ANNUAL REPORT

Development of a Model System for Testing Foliar Fertilizers, Adjuvants and Growth Stimulants.

CDFA contract number: 06-0624

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Period covered: Jan-December, 2007

Objectives:

1. Develop a model system for testing the efficacy of a broad range of foliar chemicals.
2. Conduct tests of materials of greatest relevance to growers under standardized conditions. Undertake focused field verification.
3. Define application conditions for optimal product use.
4. Conduct preliminary research on effect of plant anatomy (stomatal density and distribution, cuticle composition, leaf waxes and hairs etc) on foliar efficacy as a prelude to development of new and targeted formulations. (Future Goal)

Abstract: The goal of this project is to develop and test a model system to improve our understanding of the use and effectiveness of a broad range of foliar fertilizers, adjuvants and growth stimulants so that growers can make informed decisions to improve production efficiency and profitability. The difficulty in conducting field research has resulted in a situation where our understanding of the function and efficient use of the plethora of foliar chemicals in the market today is inadequate. This project addresses this challenge by providing an efficient and practical means to screen materials and application techniques and explore the factors that influence effectiveness of foliar chemicals.

The first year of experimentation has successfully developed the model test system and indicates that it is an effective and replicable approach to screen large numbers of materials. A total of 20 Zn materials have been screened in 5 replicate experiments a task that would have been extremely difficult under field conditions. The test plant (Arabidopsis) seems to be an ideal choice as it is highly responsive to Zn and has the ability to differentiate between materials with some resolution. Results clearly suggest that complex Zn formulations and formulations with a high POD are relatively more effective than inorganic Zn sources.

Results also suggest that Zn is quite phloem mobile within plants once it has been absorbed and that the primary limitation to the effectiveness of foliar Zn applications is the rate with which the material can pass through the leaf cuticle to the symplasm of the leaf. Research that targets this limitation is underway. In the coming year we will validate the relative efficacy of these materials in field trials in Almond and tomato and

will extend the model to the examination of Calcium based materials. Additional trials of additives designed to enhance leaf penetration will be conducted.

Introduction: Foliar fertilization and the application of foliar stimulants, adjuvants and other non-pesticide materials (foliar chemicals) have become a central practice of many agricultural producers. Our understanding of these products, is however, remarkably poor and for the majority of foliar fertilizers (especially the more specialized materials) used in Californian agriculture there is very little information on nutrient uptake, nutrient use efficiency, nutrient transport or the application conditions that optimize efficiency and return on investment.

Foliar chemicals are used for a number of reasons; some of which have a clear physiological and production rationale while others are of doubtful utility. Valid reasons for the use of foliars include the correction of low nutrient availability in soils (e.g. Fe deficiency in high pH soils), overcoming limitations induced by environment (foliar Zn in spring), overcoming excessive nutrient demand during fruit growth (N, K in nuts), targeted fruit quality enhancement and the need to ensure time critical delivery of nutrients to specific tissues (B to flowers and fruit, Ca to fruit). It is well documented that plant response to foliar applied nutrients in the field is an extremely complicated process, which depends on the nutrient status of the plant, species as well as timing of application and environmental factors (Weinbaum, 1988). As a result, contradictory results are often found with the same chemical and the same plant species but with different locations and under various conditions (Buchholz et al., 1998; Weinbaum, 1988). Field trials of foliar fertilizers have frequently been difficult to replicate, hard to interpret and hence poorly adopted by growers.

Studies of foliar fertilizers conducted under controlled laboratory conditions, and frequently with excised tissues, have made significant contributions to our understanding of the principles involved in foliar uptake and can provide valuable insight into predicted field responses. Over the past decade the most significant advances in our understanding of foliar chemicals has been made by the German researchers Schonherr and Bucholz (see references in Fernandez and Ebert, 2005), who have determined that the cuticular membrane is the most important, or the sole pathway, for the foliar nutrients (Marschner, 1995). Recent evidence, however, suggests that stomatal penetration of aqueous solutions may be an important pathway for nutrient uptake that was overlooked in laboratory based experimentation in which stomata were absent or not considered (Fernandez and Ebert, 2005). While advances in fundamental understanding have the potential to greatly improve field application, there have been remarkably few attempts to use this information to explain field responses. The following are some recent examples where integrated field and laboratory research has been used to address foliar nutrition challenges in Californian tree crops.

Picchioni et al (1995) demonstrated in laboratory trials that the physical retention of foliar product on the leaf surface, which is a result of leaf surface-tension properties, leaf hairs and specific leaf area, was a primary determinant of foliar responsiveness of various

species to foliar nutrient applications in the field. Subsequent field research confirmed these findings and provided a rationale for product selection and application technique. In Pistachio and Walnut, Zhang and Brown (1999) demonstrated that penetration of foliar Zn through the leaf cuticle represents a primary barrier to foliar uptake of Zn, young leaf tissue has thinner cuticles and as such has greater Zn uptake. This has been verified in field grown pistachio where spring Zn applications are now recommended. Brown et al (1996) demonstrated that the ability of a species to form a stable complex with B within the plant tissue greatly enhances B uptake and transport. This discovery has been translated into new fertilizer formulations and practices. Integrated studies such as these are uniquely valuable but extremely laborious have historically been compromised by the need to bring field tissues into the laboratory which limits the time of year in which studies can be conducted and introduces potential artifacts. Ultimately, advances in our use and understanding of foliar chemicals cannot be made unless experimentation is made under controlled conditions with consideration of the plant biology.

The range of foliar products available on the market has grown dramatically in recent years and now includes many products that purportedly act as 'biostimulants' or growth 'enhancers' as well as fertilizer materials with 'nutrient carriers' that have not received adequate independent testing. Unfortunately, there are also many products on the market that have very little clear physiological rationale and no verifiable research basis. There are also a vast number of products available, for example a major supplier of fertilizers in California, for example, lists 18 foliar Zn products and an additional 35 in which Zn is a component. Peer reviewed research is available to support the use of a limited number of 'traditional' materials in various crops, however these results are frequently contradictory. For the majority of non-traditional products, only scattered, and generally proprietary evidence of efficacy exists, comparative data that clearly identifies the relative benefits of any particular compound in comparison to other materials is rare. The matter is further complicated since few trials have been conducted under controlled conditions where the basis for the superiority of a material could be defined. Without the use of controlled conditions, extrapolation of results from one location or crop to another cannot be made. Repeating large foliar trials at multiple sites and under the multitude of environments is, however, clearly impractical.

Work Description of Tasks Scheduled for 2007:

Task 1: Develop a model system for testing the efficacy of a broad range of foliar chemicals.

During the model development (subtask 1.1), we noticed that Arabidopsis growth was not ideal in the proposed sand culture system. Therefore, we found that it was necessary to spend some additional time testing and modifying alternate growth medium (Task 1.2). This has now been resolved and a stable and reproducible system is now in place. This task is reported first.

1.2 Subtask: Establish growth conditions and derive response curves for each relevant nutrient commencing with nutrients of most importance to Californian agriculture, initially N, K, Ca, Zn, Fe, B.

For the development of a rapid test system to screen the many hundreds of foliar nutrient products and treatment combinations we want to develop a growth system that maintains plants at 'marginally deficiency' in the element of interest with other elements maintained at sufficiency. The choice to establish treatment levels for the element of interest at 'marginal nutrient deficiency' was made following careful consideration of existing literature and practical considerations and is designed to ensure uniformity of growth, maximal nutrient transport while avoiding the complication of high background nutrient status.

To establish the growth conditions that result in 'marginal deficiency' we first develop a nutrient response curve by growing plants at a range of concentrations from deficient through sufficiency.

We used the quartz sand (about 2 mm diameter) as the culture medium to generate the growth curve for K (concentration used: 0.01, 0.1, 0.25, 0.5, 0.75, and 1.5 mM) and for Zn (concentration used: 0, 0.005, 0.05, 0.1, 0.25, and 1 μM).

As such, the objectives were modified to include medium selection first. We used four kinds of culture medium: 1), Sunshine potting mix #1, 2), vermiculite, 3), sand, and 4), mixture of half Sunshine potting mix #1/half sand. Two goals were established: first, to ensure that have optimal growth potential to ensure rapid crop response; and two, to develop a medium that is low enough in the nutrients, so that we can manipulate the test nutrients as required. The preliminary experiments demonstrated that a mixture of leached commercial potting medium and washed quartz sand provided the ideal medium.

1.1 Subtask: Establish baseline controlled plant growth system:

Arabidopsis was grown in a controlled environment growth chamber with day length of 16 hours and light intensity of 120 $\mu mol.m^{-2}.sec^{-1}$, day/night temperature: 22/20°C, relative humidity 70%. A tray of 21"x10"x2.2" is used, which can hold 36 small pots (4 plants in a row x 9 rows). During foliar application, every two rows in each tray serves as one experimental unit so that every tray can handle 4 experimental units. The whole tray was fed with water and nutrients by root submersion technique.

The original goal was to derive an optimum growth condition that will be used as a base treatment for all subsequent studies. For research involving non-nutrient foliar chemicals (stimulants etc), this baseline system will represent the control value.

Since we are dealing explicitly with plant response to foliar applications it is essential that no applied materials contact the soil surface. This requires very considerable care and specialized application methods.

1) The soil surface is protected from spray drift by covering the entire surface with a plastic cover. A plastic pipette tip is then modified to form a tube (4 mm long, top inside diameter 3.5 mm and bottom inside diameter 3 mm) which is then inserted in the middle of the pot, through the plastic cover and into contact with the soil. Seeds are planted directly into this tube. Plant emergence then occurs through the tube.

2) Once seedling growth is established, seeds are thinned to 1 per tube and lanolin is injected into the space between seedling stem and tube to prevent runoff traveling down the stem to the soil.

3) A double layer of paper towel is then placed below the lowest leaves directly on the underlying plastic cover. This is done to avoid the run off of sprayed chemicals to soil or sub-irrigation system.

The basic form of the model system is now developed. Additional fine-tuning will be performed. To verify that no leakage of foliar materials to the soil substrate is occurring we used a marker solution containing $RbCl_2$. Rubidium is highly mobile in plants and behaves in a manner similar to K. Following foliar application we will remove plants and analyze soil Rb. The presence of any Rb in the soil will be used as a measure of possible spray contamination.

Task 2: Determination of the efficacy of Zn foliar Fertilizers.

Five separate experiments have been completed to verify the efficacy of a range of common Zn foliar fertilizer products. A mixture of commercial and single salt products were tested.

First experiment: After chilling for two days, Arabidopsis seeds were planted in sand (~2 mm diameter) in 8 trays. About 2-3 seeds were seeded to each pot. Beginning one week later, ¼ Hoagland solution without Zn were fed once a week. After transplanting, the plants were fed with full strength Hoagland solution with 0.005 ppm Zn (1/10 of the Zn in Hoagland solution) once a week in order to promote quick recovery of transplanting shock.

12 days after germination (dag) later, some extra plants (not used for the main experiment) were used for a preliminary spray with 400 or 500 ppm Zn using Zn sulfate to see if Arabidopsis can tolerate these Zn rates. After one additional week all the sprayed plants appeared normal indicating 500 ppm Zn will not cause toxicity in Arabidopsis under our growth conditions.

45 dag - pots were divided in such a way that each of 8 pots served as one experiment unit. Sept. 14, eight spray treatments were made and each treatment replicated three times. 1, ck; 2, Neutral Zn; 3, Zn EDTA; 4, Zn Metalosate; 5, Zn Lignosulfonate; 6, NZn; 7, Zn Fulvic; 8, Zn sulfate. All Zn products was standardized to a concentration of 400 ppm Zn. In every treatment 0.05% L-77 was added as the surfactant. At application no reproductive tissue was present on any plant. To ensure uniform coverage, each treatment was applied 3 times with the fine mist sprayer.

Twelve days after application (57 dag), during which period flowers appeared, opened and set seed, all inflorescence/siliques were harvested. After drying and ashing, the Zn concentration in the flowers/silique was analyzed by inductively coupled plasma – mass spectrometry (ICP-MS).

Second experiment: Arabidopsis seeds were planted in 16 trays in a mixture of half sand/half sunshine mix #1 potting soil. The seeds were pre-chilled for 3 days. Beginning one week later, 1/10 Hoagland solution without Zn was fed. 24 dag, some transplanting or thinning was made, so that each pot had one plant. Plants were then fed with ¼ Hoagland solution without Zn once a week throughout the experiment. 57 dag, the 8 treatments of the same materials, rates and techniques as experiment one were made. Each treatment was repeated 8 times. 77 dag, influorescence/silique were harvest and analyzed.

Third Zn experiments: Modifications to the planting system were made to ensure that no leakage of folair materials wsa occurring. An experimental unit with 8 pots was filled with the mixture of half Sunshine mix #1 and half sand, the unit was covered with plastic sheet and in the middle of each pot a hole is punched and a cylinder with 4 mm long and 3.5 mm inside diameter on top and 3 mm diameter on the bottom was inserted into the hole and fit tightly. About 3 to 5 cubic centimeter of some fine potting material was placed under each cylinder, to ensure that seeds do not move during pipetting. Twenty trays were planted. The Arabidopsis seeds were suspended in water and pipetted into the cylinder. ¼ Hoagland solution was used to feed the plants once a week. 45 dag just prior to spray application, lanolin was used to seal the holes of the cylinder for each plant. 45 dag spray treatments were applied as previously described. In this expriment 13 treatments including 4 used in experiments 1 and 2 were applied: a, Bio-link Zn: b, Bio-min Zn: c, Pre-Natural Zn: c, BioNutrient Zn: e, Zn Metalosate: f, Elemax super Zn FI: g, Krystal Klear Zn: h, Foli-Grow NZn: I: Foli-gro Zn: J, RNA Zinc nitrate solution: K, RNA micophos: L, Availa Zn 40: M, untreated control. Each treatment was repeated 6 times. 51 dag, inforescences and siliques were harvested and analyzed as described above.

Fourth and fifth experiment: Experiment 3 was replicated. Treatments were made 31 dag with essentially the same materials with the exception that RNA microphos and Availa Zn were omitted as they were largely insoluble and poorly effective in the prior experiment. 54 dag, inflorescence/silique were harvested and analyzed like experiment one.

Task 3: Undertake focused field verification. (not scheduled until July 2008 – initiated early)

Results from task 2.1 and 2.2 suggested that Zn was apparently phloem mobile in Arabidopsis and suggeste4d the primary limitation for foliar Zn is the penetration of Zn through the cuticle and NOT subsequent within plant mobility. To test this further we commenced targeted field verification of Zn transport using two 68 Zn labeled Zn

products (Zn Sulfate and Zn Nitrate) to verify the phloem mobility of Zn in Almond and Vinca species.

Results: Task 1 and 2.

Due to subtle differences in application techniques and experimental duration a comparison of absolute numerical values between experiments is not appropriate. Here we have contrasted replicate experiments using a relative ranking which represents the degree to which the material differed from the control treatment within the same experiment. Treatments with different whole number rankings within a single experiment, differ significantly ($p>0.05$). (Fractional numbers do not differ significant from the nearest whole number).

While not all materials were replicated in all trials it is clear from Table 1 that there is a substantial consistency in materials ranking between replicate experiments. Two materials included in the final study proved to be superior to others and will be included in an additional trials to be conducted in February 2008. Three materials, RNA Zn Nitrate, Foli-gro Zn Sulfate and BioNutrient Zn+ showed some variability in effectiveness with results between trials differing by greater than 20%.

With the exception of foli-grow NZn, the inorganic Zn sources were uniformly less effective than the 'complexed or chelated' Zn sources. (The use of the term complexed or chelated is based on manufacturers claims and has not been chemically verified.) The relative superiority of NZn over other inorganic Zn forms may be associated with its relatively low point of deliquiescence (POD) which results in the NZn remaining moist on the leaf surface for extended periods.

In subsequent trials, rates of materials will be adjusted to reflect label application rates so that field efficacy can be better defined. The most effective materials and the prominent current sources will be included in future trials.

Table 1: Relative efficacy of Zn folair materials applied at a standardized rate equivalent to 400ppm in spray solution. Results are shown for 5 replicate experiments. Relative rankings of materials is provided for each experiment.

		RANKED EFFICACY (5 Replicate Experiments)				
	Experiment	**5.0**	**4.0**	**3.0**	**2.0**	**1.0**
untreated control	Test Material	1.0	1.0	1.0	1.0	1.0
Elemax Super Zn FI	A	#	1.0	1.0	-	-
RNA Microphos	B	X	X	2.0	-	-
Availa Zn	C	X	X	2.5	-	-
Neutral Zn	D	#	#	#	1.5	2.0
RNA Zinc nitrate solution	E	4.5	2.0	4.0	-	-
Zn Fulvic	F	3.0	-	-	1.5	3.0
Foli-Gro Zn Sulfate	G	4.0	2.5	2.5	3.0	3.5
Zn Lignosulfonate	H	3.5	-	-	3.0	4.0
Krystal Klear Zn	I	3.0	3.0	3.5	-	-
Pro Natural Zn	J	3.5	3.0	4.5	-	-
Bio-Min Zn	K	3.5	3.0	4.5	-	-
Bio-link	L	3.5	3.0	3.5	-	-
BioNutrient Zn+	M	3.0	5.0	3.5	-	-
foli-Grow NZn	N	4.0	3.5	4.0	5.0	5.0
Zn Metalosate	O	3.5	4.5	4.0	3.0	3.5
Zn EDTA	P	3.5	-	-	4.5	5.0
Zn Manniplex	Q	4.5	-	-	-	-
Zn Floratine (FPG-1036)	R	4.5	-	-	-	-

"X" Producted ommitted because of solubility problems
"-" Product not included in trial
"#" Product ommitted due to prior poor results

Treatments with different whole number rankings within a single experiment, differ significantly ($p>0.05$). (Fractional numbers do not differ significant from the nearest whole

Results: Task 2 and Task 3: Extended Studies

To further investigate the chemical and physical basis for the effectiveness of various materials and to verify the field effectiveness of various spray treatments a number of supplemental experiments have been conducted.

Experiment 3.1 Zn Movement in the phloem of Almond.

Stable isotope enriched Zn Sulfate was applied directly to leaves of young Almond (4yo) growing in research fields at UC Davis. The movement of the applied Zn out of the leaf

to which it was applied was then determined. Results are shown in Table 2. Zn moved readily out of the leaf to which it was applied. Analysis of the quantity of Zn applied, the quantity recovered in leaf washing and quantity transported out of the leaf provide some insight into relative efficacy of applied Zn. Only 14 µg of 100µg applied Zn (14%) was absorbed by the leaf on day 1. Of this 14 µg, only 3 µg was removed from the leaf (3%) over the subsequent 14 days. This remobilized fraction represents 3% of the total applied Zn or 30% of the absorbed Zn.

Table 2: Movement of Foliar applied 68 Zn from application site expressed as ug 68 Zn remaining in treated leaf (100 µg applied). (Almond).

Treatment	days after 68Zn application		
	1	7	14
ck	0.66 ± 0.02	0.72 ± 0.01	0.68 ± 0.04
68Zn labeled leaf	14.5 ± 1.1	14.5 ± 0.3	11.3 ± 1.4

Experiment 3.2 Zinc movement from site of foliar application. To further resolve the fate of Zn applied to leaf tissues, we again utilized 68Zn labeled Zn Sulfate applied to fully expanded leaves on a young field grown almond plant. In this instance results are reported as the degree of 68Zn enrichment in tissues above and below the point of application. Results are shown in Table 3 and indicate that proportionally greater amounts of Zn are recovered below the point of application that are recovered above the point of application. Movement below the point of application can only occur as a result of phloem transport.

Table 3: Zinc movement into tissues above and below treated leaves.

Tissue	treatment	days after 68Zn application		
		1	7	14
Tip	ck	4.52 ± 0.01	4.49 ± 0.00	4.47 ± 0.02
	68Zn treated	4.51 ± 0.02	4.59 ± 0.02	4.62 ± 0.03**
Bark: I cm above treated leaf	ck	no data	4.49 ± 0.01	4.51 ± 0.01
	68Zn treated	no data	4.60 ± 0.01	4.71 ± 0.04**
Bark: 1cm below treated leaf	ck	4.51 ± 0.01	4.51 ± 0.01	4.51 ± 0.01
	68Zn treated	4.61 ± 0.02	4.86 ± 0.07	4.93 ± 0.04**

Summary and Future Directions:

The first year of experimentation has successfully developed the model test system and indicates that it is an effective and replicable approach to screen large numbers of materials. Delays in establishment of the system resulted in the analysis of Zn foliar fertilizers only, additional materials (K and Ca) will be studied in 2008. A total of 20 Zn materials have been screened in 5 replicate experiments a task that would have been extremely difficult under field conditions. The test plant (Arabidopsis) seems to be an ideal choice as it is highly responsive to Zn and has the ability to differentiate between materials with some resolution. Results clearly suggest that complexed Zn formulations and formulations with a high POD are relatively more effective than inorganic Zn sources. It is essential to note however, that all materials used here were applied at the same rates of applied Zn (400 ppm Zn in solution). In field settings, however, these materials are often applied at very different rates ranging from 10 ppm to 400 ppm Zn in solution. Subsequent experimentation is planned to contrast materials at rates equivalent to their field application rates.

Results also suggest that Zn is quite phloem mobile within plants once it has been absorbed and that the primary limitation to the effectiveness of foliar Zn applications is the rate with which the material can pass through the leaf cuticle to the symplasm of the leaf. Research that targets this limitation is underway.

In the coming year we will validate the relative efficacy of these materials in field trials in Almond and tomato and will extend the model to the examination of Calcium and Potassium based materials. Additional trials of additives designed to enhance leaf penetration will be conducted.

Outreach Activities:

The PI presented results directly related to this project at a number of events with generally excellent turnout.

February 6th, 2007: California Chapter of Agronomy Society of America. Talk: Use of Foliar Fertilizers (200 Participants)

February 9th: Tehama County Walnut Meeting: Talk: Use of Foliar fertilizers in Walnuts. 150 participants.

Feb 17th, 2007. ASHS Annual Meeting: Talk: Management of Nutrition in Pistachio (80 attendees)

November 15th, 2007: Actagro Annual Nutrition Management Day. Talk: Management of micronutrients in tree crops. 300 Attendees.

November 27th, 2007: CDFA/WPHA Annual Conference. Micronutrients in Agriculture. 300 Attendees.

November 27th, 2007: CDFA/WPHA Annual Conference. Foliar Fertilization. 300 Attendees.

November 29th, 2007: WPHA Annual Conference. 2 Talks: Micronutrients in Agriculture and Foliar Fertilization. 75 Attendees.

December 5th, 2007: Almond Conference. Talk: CDFA Survey results: Nutritional Management in Almonds. 400 attendees.

December 6th, 2007: Almond Conference. Growers Breakfast. Rethinking management of nutrients in trees. 300 Attendees.

December 19th, 2007. Prune Research Conference. Talk: Foliar Fertilization of Prune. 30 Attendees.