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Growth of Cotton Plants on Nitrate and Ammonium Nitrogen[1]

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ABSTRACT

Vegetative and reproductive growth of cotton (*Gossypium hirsutum* L.) were evaluated in the greenhouse on media containing nitrate or ammonium or both, to test for interactions between the two N sources on growth and yield. An inhibitor of nitrification was included in all treatments. Both N sources supported vegetative growth, although there was little response to increments of nitrate above meq/liter under the conditions of the test. Combinations of the two N sources did not support better growth than either one alone, except at low total N levels, perhaps because ammonium at $\geq$ 2 meq/liter inhibited *in vivo* nitrate reductase activity.

Reproductive growth of the plants was enhanced by N; however, 9 meq/liter nitrate was less effective than lower concentrations of either source, or their combination, in promoting fruitfulness and N accumulation relative to the vegetative parts. The failure to find a synergistic interaction in response to the two sources suggests that cotton responds to N source differently from some other crop species.

Additional index words: **Leaf area, Relative Growth rate, Nitrate reductase, *Gossypium hirsutum* L., N-Serve®.**

IN recent years some investigators have shown that wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) grow and yield better on combinations of ammonium and nitrate N than on equivalent amounts of either source alone (4, 8, 18). In addition, many other crops, including cotton (*Gossypium hirsutum* L.), are believed to grow better on nitrate alone than on ammonium alone (9, 20, 5). Nitrate must be reduced by an energy-consuming process before it can be assimilated. Thus, from an energetic standpoint, one might predict lower yields to the extent that photosynthetic products are diverted for nitrate reduction.

We sought to determine, in greenhouse experiments, the responses of cotton to nitrate and ammonium N and to combinations of the two. Although it is common field practice to fertilize cotton with either nitrate or reduced N, complex microbial transformations in the soil alter the spectrum of available forms and make interpretation of responses difficult. In our experiments, these difficulties were largely obviated by the use of an inhibitor of nitrification.

MATERIALS AND METHODS

Seeds of 'Deltapine 16' cotton were germinated in pots with vermiculite and grown on deionized water in the greenhouse for 2 weeks. Plants were then thinned to two/pot and watered twice weekly with test nutrient solutions. An experiment typically lasted until flowering. The solutions were all based upon a modified half-strength Hoagland's solution, in which KNO_3 was present at 1 meq/liter and $Ca(NO_3)_2$ at 4 meq/liter. For nitrate levels $<$ 5 meq/liter, K_2SO_4 and $CaCl_2$ were substituted for the nitrate salts to give the desired concentration. For levels $>$ 5 meq/liter, both nitrate salts were increased proportionately. Ammonium was added as $(NH_4)_2SO_4$. The pH of the nutrient solution was about 6.0, and the pH of eluates from the pots was not $<$ 5.1 except when the ammonium concentration was 20 meq/liter. Dow M-3322[3], a liquid formulation of N-Serve®, 2-chloro-6-(trichloromethyl)pyridine, (6) was included in all solutions at concentrations $\leq$ 10 ppm and effectively inhibited nitrification with no observable effects on the plants. Each treatment was replicated at least six times.

Leaf area was determined weekly by the method of Ashley, Doss, and Bennett (1). The first determination was made 1 week after treatments were begun. All leaves of length $>$ 2 cm were included. This nondestructive technique allowed the monitoring of individual plants over an extended interval, and thus minimized the error inherent in such experiments. Plant height and stem diameter were also measured, but were less satisfactory as indicators of growth. The relative growth rate R_A (14) was calculated from leaf area measurements during the period of exponential growth by the relationship:

$$R_A = \frac{\ln A_2 - \ln A_1}{t_2 - t_1} = \frac{0.693}{t_d}$$

where A is leaf area, t is time, and t_d is the time for leaf area to double. Doubling times were determined graphically from semilogarithmic plots of leaf area vs. age.

Nitrate reductase activity was determined in a separate group of 3-week-old plants (1 week after first watering with nutrient solution). The shoots were excised just below the cotyledonary node and were weighed and assayed by an *in vivo* method described previously (15). Activity is expressed as μmol/g fresh wt/hour.

In separate experiments, the influence of N source in reproductive growth was studied. Plants were grown, one/pot, on a standard high-nitrate (9 meq/liter) nutrient solution of the composition given by Guinn (7). After 6 weeks, when flower

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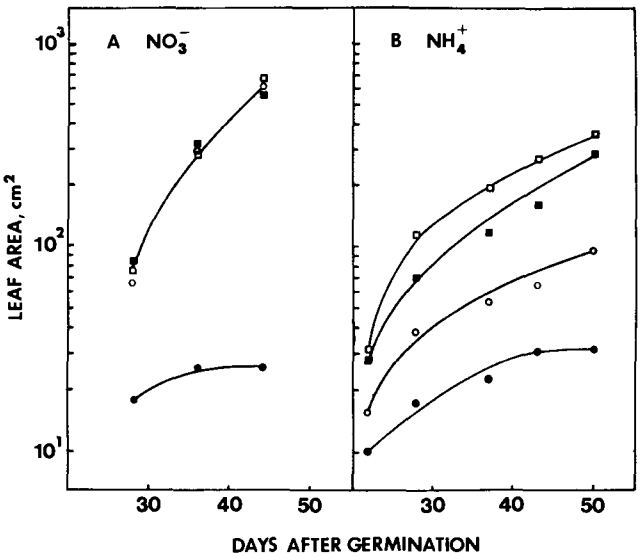

Fig. 1. Semilogarithmic growth curves for plants grown on nitrate (A) or on ammonium (B). Plants in A and in B were grown in separate experiments in July-Aug. and in Mar.-Apr., 1974, respectively. Nitrogen was present at 0 (●), 5 (○), 10 (■), or 20 (□) meq/liter.

buds (squares) had appeared, plants were selected for uniformity and watered twice weekly with test nutrient solution as before. Watering was continued for 9 more weeks, and the plants were harvested, separated into components, dried, and weighed. Lint weight was determined by difference after acid delinting. The shoots and the acid-delinted seed were separately ground and analyzed for N.

The procedure for N determination involved a micro-Kjeldahl digestion and a modified Conway microdiffusion analysis (17). Digests were diluted so that aliquots for analysis were equivalent to 0.5 mg dry tissue.

All treatment effects were evaluated by analysis of variance, followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

In tests of either N source alone at high concentrations, growth responses depended upon the source. On nitrate, plants responded to N concentrations up to 5 meq/liter, but showed little additional growth with further increases in concentration (Fig. 1). Similarly, Wadleigh (21) found very little stimulation of growth when the nitrate level was > 5.4 meq/liter. However, ammonium was effective up to the highest level tested, 20 meq/liter (Fig. 1).

Further tests were designed to measure the effects of ammonium at nitrate concentrations of either 0.5 meq/liter (low) or 5 meq/liter (saturating). In both trials there was a strong response to ammonium when given alone. The presence of 0.5 meq/liter nitrate diminished the response at low ammonium levels (Table 1); however, with 5 meq/liter nitrate there was virtually no effect of ammonium at any concentration (Table 2). These ammonium × nitrate interactions were significant at the P = 0.01 level. The results show clearly that there was no synergistic enhancement of growth by combinations of ammonium and nitrate; rather, any interactions appeared to be antagonistic (main effects less than additive).

Two explanations are possible for the observed interactions. First, both N sources might be assimilated

Table 1. Relative rates of leaf expansion during exponential growth of plants on factorial combinations of ammonium and nitrate N.

NO_3	NH_4, meq/liter					
	0	0.5	2	5	10	Mean
meq/liter	R_A, $days^{-1}$					
0	0.03	0.06	0.09	0.11	0.13	0.08 a†
0.5	0.07*	0.08*	0.09	0.12	0.12	0.10 b
Mean	0.05 a†	0.07 b†	0.09 c†	0.11 d†	0.12 d†	--

* Significantly different (P = 0.01) from the minus-nitrate treatment at that ammonium level. † Means of a column or row followed by the same letter are not significantly different at the P = 0.01 level.

Table 2. Relative rates of leaf expansion during exponential growth of plants grown on factorial combinations of ammonium and nitrate N.

NO_3	NH_4, meq/liter					
	0	0.5	1	2	5	Mean
meq/liter	R_A, $days^{-1}$					
0	0.05	0.10	0.12	0.13	0.14	0.11 a†
5.0	0.12*	0.15*	0.14	0.15	0.13	0.14 b
Mean	0.08 a†	0.12 b†	0.13 b†	0.14 b†	0.13 b†	--

* Significantly different (P = 0.01) from the minus-nitrate treatment at that ammomium level. † Means of a column or row followed by the same letter are not significantly different at the P = 0.01 level.

Table 3. Nitrate reductase activities of plants grown on factorial combinations of ammonium and nitrate N.

NO_3	NH_4, meq/liter					
	0	0.5	2	5	10	Mean
meq/liter	NRA, μmol/g/hour					
0	3.2	2.7	2.4	2.6	2.3	2.6 a*
0.5	5.2	5.5	4.4	3.7	3.3	4.4 b
Mean	4.2 a*	4.1 a*	3.4 b*	3.2 bc*	2.8 c*	--

* Means of a column or row followed by the same letter are not significantly different at the P = 0.01 level.

through the same pathway or through pathways with common rate-limiting steps. In support of this view, smooth curves can be drawn through the data of Tables 1 and 2 when plotted against total N concentration without regard for the source. This interpretation leads to the conclusion that no differences of physiological importance existed between the two N sources under the test conditions. The interpretation is unquestionably valid for low concentrations of nitrate or ammonium, but cotton plants obviously reacted differently to high levels of the two sources (Fig. 1).

The second possibility is that one N source may limit the assimilation of the other. This hypothesis also predicts less-than-additive main effects of the two factors, and thus cannot be distinguished from the first hypothesis by simple growth analysis. The rate-limiting step in nitrate assimilation is generally believed to be reduction to nitrate by the enzyme nitrate reductase (2). Nitrate reductase activity (NRA) in nitrate-treated plants decreased considerably when the ammonium concentration was ≥ 2 meq/liter (Table 3). Plants grown on ammonium alone did not have zero activity, even though nitrate was not measurable in eluates from these pots. Increasing the ammonium level to ≥ 2 meq/liter had little effect upon activity in the absence of nitrate. Again, the interaction between treatments was highly significant. Thus, at ammonium concentrations of ≥ 2 meq/liter NRA could have limited the assimilation of nitrate-N from combinations of the two sources. Earlier short-term expe-

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Table 4. Weights and N contents of cotton grown on high-nitrate nutrient solution, then switched to combinations of ammonium and nitrate.

N in test solution			Relative wt			Nitrogen		Recovery of
NO_3^-	NH_4^+	Dry wt	Boll	Lint	Seed	Shoot	Seed	applied N*
meq/liter		g/plant	% of shoot wt			g/plant	% of shoot N	%
0	0	18.0 a†	37.8 a†	8.1 a†	6.5 a†	0.06 a†	20.4 a†	--
0.5	0	19.5 a	45.1 b	11.3 ab	8.9 ab	0.09 a	27.7 b	11.9 a†
0	5	25.7 b	59.6 c	13.8 b	12.9 c	0.24 b	27.0 b	7.1 b
0.5	5	25.3 b	59.1 c	15.4 b	14.1 c	0.30 b	27.7 b	8.7 ab
9	0	37.7 c	55.3 c	12.0 ab	11.8 bc	0.40 c	22.0 a	7.5 b

* Recovery of N is based upon the increase in N over the zero-N treatment, expressed as a percentage of the total amount applied in the test solutions. † Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

riments established that amonium did not directly affect induction of nitrate reductase in green tissues of cotton (16). However, in long-term studies, secondary effects such as decreased nitrate absorption can not be ruled out (3, 10, 12).

The effects of N on yield and yield components have been thoroughly catalogued (19, 20, 21). However, only McMichael et al. (11) compared growth and yield on different N sources in carefully controlled experiments. Our second objective, therefore, was to study the effects of N source on reproductive growth. Plants were grown until squaring on a high-nitrate medium, then transferred to test solutions including the original high-nitrate solution. Vegetative growth again responded to both N sources (Table 4). In addition, N also affected the relative fruitfulness of the plants. Boll, seed, and lint weights, expressed as a percentage of total plant weight, all increased as the N supply was augmented (Table 4). Plants of the high-nitrate treatment were less efficient in supporting relative reproductive growth than those of either of the two ammonium treatments, not because total yield was less, but because vegetative growth was much greater than in the other two treatments (Table 4).

Differences among treatments did not result from differing earliness, as open flowers first appeared on plants in all treatments during the same week, and the mean number of the first reproductive node (5.7 at both the beginning and end of the experiment) did not vary significantly among treatments.

The partitioning of N between reproductive and vegetative parts displayed a pattern similar to that of relative fruitfulness. Plants transferred to a solution free of N had only 20% of total shoot N in the seed, closely followed by the high-nitrate plants at 22% (Table 4). The other plants all had about 27% of their N in the seed, regardless of the N source (s). Partitioning of N into seed was less than in mature plants (20), primarily because protein accumulation occurs most rapidly after the first month of ovule development (19). There was no evidence to indicate that either source at low concentration supported relative reproductive growth or accumulation of N better than the other.

Parr (13) defined fertilizer N use efficiency as the percentage recovery of fertilizer N by a crop. Efficiency generally declines as the amount of applied N is increased. The concept is not strictly applicable to pot-grown plants, since unused nutrients are leached out with each watering. Nonetheless, the fraction of applied N recovered by plants was slightly greater with the lowest N concentration (Table 4). Our experiments suggest that efficiency, vegetative growth, relative reproductive growth, and N partitioning between vegetative and reproductive tissues all can vary in response to N rate. In addition, we conclude that. i) The effects of N source on vegetative and reproductive growth and N accumulation were approximately additive when the sources were combined at low concentration, and less than additive at higher concentrations. ii) Ammonium, when supplied above a threshold level, tended to inhibit nitrate reduction. iii) Cotton was unlike either wheat or corn (4, 8, 18) in that it displayed no synergism in response to the two N sources.

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