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Effect of Nitrogen Source and Rate on Vegetable Amaranth Leaf Blade Mineral Nutrients, Pigments and Oxalates

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ABSTRACT

Twenty-day-old 'Hinn Choy' plants (Amaranthus tricolor L.) were transplanted on 15 x 15 cm spacings in 4-row beds with 1.2 m bed centers in a Leadvale silt loam soil. Nitrogen sources of NH_4, NO_3 and NH_4NO_3 were used at rates of 0, 100 and 200 kg/ha. The N rates were split-applied at transplanting and 1 week later. All treatments received supplemental K and P at the rate of 90 kg/ha at planting. Plants were harvested 31 days after transplanting. Plants given the NH_4 source were taller, and were higher in yield, leaf chlorophyll, total carotenoids and Mn (dry wt basis) than were plants given either NO_3 or NH_4:NO_3 (1:1) N-sources. Nitrate-N fertilizer increased leaf Fe, Cu, soluble and total oxalates, and residual soil K and NO_3, but reduced soil Mn levels. Leaf blade Ca was highest when NH_4NO_3 fertilizer was used. Increasing N-rates linearly decreased soil pH and leaf blade Ca; but linearly increased soil conductivity, NO_3, and S and leaf blade N, K, S, P, NO_3, Fe, chlorophyll, and carotenoids. Leaf blade total oxalate levels were linearly increased by increased application of NO_3-N fertilizer; whereas increasing NH_4-N fertilizer rates linearly decreased total oxalate levels.

RESUMEN

Plantas de 'Hinn Choy' (*Amaranthus tricolor* L.) de veinte días de edad fueron trasplantadas en un suelo franco limoso de Leadvale a una distancia de 15 x 15 centímetros en camas de 4 hileras con centros de cama distanciados 1.2 m. Las fuentes de nitrógeno, NH_4, NO_3 and NH_4NO_3, fueron utilizadas a las dosis de 0, 100 y 200 kg/ha. Las dosis de N fueron aplicadas divididas, al momento del transplante y una semana después de transplantar. Todos los tratamientos recibieron K y P suplementarios a dosis de 90 kg/ha al momento de plantar. Las plantas se cosecharon 31 días después del trasplante. Las plantas que recibieron NH_4 fueron más altas, tuvieron mayor rendimiento, clorofila foliar, carotenoides totales y Mn (en base a peso seco) que las plantas que recibieron fuentes de N consistentes en NO_3 or NH_4:NO_3 (1:1). El fertilizante de N en base de NO_3 incrementó la concentración foliar de Fe, Cu, oxalatos solubles y totales y el K y el NO_3 residuales en el suelo, pero redujo los niveles de Mn en el suelo. El contenido de Ca en la lámina de la hoja fue más alto cuando se utilizó NH_4NO_3 como fertilizante. El incremento de las dosis de N disminuyó linealmente el pH del suelo y el contenido de Ca de la lámina foliar; pero incrementó linealmente la conductividad y el contenido de NO_3 y S del suelo asi como el contenido de N, K, S, P, NO_3, Fe, clorofila y carotenoides en la lámina de la hoja. Los niveles de oxalato totales de la hoja se incrementaron linealmente con la aplicación de fertilizantes nitrogenados a base de NO_3, mientras que el incremento en las dosis de fertilizante nitrogenado en base a NH_4 decreció linealmente los niveles de oxalato totales.

Additional index words. Amaranthus tricolor L., mineral nutrition, oxalic acid

Vegetable amaranth (mostly *A. tricolor* and *A. dubious*), a warm season greens, is becoming more available in North American marketplaces (Makus, 1990). Leafy greens are known to accumulate anti-nutritional factors, such as nitrates and oxalates, under conditions of adverse weather and/or in combination with high levels of applied soil nitrogen (N) (Maynard and Barker, 1972; Lorenz, 1978). *Amaranthus cruentus* leaves were shown to accumulate more soluble oxalate, total oxalate, and nitrate when grown in soil of high fertility than in soils of medium fertility (Schmidt et al., 1971).

Nitrogen sources can modulate plant oxalate and nitrate concentrations (concns). Teyker et al. (1991), studying the importance of N form on maize and pigweed growth and competition, reported a 5.9 fold increase in *A. retroflexus* leaf lamina oxalate (fresh wt. basis) when $Ca(NO_3)_2$ was the N-source than with $(NH_4)_2SO_4$. When $(NH_4)_2SO_4$ was the only N-source, increasing supplemental soil N-rates from 0 to 200 kg/ha decreased oxalic acid levels in spinach leaves from 839 to 303 mg/100 g leaf fresh weight (Verma et al., 1970). Kitchen et al. (1964) observed that when spinach leaves were treated with anionic solutions, such as NO_3^-, more C^{14}-oxalate accumulated, than when cationic solutions were applied. Likewise, N-form can influence spinach leaf NO_3 concns (Barker et al., 1971). Supplemental N can increase *A. tricolor* leaf blade chlorophyll and protein and plant yield, provided there is adequate soil P (Makus, 1986; Makus, 1992).

Ammonium and nitrate fertilizer sources were found to effect soil pH and the availability of other ions essential for plant growth (Barker and Mills, 1980). Assimilation efficiency of these N-forms will ultimately influence the energy demands, cation balance, and carbon utilization by the plant.

The objective of this experiment was to determine the effect of N-source and N-rate on plant performance and the concn of several leaf blade constituents, specifically mineral nutrients, nitrates, oxalates, and pigments, in vegetable amaranth.

MATERIAL AND METHODS

On 3 Aug. 1993, three-week-old amaranth (*Amaranthus tricolor* L.) transplants of *Hinn Choy* were transplanted into a pH 7.1 Leadvale (fine-silty, siliceous Fragiudults) silt loam soil on an Agricultural Research Service site near Booneville, Ark. (Lat. 35º06'). Four rows of plants were spaced 15x15-cm apart on 0.6-m wide raised beds. Beds were on 1.2-m centers. Plant density was 215,260 plants per ha. The beds received 90 kg P/ha, 90 kg K/ha, and one-half the amount of three N-sources at rates of 0, 100 or 200 kg N/ha on the day of transplanting. The remaining N was applied one week later. Nitrogen sources were NO_3 (as calcium nitrate), NO_3:NH_4 (1:1, as ammonium nitrate) and NH_4 (as ammonium sulfate). All fertilizers were broadcast directly over the beds. Surface trickle irrigation delivered supplemental water when soil moisture fell below -20 kPa.

The seven-week-old plants were harvested on 3 Sept. Twelve plants from the middle two rows of each four-row plot were used to determine plant yield and height and to provide leaf sub-samples for analysis. Leaf blades located approx. 1/3 the distance upward from the base of the plant were then washed, frozen, lyophilized, ground through a 40-mesh (0.36 mm^2) screen and stored at -20ºC until analyzed.

Within one week of harvest, soil samples from the 0 - 15 cm profile were taken and subsequently analyzed for pH, electrolytic conductivity, organic matter, NO_3, Ca, K, P, Na, Mg, Fe, Mn, S, Cu, and Zn.

Leaf blade nitrate and P were determined colorimetrically, protein-N and S by the Leco combustion method, and mineral elements by ICP spectrometry (Plank, 1992). Chlorophylls *a*, *b*, and total carotenoid pigments were determined by the method of Welburn and Lichtenthaler (1984), and oxalates were determined by an A.O.A.C. method (1984).

The experimental design was a randomized complete block with 4 replications. Treatments were arranged as a 3 x 3 factorial, with 3 nitrogen sources and 3 nitrogen rates (for a total of 36 plots). Nitrogen form effects were compared by the Ryan 'F' test. Interactions were examined using the PDIFF option of the LSMEANS statement of PROC GLM and nitrogen rates were fitted by polynomial regression using PROC GLM of SAS Version 6.11.

RESULTS AND DISCUSSION

Application of NH_4-N or NH_4NO_3-N sources resulted in greater plant height and yield compared to the NO_3-N source (Table 1). There was a quadratic (Q) decrease in plant height with increasing application rate from the NO_3 source. The NH_4 source increased plant height at the 100 and 200 kg/ha rates, but the NH_4:NO_3 source had no effect on height at any rate (interaction not shown). Likewise, when NO_3 was the N-source, yield decreased linearly (L) as application rates increased (P<0.10), whereas the other N-sources increased yield at the highest application rates (interaction not shown). In a previous study, 'Hinn Choy' yield and plant height increased both L and Q when NH_4NO_3 applications of 0, 135 and 270 kg/ha were made (Makus, 1992). The response to the ammoniacal N-source in terms of improved yield, tends to support the general observation that calcifuges, or plants adapted to acid soils, do best when supplied with NH_4-N (Marschner, 1986).

Table 1. Effect of nitrogen form and rate on plant height and yield of 7 week-old *Hinn Choy* amaranth.

Main Effect	Plant height	Plant yield
	cm	t·ha^{-1}
N-source:		
NO_3	14.5 b	7.8 b
NO_3+NH_4	19.1 a	10.6 a
NH_4	20.5 a	11.9 a
N-rate:		
0 kg/ha	18.5	9.1
100 kg/ha	18.4	10.4
200 kg/ha	17.3	10.9
Polynomial fit:	Q**[z]	--
Interaction[y]:	**	**

[z]NO_3 source, only.

[y]** = significant at P=0.01; Q = quadratic. Lower case letters are used to separate column mean differences based on Ryan 'F' test at the probabilities shown in this and subsequent tables.

Two months after fertilizer application, soil pH and electrolytic conductivity values between NO_3- and NH_4-N sources were similar, but application of the NH_4:NO_3-N source resulted in the highest soil pH and lowest conductivity values (Table 2). Under the short two month soil incubation period, $(NH_4)_2SO_4$ did not substantially reduce soil pH. The NO_3-N source, using calcium as the carrier, did not increment soil test Ca levels at the end of the study. The 200 kg N-rate should have added 330 kg Ca/ha to the soil, but this was not reflected in the soil test for Ca. Based upon leaf blade Ca concns in an *A. tricolor* screening trial (Makus, 1984), harvested vegetable amaranth would have only removed about 38 kg Ca/ha. Soil test K levels were highest when NO_3 was used, exclusively, as the N-source. Soil NO_3 concns were increased 12.9 fold when NO_3-N was used as the N-source and application rates were increased to 200 kg/ha (data not shown). Nitrate concns were increased 3.4 and 6.4 fold when NH_4:NO_3 and NH_4 sources were used, respectively (data not shown). Sulfur concns were increased by $(NH_4)_2SO_4$ and increasing application rate, because of the SO_4 carrier, which added 114 kg S/ha for each 100 kg N applied. The other N-sources and rates had no effect on soil S concns. Soil organic matter, Ca, Fe, Na, Mn, Mg, Cu, and Zn were not effected by any treatment combination (data not shown except for Ca).

Increasing the N-rate decreased soil pH (L) and increased conductivity (L), NO_3 (L in the NO_3-N source, only), and S (L in the NH_4-N source, only). Soil pH reductions from NO_3, NH_4NO_3, and NH_4 forms at the 200 kg/ha rate were 0.28, 0.42, and 0.40, respectively, compared to the zero application rates. All N-sources increased soil conductivity with increasing application rate; however, the change in conductivity due to increased application rate was smallest when the NH_4:NO_3-N

Table 2. Effect of nitrogen form and rate on soil pH, conductivity, and several soil nutrients after harvest[Z].

Main Effect	pH	EC	Ca	K	NO_3	S
		$dS \cdot M^{-1}$	kg·ha⁻¹			
N-source:						
NO_3	7.09 b	0.129 a	3483	130 a	88.5 a	35.8 b
NH_4+NO_3	7.27 a	0.106 b	3554	116 b	44.1 b	35.0 b
NH_4	7.12 b	0.124 a	3526	120 b	39.8 b	73.7 a
	* *[y]	*	NS	* *	* *	* *
N-rate:						
0 kg/ha	7.33	0.085	3495	123	16.0	36.2
100 Kg/ha	7.17	0.108	3469	122	42.9	43.9
200 kg/ha	6.98	0.166	3598	120	113.5	64.5
Polynomial fit:	L^{**}	L^{**}	--	--	L^{**}	L^{**}
Interaction:	NS	*	NS	NS	* *	* *

[Z]No effect on soil Ca, Fe, Na, Mn, Mg, P, Cu, or Zn.

[y]NS, *, * * = Not significant or significant at P=0.05, P=0.01, respectively; L=linear.

Table 3. Effect of nitrogen form and rate on amaranth leaf blade total-N and mineral nutrients (dry wt basis).

Main Effect	Total-N	Ca	K	S	P	NO_3	Fe	Mn	Cu
	%					$\mu g \cdot g^{-1}$			
N-source:									
NO_3	4.55	4.00 b	2.87 a	0.399 b	0.285 a	1894	223 a	69 b	46.2 a
NH_4+NO_3	4.58	4.46 a	2.52 b	0.416 a	0.258 b	1449	184 b	82 b	34.2 b
NH_4	4.71	3.92 b	2.73 ab	0.425 a	0.273 ab	1622	178 b	121 a	33.8 b
	NS[Z]	*	*	* *	*	NS	* *	* *	* *
N-rate:									
0 kg/ha	4.24	4.37	2.52	0.406	0.283	939	178	91	36.7
100 Kg/ha	4.71	4.20	2.76	0.417	0.275	1791	192	85	38.3
200 kg/ha	4.92	3.79	2.84	0.418	0.256	2264	216	99	38.6
Polynomial fit:	L^{**}	L^{**}	L^{*}	--	L^{**}	L^{**}	L^{**}	L^{*}	--
Interaction:	NS	NS	NS	* *	NS	NS	NS	* *	NS

[Z]NS, *, * * = Not significant or significant at P=0.05, P=0.01, respectively; L=linear.

Table 4. Effect of nitrogen form and rate on *Hinn Choy* amaranth leaf blade pigments (dry wt basis) from 7 week-old plants.

Main Effect	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll Total	Total carotenoids
	$mg \cdot g^{-1}$			
N-source:				
NO_3	8.72 b	2.15	10.9 ab	2.66 ab
NO_3+NH_4	8.58 b	2.12	10.7 b	2.61 b
NH_4	9.06 a	2.25	11.3 a	2.75 a
	*[Z]	NS	*	*
N-rate:				
0 kg/ha	8.15	2.03	10.2	2.49
100 Kg/ha	8.91	2.19	11.1	2.71
200 kg/ha	9.30	2.31	11.6	2.82
Polynomial fit:	L^{**}	L^{**}	L^{**}	L^{**}
Interaction:	NS	NS	NS	NS

[Z]NS, *, * * = Not significant or significant at P=0.05, P=0.01, respectively; L=linear.

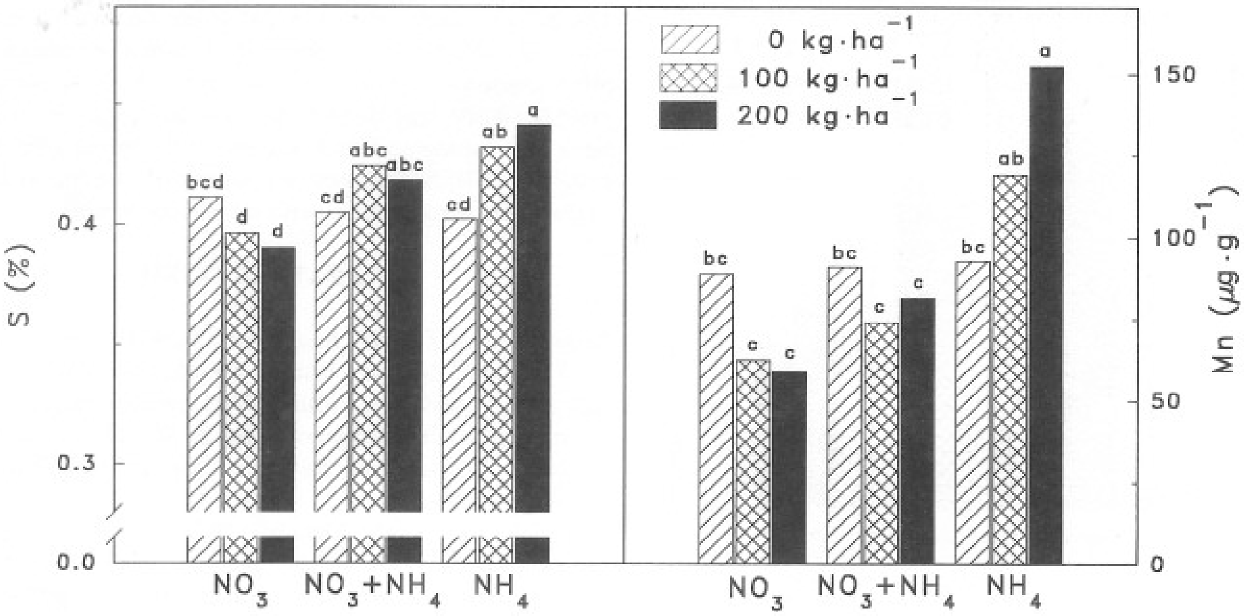

Fig. 1. Interactions between N-source and N-rate for leaf blade S and Mn concn (dry wt. basis). Lower case letters are used to separate mean differences (P=0.01).

Table 5. Effect of nitrogen form and rate on *Hinn Choy* leaf blade oxalates and the ratio of soluble to insoluble oxalates (s:i ratio) from 7 week-old plants.

	Oxalates			
	Sol.	Insol.	Total	s:i
	mg·g^{-1}			
N-source:				
NO_3	17.8 b	5.1	22.9 b	3.7 a
NO_3+NH_4	14.4 a	5.6	19.9 a	2.6 b
NH_4	14.1 a	5.2	19.4 a	2.9 ab
	* *^{Z}	NS	*	*
N-rate:				
0 kg/ha	13.7	4.9	18.6	3.0
100 kg/ha	16.5	5.3	21.8	3.2
200 kg/ha	16.1	5.7	21.8	3.0
Polynomial fit:	L^{*Y}	---	L^{*X}	---
Interaction:	0.06^{W}	NS	*	NS

^{Z}NS, *, * * = Not significant or significant at P=0.05, P=0.01, respectively; L=linear.
^{Y}NH_4:NO_3 source, only.
^{X}NH_4:NO_3 and NO_3 sources, only.
^{W}Probability of a greater 'F' value.

source was used, thus resulting in the interaction (data not shown). An explanation for the latter response may be due to the fact that the NH_4:NO_3-N source was the most efficient provider, by weight, of N and less fertilizer was required. Interactions associated with soil NO_3 and S concns were due to their contributions to the soil system from each respective carrier salt.

Application of the NO_3-N source resulted in the lowest leaf blade S concns, but highest Fe and Cu concns (Table 3). Use of NO_3- and NH_4:NO_3-N sources resulted in similar leaf blade Mn concns. Potassium, S, Fe, and Cu concns were similar when both NH_4:NO_3 and NH_4 were used as N-sources. Surprisingly, application of NH_4NO_3 and not $Ca(NO_3)_2$ resulted in the highest leaf blade Ca concns. Ammoniacal N sources resulted in leaf blades with higher mean concns of S. Total leaf blade N and NO_3, though variable, were not influenced by N-source.

Total leaf blade N, K, NO_3, and Fe concns were linearly increased and Ca and P concns were linearly decreased by increasing N-rate. Leaf blade Cu concns were not affected by N-rate. Leaf blade S and Mn concns were reduced by increasing NO_3-N rates, whereas S and Mn concns were increased by increasing NH4-N rates (Fig. 1). If NH_4:NO_3-N was used as the N-source, S and Mn leaf blade concns remained relatively constant with increasing N-rates. Leaf blade Mg, Na, and Zn concns were 0.79%, 0.28%, and 48 µg/g

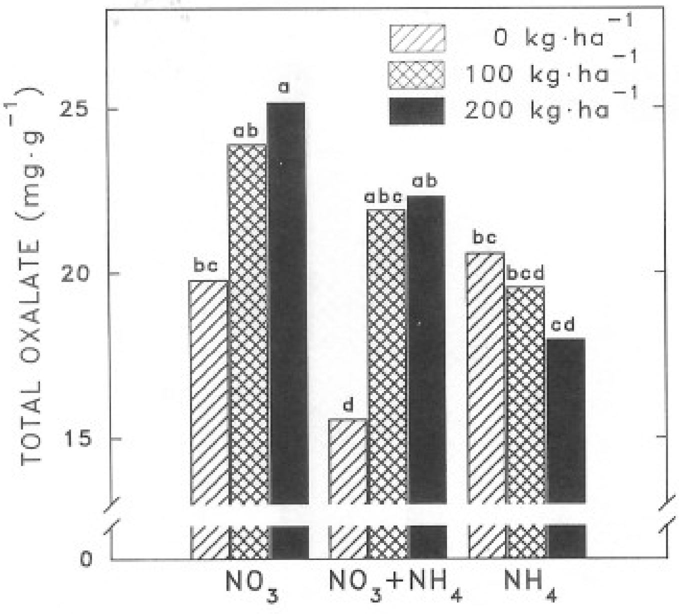

Fig. 2. Interaction between N-source and N-rate for leaf blade total oxalate concn. Lower case letters are used to separated mean differences (P=0.05).

(dry wt basis), respectively, and were not effected by N-source or rate.

The NH_4:NO_3-N source resulted in lower leaf blade chlorophyll a, total chlorophyll and total carotenoids compared to leaf blades given an NH_4-N, only, source (Table 4). Chlorophyll *b* was not affected by N-source. Increasing N-rate, linearly increased leaf blade chlorophylls *a*, *b*, total chlorophyll and total carotenoids. There were no source or rate effects on the chlorophyll *a*:*b* ratio, 4.1:1, the ratio of total chlorophyll to total carotenoids, 3.6:1, or any interactions.

Leaf blade soluble oxalate and total oxalate levels were lower if NH_4-N were the N-sources than when NO_3, only, was the N-source. (Table 5). The NH_4:NO_3-N source reduced the ratio of soluble to insoluble oxalates compared to the NO_3-N source. Insoluble oxalate concns were not effected by N-source. If N-rates were increased, the NH_4:NO_3-N source contributed to a linear increase in leaf blade soluble (P=0.06) and total oxalates. When N-rates were increased, leaf blades from the NO_3-N and NH_4:NO_3-N sources increased in total oxalate concns, whereas leaf blades from the NH_4-N source decreased in total oxalate concns as N application rate increased (Fig. 2).

Van Maercke (1973) reported that when spinach was given various forms of supplemental N, soluble and total leaf oxalates where highest in spring-grown plants given the highest rates of $Ca(NO_3)_2$, than with similar N-rates of $(NH_4)_2SO_4$ or urea N-sources. It may be possible to reduce amaranth oxalate levels further, as was done in the case of spinach, through the use of nitrification inhibitors such as dicyanodiamide and N-Serve (2-chloro-6-(trichloromethyl)pyridine) (Kick and Massen, 1973).

In a pH 7.1 silt loam mineral soil, selection of an NH_4-N source improved yield, leaf blade pigment concns, and reduced oxalate levels in vegetable amaranth cv. *Hinn Choy*. The NO_3-N source elevated leaf blade Fe and Cu concns. The NO_3:NH_4-N source generally resulted in intermediate plant responses. Although increasing N-rates increased yield (P=0.10), leaf blade N, K, P, Fe, and pigments, high N-rates also increased leaf blade NO_3 and, in the case of the NO_3- and NH_4:NO_3-N sources, increased soluble and total oxalate leaf blade concns with increasing N rates.

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