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Foliar zinc applications in *Prunus*: From lab experience to orchard management

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ABSTRACT

In almonds, and the genus prunus in general, there is little research directly relevant to improvement of zinc foliar uptake. In our lab, we have worked for several years to better understand Zn uptake, especially in almonds. This study highlights two experiments that together better elucidate this phenomenon. The first experiment included two field trials to study how Zn retranslocation from mature leaves is affected by fruiting in almonds. The second experiment was a targeted field validation of different zinc formulations, including nitrogen, in a commercially managed almond orchard for three growing seasons. Results indicate that the presence of almond fruit can increase Zn export from adjacent vegetative and woody tissue. It was also demonstrated that leaves from bearing spurs have lower Zn concentration than non-bearing spurs and that long-term Zn adequacy of bearing spurs required that applications of foliar Zn be repeated annually.

1. Introduction

Zinc is essential for the growth and production of plants where it is required in all photosynthetic tissues and is needed for cell division and chlorophyll biosynthesis (Boardman and McGuire, 1990; DalCorso et al., 2014; Mattiello et al., 2015; Sadeghzadeh, 2013). Zinc is also required for auxin metabolism (Skoog, 1940; Tsui, 1948), in fruit set (Omar et al., 2015) and pollen function (Kapoor and Takatsuji, 2006; Pandey et al., 2006; Stover et al., 1999). Zinc is considered to be the most widely-limiting micronutrient for tree fruit production, causing significant economic losses worldwide (Sadeghzadeh, 2013; Swietlik, 2002). Visual symptoms of deficiency in fruit trees include interveinal chlorosis, narrow, pointed leaves, short internodes, delayed opening of vegetative and flower buds, and significant decline in fruit production and quality (Ramos, 1997; Sadeghzadeh, 2013). In almond (*Prunus dulcis*), optimal yields require a high rate of fruit set and adequate development of fruit and seeds; delayed flowering due to zinc deficiency affects pollination and therefore leads to a reduction in fruit set (Brown and Uriu, 1996).

Zinc deficiencies are common in trees grown in alkaline soils (Sanchez et al., 2006), which are typical in fruit growing areas such as in Chile, Australia, Spain and California's Central Valley (Ortega-Blu and Molina-Roco, 2007; Sedberry et al., 1988; Sharma et al., 2013; Wear, 1956). Correction of this deficiency through soil amendment is difficult in alkaline soils, as they have a strong zinc fixation capacity, and it is typically very expensive due to the large amounts of fertilizer and soil amendments that are needed to maintain zinc availability for plants (Razeto and Salas, 1986). In strongly Zn fixing soils, foliar sprays of zinc are widely used and have been shown to be more effective, rapidly available, and ultimately lower cost than soil fertilization. Thus, Zn maintenance sprays are recommended for deciduous fruit trees in many production areas (Swietlik and Faust, 1984).

Research on the use of foliar fertilizers to correct zinc deficiencies have shown variable results. Zinc foliar applications promoted tree vigor, fruit set, and yield in apple (Wojcik, 2007) and orange (Omaima and El-Metwally, 2007). However, fruit set was not significantly affected by zinc treatment compared with the control in almond (Castro and Sotomayor, 1998). Sanchez et al. (2006) indicated that only 7% of the zinc applied to leaf surfaces was recovered in the permanent structure of peach (*Prunus persica*) trees after leaf fall. Similarly, Zhang and Brown (1999b) found that only between 3.5–6.5% of applied Zn was absorbed into tissues of walnut or pistachio and that absorption decreased significantly with leaf age. These results demonstrate that absorption from foliar Zn fertilizers has a very low efficiency. Therefore, research is still needed to improve the effectiveness of zinc foliar sprays, and thereby satisfy the zinc requirements of trees grown on soils where zinc limits plant performance.

Zinc foliar spray efficiency has been examined in some crops in conjunction with urea applications, which supply nitrogen. Studies in wheat, Kutman et al. (2011b) and Kutman et al. (2010) indicated that

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fertilization with both fertilizers had a synergistic effect on grain zinc concentration, which they suggest is the result of the formation of zinc-chelating nitrogenous compounds or increased abundance of zinc transporters. These results were consistent with those in bean (Boaretto et al., 1998). In apple, peach and nectarine, it has been reported that the combination of nitrogen, as urea, with Zn enhances Zn uptake and efficiency (Sanchez et al., 2006; Stover et al., 1999). Specifically, in apple simultaneous application of zinc and urea through soil and foliar application resulted in the highest tree yield and fruit size compared to soil-only or foliar-only (Amiri et al., 2008). However, in another study adding urea to zinc foliar sprays did not influence the effectiveness of the spray or the growth of wheat (Haslett et al., 2001). In California, almond growers have reported alleviation of zinc deficiencies when zinc and urea were applied together, but no field study has been done to test these observations. More studies are required to resolve the inconsistent results from the addition of urea to zinc sprays and to evaluate the practical implications for foliar nutrition.

In almonds, and in *prunus* in general, there is little research directly relevant to improvement of zinc foliar uptake. In our lab, we have worked for several years to better understand Zn uptake from foliar formulations. First, we conducted two field trials to study how Zn retranslocation from mature leaves is affected by fruiting in almonds and then conducted a targeted field validation of different zinc formulations, including nitrogen, in a commercially managed almond orchard for three growing seasons.

2. Materials and methods

Experiment 1. Experiments were conducted to evaluate foliar zinc retranslocation from mature leaves in the presence or absence of adjacent almond fruit. Studies were undertaken to contrast foliar applied Zn absorption and translocation at different vegetative and fruit growth stages.

Experiment 1a. Reproductive growth effects on Zn absorption and retranslocation.

This study was performed in the Pomology orchard of the University of California, Davis. In the first experiment, 13, four-year-old "Non-Pareil" almond [(*Prunus dulcis* (Mill D.A. Webb)] were used. On June 14, 2007, 80 spurs with fruit and 80 spurs without fruit were labeled.

Each leaf of 40 fruiting spurs and 40 non-fruiting spurs were submerged in a $^{68}Zn(NO_3)_2$ solution containing 0.05% L-77 as surfactant (Loveland Industries Inc. Greeley, CO) in a test tube for 5 s. The petiole of the leaf was not submerged in the ^{68}Zn solution. The remaining 40 fruiting spurs and 40 non-fruiting spurs were used as controls and were submerged in a Zn free 0.05% L-77 solution.

The $^{68}Zn(NO_3)_2$ solution was prepared as follows: 9.2 mg of ^{68}ZnO (Isotec. Inc. Miamisburg, OH) was added to 0.238 ml of 1N HNO_3 plus 3 ml of double deionized water. The 1N HNO_3 was just sufficient so that the reaction of ^{68}ZnO with HNO_3 would be complete. The suspension was then shaken overnight. Once the ^{68}ZnO was completely reacted with HNO_3, the $^{68}Zn(NO_3)_2$ solution was brought to pH 5.5 with 1N NaOH then 7.5 µl of L-77 was added. Finally, the solution was diluted to 15 ml with high purity water. The ^{68}ZnO was enriched with 95.1% of ^{68}Zn atom, and with 4.9% Zn atom% as the combination of ^{64}Zn, ^{66}Zn, and ^{67}Zn.

After treatment, sampling was made on days 1, 5, 10, and 20. Ceramic scissors were used to cut the petiole from the leaf, the leaf blade was discarded and the petiole retained for analysis. Each sample was composed of 6 petioles, each of which came from 6 different spurs. After drying, the petioles were ashed at 500 °C for 4 h, redisolved in hot 1N HNO_3 and made to a known volume and the Zn isotopes in the samples analyzed by ICP-MS. On July 12, the experiment was repeated on different branches of the same trees. 12.6 mg of ^{68}ZnO were added to 0.4 ml of 1N H_2SO_4 and 4.6 ml of H_2O and shaken overnight. 1N of NaOH was used to make pH 5.5. After pH adjustment and the addition of 10 µl of L-77, the solution was made to 20 ml for a final Zn concentration of 500 ppm The ^{68}ZnO contained 98% ^{68}Zn atom, and 2% Zn ^{64}Zn, ^{66}Zn, and ^{67}Zn. The experiment was done in essentially the same way as used on June 14, 2007. Due to fruit loss by birds some of the 10 and 20 day samples consisted of 5 petioles instead of the 6. The major difference between June and July labeling was the use of $^{68}Zn(NO_3)_2$ in June, and $^{68}ZnSO_4$ solution in July.

The results are reported as a ^{68}Zn:^{67}Zn ratio in the sampled petioles, a shift in 68:67 ratio can be used to calculate the contribution of foliar Zn applications to Zn transport through the petiole.

Experiment 1b. Vegetative growth and Zn movement

On August 9, 2007, 96 actively growing relatively uniform almond shoots were selected. 48 were randomly chosen for ^{68}Zn labeling and the remaining 48 shoots were used as the control. ^{68}ZnO (98% ^{68}Zn) was used to generate a final Zn concentration of 666 ppm pH 5.5 containing 0.05% L-77 as described above. From each ^{68}Zn-treated shoot, one recently fully mature leaf was tagged (about 5–10 cm from the shoot tip) and submerged in the $^{68}ZnSO_4$ solution for 5 s. Similarly as in the previous experiment, the petiole of the leaf was not submerged in the ^{68}Zn solution. The control was treated in the same way, but only with 0.05% L-77 solution.

After treatment, time series sampling was made at day 1, 7, and 14. At the time of sampling a 1 cm long stem excised bark piece either above or below the treated leaf was excised with a razor blade. A 1 cm stem tip with the leaf removed was also sampled. When the separation of the bark from the wood was difficult, the whole stem (including the xylem and phloem) was used for analysis. Finally, the ^{68}Zn-treated leaf-blades were collected with the 1 cm portion close to the petioles discarded. Every four shoots compose one replicate, and each treatment had four replicates. All of the samples were dry ashed and the Zn isotopes were analyzed by ICP-MS as described above.

Experiment 2. Targeted field validation

Field validation of results with select Zn products was conducted in a commercial Almond Orchard for three growing seasons at Belridge, Kern County, CA (35°N, 119°W). Almond trees var. 'Nonpareil' and 'Monterey' were planted in 1998 in an alternating pattern of one row 'Nonpareil' and one row of 'Monterey'. By the time experiment started in 2009, trees were fully mature and highly productive (average yield of 3500 kernel kg/ha/year). A randomized complete block design with six treatments and four repetitions per treatment block combination was established. Each experimental unit included a set of 10 trees, but only the 8 middle trees per experiment unit were sampled. The other trees

Table 1
Zn formulations used in the targeted field validation experiment (Experiment 1).

Material Name	Zn Concentration in final spray solution (ppm)	Comments (labeled Zn composition of undiluted product)
Zn CHO complex 2	350	7% of Zn as Zn Nitrate. 0.2% Urea nitrogen. 2.8% Nitrate nitrogen with mannitol.
Amino complex Zn 1	400	6.8% Zn as an undisclosed amino acid Zn complex.
UC Davis Formula 1	1000	25% Zn. Non-commercial product derived from Zn sulfate, Zn nitrate and Ca nitrate.
UC Davis Formula 2	1000	25% Zn. Non-commercial product. Zn sulfate, Zn nitrate and Ca chloride.
Zn Sulfate	2000	36% Zinc sulfate.
No application	N.A.	No application.