# Development of Leaf Sampling and Interpretation Methods for Almond and Development of a Nutrient Budget Approach to Fertilizer Management in Almond

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#### **Objectives:**

- Determine the degree to which leaf nutrient status varies across a range of representative orchards and environments.
- Determine the degree to which nutrient status varies within the canopy and within the year.
- Validate current CVs and determine if nutrient ratio analysis provides useful information to optimize fertility management.
- Develop a phenology and yield based nutrient model for Almond.
- Develop fertilizer response curves to relate nutrient demand with fertilizer rate and nutrient use efficiency.
- Determine nutrient use efficiency of various commercially important N and K fertilizer sources.
- Develop and extend an integrated nutrient BMP for Almond.

## Interpretive Summary:

Results of a survey of almond growers and consultants in California, suggested that leaf sampling and comparison with established standards [Critical Values (CVs)] does not provide sufficient guidance for nutrient management. Two explanations for this observation are possible. 1) The current CVs are incorrect or not useful for the decision-making process due to lack of sensitivity or inappropriate timing, 2) There are systematic errors in the manner in which critical values are used. One of the goals of this research is thus to conduct a systematic examination of leaf sampling protocols and their use in decision making. A second goal of the project is to determine the response of Almond to various rates and sources of nitrogen (N) and potassium (K) fertilizers, and to develop nutrient demand curves in order to develop more refined fertilization recommendations.

The first goal is being addressed in almond orchards in four locations in California (Arbuckle, Belridge, Madera and Modesto). Leaf and nut samples are taken at various times throughout the season to determine the degree of variability in tissue nutrient concentrations over time, space and within tree canopies. While this assessment requires data collection over several seasons (3-5 years), some preliminary data will be presented. These data indicate that leaves from spurs with local fruit load are more indicative of critical tree nutrient status than leaves from spurs without local fruit load. In addition, there is consistent evidence to postulate that the death of fruit bearing spurs can be attributable to a local nutrient deficit of N, P, K, Zn, S, and Cu throughout the season. Data also demonstrate leaf samples from spurs having two fruits (F2) collected in April can be effectively used to predict the July N (and other elements) status of the trees and provide a tool for early season diagnosis and correction of deficiencies. Representative sampling is important to monitor the nutrient status of the orchard. Status of N in a single moderately uniform almond orchard can be estimated by 4 independently collected and analyzed leaf samples while potassium is much more variable and 22 samples are required to estimate the orchard average K status with 85% confidence.

The second project goal is addressed in a fertigation trial at Belridge, Kern County, CA. This experiment is being used to develop a phenology and yield based nutrient model for Almond, to develop fertilizer response curves to relate nutrient demand with fertilizer rate and nutrient use efficiency, and to determine nutrient use efficiency of various commercially important N and K fertilizer sources. Findings from both project components will be used to develop integrated nutrient best management practices for almond. Treatments in the fertigation trial consist of four rates of nitrogen (125 lb/ac, 200 lb/ac, 275 lb/ac and 350 lb/ac), supplied by two commercially important sources of nitrogen, UAN 32 and CAN 17. There are three treatments for potassium rates (100 lb/ac, 200 lb/ac and 300 lb/ac) and three sources of potassium (SOP, SOP+KTS and KCI). Leaf and nut samples were collected from 768 individual trees in April, May, June, July and August and analyzed for essential plant nutrients. The results from the three years of data indicate that there is a significant effect of nitrogen rates on yield. Fruit nitrogen accumulation was directly correlated with N application rate while potassium rates have not shown a significant effect on yield. K sources have shown a significant

effect on yield under fan jet irrigation only. N and K rates had a significant effect on fruit and leaf nutrient concentrations, while differences between N and K sources have not been established yet. Knowledge of tree yield and nut nutrient concentration can be used to calculate orchard nitrogen removal and to plan fertilization replacement strategies. Results show that 80% of N and 70% of K is accumulated in the almond fruit by mid June, and N and K fertilizers should be applied accordingly to meet crop demand. A 1000 pound kernel yield removed 62lb and 72lb nitrogen in 2009 and 2010 respectively for the nitrogen rate treatment 275lb/ac. K removal by a 1000lb kernel yield was 70lb and 80 lb in 2009 and 2010 respectively for K rate 200lb/ac. Increasing nitrogen rate increased crop yield but nutrient use efficiency for the 350lb/ac nitrogen rate declined. Results are based on three years data and must be repeated for at least two additional years to be fully validated.

### Materials and Methods:

#### Variability trial

At each site samples were collected in year 1 from 8 to 10 year old micro sprinkler irrigated (one drip irrigated) almond orchards of good to excellent productivity planted to Non-Pareil (50%). At experiment completion, trees will have reached 11 to 14 years of age (after 3 or 5 years) representing their most productive years.

For each of the 4 almond sites (Arbuckle, Belridge, Madera and Modesto), plots are 10-15 acre contiguous blocks. Leaf and nut samples from 114 trees are collected at 5 times during the season. Sample collection is spaced evenly over time from full leaf expansion to harvest. As phenological markers, days past full bloom and stage of nut development are noted. Light interception, trunk diameter, and individual yields of these trees are also measured.

A standard leaf sampling protocol was used to determine nutrient concentrations in samples of exposed, non-fruiting spurs (NF), as well as leaves from fruiting spurs with 1 and 2 fruit (F1 and F2, respectively) to explore the sensitivity of different sampling methods as indicators of tree nutrient demand. In 2010 tissue samples were collected from NF and F2. To establish seasonal nutrient accumulation, composite nut samples were collected from each site. Both leaf and nut samples were dried and ground prior to sending them to the Agriculture and Natural Resources (ANR) Analytical Laboratory located on the UC Davis campus.

### Fertigation trial

The fertigation rate and source experiment was established in a Paramount Farms almond orchard at Belridge, Kern County, California under fan jet and drip irrigation systems. Each of the 12 treatments (**Table 1**) was replicated in five or six blocks with 15 trees per block. Treatments consisted of four rates of nitrogen (125, 200, 275 and 350 lb/ac), supplied as two commercially important sources of nitrogen (Urea Ammonium Nitrate 32% [UAN 32] and Calcium Ammonium Nitrate 17% [CAN 17]). Potassium was applied at three rates (100, 200 and 300 lb/ac as K) and supplied by three sources of potassium (Sulfate of Potash [SOP], SOP + Potassium Thiosulfate [KTS] and

Potassium Chloride [KCI]). 60% of the potassium in K rate treatments was applied as SOP in early February, while the remaining 40% was applied as KTS in four fertigation cycles. Nitrogen was applied in four fertigation cycles with 20%, 30%, 30% and 20% of total nitrogen supplied in February, April, June and October, respectively. Fifteen trees and their immediate 30 neighbors, in two neighboring orchard rows, were treated as one experimental unit. All data were collected from six trees in the middle row. A total of 768 experimental trees were selected for this experiment. Leaf and nut samples were collected from individual trees in April, May, June, July and August. A total of 5400 leaf and nut samples were collected and analyzed for N, P, K, Ca, S, Mg, B, Zn, Cu, Mn and Fe at the ANR Lab. The crop was harvested in August and individual tree yields were determined for all data trees. Yield of the remaining nine non-data trees in the data trees row was also determined to get average plot yield of fifteen trees. Four-pound samples were collected from two data trees each in each replicate to determine crack out percentage (turn over) and oven dry weight. Twenty nuts were collected at harvest from each experimental tree to determine the ratio of kernel to shell/hull and the partitioning of nutrients.

Treatment	N source	N amount (lbs/ac)	K source	K amount
				(lbs/ac)
А	UAN32	125	60% SOP / 40% KTS	200
В	UAN32	200	60% SOP / 40% KTS	200
С	UAN32	275	60% SOP / 40% KTS	200
D	UAN32	350	60% SOP / 40% KTS	200
E	CAN17	125	60% SOP / 40% KTS	200
F	CAN17	200	60% SOP / 40% KTS	200
G	CAN17	275	60% SOP / 40% KTS	200
Н	CAN17	350	60% SOP / 40% KTS	200
I	UAN32	275	60% SOP / 40% KTS	100
J	UAN32	275	60% SOP / 40% KTS	300
K	UAN32	275	100% SOP	200
L	UAN32	275	100% KCI	200

**Table 1**. Fertilization treatments.

### **Results and Discussion:**

#### Variability trial

This observational study illustrates nutrient dynamics throughout the season. Data from samples collected in 2008 (data not shown), 2009 (**Figure 1**) and 2010 (**Figure 2**) illustrate that nutrient concentrations and their variability depend on the nutrient sampled, sample type and sampling time. The patterns of nutrient change and accumulation, however, are sufficiently consistent to allow for the development of models that effectively describe and predict seasonal demand and concentration changes (see below).

The information obtained in this project adds considerably to the current understanding of the factors that determine spur longevity and potential to re-bear. Data from this project confirms that fruit presence alters spur level N and K dynamics resulting in local

deficits that may negatively impact photosynthesis and ultimately yield. Fruiting spurs can exhibit nutrient deficiencies even when non-fruiting leaves on the same tree may have "adequate" leaf concentrations. Observation and statistical analysis suggest that fruiting spurs may be better indicators of tree nutrient status than non-fruiting spurs. Further, there is a consistent and highly repeatable depletion of N, P, K, S, Zn, and Cu in fruiting spurs as crops develop. This observation, which needs confirmation, suggests that 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. We are currently testing the hypothesis that local deficit of these 6 elements are important for spur longevity and yield sustainability.

Leaf samples are characteristically collected in July in Almond. Collection of leaves earlier in the season would be useful for management by providing important information on current orchard nutrient status and enough time to correct deficiencies if any. The consistency in patterns of change in tissue nutrients over time provides an opportunity for an early season sampling to diagnose deficiencies. Further, preliminary studies suggest early season samples are better predictors of yield and tree health than July samples. July leaf nitrogen content (and likely other nutrients) can be well predicted with an early season (April) sampling. With the analysis of tissue concentrations of 11 essential plant nutrients in F2 leaves collected in April, it is possible to estimate July NF leaf concentrations with a high degree of accuracy. Results presented in **Table 2** contrast July N values predicted from April sampling against actual July N values at each of 4 sites over 3 years. Additionally, knowledge of within orchard variability allows for April sampling to be used to determine the % of trees that may be N deficient in July.

Leaf sampling is only of value if enough independent samples are collected to represent the nutrient status of the orchard as a whole. Based upon the three years of data analyses of moderately uniform and good producing orchards we have derived the minimum sampling sizes required to effectively estimate July orchard nutrient status with a given degree of confidence (**Table 3**). Thus, 4 independently collected and analyzed leaf samples can provide an estimate of average field N status with an 85% confidence level. In contrast 22 and 242 leaf samples would be required to predict field average K and Mn concentrations with 85% confidence. The collection of discrete samples (as opposed to bulked samples) also allows for an estimation of the degree of variability within the orchard. The high number of samples required for K determination is a consequence of the high variability seen in field samples across location and season. This is clearly illustrated in the large errors for K in **Figures 7 and 8**.



**Figure 1.** Nutrient behavior throughout 2009 season in leaves from non-fruiting spurs (NF), spurs with 1 fruit (F1), and spurs with 2 fruits (F2). The graphs show data collected from the Arbuckle orchard but are representative of all orchards.



**Figure 2.** Nutrient behavior throughout the 2010 seasons in leaves from non-fruiting spurs (NF), and spurs with 2 fruits (F2). The graphs show data collected from the Arbuckle orchard during 2010 season.

Results suggest that the current standard sampling protocol, which only includes leaf samples from non-fruiting spurs, may not reflect critical local tree nutrient status. The difference in response of NF and F1/F2 samples, clearly visible for N and Zn, may be of particular relevance as F1 and F2 leaves were below established leaf critical values in July.

**Table 2**: Prediction of non fruiting (NF) leaf N concentrations in July from double fruited (F2) spur leaves collected in April and estimation of field average tissue N concentrations from April F2 analysis.

Site	Year	July Nitrogen Predicted	July Nitrogen Observed	Predicted Percentage of Trees below 2.2 % at July	Observed Percentage of Trees below 2.2 % at July
Arbuckle	2008	2.4	2.3	15%	23%
Belridge	2008	2.4	2.4	3%	0%
Madera	2008	2.5	2.4	4%	10%
Modesto	2008	2.4	2.4	9%	17%
Arbuckle	2009	2.4	2.6	10%	0%
Belridge	2009	2.4	2.4	11%	3%
Madera	2009	2.6	2.4	2%	3%
Modesto	2009	2.6	2.7	1%	0%
Arbuckle	2010	2.4	2.5	14%	0%
Belridge	2010	2.3	2.7	10%	0%
Madera	2010	2.3	2.3	12%	21%
Modesto	2010	2.4	2.5	7%	0%

\*\* Leaf nutrient concentrations in samples collected in April from all sites, trees and years were used to create a mathematical model to predict July tissue values and to estimate percentages of trees that would be below currently established critical values

	Confidence level	
Nutrient Element	(%)	Number of Samples
Nitrogen	95	8
Nitrogen	85	4
Nitrogen	70	2
Phosphorus	95	17
Phosphorus	85	9
Phosphorus	70	5
Potassium	95	40
Potassium	85	22
Potassium	70	11
Calcium	95	9
Calcium	85	5
Calcium	70	3
Manganese	95	448
Manganese	85	242
Manganese	70	126

**Table 3:** Number of independent leaf samples required to estimate the true mean of a particular element in a contiguous and moderately uniform orchard.

### Fertigation trial

The accumulation of Nitrogen, Phosphorus, Potassium, Sulfur, Calcium and Magnesium in the fruit at different rates of N increased over the season as shown in **Figures 3 and 4**.

## Nitrogen

Nitrogen accumulation in the fruit was positively correlated with nitrogen supply at all sampling dates. About 80% of the nitrogen was accumulated in the fruit by mid June (111 DAFB in 2009 and 126 DAFB in 2010) as shown in **Figure 3 and** 4. At harvest 53lb and 52lb nitrogen was removed in 2009 and 2010 respectively by a 1000lb kernel yield in the 125lb/ac nitrogen rate while 62lb and 69 lb nitrogen was removed in fruit in 2009 and 2010 respectively by a 1000lb kernel yield from N rate 350lb/ac. The nitrogen removal by 1000lb kernel yield in 2010 is slightly higher than 2009 for the N rates 275lb/ac and 350lb/ac due to a slight increase in the fruit nitrogen concentration in 2010 that may have been a result of the cool and extended growing season (**Fig. 10**).

## Phosphorus

Phosphorus exhibited an annual trend that resembled nitrogen, however with increasing nitrogen supply, fruit phosphorus removal declined slightly but not significantly. The N rate 125lb/ac removed 7.8lb and 9lb phosphorus for a 1000lb kernel yield in 2009 and 2010 respectively (**Fig. 3 and 4**). The N rate 350lb/ac removed 7lb and 7.8lb phosphorus in 2009 and 2010 to yield a 1000lb kernel. The decline in phosphorus removal with increasing nitrogen supply is due to the increase in kernel percentages (larger nuts) with increased nitrogen supply.



**Figure 3**. Nitrogen, Phosphorus, Potassium, Sulfur, Calcium and Magnesium removal by almond fruit to produce 1000lb kernel yield from nitrogen rate treatments in 2009. Each point represents mean and mean error bar.



**Figure 4**. Nitrogen, Phosphorus, Potassium, Sulfur, Calcium and Magnesium removal by almond fruit to produce 1000lb kernel yield from nitrogen rate treatments in 2010. Each point represents mean and mean error bar.

#### Potassium

Fruit potassium accumulation increased over time but was not significantly influenced by K treatment suggesting that K availability at this site was not rate limiting (**Figures 5 and 6**). About 70% of the total K was accumulated in the fruit by mid June (111 DAFB in 2009 and 126 DAFB in 2010) while its total amount increased until harvest.



**Figure 5.** Potassium removal by almond fruit to produce a 1000lb kernel yield from potassium rate treatments in 2009. Each point represents mean and mean error bar.



**Figure 6**. Potassium removal by almond fruit to produce 1000lb kernel yield from potassium rate treatments in 2010. Each point represents mean and mean error bar.

## Leaf Nutrient Concentration

Leaf nutrient concentrations for the nitrogen rate treatments for 2010 are shown in **Figure 7**. Significant differences in leaf nitrogen concentration were observed between N rate treatments throughout the season in both 2009 and 2010. In 2010 leaf nitrogen concentrations were slightly higher in the beginning of the season compared to 2009 (data not shown). Significant differences have not been observed in the N source treatments (data not shown). Significant differences in leaf potassium concentration were observed between K rate treatments in both 2009 (data not shown) and 2010 (**Figure 8**). In 2009, leaf potassium content declined slightly for all the K rate treatments, while in 2010 leaf K for the higher K rates remained constant in the season. No significant differences have been observed between the K source treatments. Data illustrate the extreme degree of variability that exists in tree K concentrations and suggests that tissue sampling for K is extremely limited in its utility.



**Figure 7.** Effect of different nitrogen rates (N UAN 32) on leaf nutrient concentrations in 2010. In box plots, the central line is the median of the distribution, the edges of the boxes are the 25% and 75% quantiles, error bars, represent the 10% and 90% quantiles, and all points are outliers

The very consistent pattern of leaf nutrient concentrations over time suggests that samples of most nutrients could be effectively collected at any time of the year and be used to predict tree nutrient status at any other time of the year. For example, if a leaf exhibited a tissue N concentration of 3.5 on the 40<sup>th</sup> day after full bloom, then there is a 95% likelihood that an equivalent leaf would have a leaf N concentration of 2.35 on 156 days after full bloom.



**Figure 8.** Effect of different Potassium rates (SOP+KTS) on leaf Potassium concentrations in 2010. In box plots, the central line is the median of the distribution, the edges of the boxes are the 25% and 75% quantiles, error bars, represent the 10% and 90% quantiles, and all points are outliers

## **Fruit Nutrient Concentration**

Fruit nitrogen and phosphorus concentrations declined over time in all N rate treatments; fruit potassium showed a variable trend while there was slight decline in fruit calcium and magnesium concentration in both 2009 and 2010 (**Figures 9 and 10**). Fruit K content from K rate treatments also showed a variable trend being high in the beginning of the season, declining in the mid season, and again increasing in late season (**Figure 11**).



**Figure 9**. Nutrient concentrations in almond fruit over time for N rate treatments in 2009. Box plots illustrate the median of all samples (central line), the 25th and 75th quantiles (box edges), while error bars represent the 10% and 90% quantiles, outliers are shown as discrete points.



**Figure 10**. Nutrient concentrations in almond fruit over time for N rate treatments in 2010. Box plots illustrate the median of all samples (central line), the 25th and 75th quantiles (box edges), while error bars represent the 10% and 90% quantiles, outliers are shown as discrete points.



**Figure 11.** Potassium concentrations in almond fruit over time for K rate treatments in 2010. Box plots illustrate the median of all samples (central line), the 25th and 75th quantiles (box edges), while error bars represent the 10% and 90% quantiles, outliers are shown as discrete points.

### Yield

Nitrogen treatments had a significant effect on crop yields in the second and third year of the experiment although differences were also observed in the first year. The effect of nitrogen rate and source treatment on kernel yield in 2009 and 2010 is presented in **Table 4**. Maximum kernel yield was obtained from the highest N rate treatment (350 lb ac<sup>-1</sup>), while minimum yield was obtained from the lowest nitrogen rate treatment (125 lb ac<sup>-1</sup>) under both drip and fan jet irrigation in both 2009 and 2010. The kernel yield in 2010 was higher than 2009. Increasing nitrogen supply increased yield however decreased the nitrogen use efficiency [(NUE) (**Figure 12**)]. NUE from the N rate 275lb/ac was 81% while maintaining near maximum yields – this efficiency is among the highest ever recorded in agriculture and occurred in a low organic matter (0.4%) and low residual nitrate soil (2-4 ppm Nitrate-N in the 0-24 inch profile). NUE's of greater than 1.0 were observed in the 125 and 200 lb N treatment, however these efficiencies clearly negatively impacted yield and tree growth.

A significant effect of K rate and source on yield was not observed in 2009, however a significant effect of K source on kernel yield was observed in 2010 under fan jet irrigation (**Table 5**); K source SOP+KTS had maximum yield while KCI produced lower kernel yield. Tissue K values dropped below the critical value of 1.4% in the low K treatment for the first time in 2010 and a yield effect may be expected in 2011.

**Table 4**. Effect of nitrogen rate and source on plot mean kernel yield (lb/ac) in 2009 and 2010. Yield not connected by the same letters are significantly different.

N	UAN 32				CAN 17			
(lb/ac)	125	200	275	350	125	200	275	350
Drip	2,731	2,961	3,206	3,517	2,610	2,620	3,050	3,444
	С	bc	b	а	С	С	b	а
Fan								
Jet	2,828	3,088	3,296	3,325	3,087	3,138	3,309	3,218
	b	ab	а	а	а	а	а	а

## Kernel Yield 2009 (lb/ac)

#### Kernel Yield 2010 (lb/ac)

Ν	UAN 32				CAN 17			
(lb/ac)	125	200	275	350	125	200	275	350
Drip	2865	3,452	3,765	4,064	2,622	3,313	3,728	3,960
	С	b	ab	а	С	b	а	а
Fan								
Jet	2909	3,405	3,813	3,924	2,990	3,336	4,172	3,866
	С	b	ab	а	b	b	а	а

**Table 5.** Effect of potassium rate and source on kernel yield (lb/ac). Yield not connected by the same letters are significantly different. (N Rate = 275 lb/ac)

Κ	K rate (SOP+KTS)			K Source						
(lb/ac)	100	200	300	SOP+KTS	SOP	KCI				
Drip	3,829	3,785	3,844	3,659	3,649	3,583				
	а	а	а	а	а	а				
Fan										
Jet	3,835	3,813	3,806	3,829	3,758	3,353				
	а	а	а	а	а	b				

# Kernel Yield 2010 (lb/ac)



**Figure 12**. Cumulative nitrogen use efficiency 2008 to 2010 for nitrogen rate treatments. Box plots illustrate the median of all samples (central line), the 25th and 75th quantiles (box edges), while error bars represent the 10% and 90% quantiles, outliers are shown as discrete points. The bold line is the mean.

### **Discussion:**

This is year three of a multiyear trial and hence results must be treated with caution. Data from this project confirms that fruit presence alters spur level N and K dynamics and suggests that fruiting spurs may be better indicators of tree nutrient status than non-fruiting spurs. Early season sampling is important to diagnose and correct deficiencies in season and July nitrogen content (and likely other nutrients) can be well predicted with an early (April) sampling. Leaves from F2 in April can be used to predict the July nutrient status of NF with a high degree of accuracy. Status of some nutrients (N, P and Ca) can be estimated by collecting a reasonable number of representative samples while other nutrients are more variable (K) and more samples are required to estimate their status in the orchard while it appears infeasible to use tissue analysis as a routine monitoring tool for Mn, Fe, B and Zn. Nevertheless, tissue samples are still valuable as a means of identifying the cause of a clear visual problem.

Increasing nitrogen supply significantly increased kernel yield and nitrogen concentration in the plant tissues. 80% of the nitrogen and 70% of the potassium was accumulated in the fruit by mid June (111DAFB and 126DAFB in 2009 and 2010 respectively) suggesting that N and K fertilizer should be applied before this time to meet the crop N and K demand. K rate has not shown a significant effect on kernel yield suggesting that the trees were not K deficient even with the low K rate treatment on this type of soil. However; the tissue K value of many trees in the low K rate treatment have dropped below 1.4% and yield reduction is expected in the coming years. Trends in nutrient concentrations and fruit accumulation were evident early in the season and

persisted throughout the year and may imply that early season sampling may be useful in monitoring of tree nutrient demand. Differences in tree response to N sources have not been established, while K source have shown a significant effect on kernel yield under fan jet irrigation.

## **Recent Publications:**

Two poster presentations and corresponding short papers were presented at the XVII International Plant Nutrition Colloquium in Sacramento, August 2009.

Two posters were presented in American Society for Horticulture Science conference in Palm Spring in August 2010.

A farmer's field day was conducted in Belridge Almond Fertility Trial and more than 150 farmers and industry people participated.

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