Distribution and Transport of Foliar Applied Zinc in Pistachio

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ABSTRACT. The distribution and transport of foliar applied Zn were determined for pistachio (*Pistachio vera* L.) seedlings and mature trees using stable ^{68}Zn isotope. In seedlings, $\approx 5.4\%$ of Zn adsorbed by the leaf was transported out of the treated leaves and this Zn was detected in all other plant parts to varying extent. In mature trees, the transport of Zn occurred both acropetally and basipetally within the leaflets with more basipetal movement; however, no significant amount of Zn was transported out of the treated leaflets during the first 10 days after application. The total percentage of Zn transported to other plant parts 20 days after application was significantly greater when Zn was applied to immature leaflets (6.5%) than to mature leaflets (2.1%), though the majority of the absorbed Zn remained within the treated leaflets. The limited mobility of foliar-absorbed Zn in pistachio may partially be attributed to the high binding capacity of leaf tissue for Zn.

Foliar supply of nutrients is commonly practiced in fruit trees and vegetable crops, but its effectiveness is not consistent due to limited rates of leaf absorption and translocation. The initial absorption phase involves penetration through the cuticle and epidermal cells, while the subsequent transport of the foliar absorbed nutrients generally occurs via the phloem (Bidulph, 1954; Swanson and Whitney, 1953). The extent of redistribution of the applied nutrient is an important consideration in the use of foliar fertilization to satisfy the nutrient needs of plants. Foliar applied micronutrients generally exhibit a low mobility (Chamel, 1988; Ferrandon and Chamel, 1988) and Zn mobility varies with species (Bukovac and Wittwer, 1957; Loneragan et al., 1976). The effectiveness of foliar sprays of Zn varies greatly among species. Wallihan and Heymann-Herschberg (1956) reported that Zn was readily absorbed by, and transported from, the leaves of citrus, while Wadsworth (1970) found only 0.2% of total applied Zn was absorbed and translocated out of the treated leaves of pecan. Although various treatments including chelating molecules have been used to improve the efficiency of foliar nutrient treatments (Alexander, 1986), the effectiveness of foliar sprays of Zn remains limited in many species.

The purpose of this study was to determine the distribution and the extent of transport of foliar absorbed Zn in 'Kerman' pistachio and to optimize Zn application techniques. Previous studies of foliar Zn uptake and translocation were conducted using radioactive ^{65}Zn as a tracer. In this study we demonstrate that the accuracy and precision of the measurements using inductively coupled plasma-mass spectrometry (ICP-MS) make it possible to utilize stable isotope tracers of Zn in studies of foliar Zn uptake.

Materials and Methods

ZINC STABLE ISOTOPE PREPARATION. An enriched preparation of ^{68}Zn in the form of Zn oxide was obtained from Isotec Inc., Miamisburg, Ohio. The isotopic composition of this preparation was as follows (atom%): ^{64}Zn , 2.95; ^{66}Zn , 1.48; ^{67}Zn , 0.73; ^{68}Zn , 95.1; ^{70}Zn , 0.22. The natural isotopic composition of Zn is as follows (atom%): ^{64}Zn , 48.89; ^{66}Zn , 27.81; ^{67}Zn , 4.11; ^{68}Zn , 18.57; ^{70}Zn , 0.62. A known amount of the enriched preparation was dissolved in a few drops of 1 N acetic acid with shaking, followed by an equivalent

volume of 0.1 N H_2SO_4 . The sample was heated at 75 °C to near dryness and rediluted to the desired volume with double deionized (DDI) water. The pH of the solution was adjusted to 5.3 with 0.5 N KOH.

TRANSLOCATION AND DISTRIBUTION OF FOLIAR ABSORBED ZINC IN PISTACHIO SEEDLINGS. Since previous reports on the transport of foliar-applied Zn were contradictory (Bukovac and Wittwer, 1957; Wadsworth, 1970; Wallinhan and Heymann-Herschberg, 1956), experiments were designed to test if the inconsistency is partly due to the effect of plant or leaf age.

Pistachio seeds were germinated and grown in medium grade vermiculite in shallow flats and fertilized with 1/4 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) twice a week. Irrigation was provided as necessary. Seedlings were allowed to grow in the greenhouse until they had five to six leaves.

To study the distribution of Zn within the plant after foliar application, sixteen seedlings were chosen for uniformity. Two drops, each 40 μL of 7.5 mM ^{68}Zn solutions, were applied onto the adaxial surface of the third leaf from base. Treatment drops were applied to each side of the midvein in the mid section of the leaf. Treatments were replicated four times with 3 individual seedlings per replicate. At 2 and 10 d after application the plants were harvested and separated into treated leaves, leaves above and below the treated leaves, stems, and roots and prepared for Zn isotope analysis as described below.

TRANSLOCATION AND DISTRIBUTION OF FOLIAR ABSORBED ZINC IN MATURE TREES. During May and June, the transport of foliar absorbed Zn was investigated by applying ^{68}Zn onto intact immature and mature leaflets of five individual trees grown in the pomology orchard at the University of California, Davis. One drop of 50 μL ^{68}Zn solution (7.5 mM) was applied to the mid section of the leaflet on each side of the adaxial surface separated by the midvein. Treated leaflets (two leaflets per tree) were collected at 1, 2, 3, 5, 10, and 20 d after application and analyzed for the ^{68}Zn . The transport of Zn from foliar application was estimated by the disappearance of ^{68}Zn from the treated leaflets, which was considered to have been transported to other plant parts.

Longitudinal transport of Zn within the leaflet under field conditions, was evaluated using leaflets treated as above. Leaf blades (five replications with two leaflets per replicate) were collected at 2, 7, and 25 d after application. Each leaflet was separated into three sections (apical, middle and basal). The ^{68}Zn in these three sections was analyzed to determine the localization of foliar absorbed Zn. In another experiment, the lateral transport of foliar-absorbed Zn was

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investigated by applying two 50- μ L drops of ^{68}Zn to one side of the leaflet. Zinc isotope ratios in the leaflet were monitored in this side including the midvein, and on the opposite side 7 d after application.

CHARACTERISTICS OF ZINC BINDING TO LEAF CELLS. It is possible that the reported low translocation of Zn (Wadsworth, 1970) is due to fixation of Zn to the leaf tissue. Hence, two additional experiments were conducted to examine the capacity of leaf cells to bind Zn. A separate set of leaflets were collected from the mature trees treated with ^{68}Zn solution as described above. These leaflets were used to determine the proportion of soluble and insoluble ^{68}Zn in leaf tissue. At 40 h after application, leaflets were cut into small sections and ground in a mortar in 10 mM MES-NaOH buffer, pH 6.0, containing 0.35 M sorbitol; the ground samples were then divided into two equal aliquots and then homogenized by hand at 4 °C, either with or without 0.5 mM CaSO_4 . The homogenate was centrifuged at 500 g_n for 10 min, after which a loose pellet was formed. The pellet was filtered through a double layer of Miracloth (Calbiochem Corporation, La Jolla, Calif.). The residue was resuspended in 50 mL iced water and ultrasonicated (model 450; Branson Ultrasonics Corp., Danbury, Conn.) for 5 min at an output power of 40 W. The homogenate was then centrifuged at 1000 g_n for 10 min and refiltered through a double layer of Miracloth and washed with 50 mL of iced water twice. The filtered supernatant was collected and diluted to 250 mL (in 1% nitric acid) for Zn analysis (as water soluble Zn). The residue was collected and dried at 70 °C, then ashed and analyzed as described below. Zinc still bound in this residue after the separation process was considered 'insoluble' Zn. A separate set of leaflets without ^{68}Zn treatment were collected as the control and treated as described above.

MECHANICAL CELL ISOLATION. The affinity for Zn of pistachio leaf tissue was investigated using leaf cells isolated mechanically according to the method of Grabber and Jung (1991), which involved a series of tissue grinding and filtration procedures. The isolated cells prepared in this way were determined microscopically to be viable mesophyll cells. Prepared cells were suspended in cold 0.35 M sucrose, and were used within 1 h after final preparation. Dead cells were obtained by freezing and thawing these cells twice in liquid nitrogen.

About 10 g of cells (fresh weight) prepared in this way were placed in 100-mL test vials with 25 mL of test solution (100 μM ^{68}Zn) for 6 h. Cells were separated from the solution by centrifugation and filtration, cells were then washed with iced DDI water three times. The washed cells were collected, dry-ashed, and analyzed for ^{68}Zn (see below).

SAMPLE ANALYSIS. Leaf samples were washed with detergent (1% Liquinox, Alconox, Inc., New York) and 0.1 N HCl, rinsed three times in DDI water, and dried at 70 °C for 72 h. For all samples (leaves or isolated cells) ashing was performed overnight at 500 °C. Ash was then dissolved in 10 mL 1 N HNO_3 and heated to 110 °C for 25 min. After filtration, samples were made to 50 mL with DDI water.

A Perkin-Elmer (Norwalk, Conn.) SCIEX ELAN 500 ICP-MS system with a Meinhard nebulizer was used for all ion intensity measurements of Zn isotopes. Net intensity at 64, 66, 67, 68, and 70 mass units was monitored using total dwell time of 300 ms for isotopic ratio determinations. Ratios of ^{68}Zn intensity to the intensity of each of the other four isotopes (Zn 64, 66, 67, and 70) were calculated, and the sensitivities of each ratio to ^{68}Zn enrichment were compared. The $^{68}\text{Zn}/^{64}\text{Zn}$ and $^{68}\text{Zn}/^{66}\text{Zn}$ ratio were each less sensitive to changes in ^{68}Zn enrichment in the sample than was the $^{68}\text{Zn}/^{67}\text{Zn}$ ratio. The $^{68}\text{Zn}/^{70}\text{Zn}$ ratio was oversensitive to changes in ^{68}Zn enrichment since the lower abundance of ^{70}Zn isotope resulted in highly variable intensity readings. Thus, the ratio of $^{68}\text{Zn}/^{67}\text{Zn}$ was

chosen to calculate the ^{68}Zn enrichment in the sample. Net Zn absorption was calculated from the counts of each isotope for each sample without background correction. To correct the daily variations in the isotopic Zn ratios caused by changes in instrumental errors, the measured isotope ratio values were corrected by following the normalization procedure: the mean measured isotope ratio of the standard solution (every 10 samples), which was analyzed at intervals between samples during each instrument run, was divided by the natural abundance ratio (for $^{68}\text{Zn}/^{67}\text{Zn}$, 4.5182) to give a correction factor that was applied to the measured sample ratios obtained in that instrument run: $C_{68:67} = R_{\text{experimental } 68:67} / R_{\text{Theoretical } 68:67}$.

The mass discrimination correction factors during a typical 8 hour period of operation ranged from 0.9855 to 1.0511. National Institute of Standards and Technology (NIST) certified natural Zn standard samples (apple leaf) were measured periodically to check for drift in the Zn abundance ratio. The average coefficient of variation of duplicate isotope ratio determinations was $\approx 0.4\%$ for nonenriched samples and 2.5% for enriched samples. The amount of ^{68}Zn derived from foliar treatment was calculated from the equation based on the isotope ratio modified from Ziegler et al. (1989): $^{68}\text{Zn} = \{Zn_i \times 0.951 \times 0.0411 (R_{s68/67} - R_{c68/67})\} / 0.0073 (R_{e68/67} - R_{s68/67}) + 0.0411 (R_{s68/67} - R_{c68/67})$, where Zn_i is the total amount of Zn in μmoles in the sample, 0.0411 is the natural isotope abundance (fractional) of ^{67}Zn , 0.951 is the abundance of ^{68}Zn in the enriched preparation. $R_{s68/67}$ and $R_{c68/67}$ are the determined isotope ratios of the treated leaf samples and untreated control samples, respectively. 0.0073 is the abundance of ^{67}Zn in the enriched preparation and $R_{e68/67} (=129.58)$ is the 68/67 isotope ratio of the enriched preparation.

The element Yttrium (0.5 $\mu\text{g}\cdot\text{mL}^{-1}$) was added as an internal

Table 1. Distribution of ^{68}Zn among different plant organs following foliar application to intact leaves of 2-week-old pistachio seedlings

Zinc distribution (%)	Days after application	
	2	10
Total recovered	12.5 a ^z	14.8 b
Treated leaf ^y	99.10 a	94.64 b
Stem	0.33 a	2.68 b
Roots	0.10 a	0.75 b
Leaves		
Above treated leaf	0.12 a	1.13 b
Below treated leaf	0.12 a	0.82 b

^zMeans were compared between the 2 sampling days according to Fisher's protected LSD. Means with different letters differed significantly ($p < 0.05$). On a given day after application, only Zn distribution in the 'treated leaf' differed significantly from other organs.

^yIncludes treated area.

Table 2. Distribution (%) of recovered ^{68}Zn within the treated leaflet of intact mature pistachio trees

Leaf section	Days after application		
	2	7	25
Total recovered	18.8 a ^z	20.4 a	14.1 b
Mid section ^y	94.4 a	85.4 b	82.4 c
Apical section	2.4 c	4.7 a	4.3 b
Basal section	3.2 c	9.9 b	13.2 a

^zMeans were compared between the days after application according to Fisher's protected LSD. Means with different letters differed significantly ($p < 0.05$). On a given day after application, the Zn distribution in the midsection of the leaflet was significantly higher than that in the other two sections ($p < 0.05$).

^yIncludes treated area.

Fig. 1. Zinc recovery by pistachio leaflets after foliar ^{68}Zn application at the rate of $50 \mu\text{g } ^{68}\text{Zn}$ per leaflet. Each point is a mean of five replicates $\pm$ SE.

standard using a two-inlet sample feeding system during the analysis. Recovery experiments to verify analytical accuracy were performed by spiking the leaf samples with known amounts of isotope before and after dry ashing. The results of isotope recovery, according to the above equation, ranged from 98.1% to 103.2% for samples spiked before dry ashing and 99.95% for samples spiked during wet extraction. This suggests that no Zn is lost during dry ashing or wet extraction procedures. The accuracy and precision of the ICP-MS measurements make it possible to quantify isotope tracers of Zn in biological samples where only a small percentage of the tracer is actually found.

Zinc recovery is defined as the ^{68}Zn remaining in the leaf following washing and is expressed as the percentage of total applied ^{68}Zn . Preliminary studies with seedlings demonstrated that the percentage of ^{68}Zn that could be removed in the wash declined with time while ^{68}Zn recovered in plant tissues increased by an equivalent amount.

STATISTICAL ANALYSIS. A completely randomized design was used for all experiments. Data were subjected to analysis of variance where appropriate. Fisher's protected LSD test was used for determining the significance of mean comparison.

Results

DISTRIBUTION AND TRANSPORT OF FOLIAR RECOVERED ZINC IN SEEDLINGS. Less than 1% of the foliar recovered Zn was transported outside the treated leaves 2 d after application (Table 1). Even 10 d after application, only $\approx 5.4\%$ of the recovered Zn was transported out of the treated leaves, although ^{68}Zn was detected in all plant organs.

DISTRIBUTION AND TRANSPORT OF RECOVERED ZINC IN MATURE TREES. ^{68}Zn was found mostly in the midsection of the leaflet (where the ^{68}Zn was applied) even 25 d after application (Table 2). However, the distribution of Zn in the three sections changed significantly with time. Zn recovery in the midsection of the leaflet

was 94.4% of the total recovery 2 d after application, and decreased to 82.4% at 25 d. The change in Zn over the same time period was >4 -fold in basal but <2 -fold in apical, so it was likely that more was moving basipetally.

When Zn was applied to one side of the midvein, a significant Zn enrichment ($^{68}\text{Zn}/^{67}\text{Zn}$ ratio of 12.89) was found only in this side of the leaflet, no isotopic enrichment was observed in the other side of the leaflet ($^{68}\text{Zn}/^{67}\text{Zn}$ ratio of 4.41, which was not different from the control leaflet of 4.35). The results suggest that lateral movement of Zn across the midrib is very limited.

EFFECT OF LEAF AGE. Recovery of foliar-applied Zn in the treated leaflets increased with time and reached a maximum ≈ 3 d after application in both immature and mature leaflets (Fig. 1). The net reduction in Zn recovery, which was assumed to be due to net translocation of Zn out of the treated leaflets to other plant parts was estimated by the differences between day 3 and day 20. The amount of Zn recovered and transported from treated leaflets was significantly ($p < 0.05$) affected by leaf age. More of the applied ^{68}Zn was recovered in immature than mature leaflets. Immature pistachio leaflets transported a significantly greater amount of the recovered Zn (48%) than the mature leaflets (28%) by 20 d after application.

CHARACTERISTICS OF ZINC BINDING TO LEAF CELLS. The partitioning of Zn into soluble and insoluble fractions following foliar Zn application was determined by extraction in the presence or absence of Ca^{2+} . When Ca^{2+} was not added to the tissue extraction solution, only 9.6% of the total recovered, foliar-applied ^{68}Zn was present as a soluble component while 90% remained in the insoluble form 40 h after foliar application (Table 3). This proportion of soluble Zn was significantly lower than found in control pistachio leaf tissue. When Ca^{2+} was added to the tissue extraction solution, soluble Zn in the leaf increased to 79% of the total Zn recovered.

In a separate experiment, the affinity for Zn of mechanically isolated leaf cells was determined by submerging living or dead leaf cells in a $100 \mu\text{M } ^{68}\text{Zn}$ solution. The recovery of Zn from the treatment solution was more than 99% and 90% respectively, by living and dead cells. These results suggested high affinity for Zn in both living and dead cells.

Discussion

The mobility of foliar applied Zn varies greatly among species, ranging from only 0.2% of total applied Zn absorbed and translocated from the leaves in pecan (Wadsworth, 1970) to more than 80% in citrus and bean (Wallihan and Heymann-Herschberg, 1956; Bukovac and Wittwer, 1957). Our results demonstrated that 14 to 15% of the foliar applied Zn could be recovered 3 to 10 d after application. The absorption and transport of foliar-applied Zn from

Table 3. Forms of absorbed ^{68}Zn in mature pistachio leaf tissue 40 h after foliar application

Zinc form (%)	$^{-68}\text{Zn}^2$	^{+68}Zn	
	Control	Extraction method	
	$-\text{Ca}^{2+}$	$-\text{Ca}^{2+}$	$+\text{Ca}^{2+}$
Total recovery	---	8.1 a ^y	7.2 b
Insoluble	69.8 b	90.4 a	21.0 c
Soluble	30.2 b	9.6 c	79.0 a

²Forms of Zn in the control sample were calculated based on total Zn content in the leaf.

^yMeans were compared among extraction methods according to Fisher's protected LSD. Means with different characters differed significantly ($p < 0.05$). Extraction method significantly ($p < 0.05$) affected Zn distribution in all cases.

the treated area were much lower for mature than for immature leaflets. Mature leaflets translocated $\approx 2\%$ of the applied Zn, while immature leaflets translocated $\approx 6.5\%$. Similar patterns of the absorption and translocation of foliar-applied Zn were observed in field trials (Brown et al., 1994).

The distribution of foliar applied Zn among different plant organs was not uniform, with a tendency for the majority of the Zn to be found in the stem 10 d after application. This differential transport of Zn absorbed by the leaf is consistent with the finding of Kannan and Keppel (1976) that ^{65}Zn accumulation was greatest in the stem subtending the Zn-treated leaf. However, our results are in contrast to the high translocation of ^{65}Zn to roots of dwarf bean (*Pisum sativum*) (Ferrandon and Chamel, 1988). These differences may be species-dependent. It should be noted that the results obtained from the seedlings may not apply to mature trees, in which the presence of developing nuts may serve as a major sink for nutrients (Brown et al., 1995).

The direction of Zn transport within the leaflet was investigated by separating the treated leaflet into apical, basal and middle sections. Zn was transported both acropetally and basipetally with greater basipetal transport. This is in contrast to the finding of Kannan and Keppel (1976) that Zn transport was only basipetal in corn leaves, regardless of the site of Zn application on the leaf. The inconsistency may be attributed to the fact that different Zn concentrations were used and the nutrient movement may be concentration dependent. Using *in vivo* counting with β -sensitive semiconductor detectors, Ringoet et al. (1967) reported that foliar absorbed ^{45}Ca in oat leaves migrated acropetally at low concentration, but moved to the base of the leaf at concentrations above 20 mM.

Soluble Zn is most likely associated with the leaf symplast and is assumed to be available for transport to other plant parts. Only 9.6% of the total recovered, foliar-applied ^{68}Zn was present as a soluble component, while the majority of the newly absorbed Zn was insoluble. This may explain the limited mobility of Zn within the plants. Several investigators have suggested that Ca^{2+} must be present in the absorbing solution to maintain the integrity of the Zn absorbing mechanism (Bowen, 1969; Schmid et al., 1965). The rate of absorption rapidly declined in the absence of Ca^{2+} in excised roots and in leaf discs, while absorption continued at a constant rate for several hours when Ca^{2+} was added (Bowen, 1969; Schmid et al., 1965). In this study, when Ca^{2+} was added to the tissue extraction solution, a large portion of the insoluble Zn was released. Since tissues were extracted at low temperature (4 °C), the effect of Ca^{2+} on active Zn absorption was likely negligible. This suggests that ion exchange occurs between the soluble and insoluble Zn in the leaves and that the presence of competing cations may be important in the overall effectiveness of Zn sprays.

Zinc applied to the solution bathing isolated cells was rapidly absorbed by those cells. Living cells removed essentially all Zn present in the solutions while dead cells removed 90%. The difference in Zn recovery between living and dead cells suggests that $\approx 10\%$ of the Zn was absorbed into the cytoplasm, and probably only this portion is available for further transport. The result corresponded to the 9.6% soluble Zn found in mature leaves following Zn application.

The results suggest that the slow movement and limited redistribution of foliar-applied Zn in mature leaflets is probably due to the low penetration of Zn into leaf tissue and the high affinity of tissues for Zn. These two factors reduce the amount of Zn available for transport. Treatments that improve Zn penetration and reduce Zn complexation in cell walls might increase the effectiveness of Zn sprays.

Based on the distribution and transport of Zn determined here, we can make a rough estimate of how much a foliar Zn spray might increase tree Zn nutritional status. A field application rate of 2.24 kg·ha⁻¹ of ZnSO_4 (36% Zn) (currently recommended rate in California) will provide ≈ 4.32 g Zn per tree, assuming 187 trees/ha (7.3 $\times$ 7.3 m). An absorption and transport efficiency of 6% will provide ≈ 260 mg elemental Zn per tree. This will increase average leaf Zn level by only 1.8 $\mu\text{g}\cdot\text{g}^{-1}$ assuming an average mature tree size of 150 kg dry mass. This is consistent with the observed average increase of 2 $\mu\text{g}\cdot\text{g}^{-1}$ Zn following a single spring application in a three year field experiment in California orchards (Brown et al., 1994). This suggests that multiple sprays within a year, or sprays over several years, are needed to increase tree Zn status of pistachio by foliar application.

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