J. Amer. Soc. Hort. Sci. 124(3):312–317. 1999.

The Mechanism of Foliar Zinc Absorption in Pistachio and Walnut

Qinglong Zhang and Patrick H. Brown
Pomology Department, University of California, Davis, CA 95616

Additional index words. foliar uptake, stable isotope

Abstract. The characteristics and mechanisms of foliar Zn uptake and translocation in pistachio (*Pistachio vera* L.) and walnut (*Juglans regia* L.) were investigated using ^{68}Zn labelling in both intact and detached leaves. Following washing, mature walnut and pistachio leaves retained 8% and 12% of the total Zn applied, respectively. About half of retained Zn (3.5% and 6.5% of total Zn respectively) was absorbed into the leaf and translocated outside the treated area. Leaf age affected the Zn absorption capacity of pistachio but not walnut. Immature pistachio leaves absorbed more Zn than mature leaves. The absorption of Zn by walnut leaves at high concentrations (7.5 to 15 mM Zn) was not significantly affected by the pH of the solution. In pistachio Zn absorption was greatest at pH 3.5 and declined as pH increased to 8.5. The uptake process was not affected by light or addition of metabolic inhibitors. Foliar leaf absorption was only slightly affected by changes in temperature with an average Q_{10} of 1.2 to 1.4. This study suggests that foliar Zn uptake is dominated by an ion exchange and/or diffusion process rather than an active one. This study also demonstrates the usefulness of stable isotope labelling in studies of foliar Zn absorption.

Foliar application of nutrients has been practiced by fruit growers since early 19th century (Gris, 1844), however, research on applied and basic aspects of foliar nutrition had not been vigorously pursued until early 1950s (Tukey et al., 1952; Wittwer and Lundahl, 1951). The use of radioisotope techniques has made it possible to trace the movement of foliar applied nutrients and their metabolic pathways, and significantly improved our knowledge of the mechanisms of cuticular penetration and absorption of nutrients by leaves. Many authors have reviewed the mechanisms and physiology of uptake and the scope for foliar application of fertilizers in agriculture, with focus on fruit trees (Haynes and Gob, 1977; Swietlik and Faust, 1984; Wittwer, 1964). Foliar supply of nutrients is now commonly practiced for fruit trees and vegetable crops, although the effectiveness of foliar micronutrient sprays in general, and Zn in particular, is not always satisfactory and varies greatly among species. For example, foliar applications of Zn made before leaf drop in fall, give good correction of Zn deficiency in almonds, apricots, cherries, and pears (Weinbaum, 1989), but not in apples or pecan (Neilson and Hoyt, 1990). The amount of foliar applied Zn absorbed and translocated by the leaves of different species ranged from 0.2% of total applied Zn in pecan (Wadsworth, 1970) to 80% in bean (Bukovac and Wittwer, 1957). The reason for this significant species variability and the approaches required to optimize foliar Zn applications are poorly understood.

In California, zinc deficiency occurs in pistachio (*Pistachio vera*) and walnut (*Juglans regia*) statewide. Both soil amendments and foliar applications of Zn have been widely adopted to correct the deficiency with variable effectiveness. Little information is available concerning the effectiveness of foliar sprays of Zn in pistachio. The increasing frequency of Zn deficiency statewide (Uriu, 1990) and the ineffectiveness of current Zn application methods provided the impetus for this research. Although Uriu and Cheney (1970) reported that spring foliar sprays of Zn gave good correction of deficiency symptoms in walnut, no quantitative information concerning the characteristics, efficiency or mechanism of foliar Zn uptake was provided. Studies using radioisotopes as tracers of foliar Zn absorption have been conducted with isolated leaf cells and leaf segments by various investigators (Bajaj et al., 1970; Bowen, 1969; Smith and Epstein, 1964), however, the evidence is still insufficient to adequately define the mechanism involved in foliar Zn absorption. The role of active or passive processes in foliar Zn uptake still remains controversial (Jyung and Wittwer, 1965; Haynes and Gob, 1977). In addition, all previous studies involving leaf tissues or roots covered only narrow ranges of low concentrations of Zn (Bajaj et al., 1970; Bowen, 1969), while much higher concentrations of Zn are typically used in field spray applications in horticulture. The validity and general applicability of laboratory studies to field practices are not clear. The aims of our work were to investigate the processes involved in foliar uptake of Zn by pistachio and walnut using intact leaves. In this study, we demonstrate that the accuracy and sensitivity of the measurements using inductively coupled plasma–mass spectrometry (ICP–MS) make it possible to use stable isotope methodology in plant studies involving foliar fertilization.

Materials and Methods

Leaves at different growth stages were collected from 12-year-old pistachio ('Kerman') and 6-year-old walnut ('Gustine') trees grown at the Pomology orchard, University of California, Davis. Care was taken to avoid obviously damaged and shaded leaves. Double deionized water was used to formulate all test solutions. All chemicals and solvents used in this study were reagent grade obtained from commercial sources unless otherwise noted. Solutions were not buffered unless pH was a factor, in which case 0.1 mM sodium-citrate buffer was used for pH 3.5, 0.1 mM sodium-acetate buffer was used at pH 4 to 6, and 0.1 mM Tris-malate buffer was used for pH 7 to 8.

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

Received for publication 8 Sept. 1998. Accepted for publication 11 Feb. 1999. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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 then 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.

Exposed branches from the middle of the canopy with leaves attached, were cut from five individual trees and transported (with cut ends in distilled water) back to the laboratory for further selection. Healthy leaflets with petioles were detached from the compound leaf and the petioles were placed in glass vials containing distilled water. The vials were transferred to a growth chamber with ≈250 μmol·m^{-2}·s^{-1} PAR; and a photoperiod of 16 h with day/night temperature of 25 /20 °C. Zinc concentrations ranging from 1.5 to 30 mM were tested. A small amount (50 μL) of test solution was applied to the leaf surface in each of four restricted treatment areas with a total of 200 μL test solution applied to each leaflet. To isolate the treatment area from the rest of the untreated leaf, four plastic cylinders, 3 to 4 mm in height and 1.0 cm internal diameter, were fixed to the leaf surface with lanolin. Two cylinders were located on each side of the mid section of the leaflet avoiding the midvein. After a certain time period (usually 24 h after application unless time is a factor), the leaflets were collected, the treated area was first washed with 0.1 N HCl and then the lanolin ring was removed. Leaflets were then divided into two groups (three to five replicates each). One group of samples was analyzed for Zn content in the leaf including the treated area, and the second group was analyzed following removal of the treated area with a cork borer with internal diameter of 1.2 cm. The leaflets, other than the treated area, were washed and dried for Zn analysis (described below). The absorption of Zn was calculated by excluding the original Zn present in the leaf tissue of control (nontreated) leaves.

Leaflets used were fully expanded mature leaves and Zn was applied to the adaxial surface unless specified. Absorption experiments were conducted with Zn concentrations of 7.5 mM in the light for 24 h unless Zn concentration, light or time was the testing factor, which was specified. When Zn concentration was a factor in the experiment, it ranged from 1 to 30 mM. Leaf absorption was determined from 10 min to 24 h after application. Two inhibitors, dinitrophenol (DNP), an uncoupling agent of oxidative phosphorylation, and potassium cyanide, a respiration inhibitor, with appropriate concentrations were added to the test solution to determine the extent of Zn absorption as affected by altered plant metabolism. The effectiveness of Zn uptake from abaxial leaflets was also evaluated by applying treatment solutions to the surface of inverted leaflets. To investigate the effect of leaf age on uptake efficiency, immature leaves were collected early in the growing season when leaves were about half expanded.

Sample analysis. In all the experiments, leaf samples were washed with detergent (1% Liquinox, Alconox, Inc. New York) and 0.1 N HCl, and rinsed 3 times with DDI water, and dried at 70 °C for 72 h. 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. Total Zn concentration in the digests was determined on a TJA ATOMSCAN 25 ICP–atomic emission spectrometer (AES) (Thermo Jarrell Ash, Menlo Park, Calif.) with automatic background correction. Only Zn recovered from leaf tissue outside the treated area was considered as being absorbed. Though this method underestimates Zn uptake by excluding the treated area, it does provide assurance that all Zn measured must have been absorbed and translocated and is not simply surface contamination.

Zinc detection by ICP–MS. A Perkin-Elmer SCIEX ELAN 500 ICP–MS system (Norwalk, Conn.) 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}Zn/^{64}Zn and ^{68}Zn/^{66}Zn ratio were each less sensitive to changes in ^{68}Zn enrichment in the sample than was the ^{68}Zn/^{67}Zn ratio. The ^{68}Zn/^{70}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}Zn/^{67}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. Because ratios measured on different days or within the same day varied slightly due to changes in instrument characteristics, 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}Zn/^{67}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-h 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 ≈0.4% for nonenriched samples and 2.5% for enriched samples. The

Table 1. Foliar absorption of Zn by pistachio and walnut leaves. Each treatment was replicated four times with $ZnSO_4$ at 15 mM, pH 5.4. Leaflets were harvested 24 h after foliar application.

	Pistachio (% of applied)		Walnut (% of applied)	
Treatment	Recovery (TA[z] included)	Absorption (TA excluded)	Recovery (TA included)	Absorption (TA excluded)
Young leaf (adaxial)	---[y]	9.8	---	4.8
Mature leaf				
Adaxial	11.8	6.5	8.4	3.5
Abaxial	15.3	7.7	10.8	4.6
Fisher's protected $LSD_{(0.05)}$	3.1	2.4	2.9	2.0

[z]TA = treated area.
[y]Not determined.

amount of ^{68}Zn derived from foliar treatment was calculated from the equation based on the isotope ratio modified from Ziegler et al. (1989): $^{68}Zn = [Zn_t \times 0.951 \times 0.0411 (R_{s68/67} - R_{c68/67})]/[0.0073 (R_{e68/67} - R_{s68/67}) + 0.0411(R_{s68/67} - R_{c68/67})]$, where Zn_t 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. $Rs_{68/67}$ and $Rc_{68/67}$ 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 ppm) was added as an internal 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.

Statistics. A completely randomized design was used for all experiments. Data were subjected to regression analysis or analysis of variance where appropriate. Fisher's protected LSD test at $P = 0.05$ was used for determining the significance of mean comparison.

Results

In this study, the term recovery or uptake is defined as Zn that was both absorbed and adsorbed by the leaf surface. Zinc recovery was estimated by measuring Zn in entire leaves including the treated area, following acid washing of the leaf surface. The term 'absorption' is defined as the element absorbed by and translocated from, the treated area. This was determined by analyzing leaf ^{68}Zn after excluding the treated area. Leaf Zn absorption is expressed as a percentage of that applied to the leaf surface.

Leaf age and surfaces. Immature pistachio leaves absorbed more Zn than mature leaves ($p < 0.05$), however, leaf age did not significantly affect leaf Zn absorption in walnut (Table 1).

Leaf surface influenced Zn recovery but not Zn absorption in pistachio (Table 1). Zinc absorption was ≈50% of Zn recovery from the site of application. Leaf surface had no effect on Zn recovery or absorption in walnut. A total of 42% of the Zn recovered in walnut was translocated outside the treated area. Walnut leaves generally showed lower Zn recovery and absorption than pistachio leaves.

Effect of time. The uptake curves for recovery of applied Zn in both species were similar. Zn absorption (excluding treated area) was not observed until ≈2 h after Zn application (Fig. 1). This lag likely corresponded with the time required for the movement of Zn from the site of application to neighboring untreated tissue. Following the lag phase, Zn absorption increased up to 24 h after the application following a second order regression function which suggests that the rate of uptake decreases with increasing time.

Zinc absorption as a function of external concentration. Zn recovery showed no evidence of saturation over a 24-h period, whereas Zn absorption was saturated at ≈7.5 and 15 mM in pistachio and walnut, respectively (Fig. 2). This suggests that Zn recovered in the leaves at treatments above 7.5 mM in pistachio and 15 mM in walnut was mostly present in the treated area and was not available for subsequent retranslocation. Zinc recovery in pistachio leaves was higher than in walnut, however, there was no species difference for Zn absorption. Both pistachio and walnut

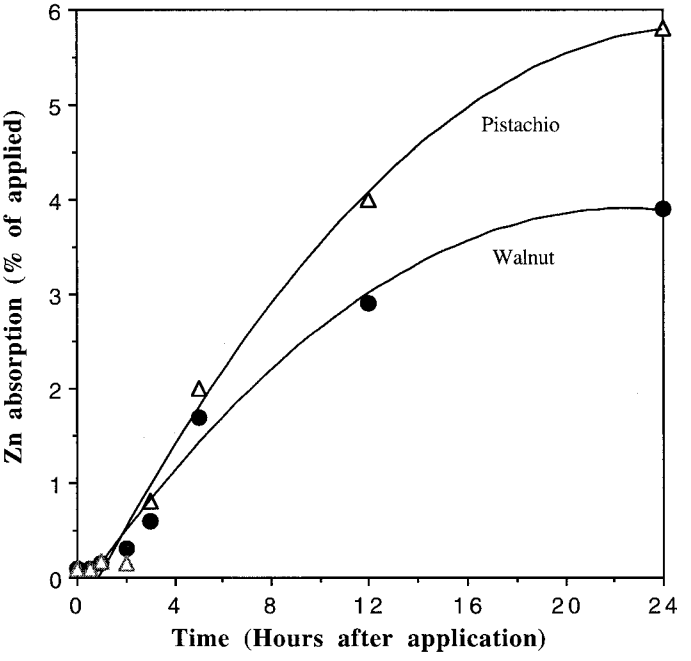

Fig. 1. Changes in Zn absorption (percent of applied) after application of Zn to the adaxial surface of mature leaves following a foliar application of 4 × 50 μL droplets of 15 mM zinc sulfate solution. Leaf Zn absorption 3 h after application can be expressed as $y = -0.409 + 0.493x - 0.009x^2$ ($R^2 = 0.995$) and $y = -0.219 + 0.366x - 0.008x^2$ ($R^2 = 0.978$) for pistachio and walnut, respectively.

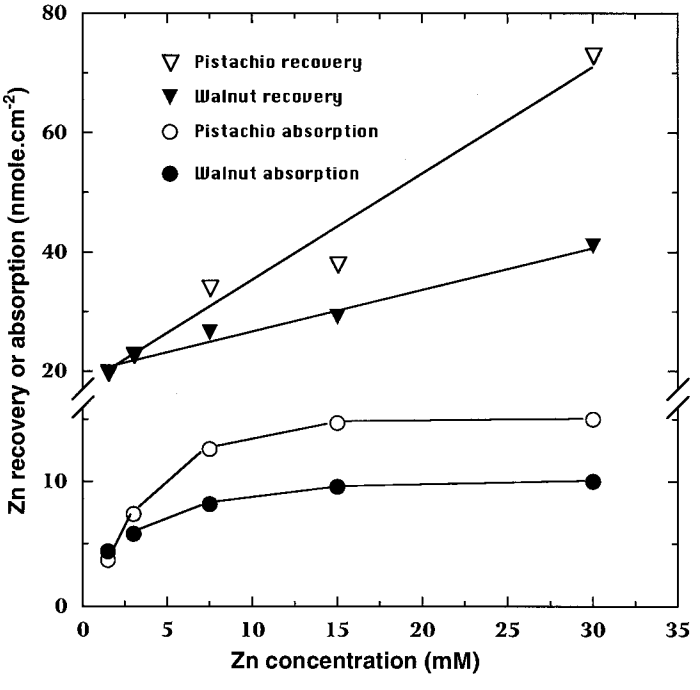

Fig. 2. Zinc recovery (treated area included) and absorption (treated area excluded) by leaves as a function of the external concentration of the treatment solution after 24 h. Pistachio recovery is best expressed by $y = 17.05 + 1.796x$ ($r^2 = 0.971$); while walnut recovery is best expressed by $y = 19.78 + 0.7x$ ($r^2 = 0.981$). Pistachio and walnut leaf absorption is best fitted by a nonlinear regression of the form $y = 15 (1 - 1.11 e^{-0.26x})$ and $y = 10 (1 - 0.75 e^{-0.19x})$, respectively.

leaves often showed phytotoxic symptoms (necrotic spots) in the treated area in the 30 mM treatments.

Effect of pH. Zn absorption decreased as pH increased from 3.7 to 8.6 in pistachio, and there was a >50% reduction in Zn absorption between the two extreme pH treatments (Fig. 3). Zn absorption was often accompanied by necrosis of the leaf tissue at the site of Zn application when pH was 3.7. In walnut there was a trend for a decrease in absorption as pH increased ($p = 0.08$).

Effect of temperature. Temperature affected pistachio leaf Zn recovery and walnut leaf Zn absorption, but not pistachio leaf Zn absorption or walnut leaf Zn recovery (Fig. 4). Zinc recovery in pistachio leaves 24 h after application ranged from 9% to 14% as temperature increased from 8 to 31 °C. Within the same temperature range, Zn absorption increased from 4% to 6%.

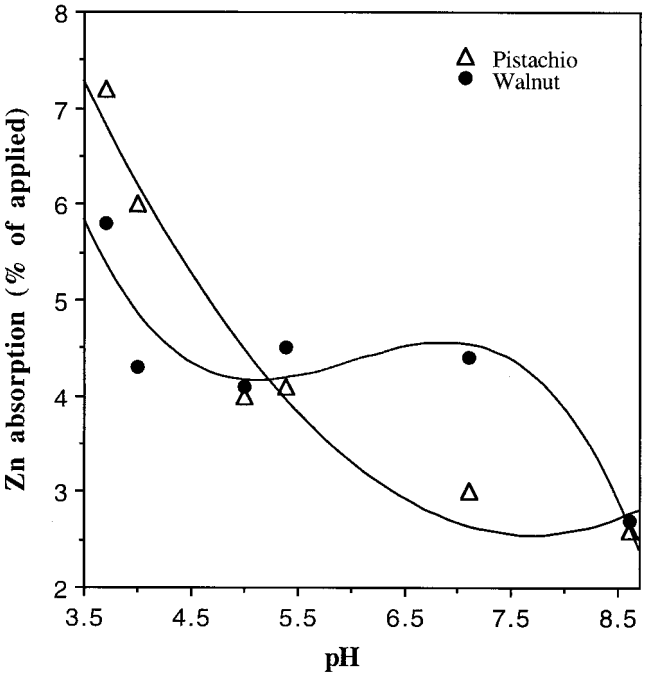

Fig. 3. Effect of pH of the zinc sulfate treatment solution (15 mM) on foliar Zn uptake. Pistachio absorption can be expressed by $y = 1.85 – 4.14x + 0.269x^2$ with $R^2 = 0.59$ ($p = 0.01$), while walnut absorption can be expressed by $y = 34.65 – 15.81x + 2.658x^2 – 0.149x^3$ with $R^2 = 0.23$ ($p = 0.08$).

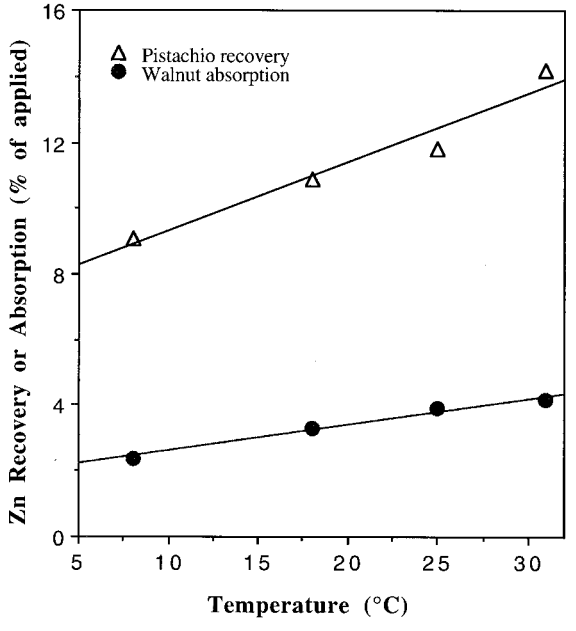

Fig. 4. Effect of temperature on leaf recovery and absorption of Zn. Pistachio recovery can be expressed by $y = 7.24 + 0.21x$ with $r^2 = 0.45$ ($p = 0.01$); Walnut absorption can be expressed by $y = 1.86 + 0.08x$ with $r^2 = 0.45$ ($p = 0.01$).

Effect of light and metabolic inhibitors. Light had no effect on Zn absorption (Table 2). Addition either of DNP or potassium cyanide into the test solution did not influence Zn absorption. The effect of metabolic inhibitors on ion absorption is generally influenced by 1) the concentration of the inhibitor and 2) time of exposure. However, subsequent experiments demonstrated that neither increasing the concentration nor the incubation time altered Zn absorption (data not shown).

Discussion

Using the microdrop technique, we demonstrated here that mature walnut recovered and translocated less Zn outside the treated area than pistachio leaves. This differential leaf uptake capacity between the two species is consistent with the results from 3 years of field trials where it was observed that foliar treated pistachio leaves contained significantly more Zn (up to 300 $\mu g \cdot g^{-1}$), than walnut leaves (<100 $\mu g \cdot g^{-1}$) following the same application at spring flush. The low recovery of foliar applied Zn in these species is in sharp contrast to reports of 60% to 90% recovery of foliar applied Zn in leaves of citrus and bean (Bukovac and Wittwer, 1957; Reed, 1983). This discrepancy likely results from differences in leaf characteristics of these species and illustrates that foliar Zn uptake capacity is highly species specific. Differences in experimental methodology can also contribute to the observed experimental results. Inclusion of the treated area in the analysis can contribute to the apparent large differential absorption of foliar applied Zn. Using an isolated cuticular membrane technique, we were able to demonstrate that the leaf cuticle in both species has a high capacity to adsorb cations such as Zn (Zhang and Brown, unpublished data). Zn retention by cuticular membrane and cell walls may account for much of the Zn remaining in the treated area. The high Zn binding capacity of cuticular membranes suggests that the treated area should not be included in the analysis as this may not reflect real Zn absorption. Ferrandon and Chamel (1988) reported that 80% of foliar absorbed ^{65}Zn was present in the treated area of bean leaves following foliar application. In a related experiment, we demonstrate that 87% of foliar applied ^{68}Zn remained in the treated leaf area 10 d after application (Zhang and Brown, unpublished data). This is consistent with the results of preliminary experiments which showed that inadequate washing methods contributed significantly to the high Zn content and anomalous results often reported in the literature.

An active uptake process is usually characterized by a steady uptake for several hours before a plateau is reached (Epstein, 1966). Here a linear relationship between Zn concentration and recovery was obtained over the experimental concentration range in this experiment and no saturation plateau was obtained. This suggests that mass flow or diffusion and not metabolic uptake was involved in Zn recovery by leaves (Epstein, 1966). Zn absorption, however, became saturated at concentrations >7.5 and 15 mM in pistachio and walnut, respectively. This saturation is consistent with a metabolic uptake process (Epstein, 1966).

The temperature coefficient (Q_{10}) is perhaps the most classical of all indices for separating active and passive uptake processes by plant tissues. According to Wittwer and Teubner (1959), the Q_{10} for active nutrient uptake processes in plants is usually above 2. An average Q_{10} of 1.2 to 1.4 for Zn absorption observed in this

Table 2. Effect of growth inhibitors and light on foliar Zn absorption by intact leaves of pistachio and walnut; 4 × 50 μL droplets of 7.5 mM of $ZnSO_4$ were applied to the adaxial leaf surface. Treatments were replicated five times and harvested 24 h after application.

	Zn absorbed (%) (TA[z] excluded)	
Treatment	Walnut	Pistachio
Light (control)	3.9	6.0
Dark	4.2	6.3
KCN (10^{-4} M)	2.8	5.6
DNP (10^{-4} M)	3.5	8.1
DNP (10^{-3} M)	4.6	7.5
Fisher's protected $LSD_{(0.05)}$	2.1	2.7

[z]TA = treated area was excluded from analysis.

experiment is consistent with a Q_{10} of 1.2 as reported by Rathore et al. (1970). Lower Zn uptake under lower temperature by leaves has been attributed to the increased viscosity of the aqueous ambient solution, which would probably result in a decreased rate of diffusion of ions (Rathore et al., 1970). The slight temperature dependence observed in these experiments is in contrast to the strong temperature dependence reported by Bowen (1969), who found a Q_{10} of >2.5 using sugarcane leaf slices. The lack of a strong temperature dependence suggests that foliar Zn absorption was largely a nonmetabolic process.

The effects of pH on Zn absorption in this study do not support the conclusion of Bowen (1969) that the optimum pH for Zn absorption is 5.0 to 6.0 and that absorption decreases when pH is above or below this range. The choice of experimental material may best explain this apparent contradiction. Bowen (1969) used leaf slices in which the cut edge of the slices had direct contact with the solution; this might be a major route for the absorption of Zn reported. Thus, pH changes in the solution in leaf disks might be expected to have greater effect on membrane permeability to Zn than the intact leaf system used in this study.

Rathore et al. (1970) concluded that Zn uptake by stem callus, leaf disks and enzymatically isolated leaf cells varied positively with increasing pH. This result may be attributed to the low concentration of Zn used (0.2 mM), which may have a different uptake mechanism from the high concentrations used here. Increased uptake at high pH may also be due to direct contact of the solution to the tissue tested in which much Zn may have been adsorbed to cell walls and cuticles. Nonselective, nonmetabolic exchange adsorption on negatively charged sites may account for a relatively large proportion of the total uptake of Zn ions because the rate of active absorption of divalent ions is usually low (Schmid et al., 1965). Such adsorption would tend to obscure the absorption of Zn (if any) by metabolically selective mechanisms. Alternatively, increased bicarbonate concentration at the higher pH used by Rathore et al. (1970) may result in precipitation of Zn as zinc bicarbonate in the cell, which would give an impression of increased Zn uptake under high pH.

Ion uptake by leaf tissue, if an active process, would be energy dependent. Light intensity is known to affect the energy-consuming mechanism for salt uptake associated with photosynthesis (Van Lookeren Campagne, 1975). A light dependence of the uptake process is one of the most indicative criteria of an energy-requiring process. The reported effects of light on Zn absorption in various plant species, tissues and organelles are inconsistent (Bowen, 1969; Christensen, 1980; Rains, 1968). The differences may be attributed to the materials used in these studies and the specific ions concerned. The resistance to solute penetration by the cuticle was minimized in studies using leaf segments (Bowen, 1969), while intact leaves were used here. In these experiment metabolic inhibitors caused no significant reduction in uptake of Zn. Neither increasing the concentration nor the incubation time of inhibitors increased the effectiveness of these inhibitors on Zn uptake. The results of our experiments support those obtained by Broda (1965) and Schmid and Hawf (1966) that neither DNP nor azide had any significant effect on Zn uptake. However, our results do not support the conclusion made by Bowen (1969) who reported that uptake of Zn by sugarcane leaf sections was reduced to 21% of control by 10^{-5} M DNP within 30 min of incubation. The absence of light dependency in foliar Zn uptake provides further supportive evidence for a nonmetabolic nature of the process.

It is now generally agreed that initial cation uptake by plants involves a passive diffusion of ions into the apparent free space, followed by exchange on the negative carboxylic groups of the cell wall pectins. The occurrence of this exchange adsorption phenomenon during the initial stage of Zn absorption has been reported by Broda (1965) and Schmid et al. (1965). Using isolated cuticular membranes we have also demonstrated that much of the foliar Zn uptake was first adsorbed to this negatively charged membrane (Zhang and Brown, unpublished data). The insensitivity of Zn absorption to light and metabolic inhibitors precludes active metabolism as the driving force in Zn absorption. Results of this experiment suggest that Zn uptake by leaves may be largely determined by physiochemical binding of the ion to the cuticle and cell wall components.

In summary, the results presented in this experiment on Zn uptake by pistachio and walnut leaves are not indicative of metabolic accumulation of ions. Zinc absorption at high concentrations (7.5 to 15 mM) was not affected by light, was insensitive to addition of metabolic inhibitors, and had a minimal response to changes of temperature. Uptake increased with increasing external concentration over a wide concentration range. These data indicate that active metabolism may not be involved in the foliar absorption processes. This experiment supports the conclusions of Bajaj et al. (1970), Rathore et al. (1970) and Broda (1965), but contradicts those of others (Bowen, 1969; Chaudhry and Loneragan, 1972; Giordano et al., 1974). The controversy concerning the mechanism of Zn uptake is probably due to variations in experimental conditions (concentration of Zn, concentration of inhibitors, presence of other competing ions). According to the criteria set by Wittwer and Teubner (1959), the results of this study indicate that foliar Zn uptake is an ion exchange and/or diffusion process.

All Zn absorption values reported here were lower than would occur in the field since the 1-cm leaf disc including and surrounding the site of application was not included. This was necessary since the inclusion of leaf tissue directly treated with Zn is misleading. However, a relatively accurate picture of *in vivo* Zn absorption and translocation by leaves can be achieved in this study by following careful and accurate experimental procedures.

Literature Cited

Bajaj, Y.P.S., V.S. Rathore, S.H. Wittwer, and M.W. Adams. 1970. Effect of dimethyl sulfoxide on $zinc^{65}$ uptake, respiration and RNA and protein metabolism in bean (*Phaseolus vulgaris*) tissues. Amer. J. Bot. 57:794–799.

Bowen, J.E. 1969. Absorption of copper, zinc, and manganese by sugarcane leaf tissue. Plant Physiol. 44:255–261.

Broda, E. 1965. Mechanism of uptake of trace elements by plants. Isotope radiation—Soil plant nutrition studies Proc. Symp. Ankara. p. 207–215.

Bukovac, M.J. and S.H. Wittwer. 1957. Absorption and mobility of foliar

applied nutrients. Plant Physiol. 32:428–434.
Chaudhry, F.M. and J.F. Loneragan. 1972. Zinc absorption by wheat seedlings: Inhibition by macronutrient ions in short term experiment and its relevance to long-term zinc nutrition. Soil Sci. Soc. Amer. Proc. 36:323–327.
Christensen, P. 1980. Timing of zinc foliar sprays. I. Effect of application intervals preceding and during the bloom and fruit-set stages. II. Effect of day vs. night application. Amer. J. Enol. Viticult. 31:53–58.
Epstein, E. 1966. Dual pattern of ion absorption by plant cells and by plants. Nature 212:1324–1327.
Ferrandon, M. and A.R. Chamel. 1988. Cuticular retention, foliar absorption and translocation of Fe, Mn and Zn supplied in organic and inorganic form. J. Plant Nutr. 11:247–263.
Giordano, P.M., J.C. Noggle, and J.J. Mortvedt. 1974. Zinc uptake by rice as affected by metabolic inhibitors and competing cations. Plant Soil 41:637–646.
Gris, E. 1844. Nouvelles expériences sur l'action des composes ferrugineux solubles, appliqés à la vegetation et specialement au traitement de la chlorose et àla denilité des plantes. C.R. (Paris) 19:118.
Haynes, R.J. and K.M. Gob. 1977. Review on physiological pathways of foliar absorption. HortScience 7:291.
Jyung, W.H. and S.H. Wittwer. 1965. Pathways and mechanisms for foliar absorption of mineral nutrients. Agr. Sci. Rev. 2nd Quart.:26–36.
Neilson. G.H. and P.B. Hoyt. 1990. A comparison of methods to raise zinc concentration of apple leaves. Can. J. Plant Sci. Rev. Can. Phytotechnol. 70:599–603.
Rains, D.W. 1968. Kinetics and energetics of light-enhanced potassium absorption by corn leaf tissue. Plant Physiol. 431:394–400.
Rathore, V.S. and S.H. Wittwer, W.H. Jyung, Y.P.S. Bajaj, and M.W. Adams. 1970. Mechanism of zinc uptake in been tissue. Physiol. Plant. 23:908–919.
Reed, D.W. 1983. Methods to assay absorption of foliar applied nutrients and removal of unabsorbed residues. J. Plant Nutr. 6:667–677.
Schmid, W.E., H.P. Haag, and E. Epstein. 1965. Absorption of zinc by excised barley roots. Physiol. Plant. 18:860–869.
Schmid, W.E. and L.R. Hawf. 1966. Effects of carbonyl cyanide phenylhydrazone on cation uptake in excised barley roots. Plant Cell Physiol. 7:145–148.
Smith, R.C. and E. Epstein. 1964. Ion absorption by shoot tissue: Technique and first findings with excised leaf tissue of corn. Plant Physiol. 39:338–341.
Swietlik, D. and M. Faust. 1984. Foliar nutrition of fruit crops. Hort. Rev. 6:287.
Tukey, H. B., R.L. Ticknor, Jr., O.N. Hinsvark, and S.H. Wittwer. 1952. Absorption of nutrients by stems and branches of woody plants. Science 116:167.
Uriu, K. 1990. Diagnosis and treatment of pistachio nutritional deficiencies, p. 4–8. In: Pistachio production. A pomology short course. Univ. Ext. Press, Univ. Calif., Davis.
Uriu, K. and D.H. Cheney. 1970. Foliar sprays for correcting Zn deficiencies in walnut. Calif. Agr. 24:10–11.
Van Lookeren Campagne, R.N. 1975. The action spectrum of the influence of light on chloride absorption in *vallisneria* leaves. Proc. Kon. Ned. Akad. Wetensch., ser. C 60:70–76.
Wadsworth, G.L. 1970. Absorption and translocation of zinc in pecan trees MS thesis. Texas A&M Univ., College Station.
Weinbaum S.A. 1989. Foliar nutrition of fruit trees, p. 81–100. In: P.M. Neumann. (ed.). Plant growth and leaf applied chemicals. CRC Press, Boca Raton, Fla.
Wittwer, S.H. 1964. Foliar absorption of plant nutrients. Adv. Frontiers Plant Sci. 8:161.
Wittwer, S.H. and W.S. Lundahl. 1951. Autoradiography as an aid in determining the gross absorption and utilization of foliar applied nutrients. Plant Physiol. 26:792.
Wittwer, S.H. and F.G. Teubner. 1959. Foliar absorption of mineral nutrients. Ann. Rev. Plant Physiol. 10:13–32.
Ziegler, E.E., R.E. Serfass, S.E. Nelson, R. Figueroa-Colon, B.B. Edwards, R.S. Houk, and J.J. Thompson. 1989. Effect of low zinc intake on absorption and excretion of zinc by infants studied with ^{70}Zn as extrinsic tag. J. Nutr. 119:1647–1653.