IMPROVING WALNUT ZINC NUTRITIONAL STATUS BY FOLIAR SPRAYS

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ABSTRACT

Field trials and lab experiments were conducted to develop an effective method of improving Zn status of walnut trees by foliar fertilization in 1993, 1994. The eight Zn formulations shown to be most effective were further investigated at mid-late spring flush stage in 1995 season when walnut leaves are expanding fastest. Treatments were conducted by applying Zn foliar sprays to individual trees and determining the Zn concentration in a) the sprayed leaf or b) a non-sprayed leaf on the treated branch. Presence of increased Zn concentrations in these non-sprayed leaves indicates that the Zn treatment used was both absorbed and translocated within the tree.

Timing: In 1993, foliar sprays of zinc at late dormant and bud break stages were not effective in improving zinc status of walnut leaves. The residual effect of foliar spray applied after harvest is minimum. Foliar sprays at early spring flush were not as effective as late spring flush sprays when the leaves are expanding fastest. Sprays after leaves mature were not effective. The most effective method of increasing Zn nutritional status of the walnut tree was application at 2 lbs ZnSO₄ per 100 gallons at mid to late spring flush, and this was validated in 1994 and 1995. The best stage for application is recognized when the color of the leaves has just turned to green from pinkish.

Formulations: Various formulations were tested compared to the standard zinc sulfate sprays over a 3 year period. Zinc sulfate based solutions are more effective than oxide derived product. Liquid forms of Zn-sulfate are as effective as powder forms. Evidence suggests that Zn-chelate(EDTA) may be helpful in enhancing retranslocation of zinc from absorption site to non-sprayed and newly emerging tissues, especially with addition of surfactant. Zn sprays are compatible with sprays of B (pH adjusted) and low concentrations of Cu/Fe. Addition of N and P are not recommended in foliar Zn sprays. Leaf Zn uptake increases as the solution pH decreased. S & A liquid Zn and zinc sulfate complexed with organic acids (pH 5.0) consistently provided more Zn uptake and translocation than other products. Significantly, the residual value of foliar Zn sprays for second year growth is limited and consecutive sprays for several years are necessary to improve the long term Zn nutritional status of walnut. Traditional Zn fertilization programs utilizing 40 lb ZnSO₄/300 gal applied in fall were effective in one of 3 years and may be beneficial in areas of chronic Zn deficiencies. The continued use of these very high rates of Zn application over the life time of the orchard is of some environmental concern.

Recommendations: Based on the results of the 3 year trial we recommend the following approach to remediation of Zn deficiency in walnut orchards: foliar sprays of Zn should be applied at mid-late spring flush at a rate of 2.0 lb ZnSO₄ (36 %) or 4.5 lb Zn-EDTA (15%) per 100 gal. water per acre. The pH of the zinc sulfate spray solutions should be adjusted to 5.0 using organic acids (malic acid preferred). Surfactants should be added if EDTA-Zn is used. Addition of Cu or Fe (0.3 lbs/100 gal.) improves effectiveness of Zn spray. Nitrogen and phosphorous are not recommended in the Zn application. The residual effect of any zinc spray is limited, therefore application of Zn should be repeated annually to have long term benefit. In areas of chronic Zn deficiency the current soil Zn recommendations (Walnut Orchard Management 1996. UC
Cooperative Extension Publication) coupled with soil acidification should be considered.

OBJECTIVE

Foliar sprays are recommended for the correction of Zn and Cu deficiency fruit production in California and may also be important in correcting B deficiency. For each of these nutrients the factors controlling their uptake into the leaf are not understood. Foliar zinc uptake has been shown by Uriu (1990) to be most effective when applied during early fall and just prior to bud break in the spring. Such practices have also been recommended for apple and other tree crops (Neilsen & Hogue 1983). In the case of Zn, late fall or early spring applications appear to be effective only when extremely high rates (>14.4 lbs. Zn/300 gal) are applied. This suggests that the efficiency of this method is extremely low. Even at these high rates results are variable and the residual effect is minimal.

The ineffectiveness of foliar zinc fertilization in fall may be due to the physical barrier of the leaf or lack of mobility of zinc after penetration through plant cuticle. The mechanism of foliar zinc uptake and its influencing factors is not clear. Zn deficiency is a major problem in many orchards of California and has been difficult to correct through soil application. The feasibility of foliar sprays of zinc in walnut has not been adequately explored. In 1993 and 1994, our research showed that there is a significant effect of the growth stage on the effectiveness of Zn foliar sprays. Several zinc sources have been shown to be effective in increasing Zn status of walnut leaves if they were applied at the right time. The purpose of this project was to study the effectiveness of various foliar zinc sprays in increasing walnut leaf nutritional status, and to gain an understanding of the mechanism of foliar Zn uptake and the factors that may influence it.

PROCEDURE

The effectiveness of foliar fertilization with different zinc carriers was investigated in 1995 season. A completely randomized design involving 8 Zn treatments and a control, with five replications, was established in a ten year-old walnut orchard in San Joaquin County. Trees of more or less same size within the experimental block were chosen. Each treatment was separated by at least one control tree. Sprays were applied at mid spring flush (April 12, 1995), that is the stage at which walnut leaves has just turn to green in color and are expanding fastest. Zinc solutions included zinc sulfate based complex, new products such as zinc borate and zinc citrate, and organic chelate (Table 1). Each tree was sprayed with about two and half gallons of solution using a gasoline powered sprayer.

In order to evaluate the mobility of Zn absorbed by the leaves, four vegetative buds or twigs of each tree were chosen randomly and covered with plastic bags before spraying. Each treatment was sprayed to run off. On 5/20/1995, leaf samples were taken from (1) bag-covered branches, and (2) branches receiving sprays. Leaves were returned to the laboratory and washed in mild detergent tap water, 0.2 N HCl solution for 10 minutes, and finally rinsed with double deionized water. Leaf samples were then dried in a mechanical convection dryer at 70°C for 72 hours and ground in a stainless steel bladed Wiley mill to pass a 20 mesh stainless steel screen. Zn concentration in these leaf samples were analyzed by ICP-AES. The trees receiving foliar sprays were visually assessed for phytotoxicity on leaves, no significant phytotoxic symptoms were observed.

The Zn uptake capacity of walnut leaves of different physiological ages as related to the development of epicuticular wax on the leaf surface was evaluated in 1995. The leaf areas of detached leaves was measured using a Delta-T area meter( Decagon, Inc., Pullman, Wash.). The epicuticular waxes were then extracted separately from adaxial and abaxial surface by dripping a
known area of leaflets (3 replicates with 30 pieces each) into a 1000 ml beaker with successive chloroform solutions delivered from a burette over leaves held vertically by the petiole for two minute each leaflet. The chloroform mixed with surface wax were dried with sodium sulfate, filtered and evaporated under N₂. The residue were dried and weighed. Quantity of wax is expressed as µg/cm². The cuticular membranes of walnut were isolated chemically and their Zn retention capacity was investigated by immersing isolated cuticular membranes in Zn containing solution.

The long term retranslocation of foliar absorbed Zn was investigated by applying the stable isotope ⁶⁸Zn onto the intact leaves of trees grown in the field. Zn solution at 250 ppm was applied as a 50 µl drop on the confined areas (two lanolin rings on the mid-section of the leaf) at each side of the adaxial surface separated by the midvein. Treated leaflets (five each time) were harvested at designated time and analyzed for ⁶⁸Zn concentration in each leaflets. The ⁶⁸Zn from foliar application was estimated using the isotope ratio determinations by ICP-MS. The disappearance of ⁶⁸Zn in the treated leaves with specific time was considered to have been retranslocated to other plant parts.

RESULTS

Results of experiments conducted in 1993 and 1994 have been summarized previously (1993-1994 annual report). The key results of 1995 experiment are as follows: **Residual effect of 1994 spring flush spray:** Foliar Zn sprays in spring 1994 significantly increased walnut leaf Zn concentration in May 1994 for both non-sprayed and sprayed leaves (not shown, see 1994 report). Walnut leaves from trees that received spring flush Zn spray in 1994 were collected on May 28, 1995 to evaluate the residual effect of such spray in the second year. Walnut leaf zinc concentrations were generally increased by 1 to 5 µg/g in May 1995 (data not shown). This indicates that spring flush zinc spray had only a very small effect on leaf zinc concentration of the following year.

Very little phytotoxic symptom was observed in any treatments.

**Effect of carrier:** Figure 1 shows the result of tissue analysis for both covered leaves and sprayed leaves sampled on May 22, 1995. Trees received a variety of treatments on April 12, 1995 as described in Table 1. Leaf Zn concentration without spray treatment averaged about 24 µg/g in May and 18 µg/g in July. The detected differences in zinc concentration between covered leaves in sprayed trees and leaves in non-sprayed trees is a measure of Zn translocation. Trials in 1993 and 1994 had demonstrated that the translocation of zinc from sprayed parts of the plant to covered leaves was greatest when solutions were sprayed at mid to late spring flush stage, when leaf area expansion was fastest. When Zn sprays were applied at mid spring flush in 1995 season (April 12, 1995), only three treatments (E, F, G) out of the eight treatments significantly increased non-sprayed leaf zinc concentration at 10% level. Contrary to 1993 and 1994 results, zinc sulfate without buffer at 700 ppm in 1995 did not significantly increased covered leaf Zn. The concentration difference used (700 vs. 900 ppm) may partially account for the different results obtained in these growth seasons. More importantly, it confirms the variable effectiveness of foliar Zn spray in fruit trees as reported by many investigators. Such variability is a result of various factors such as leaf characteristic and its physiological age, environmental conditions and spray solution characteristics etc..

Since commercially available powder forms of zinc sulfate (36 %) do not readily dissolve in
water, liquid forms of Zn may have advantages as a foliar fertilizer. Two liquid forms of Zn fertilizers were tested in 1995, liquid zinc sulfate (12% Zn) was used as an alternative for powder zinc sulfate, Ctags® liquid Zn (5% Zn) with N as stimulant for foliar penetration was compared. The results in 1995 season demonstrated that both liquid zinc sulfate and Ctags® liquid Zn were not superior to the powdered forms of zinc sulfate as a foliar fertilizer.

Sprays of zinc sulfate added with 500 ppm Cu depressed the effect of zinc sulfate spray on walnut leaf Zn content in 1993, whereas addition of 300 ppm B and Cu together showed slightly beneficial effect over zinc sulfate alone in 1994.

Addition of boron as Solubor® in Zn sprays (adjusted to pH 5) significantly increased walnut Zn concentration in the covered leaves in 1993 and 1994 trials. However, direct addition of Solubor® to the Zn spray solution without adjusting pH caused severe Zn precipitation and thus reduced the effectiveness of spray. Adjusting pH of spray solutions in the field is difficult if solubor is used. In order to simplify the treatment, Zn complexed with boric acid into a new product --zinc borate is now available. Another fully complexed product, Zinc citrate was tested in 1995. Both of zinc borate and zinc citrate were applied without pH adjustment. Figure 1 shows that they are not effective in enhancing leaf Zn concentration. The ineffectiveness of both products is due to their low solubility.

We also included several experimental zinc complexes which were designed to enhance zinc translocation from the absorption site. Trees receiving experimental Complexes F (zinc sulfate plus malic acid pH 4.9) and G (zinc sulfate plus a series of organic acids and amino acids) show slightly higher zinc concentration in leaves than those zinc sulfate alone, however, the difference is not statistically significant. Treatments E (EDTA-Zn), F, and G also significantly increased leaf Zn concentration of non-sprayed leaves in July 1995 at 10% level (figure 2). An increase of average of 7 to 14 µg/g Zn were observed in these treatments.

Though the organic form of zinc (EDTA) were shown to be the least effective in Zn recovery in the sprayed intact leaves, detached leaves and in isolated cuticular membranes, EDTA-Zn sprayed with surfactant was effective at enhancing Zn status of covered-leaves in field trials in 1994. In 1995, EDTA-Zn sprayed at 800 ppm added with surfactant also significantly increased the covered-leaf Zn concentration. Brennan (1991) reported that foliar applied Zn-EDTA was 1.4-1.7 times more effective than zinc sulfate applied at the GS-14 growth stage in wheat. This may suggest that leaf uptake of Zn-EDTA is low, while translocation of the foliar-absorbed Zn is higher than any inorganic forms of Zn. This phenomenon has also been reported in pea (Pisum sativum) by Ferrandon and Chamel (1988). Stewart et al. (1969) also indicated that Zn-EDTA was effective for foliar application in citrus. Thus we conclude that EDTA-Zn with surfactant is marginally more effective than zinc sulfate as a foliar fertilizer for walnut, though differences in cost of these materials must also be considered.

**Effect of addition of surfactants:** In 1994, we demonstrated that short term penetration of Zn into walnut leaves was enhanced with addition of surfactants in the solution. Sylgard® 309, a superior silicone adjuvant was added at 0.1% in all spray solutions in the field trial of 1995. The significant effect of Zn-EDTA spray in 1995 may indicate that surfactant is an important additive if
EDTA is used as carrier. When zinc sulfate is used, the surfactant Sylgard® 309 may significantly reduce total retention of spray solution by the leaves, thus, reducing the effectiveness of foliar zinc spray.

The first barrier to Zn penetration into walnut leaves is the presence of large amounts of cuticular waxes on the walnut leaf surface. However, the amount of cuticular waxes on the surface is not the only determinant of foliar Zn uptake. Leaf characteristics other than the amount of waxes play an important role in determining the differential uptake capacity with different physiological ages of the leaf. Immature leaves have higher Zn uptake capacity than mature leaves (fig. 3) even though immature leaves have higher surface wax content (80 µg/cm²) than the mature ones (30 µg/cm²). Thus, the relationship between surface wax content in the cuticle (fig. 4) and their Zn uptake capacity suggests that the chemical properties of surface waxes may be more important than the amount of wax present. Of course, the low foliar Zn uptake in walnut leaves can be the result of a single or a combination of chemical and physical properties of walnut leaves. Identification of these characteristics that prohibit Zn uptake would be very useful in determining the strategy to overcome the problem.

DISCUSSIONS AND CONCLUSIONS

In 1993, we demonstrated that foliar sprays of zinc at late dormant and bud break stages were not effective in improving zinc status of walnut leaves. The residual effect of foliar spray applied after harvest is minimum. Foliar sprays at early spring flush were not as effective as mid to late spring flush sprays when walnut leaves are expanding fastest. Sprays after leaves mature were not effective. The most effective method of increasing Zn nutritional status of the walnut tree was application at mid to late spring flush. The stage is recognized when the color of the leaves has just turned to green from pinkish.

The lack of effect from foliar sprays at early spring flush is probably not related to the presence of large amount of surface waxes as a barrier to solute penetration. This is because short-term experiments showed that immature leaves (that uptake more Zn than the mature counterparts) have a higher surface wax content than mature leaves. Thus, the ineffectiveness of foliar spray at early spring flush may be a result of the limited amount of leaf surface area available, in addition to the dilution effect due to rapid leaf expansion. The limited mobility of foliar-absorbed Zn in walnut after leaves mature could be due to either the high binding capacity of mesophyll cells or the immobilization of Zn in the phloem. The Zn affinity test using isolated walnut leaf cells suggests that both live and dead leaf cells have a very high affinity for Zn which would limit the amount of Zn that can be retranslocated to other plant parts.

We found that a large amount of surface cuticular waxes on walnut leaf surface and probably their chemical properties, severely limit the penetration and uptake of foliar applied Zn. This barrier to zinc penetration can be mitigated by the addition of specific surfactants which are capable of significantly reducing surface tension, e.g. Sylgard® 309, Silwet®L-77, and Kinetics®. Use of surfactants in spray solution is especially important when chelated products were applied foliarily because organic chelates of Zn (Zn-EDTA) are less effective at entering the leaf. Although surfactants stimulate penetration of zinc sulfate through leaf surfaces in short term experiment, its beneficial effect is probably compromised by the reduction in solution retention on the leaf surface
caused by the overspreading of spray solutions.

Results of this three year study suggest that foliar Zn sprays should be applied at mid to late spring flush when the leaves are expanding fastest. At this period, walnut trees provide enough leaf area for spray retention and at the same time leaves at this physiological age still possess high Zn uptake capacity. Regardless of time of application the effectiveness of foliar sprays of Zn will be variable from year to year. In all cases the effectiveness of zinc foliar sprays was quite low in subsequent year and sprays would have to be repeated annually to have long term benefit. The standard foliar formulations of 36% zinc sulfate and 12% liquid zinc sulfate (unbuffered) in these experiments were effective in the first two years trial but not in the third year. Application of zinc sulfate pH adjusted to about 5.0 with organic acids and amino acids consistently showed a significant effect in enhancing leaf Zn concentration. Fully complexed product zinc citrate was not superior to zinc sulfate alone. The effectiveness of zinc sulfate added with malic acid and other organic acids may suggests that the presence of some undissociated organic acid in the solution may be helpful for Zn penetration and retranslocation in the plant.

Addition of B as Solubor® (pH adjusted) effectively increased Zn uptake in the first two years of the experiments. The effect of addition of other micronutrients such as Cu and Fe on leaf zinc uptake depended on the relative concentration ratio of these cations. Addition of low concentrations of Cu or Fe (at concentration ratio of 1:5 of Cu/Fe:Zn) were effective in increasing Zn uptake in 1994, while the presence of high concentrations of Cu (1:2 ratio) in 1993 depressed foliar Zn uptake. This suggested that foliar Zn application is compatible with addition of boron and low concentrations of copper. However, when boron was totally complexed as zinc borate, leaf Zn uptake decreased. Adjusting solution pH is especially important if B were applied with Zn since addition of Solubor into zinc sulfate can caused severe precipitation of Zn.

Use of EDTA-Zn with surfactant significantly increased walnut leaf Zn concentration in both 1994 and 1995 trials. EDTA-Zn was marginally superior to zinc sulfate alone. Considering the high cost of organic chelates as opposed to zinc sulfate, the advantage of the former over the latter must be sufficient to justify its use. EDTA-Zn also has the benefit of low phytotoxicity.

In summary, we recommend that foliar Zn sprays should be applied at mid-late spring flush at a rate of 2.0 lb ZnSO₄ (36%) or 4.5 lb Zn-EDTA (15%) per 100 gal. water per acre. The pH of the zinc sulfate spray solutions should be adjusted to about 5.0 using organic acids (malic acid preferred). Surfactants should be added if EDTA-Zn is applied. Sprays of zinc must be repeated annually to have long term benefit. Addition of other micronutrients can be beneficial providing they are used in low amount (0.3 lb elemental Cu or Fe/100 gal). Nitrogen and phosphorous should not be combined with Zn spray.
LITERATURE CITED


Table 1. Walnut foliar sprays at spring flush and its phytotoxic ratings in 1995

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>Concentration ppm (lbs/100 gal.)</th>
<th>Phytotoxic rating</th>
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<tr>
<td>A</td>
<td>6.0</td>
<td>700 (1.3 gal)</td>
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<td>liquid ZnSO₄ (12%) +0.3% N &amp; Cu (0.01%)</td>
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<td>B</td>
<td>5.9</td>
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<td>ZnSO₄ (36%)</td>
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<tr>
<td>C</td>
<td>6.1</td>
<td>800 (4.0)</td>
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<td>Zinc Borate (17%)</td>
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<tr>
<td>D</td>
<td>5.6</td>
<td>800 (6.1)</td>
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<tr>
<td>Zinc Citrate (11%)</td>
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<td>E</td>
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<tr>
<td>Zn-EDTA (15%)</td>
<td></td>
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</tr>
<tr>
<td>F</td>
<td>4.9</td>
<td>700 (1.6)</td>
<td>1</td>
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<tr>
<td>ZnSO₄ (36%) +60 ppm Malic acid</td>
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<tr>
<td>G</td>
<td>4.5</td>
<td>700 (1.6)</td>
<td>1</td>
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<tr>
<td>UCD complex (36%)</td>
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<tr>
<td>F</td>
<td>5.4</td>
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<td>Ctags® liquid Zn(5%)</td>
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<td>H</td>
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<td>Control</td>
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*--- PHYTOTOXIC RATING FROM 0 (NO NECROTIC SPOTS) TO 5 (SEVERE LEAF BURN)
0.1% SURFACTANT SYLGARD 309 WERE ADDED TO ALL ABOVE TREATMENTS
Figure 1. Effect of foliar Zn spray at mid spring flush in April, 1995 on leaf Zn content one month after application. Zn concentration in covered leaves with a * differ significantly from control at 10% level.

1--Treatments are specified in table 1
Figure 2. Effect of foliar Zn spray in April, 1995 on walnut leaf Zn content in July, 1995. Values with a * differs significantly from control at 10 % level.
Figure 3. Changes of foliar absorbed Zn in walnut leaves. Zn isotope was applied as microdrops onto leaf surface.
Figure 4. Relationship between leaf area and leaf epicuticular wax content during the development of pistachio and walnut leaves.