Zinc Content and Fruit Quality of Pecan as Affected by Application of Zinc Sulfate

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Abstract. Pecan [Carya illinoinensis (Wangenh.) K. Koch] is an important nut tree species, and Zn nutrition is critical for its growth and nut production. The aim of this study was to provide a perspective on Zn accumulation in pecan fruit over time and to determine the effects of Zn application on fruit quality. Two concentrations of ZnSO4 (0.4% and 1.6%) were sprayed onto the shuck or the nearest pair of leaflets. Purified water was sprayed similarly as the control. The results show that Zn application to the shuck and leaves increased the Zn concentration and content in embryos. The greater the Zn concentration of the Zn solution sprayed, the greater the Zn concentration in the embryo. The greatest zinc concentration in the embryo was found during the early stage of embryo development. In the treatment during which 1.6% ZnSO4 was sprayed onto the shuck, the Zn concentration in the early embryo was 242.91 mg·kg–1, which was 2.2 times that of the control. Thereafter, embryo Zn concentration decreased gradually until maturity. The treatments could be ranked, from greatest Zn concentration in the mature embryo to least, as follows: 1.6% ZnSO4 on shuck (66.36 mg·kg–1) > 1.6% ZnSO4 on leaflets (64.28 mg·kg–1) > 0.4% ZnSO4 on shuck (55.51 mg·kg–1) > 0.4% ZnSO4 on shuck (49.67 mg·kg–1) > control (47.34 mg·kg–1). A model was presented that showed Zn was transported from the shuck and leaves to the stalks through the conducting tissue, and then to the embryo through the embryo sac. The application of 0.4% ZnSO4 to the shuck resulted in the greatest oil content (74.05%), which was 10% greater than that in the control. Applications of 0.4% ZnSO4 to the shuck and leaflets resulted in a greater proportion of oleic acid (~69%) and a lower proportion of linoleic acid (~20%), palmitic acid (~6.0%), and linolenic acid (~1.1%). The results of this preliminary investigation are useful for exploring the mechanism of action for Zn on pecans.

Zn is an essential micronutrient for plants, and it plays an important role in physiologic metabolism, hormonal regulation, and membrane structure and function (Ojeda-Barrios et al., 2014). It is also a cofactor for a variety of enzymes with roles in protein synthesis, membrane stability, cell division, and metabolism (Hajiboland and Amirzad, 2010). The application of Zn fertilizer at appropriate rates can enhance the fruit quality of fruit trees significantly (Davarpanah et al., 2016). Saadati et al., (2013) studied the effects of Zn foliar spray on olive trees at the fruit ripening stage and found that Zn application increased the oil content, decreased the soluble carbohydrate content, and increased the ratio of unsaturated fatty acids to saturated fatty acids. Razzaq et al. (2013) found that foliar application of 0.4% ZnSO4 in ‘Kinnow’ mandarin trees increased the total number and weight of fruit per tree at harvest. Pecan [Carya illinoinensis (Wangenh.) K. Koch] belongs to the Juglandaceae family and originates from North America (Hal, 2000). Pecans are a delicious and nutritious food rich in unsaturated fatty acids, protein, fiber, minerals, vitamins, and many other bioactive substances, including phytoestrogens and phenolic antioxidants (Huang et al., 2017). The Zn content of pecan kernels is high, so they serve as a natural Zn supplement for humans (Glenny and Allen, 2007). Zn nutrition is critical for pecan growth and nut production (Ojeda-Barrios et al., 2014; Walworth et al., 2006). It is instrumental in improving fruit set because it is involved in the metabolism of auxin, which is responsible for fruit set and growth (Núñez-Moreno et al., 2009). Zn deficiency causes pecan leaf disease and reduction in nut yield, resulting in serious economic losses (Hu and Sparks, 1990; Sparks, 1993). Recent studies have shown that the application of 0.4% ZnSO4 to the leaves of pecan seedlings promoted their growth and photosynthesis (Ashraf et al., 2013; Hussain et al., 2007; Yazdi and Khorsandi, 2008). However, the effect of Zn on the development of pecan fruit after Zn application has not been investigated. Considering the importance of Zn in the growth and nut production of pecan, the aim of this study was to provide a perspective on Zn accumulation in the pecans over time and to increase Zn and oil content in the pecan embryo by Zn application.

Materials and Methods

Experimental site

The study was conducted in 2016 at an orchard in Xinchang, Zhejiang Province, China (lat. 30°15′N, long. 119°58′E; elevation, 50 m). The average annual rainfall in this region is 1478 mm, and the minimum and maximum temperatures are –9℃ and 40℃, respectively. The soil texture is red yellow earth with a clay bottom (Shi et al., 2004), and its chemical properties are as follows: The concentrations of total Zn (perchloric acid and hydrofluoric acid method) (Chapman et al., 1949) and available Zn (0.1 mol·L–1 hydrochloric acid) in 0 to 20 cm of soil were 107.62 mg·kg–1 and 2.61 mg·kg–1, respectively. The concentrations of total Zn and available Zn in 20 to 40 cm of soil were 110.72 mg·kg–1 and 1.39 mg·kg–1, respectively. pH was 4.8 (2.5:1 soil:water).

Plant materials and treatments

The experiment was performed on 13-year-old trees of the pecan cultivar Western. The orchard owner managed all orchard operations except fertilizer application during the experiment. Three trees with more than five scaffold branches with many fruit clusters were selected for the Zn application treatments, and artificial pollination was conducted on 1 May. Leaf nutrient concentrations of the three trees at 85 d after pollination (DAP) are shown in Table 1. “Rating” in the table refers to a comparison of our results with those of Pond et al. (2006). Leaf Zn concentration of the three trees was normal. Two concentrations of ZnSO4 (0.4% and 1.6%) were sprayed onto the shuck or the nearest pair of leaflets to the fruit. Purified water was sprayed similarly as the control. Each solution was sprayed using a 1-L spray until the solution ran off; plastic film was used to prevent the solution from being sprayed onto other tissue. Three replicates were used, and each replicate was sprayed off separately. The method is described in “Metal concentrations.”

Table 1. Leaf nutrient concentration before initiation of Zn treatments (at 85 d after pollination). The method is described in “Metal concentrations.”

| Element | Result | Rating |
|---------|--------|--------|
| Zn (mg·kg–1) | 172.63 ± 40.16 | Normal |
| Cu (mg·kg–1) | 6.85 ± 3.16 | Normal |
| Fe (mg·kg–1) | 44.25 ± 6.88 | Normal |
| Mn (mg·kg–1) | 691.46 ± 233.80 | High |
| K (%) | 1.35 ± 0.23 | Normal |
| Mg (%) | 0.61 ± 0.03 | High |
| Ca (%) | 1.67 ± 0.1573 | Normal |

*Rating compares our results with those of Pond et al. (2006).
consisted of one tree. The trees were sprayed before 10 AM to avoid rapid evaporation of water from the solution. Treatments were applied twice during the fruitlet stage: at 85 DAP and 95 DAP.

**Plant measurements**

*Fruit sampling.* Fruit samples were selected randomly, harvested from the tree, and then transferred to the laboratory. Fruit was harvested at 105 DAP (early cotyledon stage) and then every 15 d until 165 DAP (maturity). Ten fruit per time per tree were harvested for analysis. The 30 fruit were separated into shuck, shell, liquid endosperm, and embryo.

*Dry weight.* The shuck, shell, and embryo were oven-dried at 65 °C to a constant weight and then weighed using an electronic balance with an accuracy of 0.001 g. Dried shuck, shell, and embryo were ground and stored in air-tight containers until analysis.

*Metal concentrations.* Dried shuck, shell, embryo samples (0.20 g dry weight), and liquid endosperm (2.00 g) were dissolved in 98% sulfuric acid, digested at 250 °C, clarified by adding H₂O₂, and then diluted with deionized water to 50 mL before determining the concentrations of Zn, Cu, Fe, K, Mn, Mg, and Ca using an atomic absorption spectrometer (iCE 3300; Thermo Fisher Scientific, Yokohama, Japan) (Khan et al., 2012).

**Statistical analysis**

Data were analyzed using SPSS ver. 17 (SPSS Inc., Chicago, IL). One-way analysis of variance was used for processing the results; the least significant difference test (α = 0.05) was used to determine the mean separation among treatments. Three replicates were used and each replicate consisted of one tree. The values of total Zn content and total Zn content in the whole fruit were determined using the following equations:

\[
\text{Total Zn content} = \text{Mean dry weight} \times \text{Mean Zn concentration}
\]

\[
\text{Total Zn content in whole fruit} = \frac{\text{Total Zn content (Shuck + Shell + Embryo)}}{3}
\]

**Results**

**Fruit appearance during pecan embryo development**

Figure 1 shows pecan embryos developed between 105 and 165 DAP in the control. During the early cotyledon stage (105 DAP), the embryo was watery and transparent, with a diameter of less than 5 mm. The embryo was located far from the stalk end and close to the embryo sac, which was filled with liquid endosperm. During the midcotyledon stage (120 DAP), the embryo diameter increased to about 10 mm. The embryo grew toward the base, and some endosperm remained. During the late cotyledon stage (135 DAP), the embryo diameter increased to 15 mm. The embryo developed a flavescent surface surrounding the fleshy insides, and the endosperm was completely absorbed. At the full cotyledon stage (150 DAP) and at maturity (165 DAP), the embryo grew slightly and the shuck began to shrink.

**Dry weight**

As shown in Fig. 2A, the shuck weight increased from 1.38 g/nut (105 DAP) to 3.42 g/nut (165 DAP) in the control. The application of 0.4% ZnSO₄ to the shuck or leaves resulted in more shuck weight than in other treatments at 135 DAP. As shown in Fig. 2B, the shell weight decreased from 3.48 g/nut (105 DAP) to 1.91 g/nut (165 DAP) in the control. The Zn treatments did not affect shell weight significantly. As shown in Fig. 2C, embryo weight increased rapidly from 120 to 135 DAP, and then increased slowly. The application of 0.4% ZnSO₄ to the leaves resulted in greater embryo weight than seen in other treatments at 150 DAP.

**Zn concentration in pecan fruit**

*Zn concentration in the shuck.* As shown in Fig. 3A, the Zn concentration in the pecan fruit shuck in the control was about 100 mg·kg⁻¹. As the fruit developed, the Zn concentration increased first and then decreased, reaching the greatest value at 120 DAP (134.29 mg·kg⁻¹) in the control. There was a significant difference among the means within a sampling date. The application of 1.6% ZnSO₄ to the shuck increased the Zn concentration in the shuck to 176.28 mg·kg⁻¹ at 165 DAP, which was about double that in the other treatments.

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**Fig. 1.** Changes in shape of pecan embryo and fruit 105 to 165 d after pollination (DAP).

**Fig. 2.** Dry weight of the (A) shucks, (B) shells, and (C) embryos. Symbols represent mean values and short vertical lines indicate se. The significance of the effect of the developmental stage was tested by analysis of variance (*P < 0.05, **P < 0.01). The unit of measure on the x-axis is days after pollination.
**Zn concentration in the shell.** As shown in Fig. 3B, the Zn concentration in pecan shells in the control was about 35 mg·kg⁻¹. As the fruit developed, the Zn concentration decreased, increased, and then decreased again, with the greatest value at 135 DAP (54.42 mg·kg⁻¹). The application of 1.6% ZnSO₄ to the shuck increased the Zn concentration in the shell to 74.39 mg·kg⁻¹ at 135 DAP, which was greater than that seen in other treatments. The applications of ZnSO₄ increased the Zn concentration in the shell slightly at 165 DAP.

**Zn concentration in the endosperm or embryo.** As shown in Fig. 3C, at 105 DAP, the Zn concentration in the endosperm was very low (about 3 mg·kg⁻¹). The Zn treatments did not affect the Zn concentration in the endosperm significantly. At 120 DAP, the Zn concentration in the embryo was significantly greater. After application of 1.6% ZnSO₄ to the shuck, the Zn concentration in the embryo was 242.91 mg·kg⁻¹, which was about 2.2 times that of the control. When the embryo developed to 135 DAP, the Zn concentration decreased rapidly (Zn concentration in the control, 67.62 mg·kg⁻¹). At 165 DAP, the Zn concentration in the control decreased to 47.34 mg·kg⁻¹. The treatments could be ranked from greatest Zn concentration to least: 1.6% ZnSO₄ on shuck > 0.4% ZnSO₄ on shuck > 0.4% ZnSO₄ on leaflets > 1.6% ZnSO₄ on shuck > control. As shown in Fig. 4A, the Zn content in the whole fruit (165 DAP) in the control was about 7.07 mg·kg⁻¹, which was less than that seen in the other treatments, and the application of 0.4% ZnSO₄ to the shuck resulted in the lowest Mn and K concentrations (0.31%, respectively). There was a significant negative correlation between the Zn and Fe and Zn and Mn in the endosperm and embryos at 135 DAP (correlation coefficient, 0.978). There were significant negative correlations between Zn and Fe and Zn and Mn in the embryo at 135 DAP (correlation coefficients, 0.942 and 0.920, respectively). There was also a significant positive correlation between Zn and Cu at 150 and 165 DAP.

**Total Zn contents in the shuck, shell, embryo, and whole fruit.** As shown in Fig. 4A, the Zn content in the shuck increased from 108.96 μg/nut (105 DAP) to 258.26 μg/nut (165 DAP) in the control. The application of ZnSO₄ to the shuck can increase the Zn content in the shuck. The application of 1.6% ZnSO₄ to the shuck increased the Zn content in the shuck to 590.83 μg/nut at 165 DAP, which was about double that seen in the control. As shown in Fig. 4B, the Zn content in the shell decreased from 150.46 μg/nut (105 DAP) to 45.50 μg/nut (165 DAP) in the control. Zn application to the shuck and leaves increased the Zn concentration in the shell at 165 DAP. As shown in Fig. 4C, Zn application to the shuck and leaves increased the Zn content in the embryo at 165 DAP. The treatments could be ranked from greatest Zn concentration to least: 1.6% ZnSO₄ on shuck > 1.6% ZnSO₄ on leaflets > 0.4% ZnSO₄ on shuck > 0.4% ZnSO₄ on leaflets > 1.6% ZnSO₄ on shuck > control. As shown in Fig. 4D, Zn application to the shuck and leaves increased the Zn content in the whole fruit at 165 DAP. The applications of 1.6% ZnSO₄ to the shuck resulted in more Zn content in the whole fruit than seen in other treatments between 105 and 165 DAP.

**Metal concentrations in the endosperm and embryo.** As shown in Fig. 5, at 105 DAP, the Cu, Fe, Mn, K, Mg, and Ca concentrations in the endosperm were very low. As the embryo developed, the metal concentrations increased at 120 DAP and then decreased at 135 DAP. At 165 DAP, the Cu concentration (Fig. 5A) in the control was 7.07 mg·kg⁻¹, which was less than that seen in the other treatments, the Mn (Fig. 5C) and K (Fig. 5D) concentrations in the control (125.21 mg·kg⁻¹ and 1.15%, respectively) were greater than those seen in the other treatments, and the application of 0.4% ZnSO₄ to the shuck resulted in the lowest Mn and K concentrations (42.09 mg·kg⁻¹ and 0.64%, respectively) in all treatments. The Zn treatments did not affect the concentration of Fe (Fig. 5B), Mg (Fig. 5E) and Ca (Fig. 5F) significantly in the embryo at 165 DAP. And the Fe, Mg, and Ca concentrations in the control were 51.83 mg·kg⁻¹, 0.22% and 0.31%, respectively.

**Relationships between Zn and elements in endosperm and embryos.** As shown in Table 2, there was a significant negative correlation between the Zn and Ca concentrations in the endosperm at 105 DAP (correlation coefficient, 0.978). There were significant negative correlations between Zn and Fe and Zn and Mn in the embryo at 135 DAP (correlation coefficients, 0.942 and 0.920, respectively). There was also a significant positive correlation between Zn and Cu at 150 and 165 DAP.
Significant at $P < 0.05$ or $P < 0.01$, respectively. Confidence interval, 95%.

**Discussion**

Zn is absorbed by plants in the form of $Zn^{2+}$, and it has moderate mobility in plants compared with that of other trace nutrients (Fargasová, 2004; Wadsworth, 1970). Comparisons of Zn concentrations among the various parts of the pecan fruit showed that Zn application had the strongest effect on the Zn concentration in the embryo. Zn applications did not increase the Zn concentration in the endosperm at 105 DAP ($\approx 3$ mg kg$^{-1}$). This indicated that the Zn concentration in the endosperm was very low, and so the developing embryo depended on transported Zn for its growth and development. Foliar application of ZnSO$_4$ increased the Zn concentration in the embryo significantly, but not in the shuck, indicating that the Zn applied to the leaves was transported and targeted to the region of vigorous growth in the fruit. Zn can be loaded into the phloem tissue and then is subjected to long-distance phloem transport to those tissues where it is required (Mengel, 2002). Based on our results, we provide a model for Zn transport to the embryo in pecan fruit at the early stage of development (Fig. 6). The Zn applied to the shuck and leaves was transported through the conducting tissue to the stalks, and then to the embryo through the embryo sac.

At 135 DAP, the Zn concentration in the shell generally increased over time through the season, which was unrelated to treatments, probably because of the rapid hardening of the shell at $\approx 135$ DAP. With the fast accumulation of organic matter and the embryo rapidly expanded, the metal concentrations in the embryo decreased quickly. The Zn concentration in the embryo in the control decreased from 106.44 mg kg$^{-1}$ at 120 DAP to 67.62 mg kg$^{-1}$ at 150 DAP, and there was little difference among treatments at 150 DAP. The result of this Zn concentration change is consistent with foliar Zn applications during seed development in wheat (Ozturk et al., 2006). The greatest Zn concentration in the seeds was found in the first stage of seed development; thereafter, seed

Table 2. Correlation coefficients (Pearson $r$ values) between Zn and other metal elements in pecan endosperm and embryo.

| Element | 105 DAP | 120 DAP | 135 DAP | 150 DAP | 165 DAP |
|---------|---------|---------|---------|---------|---------|
| Cu      | 0.553   | -0.582  | 0.338   | 0.997** | 0.944*  |
| Fe      | -0.102  | -0.942* | 0.733   | 0.520   | 0.375   |
| Mn      | 0.606   | -0.920* | 0.217   | 0.633   | -0.085  |
| K       | 0.112   | 0.426   | 0.665   | 0.814   | 0.288   |
| Mg      | -0.018  | 0.705   | 0.650   | 0.481   | 0.331   |
| Ca      | -0.978**| -0.571  | 0.020   | -0.173  | -0.348  |

* Significant at $P < 0.05$ or $P < 0.01$, respectively. Confidence interval, 95%.

**Discussion**

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Table 3. Effects of zinc applications on oil content and fatty acid composition of mature embryo (measured as a percentage).

|                  | Control | Zn 0.4% + leaf | Zn 0.4% + shuck | Zn 1.6% + leaf | Zn 1.6% + shuck |
|------------------|---------|----------------|----------------|----------------|----------------|
| Oil content      | 64.71 ± 0.12 c | 70.16 ± 0.06 b | 74.05 ± 0.12 a | 66.99 ± 0.01 c | 66.55 ± 0.11 c |
| Palmitic C16:0   | 6.31 ± 0.22 a  | 5.89 ± 0.39 b  | 6.05 ± 0.37 b  | 6.40 ± 0.02 a  | 6.35 ± 0.08 a  |
| Stearic C18:0    | 2.96 ± 1.63 a  | 2.94 ± 0.39 a  | 3.60 ± 0.43 a  | 3.08 ± 0.32 a  | 3.02 ± 1.49 a  |
| Oleic C18:1      | 65.66 ± 1.14 b | 68.90 ± 1.01 a | 69.34 ± 0.82 a | 64.40 ± 0.27 bc| 63.22 ± 1.59 c |
| Linoleic C18:2   | 23.36 ± 0.09 b | 20.78 ± 0.02 c | 20.19 ± 0.06 c | 24.30 ± 0.02 ab| 25.49 ± 0.09 a |
| Linolenic C18:3  | 1.44 ± 0.10 a  | 1.14 ± 0.07 b  | 1.12 ± 0.09 b  | 1.47 ± 0.08 a  | 1.50 ± 0.01 a  |
| Arachidic C20:0  | 0.01 ± 0.05 a  | 0.11 ± 0.05 a  | 0.05 ± 0.03 a  | 0.08 ± 0.02 a  | 0.16 ± 0.01 a  |
| Eicosenoic C20:1 | 0.25 ± 0.31 a  | 0.26 ± 0.71 a  | 0.27 ± 0.37 a  | 0.28 ± 0.02 a  | 0.27 ± 0.20 a  |
| Unsaturated oil  | 90.84 ± 0.29 a | 90.93 ± 0.69 a | 90.92 ± 0.34 a | 90.38 ± 0.02 a | 90.47 ± 0.19 a |
| Saturated oil    | 9.18 ± 2.15 a  | 9.08 ± 1.55 a  | 9.10 ± 2.23 a  | 9.61 ± 0.25 a  | 9.52 ± 0.25 a  |

The means refer to three repetitions ± SD. Different letters indicate significant differences among treatments at $P < 0.05$ by least significant difference.
Zn concentration decreased gradually until maturity. The Zn contents in the shuck and embryo increased, and the Zn content in the shell decreased in the fruit over time (Fig. 4). This indicates that Zn in the shell may be transported to the shuck and embryo as the fruit develops. In addition, Zn application to the shuck and leaves increased the Zn content in the whole fruit between 150 and 165 DAP. This indicates that Zn in the branch will be transported to the fruit during the late fruit development. The additional Zn in the fruit of Zn-treated plants probably came from the ZnSO₄ fertilizer that was applied, or soil-derived Zn. To demonstrate that the additional Zn in the pecan fruit actually came from the applied Zn treatments would have required another experiment. For example, using isotopically labeled ZnSO₄ would have allowed us to track the transport of the Zn.

Our data show that Zn applications can increase Zn concentration in the embryo and affects the concentration of other metals. There were significant negative correlations between Zn and Fe and Zn and Mn in the embryo at 135 DAP. This may be the result of the high concentration of Zn in the early stage of embryo development, resulting in a deficiency of Fe and Mn. Sagardoy et al. (2009) suggested Zn-induced Fe deficiency based on the fact that the two metals have similar ion radii. And Ruano et al. (1987) reported that a large Zn supply strongly decreases the Mn concentration of plants. At 150 to 165 DAP, Zn correlated positively with Cu, and the concentrations of Zn and Cu decreased as the oil content increased, indicating that Zn and Cu have similar accumulation patterns in the kernel.

In our study, the application of 0.4% ZnSO₄ to the shuck resulted in the greatest oil content (74.05%), which was 10% greater than that of the control. These results indicate that the application of Zn at appropriate concentrations can increase the oil content. These findings are consistent with those of Upadhyay and Badyal (2008), who found that Zn-treated pecan trees produced more and greater quality nuts. Recent studies have shown that improvements in fruit quality are achieved mainly through improvement in photosynthesis (Hajiboland and Amirzad, 2010; Hu and Sparks, 1991; Kim et al., 2002; Ojeda-Barrios et al., 2012; Tavallati, 2017). Another study found that the application of 0.4% ZnSO₄ to the leaves of pecan seedlings promoted their photosynthesis (Ashraf et al., 2013). Therefore, we speculate that application of 0.4% ZnSO₄ to the shuck promoted photosynthesis, leading to greater oil content.

Conclusions

Our results showed that Zn application to the shuck and the leaves can increase the Zn concentration and content in embryos. Zn was transported from the shuck and leaves through the conducting tissue to the stalks, and then to the embryo through the embryo sac. The greater the concentration of the Zn solution sprayed, the greater the Zn concentration in the embryo. The application of 0.4% ZnSO₄ to the shuck resulted in the greatest oil content (74.05%), which was 10% greater than that of the control. The results of this preliminary investigation are useful for exploring the mechanism of action for Zn on pecan.

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