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Some Factors Limiting Nitrate Reduction in Developing Ovules of Cotton[1]

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ABSTRACT

Ovules of developing cotton (*Gossypium hirsutum* L.) bolls had significant nitrate reductase activity, although considerably less than in leaves. The capability of the ovules to reduce nitrate (total nitrate reductase activity) increased during development, whereas the absolute rate of nitrate assimilation *in situ* reached a maximum at about 16 days after anthesis and then declined. Nitrate was present only in extremely small quantities in ovules, severely limiting their rate of nitrate reduction *in situ*. Transpiration from bolls was extremely slow, suggesting that ovules lack a mechanism for rapid replenishment of consumed nitrate. Estimated rates of ovular nitrate reduction could account for $< 5\%$ of the accumulation of reduced nitrogen.

Additional index words: **Gossypium L., Nitrate reductase, *In vivo* assay, Transpiration.**

MATURE seeds of cotton (*Gossypium hirsutum* L.) store large amounts of protein, enough that the embryos can contain up to 7% N by dry weight (10). More than half the N of a cotton crop near maturity resides in the bolls (fruits), with the seed containing the greatest part of the boll N (11). To understand the development of high-quality seed, one must know where the N deposited in the seed originates in the mother plant.

During the growing season, most N for field plants is available in the nitrate form. Nitrate is absorbed and reduced by plants to the level of ammonia. The activity of this pathway can be followed by measurement of nitrate reductase activity (NRA), which is believed to limit nitrate assimilation under most conditions (1). NRA occurs mainly in green leaves of cotton plants with a minor amount in the roots (7). However, a recent study of the enzyme in germinating seeds (7) implied that it might also occur in developing ovules. Several investigators have found significant rates of nitrate reduction in developing fruits or associated structures of other crop species (2, 5, 6, 9,). The present work was begun to determine whether nitrate is reduced in ovules or other tissues of cotton bolls, and whether such reduction contributes significantly to the N stored in the seed.

MATERIALS AND METHODS

'Deltapine 16' cotton was grown in pots in the greenhouse and watered with a nutrient solution described by Guinn (4). Flowers were tagged on the day of anthesis. Bolls of the proper age were excised and stored between wet paper towels until transported to the laboratory for dissection and analysis. This storage period never exceeded 20 min. Ovules were removed from the bolls, and the fiber was stripped off by hand before weighing and assaying. When stripping was done gently, the outer layer (outer integument) of the seed coat appeared to be uninjured; however, some fuzz fibers were not completely removed, especially on ovules older than 30 days. NRA measurements were not affected by the fuzz.

NRA was determined with an anaerobic *in vivo* assay technique. The temperature of the assay medium (30 C) was an approximation to the diurnal mean temperature of the greenhouse in which the plants were grown. For simple comparisons of total NRA, the assay medium routinely included 30 m*M* nitrate. However, in studies involving assessment of both potential and actual rates of nitrate reduction *in situ*, assays were also conducted with no exogenous nitrate (8). All assays without added nitrate were terminated after 25 to 30 min. Activity slowly declined during the assay as the endogenous nitrate became depleted, especially in ovules older than 24 days, and the reported rates necessarily underestimate the initial rates by a small amount.

Nitrate and protein of ovules were determined by methods reported elsewhere (8).

Resistances to transpiration were measured with a diffusive resistance meter (12), Lambda Instruments Co.[3], Lincoln, Neb.). The tubular chamber was hand-held in place against the curved boll surface during a run. Readings were corrected for the increased surface area of the bolls (assumed to be spherical) relative to a flat surface. However, errors resulting from the position of the curved evaporating surface relative to the sensing element were neglected. Resistances were measured only with leaves and bolls in full sunlight and on well-nourished greenhouse plants. Leaf and boll temperatures were all in the range of 32 to 34.5 C.

RESULTS AND DISCUSSION

Table 1 shows the distribution of NRA among the ovules, carpel walls, and bracts of 16-day-old bolls. Although the subtending leaves displayed much greater activity than any other part assayed, the ovules also had significant activity. The bracts and carpel

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[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, and it does not imply approval to the exclusion of other products that may also be suitable.

Table 1. NRA of tissues of 16-day-old bolls.

Tissue	Nitrate reductase activity*		
	μmol/g/h	μmol/boll/h	% of total boll activity
Bracts	0.32	0.22	14
Carpel wall	0.05	0.37	24
Ovules	0.13	0.98	62

* For comparison, mean activity of the subtending leaves was 3.1 μmol/g/hour (7.6 μmol/leaf/hour). Assays were conducted with added nitrate in the medium.

Table 2. NRA of ovules given nitrate at several concentrations.

Nitrate concentration	Nitrate reductase activity*	
	Ovules from excised bolls	Isolated ovules
mM	μmol/ovule/h	
0	0.04	0.09
1	0.03	0.08
10	0.03	0.22
100	0.09	0.67
300	-	0.39

* Either excised bolls or isolated ovules were incubated for 24 hours in the solutions before assay. Bolls were 30 to 32 days old at time of removal from the plants. Assays were conducted with added nitrate in the medium.

Table 3. Resistances to water loss shown by various parts of greenhouse-grown cotton plants.

Plant part	Plant water status	Resistance
		sec/cm
Leaf		
Lower surface	Turgid	<2
Upper surface	Turgid	<2
Lower surface	Wilted	13 to 15
Boll	Turgid	25 to 44

wall did not appear to contribute greatly to nitrate reduction and were not characterized further. Ovules, however, appeared to be able to reduce significant amounts of nitrate, more because of their mass than because of their specific activity (Table 1).

NRA of ovules depended strongly on age. On a per-gram basis, total NRA (assayed with nitrate in the medium) declined until reaching a minimum at 15 days after anthesis and then increased very rapidly until the end of the experiment at 32 days (Fig. 1A). On a per-ovule basis, activity increased continuously for the 32 days (Fig. 1B). Activity with only endogenous nitrate as substrate (8) showed quite a different pattern, however. On a per-gram basis, it declined continuously during development and was negligible by 32 days (Fig. 1A). On a per-ovule basis, it increased slowly to a maximum at 15 to 18 days then declined to nearly zero (Fig. 1B).

The ratio of activity assayed without nitrate to that assayed with nitrate in the medium indicates the efficiency with which the tissue uses its capacity for nitrate reduction (8). In developing ovules, the efficiency of nitrate reduction decreased greatly starting 16 days after anthesis (Fig. 1). This decline in efficiency resulted from a very large increase in total NRA, without a corresponding change in the true rate of assimilation. Analysis for nitrate in ovules showed that, although the total amount increased during development, its concentration declined (Fig. 2). Compared to the nitrate levels normally found in leaves or other nitrate-storing organs, those of ovules were very low at all ages, but particularly so 10 days or more after anthesis. Because NR is considered to be substrate-inducible (1), it was unusual to find

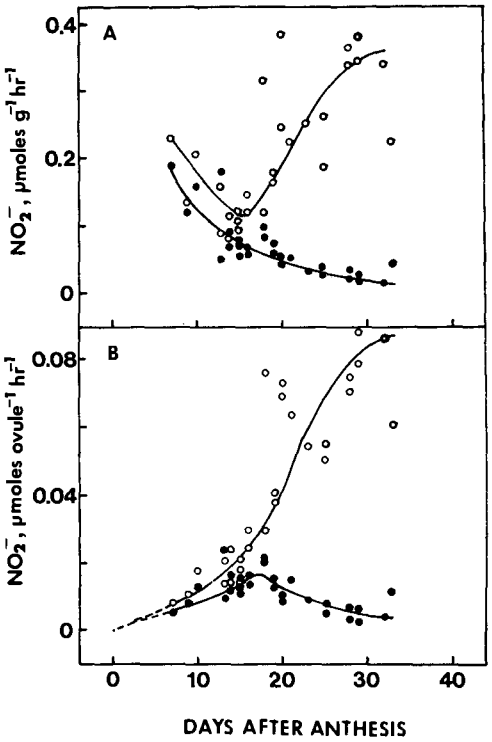

Fig. 1. NRA of developing ovules, expressed on a A) per-gram or B) per-ovule basis. The ovules from each boll were divided into two groups and assayed either with (○) or without (●) nitrate in the assay medium. In B, activity is projected back to the origin for determination of the areas under the curves (Fig. 3).

large increases in enzyme activity even as nitrate concentration declined almost to zero (Figs. 1, 2). Factors affecting the sensitivity of NR induction to nitrate level in ovules are not yet known. However, our preliminary data suggest that the increase in activity coincides with a period of high sucrose concentration in ovules.

Integration of the lower curve of Fig. 1B with time yielded a cumulative estimate of the true amount of nitrate assimilated by the ovules (8). This quantity represented $< 5\%$ of the reduced N accumulated during the first 20 days after anthesis (Fig. 3). Thus, virtually all the reduced N of ovules was probably imported from other sites in the plant. The capacity of the ovules for nitrate reduction, however, obtained by integration of the upper curve of Fig. 1B, represented $> 20\%$ of the amount of accumulated reduced N.

The availability of nitrate not only limited the efficiency of ovular NR, but also strongly affected the amount of enzyme activity present. When 30 to 32-day-old bolls were excised and the peduncles kept in nitrate solutions for 24 hours, activity was more than doubled over that of the water control (Table 2). Similarly, activity of isolated ovules, incubated directly in the nitrate, was increased up to sevenfold over that of the control (Table 2). Thus increased nitrate availability to the ovules caused the induction of additional NRA as well as the provision of more substrate to be reduced. Any increase in reduced N

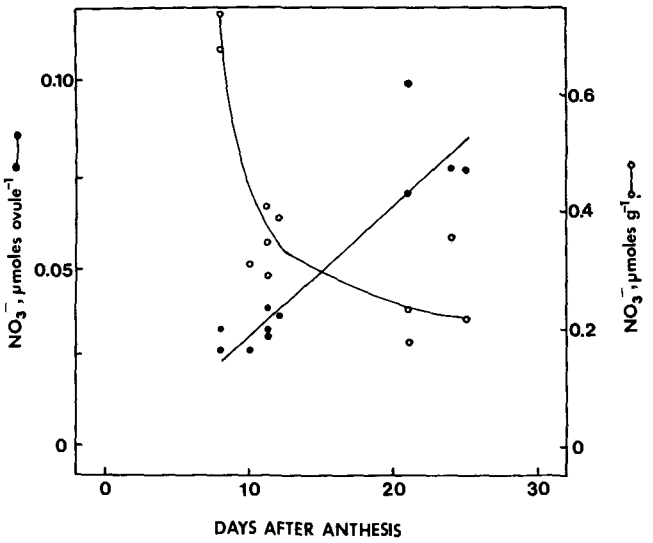

Fig. 2. Nitrate content (●) and concentration (○) in developing ovules.

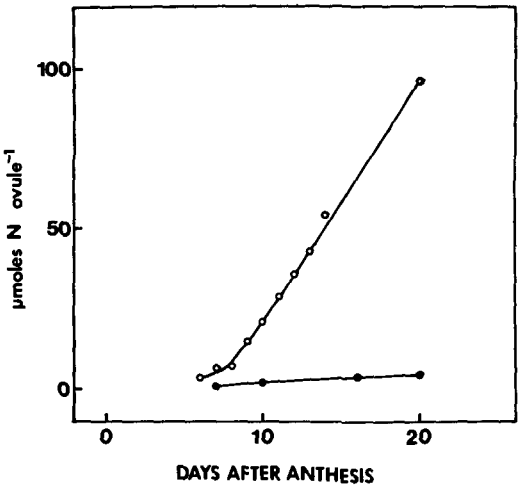

Fig. 3. Reduced N content of ovules during development (○) and total amount of nitrate assimilated by the ovules themselves (●). The latter quantity was derived from Fig. 1B by graphical integration.

in the ovules would presumably be deposited there as storage protein. However, little is known about controls on the translocation of reduced N into the boll, and our experiments do not prove that a net increase in accumulation would be realized from greater ovular nitrate reduction.

Nitrate is carried in the plant in the xylem sap and frequently accumulates in transpiring organs. Cotton bolls had extreme resistance to water loss and thus can be considered almost nontranspiring (Table 3). A lack of nitrate movement into the boll, combined with nitrate consumption in the ovules, would explain its depletion to extremely low levels during ovule development (Fig. 2). Despite the failure of bolls to transpire at appreciable rates, Elmore (3) found relatively large numbers of stomata on their surfaces. This anomaly suggests that the stomata were almost completely closed, or perhaps occluded by wax.

In cotton, stomatal number in leaves and rate of water loss are affected strongly by both environment and genetic background (Bruce Roark, personal communication). These characters in bolls might also be affected by environmental or genetic factors. The supply of nitrate to the bolls via the transpiration stream thus is an aspect of development that might be experimentally approachable. How increased nitrate movement into bolls would alter its availability in ovules, however, is not yet clear.

Nitrate reduction requires much energy, and incorporation of reduced N into amino acids requires carbon skeletons. Thus, if nitrate reduction and amino acid synthesis in the ovules were greatly increased, embryo and fiber might be forced to compete for available carbohydrates. Indeed, Wadleigh (13) showed that a greater nitrate supply to the whole plant stimulated embryo growth and protein content of the ovules much more than it promoted fiber growth, even though vegetative growth was also strongly increased. Thus, even though ovular nitrate reduction is limited simply by boll transpiration, there are serious agronomic problems associated with an increase in the rates of these processes. The relationship of ovular nitrate reduction to yield and protein accumulation deserves further study.

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