# Nitrogen management through intensive on-farm monitoring

### **FREP Contract # 94-0362**

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# **Objectives:**

- 1. Document the accuracy, labor intensity and cost of on-farm monitoring techniques for soil and crop N status.
- 2. Develop baseline data on N uptake rates and tissue N sufficiency levels for the important cool season crops.
- 3. Demonstrate the effect of "best management practices" (drip irrigation and fertigation, intensive monitoring) on crop yield, and water and nitrogen use.
- 4. Conduct outreach activities to disseminate research results.

### **Summary:**

A series of trials were conducted in drip-irrigated commercial fields near Santa Maria to:

- Evaluate vegetable crop productivity at reduced N application rates.
- Document soil and plant N dynamics in cool-season vegetable production under typical coastal conditions.
- Evaluate the accuracy, labor requirement and cost of on-farm monitoring techniques for soil and plant N status.

Eight drip-irrigated fields in long-term vegetable rotations, representing a range of soil texture, were monitored through typical crop rotations (including broccoli, cauliflower, celery and lettuce) from summer, 1994 through fall, 1995. At the beginning of each crop in each field, 4 replicate plots were fertilized by a banded preplant

application of slow-release fertilizer at 100 lb N/acre. These plots, which also received all N applications delivered through the drip irrigation system, served as a high N >reference= treatment against which the rest of the field (designated >field management= treatment) was compared with respect to crop performance and N status of crop and soil. An intensive program of crop and soil monitoring was initiated at crop establishment, including soil NO<sub>3</sub>-N determination (by an on-farm >quick test= procedure and conventional laboratory analysis), petiole NO<sub>3</sub>-N (by on-farm sap analysis and conventional laboratory technique) and relative chlorophyll content (by leaf absorbance meter). The results of these on-farm monitoring techniques, together with the visual comparison of field management and reference plots, helped guide fertility decisions.

In nearly all fields monitored, seasonal N applications considerably lower than industry norms were used without reducing marketable yield (compared to the high N reference plots); in only one field (of fall broccoli) was yield significantly affected, the effect being a slight delay in maturity. Total plant biomass was equivalent in both N treatments in all fields, as was total N uptake; the only consistent result of the increased N rate of the reference plots was to enlarge the pool of soil NO<sub>3</sub>-N. A consistent seasonal pattern of soil NO<sub>3</sub>-N was observed. At the start of the summer/fall 1994, season soil NO<sub>3</sub>-N concentration was >30 ppm in all fields; these levels were maintained throughout the season, diminishing substantially only during heavy winter rains. Despite conservative fertilization, soil NO<sub>3</sub>-N increased throughout the spring, 1995 season; by fall, NO<sub>3</sub>-N concentration was back to levels similar to the previous year. Mineralization of soil organic nitrogen was significant contributor to the development and maintenance of elevated soil NO<sub>3</sub>-N. Rates of 1.4 to 2.2 lb N/acre day were measured by various anaerobic and aerobic incubation techniques.

On-farm monitoring techniques, although not as accurate as conventional laboratory analysis, provided useful information in a timely and cost efficient manner. Petiole NO<sub>3</sub>-N analysis documented that adequate plant N nutrition was maintained, but could not reliably be used to infer soil N status; soil NO<sub>3</sub>-N testing was required to assess soil mineral N supply. A routine monitoring program of soil NO<sub>3</sub>-N testing (2-3 times per season) to assess N sidedress requirement and companion petiole NO<sub>3</sub>-N analysis to ensure plant N sufficiency would be cost effective. This study suggests that N rate reductions of 50-100 lb N/acre below average industry application rates are possible, with a cost savings of 5-10 times the cost of monitoring.

### **Procedures:**

In cooperation with Betteravia Farms of Santa Maria, California a major grower of cool-season vegetables, eight commercial fields were selected for study in spring, 1994. These fields, all in long-term vegetable rotations, represented a wide range of soil texture and organic matter content. All fields were drip-irrigated through semi-permanent subsurface systems; drip-irrigated fields were chosen because the ability to apply nitrogen fertilizer at any time was necessary for this pilot study. Beginning with summer-planted crops in 1994 these fields were monitored through normal crop

rotations until fall, 1995. Broccoli, cauliflower, celery and lettuce were included in the rotation.

At the beginning of each crop, each field was divided into quadrants; in each quadrant a plot (4 rows wide, the length of the field) was fertilized immediately before planting with a banded application of slow-release N at 100 lb N/acre. These plots, which also received all drip-applied (fertigated) N delivered during the growing season, served as an N sufficiency reference against which the rest of the field was compared with respect to crop performance and N status of crop and soil. The N treatments will hereafter be designated >field management= or >reference=. No whole-field preplant N applications were made.

After crop establishment (defined as 7-10 days after transplanting, or after the removal of sprinklers following the emergence of direct-seeded crops) composite soil samples (0-12 inches) were collected from both N treatments in each quadrant (8 samples per field). Samples were analyzed for mineral N (NH<sub>4</sub>-N + NO<sub>3</sub>-N, in 2N KCI extracts of moist soil, by diffusion conductivity technique), organic matter content (dichromate reduction method) and total soil N (Kjeldahl digestion method). Relative net N mineralization potential was evaluated by three techniques:

- a) potentially mineralizable N (PMN) by anaerobic incubation (Keeney, 1982) at 40°C for 7 days
- b) 4 week aerobic incubation of field-moist soil at constant moisture and temperature (30°C)
- c) 4 week in-field incubation of field moist soil (Nadelhoffer et al, 1985) in 1 mil-thick ziplock bags, buried 4-6 inches deep.

Changes in NH<sub>4</sub>-N (PMN) and total mineral N (aerobic incubations) were determined on 2N KCI extracts.

At three times during the cropping season, and at harvest, additional soil (0-12 inches) and plant samples (petiole/midrib and whole plant) were collected. Soil was evaluated for NO<sub>3</sub>-N concentration (2N KCI extraction). Petiole/midrib tissue was ovendried, ground, extracted in 2% acetic acid and analyzed for NO<sub>3</sub>-N concentration by the cadmium reduction technique. Whole plants were dried and evaluated for total biomass and total N concentration (combustion technique, Sweeney, 1989).

In-season soil and petiole/midrib samples were split, with half utilized for the conventional laboratory analysis just outlined; the remaining portion was analyzed onfarm by Betteravia Farms personnel by several >quick test= techniques:

- a) Petiole sap analysis by >Cardy= nitrate selective electrode (Hartz et al, 1993b)
- b) Relative chlorophyll content by Minolta SPAD 502 leaf absorbance (chlorophyll) meter (Schepers et al, 1992)
- c) Soil NO<sub>3</sub>-N concentration, using a volumetric extraction of moist soil (Hartz, 1994).

The results of these on-farm monitoring techniques were made available to the farm manager; these results, together with the visual comparison of field and reference plots, provided information to help guide N fertigation decisions.

#### Results:

A total of 18 field trials were conducted during the study; 8 during the 1994 summer/fall season, 8 in the 1995 spring/summer season, and 2 in summer/fall, 1995. The management of two of the fields planted in the spring of 1995 was complicated by the heavy rain, and received N applications in excess of regional norms; results from those fields are not presented.

# Soil characteristics

The fields chosen, which ranged in texture from sand to clay loam, had substantial differences in organic matter and total N content (Table 1). Although considerable variation existed among fields and estimation techniques, as a group these soils showed considerable N mineralization potential. The values presented represent the mean of 4 replicate soil samples (0-12 inches) collected a crop establishment, 4-8 weeks following incorporation of previous crop residue. Potentially mineralizable N (PMN, anaerobic incubation for 1 week at 40°C) averaged 0.36 ppm/day; 4 week aerobic incubation at constant temperature, or in-field, averaged 0.44 and 0.54 ppm/day, respectively. At an average bulk density of 1.5g/cm<sup>3</sup> these estimates represented the equivalent of approximately 1.4 to 2.2 lb net N mineralization/acre day. Caution should be used in the interpretation of these values. PMN is an indirect measure of an aerobic process (N mineralization); aerobic incubation techniques are also problematic, since results are affected by the disturbance and handling of the soil, and its moisture and aeration status. However, taken in total, these data clearly show that net N mineralization of these soils could contribute substantially to crop fertility; soil nitrate data collected over the course of this study confirmed the significance of N mineralization.

## Crop productivity, nitrogen uptake and water application

No recent comprehensive survey of N fertilization practices of coastal vegetable growers exists, but informed contacts within the industry suggest that average per acre N application rates exceed 180 lb for iceberg lettuce, 250 lb for broccoli, and 300 lb for cauliflower and celery, with some growers using up to 50% more than these averages. In most of the fields monitored in this study substantially lower seasonal N rates were applied (Table 2). Comparison of reference plots (which received the additional 100 lb N/acre as preplant slow-release fertilizer) with the field management plots showed statistically equal marketable yield in 15 of 16 fields; the comparison presented were based on yield at first harvest. There were no evident differences in product quality (size or grade). At no time were visual differences in plant vigor between N treatments evident in any field; these observations were supported by measurement of total aboveground plant biomass, which showed no significant differences between N treatments in any field.

In the only field where yield differences were significant (broccoli in field 706, summer/fall, 1994) the treatment effect appeared to be slightly earlier maturity in the reference plots. Plant biomass at first harvest was equal between treatments and yield differences were eliminated by subsequent harvests. That field was targeted for production of export-quality broccoli crowns; the grower had minimized N application (only 102 lb/acre) in an attempt to >harden the plants to enhance post-harvest quality. Even this extreme treatment had minimal effect on overall plant productivity; the commercial yield of this field was 630 cartons per acre.

More surprising than the lack of yield or crop biomass response to the additional N fertilization of the reference plots was the equivalent total N accumulation in plant biomass between N treatments (Table 2). Across all crops monitored field management plots averaged 98% of the biomass N at harvest present in the corresponding reference plots. The primary result of the additional N fertilization was to enlarge the soil NO<sub>3</sub>-N pool, increasing NO<sub>3</sub>-N leaching potential.

The sampling protocol allowed the development of N uptake and partioning information (Table 3). Total biomass N accumulation and harvest removal are similar to previously reports (Stivers et at, 1993) for broccoli, cauliflower and lettuce, somewhat lower for celery. Maximum daily rates of N uptake were marginally lower than those recently reported by Doerge et al. (1991) under Arizona desert conditions. These differences undoubtedly relate primarily to the cooler weather of the central coast. In general, rates of N uptake >2 lb/acre day occur only during the final month before harvest, paralleling vegetative growth rates. The influence of residue incorporation on subsequent N mineralization is obvious, given the succulent nature and high N content of the residue. This is particularly true of broccoli and cauliflower, where residue may contain 150 lb N/acre.

This study also clearly documented the water conservation potential of drip irrigation. Water application from crop establishment (excluding sprinkler application for seedling establishment) to first harvest averaged approximately 7 inches. Again, no comprehensive data are available, but average water application by conventional means (furrow and/or sprinkler) is at least 25% higher.

# Soil and crop N monitoring

Large amounts of soil NO<sub>3</sub>-N were present at crop establishment in most fields and persisted through the cropping cycle. Table 4 lists soil NO<sub>3</sub>-N concentration at the third sampling date, 2-3 weeks pre-harvest. The influence of the additional N fertilization of the reference plots enriching the soil NO<sub>3</sub>-N pool was clear. However, petiole NO<sub>3</sub>-N concentration did not consistently reflect the differences in soil N availability, either among fields or between N treatments in a given field. All petiole NO<sub>3</sub>-N levels measured were with the sufficiency levels suggested by Doerge et al (1991); >excessive= levels were not found, despite very high late-season soil NO<sub>3</sub>-N levels. The practical implication of these data is that petiole NO<sub>3</sub>-N analysis, although a useful indication of crop N status, does not provide the information on current soil NO<sub>3</sub>-

N status necessary to delay or reduce additional N application; there is no substitute for soil NO<sub>3</sub>-N monitoring.

A reasonable correlation between petiole sap NO<sub>3</sub>-N and laboratory analysis of dry petiole tissue was found for cauliflower (Fig. 1), the only crop where a sufficiently large data set was developed to give confidence in the result. There were no evident growth stage effects, in agreement with previous research (Hartz et al, 1993b). Variation from the general relationship was not random, however; all replicate measurements from a given field/sampling date tended to be tightly clustered, whether close to, or substantially different from the overall relationship. This pattern suggested that inaccurate daily calibration of the >Cardy= meter, or improper collection and/or handling of samples may have been a factor.

Relative chlorophyll content (RCC), as measured by the Minolta SPAD 502 leaf absorption meter, showed virtually identical values for field management and reference plots (Table 4); this confirms the equivalent N status of plants from both N treatments. There was substantial variability in RCC among fields, even though N was not limiting in any field; varietal or site specific variables obviously confounded RCC measurements, making the development of RCC sufficiency guidelines problematic. The comparison of a field with an in-field reference of known N sufficiency may be the most appropriate use of this technology (Schepers et al., 1993a). The quick, non-destructive nature of this measurement is a plus, the cost of the meter (>\$1200) a drawback.

The soil NO<sub>3</sub>-N >quick test= procedure consistently indicated the presence of substantial NO<sub>3</sub>-N levels (Fig. 2) but as conducted by on-farm personnel, was considerably less accurate than in previous research (Hartz, 1994). Much of this inaccuracy can be attributed to specific factors, and can be corrected. As a volumetric extraction procedure, the moisture content of the soil can significantly influence the soil weight: liquid volume ratio of the blend. For simplicity the farm personnel used a conversion factor based on an average soil moisture content of 17%; given the wide range of soil texture encountered, actual moisture content ranged from 8-24%; correcting for estimated soil content would improve accuracy.

The other main factor was the relatively high soil NO<sub>3</sub>-N levels encountered. The test procedure was structured to produce approximately a 2:1 extract: soil blend by weight. When soil NO<sub>3</sub>-N levels are high, the NO<sub>3</sub>-N concentration of the resulting solution is also high, making accurate visual determination of color development on the NO<sub>3</sub>-N test strips difficult. Substantial errors can result from misinterpretation of the strip color; also, color perception among individuals varies substantially. In future work, the test will be modified to produce a more dilute extract (4:1) and the color of the test strips will be electronically read by a simple battery-operated colorimeter now available for on-farm use.

A definite pattern of soil NO<sub>3</sub>-N concentration became evident over the course of the study (Fig. 3). At the establishment of the summer/fall, 1994, crop, soil NO<sub>3</sub>-N was >30 PPM in all fields. Soil NO<sub>3</sub> -N remained high throughout the growing season.

except in fields still in production when winter rains began. Precipitation from November-March, totaling approximately 22 inches, effectively leached the top foot of soil. However, for fields planted in April or later, NO<sub>3</sub>-N levels had rebounded substantially (in the absence of pre-plant fertilization). Despite conservative fertilization, NO<sub>3</sub>-N levels generally increased through the spring season. For the fields monitored through the 1995 summer/fall season, soil NO<sub>3</sub>-N concentration increased further, to approximately the level of the previous fall.

This strong seasonal pattern was additional evidence that mineralization of soil organic N was a significant factor in the maintenance and enlargement of the soil NO<sub>3</sub>-N pool. Together with the crop response data, it also suggested that substantial reduction in N application, even lower than the conservative rates employed in this study, are possible without sacrificing plant productivity. This is particularly true for summer plantings which begin the season with high soil NO<sub>3</sub>-N concentration. For an industry which has historically concentrated on tissue testing as the primary measure of N status, increased emphasis on soil NO<sub>3</sub>-N monitoring is warranted.

The cost/benefit relationship of on-farm monitoring of soil and plant N status is very favorable. For a 20 acre field, collection of composite soil and petiole samples would take less than 1 hour; processing and analyzing them less than 30 minutes. The Cardy nitrate-selective electrode meter costs approximately \$300; given the average life of the electrode itself (200-300 samples) and its replacement cost (about \$70), cost per sample should not exceed \$1.50. The cost of the electronic colorimeter to read the nitrate test papers is approximately \$500, the strips under \$1.00 each; per sample cost for this upgraded version of the soil nitrate >quick test= should not exceed \$2.50.

A realistic monitoring program for conventionally-irrigated vegetables would be three soil nitrate tests (before each of two sidedressings, and one 3-4 weeks from harvest) and two petiole tests (done in conjunction with the last 2 soil analyses); this would require a total of about 4 hours for sample collection, preparation and analysis, with prorated analytical costs of about \$10.50. This routine work is unlikely to be performed by farm management, but rather by lower level wage labor. At \$15 per hour (wages, benefits and vehicle use) total seasonal monitoring cost would be approximately \$70 per 20 acre field, or about \$3.50 per acre. Per acre costs for smaller fields would be correspondingly higher, as would be the case if the program was conducted by an external consultant, or if traditional laboratory analysis was use in lieu of the on-farm techniques.

By comparison, potential cost savings are much greater. The results of this study suggest that N rate reduction of 50-100 lb N/acre are possible for growers currently using industry average application rates. At current N prices (an average of > \$.40/lb N for the commonly used fertilizer materials) per acre savings of \$20-40 are possible. Expressed another way, saving 50 lb N/acre on just 50 acres would cover the costs of the Cardy meter and the colorimeter.

#### **Outreach Activities:**

A number of educational activities have taken place or are planned to disseminate information relating to N management of vegetable crops, with an emphasis on the N-intensive cool season crops. The Project Leader has, over the duration of this project, made presentations at 10 educational meetings of industry, university and other governmental personnel, the information presented drawn completely on in part from results of this project:

- Grower meeting, Coachella, November 29, 1994.
- Pacific Northwest Vegetable Growers Association Annual Meeting, Pasco, Washington, December 8, 1994.
- Grower meeting, Hollister, February 21, 1995.
- Grower meeting, Guadalupe, February 22, 1995.
- Small Farms Conference, Sacramento, February 28, 1995.
- American Society for Horticultural Science, Montreal, August 1, 1995.
- In-service training for NRCD personnel, Lodi, October 17, 1995.
- Grower meeting, Guadalupe, November 9, 1995.
- UC Statewide Water Quality Conference, Fresno, November 27, 1995.
- CDFA-FREP Annual Conference, Parlier, December 7, 1995.

Cumulative attendance was approximately 700. Additional meetings are scheduled for 1996: Modesto (January 18), Hollister (February 1), Visalia (February 20), Santa Rosa (February 27) and Salinas (February 28).

In addition to research summaries distributed at several of these meetings, elements of this research have been featured in articles in several trade publications (Vegetable magazine, September, 1994; California Farmer, October, 1995; California-Arizona Farm Press, September 16, 1995); another feature article in Farm Press summarizing the project is scheduled in early 1996. The layman=s summary report will be modified and submitted to California Farmer in January, 1996.

As an adjunct to the research, a consultant was engaged to survey the industry regarding grower practices and attitudes, and to evaluate barriers to adoption of improved N management practices. The survey work is continuing; a preliminary report is presented in Appendix 1.

Table 1. Soil characteristics and net N mineralization estimates.

		Soil characteristics		Anaerobic	Aerobic Mineralization estimates (ppm N/day)		
Field	Organic matter (%)	Total N (%)	C/N Ratio	PMN² (NH <sub>4</sub> -N/day, ppm)	Constant 30°C	In-field	
	0.40	0.14	0.0	0.50	0.04	0.00	
60 3	2.13	0.14	9.0	0.56	0.91	0.86	
60	1.39	0.09	8.9	0.44	0.71	0.36	
7							
70 3	1.63	0.09	10.1	0.22	0.27	0.56	
70 4	1.46	0.09	9.6	0.21	0.00	0.04	
70 6	1.71	0.10	9.8	0.31	0.28	0.24	
1506	1.28	0.10	7.5	0.43	0.26	0.39	
151 6	1.01	0.07	8.1	0.39	0.17	-	
151 7	0.82	0.07	7.0	0.34	0.91	1.31	

<sup>&</sup>lt;sup>a</sup> potentially mineralizable N, by anaerobic incubation.

Table 2. Crop production,<sup>z</sup> and seasonal nitrogen and water application.

			% (	of reference pl		1		
Crop	Season	Fiel '	Marketable Yield			N application (lb/acre)	Irrigation Volume (in)	
Cauliflows r	e S/F 1994	603	119	96	92	170	5.9	
		607	95	100	97	175	5.8	
		703	88	108	111	175	3.9	
		704	99	113	101	163	5.8	
		150 6	108	91	86	173	6.7	
		151 7	103	104	104	295	9.8	
	S/S 1995	704	96			190	7.4	
	S/F 1995	607	123	97	94	214		
		150 6	97	100	94	290		
Broccoli	S/F 1994	706	52*	100	104	102	5.9	
		151 6	107	105	97	205	9.8	
	S/S 1995	603	90	108	104	199	8.4	
Lettuce	S/S 1995	607	101	87	92	210	5.1	
		703	108	101	100	132	3.5	
		150 6	109	89	87	216	7.6	
Celery	S/S 1995	706	101	107	100	300	10.1	

<sup>&</sup>lt;sup>Z</sup> crop performance of field management plots in comparison to reference plots <sup>y</sup> S/F = summer/fall, S/S = Spring/summer \* significantly different from the reference plots at p = .05

Table 3. Crop N uptake and removal.

Crop	Total biomass N (lb/acre)	Maximum N uptake rate (lb/acre*day)	Harvest removal (lb N/acre)	% N in Residue
Cauliflower	180-230	4-6	50-70	4.5
Broccoli	180-220	4-6	50-70	4.0
Celery	140-160	4-5	90-110	3.0
Lettuce	60-90	3-4	40-50	3.5

Table 4. Preharvest soil and plant nitrogen status at the 3<sup>rd</sup> in-season sampling, 2-3 weeks pre-harvest. <sup>Z</sup>

		5	Soil NO3-N		Petiole NO3-N		Relative chlorophyll	
Crop	Season	Bro <del>d</del> ucti	Fiel	Refere	Field	Refere	Field	Reference
Cauliflow er	S/F 1994	603	53	73	8000	8400	56.3	53.6
		607	31	87	1070 0	12700	47.5	46.9
		703	5	6	3400	4100	39.0	38.4
		704	39	41	8900	8800	52.1	52.9
		1506	45	75	9800	9800	53.9	51.6
		1517	50	81	1070 0	9300	56.8	58.3
	S/S 1995	704	21	28	5300	6400	49.6	50.7
	S/F	607	29	33	6400	6200	-	-

	1995							
		1506	45	52	9200	8700	-	-
Broccoli	S/F 1994	706	30	77	6400	7900	66.3	66.1
		1516	41	49	7700	6900	66.4	66.3
	S/S 1995	603	13	44	9100	9100	71.8	71.7
Lettuce	S/S 1995	607			5900	5700	35.2	34.2
		703	38	43	6100	6400	37.3	36.0
		1506	21	31	5400	6400	35.9	35.5
Celery	S/S 1995	706	33	49	7400	6400	35.7	36.0

<sup>&</sup>lt;sup>Z</sup> mean of 4 replicate samples

#### **Literature Cited**

Doerge, T.A., R.L. Roth and B.R. Gardner. 1991. Nitrogen fertilizer management in Arizona. Univ. of Arizona Special Publication.

Hartz, T.K. 1994. A quick test procedure for soil nitrate-nitrogen. Commun. Soil Sci. Plant Anal. 25:511-515.

Hartz, T.K., M. LeStrange and D.M. May. 1993a. Nitrogen requirements of drip-irrigated pepper. HortScience 28:1097-1099.

Hartz, T.K., R.F. Smith, M. LeStrange and K.F. Schulbach. 1993b. On-farm monitoring of soil and crop nitrogen status by nitrate-selective electrode. Commun. Soil Sci. Plant Anal. 24:2607-2615.

Keeney, D.R. 1982. Nitrogen-availability indices. In: Methods of soil analysis, Part 2. Chemical and microbiological properties. Monograph 9, Amer. Soc. Agron., Madison, WI.

Nadelhoffer, K.J., J.D. Aber and M.M. Melino. 1985. Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecology 66:1377-1390.

Schlepers, J.S., D.D. Francis, M. Virgil and F.E. Below. 1992. Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. Commun. Soil Sci. Plan Anal. 23:2173-2187.

Stivers, L.J., L.E. Jackson and G.S. Pettygrove. 1993. Use of nitrogen by lettuce, celery, broccoli and cauliflower: a literature review. CDFA-FREP Special Publication.

Sweeney, R.A. 1989. Generic combustion method for determination of crude protein in feeds; collaborative study. J. Assoc. Off. Anal. Chem. 72:770-774.