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TRACE ELEMENTS ANALYSIS

Preparation of Plant Tissues for Micronutrient Analysis. Removal of Dust and Spray Contaminants

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Procedures for removal of contamination from dust and spray residues from plant tissues prior to analysis for micronutrients are described. Three herbaceous plants—rye, tomato, and sugar beet—were dusted with soil or sprayed with a simulated Bordeaux spray containing copper, iron, manganese, molybdenum, and zinc. Effectiveness of removal of contamination by dilute hydrochloric acid or dilute hydrochloric acid plus detergent was measured. Dust contamination was removed satisfactorily by both wash solutions. Spray contamination was difficult to remove by washing. The wash solutions and procedures used did not result in loss of elements from within the tissues.

REPORTS on the need for removal of surface contamination from plant tissue prior to determination of micronutrients have largely been confined to work with iron in tissues such as leaves of citrus (*7*) and pears (*2*). Jacobson (*2*) considered it necessary to wash corn and tobacco leaves with dilute acid prior to iron determination, and Jacobson and Oertli (*3*) washed sunflower leaves with 2% Dreft detergent solution for the same purpose. Nicholas, Lloyd-Jones, and Fisher (*6*) studied methods of removing surface contamination from tomato tops and showed that immersion of fresh tissue in a detergent (0.37% Teepol) or in detergent and then 0.25*N* hydrochloric acid, followed by distilled water rinsing, removed iron contaminant from leaf surfaces without causing loss of potassium, phosphorus, manganese, or internal iron (tagged with iron -59).

This paper describes studies designed to obtain additional information, for three herbaceous species, on the efficiency of procedures for removing contamination with manganese, copper, zinc, and molybdenum as well as iron. This information is of consequence because of increasing recognition of the need for reliable chemical analyses in order to assess potential micronutrient deficiencies in annual crops where the analytical hazard from soil and spray residue contamination is large.

Because of the nature of the foliar sample—usually small and easily bruised leaves—it did not appear that procedures recommended for cleaning surfaces of tree leaves would be generally applicable. Smith, Reuther, and Specht (*7*) and others have recommended either scrubbing citrus leaves with a fiber-bristled hand brush or wiping with moistened clean cloth, and Jacobsen (*2*) rubbed pear leaves with the fingers in 0.3*N* hydrochloric acid. These procedures would be time-consuming when applied to tissues such as grasses or tomato leaves and also subject to the risk of damaging the tissue and permitting leakage of ions from the interior. Therefore, the procedure of Nicholas *et al.* (*6*), of simply immersing and agitating the samples in the washing and rinsing solutions, was studied to determine whether surface contamination could be removed from the species under consideration.

The question of whether elements are leached from within plant materials is usually raised when decontamination by washing is suggested. In a recent review of foliar leaching, Tukey, Tukey, and Wittwer (*8*) presented data which show that the continuous leaching of plant leaves with distilled water for periods up to 24 hours results in the loss of appreciable quantities of some absorbed nutrients. However, their data show that the leaching losses are a direct function of time, and that losses to be expected even in 2 hours' leaching from detached leaves appear to be 5% or less, depending upon the element and plant species studied. Their results therefore support the conclusions of Nicholas *et al.* (*6*) and of the authors (as will be shown) that there is negligible hazard of leaching losses during washing procedures of short duration (2 minutes in this study).

Materials and Methods

The three species used—sugar beet, rye, and tomato—represent a variety of leaf types. The plants were cultured on nutrient solutions according to the procedure of Johnson *et al.* (*4*) in a relatively dust-free greenhouse (filtered ventilating air and concrete floor), and were about 2 months old when contaminated with dust or spray. Six 18-liter containers of nutrient solutions were provided for each species. For tomato and sugar beet, six plants were grown in each container; for rye there were 18 plants per container.

Within each species, plants were randomized among containers just before application of contaminants. This precaution to minimize the effects of possible variation between culture solutions was perhaps unnecessary, as the media were carefully made up and growth for a given species was visibly uniform.

Contamination Treatments

The plants of two containers of each species were retained uncontaminated as controls. Those of two other containers were sprayed with a Bordeaux-type spray containing 1 gram per liter

of copper, zinc, manganese, iron, and molybdenum, prepared by dissolving ammonium molybdate in water together with ferrous, manganous, and zinc sulfates, adding an amount of commercial Bordeaux dry mixture (Bordox) equivalent to 1 gram of copper, and finally neutralizing to pH 7 with calcium carbonate.

The spray used was about one third as concentrated (0.1% w./v. of each element) as commercial Bordeaux sprays (approximately 0.3% copper, w./v.). The application was made to the runoff point. Therefore the degree of contamination is approximately that which might be encountered in agricultural practice. The composition of this spray material is unusual; the purpose of preparing such a contaminant was to provide a severe test of the washing procedures.

Plants in the remaining two containers were lightly sprayed with a fine mist of water and then dusted with fine peat soil while moist. The soil contained 16,300 p.p.m. of iron, 260 p.p.m. of manganese, 53 p.p.m. of copper, 45 p.p.m. of zinc, and 3.5 p.p.m. of molybdenum. The apparent contamination from iron indicated that the amount of the soil adhering to the plant tissue was in the order of 4 to 10 mg. per gram of dry tissue.

Harvesting and Washing

Each container was harvested separately, thus providing duplication of each contamination treatment. All plants were cut off 1 inch above the root crown. Blades of the tomato plants were separated from petioles and used as sample material, the remainder of the plant being discarded. With sugar beet, both blades and petiole were sampled and processed separately. All the blade material of the rye was used. Fresh weights of samples were from 60 to 120 grams, depending on species. Of the plants in each container for each species, one third were harvested and dried for analysis without washing (treatment 1 of Figure 1); one third were harvested, washed in 0.1N hydrochloric acid, rinsed twice with distilled water and finally with borosilicate glass–redistilled water (treatment 2), then dried for analysis. The remaining plants in each pot were harvested, washed in a solution of 10 ml. of liquid detergent in 3 liters of 0.1N hydrochloric acid, and rinsed twice with distilled water and finally with borosilicate glass–redistilled water, (treatment 3), before being dried for analysis. The samples were agitated in 3 liters of wash solution for 30 seconds. Rinses with distilled water were also of 30-second duration. Washing times of 20 to 90 seconds have been shown by Nicholas *et al.* (*6*) to be adequate, with no loss of nutrients at the longer time.

Samples were drained on aluminum screen trays and dried at 75° C. for 16 hours.

Blades of rye were cut into small pieces with new, clean, stainless steel scissors prior to ashing. Blades of sugar beets and tomato were hand crushed in kraft bags prior to sampling or ashing.

Analytical Methods

Iron was determined by the *o*-phenanthroline method, manganese by periodate oxidation, zinc by the Zincon method, copper by the diethyldithiocarbamate method, and molybdenum by the thiocyanate method (*5*).

Five-gram (dry weight) samples were taken for wet ashing, with nitric and perchloric acid, the ash solution was made to 250 ml., and suitable aliquots were taken for determination of the elements.

Results and Discussion

Mean values for analytical results are presented graphically in Figure 1. The analytical values were subjected to statistical analysis after logarithmic transformation. In the following discussion statements of differences in apparent elemental concentration resulting from contamination and washing treatments are based on evidence of statistical significance at the 5% probability level.

Dust Contamination Removal

Contamination of plant samples by soil dust, at least in the case of the peat dust used here, resulted in large increases in apparent concentrations of iron in unwashed samples in all cases. Washing with either 0.1N hydrochloric acid or detergent in 0.1N acid reduced the apparent iron content of the samples to that of the uncontaminated samples. Washing of samples of foliage from these species with either acid alone or acidified detergent solution did not remove iron initially present within the tissues as shown by comparison of treatments 1, 2, and 3 for the initial (uncontaminated) samples.

Copper contamination was significant only with dusted and unwashed sugar beet blades. Both washing treatments were effective in reducing the copper concentration to their initial values—compare treatments 2 and 3 of the dusted group with treatments 1, 2, and 3 of the initial group.

Within the limits of biological and analytical variation the dust did not lead to a significant contamination of any of the tissues with manganese, molybdenum, or zinc. Analytical values for any of these elements after either washing technique were not significantly different from those of the initial (uncontaminated) samples.

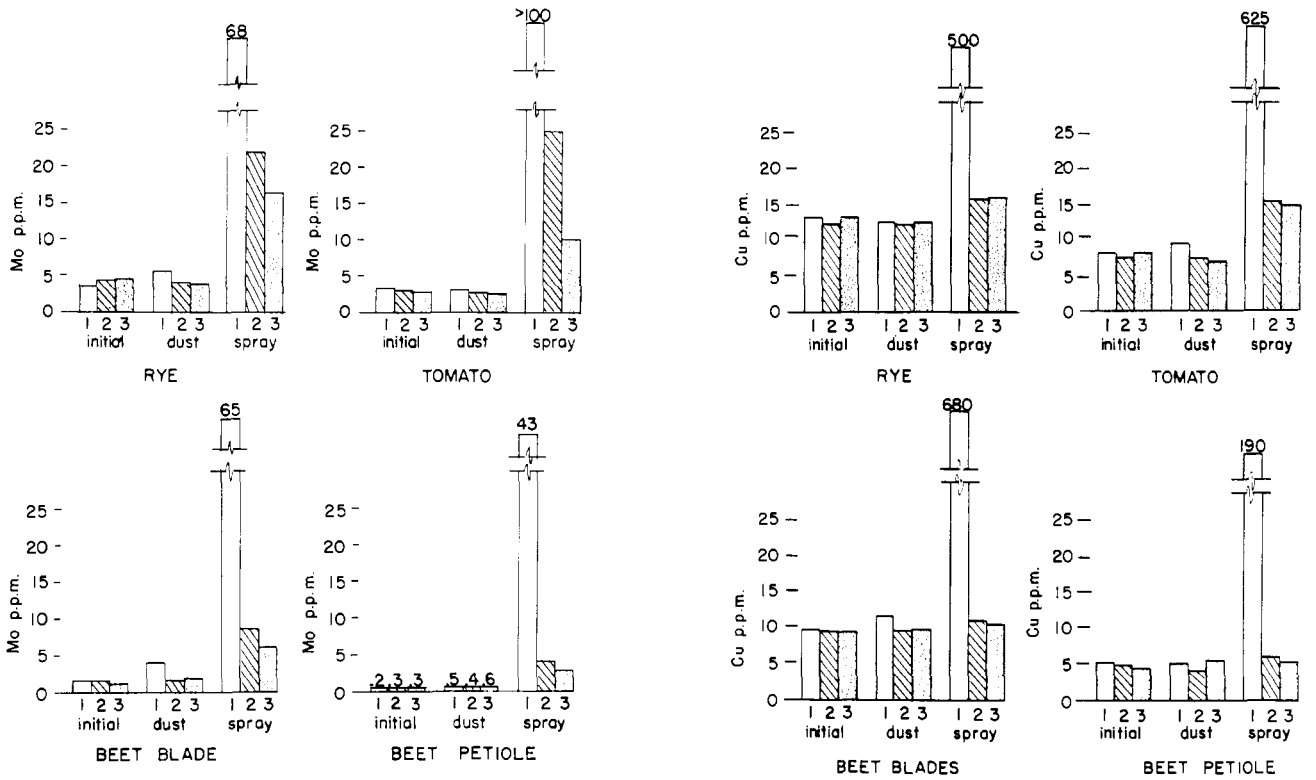
Removal of Spray Contamination

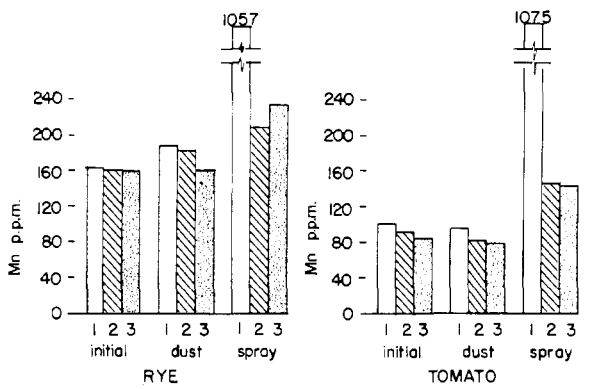
In normal practice samples would be avoided which were obviously contaminated by spray material. However, in some instances local spraying history might be unknown, or it might be necessary to attempt investigation of the effectiveness of spray application of increasing the micronutrient content in plants. Thus it was of interest to investigate the usefulness of the washing procedures in removing spray residues. The concentrations of the elements remaining on the sprayed and unwashed foliage were large, ranging from 43 p.p.m. for molybdenum to over 1000 p.p.m. for manganese. Evidently the various elements were distributed differently between solid and liquid phases, and hence between spray and sediment in the sprayer, and between runoff and residue on the plant.

Estimation of the extent of removal of surface contamination from spray residues is complicated by the probability that some of the applied compounds are actually absorbed by the tissues and thus not amenable to removal by washing. However, in many instances the washing procedures were remarkably effective in reducing the apparent micronutrient concentrations to levels near that of the initial (uncontaminated) samples as is shown in Figure 1. The extent of the removal depends on the particular element and the nature of the foliar sample.

Iron, copper, and zinc contaminants from spray applications were removed effectively by the washing procedures from sugar beet blade and sugar beet petiole. Tomato blades and rye, probably because of their hairy or rough surfaces, appear to retain all elements from the spray in rather large amounts despite washing. There is the possibility that a portion of the applied spray is actually absorbed by the plant. It is difficult to assess the distribution between surface and interior content of the elements from the data of this experiment.

The apparent concentrations of molybdenum and manganese in the sprayed samples were reduced by both washing procedures as compared with the concentrations in the unwashed samples. However, except in sugar beet blades, the apparent concentration of manganese remaining after washing was considerably higher than that in the uncontaminated foliage samples. Much of the molybdenum from the spray application was removed by each of the washing treatments, but the final apparent molybdenum concentration in all tissues after washing remained severalfold higher than that in the uncontaminated material. Effectiveness of foliar application of molybdenum in supplying molybdenum-deficient plants has been





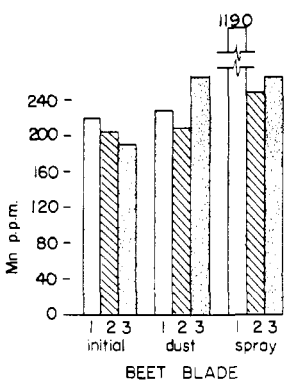


Figure 1. Efficiency of washing procedures in removing copper, iron, manganese, molybdenum, and zinc from contaminated foliage samples

Initial. Foliage samples taken from relatively dust-free greenhouse
Dust. Similar samples contaminated by dusting moistened foliage samples with peat soil
Spray. Similar samples sprayed with a simulated Bordeaux spray containing copper, iron, manganese, molybdenum, and zinc
Washing treatment numbers
1. No washing
2. Agitated 30 seconds with 0.1*N* HCl, washed twice with distilled water, and once with borosilicate glass–redistilled water
3. Agitated 30 seconds with 0.1*N* HCl and detergent, rinsed twice with distilled water, and once with borosilicate glass–redistilled water

observed for many years. Much of the increase in molybdenum concentration in the sprayed and washed tissues probably can be explained on the basis of rapid absorption of the element.

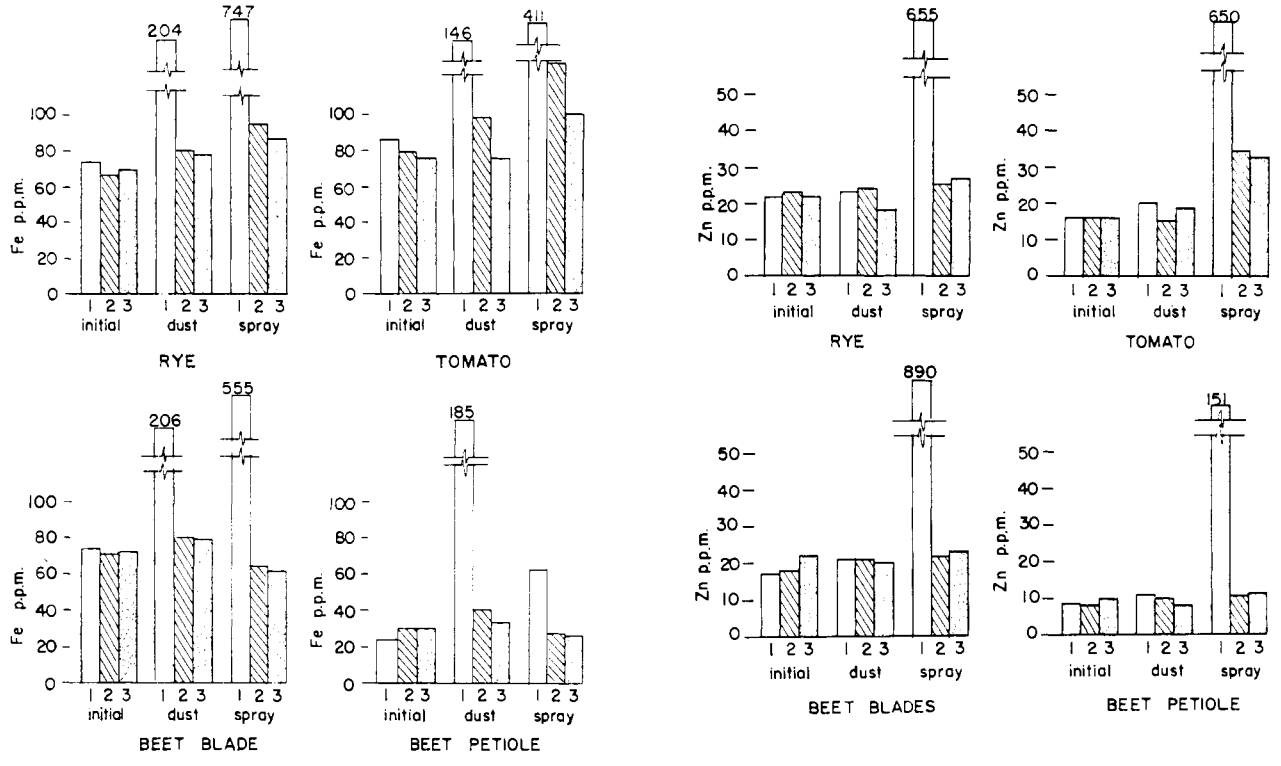
Summary

Contamination from aqueous sprays was less effectively removed by either of the wash solutions than was dust contamination. Therefore analyses of tissue known to have been contaminated by sprays should be suspect even after washing.

With the soil used in this experiment, iron contamination was the only one of statistical significance and this was removed by either washing technique. However, soils may vary widely in their micronutrient content (*1*) and errors in the apparent concentration of any or all of the elements arising from dust contamination must be recognized as a possibility. These experiments show that internal concentration of none of the five elements is reduced by the washing procedures when applied to diverse tissues. This conclusion is supported by the work of Nicholas *et al.* (*6*), and is not discordant with the data of Tukey *et al.* (*8*), since the period of immersion of the plant material in the washing and rinsing solutions was short in contrast to the long leaching studies of Tukey *et al.* (*8*). Therefore, it is recommended that all tissues be washed in preparation for all micronutrient analyses.

These experiments indicated that dilute acid alone was as effective as acid plus detergent in removing surface contamination. However, as noted by Jacobson (*2*), the dilute acid would not be expected to dissolve iron oxide or iron silicate. Thus the efficiency of the washing is largely a matter of dispersing dust particles in the washing solution. With field samples where there has been considerable build-up of layers of dust and perhaps spray materials, the use of the combined acid and detergent wash solution should prove superior. Because no leaching of ions occurs with this wash solution, it is recommended for routine use.

Although emphasis has been placed on procedures for minimizing spurious results from surface contamination in developing correlations between analytical values for micronutrient concentrations and foliar symptoms of deficiency, it is not implied that proper washing procedures alone will ensure good correlations. Proper removal of surface contamination is only one re-



quirement for attainment of this goal, others being proper sampling, selection of proper tissue, and adequate analytical methods.

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PLANT COMPOSITION AND VIGOR

Hemicelluloses and Winter Hardiness in Raspberry Canes

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In a general study of winter hardiness in plants, hemicelluloses from winter-hardy and nonwinter-hardy varieties of raspberry canes were isolated and studied to determine a possible relationship. The hemicelluloses were found to be similar in quantitative and qualitative aspects. Xylose was present in by far the greatest amounts. The data on the present basis do not seem to indicate a positive relationship.

VARIETIES OF RASPBERRIES vary in their ability to survive when exposed to extremely low temperatures. The resistance of a given variety is not necessarily constant for the winter season. It may be lost and regained during the same season. The phenomenon of winter hardiness is not generally understood. Excellent reviews on the hardiness of plants have been published by Dexter (*2*) and Levitt (*4*). Hardiness has been related to many plant substances, including hemicelluloses. Although these substances are sufficiently hydrophilic to play an important role in the water relationships of the plant, published reports vary as to the importance of their role in winter hardiness (*1, 5, 8*). To investigate further the possibilities of an indirect relationship, a chemical investigation has been conducted on the hemicelluloses of hardy (Latham) and tender (Milton) varieties of raspberry canes.

Material and Methods

Representative samples of both Latham and Milton canes were collected approximately every two weeks from November through February, dried in a forced draft oven at 75° C., and composited later. Approximately 500 grams of each were extracted successively with a