A: Cover Page
CDFA Fertilizer Research and Education Program
2010

Development of leaf sampling and interpretation methods for Almond and Pistachio. (Request for two year funded continuation to 07-671)

Project Leader: Patrick Brown, Professor, Department of Plant Sciences, One Shields Ave., University of California, Davis, CA 95616-8683, 530 752-0929, phbrown@ucdavis.edu
Cooperators: Bruce Lampinen, Professor, Department of Plant Sciences, One Shields Ave., University of California, Davis, CA 95616-8683, 530-752-2588, bdlampinen@ucdavis.edu
Richard Plant, Professor, Department of Plant Sciences, One Shields Ave., University of California, Davis, CA 95616-8683, 530 752-1705, replant@ucdavis.edu
John Edstrom, University of California Cooperative Extension, 100 Sunrise Blvd
Colusa, Ca. 95932, Office (530) 458-0570, jpedstrom@ucdavis.edu
Roger Duncan, University of California Cooperative Extension, 3800 Cornucopia Way
University of California Cooperative Extension, Suite A. Corner of Service and Crows Landing, Suite A, Modesto, CA 95358, (209) 525-6800, raduncan@ucdavis.edu
Blake Sanden, Farm Advisor, UCCE Tulare County, University of California Cooperative Extension, 1031 S. Mt Vernon Ave. Bakersfield, CA 93307, (661) 868-6218, blsanden@ucdavis.edu

Project location: Selected representative Almond Orchards **Project duration:** Two years.
Description of target audience: Growers of >1 million acres of Almonds. 400,000 acres of Pistachio. Farm Advisors, Consultants, Fertilizer Industry.

Supporter: Paramount Farming Company

CDFA Funding Request: Year 1: $48,823, Year 2: $ 46,670

Other Funding for Related Research not directly funding this proposal: **Almond Board of California**: Yr 1: $32,000, Yr 2: $32,000. Contact: Robert "Bob" Curtis - Senior Manager, Production Research, 1150 9th Street, Suite 1500 - Modesto, CA 95354 USA - 209 343.3216 - Cell 209.604.0385. **Yara Fertilizers**: Yr 1: $25,000, Yr 2:$25,000 Contact: Sebastian Braum, PhD, Manager, Marketing Support & Agronomic Services, Yara North America, Inc. 3840 Ginger Court, Auburn CA 95602, 530-878-3934, sebastian.braum@yara.com. **Administrative Committee for Pistachios:** Yr 1: $34,000, Yr 2:$34,000 Bob Klein, Ph.D. Manager, Administrative Committee for Pistachios. 4938 E. Yale Avenue, Suite 102 Fresno, CA 93727.

B: EXECUTIVE SUMMARY

A majority of growers and consultants participating in the CDFA-FREP funded surveys of growers, felt that UC Critical Values (CV's) were not appropriate for current yield levels, were not useful early in the season and did not provide sufficient guidance for nutrient management. Two explanations for this observation are possible, 1) the current CV's are limited in application and are possibly incorrect, or 2) that there are systematic errors in the manner in which critical values are used. While it is not known if UC CV's are incorrect (this will be verified), it is known that they have not been validated for early season use and it is clear that there has been a systematic error in the way leaf sampling and CV's have been used. Currently, standardized leaf samples from random trees scattered through the orchard are collected, analyzed for nutrients and compared with established CV's. If the resultant mean field nutrient concentration is equal or greater than the CV then the field is deemed to be sufficient. In high value crops, however, this is an invalid approach since it will result in half of the field being below the critical value. Growers, who have observed that a higher 'CV' is beneficial are in effect, bringing a greater percentage of individuals trees above the CV.

We conclude, that the 'problem' with current CV's is not that they are necessarily wrong, but that they do not account for within-field, within-canopy, between season or within-season variability. Current leaf sampling also does not allow for early season tissue analysis. Preventing the occurrence of a deficiency in any part of an orchard or canopy, at any time of the year, is essential to high productivity and fertilizer use efficiency and is the goal of good growers. Unfortunately, the tools to achieve this economically and in an environmentally sound manner are not available and over-fertilization is currently the only tool growers have to ensure optimal field productivity. The recent CDFA-FREP nutrition focus group demonstrated that growers are aware of this problem and have a clear desire to find a better approach.

This project aims to correct this situation by developing new approaches and interpretation tools that better quantify field and temporal variability, are sensitive to yield and provide for in-season monitoring and fertilizer optimization in Almond and Pistachio. This project will also offer the unique opportunity to verify current CV's and determine the utility of nutrient ratios as a diagnostic tool. (*As this is a request for project extension the following list of objectives includes a statement of current completed activities and proposed new activities.*)

1. Determine the degree to which leaf nutrient status varies across a range of representative orchards and environments *(third year of data will be collected in 2010, analyzed in 2011and incorporated in to new guidelines in 2011/2012)*
2. Determine the degree to which nutrient status varies within the canopy and within the year. *(third and fourth year of data from current fields will be collected in 2010/11, analyzed in 2011, supplemental trial commenced 2011 and incorporated in to new guidelines in /2012)*
3. Validate early season leaf analysis protocols and relationship with yield, validate current CV's and determine if nutrient ratio analysis provides useful information to optimize fertility management *(data collected 2010 and 2011. Supplemental trial commenced 2011. Analysis, interpretation integration in 2012)*
4. Test utility of use of fruiting spur leaf analysis under variable N and K treatments, validate as an indicator of tree nutrient status, monitor role of fruiting spur leaves in yield, monitor relationship between spur nutrient status and spur survival in Almond *(new activity for 2011-2012)*
5. Develop and extend an integrated nutrient BMP for Almond and Pistachio.

C: JUSTIFICATION:

Justification for Project Extension: This is a request for two years continuation funding for proposal 07-671 which was established as a field experiment in 2008. Field research in Almond requires a minimum of 4-5 years field data. By the completion of the current research project (07-671) we will have collected 3 years of data. We request support for an additional 1 or 2 years of data collection, validation (depending upon the specific objective) analysis and presentation. Results obtained to date have been very promising and we are confident a robust set of conclusions will be obtained.

A summary of the key activities and findings to date is provided below (see annual report for projects 07-671 for more details). ***The text in italic summarizes the ongoing activities and the specific request for renewal funding.***

The most significant new activity proposed here is the addition of Objective 4 which is designed to explicitly test and extend the findings of the first three year funding cycle.

Four fields trials were established in 2008 each site monitors the yield and nutrient status, annual nutrient demands and seasonal patterns of nutrient assimilation of 114 trees. 5464 leaf and nut samples have now been collected and analyzed for full nutrient suite and yield (5064 leaves and 400 nuts). 912 yield records that will be used to derive nutrient response curves. ***To construct sound yield x nutrient relationships a minimum of 4 years of data collection (one additional year) is required. This data will then be subjected to regression analyses and geostatistical procedures to derive response curves and multiple element relationships with yield. We request funding for 1 additional year of data collection and 1 year of analysis and BMP development.***

Data from these field sites has been used to calculate the number of independent leaf samples that would be required to accurately determine the true field nutrient mean. At these sites the field mean for nitrogen could be defined with 95% confidence if 8 independent and equally spaced samples had been collected while an estimated 40 and 65 samples would be required to obtain true field mean values for K and Mn. ***Field collection will be completed in 2010 and newly developed sampling methodologies will be validated with data collected in 2011 and 2012.***

Analysis of nutrient dynamics in the three different leaf types sampled (non-fruiting, single fruited and double fruited) collected over the full season suggests that leaves on fruiting spurs may exhibit nutrient deficiencies even when non-fruiting leaves on the same tree may have "adequate" leaf concentrations. In the existing project we have analyzed eleven nutrients as predictor values, three different spur categories at four dates with individual tree yield records. To fully interpret this data we are employing new multivariate statistical techniques (principal component analysis, canonic analysis, multilinear regressions, kriging, and variograms). This data will be strengthened by the addition of further sampling. ***The first two years of data collection by this project suggests that fruiting spur leaves are more sensitive indicators of nutrient status than non-fruiting spur leaves. This is a clear departure from current approaches. An additional one or two years of data will be collected to validate this finding. Findings will be tested at a new site in 2011-12. The new experimental objective 4 will further allow the validation of this result across a wider range of N and K status. (commence 2011 complete 2012).***

An important observation made in years 1-3 will be tested with a new research objective (Objective 4). Spurs serve as the fundamental bearing units in almond (Heerema et al., 2008) because mature almond trees bear a high percentage of fruit on these short shoots, with only a small percentage (fewer than 15%) of fruit born laterally on long 1-year-old shoots. Results in years 1 and 2 suggest that fruiting spurs exhibit a clear deficiency of N and K as well as P, K, S, Zn, and Cu in contrast to non-fruiting spurs and suggest that this local deficit influences spur survival (Fig 1). This specific and highly reproducible result demonstrates that spurs on almond behave independently and suggest that further analysis of spur behavior is warranted. ***A new project activity has been commenced to test this observation by utilizing an ongoing experimental orchard in which N and K fertilizer rate trial has been established (CDFA project 06-670). The availability of trees with distinct differences in N and K status not only allows direct testing of this theory but also increases the diversity of tree nutrient status for all associated objectives (1-5). (Initiate 2010, complete 2012).***

Results of the current project suggest that there is a predictable and proportional change in seven essential nutrients (N, P, B, Zn, Ca, Mg, Cu) across the season. This pattern of behavior is consistent across sites and within N treatment regimes and indicates that early season samples can reliably be used to predict late season tissue values. This will allow growers to now reliably utilize early season samples and adjust in-season fertilization. ***The development of a protocol to use early season leaf samples to predict in-season demand would be of great value to the industry. Results to date suggest this is possible. The new experimental objective 4 will further allow the validation of this result across a wider range of N and K status. Funding is requested for an additional year of sampling (2011) and integration/analysis (2012).***

Problem: In tree crop production in California, leaf sampling and critical value analysis represents the primary tool for fertilizer decision making (Brown and Uriu, 1996). A recent focus group activity among leading growers, consultants and a subsequent survey of almond growers, however, demonstrated that > 90% of all respondents felt that UC Critical Values (CV's), especially for N and K, were not appropriate for current yield levels. A vast majority of growers also noted that CV's are of no use early in the season when in-season adjustments could still be made, and many noted that even if a sound leaf sample is taken that the analysis cannot be used to determine a specific fertilization response.

Ideally, critical values are established by carefully controlled experiments in which the relationship between yield and nutrient concentration is closely followed. The majority of critical values relating to almond and pistachio, however, have been determined on the basis of visual symptoms, not from yield reduction (Beutel et al., 1978; Brown and Uriu, 1996). Yield based determination of critical values in Almond, for example, are only available for N (Uriu, 1976; Meyer, 1996; and Weinbaum et al, 1980, 1990), K (Meyer, 1996; Reidel et al, 2004) and B (Nyomora et al, 1999) and to our knowledge there are no yield based CV's established for the essential elements P, Mg, Ca, S, Cu, Zn, Fe, Mn, Mo, Ni, or Cl.
The poor validation of current almond and pistachio CV's is further exacerbated by the very significant constraints to tissue sampling in a perennial species. The difficulties in obtaining a meaningful leaf sample have long been known. Lilleland and Brown (1943), when studying the P nutrition of peach trees, found that the composition of morphologically homologous leaves taken from adjacent trees receiving the same fertilizer treatment differed considerably. This was also observed by Thomas (1945) in apple trees, and Steyn (1959) in citrus trees and pineapple.

Many factors contribute to the variability among tissue samples even within a single tree. In Olive, Perica (2001) found that the largest single influence on N concentration was light exposure. Sanchez (1991) observed that 'Comice' pear fruits in west and south quadrants had more N than those from north and east locations. Fruits from the top and middle canopy levels had more N than fruit from the bottom level and peripheral leaves always had significantly higher leaf N than that of mid-stem or interior leaves (Sanchez and Righetti,1990). Crop load also influences nutrient variability. Righetti et al. (1990) noted that the leaves in bearing trees had significantly lower K than non-bearing trees in the growing season. Similar trends were also noticed in French prune (Righetti et al., 1990). In almond, both light exposure and crop load have important implications at least in leaf N. Heerema (2005) clearly demonstrated that non-fruiting leaves have higher N content than fruiting spur leaves, while exposed leaves have higher N than non-exposed leaves.

The very great deal of variability in leaf nutrient concentrations seen in tree crops has resulted in the development of.. "standardized sampling techniques that cannot be over emphasized, since criteria for elemental analysis and interpretation have been established for specific plant conditions" (Jones and Case,1990). While it is true that the use of a standardized sampling protocol is essential if you are to contrast results with a pre-determined standard, this does not necessarily imply that such leaf samples are either the most sensitive or the most relevant indicators of tree nutrient status or potential for response. The choice of a July, non-fruiting, exposed spur leaf for nutrient analysis in Almond is clearly a compromise selected to ensure low variability, there has been no study (to our knowledge) that specifically attempts to determine the relative sensitivity of this standard leaf with any other leaf type, or time of sampling.

In addition to within-tree variability in leaf nutrient status, there is also a great deal of within orchard and between orchard variability that occurs as a consequence of variability between trees, changes in soil conditions and local micro-climate. Typically, this within field variability is not considered in sampling and as a consequence can lead to incorrect interpretation. For an individual plant the CV represents the minimum nutrient concentration in that individual that is required to attain 95% full yield. In a population of plants, however, the CV is the nutrient concentration of the population that results in 95% of all individuals attaining full yield. This population CV will always be greater than the CV of the individual by an amount determined by the variability in the population. Estimating that variability, is therefore essential if the true field CV is to be determined. None of the current texts or guidelines on nutrient management in tree species recognizes this issue and as a consequence we have been misusing the single most 'trusted' tool for nutrient management in tree crops (Beutel et al, 1978).

CDFA/FREP Goals: This project specifically addresses two areas of special emphasis for the 2007 call: 1) Nutrient uptake by tree crops and 2) Guidelines for orchard fertilization patterns. This project also aims to develop improved diagnostic tools and site specific management technologies. In combination with the partner project submitted to this call (Brown et al: Development of a Nutrient Budget Approach To Fertilizer Management In Almond), these projects will also determine crop nutrient requirements, critical values and efficiency of several fertilization practices.

Impact: In the absence of a reliable nutrient monitoring system and integrated fertilization program, growers of >1.4 million acres of Pistachio and Almond have inadequate scientifically validated tools on which to make sound fertilizer decisions. The development of tools and approaches that can be effectively used to monitor plant nutrient status and design efficient fertilizer strategies is a crucial first step toward responsible and profitable fertilizer use. Efficient and responsive fertilizer strategies are essential if we are to protect the Californian environment from non-point fertilizer pollution and is an economic imperative as consumers increasingly demand sustainability and responsible production techniques.

Long Term Solutions: This project will provide a robust new approach to leaf nutrient sampling and interpretation that reflects spatial and temporal and yield dependent variability. The **target audience** for this work is the Almond and Pistachio industry. **Success** will be measured by the reliability of new sampling and analytical protocols and subsequent field-testing, by the nature and effectiveness of outreach activities and by the adoption of new practices by the industry. This project has been developed collaboratively with all major stakeholders (growers, consultants, fertilizer companies, farm advisors, industry, regulatory agencies and analytical laboratories), through this and effective dissemination of our results, we expect excellent buy-in and adoption. This project is well supported by Almond Board, Committee on Pistachio and Yara NA who are funding related research on this topic.

Related Research: Our goal here is to improve the effectiveness of current sampling protocols and to provide guidance on the collection and interpretation of early season tissue sampling protocols and to develop nutrient best management practices. This project is a component of a larger integrated study examining strategies for more efficient management of nutrients and water in tree crops. The importance of this research to stakeholders is illustrated by the extent of funding for related projects Yara: $25,000 per year 2007-12; Almond Board: $32,000 per year 2007-2012; Pistachio Research Committee: $34,000 per year 2007-12.

A large-scale survey of within-field, between-field, within-tree, within year and between-organ nutrient concentration and variance has been conducted for three years (harvest 2010) in mature Almond and Pistachio orchards and will be extended for an additional year (2011). The interaction between yield and nutrient status is being examined at four pistachio and three almond sites and will be examined for two more years (2010/11). Principles developed will be validated in a new orchard (2011). Statistical techniques adapted from research in precision agriculture will be used to design new sampling protocols to better interpret tissue analyses and to determine the accuracy of current UC critical values. Perennial tree research requires a minimum of two complete two-year production cycles to account for carryover and alternate bearing tendencies. An extension of the current project into harvest year 4 is essential and will further enrich the dataset and allow the more accurate determination of yield x nutrient status. On the basis of year 1-3 results suggesting the importance of fruiting spur leaves for nutrient diagnosis a new trial will be implemented at a new site to specifically validate the potential for fruiting spur leaf samples to be used for early season diagnosis.

Knowledge Base: We will utilize this information to develop more rigorous critical values and if indicated develop approaches to use of nutrient ratios and site specific critical values. Collaboratively, a new nutrient BMP will be developed from an integration of this project and the parallel project (Brown et al: Development of a Nutrient Budget Approach To Fertilizer Management In Almond). The combination of nutrient budget determination, nutrient response information, improved sampling and monitoring strategies and yield determination provides a theoretically sound and flexible approach to ensure high productivity and good environmental stewardship. The output of this activity will be a new paper and computer based model that will help growers define and optimize their fertilization strategies based upon a sound understanding of nutrient budget demands of the tree as influenced by environment, crop load, location and yield. We expect to also refine current leaf CV's, investigate the utility of nutrient ratios and define the optimal rate of N application and effect of nutrient source. Research will emphasize N and K but will include an analysis of all essential elements commonly applied in California.

Grower Use: The 2008 CDFA funded survey of almond and pistachio growers simultaneously illustrated that growers were uncomfortable with existing nutrient management strategies and were interested in improved sampling and monitoring strategies (Lopus et al California Agriculture, 2010 June issue in press). The greatest specific incentive that will contribute to grower adoption is the pressure from key buyers to ensure that Almond and Pistachio is being produced in an environmentally sound manner. With much of the Almond and Pistachio crop going to Europe and Japan where 'sustainability' and 'eco-friendly' production are market considerations, the need to be seen as good stewards is becoming more essential.

We are however fully aware of the constraint in getting growers to adopt new approaches. We hope to overcome this by gaining early buy-in to our projects from all stakeholder, through strong collaboration with Farm Advisors and Industry, frequent presentation and workshops and the production of good, reliable, easy to use tools. Dr Brown makes numerous presentations each year and will be emphasizing this work heavily, presentations at UC, industry and fertilizer company events are planned.

D: OBJECTIVES

1. Determine the degree to which leaf nutrient status varies across a range of representative orchards and environments *(third year of data will be collected in 2010, analyzed in 2011and incorporated in to new guidelines in 2011/2012)*
2. Determine the degree to which nutrient status varies within the canopy and within the year. *(third and fourth year of data from current fields will be collected in 2010/11, analyzed in 201, supplemental trial commenced 2011 and incorporated in to new guidelines in /2012)*
3. Validate early season leaf analysis protocols and relationship with yield, validate current CV's and determine if nutrient ratio analysis provides useful information to optimize fertility management *(data collected 2010 and 2011. Supplemental trial commenced 2011. Analysis, interpretation integration in 2012)*
4. Test utility of use of fruiting spur leaf analysis under variable N and K treatments, validate as an indicator of tree nutrient status, monitor role of fruiting spur leaves in yield, monitor relationship between spur nutrient status and spur survival in Almond *(new activity for 2011-2012)*
5. Develop and extend an integrated nutrient BMP for Almond and Pistachio.

E: WORKPLAN

This task list is an extension of the first three year project (07-671) submitted to CDFA in 2007 at which time the suggestion that this would be a 5-year project was presented. Here we request an additional 2-year term (2011-2012) since it is clear that nutrition research in tree species demands a long term approach.

Workplans (the following work plans integrate both the existing and proposed extension period)

Objective 1/Task 1: Determine the degree to which leaf nutrient status varies within and across a range of representative orchards and environments.

Task 1.1 Continue trials commenced in 2008 harvest will be conducted in 2010 and results used to determine if additional years are required.

Initiated August 2007: Complete November, 2010/11

All trials were initiated in 8 or 9 yo microsprinkler irrigated Almond Orchards and 12-14 yo Pistachio of good to excellent productivity. Almond is planted to Non-Pareil (50%) on Nemaguard rootstock in soils representative of the region and a large percentage of Almond acreage. At experiment completion, trees will have reached 14 yo (after 5 years) representing their most productive years. In addition trials have been established in 4 Pistachio orchards (Madera, Fresno, Kings and Kern). The results of Nonpareil are likely to be highly relevant to other almond cultivars. Pistachio research is being conducted in Kerman cultivar.

Task 1.2 Initiate and conduct sample collection:

Initiate Jan, 2008: Complete November, 2010/11

In four, 8-9 yo mature Nonpareil (NP) orchards growing under excellent management conditions in four major growing regions, and four Pistachio orchards (14 yo) we have established an extensive Grid-Sampling protocol using techniques developed for GIS (with Richard Plant, a leading agronomic statistician). At 49 grid points uniformly distributed across a 10 acre block of trees, May and July leaf nutrient status, light interception, trunk diameter and tree yield will be determined in each Nonpareil tree. At 25 of these grid points, the nutrient status and yield of 2 neighboring trees will also be collected as independent data points. Initially, non-fruiting spur leaves (and subterminal leaves in pistachio) in exposed positions will be selected for these samples, however, depending on the early results of Task 2 below, sampling protocols may be adjusted. Two statistical techniques 'nugget sampling' and 'modified Mantel' statistics will be used, this approach allows for partitioning of variance in nutrient status due to environment, due to genetic variability and 'random' variability and allows for determination of the interactions and dependencies between nutrition and yield and the nature of spatial variability within an orchard .

Task 1.3 Tissue analysis

Initiated March 2008, ongoing through 2011/12.

Tissue determination for the major elements (N, P, K, S, Ca, Mg, B, Zn, Fe, Mn, Cu) in all leaf samples will be processed by the DANR analytical laboratory at UC Davis. The results of tissue analysis will be interpreted with reference to individual tree yield and environmental variability. This approach is unique as previously researchers have not considered the strong interactions that occur between yield and leaf nutrient concentration. Not only does enhanced nutrition potentially enhance yield (positive correlation), but high yields clearly decrease leaf nutrient status through competition (negative correlation). This iterative interaction has undoubtedly confused previous research in this area and will be addressed here.

Task 1.4 Determine tree yield

Initiated August, 2008 Completed 2010/11

In all experiments described here, individual tree harvest will be performed three days prior to commercial field harvest by selectively shaking individual experimental trees then raking and weighing by hand. To facilitate this we will use multiple supervised teams of 3-4 laborers and UC personnel at each site. Experience suggests 100 trees can be harvested in a single day in this fashion. A total of eight orchards and in excess of 1000 trees will be managed in this way.

Task 1.5 Statistical Analysis

Initiated July 2008, completed 2011/12.

In this experiment and in the second project proposed by us to this program (CDFA-FREP Brown et al: Development of a Nutrient Budget Approach To Fertilizer Management In Almond) we will use a combination of linear and non-linear statistical approaches utilizing both individual tree analysis and blocked treatments, replicated over several years in a mixed heirachical model. (Dr Richard Plant a leading agronomic statistician and PI on this grant is being consulted on the design and analysis of this experiment.) Effects of climate, location in the field and environment on patterns of nutrient uptake, in-field variability and budget will be determined by cross site comparison.

Where spatial data is involved, data will be geostatistically interpolated to develop maps of nutrient status for each element. These maps will be used to estimate the distribution of nutrient concentration in the field. Based on these distributions and spatial relationships a sampling plan will be developed that will permit growers to determine with a high level of probability that an acceptable percentage of their trees meets or exceeds the UC CV. While this initial experiment involves complex statistics and extensive sampling the expectation is that this basic information will allow us to develop a practical grower -friendly protocol. Basically, once the mean and variance of nutrients in a typical almond orchard is known, then a single composite sample of adequate size can provide all required information to select a target mean leaf nutrient value. This approach may have to be modified slightly for location and adjusted for economic return.

Task 2/Objective 2: Determine the degree to which nutrient status varies within the canopy and within the year and develop tissue-sampling protocols that provide early season measures of nutrient status.

The current leaf sampling standard of collecting non-fruiting exposed spur leaves in the July was chosen because it was necessary to combat the extreme variability in nutrient status that exists spatially and temporally in trees (see Righetti et al. 1990). It was never rigorously verified that this was a highly reproducible, sensitive or effective approach, only that it limited variability. Indeed it is possible that the current sampling strategy is highly reproducible but also highly insensitive to nutrient status. Several researchers have observed that once an almond leaf exceeds 2.2% N that additional fertilizer has only a slight effect on leaf N concentration (Uriu, 1976; Meyer, 1996; and Weinbaum et al, 1980, 1990). Indeed, Weinbaum observed in several experiments that a doubling of fertilizer application from 250 to 500 lbs per tree had no significant effect on leaf N concentration (Weinbaum, 1990). This observation has generally been interpreted as evidence that little of this additional N was acquired by the tree. This assertion might be incorrect, however, and an alternative explanation is simply that non-fruiting, exposed spur leaves sampled in late July are a poor indicator of tree N status.

Observation tells us that spur leaves associated directly with fruit are the first to show deficiencies, and as such may be the most sensitive indicators of a whole tree nutrient stress.

Reidel et al, 2004; demonstrated that spur leaf nutrient status correlates with current yield and influences future yield. Hereema (2005), contrasted leaves collected from fruiting and non-fruiting spurs and demonstrated that fruiting greatly decreases leaf nitrogen status, but he did not conclude that there is clear benefit to using these leaves. Hereema (2005) did not however, examine leaves early in the season when growers would be interested in an early indicator. Ultimately, our goal is to develop a method of sampling trees that best reflects the current nutrient status of the tree, predicts possible shortfall and guides in-season fertilizer practice. Attempts to use dormant tissue sampling as a measure of tree nutrient storage is not likely to be useful for high yielding Almond since total stored N appear to account for less than 10% of annual tree N demand. Overall, there has been inadequate rigorous research to determine if there is a meaningful or interpretable relationship between the various leaf types and either whole tree nutrient status or yield.

Results of the first two years of this experiment are extremely promising and we fully expect that a new more sensitive and relevant sampling strategy will be validated (see 07-671 annual report).

Task 2.1 Conduct multiple leaf sampling protocols to determine if there is a more sensitive indicator to tree nutrient status.

Initiated Jan 2008, current field sites completed 2010 unless otherwise indicated.
Validate at new field site with wider range of tissue N and K status (see Objective 4 below).
Initiated June 2010, complete Dec 2012.

Twenty trees, on which yield will be determined in the approach above, will be selected at each site for multiple within-year and within-canopy tissue sampling (80 trees in 4 almond orchards and 80 trees in one Pistachio Orchard will be sampled.). Yield will be determined on all individual trees. Leaves will be collected from central leaves on 1-3 year old well exposed spurs at 5 dates during the year from March till 1-month post harvest. Leaves from three spur types in almond and three positions in Pistachio will be collected and analyzed separately: in Almond, non-fruiting spur leaves, and fruiting spur leaves from spurs with either 1 or 3 fruits will be sampled. The specific phenological stage of the tree at each sample date will be determined by noting the stage of fruit development characteristics and days past full flowering. A total of 1500 (100 trees x 5 dates x 3 spur types) leaf samples will be collected. Yield on each of these trees will be determine in 1.4 above along with an analysis of local nutrient variability determined in 1.2 above. Leaves will be analyzed for the full suite of important elements

Task 3: Objective 3: Validate early season leaf analysis protocols and relationship with yield, validate current CV's and determine if nutrient ratio analysis provides useful information to optimize fertility management.

Task 3.1 Collect yield x leaf nutrient data for all essential elements and at multiple times through growing season. Relate to yield and nutrient ratios.

Initiate August 2010: Complete Dec, 2012
Validate at new field site with wider range of tissue N and K status (see Objective 4 below).
Initiated June 2010, complete Dec 2012

Results from year 1 and 2 data clearly suggests that early season leaf analysis is a strong predictor of both late season nutrient status and yield. Indeed the relationship between early season analysis and yield appears to be stronger than is evident from traditional sampling.

Initial regression analyses suggests, however that prior year yield also interacts with this relationship in a complex fashion. To fully explore these relationships a minimum of 4 years of yield and nutrient data are required. Results obtained in objective 2 above will be analyzed using a variety of statistical techniques with the goal of developing models that effectively predict July/August tissue values from March/April samples.

In the combination of experiments described here, >500 individual trees will be monitored for yield and nutrient status each year over a 4 year period at 8 sites. This represents by far the largest data base of yield x nutrition ever collected and will be used to help redefine or validate existing Critical Values. Furthermore, this will allow us to analyze nutrient ratio x yield effects as a potential basis for application of the DRIS system of nutrient ratio analysis in Almond. This pool of data will be analyzed using a variety of statistical and graphical approaches to partition variance, identify and classify data clusters, identify and model data trends, and ultimately estimate nutrient optimums (Boundary Layers, DRIS analysis, Mitscherlic response fitting etc). Results from the first three years of experimentation will be analyzed and targeted re-sampling will occur to validate newly developed models 2011/12 to further validate and refine the process.

Task 4: Objective 4: Test utility of use of fruiting spur leaf analysis as an indicator of tree nutrient status, monitor the relationship between spur nutrient status and spur survival in Almond *(new for 2011-2012)*

Data from this experiment in 2008 and 2009 suggest that fruiting spurs are more sensitive indicators of nutrient status than non-fruiting spur leaves. Further, prior work by Basile et al (2003) and Heerema et al (2008) suggest that the survival of fruiting spurs is a key determinant of return bloom and yield. Spurs serve as the fundamental bearing units in almond (Heerema et al., 2008), because mature almond trees bear a high percentage of fruit on these short shoots, with only a small percentage (fewer than 15%) of fruit born laterally on long 1-year-old shoots. Fourty five % of all productivity is carried on spurs with one fruit with 35% carried on spurs with two fruits. As a result, maintenance of the total number of living spurs per tree and ensuring their productivity is extremely important. Heerema et al. (2009) demonstrated that the interaction between tree N treatment and spur fruiting status was significant. In shaded canopy positions, leaf abscission rates on single-fruited spurs from May 31 to Sept. 28 were much lower on 'high N' (57%) than 'low N' (75%) trees.

Like N, potassium nutrition has been suggested as a critical factor for spur viability (Basile et al., 2003). Basile et al., (2003) observed that K deficiency resulted in premature leaf senescence and abscission. Potassium deficiency negatively affected the yield of almond trees by increasing the mortality of fruit-bearing spurs and reducing flowering of K-deficient spurs. Late in the season, tree light interception declined in non-fertilized trees compares with the high K trees. This effect could clearly be a consequence of premature leaf abscission, which was observed on fruiting spurs as the season progressed, and was exacerbated by the harvest process. Almond leaves located close to developing fruits had lower K concentration than leaves located on vegetative spurs suggesting that fruits draw K nutrition from nearby leaves.

In almond trees, spurs behave as semi-autonomous units (behaving independently of each other and the tree as a whole) with the autonomy of the spur unit increasing as yield increases. Survival of the individual spur is largely dependent upon local exposure, age of the spur and local fruit load. The semi-autonomous nature of individual spurs on a tree offers an opportunity for within-tree replication thereby reducing experimental error and tree-tree variability. To adequately conduct research in this system, however, it is essential that very careful attention be paid to the selection and replication of the experimental unit (spurs) on the tree.

Task 4.1 *Monitor spur leaf nutrient status through year and spur annually.*
Initiate 2010: Complete Dec, 2012

To further validate our observation that spur nutrient status reflects tree nutrient stress and predicts future yield we will initiate a new experiment in the N and K rate trial experiment underway at Belridge under the direction of Dr Brown (CDFA project 07-670). In this trial a large number of trees with clearly divergent N and K status have been established.

In four trees each in three K treatment rates (K125, K200, K300) and four N rates (N125, N200, N275, N350), sixteen spurs (8 single fruited, 8 double fruited) from within a well exposed section of each experimental tree will be individually tagged. This design will be replicated in four blocks for a total of 24 spurs per tree x 4 trees per block x 4 blocks (n=384) for each of 7 treatment combinations (n=2,658). These spurs will be monitored for survival (2011) and re-tagged for repeat survival measures in 2012 (dead spurs will be replaced).

In these same trees a single composite leaf sample will be collected from 4 spurs in each fruiting class and analyzed 5 times through the year for nutrient status (4 replicates per block x 4 blocks x 5 times x 7 treatments (n= 560)).
As part of an ongoing experiment these trees will be monitored for yield. The goal of this experiment is to replicate the early season sampling protocols (task 2 and 3) across a broader range of nutrient concentrations and to follow the effect of spur nutrient status on spur survival and tree yield.

Task 5. Develop new sampling and interpretation approaches that provide growers with a rational, and timely sampling protocol to optimize yield and return. Develop and extend an integrated nutrient BMP for Almond

Initiate Jan 2012: Complete Dec, 2012

Ultimately, our specific goal in this current project is to provide growers with information needed to determine what their target mean nutrient concentration should be to guarantee that 90%, 95% or 99% of their orchard is above a prescribed value. We will also develop the means to sample leaves early in the season to allow for more effective in-season nutrient management. We will also provide critical information on the role of fruiting spur leaf nutrition on spur survival and yield sustainability. We will utilize this information to develop more rigorous critical values and if indicated develop approaches to use of nutrient ratios and site specific critical values. Collaboratively, a new nutrient BMP will be developed from an integration of this project and the parallel project (Brown et al: Development of a Nutrient Budget Approach To Fertilizer Management In Almond). The combination of nutrient budget determination, nutrient response information, improved sampling and monitoring strategies and yield determination provides a theoretically sound and flexible approach to ensure high productivity and good environmental stewardship. The output of this activity will be a new paper and computer based model that will help growers define and optimize their fertilization strategies based upon a sound understanding of nutrient budget demands of the tree as influenced by environment, crop load, location and yield. We expect to also refine current leaf CV's, investigate the utility of nutrient ratios and define the optimal rate of N application and effect of nutrient source. Research will emphasize N and K but will include an analysis of all essential elements commonly applied in California.

Given the very large amount of data to be collected in this project a substantial amount of time will be devoted to a well integrated and highly accessible summary of activities and recommendations. Data and presentations will be posted to a website and an easy to use interactive decisions support system will be developed. A number of industry and science focused publications are expected.

F: Project Management, Evaluation and Outreach

Dr. Patrick Brown will provide overall coordination of all activities working closely with the responsible farm Advisor in each county. Major activities associated with Task 2 and 3 will be managed on a day-to-day basis by the Farm Advisor hired Field assistant located in the county in which the trial is located. The allocation of specific funds to this staff person and the dedication of additional funds to ensure Farm Advisor and their staff to participate is essential.
Each farm Advisor will assume coordinating responsibility for trials located in their county.
Dr. Bruce Lampinen will be a key advisor in all activities and will play a significant role in light interception measurements, project design and coordination.
Dr. Plant will supervise all statistical analyses.
A graduate student with expertise in tree nutrition and family ties to the Californian almond industry will be dedicated to this project. As a component of his thesis research this student will complete a thorough literature review of approaches to tree nutrient management and will in collaboration with Dr Brown write academic and 'farm press' summaries of these findings. Two additional Spanish speaking masters students will assist with all harvest activities.
Day laborers will be used extensively to assist with field harvest and avoid compromise with grower harvest schedule. Grower collaborators will be selected based upon their historical commitment to research, availability of harvest equipment and day labor pools.

Evaluation of project performance will be conducted on an ongoing basis. Conference calls are scheduled each 4 months among all participants to ensure progress is being maintained. Annual reports of activities will be submitted to CDFA and to the co-sponsors.

Outreach is a critical component of this project and will be actively pursued at all stages of project activity.

This project is derived in large part from the CDFA and industry sponsored focus groups and surveys currently underway by this project team. The involvement of >50 industry leading growers, consultants and farm advisors in the focus groups that helped initiate this project has helped raise the awareness of this activity. Further, the industry nutrition surveys recently mailed (>2,800) and the advertised web-based survey each contain specific explanations of our goals in new nutrient management in Almond and contain carefully phrased questions that will alert growers to the potential issues with current practices, thus whetting their appetites for new approaches.

We have found that involving stakeholders early in the process enhances buy-in in all projects, when stakeholders know what we are planning they tend to be more receptive to the results. The Almond Board and Yara fertilizers and Pistachio Research Committee have pledged a significant commitment to this and the associated project. The involvement of these entities will encourage grower recognition and fertilizer industry attention.

Annually, Dr Brown and Farm Advisors will present this ongoing research and ultimately the outcomes of this project at numerous events. Dr. Brown for example, typically presents 3-5 large audience presentations annually to meetings of the Almond Research Conference, Western Plant Heath Association, Cal-ASA Plant and Soil conferences, Pistachio Conferences, almond industry events, FREP events, regional Almond Meetings (eg SJV Almond Day, Nickels Field Day), Chemical Industry Grower Days (Actagro, Tesenderlo Kerley, Yara), PCA/CCA events.
As specific project results are developed, they will be distributed widely to the primary audience through the meetings described above and through press releases and ultimately training courses. The FNRIC site at UC Davis will develop a project web page for this activity and ultimately will host any subsequent computer based product.

REFERENCES

Beutel J, Uriu K, Lilleland O. 1978. Leaf analysis for California deciduous fruits. In: Reisenauer HM (ed.) soil and plant-tissue testing in California. Pp 11-14.

Brown PH, and Uriu K. 1996. Nutrition deficiencies and toxicities: diagnosing and correcting imbalances. In: Almond production manual. University of California, Division of Agriculture and Natural Resources. Publication 3364.

Heerema RJ. 2005. Compartmentalization of carbon and nitrogen stresses within almond (*Prunus dulcis* (Mill.) D.A. Webb) Spurs. Ph.D. Dissertation, University of California, Davis.

Jones JB, Jr, Case VW. 1990. Sampling, handling, and analyzing plant tissue samples. In: Westerman RL (ed.) Soil testing and plant analysis. 3rd ed. Soil Science Society of America, Inc. Book Series 3. Madison, Wisconsin. Pp 389-427.

Lilleland O, Brown JG. 1943. Phosphate nutrition of fruit trees. Proc. Amer. Soc. Hort. Sci. 41: 1-10.

Meyer RD. 1996. Potassium fertilization/foliar N/P/K/B studies. In: Almond Board of California. 1972-2003. Years of discovery. pp 291-292.

Nyomora AMS, Brown PH, Krueger B. 1999. Rate and time of boron application increase almond productivity and tissue boron concentration. HortScience 34: 242-245.

Perica S. 2001. Seasonal fluctuation and intracanopy variation in leaf nitrogen level in olive. J. Plant Nutr. 24: 779-787.

Reidel EF, Brown PH, Duncan RA, Heerema RF, Weinbaum SA. 2004. Sensitivity of yield determinants to potassium deficiency in 'Nonpareil' almond (Prunus dulcis (Mill.) DA Webb). J Hort. Sci Biotech. 79: 906-910.

Righetti TL, Wilder KL, Cummings GA. 1990. Plant analysis as an aid in fertilizing orchards. In: Westerman RL (ed.) Soil testing and plant analysis. 3rd ed. Soil Science Society of America, Inc. Book Series 3. Madison, Wisconsin. Pp 563-601.

Sanchez EE, Righetti TL. 1990. Tree nitrogen status and leaf canopy position influence postharvest nitrogen accumulation and efflux from pear leaves. J. Amer. Soc. Hort. Sci. 115: 934-937.

Sanchez EE, Righetti TL, Sugar D, Lombard PB. 1991. Nitrogen variability between Comice pear fruits from trees having high and low nitrogen status. J. Hort. Sci. 66: 43-50.

Steyn WJA. 1959. Leaf analysis. Errors involved in the preparative phase. J. Agric. Food Chem. 7: 344-348.

Steyn WJA. 1961. The errors involved in the sampling of citrus and pineapple plants for leaf analysis purposes. In: W. Reuther (ed.). Plant analysis and fertilizer problems. Amer. Inst. Biol. Sci., Washington, DC. Pp. 409-430.

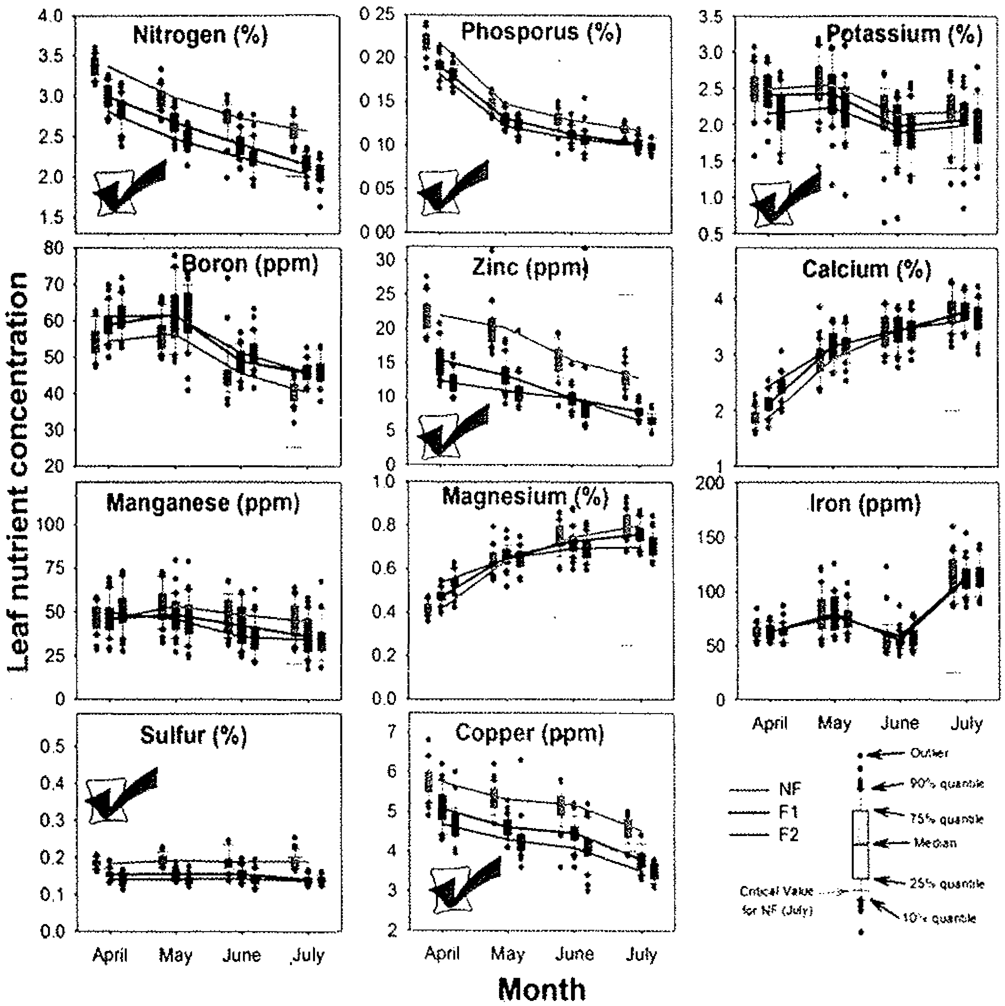
Thomas W. 1945. Foliar diagnosis. Soil Sci. 59: 353-374.

Uriu K. 1976. Nitrogen rate study. In: Almond Board of California. 1972-2003. Years of discovery. p 287.

Weinbaum SA, Broadbent FE, Wicke W, Muraoka T. 1980. Nitrogen timing study. Almond Board of California. 1972-2003. Years of discovery. p 288.

Weinbaum SA, Carlson RM, Brown PH, Goldhamer DA, Micke WC, Asai W, Viveros M, Muraoka TT, Katcher J, Teviotdale B 1990. Optimization of nitrogen use. In: Almond Board of California. 1972-2003. Years of discovery. pp 289-290.

Figure 1: Changes in leaf nutrient concentrations in leaves from non-fruiting (NF), single fruited (F1) and double fruited spurs. Data for Arbuckle 2009 is shown however the same result was observed at all 4 experimental sites in both years analyzed to date. Elements (N, K, P, Zn, S, Cu) differ significantly between NF and F1 or F2 spurs.



Arbuckle 2009