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Nitrogen Form and Solution pH Effect on Organic Acid Content of Cranberry Roots and Shoots

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Abstract. **The effect of N form and solution pH on the carboxylic and phenolic acid content of cranberry *(Vaccinium macrocarpon* Ait. cv. Searles) shoots and roots was determined in a greenhouse experiment. The predominant carboxylic acids measured were malate and citrate. Protocatechuic acid was the dominant phenolic acid detected. Total organic acid concentrations were unaffected by N form supplied. In shoots, higher total concentrations of organic acids were found at pH 4.5 than at 6.5 in the shoot, but there was little pH effect in the roots.**

A unique characteristic of many Ericaceous plants is their adaptation to ammonium-N nutrition and low soil-pH conditions. Previous work has shown that cranberries respond better to NH_4-N than to NO_3-N (Greidanus et al., 1972), although NO_3-N has sometimes been beneficial (Dirr, 1974; Leschyson and Eaton, 1971). Our experiments showed that cranberry fresh weight accumulation and final dry weight were higher when plants were supplied with either NH_4-N or NH_4-N/NO_3-N than with NO_3-N alone (Rosen et al., 1990).

Mechanisms for tolerance of NH_4-N by *Vaccinium* spp. are not known, but may be related to a lower cation requirement or a greater ability to synthesize organic acids in the absence of NO_3-N compared to nontolerant plants (Salsac et al., 1987). For most plants, shoot concentrations of carboxylic acids are higher with NO_3-N nutrition, due to the requirement for charge balance for the cations accompanying nitrate uptake once the nitrate is reduced and assimilated (Haynes and Goh, 1978; Kirkby and Mengel, 1967; Marschner, 1986). In ammonium-fed plants, organic acids are typically at low levels due to amino acid formation (Haynes and Goh, 1978). Phenolic content of plants is also affected by N supply (Marschner, 1986).

The objectives of this research were to determine the effect of N form and pH on the organic acid constituents of cranberry roots and shoots. Because we saw differences in plant response to N form, we expected to see corresponding differences in carboxylic and phenolic acid production.

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Materials and Methods

The cranberry clone 'Searles' was grown as described previously (Rosen et al., 1990). Stem cuttings were rooted for 6 weeks in 1-liter jars with a combined N source and then transferred to 7-liter black plastic pots containing a N-deficient nutrient solution of the following composition (in mM): $CaSO_4 \cdot 2H_2O$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.75; K_2SO_4, 0.5; $Ca(H_2PO_4)_2$, 0.13; (in µM): FeNaEDTA, 80; H_3BO_3, 46; $MnCl_2 \cdot 4H_2O$, 9; $ZnSO_4 \cdot 7H_2O$, 0.8; $CuSO_4 \cdot 5H_2O$, 0.3; $H_2MoO_4 \cdot H_2O$, 0.1. Treatments were imposed in a randomized complete-block design with four replications, with NH_4-N only, NH_4/NO_3-N and NO_3-N only in a factorial combination, with pH treatments of 4.5 and 6.5. All N levels were adjusted to 2 mM using $(NH_4)_2SO_4$ in the NH_4-N only solution, NH_4NO_3 in the NH_4/NO_3-N solution, and $Ca(NO_3)_2$ in the NO_3-N only solution. Solutions were changed every 14 days and pH adjusted daily. To inhibit nitrification, 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) was added to all solutions at the rate of 4 µM. Treatments were initiated 22 Apr. 1987, and the experiment was terminated 30 Sept. 1987.

Plants were separated into roots and shoots, and roots were briefly rinsed in deionizedwater. Tissue samples were dried at 60C and ground in a Wiley mill to pass through a 425-µM 40-mesh screen. A 1-g dried sample of roots or shoots was extracted with 15 ml of 70% ethanol, shaken for 24h, and centrifuged at 12,096 × *g* for 10 min. The pellet was resuspended in fresh ethanol, shaken, and centrifuged for a total of three times. Collected supernatant was pooled and recentrifuged to eliminate insoluble precipitates, then evaporated to dryness. The dried residue was resuspended in 5 ml of water, then prepared for injection on high-performance liquid chromatography by sequential solid-phase extraction through two 500-mg CH columns (BondElut, Varian, Harbor City, Calif.).

The carboxylic and phenolic acid compositions were determined using a Spectra-Physics HPLC (SP8800 pump and SP4290 integrator; San Jose, Calif.) fitted with an HPX-87 column and guard column (Bio-Rad Laboratories, Richmond, Calif.). Analyses were performed using a 5% to 30% acetonitrile/0.008 N H_2SO_4 gradient at 0.8 $ml \cdot min^{-1}$ and 45C column temperature. Peaks were detected with an ultraviolet detector at 210 nm and identified by cochromatography with known standards. Succinic and shikimic acid standards coeluted with a retention time of 8.53 min. Data were analyzed using PROC ANOVA (SAS Institute, 1985), and means were separated using Duncan's multiple range test at $P \leq 0.05$ where appropriate.

Results

Citric and malic acids were the dominant carboxylic acids in shoots and roots of cranberry plants. Protocatechuic acid was the dominant phenolic acid. Oxalic acid was not detected in shoot extracts. In shoot extracts, there were no significant differences in concentrations of organic acids for different N forms, but citric, t-aconitic, and fumaric acid concentrations were significantly higher under pH 4.5 than 6.5 (Table 1). Succinic and/or shikimic acid followed the same trend, with significantly higher concentrations at pH 4.5 than 6.5 (data not shown). Results reported for blueberry indicate that this chromatographic peak is likely to be primarily composed of shikimic acid (Neuendorff and Patten, 1989), in which case shikimic acid would be a dominant phenolic acid. The concentration of t-aconitate was higher at pH 4.5 than 6.5 for the NO_3 and NH_4 treatments (data not shown), but lower for the combined N treatment, resulting in a significant interaction.

In root extracts, only syringic and benzoic acids were at higher concentrations at pH 4.5 than at 6.5, and these acids were present in very small amounts (Table 2). Nitrogen form affected fumaric, oxalic, protocatechuic, and vanillic acid concentrations, but there were no differences for the dominant acids. Only for syringic acid was there a significant treatment interaction, in which root concentrations were higher at pH 4.5 than 6.5 for the NO_3-N source, but lower for the combined and NH_4-N source (data not shown).

The only difference among treatments in concentrations of total carboxylic acids, phenolic acids, or the combined organic acids was due to a pH effect in the shoots, primarily due to the increased concentration of citrate at pH 4.5.

Discussion

Because N form affected 'Searles' cranberry growth (Rosen et al., 1990), we expected an effect of N source on the organic acid content. In typical N metabolism, organic acids accumulate in nitrate-fed plants to maintain charge balance due to excess cation uptake and subsequent reduction of NO_3 (Haynes and Goh, 1978). In addition, there is often an increase in the pH of the nutrient solution due to excretion of hydroxide or bicarbonate. In our previous work, plant weight was depressed

Table 1. Solution pH and N form effects on concentrations of carboxylic and phenolic acids in cranberry shoot extracts.

	Carboxylic acids				Phenolic acids								Total measured organic acids
	Malic	Citric	t-Aconitic	Fumaric	Proto-catechuic	Syringic	4-Hydroxy-benzoic	Gallic	Benzoic	Vanillic	Ferulic	p-Coumaric	
							µmol/g shoot dry wt						
N form[z]													
NH_4	136	56	0.66	0.48	1.4	0.002	0.25	0.008	ND[y]	0.017	0.012	0.012	196
NH_4/NO_3	177	43	0.46	0.58	1.6	0.004	0.18	0.061	0.021	0.061	0.026	0.026	223
NO_3	157	38	0.13	0.60	2.2	0.004	0.04	0.078	0.004	0.021	0.024	0.024	197
pH													
4.5	238	83	0.60	0.89	1.9	0.006	0.21	0.107	0.015	0.054	0.014	0.014	324
6.5	92	13	0.23	0.28	1.7	<0.001	0.09	0.008	0.004	0.018	0.028	0.028	108
Significance	NS	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS	*
pH × N form													
Significance	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Effects due to N form were nonsignificant for any organic acid.
[y]ND = not detected.
NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

when N was supplied as NO_3, and rates of uptake were 30 to 100 times lower than for NH_4-N (Rosen et al., 1990). There was no change in the pH of the nutrient solution of the nitrate-fed cranberry plants, nor in the rate of cation uptake (milliequivalents K + Ca + Mg per gram dry weight was about equal among N treatments). As we concluded previously, there may have been minimal uptake of NO_3, especially at pH 6.5, so the plants did not respond with increased synthesis of organic acids.

Although plants that are not adapted to ammonium nutrition generally have a lower organic acid content (Haynes and Goh, 1978) and often display Ca and Mg deficiencies, it appears that cranberry, like many other NH_4-adapted species, can fix CO_2 into organic acids sufficiently to compensate for the uptake of excess cations, including NH_4^+, and to provide carbon skeletons for detoxification and a supply of amino acids and amides to shoots (Salsac et al., 1987; Woolhouse, 1966). In some cases, the demand for carbohydrate supply with NH_4-N nutrition can be so high that there is little to invest in root growth, with subsequent decreases in root mass (Lewis et al., 1990). Restricted root growth in NH_4-fed plants can be ascribed to the cumulative effect of increased respiration, greater allocation of C to nitrogenous compounds, and the increased export of C from root to shoot compared to that occurring in NO_3-fed plants. In our previous experiments, root mass was lowest with the NH_4-N form (Rosen et al., 1990), but cation uptake and organic acid content were maintained.

At low substrate pH, the efficiency of the proton efflux pump at the plasma membrane decreases, and downhill transport of protons into the cytoplasm increases (Marschner, 1986). Electrochemical potentials of root cells can decrease from –150 mV at pH 6 to –100 mV at pH 4 (Dunlop and Bowling, 1978). The result is that cation uptake is generally inhibited at low pH. Thus, one would predict lower organic acid concentrations because there is lower cation uptake and consequent requirement for charge balance.

In this experiment, we found significantly higher levels of some organic acids at low pH (4.5 vs. 6.5). Previous research demonstrated that shoot uptake of K, Ca, and Mg was about equivalent (Rosen et al., 1990), which indicates that cranberry plants are able to maintain their cation content even under low-pH conditions. If higher rates of dark CO_2 fixation (as have been shown to occur in response to NH_4 nutrition; Salsac et al., 1987) can occur at low pH, cation uptake could be maintained despite lower cell electrochemical potentials. Perhaps higher organic acid content may provide a means to maintain negative cell potentials at low pH.

Although cranberry growth was affected by N source (Rosen et al., 1990), the organic acid concentrations of shoots and roots did not change substantially. Apparently, NO_3 uptake was not great enough to result in an accumulation of organic acids as is expected with NO_3 nutrition. Higher organic acid concentrations, however, were observed at low pH, possibly due to increased dark CO_2 fixation to maintain negative cell potential and cation uptake.

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Table 2. Solution pH and N form effects on concentrations of carboxylic and phenolic acids in cranberry root extracts

	Carboxylic acids					Phenolic acids								Total measured organic acids
	Malic	Citric	t-Aconitic	Fumaric	Oxalic	Proto-catechuic	Syringic	4-Hydroxy-benzoic	Gallic	Benzoic	Vanillic	Ferulic	p-Coumaric	
								µmol/g shoot dry wt						
N form														
NH_4	36	63	0.33	0.10 b[z]	1.9 a	0.57 b	0.19	0.16	ND b[y]	0.068	0.052 a	ND	ND	102
NH_4/NO_3	65	101	0.63	0.24 a	2.7 a	0.70 ab	0.26	0.02	ND b	0.091	0.042 ab	ND	ND	171
NO_3	38	52	0.43	0.07 b	ND b	1.21 a	0.19	0.02	0.034 a	0.024	0.020 b	0.026	0.027	92
Significance	NS	NS	NS	*	**	*	NS	NS	**	NS	*	NS	NS	NS
pH														
4.5	48	63	0.44	0.11	1.8	0.6	0.32	0.02	0.024	0.105	0.036	ND	ND	114
6.5	47	82	0.50	0.17	1.2	1.0	0.13	0.08	0.003	0.023	0.037	0.017	0.018	131
Significance	NS	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS
pH × N form														
Significance	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS	NS	NS	NS

[z]Mean separation within columns by Duncan's multiple range test, $P < 0.05$.
[y]ND = not detected.
NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

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