out by atomic absorption spectrophotometer.

Results and Discussion

Fe, Mn and Zn contents. Contents of Fe, Mn and Zn were significantly increased in 'Pineapple' orange leaves over the control by the inclusion of these elements in different nutritional formulations (Table 2). These results confirm that mineral elements can be absorbed through the leaves as reported earlier (18, 19, 27). The use of urea as a penetrating agent, and PEG-1000MO as a surfactant, which have been reported to increase Fe absorption (5, 17, 22), resulted in a markedly higher uptake of Fe, Mn and Zn in leaf tissues. However, samples from leaves treated with Formulation #3, which contained 50 mM FeEDTA plus both Mn and Zn, showed 29% more increase in Fe than samples taken from leaves treated with Formulation #1 which contained the same amount of Fe but lacked Mn and Zn. It was evident that foliar application of a nutrient solution containing Fe, Mn and Zn corrected the visual symptoms of Fe deficiency and also increased concentrations of these three elements in the leaves. Leaves which had been chlorotic prior to treatment with Formulation #3 revealed increases of 64, 34 and 21% in Fe, Mn and Zn, respectively, after 96 hr. There were only 34%, 11% and 1% increases in Fe, Mn and Zn, respectively, when plants were treated with Formulation #1. It has been generally accepted that certain microelements can induce or prevent chlorosis. Mn seems to be definitely related to Fe in the induction of chlorosis. On the other hand, Zn has been found to cause symptoms that resemble Fe chlorosis (8, 11). The data clearly illustrate that the physiological activity of Fe in citrus seedlings was determined by the relative supply of Mn and Zn and that the visual recovery from chlorosis depended on the availability of Mn and Zn in the leaf tissue rather than that of Fe alone.

Chlorophyll content. Chlorophyll content was increased 34% by the addition of Fe to chlorotic 'Pineapple' seedlings (Table 2). Inclusion of Mn in Formulation #2 did not increase significantly the level of chlorophyll in the treated leaves. Addition of Fe, Mn and Zn in the nutrient solution did result, however, in an 80% increase in leaf chlorophyll over the control. Fe is not a part of the chlorophyll molecule, but it is necessary for the formation of an Fe-prophyrin complex which is required for chlorophyll synthesis (24, 31). Various investigations have been directed toward determining the relation between Fe and chlorophyll content of the leaf, since chlorophyll is obviously linked to Fe deficiency. There seemed to be a direct correlation in some cases (1, 14). Data obtained in this report confirmed these findings. However, while visual symptoms of Fe deficiency disappeared in 'Pineapple' orange leaves treated with Fe, Mn and Zn, chlorophyll and Fe contents were not at the same level as in the healthy green leaves. Chlorophyll and Fe were much higher in leaves treated with Fe, Mn and Zn than in those treated with Fe alone. Disappearance of chlorotic symptoms or

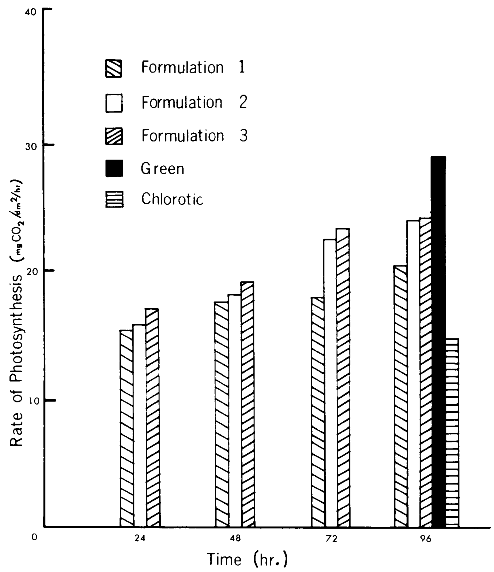

Fig. 1. Photosynthetic rate of 'Pineapple' orange seedlings during 96-hr period following treatment with different Fe formulations.

Table 3. Effects of different nutritional formulations on photosynthesis and the Hill reaction activity of 'Pineapple' orange leaves 96 hr after treatments.[z]

Formulation	Photosynthesis (mg CO_2 dm-2 hr-1)[y]	Hill activity ΔOD at 620 nm/mg chl. hr[y]
# 1	20.33 b	367.6 b
# 2	23.91 c	469.9 c
# 3	24.16 c	474.0 c
Green	28.92 d	546.1 d
Chlorotic	14.53 a	305.1 a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Avg of 9 mature leaves per treatment.

the increase in Fe and chlorophyll levels in the leaves are not absolute indicators of complete recovery of the leaf and of its optimum function for growth and development.

Photosynthetic activity. Chlorosis of 'Pineapple' orange leaves resulting from Fe deficiency markedly influenced the rate of photosynthesis. Data on the effects of applying chelated elements to Fe-deficient leaves are presented in Table 3. The rate of photosynthesis increased progressively during the 96 hr period after the chlorotic leaves were treated (Fig. 1). Application of Mn and Fe chelate to chlorotic leaves resulted in a 65% increase in the rate of photosynthesis, while Fe alone brought about a 40% increase. Leaves treated with Zn plus Fe and Mn were unable to sustain significantly higher rates of photosynthesis than those obtained without the addition of Zn. This, of course, did not rule out the importance of the part played by Zn for maintaining optimum rates of photosynthesis. One possible explanation of the negligible increase have been, however, that the higher levels of Zn in the chloroplasts were not really critical for maintaining higher rates of photosynthesis. This confirmed the previous reports by the authors (4).

A reduced rate of photosynthesis has been associated with reduction in the Fe, Mn, and Zn contents of green tissue (7, 30). It has been shown that Mn content changed significantly in citrus leaves when Fe levels in the nutrient solution were altered (20, 28). Under certain conditions, application of Fe alone also induced Mn deficiency symptoms in citrus leaves. It is conceivable that the addition of Fe chelates alone by foliar application to Fe-deficient plants may not raise substantially photosynthetic efficiency, although it will increase Fe content in the leaves. Balanced nutrition is needed to obtain optimum rates of photosynthesis providing that other factors also are optimal.

The Hill reaction activity. The Hill reaction of photosynthetically active chloroplasts isolated from 'Pineapple' orange leaves increased 55% over a 96 hr period after treating the leaves with Formulation #3 (Table 3). Addition of Fe alone increased the Hill activity by only 20% during the same interval. Similar to photosynthetic tests, incorporation of Zn with both Fe and Mn slightly increased the Hill reaction. This demonstrated an interrelation among photosynthesis, the Hill reaction and the level of nutrient elements.

There is incomplete information regarding the effects of mineral elements on the Hill reaction. The major part of Fe in the leaf is located in the chloroplasts and is necessary for maintenance of functional chloroplasts (9). Isolated chloroplasts did not accumulate Mn but rather Fe and Cu (35). Our findings agreed with those of Spencer and Possingham (29), who found that Fe deficiency caused impairment of the Hill reaction. The degree of impairment was related to Fe level. The phytolysis of water and evolution of oxygen in the Hill reaction is a Mn-dependent process (10, 23, 30, 35). The rate of the Hill reaction of isolated tomato chloroplasts was proportional to both the level of Mn supplied externally and, more importantly, to the Mn content in the isolated chloroplasts (29). The effects of mineral element deficiencies on the ultrastructure of the chloroplasts of different crops have been established recently. Deficiencies of various elements caused significant morphological changes in chloroplast structure that were related to functional changes as measured by photosynthesis (3, 6, 10, 34). Highly organized chloroplast structure, as in the case of healthy plants, appeared to be a prerequisite for the Hill reaction activity *in vivo*. Bogorad et al. (6) and Whatley (34) found distinctive chloroplast types which have a granular disorganized appearance associated with conditions of Fe deficiency. Application of Mn with Fe for correction of Fe deficiency resulted in higher Hill reaction rates than those obtained when Fe was applied without Mn. Suboptimal Fe effect could be only on certain chloroplasts in a population. On the other hand, suboptimal Mn effect could influence nearly all chloroplasts in the same population, since it is a component of the Hill reaction. This could explain the greater decrease in the Hill reaction activity with the ommission of Mn rather than of Fe in the nutrient solution.

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Inheritance of 1-octen-3-ol Concentration in Frozen Pods of Bush Snap Beans, *Phaseolus vulgaris* L.[1]

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Additional index words. flavor components

Abstract. **Inheritance of the volatile flavor component 1-octen-3-ol in 'OSU 58-110' x 'Bush Romano FM-14' was determined, using gas-liquid chromatographic technique with gas-entrainment on-column trapping. The low concentration of 1-octen-3-ol characteristic of '58-110' was dominant in the F_1, F_2 and backcrosses. The range of concentration in each parent and the F_1 was about 80 ppb. The concentration in the F_1 was nearly identical to that of the low parent. F_2 and backcross data were continuous over a wider range of concentration than the parents or F_1. A definite bimodal distribution was not apparent.**

Flavor differences among green bean cultivars have become increasingly important to breeders. In areas where the canning industry was formerly based on the 'Blue Lake' pole cultivar, the flavor of 'Blue Lake' is preferred or required in bush cultivars by some processing firms. In many other cases the flavors of other bush cultivars, such as 'Tendercrop' and its derivatives, are considered satisfactory.

Stevens and Frazier (10) established the importance of several volatile compounds in green bean flavor. They found that 'FM-1L Blue Lake' contained 10 times as much 1-octen-3-ol as 'Gallatin 50', but only 2/3 as much as the pole cultivar 'Romano'. Both 'Gallatin 50' and 'Romano' contained 6 times as much linalool as 'FM-1L Blue Lake'. 1-octen-3-ol concn was

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