Communications in Soil Science and Plant Analysis, 36: 2923–2931, 2005

ISSN 0010-3624 print/1532-2416 online
DOI: 10.1080/00103620500306098

Sample Washing Procedures Influence Mineral Element Concentrations in Zinc-Sprayed Apple Leaves

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Abstract: Widespread use of postbloom zinc (Zn) nutritional sprays in tree fruit and nut orchards can lead to substantial surface contamination of apple leaf samples by Zn spray residue, which complicates diagnosis of tree Zn status and Zn nutritional requirements. Detergent washing alone substantially reduced the Zn, iron (Fe), and aluminum (Al) concentrations of Zn-sprayed Golden Delicious apple (*Malus domestica* Borkh.) leaves compared to unwashed leaves. Adding a 0.1*M* HCl washing step further reduced leaf Zn concentration, but had no additional effect of leaf Fe and Al concentrations. There was evidence for nitrogen (N) and sodium (Na) contamination of the leaf samples, possibly from chemical components of the detergent washing solution. The results indicate that the detergent washing is critical for eliminating Fe or Al contamination introduced by dust or soil adhering to leaves collected from trees grown in dusty environments or impacted by soil splash. Adding the acid wash should improve the estimate of the physiologically meaningful Zn concentration in Zn-sprayed leaf tissue; however, the resulting leaf Zn concentrations may still be biased by a small and possibly variable amount of Zn spray residue.

Keywords: *Malus domestica*, apple, tree fruit, mineral nutrition, foliar spray, micronutrient, zinc, sample preparation

Received 29 November 2004, Accepted 14 July 2005
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INTRODUCTION

Leaf analysis is a well-established procedure to assess the mineral element status of plants and prediction of plant responses (Jones et al., 1991). Proper interpretation of leaf nutrient concentrations requires confidence that the leaf samples are not contaminated with nonphytoactive surface residues containing elements of interest. Iron, Al, and silicon contamination is common on leaves from plants grown in dusty environments or that are low to the ground. Adhering dust or soil often but not always is readily removed by water or detergent washing procedures.

Contamination derived from foliarly applied nutrient sprays is more problematic. Zinc contamination of fruit and nut tree leaves is very common because foliar application of Zn is a widely used and often effective way to supply plant-available Zn, particularly when soils have high Zn-fixing capacity (Childers, 1966). Postharvest and dormant sprays of high rates of Zn traditionally used on deciduous fruit trees often have been found to be ineffective or only marginally effective at enhancing tree Zn status, and have been supplemented or replaced by postbloom sprays of Zn at lower rates and safer formulations (Hoffman and Samish, 1966; Orphanos, 1975; Neilsen and Hogue, 1983; Sanchez and Righetti, 2002; Swietlik, 2002). As a result, there is greater likelihood of Zn spray contamination of leaves and increasing need to better address this problem.

Some references conclude that knowledge of Zn concentrations in Zn-sprayed leaves has limited or no utility (Stiles and Reid, 1991; Viveros, 2000; Obreza and Zekri, 2003; Beede, 2004). An alternative approach is to develop procedures that effectively remove any Zn spray residues prior to analysis. Detergent washing of Zn-sprayed leaves substantially reduces measured Zn concentrations compared to unwashed leaves. A further precaution is to incorporate a dilute hydrochloric acid (HCl) wash after the detergent wash. This added step reduced leaf Zn concentrations in some cases (Orphanos, 1975, 1977; Smith et al., 1950; Smith and Storey, 1976; Crowley et al., 1996), but conferred no additional benefit in others (Ashby, 1969; Labanauskas, 1966). The acid wash does not appear to remove Zn inside leaf tissue. In leaves not sprayed with Zn, unwashed, detergent-washed, and detergent + acid-washed leaves exhibited identical Zn concentrations (Smith and Storey, 1976; Crowley et al., 1996; Ashby, 1969).

Ashby (1969) and Smith and Storey (Smith and Storey, 1976, 1979) felt that the detergent + HCl washing procedure was highly efficient at removing surface Zn residues on Zn-treated leaves; however, most authors have concluded that some external residues were likely to remain on or embedded in the washed leaf surfaces (Orphanos, 1975, 1977; Smith et al., 1950; Labanauskas, 1966; Chamel et al., 1982). Portions of radiolabeled (0.2 to 4.2% of total applied) or heavy isotopic Zn (5 to 14% of total applied) placed as individual droplets onto avocado, pistachio, and walnut leaf surfaces were retained by the leaves against removal by detergent followed by HCl washing (Crowley et al., 1996; Zhang and Brown, 1999a, 1999b). Small amounts of the exogenous Zn were detected in tissues not directly contacted by the droplets, indicating that not all of the retained Zn occurred simply as surface residue. The amount of added Zn recalcitrant to detergent + HCl removal increased with residence time of the Zn-containing droplets on the leaf surface in detached leaves, potted seedlings, and field-grown trees (Zhang and Brown, 1999a, 1999b). This amount began to decline in the field-grown pistachio trees 4 days after application, which was interpreted as Zn export out of the treated leaves. Twenty-eight to 48% of the exogenous leaf Zn moved out of the leaves by 20 days after application. These results confirm that some of the foliarly applied Zn not removed by detergent + HCl-washing resides within the plant tissue and suggests that Zn in residues adhering to leaf surfaces may continue to be absorbed into the tissue as the growing season progresses.

Crowley et al. (1996) pointed out that an intrinsic problem is how to quantitatively partition leaf Zn recalcitrant to detergent + acid washing into residual and physiologically active components. In spite of this limitation, they and numerous other authors recommended or used the Zn concentration of detergent + acid-washed leaves to diagnose Zn nutritional requirements (Smith and Storey, 1976; Herrera, 2000) or to quantify the relative effects of Zn spray products or spray adjuvants on leaf Zn status (Hoffman and Samish, 1966; Orphanos, 1975, 1977, 1982; Crowley et al., 1996; Smith and Storey, 1979; Labanauskas and Puffer, 1964).

Although the detergent + acid-washing procedure appears to be an imperfect solution, it should give a more meaningful assessment of the physiologically active Zn concentration in Zn-sprayed leaf tissue than does detergent washing alone. The current study was conducted to determine the influence of detergent and sequential detergent + HCl washing procedures on mineral element concentrations in leaves collected from apple trees grown under field conditions and receiving postbloom Zn sprays.

MATERIALS AND METHODS

The study was conducted in an irrigated Golden Delicious/EMLA.9 apple orchard planted in 1985 in Wenatchee, Washington, USA. The natural environment is semi-arid big sagebrush (*Artemisia tridentata*) and bluebunch wheatgrass (*Agropyron spicatum*) steppe. Annual precipitation averages 265 mm. Average January and July temperatures are −1.7 and 22°C, respectively. The soil is classified as a Burch loam (coarse-loamy, mixed, superactive, mesic Aridic Haploxerolls). The trees were planted at a 1.2-m × 3.35-m spacing (2244 trees per ha) and were supported by a metal conduit-wire trellis system. The trees were irrigated using a permanent undertree high-pressure/high-volume sprinkler system. Herbicides were used to maintain a weed-free strip within the tree rows. Chemical and hand-thinning of fruit and control of insect and disease pests were carried out using commercial practice.

Thirty-nine experimental plots of single Golden Delicious apple trees were identified in four adjacent tree rows. The plots are physically separated within-row by five guard-trees, while experimental trees in adjacent rows are offset by three guard-trees. Thirteen treatments (12 Zn spray products plus a water-sprayed control) were imposed on the 39 plots using a randomized complete block design with three replications.

In 2000, solutions or suspensions of the 12 Zn-containing spray products were prepared in polyethylene containers, with individual containers prepared for each experimental plot. Each container held 0.25 g Zn and 1.2 L tap water (average amount established empirically on guard trees as that required to bring the single tree of each experimental plot to drip). The entire content of each container was applied to each plot using a portable hand-pump sprayer. The effective Zn rate was 0.56 kg/ha per spray. The sprays were applied on 23 May and again on 16 June 2000.

Plant Tissue Analyses

On 8 Aug. 2000, 30 leaf samples were sampled randomly from each experimental tree in each experimental plot, selecting mid-shoot leaves on the current season's growth. The 30-leaf sample was split randomly into three subsamples of 10 leaves each (Groups A, B, C). Group A (unwashed) leaves were oven-dried at 65°C without washing. Group B (detergent-washed) leaves were individually washed by hand using sequential detergent wash (8 L of 0.5% detergent), tap water rinses (immersed in 8 L tap water followed by flowing tap water rinse), and a final flowing deionized water rinse, and were oven-dried at 65°C. Group C (detergent plus acid wash) leaves were individually washed by hand using the sequential 0.5% detergent wash and tap water rinses as in Group B, followed by washing in 8 L of 0.1 *M* HCl in a container, then sequential rinses with flowing tap water and flowing deionized water, and were oven-dried at 65°C. Both the adaxial and abaxial surfaces of the Group B and C leaves were gently rubbed by the operator's gloved fingers in the detergent and acid baths during the cleaning process. The detergent + acid wash cleaning procedure is similar to that used for pecan leaves by Smith and Storey (1976) except that Liquinox was used in place of Alconox (both Alconox, Inc., White Plains, NY) to avoid phosphorus contamination. All dried plant tissue samples were ground in a stainless steel Wiley mill and analyzed for N, P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Na concentrations. Plant N was determined by total Kjeldahl digest and flow injection colorimetry, and the other mineral elements by wet-digestion, followed by assay using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Plant tissue analytical data are reported on a dry mass basis.

Statistical Analyses

The leaf analysis data for the Zn-sprayed trees were pooled and analyzed by paired t-test by plot (Steel and Torrie, 1980). The data for the water-sprayed control treatment were analyzed separately by paired t-test to avoid the confounding effect of Zn spray residues. Statistical analyses were carried out using the computer program SAS for Windows, Release 8.0 TS Level 00M0 (SAS Institute, Inc., Cary, NC). Statistical significance was defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the mineral element concentrations measured in the unwashed Zn-sprayed leaves, the absolute changes in concentrations caused by the differing sample washing procedures, and the associated significance level of the paired t-test. The observed concentrations were consistent with values considered satisfactory for apple fruit production, with the exceptions of Cu, which was marginally low, and Zn, which was very high (Jones et al., 1991; Bould, 1966). No visual symptoms characteristic of nutrient deficiency or excess were present on the trees at any time. The Zn concentration of the unwashed leaves from the water-sprayed control treatment averaged 19.5 mg kg^{-1}, which would be considered marginally low. Mineral element concentrations other than Zn in the water-sprayed control leaves were consistent with those of the Zn-sprayed leaves.

Detergent and detergent + acid washing substantially reduced leaf Zn, Fe, and Al concentrations relative to the unwashed leaves (Table 1). The loss of Fe and Al is consistent with the removal of Fe-containing aluminosilicate dusts coating the leaf surfaces (Jones et al., 1991). Adding the HCl rinse did not further enhance Fe and Al loss, suggesting that the detergent wash alone removed all of the adhering dust. Other authors (Orphanos, 1977; Smith and Storey, 1976; Ashby, 1969; Bradfield and Bould, 1963) have reported the same result for fruit and tree nut crop leaves.

In contrast to Fe and Al, the detergent + acid wash removed an additional amount of Zn relative to the detergent wash. Other authors (Orphanos, 1975, 1977; Smith and Storey, 1976; Crowley et al., 1996) also observed this effect for leaves sprayed with Zn during the growing season and concluded that the difference represented loss of surface-adsorbed Zn spray residues recalcitrant to detergent removal alone. Ashby (1969) failed to find an additional benefit of adding an HCl wash to a detergent wash for leaf Zn concentrations of detached apple, cherry and peach leaves that were dipped for 2 sec in a Zn-containing solution and allowed to air-dry for 2 h. Zinc concentrations in the detergent and detergent + acid washed Zn-dipped leaves did increase relative to the unwashed undipped control leaves, which Ashby attributed to Zn uptake by the Zn-dipped leaves and not to adhering surface residues. All of the cited

Table 1. Effect of detergent washing and detergent plus acid washing on mineral nutrient concentrations in leaves sampled from Zn-sprayed apple trees

		Unwashed vs. detergent wash		Unwashed vs. detergent + acid wash		Detergent wash vs. detergent + acid wash	
Element	Mean concentration, unwashed leaves	Gain (+)[a] or loss (−)	Prob.[b]	Gain (+) or loss (−)	Prob.	Gain (+) or loss (−)	Prob.
N	20.3 g kg^{-1}	+0.6	0.0014**	+0.4	0.0439*	−0.3	0.0866
P	1.4 g kg^{-1}	−0.1	0.0577	−0.0	0.0507	+0.0	0.5674
K	15.5 g kg^{-1}	+0.5	0.1787	+0.7	0.0458*	+0.2	0.4169
Ca	17.0 g kg^{-1}	−0.2	0.7469	−0.5	0.2896	−0.3	0.4581
Mg	3.9 g kg^{-1}	+0.0	0.8890	−0.0	0.6255	−0.0	0.4986
S	1.8 g kg^{-1}	−0.1	0.0125*	−0.1	0.0146*	+0.0	0.7989
Zn	161.5 mg kg^{-1}	−62.1	< 0.0001***	−75.5	< 0.0001***	−13.4	0.0024**
B	35.7 mg kg^{-1}	+1.9	0.0599	+1.9	0.0748	+0.0	0.9850
Mn	73.8 mg kg^{-1}	−2.6	0.2388	−3.5	0.1102	−0.9	0.7980
Cu	5.4 mg kg^{-1}	−0.2	0.2192	−0.1	0.4262	+0.1	0.5483
Fe	129.8 mg kg^{-1}	−61.0	< 0.0001***	−61.5	< 0.0001***	−0.5	0.7980
Al	188.4 mg kg^{-1}	−83.2	< 0.0001***	−77.7	< 0.0001***	+5.5	0.1561
Na	34.4 mg kg^{-1}	+5.6	0.0751	+4.2	0.1479	+4.8	0.2271

[a]Absolute change in elemental concentration of leaf sample due to differential washing procedure. Positive value indicates concentration increase; negative value indicates concentration reduction. Some very low values appear as zero because of rounding.

[b]Level of significance for paired t-test (n = 36 except for Na, for which n = 27, 28, and 34, respectively, for the three comparisons).

*, **, *** indicate effect of washing treatment was significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

authors reported that detergent and detergent + acid washing caused no reduction in Zn concentrations of unsprayed leaves. In the current study, neither washing procedure changed leaf Zn concentrations of the water-sprayed control treatment ($P = 0.5034$ for unwashed vs. detergent; $P = 0.9522$ for unwashed vs. detergent + acid).

There were small, but significant, increases in leaf N concentrations for the detergent and detergent + acid washed leaves. There also were substantial but not significant increases in leaf Na concentrations in the washed leaf samples (the relative analytical error for leaf Na concentration is high because ICP-AES has low sensitivity for Na and the sample leaves contained low Na concentrations). These results raise the possibility that the Liqui-nox, which contains considerable Na, sulfur (S) as sulfonate, and N as amide, may have slightly contaminated the washed leaf samples with N and Na. Increases in leaf N concentration were not observed for detergent or detergent + acid washed leaves from other studies where different detergent sources and a lower detergent concentration (ca. 0.1%) were used (Smith and Storey, 1976; Ashby, 1969; Bradfield and Bould, 1963). Leaf S concentration in the current study decreased slightly ($-0.1\ \mathrm{g\ kg^{-1}}$), but significantly with washing; however, the analytical sensitivity for the S analysis is $\pm 0.1\ \mathrm{g\,kg^{-1}}$, so the significant t-test results for S likely are not meaningful.

With one exception, the washing treatments had no effect on the leaf concentrations of any other measured elements. The exception was an increase in leaf potassium (K) concentration for the detergent + acid wash. While this likely is a random statistical artifact, Smith and Storey (1976) also found that their washing procedures sometimes caused increases in leaf K concentration. Other authors (Orphanos, 1977; Ashby, 1969; Worley, 1993) did not find this effect. Soaking of leaves in 1 *N* HCl for 10 to 20 min, as opposed to brief rinsing, sometimes caused loss of K, magnesium, manganese, and additional Zn from Zn-sprayed apple leaves (Orphanos, 1977).

The washing procedures introduced relative deviations of less than 5% from the mean concentrations of the macro- and secondary plant nutrients in the unwashed leaves. In contrast, the detergent wash removed 47% and 44% of the Fe and Al, respectively, a congruence that would be consistent with dust removal. Detergent and detergent + acid washing, respectively, removed 38% and 47% of the Zn. These results emphasize the value of detergent washing of plant tissue samples collected from environments in which they may have become contaminated with dust or soil. They also suggest that incorporating an acid wash aids in removing additional Zn residues from plant tissue surfaces.

While its efficiency may be imperfect and additional validation work is warranted, use of the detergent + acid washing procedure does appear to provide a more accurate assessment of the physiologically meaningful Zn concentration in Zn-sprayed leaves than does detergent washing alone.

ACKNOWLEDGMENTS

This research was conducted under Project WNP0747, College of Agricultural, Human, and Natural Resource Sciences, Washington State Univ., Pullman. Funding support was provided by the College and by the Washington Tree Fruit Research Commission. I thank Jennifer Moore, and Casimir Lorentz for their technical support.

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