CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
FERTILIZER RESEARCH AND EDUCATION PROGRAM

Final Report – 2008-2010

Project Title: Comparing the Efficiency of Different Foliarly-Applied Zinc Formulations on Peach and Pistachio Trees by Using ^{68}Zn Isotope

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Introduction

Zinc (Zn) deficiency is a major concern in California fruit and nut orchards. Peach has been identified as particularly prone to this disorder. The problem is so widespread that foliar Zn sprays are applied on a routine basis even when no deficiency symptoms are observed. Rates of application can be very high, especially in pistachio orchards where recommendations of 40 lbs zinc sulfate/acre have been published. Since only a small fraction of the applied amount is needed to correct a deficiency, most of the Zn is simply wasted. This is not only a financial burden on the grower (especially since early 2006 when zinc prices skyrocketed) but is also an environmental problem that is not easy to remedy. Zinc is a heavy metal that will slowly build up in the soil and can eventually become a contaminant. Thus there is a great need to improve the efficiency of zinc foliar sprays.

We had a zinc project with FREP that ended in 2007. This project allowed us to approach the objective of improving fruit and nut tree Zn nutrition from many different angles. First, we made good progress on sampling for Zn within a peach tree and have an improved procedure for determining tree Zn status. Second, we tried several techniques for improving root uptake efficiency and have a very promising approach with newly planted trees. Finally, we focused a lot on improving foliar Zn uptake. This is the area we feel has the greatest potential for improving Zn uptake efficiency. Using a labeled ^{68}Zn isotope, we have been able to quantify the amount of zinc sulfate that gets into permanent structures of the tree from a fall foliar application, and also trace its movement into roots and eventually into new growth in the spring.

There is one area of zinc nutrition where we have not made as much progress as hoped – a comparison of different Zn formulations. As part of our project, Patrick Brown at UC Davis compared foliar sprays of 7 Zn formulations on Arabidopsis, a small plant that can be grown quickly and easily in growth chambers. He found some formulations supplied Zn to the plant more efficiently than others. Since Arabidopsis is quite different from fruit trees in several ways, we decided to try a similar experiment on peach seedlings in the greenhouse. In our first trial we found no statistical differences among formulations. Because only about 5% or less of the foliar-applied zinc gets out of the leaf and into the rest of the plant, this small amount becomes difficult to detect. We have concluded that the best way to detect this small amount, and the likely subtle differences among formulations, is by incorporating the ^{68}Zn isotope into each formulation. This approach allows us to more precisely quantify uptake efficiency of the different formulations with both peach and pistachio.

Statement of Objectives

1. To incorporate the ^{68}Zn isotope into some commonly used zinc formulations such as sulfate, EDTA chelate, oxide, amino acid or poly amine complex, citrate, lignosulfonate, fulvic acid, neutral-52%, nitrate etc.
2. To test the foliar uptake efficiency of these formulations on peach and pistachio seedlings with and without different types of surfactants.
3. Using the best treatments from objective 2, treat young peach and pistachio trees with ^{68}Zn in the field.
4. To test the most efficient Zn treatments in commercial peach and pistachio orchards.

Executive Summary

The focus of this project was to compare the uptake efficiency of different foliar zinc formulations on peach and pistachio trees. A protocol was developed using peach seedlings that was very effective at sorting out some of the more commonly used formulations. Through a series of experiments 12 different formulations were tested and separated into five categories of effectiveness. It was concluded that zinc nitrate is the most effective material, but zinc sulfate, which is considerably less expensive, is the most cost effective. A field test in a commercial orchard using labeled ^{68}Zn sulfate vs. ^{68}Zn nitrate showed no difference between the two formulations, supporting our conclusion that zinc sulfate is the material of choice. There was no benefit from adding a surfactant to the solution. Experiments to determine the best timing for fall applications of zinc sulfate were inconclusive. Experiments with pistachio trees proved to be more challenging as it was much more difficult to get zinc into these plants. Nevertheless, a seedling procedure was developed for comparing formulations and very similar results were obtained. The field experiment with pistachio was inconclusive.

Work Description and Results, Discussion and Conclusions

Task 1. Incorporate the ^{68}Zn isotope label into different zinc formulations (Objective 1). The ^{68}Zn label was incorporated into five different formulations. The material came from the manufacturer as Zn oxide. Some of this was then converted to Zn sulfate by a chemist at UC Davis in 2006. Then, working with a chemist from Monterey Ag Resources we incorporated the label into three more products – Zn EDTA in 2008 and Zn nitrate and Zn chloride in 2009. These products showed a wide range of zinc uptake efficiency in peach and pistachio seedling tests. In 2009 and 2010 we took some of these labeled materials to the field (see Objective 4). We had originally planned to incorporate the label into other formulations, but our seedling tests showed they all had lower uptake efficiency than sulfate, nitrate and chloride, so we decided it was not necessary.

Task 2. Test the formulations with ^{68}Zn label on peach and pistachio seedlings in the greenhouse (Objective 2).

Subtask 2.1. Test the formulations with ^{68}Zn label on peach seedlings in the greenhouse. As we started to run tests with the labeled formulations, we discovered a procedure with non-labeled materials that was very effective at separating out the different formulations and was considerably less expensive. Thus, we focused on this procedure for over a year and ran several experiments that allowed us to compare 12 different formulations (Table 1). The procedure involved Nemaguard peach seedlings grown in a greenhouse under conditions that induced noticeable zinc deficiency. Foliar sprays of zinc formulations then overcame these symptoms within 20 to 30 days. The degree of recovery demonstrated the relative effectiveness of the material.

Table 1. Zinc formulations used in experiments to treat Nemaguard peach seedlings showing symptoms of zinc deficiency.

Formulations	% Zn	Name	Company	Comments
Experiment 1				
Zinc Sulfate	36	Zn Sulfate	AG Specialties	Widely used in orchards
Zinc EDTA	9	Sequestar 9% Zinc Chelate solution	Monterey Ag. Resources	Derived from Zn $(NH_4)_2$ EDTA
Zinc Oxysulfate	52	Neutral or Basic Zinc	Monterey Ag. Resources	Mostly insoluble (ZnO). Has 1.25% soluble Zn $(ZnSO_4)$
Zinc Leonardite	6.5	Actagro 6.5% Zinc	Actagro	Zn from $ZnSO_4$ + organic acids derived from leonardite
Zinc Polyamine	5.8	Zinc PolyAmine	Northwest Agricultural Products (NAP)	Derived from zinc sulfate; chelated with organic and amino acids.
Experiment 2				
Zinc Sulfate	36	Zn Sulfate	AG Specialties	Widely used in orchards
Zinc Nitrate Mix	3.8	Formula 1	Patrick Brown's Mixture	Mixture of Zn $(NO_3)_2$ and other chemicals
Zinc Carbohydrate	6	Zicron-F	Floratine Biosciences	Derived from zinc sulfate monohydrate
Zinc Oxide Suspension	39.8	Zintrac	Pace International (Leffingwell)	Milky suspension of insoluble ZnO
Experiment 3				
Zinc Sulfate	36	Zn Sulfate	AG Specialties	Widely used in orchards
Zinc Nitrate Mix	3.8	Formula 1	Patrick Brown's Mixture	Mixture of Zn $(NO_3)_2$ and other chemicals
Experiment 4				
Zinc Sulfate	36	Zn Sulfate	AG Specialties	Widely used in orchards
Zinc Nitrate	22.0	Lab Grade Zn $(NO_3)_2 \bullet 6H_2O$	J.T. Baker	
Zinc Chloride	48.0	Lab Grade Zn Cl_2	EMD Chemicals	Very corrosive
Zinc Phosphite	6.5	VZP	Agro-K	Derived from Zn carbonate and phosphorous acid
Zinc Glycine	7	Biomin	JH Biotech	Derived from Zn sulfate, citric acid and glycine

Details of the procedure are as follows: Nemaguard seedlings were grown in washed beach sand and the cotyledons were removed shortly after emergence to cut off nutrient reserves. They were fertilized with a 10% Hoagland solution minus Zn to keep them growing steadily but not so vigorously that secondary shoots started to push. Once the seedlings were about 12 to 16 inches in height, they began to show typical zinc deficiency symptoms of narrow, pointed, chlorotic leaves at the shoot tip. Often, lateral shoots started to grow as well and showed the same symptoms. For treatment, the plants were brought into a lab where they were sprayed thoroughly from a spray bottle. No surfactants were added to the solution so beads were clearly visible on leaves, stem and in the axils of the leaves. The plants were returned to the greenhouse where they were grown under 40% Hoagland solution to help promote vigorous growth and stimulation of lateral shoots. The effect of the zinc was to promote larger primary leaves, greater secondary growth and a higher zinc concentration in the new growth. Four main experiments using this procedure were conducted (Table 2).

Table 2. The effectiveness of zinc formulations at overcoming zinc deficiency symptoms in peach seedlings. All materials sprayed on seedlings with solutions containing 500 ppm Zn unless otherwise noted.

	Formulation	Primary Leaf Area (cm^2)	Lateral Shoot Leaf Area (cm^2)	Zinc in New Growth (ppm)
Experiment 1				
	Untreated Control	5.8 b*	66 c	8.1 c
	Zinc Sulfate	8.9 a	274 a	12.4 a
	Zinc EDTA	9.1 a	160 b	8.8 c
	Zinc Oxysulfate-52%	8.6 a	159 b	9.9 bc
	Zinc Leonardite	8.8 a	189 b	9.7 bc
	Zinc Polyamine	8.6 a	241 a	11.0 ab
Experiment 2				
	Untreated Control	4.8 c	51 b	3.7 c
	Zinc Sulfate	10.1 a	213 a	9.5 b
	Zinc Nitrate	10.5 a	271 a	14.8 a
	Zinc Carbohydrate	10.4 a	209 a	9.5 b
	Zinc Oxide Suspension	8.1 b	77 b	6.0 c
Experiment 3				
	Untreated Control	3.8 b	49 b	8.2 b
	Zinc Sulfate – 250 ppm Zn	13.7 a	59 b	9.8 b
	Zinc Sulfate – 500 ppm Zn	16.4 a	104 b	10.2 b
	Zinc Nitrate – 250 ppm Zn	13.4 a	287 a	9.8 b
	Zinc Nitrate – 500 ppm Zn	15.8 a	322 a	17.5 a

Experiment 4

Untreated Control	2.5 c	26 d	
Zinc Sulfate	6.5 b	68 cd	Data
Zinc Nitrate	11.3 a	189 b	Lost
Zinc Chloride	13.0 a	284 a	
Zinc Phosphite	5.0 bc	42 d	
Zinc Glycine	6.6 b	94 c	

* Within each experiment, values in columns followed by different letters are significantly different from each other at p = 0.05.

Once we had conducted these experiments with non-labeled materials, the last step was to conduct an experiment using this same procedure but with the five labeled materials listed under task 1. The results of this experiment are shown in Table 3.

Table 3. Growth response and Zn uptake of Zn deficient peach seedlings treated with different ^{68}Zn labeled formulations applied to the leaves.

	Treatments						Significance
	Control	Sulfate	Nitrate	Chloride	Oxide	EDTA	(p value)
Lateral Shoot Leaf Area (cm^2)	2.2 c[z]	177 a	144 a	166 a	66 b	55 bc	.001
^{68}Zn in New Growth (µg)	0 c	12.9 a	11.4 a	11.5 a	6.5 b	5.2 b	.001
^{68}Zn in Roots (µg)	0 b	1.6 a	1.4 a	1.8 a	.1 b	.1 b	.001
^{68}Zn Uptake (%)	0 c	14.5 a	12.9 a	13.4 a	6.6 b	5.3 b	.001

[z] Values in rows followed by different letters are significantly different by Duncan's Multiple Range Test at the p-value indicated.

Based on all the experiments we conducted (including some minor ones not reported here), we arrived at the following general conclusions (see Table 4). Zinc chloride is the most effective material for supplying zinc to peach trees. However, to our knowledge, it is not currently used as a foliar fertilizer. Furthermore, it can be very phytotoxic and in a few tests it performed no better than some other formulations. Therefore, we do not see the need for pursuing it as a new fertilizer material. Zinc nitrate is the next best formulation and has been used in the past for foliar fertilization of various crops. It can be effective, but should be used with caution due to its high potential for phytotoxicity. It should probably be avoided when fruit are on the tree. Next on the list is zinc sulfate, which is widely used in fresh fruit orchards. It would generally be considered the material of choice because it is less phytotoxic and only slightly less effective than zinc nitrate. Furthermore, it is one of the least expensive materials and would therefore be the most cost effective formulation of any we have tested. All the other formulations can supply zinc to a peach tree but would be considered much less desirable due to higher costs and/or lower effectiveness. If phytotoxicity is of particular concern, materials further down the list should probably be used even though they are less effective.

Table 4. Ranking of effectiveness of zinc formulations based on peach seedling experiments. Phytotoxicity was evaluated on both peach seedlings and in stone fruit orchards sprayed with solutions containing 500 to 1,000 ppm zinc.

Ranking	Formulation	Anion Size	Solubility (g/100 H_2O)	Phytotoxicity
Most Effective	Zinc Chloride	35	432	High (58*)
Almost As Good	Zinc Nitrate	62	324	High (54)
	Zinc Nitrate Mix	62 & 96	324	High (59)
Next Best	Zinc Sulfate	96	50	Moderate (12)
	Zinc Carbohydrate	96 & ?	High	Moderate
	Zinc Polyamine	96 & 75-204	High	Moderate
	Zinc Glycine	96 & 75		Moderate (15)
Less Effective	Zinc EDTA	292	High	Low
	Zinc Leonardite	1000+	High	Low
	Zinc Oxysulfate	16 & 96	1.3	None
Least Effective	Zinc Phosphite	79	?	Low (17)
	Zinc Oxide Suspension	16	Insoluble	None

* Percent of leaves showing obvious phytotoxicity in a controlled experiment on Summer Fire nectarine.

Subtask 2.2. Test the formulations with ^{68}Zn label on pistachio seedlings in the greenhouse. Once we had refined the peach seedling procedure, we focused attention on developing a protocol for testing pistachio seedlings in the greenhouse using unlabeled materials. We started with the peach seedling procedure but soon found some distinct differences between the two species. It was obvious it is considerably more difficult to get zinc into pistachios than into peach. Eventually, we arrived at an effective protocol and conducted several experiments. Our results with pistachio were quite similar to peach (Tables 5, 6, 7). When we conducted the experiment with all five labeled materials, our results were almost identical to the peach experiment (Table 7). Thus, just as with peach, our conclusions are that zinc nitrate and zinc sulfate are the most promising materials to pursue in field trials with pistachio.

Table 5. Growth response, phytotoxicity and Zn uptake of Zn deficient pistachio seedlings treated with different Zn formulations (Experiment 1).

	Treatments					Significance (p value)
			Foliar Treatments			
	Control	Root Zn	Sulfate	Nitrate	Phosphite	
Growth in height (cm)	4.2	10.0	.9	6.8	3.6	NS
Lateral Shoot Growth (cm)	.3 b[z]	16.2 a	9.7 ab	1.2 b	1.7 b	.02
Lateral Leaf Area (cm^2)	.2 b	45.6 a	19.4 ab	.6 b	1.8 b	.06
Phytotoxicity (0-3)	.7 b	.5 b	.3 b	2.2 a	1.5 a	.001
Zn in New Growth (ppm)	3.7 b	6.0 b	5.5 b	9.3 a	7.5 ab	.01
Zn in Roots (ppm)	5.0 b	24.0 a	5.3 b	6.2 b	4.8 b	.001

[z] Values in rows followed by different letters are significantly different by Duncan's Multiple Range Test at the p-value indicated.

Table 6. Growth response and Zn uptake of Zn deficient pistachio seedlings treated with different Zn formulations to the leaves (Experiment 2).

	Treatments						Significance (p value)
	Control	Sulfate	Nitrate	Amino Acid	Oxide	EDTA	
Growth in height (cm)	1.7 b[z]	4.9 b	10.8 a	2.2 b	1.9 b	1.7 b	.001
Zn in New Growth (ppm)	4.5 b	---	7.6 a	---	---	---	.01
Zn in Roots (ppm)	8.3 bc	10.3 ab	11.4 a	7.3 c	10.1 ab	8.7 bc	.05

[z] Values in rows followed by different letters are significantly different by Duncan's Multiple Range Test at the p-value indicated.

Table 7. Growth response and Zn uptake of Zn deficient pistachio seedlings treated with different ^{68}Zn labeled formulations (Experiment 3).

	Treatments						Significance
	Control	Sulfate	Nitrate	Chloride	Oxide	EDTA	(p value)
Growth in height (cm)	7.2 b[z]	11.4 a	10.8 a	13.8 a	6.3 b	5.2 b	.001
^{68}Zn in New Growth (µg)	0 c	3.9 ab	3.0 b	4.2 a	.1 c	.2 c	.001
^{68}Zn in Roots (µg)	0 c	1.9 a	1.9 a	2.0 a	.1 c	.6 b	.001
^{68}Zn Uptake (%)	0 c	3.6 ab	3.1 b	3.9 a	.1 c	.5 c	.001

[z] Values in rows followed by different letters are significantly different by Duncan's Multiple Range Test at the p-value indicated.

Task 3. Test the best formulation/surfactant combinations on young peach and pistachio trees in the field (Objective 3). We had done some work with potted plants in the previous FREP project (see 2007 FREP report). Once we had spent considerable time on tasks 1 and 2, we decided to focus on task 4 in order to complete the project within the time limit.

Task 4. Test the most efficient Zn treatments in commercial peach and pistachio orchards (Objective 4). Two experiments were conducted in commercial peach orchards. First, since Zn nitrate often out-performed Zn sulfate in our seedling experiments, we decided to compare these two materials in a mature (about 10 year old) Summer Fire nectarine orchard. In early October of 2009, individual trees (5 per treatment) were sprayed with 100 ml of labeled formulations of either zinc sulfate or zinc nitrate. Each solution contained 864 ppm of ^{68}Zn. Flower and new leaf samples were taken in the spring of 2010 and analyzed for ^{68}Zn. The results of this experiment showed no difference in zinc uptake between the sulfate and nitrate formulations (Table 8). This experiment also demonstrated that it is possible to use a relatively small amount (100 ml) of labeled zinc solution on full grown trees and still pick up the ^{68}Zn signal in new growth, even though it was only 0.03% of the amount applied. Based on the results of this experiment, we decided to use only zinc sulfate for the final experiment in 2010.

Table 8. Recovery of ^{68}Zn applied to Summer Fire nectarine trees in early October, 2009. Labeled ^{68}Zn applied as either sulfate or nitrate in an 864 ppm Zn solution at 100 ml/tree. Recovery measured in flowers and new growth collected in March, 2010.

	Treatments			
Parameter	Untreated Control	^{68}Zn Sulfate	^{68}Zn Nitrate	Significance
^{68}Zn in Flowers (μg)	0 b*	18.0 a	17.8 a	.001
^{68}Zn in Young Leaves (μg)	0 b	7.3 a	5.5 a	.0001
Total ^{68}Zn Recovered (μg)	0 b	25.2 a	23.3 a	.0004
Percent of Applied (%)	0 b	0.03 a	0.03 a	.0004

*Different letters in a row indicate significantly different values at the significance level indicated.

The final experiment was set up on some mature (about 15 year old) O'Henry peach trees at the Kearney Ag Center to test the best timing for fall applications and to see if a surfactant improves zinc uptake. Foliar applications of ^{68}Zn sulfate were made on two dates, Sept 30 and Nov 11, 2010. No leaf senescence had occurred on the first date, and was just starting on the second date. Leaves then proceeded to drop quickly and were completely gone by early December. Thus, active leaves were present for nearly two months after the first application, but substantially less than a month after the second. On each date two treatments were made: one with zinc sulfate alone and one with a silicone based surfactant (L77) added to the solution. This surfactant has been reported to break down the surface tension of solutions to the point of allowing penetration directly through the stomates. One of our greenhouse experiments showed a slight benefit from adding this material to a zinc sulfate solution. For each treatment, 100 ml (about ¼ pint) was sprayed on the lower half of the canopy. No appreciable runoff was observed in any treatment even though the surfactant helped spread the solution more extensively over the treated leaves. Each of these four treatments plus an untreated control was replicated on five individual trees for a total of 25 trees in the experiment. Samples were taken of dormant roots, flowers and new leaves (mid-March) from both the treated (lower canopy) and untreated (upper canopy) portions of the tree and of mid summer mature leaves. Each sample was analyzed for the ^{68}Zn label.

The results (Table 9) show no clear benefit from using the L77 surfactant and indicate better zinc uptake from the later application time (November) compared to the earlier timing. This is exactly opposite from the results we obtained on small potted plants where the earlier timing was more effective. Thus, the question of optimum timing for foliar zinc sulfate applications remains unresolved at the end of this project.

Table 9. Recovery of ^{68}Zn applied to O'Henry peach trees as ^{68}Zn sulfate on either Sept. 30 or Nov. 11, 2010 with or without L77 surfactant. ^{68}Zn applied in an 864 Zn solution at 100 ml/tree.

	Treatment with ^{68}Zn Sulfate					
Parameter	Untreated control	9/30	9/30 + L77	11/11	11/11 + L77	Significance
^{68}Zn in Roots (μg) Dec. 2010	0 b	2.2	1.4	1.8	-0.7	NS
Treated Area						
^{68}Zn in Flowers (μg) March 2011	0 c	5.3 b	5.4 b	9.8 a	13.0 a	.0001
^{68}Zn in Leaves (μg) March 2011	0 c	1.9 bc	2.2 b	4.1 b	6.6 a	.0001
^{68}Zn in Leaves (μg) June 2011	0	1.6	1.5	1.8	1.6	NS
Above Treated Area						
^{68}Zn in Flowers (μg) March 2011	0 b	1.6 a	0.3 ab	-1.0 b	0.7 ab	.06
^{68}Zn in Leaves (μg) March 2011	0 ab	1.7 a	-0.9 b	0.2 ab	1.3 a	.06
Total ^{68}Zn Recovered (μg)	0 c	14.4 ab	9.9 b	16.6 ab	22.4 a	.0004

For pistachio, we used a slightly different approach since it is generally much more difficult to get zinc taken up by the leaves. Some of our seedling tests showed zinc nitrate to be considerably more effective than zinc sulfate. Therefore, we wanted to test the effectiveness of both these formulations in the field, as well as a timing test similar to the peach orchard. Thus, the treatments in this experiment were two formulations (^{68}Zn nitrate and ^{68}Zn sulfate) and two dates (Sept 30 and Nov 2). Each of these four treatments plus an untreated control was replicated on tagged branches of five individual trees for a total of 25 trees in the experiment. Samples of new growth were taken in the spring and analyzed for the ^{68}Zn label. Unfortunately, many samples were lost when a pruning crew accidently cut out some of the treated branches. No clear difference between materials or timings could be determined.

Outreach Activities Summary:
Our work on zinc was reported at many meetings during the time period of the project:
2008
January 17 – Sutter/Yuba Counties grower meeting – 30 in attendance
February 5 – Plant and Soil Conference in Visalia – 20 in attendance
February 15 – International Fruit Tree Association – 140 people
April 17 – Fresno State University nutrition class – 9 students
November 12 – FREP/WPHA annual meeting – 175 in attendance
December 3 – Winter Tree Fruit meeting at Kearney Ag Center – 120 in attendance
December 4 – Fresno State University crop physiology class – 20 students
December 10 – Western Fluid Fertilizer Technology Workshop in Fresno – 50 people
2009
January 29 – Pomology Extension Continuing Conference – 30 in attendance
February 13 – San Benito County grower meeting – 30 in attendance
March 16 – Fresno State University pomology class – 15 students
October 28 – Farm Advisor Training – 40 in attendance
December 2 – Winter Tree Fruit meeting at Kearney Ag Center – 85 in attendance
December 3 – Northern San Joaquin Valley Cling Peach Day – 40 in attendance
2010
January 20 – Western Colorado Hort Society meetings – 100 in attendance
January 27 – Sutter/Yuba County grower meeting – 40 in attendance
January 28 – North San Joaquin Valley Almond Day – 250 in attendance
March 2 – El Dorado/Amador County grower meeting – 35 in attendance
Nov 19 – Boise Idaho orchard managers – 12 in attendance
Dec 2 – Sutter/Yuba County PCA/CCA training – 25 in attendance
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