Table 5. Production comparisons between standard 'Valencia' and 'Rohde Red Valencia' varieties by tree-age.

	Boxes per Acre[1]			Pounds solids per acre		
Tree Age	Valencia	Rohde	Difference[2] (T-statistic)	Valencia	Rohde	Difference[2] (T-statistic)
10	380.9	na	na	2408.9	na	na
3-yr mean[3]	409.4	441.3	-31.9	2585.2	2836.4	-251.2

[1]Boxes are 90 pounds each.
[2]Difference between the reported means of tree-age yield. T-statistic, reported in parentheses, is calculated for a difference-in-means test. At the 95% confidence level, a critical T-value greater than 1.8 implies a rejection of the null hypothesis, concluding that mean yields between groups are not equal.
[3]Mean calculated from years 7, 8, and 9.

were significantly more than 'Valencia' through year seven. While pound solids from 'Hamlin' in the eighth and ninth year remained numerically greater than 'Valencia', the difference was no longer statistically significant (i.e. failed to reject the null hypothesis).

Rootstock effects were tested by calculating separate yield means for 'Hamlin' and 'Valencia' on both Carrizo and Swingle rootstocks. Table 3 presents mean boxes per acre by tree age for each scion variety/rootstock combination. Except for six and eight-year-old 'Valencia', Carrizo rootstocks yielded more boxes per acre than Swingle rootstock. However, the null hypothesis that there are no yield differences between Carrizo and Swingle rootstocks could not be statistically rejected. In other words, at the 95% confidence level, yields differences between Carrizo and Swingle rootstocks were not significantly different from zero. Table 4 presents similar information with respect to pound solids per acre. For the years where there were sufficient data, pounds solids per acre were generally more from Carrizo rootstock as compared to Swingle rootstocks. However, yield differences were not statistically different from zero. The one exception occurred on six-year-old 'Valencia's where Swingle produced more pound solids than Carrizo. However, there could be unexplained anomalies because on five and seven-year-old 'Valencia', Carrizo outperformed Swingle.

Limited yield information was available for 'Rohde Red Valencia'. Table 5 compared production from Rohde to standard 'Valencia'. Rohde and standard 'Valencia' varieties appear to produce an equal number of boxes per acre. However, there was some evidence that during years five though seven Rohde's produced more pound solids per acre.

Future Work

Currently, all study blocks are located in either Hendry or Collier Counties. As the study continues, it is planned to increase the number of study blocks to include citrus acreage in Charlotte, Glades, and perhaps Lee Counties.

Annual collection of production data from the study blocks will continue. As the study blocks "mature", the yield-age profile will be extended. During the next five years, it is hoped that tree-age peak yields and typical production profiles can be characterized for each scion/rootstock combination. In particular, it is hoped that hedging practices be incorporated into the analysis to examine their effect on the tree-age yield profile. Finally, if the study is allowed to continue indefinitely into the future, it may be possible to place parameters on the expected life span of a citrus grove in southwest Florida.

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SURFACE DECONTAMINATION OF CITRUS LEAVES FOR MACRO AND MICRO NUTRIENT ANALYSIS

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Abstract. **Mineral nutrition status of the crop plants can be evaluated by leaf analysis. The value of leaf analysis largely depends on careful consideration of sampling, decontamination, and preparation of extract for various analysis. The recommended physiological stage for citrus leaf sampling is about 5- to 7-mo-old spring-flush. In most cases, foliar application of micro-nutrient is made during the flush emergence stage and**

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later in summer. In this study, we evaluated the effects of recommended leaf washing procedure, i.e., detergent plus acid washing followed by several rinses in deionized water, vs. rapid rinse in deionized water alone on the concentrations of macro- and micro-nutrients in the leaves. The results showed no significant differences in most macro and micro-nutrient concentrations, except Cu, between the detergent plus acid washing vs. rapid rinse procedures. Therefore, under the conditions when the micro-nutrients foliar application was made in early flush stage and the leaves are sampled about 6 mo later and prior to the summer micro-nutrient application, a rapid rinse in deionized water was satisfactory for leaf surface decontamination of most mineral elements, except Cu. However, the critical time interval between the foliar spray of micro-nutrients and time of leaf sampling to minimize carry over of micro-nutrient residue on the leaf surface cannot be derived from this study.

Leaf analysis is an important technique to evaluate the mineral nutrition status of crop plants (Mills and Jones, 1996). Sampling the leaves at desirable physiological stage of growth, decontamination of leaves, ashing and analytical techniques influence the precision of analysis and, in turn, the evaluation of mineral nutrition status of the crop in question. Among the above factors the decontamination procedure is most variable and could have considerable impact on the analytical results, particularly for perennial tree crops.

The recommended stage of growth for sampling citrus leaves for mineral analysis is 5- to 7-mo-old spring-flush (Obreza et al., 1993; Smith, 1966; Tucker et al., 1995). In Florida, the sampling is generally done from non-fruiting terminals. The decontamination of leaf is considered as an important step, particularly if micro-nutrient analysis is required, since the micro-nutrients are applied as foliar spray at various times after the spring-flush leaves are fully open. The residue of micro-nutrients outside the leaves, if not removed prior to ashing and analysis, could considerably over estimate the micro-nutrient status in the leaves. The carry over of micro-nutrient residue on the leaves largely depends on the time of foliar application in relation to the leaf sampling time for nutrient analysis.

The leaf decontamination procedure includes washing the leaves in detergent solution by rubbing both the side of the leaves using cheesecloth, followed by 20 sec rinse in 5% HCl and several rinses in distilled water. This is a tedious procedure and labor intensive, therefore, most commercial laboratories do not follow this procedure.

The aim of this study was to evaluate the difference in concentrations of various mineral elements following: either (i) quick rinse in distilled water or (ii) detergent-acid cleaning procedure.

Materials and Methods

This study was conducted in an 8-yr-old commercial grove of Navel orange trees on Swingle citrumelo rootstock planted in double row beds at 25 × 15 ft spacing in a block with Manatee loamy fine sand and Riviera fine sand. The soil pH was 8.0 (in water) with approximately 21,000 lb/ac extractable Ca (Mehlich 3 extraction; Mehlich, 1984). The calcareous nature of this soil was quite apparent from the high pH and Ca and bicarbonate contents. The trees showed visible symptoms of moderate to severe deficiencies of micro-nutrients. This study was part of a main study on the evaluation of various source of micro-nutrients for correction of micro-nutrient deficiencies in commercial citrus groves (Alva and Tucker, 1992). The study included 7 sources of micro-nutrients, i.e., nitrate forms, alpha keto acid, lignin sulfonate, gluco heptanate, NZN, dry mix, and EDTA-chelated forms. Each formulation was applied to provide 2 rates of micro-nutrients: i.e., (i) 0.5 lb each of Mn and Zn, and 0.125 lb of Fe per acre, and (ii) 2× of the low rates. The annual dose was applied in 2 equally divided doses. The first application was made in February, i.e., flush emergence stage, and the second application was made in summer after the mature flush was sampled for mineral nutrient analysis. A commercial speed blast sprayer calibrated at 167 gal/ac was used to apply the material. The sprayer was adjusted to make one sided application with both sides of the tree being sprayed. All treatments received a uniform application of Cu using Cu hydroxide (23% Cu) at 2 gal of the product per 500 gal spray solution. A control treatment was included which received only Cu spray. A completely randomized design was followed with 5 replications. The experiment was conducted for 2 yr.

Leaf Sampling and Analysis. Six-mo-old spring-flush leaves were sampled in August prior to the application of second spray each year. Within each of the non-fruiting terminal chosen for leaf sampling, 2 leaves were picked and split into 2 separate samples for washing procedure evaluation. Approximately 50 leaves were sampled for each split sample per plot. The following two washing procedures were evaluated in this study: (i) rinsed in deionized water only with rapid swirling action and, (ii) washing the leaves in Liqui-nox detergent (Alconox, Inc.) solution by scrubbing both sides with cheesecloth, followed by several rinses in tap water. The leaves were then rinsed in 5% HCl for 30 sec followed by rinses in deionized water. For convenience in discussion, the above procedures are referred to as rinsed and washed, respectively.

The leaves were dried at 158°F for 48 hr, ground to pass through 40 mesh screen, and 0.5 g ground tissue sample was dry ashed in a muffle furnace at 932°F for 6 hr. The ash was dissolved in 10 mL 1M HCl and concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, and Al were measured using a inductively coupled plasma argon emission spectroscope (ICPAES, Perkin Elmer Corporation). The concentration of N was determined by the micro-Kjeldahl method.

Results and Discussion

The concentration of most macro- and micro-nutrients, with the exception of Cu, showed no significant difference between the wash and rinse treatments over 2 yr (Table 1). The purpose of tedious washing procedure is to ascertain removal of residue from the leaf surface which could over estimate the actual concentration of an element within the leaves. The results of this study show that there was no evidence of micro-nutrient residue on the surface of the leaves sampled 6 mo after the micro-nutrient spray, which was made during the young flush-stage.

This is in contrast to the results of Futch and Gallaher (1996) who reported a significantly lower Zn concentrations in the leaves which were washed using detergent plus acid or detergent plus EDTA as compared to those in the leaves which were washed in water alone or unwashed, suggesting that the former washing procedures were more effective in removing the surface residues on the leaves. In their study, the washing procedure evaluation was conducted 7 and 28 days after spraying $ZnSO_4$ solution. The short interval between spraying the micro-nutrient solution and sampling for leaf analysis may have contributed to leaf surface Zn residue for the Zn concentration measured in leaves which were unwashed or washed only in water. In contrast, in the present study, the leaf sampling was done 6 mo after the micro-nutrient spray application. Therefore, there was no surface residue on the leaves during the leaf sampling. This study does not address the critical time interval between spraying the micro-nutrients and leaf sampling for micro-nutrient analysis to eliminate contribution of leaf surface residue for leaf composition. However, in most cases the micro-nutrient are generally sprayed in the early spring during flush emergence stage and in summer. Therefore, if the timing of leaf sampling is adjusted prior to the summer spray of micro-nutrients, the early spring spray would have minimal residue carry over of micro-nutrients on the surface of the leaves.

The concentration of macro-nutrients showed very little difference between the washed and rinsed leaves. Since these nutrients are soil applied the only potential source of contamination is by dust particles on the leaves. The results of this study show that a rapid rinse procedure is equally effective as the detergent plus acid wash procedure for removal of dust particles and other macro-nutrient contaminants on the leaves.

Table 1. Effects of detergent plus acid washing followed by several rinses in deionized water (wash) vs. rapid rinsing in deionized water (rinse) of leaves on concentrations of various mineral elements in 6-mo-old spring-flush of Naval orange trees on Swingle citrumelo rootstock, which received micro-nutrient foliar application during flush emergence stage.

	Concentration in 6-mo-old spring-flush									
Leaf pretreatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Al
	% dry wt basis					ppm dry wt basis				
	1991									
Wash	2.65 ± 0.03[†]	0.17 ± 0.004	1.64 ± 0.05	3.11 ± 0.06	0.26 ± 0.004	14.2 ± 1.1	45.5 ± 1.9	5.9 ± 0.4	12.0 ± 0.5	90.5 ± 2.0
Rinse	2.70 ± 0.05	0.17 ± 0.004	1.59 ± 0.05	3.36 ± 0.07	0.26 ± 0.004	17.4 ± 1.9	47.9 ± 2.0	6.6 ± 0.4	13.4 ± 0.4	94.2 ± 2.0
T - test	NS[‡]	NS	NS	NS	NS	**[§]	NS	NS	NS	NS
	1992									
Wash	2.53 ± 0.04	0.15 ± 0.002	1.55 ± 0.04	3.06 ± 0.04	0.23 ± 0.004	31.7 ± 1.7	46.5 ± 1.0	10.5 ± 0.6	16.2 ± 0.5	202.3 ± 4.4
Rinse	2.61 ± 0.08	0.15 ± 0.002	1.52 ± 0.05	3.07 ± 0.04	0.23 ± 0.004	92.1 ± 2.5	46.3 ± 1.0	10.3 ± 0.6	17.7 ± 0.5	209.3 ± 4.5
T - test	NS	NS	NS	NS	NS	**	NS	NS	NS	NS

[†]Mean ± standard error (n = 5).
[‡]Non-significant.
[§]Significant at P = 0.01.

The Cu concentration was significantly lower in the washed as compared to the rinsed leaves in both years. This is an indication of the leaf surface residue of Cu from fungicide sprays done during various times after the micro-nutrients spray prior to the leaves being sampled for analysis. The detergent plus acid washing procedure was effective in removing a portion of the Cu residue from the leaf surface as compared to the rapid rinse alone using deionized water.

Conclusions

In commercial citrus grove conditions, foliar application of micro-nutrients (Fe, Mn, and Zn) made during the young flush stage (early spring), showed no evidence of residue carryover on the leaf surface when the leaves were sampled after about 6 mo. Under this condition, a rapid rinse of the leaves with deionized water alone was quite an effective step for surface decontamination to prepare the leaves for both macro- and micro-nutrients analysis.

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