

A COMPARISON OF CITRUS LEAF WASH METHODS FOR REMOVAL OF ZINC NUTRITIONALS SPRAYS

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Abstract. Various methods have been utilized to decontaminate citrus leaves prior to analyzing for nutritional status. Sources of contamination can be dust or dirt particles and chemical sprays which have been applied to the citrus tree. Valencia orange (*Citrus sinensis* L.) trees were sprayed with zinc oxide at 5 lb Zn/acre and basic zinc sulfate at 2.5 and 5 lb Zn/acre rates. After the trees were sprayed, leaves were analyzed 7 and 28 days after treatment (DAT) to compare 6 wash treatments for effective removal of Zn from external leaf surfaces. At 42 days after treatment, one of the 6 hand-wash methods was compared to a jar wash treatment. Detergent plus HCl acid wash or washes containing EDTA were effective in removal of Zn nutritionals. These wash treatments were also most effective in removal of all nutrients contaminating the leaf surfaces. A jar wash was equally effective in removal of surface material as compared to hand washing and is easier and quicker. Leaf absorption of basic Zn sulfate at 2.5 lb Zn/acre or 5 lb Zn/acre was equal to Zn oxide at 5 lb Zn/acre for the 7 and 28 days after treatment. However, by 42 days after spraying the leaves, the Zn oxide treatment was superior for Zn absorption. Large amounts of rainfall and the almost complete solubility of basic Zn sulfate appears to have aided in washing the compound off the leaves. This was likely the cause for the lower absorption of Zn from this nutritional source than was expected.

Leaf analysis for various elements has been widely used to determine the nutritional status of citrus since the 1950's (Embleton et al., 1973; Reitz and Long, 1952; Reuther and Smith, 1954; Smith et al., 1949; Smith, 1966). Leaf analysis has given citrus growers the opportunity to evaluate their nutritional program by comparing the results to established standards which have been developed by research studies over the past 40 years.

Leaf nutritional standards have been established for: (1) primary nutrients of N, P and K, and (2) micronutrients including Cu, Mn, B and Zn. Other elements including P, Ca, Cu and Mg can be tested for in the leaves or soil. Elements in the leaves that are reported as either percent or g/kg are N, P, K, Ca, Mg, and S. Elements expressed in parts per million (ppm) or mg/kg are Mn, Zn, Cu, Fe, B, Cl, and Mo (Obreza et al., 1992). For test results to be meaningful, growers must follow established methods for leaf collection, decontamination, and analysis (Hanlon et al., 1995; Jones et al., 1991; Obreza et al., 1992).

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Recommendations state that analysis of leaves which have been sprayed with nutritional elements, particularly micronutrients, must be thoroughly washed with detergent solution and rinsed in distilled water prior to drying (Smith, 1966). It has been previously reported that leaves recently sprayed with Cu, Zn, or Mn should not be analyzed for these elements, even if washed, as it is impossible to eliminate interference from surface contamination (Hanlon et al., 1995; Smith, 1966; Obreza et al., 1992).

The objectives of this study were:

- 1) To determine the most effective wash method to be used for the removal of Zn residue prior to analysis.
- 2) To evaluate the Zn concentration in leaves which were sprayed with either Zn oxide or basic Zn sulfate.
- 3) At 42 days after treatment (DAT) the wash method which provided the best comparison to the control was selected and compared to a jar wash method to determine the effectiveness of this jar wash decontamination method.

Materials and Methods

Twenty potted Valencia citrus (*Citrus sinensis* L.) trees on sour orange (*Citrus aurantium* L.) rootstocks were obtained from a commercial citrus nursery just prior to the beginning of the experiment. The trees had been under a commercial citrus nursery production program for 3 yr, were well maintained, grown in 10-gal containers and were approximately 5 to 6 ft tall. The potted trees were placed into a randomized complete block design experiment consisting of four trees of equal size per replication. Trees were watered as needed. The study was conducted at the University of Florida in Gainesville.

Each tree was sprayed on 8 Sep. 1994 with 500 ml of deionized water containing either 0.360 g of Zn oxide (5 lb Zn/acre) or 0.262 g basic Zn sulfate (2.5 lb Zn/acre) or 0.524 g of basic Zn sulfate (5 lb Zn/acre). Basic Zn sulfate contained 54.6% Zn and Zn oxide contained 79.54% Zn. Control trees were sprayed with 500 ml of deionized water. An electric, hand held Wagner 120 power paint sprayer was used to spray each tree with all possible effort being taken to assure complete coverage of both the upper and lower sides of leaves. Rates of 5 lb Zn were based upon IFAS Bulletin 536D (Koo et al., 1984) and the current replacement publication for Bulletin 536D which is University of Florida SP169 (Tucker et al., 1995). Treatment trees were assumed to cover 5.5 square ft (2 ft 4 inches × 2 ft 4 inches) or 0.000126 acre.

Trees remained outside during the study period and received the following amounts of rainfall during the study period.

After sprays were applied, 6 samples of 40 leaves each were tagged on each tree. Tags were numbered from 1 to 6 corresponding to the six wash treatments. On each sampling date, 20 leaves were sampled per tree. The 20 leaves collected at the 7-day collection time (15 Sep. 1994) were chosen from the marked twigs. The remaining leaves were selected at the 28-day collection time (6 Oct. 1994).

Wash Treatments

1. Control leaves analyzed without washing.

2. *Hand washing with deionized water.* Leaves were thoroughly washed by rubbing each leaf between the fingers using 500 ml of deionized water. Wash time took approximately 2 to 3 min per 20 leaves. Leaves were rinsed twice in clean deionized water (500 ml each rinse) by rubbing each leaf between the fingers for about 30 sec per each 20-leaf sample.

3. *Hand washing with 0.1% detergent and rinsing with deionized water.* Leaves were washed thoroughly by rubbing each leaf between the fingers using 500 ml of 0.1% Liqui-nox detergent. Washing time took approximately 2 to 3 min per 20 leaves. Leaves were rinsed twice in clean deionized water (500 ml each rinse) by rubbing each leaf between the fingers for about 30 sec per 20-leaf sample.

4. *Hand washing with 0.1% Liqui-nox detergent, rinsing with deionized water, hand washing in 3% by volume HCl and rinsing with deionized water.* Leaves were washed thoroughly by rubbing each leaf between the fingers using 500 ml of 0.1% Liqui-nox detergent. Wash time lasted approximately 2 to 3 min per 20 leaves. Leaves were then rinsed once by rubbing each leaf between the fingers in clean deionized water (500 ml each rinse) for about 30 sec per each 20 leaves. After rinsing, the leaves were then hand washed thoroughly by rubbing each leaf between the fingers using 500 ml of 3% HCl by volume taking about 2 to 3 min per 20 leaves and rinsed using 500 ml of deionized water.

5. *Hand washed with deionized water, twice in ammonium citrate acid/EDTA solution and rinsed once with deionized water.* Leaves were washed thoroughly by rubbing each leaf between the fingers using 500 ml of deionized water, with a wash time taking approximately 2 to 3 min per 20 leaves. Leaves were hand washed thoroughly by rubbing each leaf between the fingers using 250 ml of citrate acid/EDTA solution for about 2 to 3 min followed by a second wash using 250 ml of citrate acid/EDTA solution for 30 sec and rinsed with 500 ml of deionized water.

6. *Hand washed with 0.1% Liqui-nox detergent, deionized water, twice in ammonium citrate acid/EDTA solution and then once with deionized water.* Leaves were washed thoroughly by rubbing each leaf between the fingers using 500 ml of 0.1% Liqui-nox detergent for 2 to 3 min per 20 leaves and washed in deionized water for 2 to 3 min. Leaves were hand washed thoroughly by rubbing each leaf between the fingers using 250 ml of citrate acid/EDTA solution for 2 to 3 min followed by washing a second time using 250 ml of citrate acid/EDTA solution for 30 sec and rinsed using 500 ml of deionized water.

7. *Jar wash method.* Jar wash method was done (42 days after spray treatment on 20 Oct. 1994) by placing the leaf sample in a wide mouth screw top jar which contained 1 liter of 3% HCl by volume plus 0.1% Liqui-nox detergent solution. The container was shaken vigorously for 2 min and then flushed with deionized water. Leaves were rinsed twice with deionized water for 2 min each rinse. The jar wash method offers considerable time savings advantage and would be an easier method to use in a commercial setting.

All leaves were washed within 24 hr after collection. If leaves could not be washed within 4 to 6 hr after collection, they were stored in a refrigerator. After washing, leaves were placed in a forced air oven at 70°C for 48 hr.

After drying, the 20-leaf samples were ground in a Wiley mill grinder. The grinder was cleaned between each sample with a brush and vacuum machine to remove any leaf particle prior to the next sample being ground. The ground leaf sample was collected and stored in sterile airtight plastic bags. Each ground leaf sample was redried at 70°C prior to analysis of material for N, P, K, Fe, Zn, Mn, Cu, Mg, and Ca concentrations.

Plant samples weighing 1.0 g were placed into 50 ml Pyrex beakers and ashed in a Thermolyne muffle furnace at 480°C for approximately 6 hr. Cool ash contents were hydrated with approximately 20 ml deionized H₂O, mixed with 2 ml of concentrated HCl and gently heated to dryness on a hot plate. This water/acid treatment was repeated a second time and brought to a vigorous boil on the hotplate. It was then removed and allowed to cool to room temperature. This solution was transferred to a 100 ml volumetric flask and brought to volume with deionized H₂O for a solution strength of 0.1 N HCl. Solutions were analyzed in the IFAS Extension Soil Testing Lab, University of Florida, for P (colorimetry), K (flame emission), and Ca, Mg, Cu, Mn, Fe, and Zn (atomic absorption) concentrations on a Perkin-Elmer Atomic Absorption Spectrophotometer.

Leaf N Analysis

A 0.100 g ground leaf sample and two glass boiling beads were placed in a 100 ml Pyrex test tube under a hood with 3.2 g of prepared catalyst (9:1 K₂SO₄:CuSO₄) and 10 ml concentrated H₂SO₄, mixed, placed into an aluminum digester block and digested at 370°C for 210 min (Gallaher et al., 1975). Tubes were capped with small funnels which allowed gases to escape while preserving refluxing action. Cool, digested solutions were mixed with approximately 25 ml of deionized water and allowed to cool. Solutions were mixed again with deionized water and brought to 75 ml volume, transferred to square Nalgene storage bottles, sealed, mixed, and stored.

Nitrogen was analyzed on a Technicon Autoanalyzer II (manifold, colorimeter) linked to an automatic Technicon Sampler IV, an Alphen Corporation Proportioning Pump III, and strip chart recorder with a standard laboratory control plant sample used as a check with each 39 samples.

Results

Leaves were found to be in the optimum range or higher (Koo et al., 1984; Hanlon et al., 1995) for the elements of Zn, Mn, Cu, Fe, N, P, K, and Mg. The only element found to be in the low range of 1.5 to 2.9% (Koo et al., 1984; Hanlon et al., 1995) was Ca.

Leaf Zinc

As shown in Table 1, the wash treatments used in the study were effective in removing Zn from the surface of leaves when compared to unwashed leaves which received basic Zn sulfate. At 7 and 28 days after treatment, the removal of Zn oxide was improved by using wash methods number 3 (Det. & H₂O), 4 (Det. & H₂O & acid), 5 (H₂O & EDTA), or 6 (H₂O & Det. & EDTA) as compared to the unwashed or washed with H₂O alone. However, wash 4 (Det. & H₂O & acid) and the two washes which contained EDTA (wash 5 & 6) were significantly better. At 42 days after treatment, both wash methods of either rubbing the leaf between the finger (wash method 4) or placing the leaves in a solution inside a jar which was shaken proved equally effective for removal of all Zn products.

During the study period, Zn levels of both products at all rates in the unwashed treated leaves decreased over time. This decrease could be, in part, due to the heavy rains received at the site over the study period. The cumulative rainfall amounts were 0.45, 7.75, and 9.15 inches respectively for 7, 28, and 42 DAT.

Table 1. Citrus leaf Zn concentration from application of Zn nutritionals and wash treatments at three dates after spray applications.

Wash treatment	Zinc nutritionals			Control	Average
	5 lb basic ZnSO ₄	2.5 lb basic ZnSO ₄	5 lb ZnO		
----- ppm -----					
7 days after treatment					
None	98.5 a x	76.3 a xy	251.8 a w	51.0 a y	119.4
Water	55.8 b wx	41.8 b xy	74.0 b w	29.8 a y	50.3
Det. & water	39.5 b wx	37.0 b wx	60.3 bc w	27.8 a x	41.1
Det. & water & acid	35.8 b w	30.0 b w	30.8 d w	26.5 a w	30.7
Water & EDTA	41.3 b w	44.8 b w	34.8 d w	33.0 a w	38.4
Water & Det. & EDTA	40.8 b w	38.0 b w	40.0 cd w	35.5 a w	38.1
Average	51.9	44.6	81.9	33.6	
LSD _{a&b} = 25.39					
CV _s = 59.25%					
CV _b = 33.87%					
28 days after treatment					
None	46.3 a x	46.5 a x	102.0 a w	33.3 b x	57.0
Water	44.3 ab x	44.3 a x	72.0 b w	27.0 b y	46.9
Det. & water	33.5 abc wx	32.8 a x	49.5 c w	49.5 a w	33.8
Det. & water & acid	25.8 c x	16.0 b x	42.8 c w	21.3 b x	26.4
Water & EDTA	29.8 bc x	35.3 a wx	49.3 c w	26.8 b x	35.3
Water & Det. & EDTA	38.0 abc w	35.0 a w	49.8 c w	49.8 a w	37.1
Average	36.3	35.0	60.9	25.5	
LSD _{a&b} = 16.15					
CV _s = 54.42%					
CV _b = 28.99%					
42 days after treatment					
None	46.8 a x	35.3 a xy	95.5 a w	29.3 a y	51.7
Det. & water & acid (H)	35.8 a x	30.3 a x	53.3 b w	26.3 a x	36.4
Det. & water & acid (J)	32.8 a x	28.0 a x	53.5 b w	29.0 a x	35.8
Average	38.4	31.2	67.4	28.2	
LSD _{a&b} = 14.88					
CV _s = 52.45%					
CV _b = 24.70%					

Water = deionized; Det. = detergent; Acid = HCl; EDTA = Ammonium citrate acid/EDTA solution; H = hand washed; J = jug washed; Values (a,b,c,d,) in columns within a sampling date among wash treatments not followed by the same letter are significantly different according to LSD at the 0.05 level of probability. Values in rows within a sampling date among Zn nutritionals not followed by the same letter (w,x,y) are significantly different according to LSD at the 0.05 level of probability. CV_s = among Zn nutritionals. CV_b = among wash treatments.

Records of Events

Days from spray	Date	Rainfall (inches)	Treatment and sampling
0-7	9/8		Trees sprayed
	9/11	0.45	
	9/14	0.40	7-day sampling
	9/15	0.30	
7-28	9/16	2.50	28-day sampling
	9/20	1.50	
	9/25	0.25	
	10/1	1.00	
	10/3	1.50	
	10/4	0.25	
	10/6		
28-42	10/9	0.50	42-day sampling
	10/12	0.90	
	10/20		

The wash treatments were effective in removing basic Zn sulfate from the surface of the leaf. For Zn oxide, wash treatments including acid or EDTA were the best at removing Zn oxide from the leaf surface. This data supports the recommendation by Smith (1966) that analysis of leaves which had been sprayed with nutritional elements must be thoroughly washed. However, our data suggests that leaves need to be washed with detergent and a solution of 3% HCl by volume for maximum removal of leaf surface nutrients.

The suggestion that leaves that have been sprayed with Cu, Zn, or Mn should not be analyzed for these elements even if washed (Koo et al., 1984; Obreza et al., 1992) is questionable in light of our data. Our data suggests that the detergent plus HCl acid wash appeared to be very effective in removing Zn nutritionals applied to the foliage when compared to control treatments. Our data also showed that washes containing EDTA were as effective as the acid wash in removal of leaf surface Zn and other plant nutrients.

When comparing basic Zn sulfate and Zn oxide as sources of Zn for nutritional sprays, no consistent differences occurred for the majority of the washes at either 7 or 28 DAT. By the 42 DAT time period, Zn oxide was at significantly higher levels than the Zn sulfate. Citrus trees used in this study had high levels of Zn, Mn, Cu, and Fe nutrients on the surface of the leaves prior to application of Zn nutritionals due to the nursery spray program.

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EFFECTS OF APPLICATION TIME AND SPRAY VOLUME ON DEPOSITION

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Abstract. The objective of the research was to characterize the influence of weather parameters on spray application and determine the effects and interactions of the application time and spray volume on deposition. Spray applications were made in August, November, and February to plots of Dancy tangerine trees. In each month, the trees were sprayed at 6 nominal times (2, 6, and 10 a.m. and 2, 6, and 10 p.m.), 3 volume rates (470, 1890, and 4700 L/ha (50, 200, and 500 gpa)), and 4 replications. Spray mixtures contained a manganese tracer and spray deposition was sampled at 3 heights (1.2, 2.4, and 3.6 m (4, 8, and 12 ft)) and six radial locations at each height. Deposition was

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Trade and company names are for providing specific information only. Their mention does not imply an endorsement by the University of Florida.

- Embleton, T. W., W. W. Jones, C. K. Labanauskas and W. Reuther. 1973. Leaf analysis as a diagnostic tool and a guide to fertilization. p. 183-210. *In* W. Reuther (ed.), *The Citrus Industry Volume III*. Univ. Calif., Berkeley.
- Gallaher, R. N., C. O. Weldon and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. *Soil Sci. Soc. Amer. Proc.* 39:803-806.
- Hanlon, E. A., T. A. Obreza, and A. K. Alva. 1995. Tissue and Soil Analysis. *In Nutrition of Florida Citrus Trees*, Ed. D. P. H. Tucker, A. K. Alva, L. K. Jackson, T. A. Wheaton. Univ. of Fla., Fla. Coop. Ext. Serv., SP 169, p. 13-16.
- Jones Jr., J. B., B. Wolf and H. A. Mills. 1991. *Plant Analysis Handbook*. Micro-Macro Publishing, Inc., Athens, GA.
- Koo, R. C. J., J. I. Stewart, C. A. Anderson, D. V. Calvert and H. K. Wutscher. 1984. Recommended fertilizers and nutritional sprays for citrus. *In* R. C. J. Koo (ed.), *Fla. Agric. Exp. Stn. Bul.* 536D. 33 p.
- Obreza, T. A., A. K. Alva, E. A. Hanlon and R. E. Rouse. 1992. Citrus grove leaf-tissue and soil testing: sampling, analysis, and interpretation. *Fla. Agric. Exp. Stn. Fact Sheet* SL115. 4 p.
- Reitz, H. J. and W. T. Long. 1952. Mineral composition of citrus leaves from the Indian River area of Florida. *Proc. Fla. State Hort. Soc.* 65:32-38.
- Reuther, W. and P. F. Smith. 1954. Leaf analysis of citrus. Chapter 7. *In* N. F. Childers (ed.), *Mineral Nutrition of Fruit Crops*. Horticultural Publications, Rutgers Univ., New Brunswick, NJ, Somerset Press, Somerville, NJ.
- Smith, P. F., W. Reuther and A. W. Specht. 1949. The influence of rootstock on the mineral composition of Valencia orange leaves. *Plant Physiol.* 24(3):455-461.
- Smith, P. F. 1966. Leaf analysis of citrus. Chapters 7 & 8. *In* N. F. Childers (ed.), *Fruit Nutrition*. Horticultural Publications, Rutgers Univ., New Brunswick, NJ, Somerset Press, Somerville, NJ.
- Tucker, D. P. H., A. K. Alva, L. K. Jackson and T. A. Wheaton. 1995. *Nutrition of Florida Citrus Trees*. University of Florida SP169.
- Smith, P. F. 1966. Leaf analysis of citrus. Chapters 7 & 8. *In* N. F. Childers (ed.), *Fruit Nutrition*. Horticultural Publications, Rutgers Univ., New Brunswick, NJ, Somerset Press, Somerville, NJ.
- Tucker, D. P. H., A. K. Alva, L. K. Jackson and T. A. Wheaton. 1995. *Nutrition of Florida Citrus Trees*. University of Florida SP169.

determined by colorimetry. Weather data including temperature, relative humidity, wind velocity, and wind direction were collected during the applications and leaf surface wetness was characterized by visual judgement.

Application time and spray volume had significant effects on spray deposition. Overall, night-time applications (lower temperatures and higher humidities), made under dry leaf conditions, had higher deposition than day-time applications. However, wet leaf conditions could reduce deposition by increasing runoff from the leaves.

Spray application is a complex process and can be influenced by many variables. The magnitude and uniformity of spray deposition depend on the canopy geometry (Hall et al., 1991), pesticide properties (Sundaram and Sundaram, 1987), spray equipment design (Furness and Pinczewski, 1985), application parameters (Randall, 1971), and weather conditions (Threadgill and Smith, 1975).

Droplet size spectrum is an important factor in spray application and can affect spray deposition (Salyani, 1988) and drift (Akersson and Yates, 1988). Large droplets tend to bounce off the leaf surface and fall on the ground while small droplets are drift-prone and may move out of the application site. Retention of a certain droplet size on the leaf surface is, in turn, dependent on the physical properties of the pesticide, surface characteristics of the target, and atmospheric conditions. For a particular sprayer design, operating parameters, and droplet size spectrum, the amount of on-target spray deposition and drift can be affected significantly by weather conditions.