

# Relationships of Nitrogenous Compounds in Petiole Sap of Tomato to Nitrogen Fertilization and the Value of these Compounds as a Predictor of Yield

Peter C. Andersen, Fred M. Rhoads, Steven M. Olson, and Brent V. Brodbeck

University of Florida, North Florida Research and Education Center—Quincy, Route 3, Box 4370, Quincy, FL 32351

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**Abstract.** ‘Colonial’ tomato (*Lycopersicon esculentum* Mill.) plants were grown on raised beds with black polyethylene mulch, drip irrigation, and preplant-N rates of 0, 67, 134, 202, or 269 kg·ha<sup>-1</sup>. Petiole sap was collected 7 and 13 weeks after transplanting. Concentrations of NO<sub>3</sub>-N, free amino acids, total amino acids, and total-N (the sum of NO<sub>3</sub>-N and amino acid-N) were examined as functions of the rate of N fertilization. Also, each of these compounds was used as an independent variable as a predictor of fruit yield. Seven weeks after planting, the concentrations of NO<sub>3</sub>-N and 15 of 18 of the free amino acids were correlated with the rate of N fertilization, but concentrations of bound or total amino acids were not. The amount of NO<sub>3</sub>-N accounted for 37% of the total-N in the 0 kg·ha<sup>-1</sup> treatment, and up to 83% in the 202 kg·ha<sup>-1</sup> treatment. NO<sub>3</sub>-N was highly correlated with total-N for both nonhydrolyzed and hydrolyzed sap ( $R^2 = 0.98$ ). Thirteen weeks after transplanting, neither the concentration of NO<sub>3</sub>-N nor that of amino acids, other than asparagine, glutamine, and proline, were significantly related to the rate of N fertilization. On both dates, concentrations of glutamine plus glutamic acid were correlated with rate of N fertilization whether expressed as absolute values or as percentage values. N fertilization rate and the concentration of NO<sub>3</sub>-N or total-N were related to total fruit yield ( $R^2 = 0.69$  to 0.74), and marketable fruit yield ( $R^2 = 0.78$  to 0.82). N-fertilization rate and petiole sap concentrations of NO<sub>3</sub>-N or total-N were also correlated with the N contained in total or marketable yield. Petiole sap variables measured 13 weeks after transplanting were not significantly correlated with fruit yield or the quantity of N contained in the fruit. Free, bound, or total amino acids in petiole sap were not as well correlated with fruit yield parameters as were N-fertilization rate, NO<sub>3</sub>-N, or total-N in petiole sap.

Although N is an important nutrient for plant growth, it is also the most difficult nutrient to optimize because of susceptibility to leaching, denitrification, and volatilization. Uptake of N from the soil is influenced by its chemical and spatial availability, the number and activity of uptake/transport sites at the root surface, transport systems that move substances to the shoot, and by the number and strength of plant organs that serve as sinks (Engels and Marschner, 1995). The numerous processes affecting N availability and the difficulty in predicting crop-N demand make it difficult to optimize preplant-N fertilization rates (Scaife and Stevens, 1983). For example, Hills et al. (1983) found that N-uptake effi-

ciency by tomato was <30% of the N applied.

Laboratory leaf analysis of N often requires a week or more turnaround time—too long for growers to adjust the nutritional status of many vegetable crops. As an alternative, Emmert (1934) quantified NO<sub>3</sub> in conducting tissue of tomato and lettuce (*Lactuca sativa* L.) as an indicator of N availability and yield. Gomez-Lepe and Ulrich (1974), working with greenhouse-grown tomato plants in solution culture, found that petioles from recently matured leaves were better indicators of the N status of the plant than were the root, stem, or leaf. Movement of NO<sub>3</sub>-N was primarily unidirectional to the leaf blade with little transport via phloem to the stem. A threshold concentration of 500 mg·L<sup>-1</sup> (36 mM) was established for petiole NO<sub>3</sub>-N, below which growth was inhibited. Later, Coltman (1987) established 800 mg·L<sup>-1</sup> (57 mM) NO<sub>3</sub>-N as the critical concentration to support maximum yield of greenhouse-grown tomatoes.

Several investigators have assessed techniques to quantify N status in vegetable crops (Coltman, 1987; Hochmuth and Hochmuth, 1991; Hochmuth et al., 1988, 1993; Prasad and Spiers, 1982, 1984, 1985; Scaife and Stevens,

1983). Locascio et al. (1997) compared the nitrate sap test and laboratory leaf-N analysis as predictors of fruit yield of tomato plants. Six weeks after planting, the nitrate sap test results were as well correlated with total yield as were results of leaf-N analysis; however, later in the season leaf-N analysis was a better indicator of yield. Over the last 10 years the nitrate sap test has emerged as a commonly used method to assess N-status of a variety of annual crop species, and “quick test” kits have been developed (Hochmuth, 1994). Although ion-specific electrodes only provide approximations of ion concentration in biological samples, good correlations between applied N and sap nitrate concentration and between sap nitrate and yield have often been reported (Hochmuth, 1994; Hochmuth et al., 1988, 1993; Locascio et al., 1997; Prasad and Spiers, 1982, 1984, 1985; Rhoads et al. 1996; Scaife and Stevens, 1983).

However, little information is available concerning the concentration of other N-containing compounds in petiole sap or the relationship between these N-containing compounds and fruit yield. Although nitrate sap tests are rapid and convenient, correlations between other plant sap components and applied N or yield may be significantly improved, and a comparison of the relationships between rate of N-fertilization and petiole sap components vs. yield would provide an assessment of the value of the concentration of other N-containing compounds as predictors of yield.

The objectives of this study were to: 1) test the influence of N fertilization on the concentrations of NO<sub>3</sub>-N and other N-containing compounds in petiole sap; 2) discern the relationship between NO<sub>3</sub>-N and total-N vs. other N-containing compounds in petiole sap; 3) quantify the relationships between concentrations of NO<sub>3</sub> and other N-containing compounds in petiole sap and total yield, marketable yield, N in total yield, and N in marketable yield; and 4) evaluate the correlations between rates of N fertilization and N-containing compounds in petiole sap vs. fruit yield.

## Materials and Methods

‘Colonial’ tomato plants were transplanted 29 Mar. 1996 utilizing a black polyethylene mulch planting system with raised beds 0.91 m wide and 1.83 m apart (Hochmuth and Maynard, 1996) at the North Florida Research and Education Center, Quincy. Soil type was a Orangeburg loamy fine sand (Typic Paleudult: Siliceous, Thermic). Preplant-N (ammonium nitrate) was applied by modified broadcast method in the bed area at five rates (0, 67, 134, 202, 269 kg·ha<sup>-1</sup>). All treatments received triple superphosphate (0–46–0) at 224 kg·ha<sup>-1</sup> and muriate of potash (0–0–50) at 336 kg·ha<sup>-1</sup>. Prior to transplanting, the raised beds were treated with 98% methyl bromide–2% chloropicrin (trichloromethane) at 225 kg·ha<sup>-1</sup>. All plants received drip irrigation daily. Drip irrigation tubing with emitters spaced 0.3 m apart was positioned 5 cm from the center of the bed. Each single-row plot contained 18 plants spaced 51 cm apart. Only the 12 center

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plants were harvested for fruit yield. Each treatment was replicated four times. Wooden stakes and twine were used to retain plants in an upright position. Fruit were harvested three times and were graded into medium, large, and extra-large fruit and culls. Total yield included all graded tomatoes, including culls, while marketable yield did not include culls.

The fifth or sixth leaf from the apex of each of 10 tomato plants per experimental unit was cut at the base of the petiole on each sampling date and brought to the laboratory for determination of petiole sap  $\text{NO}_3\text{-N}$  and free and total amino acid concentration. Total-N was calculated as the sum of  $\text{NO}_3\text{-N}$  and total amino acid-N. Samples were taken 7 weeks (15 May, 2 weeks before first harvest) and 13 weeks (26 June, last harvest) after transplanting. Petioles of the leaves were cut into several 1-cm pieces and a total of  $\approx 2$  mL of the sap was expressed with a hydraulic press at a pressure of 8.9 MPa. A few drops of sap were placed on a Cardy nitrate ion meter equipped with a digital display (Cardy ion meter; Horiba Inc., Horiba, Japan). Immediately thereafter, the remaining sap was frozen in 5-mL cryogenic vials and placed in liquid  $\text{N}_2$ . Samples were stored at  $-20^\circ\text{C}$  prior to amino acid analysis.

Samples were divided for separate determinations of free amino acid and total amino acid concentrations. Analysis for free amino acids proceeded according to Andersen et al. (1992). After filtering, samples were lyophilized and derivatized by adding 2 ethanol : 2 triethanolamine (TEA) : 1 water. A 7 ethanol : 1 TEA : 1 water : 1 phenylisothiocyanate mixture was added and reactions were allowed to proceed for 20 min under  $\text{N}_2$ . Sample analyses were in 5 mM sodium phosphate buffer with 6% acetonitrile on a Waters HPLC system (Waters Division, Millipore Corp., Milford, Mass.) with a Pico Tag (Waters Division, Millipore Corp.) column. The analysis of total amino acids proceeded in a similar manner except that samples were not filtered, but were hydrolyzed prior to derivatization. Hydrolysis was accomplished by subjecting

samples to constantly boiling HCl (6 M) at  $110^\circ\text{C}$  for 24 h under  $\text{N}_2$ .

Statistical analysis was by regression (five levels of N fertilization) using general linear models procedures of the statistical analysis system (SAS Institute, 1985). First, the chemical variables were treated as dependent variables and were modeled in relation to N fertilization rate. Each level of N fertilization was replicated four times. Second, total yield, marketable yield, N contained in total yield, and N contained in marketable yield were considered as dependent variables and were statistically analyzed in relation to each of the components of petiole sap ( $\text{NO}_3\text{-N}$ , and individual and total amino acids). In both cases, each of the experimental units were treated independently in the regression procedure ( $n = 20$ ). Data for each sample date were analyzed separately. Linear, quadratic and cubic relationships were assessed at 5%, 1%, 0.1%, and 0.01% levels of significance. In the event that an equal level of significance ( $P$  value) was obtained for a given comparison, a 0.10 or greater increase in  $R^2$  was used as the determinant to use the next highest order polynomial.

## Results

Seven weeks after transplanting, the concentrations of the free amino acids in petiole sap of tomato were linearly related to rate of N fertilization, with many  $P$  values ranging from 0.01 to 0.0001 (Table 1). Only the concentrations of tyrosine, methionine, and cysteine were not correlated with fertilization rate. The highest coefficient of determination was found for proline ( $R^2 = 0.61$ ). Thirteen weeks after transplanting, only concentrations of asparagine ( $R^2 = 0.56$ ), glutamine ( $R^2 = 0.51$ ), and proline ( $R^2 = 0.36$ ) were related to N-fertilization rate (data not shown).

The significance of the relationship of amide concentrations to N fertilization on both dates prompted a more complete investigation of the amides and their respective acids in nonhydrolyzed and hydrolyzed samples (Table 2). Al-

though the concentrations of asparagine + aspartic acid sometimes were correlated with rate of N fertilization (data not shown), glutamine + glutamic acid (or the percentage of these compounds divided by total amino acids) produced the most consistent correlations. On both dates, data for free amino acids produced higher coefficients of determination ( $R^2 = 0.46$  to  $0.75$ ) than did data for total amino acids.

Concentrations of  $\text{NO}_3\text{-N}$  concentration in sap varied between 8 and 88 mM 7 weeks after transplanting;  $\text{NO}_3\text{-N}$  accounted for the great majority ( $>77\%$ ) of total-N in the petiole sap for all N treatments except the  $0\text{ kg}\cdot\text{ha}^{-1}$  rate (Table 3). The concentration of  $\text{NO}_3\text{-N}$  was better correlated with rates of N fertilizer than were those of individual amino acids (Table 1) or total amino acids (Table 3). Only the concentration of free amino acid (or free amino-N) in petiole sap were significantly correlated with N fertilization rate; concentrations of bound or total amino acids were not correlated on either date. Thirteen weeks after transplanting, the concentration of  $\text{NO}_3\text{-N}$  varied little (18–25 mM), and was not related to rate of N fertilization (Table 3). On this date, only the concentrations of free amino acids and free amino-N were correlated with rates of N fertilization.

Seven weeks after transplanting the concentration of  $\text{NO}_3\text{-N}$  explained 98% of the variation in total-N for both nonhydrolyzed and hydrolyzed samples of petiole sap (Table 4). Coefficients of determination for the relationship between amino acid-N and total-N were 0.81 for nonhydrolyzed samples vs. 0.22 for hydrolyzed samples. A similar pattern was noted for the relationship between  $\text{NO}_3\text{-N}$  and amino acid-N (Table 4). Thirteen weeks after transplanting, the concentrations of  $\text{NO}_3\text{-N}$  and total-N were still well correlated, although the coefficient of determination was much higher for nonhydrolyzed ( $R^2 = 0.90$ ) than for hydrolyzed ( $R^2 = 0.63$ ) samples (data not shown). Similarly, the relationships between the concentrations of amino acid-N and total-

Table 1. Amino acid profile of nonhydrolyzed sap (free amino acids;  $\mu\text{M}$ ) from tomato petioles collected 7 weeks after transplanting as a function of N fertilization rate.

Amino acid	N applied ( $\text{kg}\cdot\text{ha}^{-1}$ )					Regression equation	$R^2$	Significance
	0	67	134	202	269			
ASP	85	127	192	121	204	$99.6 + 0.362x$	0.31	*
GLU	76	78	135	160	211	$61.9 + 0.525x$	0.48	***
ASN	56	181	271	354	1072	$-36.2 + 3.29x$	0.32	**
GLN	128	472	967	945	287	$-115 + 9.16x$	0.48	***
GLY	24	26	28	43	71	$19.3 + 0.150x$	0.43	**
HIS	57	32	41	25	73	$57.5 - 0.54x + 0.0018x^2$	0.46	**
ARG	480	573	956	628	944	$519 + 1.54x$	0.37	**
THR	32	50	66	62	125	$27.3 + 0.305x$	0.44	**
ALA	106	110	164	103	171	$106 + 0.199x$	0.22	*
PRO	82	186	329	213	522	$85.3 + 1.40x$	0.61	****
TYR	8	4	9	3	3	---	---	NS
VAL	43	73	92	68	129	$47.2 + 0.263x$	0.46	**
MET	11	9	18	10	12	---	---	NS
CYS	2	2	3	2	2	---	---	NS
ISO	14	36	38	35	78	$14.6 + 0.196x$	0.37	**
LEU	27	41	62	45	76	$29.9 + 0.158x$	0.48	***
PHE	14	26	38	32	45	$17.4 + 0.105x$	0.54	****
LYS	8	13	22	19	27	$9.14 + 0.068x$	0.55	****
Total	803	1468	2492	2227	5692	$431 + 16.2x$	0.49	****

ns, \*, \*\*, \*\*\*\* Nonsignificant or significant at  $P < 0.05$ , 0.01, 0.001, or 0.0001, respectively.

Table 2. The concentration of glutamine + glutamic acid, and the concentration ratio of glutamine + glutamic acid to total amino acids in tomato petiole sap collected 7 and 13 weeks after transplanting as a function of N fertilization.

Variable	Weeks after transplanting	N applied (kg·ha <sup>-1</sup> )					Regression equation	R <sup>2</sup>	Significance
		0	67	134	202	269			
<i>GLN + GLU (μM)</i>									
Free	7	204	550	1101	1106	3082	-51.3 + 9.69x	0.49	***
Total	7	1447	1891	2717	2999	3339	1500 + 7.29x	0.25	*
Free	13	838	1164	1239	2392	2876	641 + 7.91x	0.46	**
Total	13	1683	1632	1737	1578	2393	---	---	NS
<i>(GLN + GLU)/Total AA (%)</i>									
Free	7	24.6	40.0	43.7	50.4	60.0	27.5 + 0.102x	0.75	****
Total	7	10.6	10.6	11.2	10.1	11.0	13.3 + 0.340x	0.31	*
Free	13	14.0	19.5	25.8	30.5	32.7	15.3 + 0.70x	0.58	****
Total	13	13.0	14.8	14.6	15.9	15.3	13.6 + 0.0085x	0.23	*

ns, \*, \*\*, \*\*\*, \*\*\*\* Nonsignificant or significant at  $P < 0.05, 0.01, 0.001, \text{ or } 0.0001$ , respectively.

Table 3. The concentrations and percentages of various compounds in tomato petiole sap collected as a function of N fertilization.

Compound	N applied (kg·ha <sup>-1</sup> )					Regression equation	R <sup>2</sup>	Significance
	0	67	134	202	269			
<i>7 weeks after transplanting</i>								
<i>Concentration (mM)</i>								
Nitrate-N	7.67	55.69	73.45	88.35	87.98	23.95 + 0.278x	0.65	****
Free amino acids	0.80	1.47	2.49	2.23	5.69	0.431 + 0.162x	0.49	***
Bound amino acids	9.88	10.73	12.71	10.40	8.93	---	---	NS
Total amino acids	10.68	12.19	15.20	14.08	14.62	---	---	NS
Free amino-N	3.06	4.49	7.66	6.10	13.59	2445 + 0.035x	0.54	***
Bound amino-N	10.76	11.27	12.06	10.04	7.74	---	---	NS
Total amino-N	13.82	15.75	19.71	18.25	19.12	---	---	NS
Total-N	21.48	71.45	93.16	106.63	107.10	38.68 + 0.308x	0.65	****
Percent								
Nitrate-N/total-N	36.91	77.30	77.68	82.74	81.97	52.20 + 0.142x	0.53	***
Total amino-N/total-N	63.09	22.70	22.32	17.26	18.03	47.80 - 0.142x	0.53	***
<i>13 weeks after transplanting</i>								
<i>Concentration (mM)</i>								
Nitrate-N	22.58	20.97	18.15	25.40	20.97	---	---	NS
Free amino acids	5.52	5.58	4.86	7.72	8.89	4.71 + 0.136x	0.26	*
Bound amino acids	7.44	5.44	7.25	4.37	6.99	---	---	NS
Total amino acids	12.96	11.01	12.11	10.18	15.90	---	---	NS
Free amino-N	6.85	7.01	6.23	10.40	12.54	5.65 + 0.225x	0.32	*
Bound amino-N	9.67	6.85	9.06	5.12	7.95	---	---	NS
Total amino-N	16.52	13.86	15.29	12.78	20.50	---	---	NS
Total-N	39.10	34.83	33.43	38.19	41.46	---	---	NS
Percent								
Nitrate-N/total-N	57.89	60.43	54.90	65.07	51.97	---	---	NS
Total amino-N/total-N	42.11	39.57	45.10	34.93	48.50	---	---	NS

ns, \* Nonsignificant or significant at  $P < 0.05$ , respectively.

N and between NO<sub>3</sub>-N and total-N concentrations were better for nonhydrolyzed than for hydrolyzed samples (data not shown).

Total fruit yield was related to N fertilization rate in a quadratic manner with a maximum at 202 kg·ha<sup>-1</sup> (Fig. 1). Marketable yield mirrored total yield, except that marketable yield ( $R^2 = 0.81$ ) was better correlated with N fertilization than was total yield ( $R^2 = 0.74$ ). The concentrations of NO<sub>3</sub>-N (Fig. 2) or total-N (Fig. 3) in petiole sap were as closely associated with total yield as was the rate of N fertilization ( $R^2 = 0.69$  to  $0.74$ ). Similarly, ≈80% of the variation in marketable yield was explained by a model incorporating either NO<sub>3</sub>-N or total-N as independent variables. Essentially identical yield predictions were obtained by using results from hydrolyzed and nonhydrolyzed samples in the models (data not shown). In all cases, second-order polynomials most accurately described the relationships between the independent variables and total and marketable fruit yield. Neither total nor marketable fruit yield was correlated with free or total amino acids in petiole sap (data not shown). The rate of N fertilization explained

Table 4. Equation and statistics describing the relationships between nitrate-N, amino acid-N, and total-N in tomato petiole sap collected 7 weeks after transplanting. All units are mm.

Variables	Regression equations	R <sup>2</sup>	Significance
<i>Nonhydrolyzed sap</i>			
NO <sub>3</sub> -N and Total-N	0.934 + 0.870x	0.98	****
Amino acid-N and Total-N	6.39 - 0.211x + 0.00259x <sup>2</sup>	0.79	***
NO <sub>3</sub> -N and Amino acid-N	6.80 + 9.83x - 0.216x <sup>2</sup>	0.51	***
<i>Hydrolyzed sap</i>			
NO <sub>3</sub> -N and total-N	11.54 + 0.928x	0.98	****
Amino acid-N and total-N	11.54 + 0.0725x	0.22	*
NO <sub>3</sub> -N and Amino acid-N	---	---	NS

ns, \*, \*\*, \*\*\*, \*\*\*\* Nonsignificant or significant at  $P < 0.05, 0.001, \text{ or } 0.0001$ , respectively.

71% of the variation in N content in total fruit yield, whereas from 55% to 57% of the variation in total crop N was explained by concentrations of NO<sub>3</sub>-N or total-N in petiole sap (Table 5). The coefficients of determinations were similar ( $R^2 = 0.72$  to  $0.76$ ) for the quadratic relationships between marketable yield and N-fertilization rates, and between the NO<sub>3</sub>-N, and total-N in petiole sap and marketable yield. Free or total amino acids were not correlated with N contained in total or marketable fruit yield (data not shown).

## Discussion

The major value of the chemical profile of petiole sap from a practical standpoint is its use as an indicator of fruit yield. This experiment was not designed to show a threshold NO<sub>3</sub>-N level below which a reduction in yield may occur (Hochmuth, 1994; Locascio et al., 1997; Rhoads et al., 1996), but to evaluate the utility of chemical variables in petiole sap as predictors of yield. Seventy-four percent and 81% of the variation in total and marketable

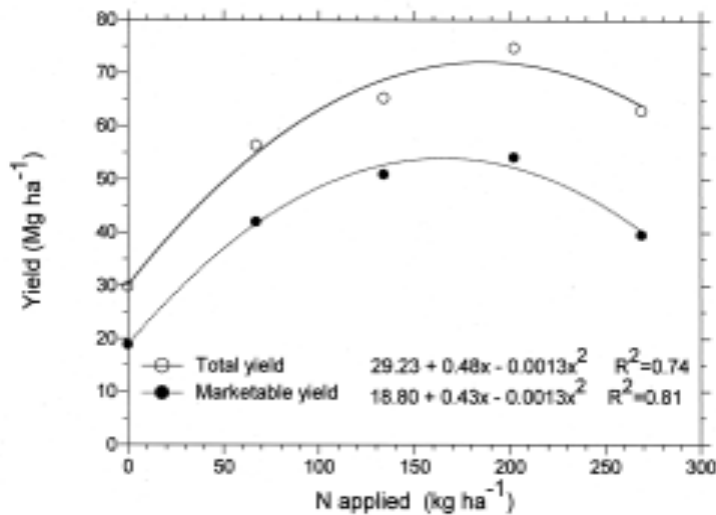


Fig. 1. Total yield and marketable yield as a function of N applied to tomato. Mean values are presented.

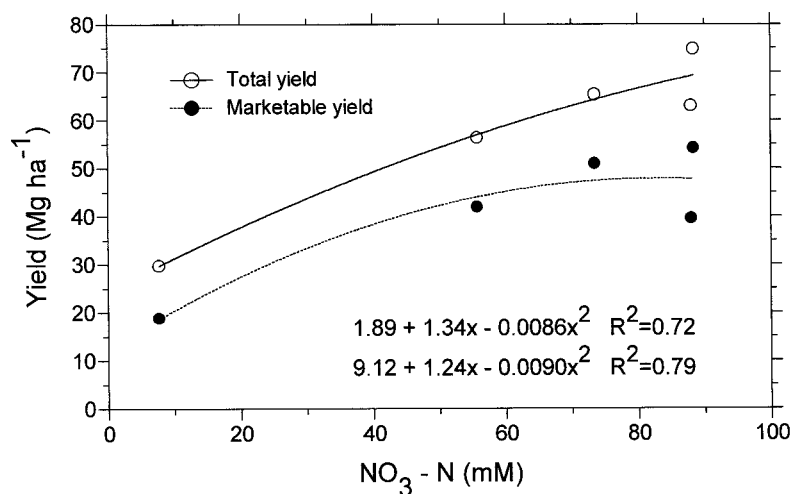


Fig. 2. Total yield and marketable yield as a function of  $\text{NO}_3\text{-N}$  in petiole sap collected 7 weeks after transplanting. Mean values are presented.

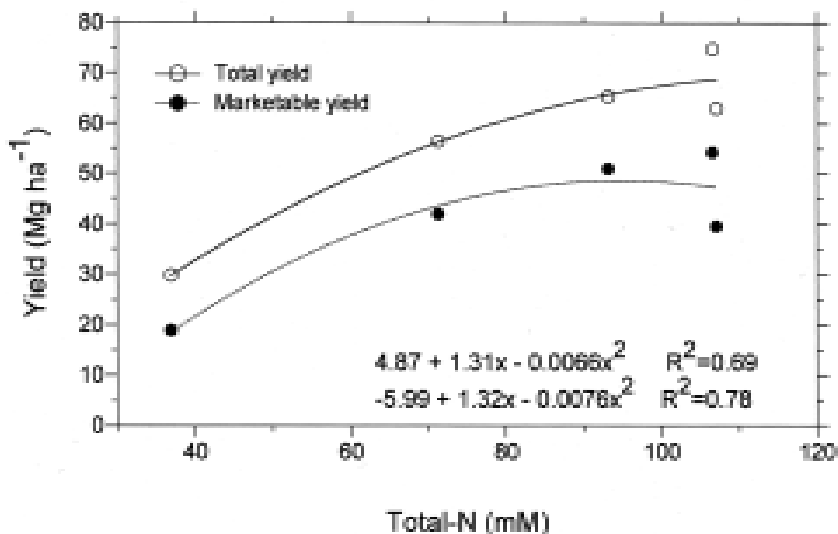


Fig. 3. Total and marketable yield of tomato as a function of total N in petiole sap collected 7 weeks after transplanting. Mean values are presented.

yield, respectively, could be explained simply by rates of N fertilization (Fig. 1). The concentrations of  $\text{NO}_3\text{-N}$  (Fig. 2) or total-N (Fig. 3) measured in petiole sap 7 weeks after planting explained 69% to 72% of the variation in total yield, and 78% to 79% of the variation in marketable yield, which was roughly equivalent to a model based solely on the rate of N fertilization.

Given the high correlations between fruit yield and rate of N fertilization, why should one consider using petiole sap analysis as an indicator of yield? In our experiment, the application of N-fertilizer was probably more accurate than under grower conditions and the experimental conditions were such as to ensure that N was limiting for some treatments. Also, plant response to a given rate of N-fertilizer is dependent on both edaphic and climatic conditions, which vary with year and location. The value of the  $\text{NO}_3\text{-N}$  sap test is that it provides a rapid indication of the N status of the plant that takes into account edaphic and climatic factors.

Yield was not linearly related to independent variables. Quadratic responses between total yield or marketable yield and rates of N fertilization, and between yield and concentrations of  $\text{NO}_3\text{-N}$  or total-N in petiole sap, reflected the fact that yields were maximized at less than maximum values of these variables (Fig. 1). The data showing maximum yield at  $202 \text{ kg}\cdot\text{ha}^{-1}$  are in agreement with Univ. of Florida recommendations (Hochmuth and Maynard, 1996). The amount of N contained in the fruit (Table 5) was not as well correlated with N fertilization as was fruit yield (Fig. 1), indicating that N may have had more of an effect on the physiological mechanisms responsible for fruit yield than on the actual quantity of N contained in the fruit.

The lack of correlation between  $\text{NO}_3\text{-N}$  concentration and fruit yield late in the season was not unexpected based upon previous research (Hochmuth, 1994; Locascio et al., 1997; Rhoads et al., 1996); however, the relationship between the other chemical variables in petiole sap and fruit yield late in the season was previously unknown. Seven weeks after transplanting, the concentrations of total amino acids and a few individual amino acids were correlated with fruit yield, but the significance was only  $P < 0.05$  and  $R^2 < 0.35$  (data not shown). Thirteen weeks after transplanting, the concentration of no individual amino acids was correlated with total yield, marketable yield, or the N contained in total or marketable yield (data not shown). Thus, neither amino acids nor amino-N is useful as a predictor of yield. The concentrations of  $\text{NO}_3\text{-N}$  or total-N in petiole sap were roughly equivalent in predictive capacity. That any test involving total-N (or amino acids) in petiole sap will be a practical replacement for a  $\text{NO}_3\text{-N}$  test is unlikely. Total-N could be measured using a CHN analyzer, although the time required would be greater than for a nitrate sap test. The CHN analysis, a laboratory procedure, would provide little advantage over a standard leaf analysis, which the petiole sap test was designed to replace.

Table 5. Equation and statistics describing total yield, marketable yield, nitrogen in total yield, and nitrogen in marketable yield as a function of soil N fertilization, and concentrations of nitrate and total N in petiole sap of tomato collected 7 weeks after transplanting.

Variables	Regression equations	R <sup>2</sup>	Significance
<i>Nitrogen in total yield</i>			
Soil N fertilization (kg·ha <sup>-1</sup> )	23.6 + 0.454x - 0.000959x <sup>2</sup>	0.71	****
Sap contents			
Nitrate-N (mm)	25.6 + 0.527x	0.57	***
Total-N (mm)	19.8 + 0.486x	0.55	****
<i>Nitrogen in marketable yield</i>			
Soil N fertilization (kg·ha <sup>-1</sup> )	14.6 + 0.406x - 0.00106x <sup>2</sup>	0.76	****
Sap contents			
Nitrate-N (mm)	5.97 + 1.16x - 0.0075x <sup>2</sup>	0.72	****
Total-N (mm)	-9.02 + 1.26x - 0.00667x <sup>2</sup>	0.72	****

ns, \*\*\*, \*\*\*\* Nonsignificant or significant at P < 0.001 or 0.0001, respectively.

Nitrogen fertilization had a profound influence on petiole sap chemistry at 7 weeks after planting, but not at 13 weeks after planting. Concentrations of NO<sub>3</sub>-N ranged up to 88 mm on 15 May compared with only 25 mm on 26 June. Only in the control treatment (0 N kg·ha<sup>-1</sup>) was most of the N in reduced form (amino acids) in petiole sap. The application of ammonium nitrate in the other N-fertilization treatments increased the NO<sub>3</sub><sup>-</sup> content to a level that exceeded the capacity of nitrate reductase, glutamine synthase, and glutamine synthetase to reduce NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> and NH<sub>4</sub><sup>+</sup> to amino acids.

Of particular interest are glutamine + glutamic acids or their ratio to total amino acids. These variables were significantly affected by rate of N fertilization on both dates in both hydrolyzed and nonhydrolyzed samples (Table 2). Glutamine is a neutral amino acid with a high N : C ratio, is mobile in phloem and xylem and accounts for a high proportion of the organic-N transported in the xylem fluid of many plant species (Andersen et al., 1989, 1992; Pate, 1980; Sauter and van Cleve, 1992; Shelp and Da Silva, 1990). Also, researchers using <sup>15</sup>NO<sub>3</sub><sup>-</sup> or <sup>15</sup>NH<sub>4</sub><sup>+</sup> as precursors have demonstrated that <sup>15</sup>N is incorporated into the amide moiety of glutamine, and later into other amino acids (Kato, 1980; Pate, 1980). Glutamine is the major N carrier among the amino acids, and may play a secondary role to nitrate in N transport and N storage.

The pool of mobile N-containing compounds provided the best correlation between concentration of N compounds and rate of N fertilization. The enhanced significance associated with free (nonhydrolyzed) amino acids vs. total (hydrolyzed) amino acids in petiole sap in relation to N fertilization was consistent, and was due to the variable contribution of bound amino acids. Total amino acids include the free amino acids plus those derived from soluble and structural proteins. Many of the bound amino acids are insoluble, attached

to pieces of cells or exist as part of cell organelles, and were not correlated with the amount of N applied.

Other studies have provided coefficients of determination between NO<sub>3</sub>-N and yield similar to that reported here (Locascio et al., 1997; Rhoads et al., 1996). The NO<sub>3</sub>-N sap test appears to combine high speed and convenience with a fair degree of accuracy in predicting yield. While the composition of petiole sap may provide estimates of potential tomato yield, use of this technique in physiological research has limitations. Concerns of ion specific electrodes, such as the Cardy nitrate ion meter, include the tissue-dependent variation from actual ion concentration and the non-tissue-specific nature of expressed petiole sap. Despite these limitations, the chemical analysis of NO<sub>3</sub>-N in petiole sap has shown consistently good correlations with yield, and this ion-specific meter may be of value in understanding the nutritional status of tomatoes. Also, results of this study and previous studies have documented that there is a fairly narrow window (perhaps 6 to 10 weeks after transplanting) during which the nitrate sap test is a useful indicator of yield potential. After this time a leaf-N analysis (Locascio et al., 1997) may be more valuable.

Literature Cited

Andersen, P.C., B.V. Brodbeck, and R.F. Mizell, III. 1989. Metabolism of amino acids, organic acids and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata*. Entomol. Expt. Appl. 50:149-159.  
 Andersen, P.C., B.V. Brodbeck, and R.F. Mizell, III. 1992. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. J. Insect Physiol. 38:611-622.  
 Coltman, R.R. 1987. Sampling considerations for nitrate quick tests of greenhouse-grown tomatoes. J. Amer. Soc. Hort. Sci. 112:922-927  
 Emmert, E.M. 1934. Tests for phosphate, nitrate and soluble nitrogen in conducting tissue of tomato and lettuce plants as indicators of avail-

ability and yield. Kentucky Agr. Expt. Sta. Circ. 43.  
 Engels, C. and H. Marschner. 1995. Plant uptake and utilization of nitrogen, p. 41-82. In: P.E. Bacon (ed.). Nitrogen fertilization in the environment. Marcel Dekker, New York.  
 Gomez-Lepe, B.E. and A. Ulrich. 1974. Influence of nitrate on tomato growth. J. Amer. Soc. Hort. Sci. 99:45-49.  
 Hills, F.J., F.E. Broadbent, and O.A. Lorenz. 1983. Fertilizer nitrogen utilization by corn tomato or sugarbeet. Agron. J. 75:423-426.  
 Hochmuth, G.J. 1994. Plant petiole sap testing. Florida Ext. Serv. Circ. 1144.  
 Hochmuth, G.J., P.R. Gilreath, E.A. Hanlon, G.A. Clark, D.N. Maynard, C.D. Stanley, and D.Z. Haman. 1988. Evaluating plant N status with plant sap quick-test kits. Proc. Tomato Inst. Florida Coop. Ext. Serv. Spec. Ser. SS-VEC-801:6-14.  
 Hochmuth, G.J., R.C. Hochmuth, M.E. Donley, and E.A. Hanlon. 1992. Eggplant yield in response to potassium fertilization on sandy soil. HortScience 28:1001-1005.  
 Hochmuth, G.J. and D.M. Maynard. 1996. Vegetable production guide for Florida. Univ. Florida SP-170.  
 Hochmuth, R.C. and G.J. Hochmuth. 1991. Nitrogen requirements for mulched slicing cucumbers. Soil and Crop Sci. Soc. Florida Proc. 50:130-133.  
 Kato, T. 1980. Nitrogen assimilation by a citrus tree. 2. Assimilation of labeled ammonium by detached leaves in light and dark. Physiol. Plant. 50:304-308.  
 Locascio, S.J., G.J. Hochmuth, F.M. Rhoads, S.M. Olson, A.J. Smajstrla, and E.A. Hanlon. 1997. Nitrogen and potassium application scheduling effects on drip-irrigated tomato yield and leaf tissue analysis. HortScience 32:230-235.  
 Pate, J.S. 1980. Transport and partitioning of nitrogenous solutes. Annu. Rev. Plant. Physiol. 31:313-340.  
 Prasad, M. and T.M. Spiers. 1982. Evaluation of a simple sap nitrate test for some ornamental crops, p. 474-479. In: Plant Nutrition, Proc. 9th Intl. Plant Nutr. Colloq. Commonwealth Agr. Bureau Slough, U.K.  
 Prasad, M. and T.M. Spiers. 1984. Evaluation of a rapid method for plant sap nitrate analysis. Commun. Soil Sci. Plant Anal. 15:673-679.  
 Prasad, M. and T.M. Spiers. 1985. A rapid nitrate sap test for outdoor tomatoes. Scientia Hort. 25:211-215.  
 Rhoads, F.M., S.M. Olson, G.J. Hochmuth, and E.A. Hanlon. 1996. Yield and petiole-sap nitrate levels of tomato with N rates applied preplant or fertigated. Soil Crop Sci. Soc. Florida Proc. 55:9-12.  
 SAS Institute. 1985. SAS/STAT guide for personal computers. Vers. 6 ed. SAS Inst., Cary, N.C.  
 Sauter, J.J. and B. van Cleve. 1992. Seasonal variation of amino acids in xylem sap of "*Populus xcanadensis*" and its relation to protein body mobilization. Trees 7:26-32  
 Scaife, A. and K.L. Stevens. 1983. Monitoring sap nitrate in vegetable crops: Comparison of test strips with electrode methods, and effects of time of day and leaf position. Commun. Soil Sci. Plant Anal. 14:761-771.