EFFECTS OF ROOT AERATION, PLANT AGE, ROOT TEMPERATURE, AND PHOTOPERIOD ON THE RATIO OF NITRATE TO REDUCED NITROGEN TRANSPORTED IN THE XYLEM SAP OF COTTON (*GOSSYPIUM HIRSUTUM* L. CV. WESTBURN)

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1966

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
For the Degree of
MASTER OF SCIENCE
August, 1969

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ACKNOWLEDGEMENTS

I wish to acknowledge the Department of Botany and Plant Pathology and Oklahoma State University for the use of their research facilities and the privilege of pursuing an advanced degree.

Special gratitude is extended to my major advisor, Dr. Gene Guinn, whose advice, counsel, and patient encouragement were of invaluable aid, not only in the completion of this study, but in perpetuation of my academic endeavor.

I am also indebted to the other members of my committee, Dr. Eddie Basler and Dr. John Thomas, for their time and talent.

Special thanks is due my wife, DiAnne, whose patience and moral support were a constant source of comfort.

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CHAPTER I

INTRODUCTION

The effect of environment on plant metabolism is of great current interest. Of special interest is the effect of various environmental conditions on nitrogen metabolism, since nitrogen metabolism and protein synthesis are inseparably related. Nitrogen metabolism of roots is being studied more intensely because it is becoming evident that the root plays a much greater role in plant activity than just an adsorbing organ for water and essential mineral elements. The root system is being given more and more attention as an organ which possesses a complex metabolic activity. This activity is very diversified and is essential, not only for the roots themselves, but also for the entire plant. The role of roots in the primary synthesis and transformation of nitrogenous compounds is particularly significant. This is expressed in their ability to reduce nitrates; to synthesize amino acids, amides, and proteins; to synthesize allantoin and nucleic acids; to have a part in the formation of nicotine; and possibly even the synthesis of forerunners of chlorophyll (23). Recently, root absorption, and particularly excretion, has been studied (12, 16, 17).

Nitrate is the principal form of nitrogen absorbed by higher plants (7, 25, 28). However, before it can be incorporated into organic nitrogenous compounds, it must be reduced to ammonia. Ammonia has been shown to be directly incorporated into organic nitrogenous substances

via glutamate and glutamine (10, 37). The first step in this reduction process is the conversion of nitrate to nitrite by the enzyme nitrate reductase (14). Decreased nitrate reduction under stress may influence the quantity and/or quality of other nitrogen fractions and of total nitrogen of a plant. Decreased nitrate reductase activity has been attributed to decreased light, low moisture, low fertility, genotype differences, and possibly other factors (25).

The evidence in favor of nitrate reduction occurring in roots of plants is considerable. The findings of Bollard (6) that, at most, only traces of inorganic nitrogen could be detected in the xylem sap of a wide range of plants, and the demonstration by Sanderson and Cocking (28) that excised tomato roots thrive in sterile nutrient solution with nitrate as the sole source of nitrogen, are but two of many relevant experimental findings. Nevertheless, the presence of nitrate reductase in roots does not seem to have been demonstrated satisfactorily.

Evans and Mason (14) reported extremely low rates of activity in extracts of wheat, corn, and soybean roots where activity was about 6, 1, and 2 mμmoles nitrate reduced per milligram dry weight of tissue per hour. The levels of activity in cauliflower roots (9) and corn roots (19) were reported simply as being very low. Vaidyanathan and Street (33) reported barely perceptible rates in aqueous extracts of excised tomato roots. Cresswell, as quoted by Sanderson and Cocking (28), examined root extracts of several species of plants and found either low rates of activity, which he felt could be accounted for by the presence of denitrifying bacteria, or no activity at all.

Tissue tests for nitrate are often used in an effort to establish available nitrogen levels in plants and soils. The validity of such tests depend upon the ratio of nitrate to organic forms of nitrogen transported from the roots to the aerial portions (3). Because of conflicting reports on the form of nitrogen transported in other plants, it seemed desirable to establish the ratio in cotton. Since nitrate reductase is inducible (1, 5, 20, 38), it also seemed important to establish some internal and environmental parameters of this tissue testing procedure.

CHAPTER II

METHODS AND MATERIALS

The effects of plant age, root temperature, photoperiod, and root aeration on the nitrogen composition of the xylem sap of cotton were examined in this study. The plant used was Gossypium hirsutum L. cv. Westburn. All experiments were conducted under greenhouse conditions except the day-length experiment which was conducted in a growth chamber. All the experimental plants were grown in a modified Hoagland's (24) nutrient solution in twelve-liter plastic buckets. Each bucket contained four plants which were supported in holes through marine plywood lids.

The plants were germinated in vermiculite and allowed to grow until the cotyledons began to unfold. This generally took about five days. When the plants had reached this stage, they were separated and selected for uniform development and placed in the nutrient solutions.

The greenhouse was equipped with tanks designed for controlling root temperatures. These tanks were designed to hold 16 buckets each. Water was placed in the tanks only for the root temperature experiment. In all the other experiments, the tanks were left dry so that the root temperature was determined by the greenhouse temperature. The lids were adjacent to one another allowing very little light to penetrate to the roots of the plants. This simulated the proper conditions for normal root development. All the buckets in these tanks were

continuously aerated to assure adequate oxygen content in the nutrient solution.

Procedure for Xylem Sap Collection

The collection of the xylem sap exudate included two methods. The voluntary flow method, which has been outlined by other workers such as Crafts (11), and VanOverbeek (34), was used to collect the sap for three of the experiments: root temperature, photoperiod, and plant age. For the aeration experiment, it was necessary to use a modification of Eaton's (13) and Grossenbacker's (18) method of sap collection. Sap in cotton will not flow freely if the roots are subjected to anaerobic conditions. It was thus necessary to collect the sap for this experiment by using a suction technique.

In the free flow method of collection, the plants were decapitated about 1 inch below the cotyledons. Using a razor blade, four one-half inch longitudinal incisions were made around the terminal end of the stump. The epidermal, cortical, and phloem tissues were then peeled back, exposing only the central cylinder of xylem and pith. The stumps were then rinsed with distilled water, and three-inch lengths of latex tubing were tightly fitted around the xylem cylinder. The xylem exudate was then collected, over a period of approximately eight hours, from the reservoir formed by the tubing. Longer collection times were avoided for fear of recording starvation effects of the roots. Every two hours the exudate was collected and transferred to a freezer to prevent biological changes due to microorganisms.

The suction method of sap collection was similar in procedure to that of the free-flow method. The only variation in the method was

that 10-ml. pipettes were fitted to the plastic tubings, and the suction side of a separate aeration pump was connected to the distal portion of the pipettes. The suction method of sap collection was faster than the free flow method, thus cutting collection time to about four hours.

Experiment 1. Age and its effect on xylem transport of nitrate and reduced nitrogen.

The purpose of this experiment was to determine whether plant age affects xylem sap composition with respect to nitrate nitrogen and reduced nitrogen. The experimental design was set up to include three plant ages: 35, 50, and 90 days old. Three replications were used for each age.

The experiment ran from July 19, to September 18. It can, therefore, be considered that the plants were subjected to moderately long-day conditions. The temperatures in the greenhouse ranged from a maximum of 38^{o}C during the day to a minimum of 21^{o}C at night. At all three ages the sap was collected under voluntary flow from 10 a.m. until 5 p.m.

Experiment 2. Aeration and its effect on the xylem sap composition from excised cotton roots.

This experiment involved the study of root metabolism under aerobic and anaerobic conditions. The two treatments, aerated and not aerated, were replicated four times.

The plants were allowed to grow for 37 days. Twenty-four hours prior to the start of sap collection, aeration was discontinued in Treatment 2. Treatment 1 was continously aerated until the end of collection. The sap was extracted under suction in both treatments.

The collection of the nonaerated plants was done from 1:30 to 5 p.m. The aerated plants were collected from 5:30 to 10:30 p.m. that same day. This procedure was followed because not enough apparatus was available to collect sap from 64 plants at one time.

The experiment ran from July 19, to August 27. Thus, the plants were exposed to relatively long-day conditions. The greenhouse temperatures remained in the range of 21°C to 38°C, similar to those in the plant age experiment.

Experiment 3. The effect of root temperature on the nitrogen composition of xylem sap.

This experiment was designed to study the effect of root temperature on xylem sap composition. Root temperatures of 20, 25, and 30°C were maintained within ± 0.8°C in thermostatically controlled water tanks. The root temperature treatments were replicated three times.

The sap in all three treatments was collected after 68 days of growth. The experiment ran from August 23, to October 30; thus, the plants were subjected to approximately equal day and night lengths. The plants in all three treatments were continuously aerated.

Experiment 4. The effect of photoperiod on nitrogen composition of xylem sap.

In this experiment the effect of short and long day-length on sap composition was investigated. The plants were grown in growth chambers where the temperature and day-length could be controlled. The short-day plants were subjected to an 8 hour day-length, the long-day plants to a day-length of 16 hours. The daytime temperature was held at 30°C, while the night temperature varied from 18 to 20°C.

The light intensity in the growth chamber was approximately 3500 foot candles. Xylem sap was collected from both long and short-day plants after 50 days of growth.

The short-day treatment was replicated three times, but the long-day treatment was replicated four times. The statistical analysis for this experiment was a Student's t test corrected for unequal replications.

Determination of Reduced and Nitrate Nitrogen

The nitrate test used for this study was one described by West and Ramachandran (35) which they used to determine the nitrate content of polluted air and water. This procedure is a spectrophotometric method employing chromotropic acid (1,8-dihydroxy-3,6-naphthalene disulfonic acid) as the chromophore. A Klett-Summerson photoelectric colorimeter, equipped with a 420 mμ filter, was used for the photometric determinations. This test is well suited for nitrate determinations of plant sap because it is free of significant interferences, expecially chlorides, nitrites, and oxidizing agents which are often troublesome in many of the other testing procedures. The test gave reproducible results and followed Beer's law over a range of 0.2-20 mg. of nitrate/liter.

The test for reduced nitrogen was adopted from the method of Fels and Veatch (15). The test was extremely sensitive and followed Beer's law on a 0.2 ml. sample over a range of 0 to 1 mM of reduced nitrogen. The standard amino acid solution was 75.1 mg. of glycine/liter or 1 mM. Ninhydrin was used to develop the color for the spectrophotometric determinations at 560 mμ. Although the present procedure does

not have the precision of the classic Kjeldahl determination of nitrogen, it was more sensitive and less laborious. The test proved very satisfactory in accuracy and reproducability.

Chromatographic Procedures

The amino acids in the xylem sap were purified and separated by a modification of a procedure outlined by Thompson and Morris (30, 31). Ten ml. of sap was run through Dowex 50 (50-100 mesh) which had previously been charged to the hydrogen form. The amino acids were eluted with three 25-ml. portions of 2N NH_4OH. The eluate was evaporated to dryness on a rotary evaporator. The amino acids were then dissolved in 2 ml. of distilled, deionized water.

The purified amino acids were separated and identified by descending two-dimensional paper chromatography. The mobile phases used were butanol, acetic acid, and water (4:2:1) in the first dimension and fused phenol (80% in water) in the second dimension. No attempt was made at quantitation of the amino acids other than visual comparisons.

CHAPTER III

RESULTS AND DISCUSSION

Experiment 1. Age and its effect on xylem transport of nitrates and reduced nitrogen.

It is known from numerous data in the literature that the nitrogen metabolism of plants is very labile and changes in relation to many factors, both external and internal.

One of the most important of the internal factors is the age of the plant. The present experiment was devoted to a study of the significance of plant age in the reduction of nitrate in roots.

The significance of plant age in nitrogen metabolism may be seen from a comparison of the data in Figures 1 and 2, and Table I. Figure 1 shows a significant decrease in nitrate nitrogen in sap with plant age. This data contradicts Hay et al. (22) who found no uniform increase or decrease in nitrates with plant age. Their data were very erratic, and they attributed this irregularity to diurnal variation. In my study, the irregularity of nitrates due to diurnal variation would not have been observed since sampling was done at the same time of day in each treatment. The differences in nitrate nitrogen among the three treatments were found to be statistically significant at the 5% level.

The apparent decline in nitrates with plant age could be misleading. It was observed that older root systems exuded more sap than

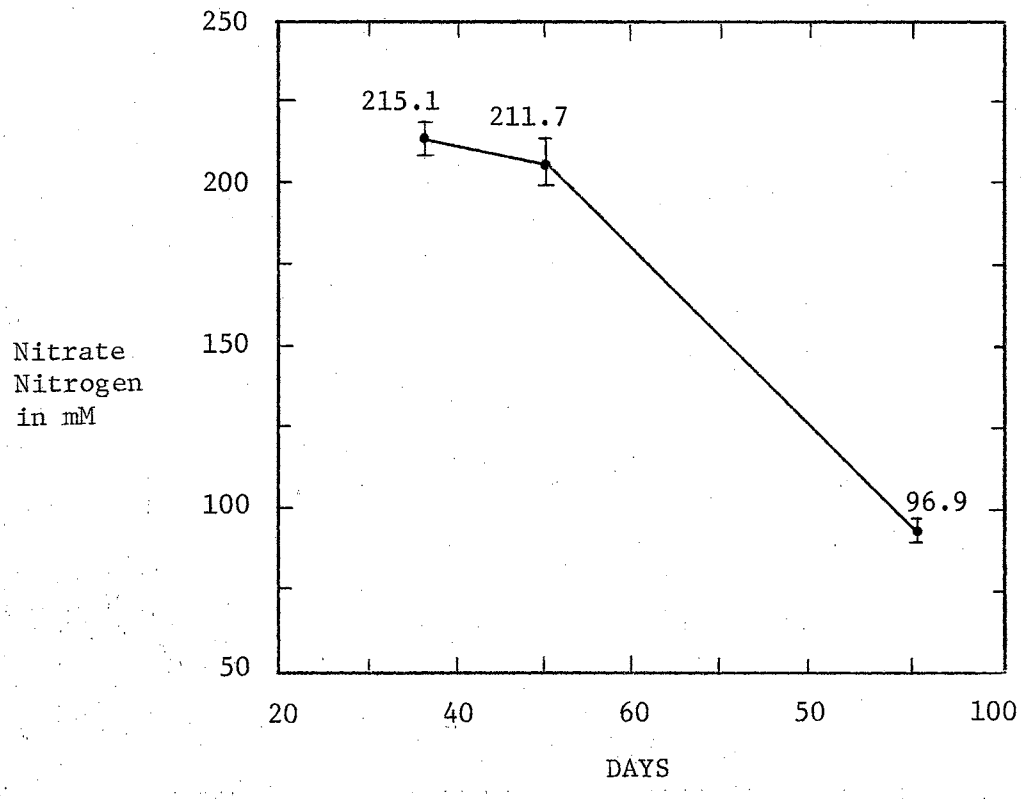

Figure 1. A comparison of the variation, due to age, in the nitrate nitrogen composition of the xylem sap of cotton. The sampling of the three treatments occurred at 35, 50, and 90 days respectively. The verical lines represent the standard error of thre treatment mean.

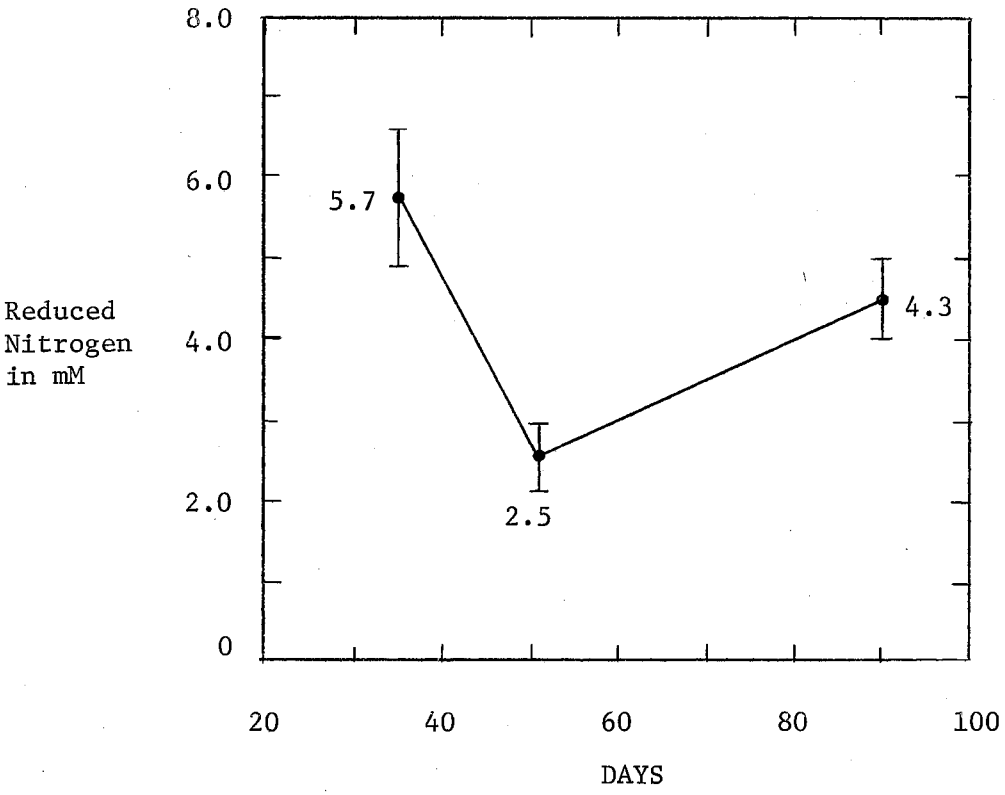

Figure 2. The behavior of the reduced nitrogen concentration in the xylem sap of cotton in relation to plant age. The vertical lines represent the standard error of the treatment mean.

TABLE I

THE AMINO ACID COMPOSITION OF THE XYLEM SAP OF COTTON UNDER NORMAL CONDITIONS AT 35, 50, AND 90 DAYS OF GROWTH

35 Days (Treatment 1)	50 Days (Treatment 2)	90 Days (Treatment 3)
Alanine	Alanine^{--}	----------
Asparagine	Asparagine^{-}	Asparagine^{+}
Aspartic Acid	Aspartic Acid	Aspartic Acid^{+}
γ-Aminobutyric Acid	γ-Aminobutyric Acid^{-}	γ-Aminobutyric Acid
Cystein	----------	Cystein^{--}
Glutamic Acid	Glutamic Acid^{-}	Glutamic Acid
Glutamine	Glutamine^{--}	Glutamine
Isoleucine	----------	----------
Ornithine	----------	----------
Serine	Serine^{--}	Serine
Threonine	----------	Threonine
Valine	----------	----------

\- Decrease in quantity

-- Extreme decrease in quantity

\+ Increase in quantity compared to the 35-day treatment

younger root systems. It seems likely, therefore, that even though the concentration of nitrate in the sap of 90-day-old plants is lower than that of the sap of 30-day-old plants, the total amount of nitrate taken up and transported may actually be greater in the older root system.

The results of plant age on the reduced nitrogen content of the sap are shown in Figure 2. It can be seen that reduced nitrogen content of xylem sap varied with plant age. There was a decrease from 5.7 mM reduced nitrogen in Treatment 1 to 2.5 mM in Treatment 2. From Treatment 2 to Treatment 3, there was an increase to 4.3 mM per liter. These variations in reduced nitrogen were statistically significant at the 5% level.

Avundzhyan's (4) results with Polygonum orientale L. were compared with my results. Avundzhyan's data can be found in Figure 3. He found that reduced nitrogen fluctuated in relation to plant age. It can be seen from his data that reduced nitrogen accumulated in the xylem sap to a concentration of about 9.3 mM during cotyledon formation, 8.5 mM during the vegetive stage, 10.9 mM at flower initiation, and was down to 1.8 mM during the aging process. He did not stipulate the length of time involved in the aging phase.

Figure 2 exhibits the behavior of the reduced nitrogen concentration of xylem sap in relation to plant age. The sap from Treatment 1 was collected during the formation of the second set of leaves. Treatment 1 would then be comparable in development to the values between A and B in Figure 3. The sap from Treatment 2, which was collected during the vegetive stage, showed a decline in reduced nitrogen similar to B in Figure 3. The sap from Treatment 3 was collected during the latter part of the flowering stage and would be comparable

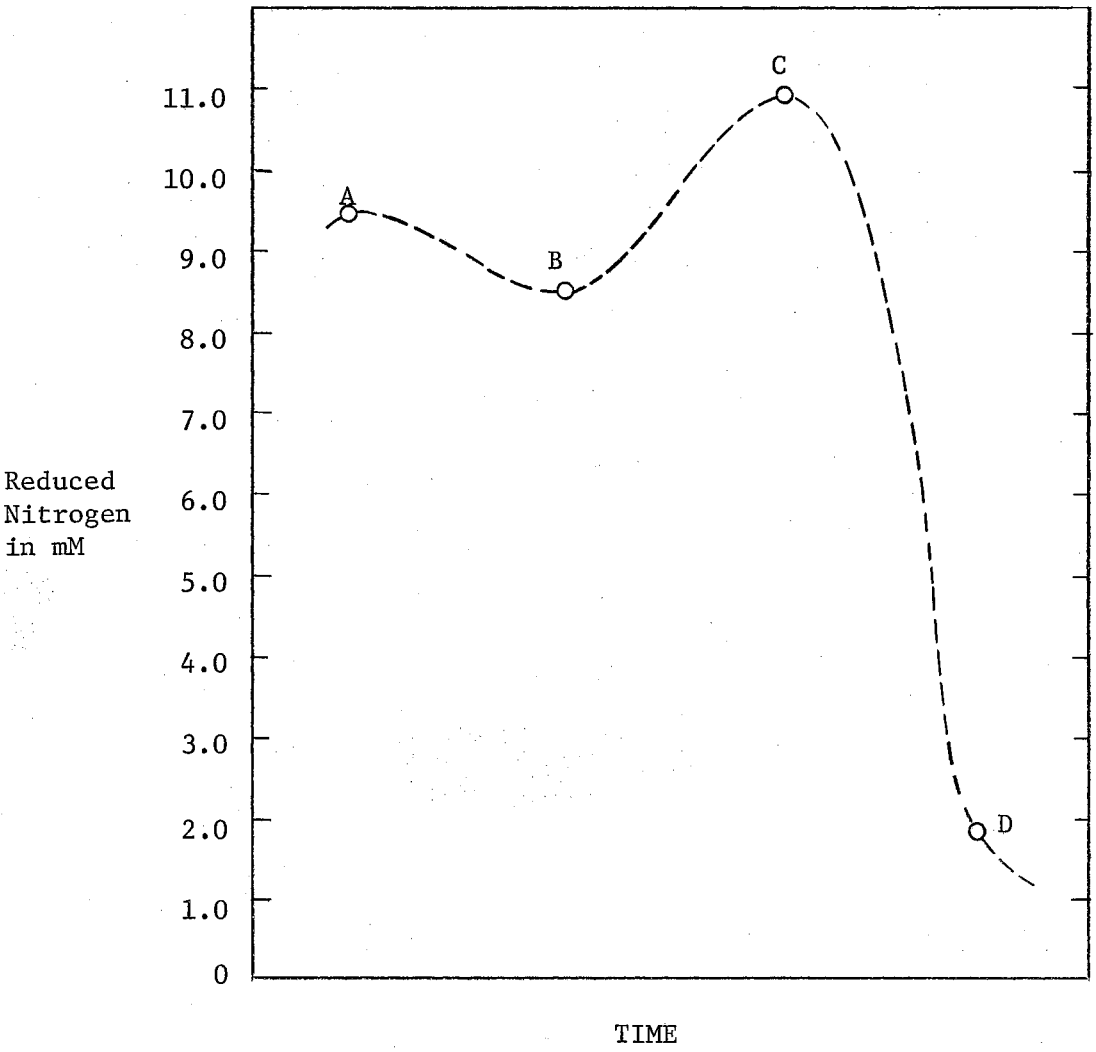

Figure 3. The pattern of behavior of the reduced nitrogen in the exuded sap of Polygonum orientale L. during ontogenesis by E. S. Avundzhyan.
A = cotyledon formation.
B = vegetive stage.
C = budding and flower initiation.
D = aging.

to the values between C and D in Avundzhyan's data. The correlation between Gossypium and Polygonum seems to be valid because the data from this experiment and the data from Avundzhyan's study are similar.

The results of this experiment also agree with the findings of Afridi and Hewitt (2). They sampled cauliflower plants to include all the leaves up to 15 weeks. The results showed that plants grown with nitrate demonstrated a rapid increase in reductase activity up to 5 weeks followed by little change until 10 weeks and then a marked decline. Possibly this variation of nitrate reductase activity with age found in leaves also occurred in cotton roots.

Table I presents the amino acids which were tentatively identified in each treatment. It should be noted that quantitation of these amino acids was by visual estimation and not photometric determination. The comparative quantity of total amino acids in all three treatments corresponded with the reduced nitrogen tests.

It is understandable that the quantity and quality of amino acids would be high during early seedling growth and cotyledon formation. The amino acids could very well be playing a major role in the ontogeny of the plant. The decrease in the reduced nitrogen content during the vegetive stage may not be so easily explained. It may be possible that the growth and development during this stage is not so rapid and varied as in earlier growth. It is well known that flowering is a drain on plant constituents. It is logical, therefore, for the greatest quantity of amino acids to occur during this stage of growth. This was shown in Avundzhyan's data. The decrease in reduced nitrogen during the aging process can also be explained. As the plant begins to age, and vital processes begin to slow down, it would be expected that the

translocation rate of raw material from the roots to the areas of utilization in the aerial portions would exhibit a corresponding decrease.

The results of this experiment indicate that the quantitative and qualitative composition of the amino acids delivered by the roots to the above-ground organs of the plant varies with plant age.

Experiment 2. Aeration and its effect on the xylem sap composition from excised cotton roots.

This portion of the present study was concerned with nitrogen metabolism under two conditions of aeration. This was of interest since in crop production much significance is ascribed to the aeration of the root growth zone, which to a large extent determines normal nutrition and development of the plant. Total anaerobiosis was not attempted as it was only planned to explore the changes in root nitrogen metabolism under low levels of oxygen availability. Such partial anaerobiosis could possibly occur in natural conditions such as in swampy or very compact soils or briefly after rains or irrigation.

The results of this experiment are presented in Table II. It can be seen that the nitrate content of the sap decreased under partial anaerobiosis, and the reduced nitrogen increased under the same conditions. These differences were found to be statistically significant at the 10% level.

Since nitrate was the only source of nitrogen available to these plants, it seems reasonable to assume that as the reduced nitrogen goes up, the concentration of nitrates should correspondingly decrease. My results agree with the findings of Grineva and Karimova (17). These investigators worked with maize roots. They found that nitrate

translocation rate of raw material from the roots to the areas of utilization in the aerial portions would exhibit a corresponding decrease.

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TABLE II

THE EFFECT OF AEROBIC AND ANAEROBIC ROOT CONDITIONS ON THE NITRATE NITROGEN AND REDUCED NITROGEN CONTENT OF THE XYLEM SAP OF COTTON

Composition	Aerated (Treatment 1)	Nonaerated (Treatment 2)
Nitrate Nitrogen	90.4 mM	30.1 mM
Reduced Nitrogen	2.6 mM	4.6 mM
Amino Acids	Alanine	Alanine^{++}
	Asparagine	----------
	Aspartic Acid	Apartic Acid
	γ-Aminobutyric Acid	γ-Aminobutyric Acid^{++}
	Glutamic Acid	Glutamic Acid^{+}
	Glutamine	Glutamine^{++}
	Glycine	Glycine^{+}
	Ornithine	Ornithine
	Serine	Serine^{-}
	Threonine	Threonine^{+}

\+ Increase in quantity

++ Extreme increase in quantity

\- Decrease in quantity

reduction was much more intensive under conditions of oxygen deficiency. They also found that nitrate utilization increased under anaerobic conditions. They postulated that nitrates may substitute for oxygen as the final hydrogen acceptor in terminal oxidation. Ohnishi (25) found that as electrons are transferred from a substrate to nitrate, there is a concomitant formation of ATP. Ohnishi was working with cell free extracts of Pseudomonas denitrificans. Thus, nitrates may be used as the terminal hydrogen acceptor in place of oxygen under anaerobic conditions. This may be one of the explanations for the decrease of nitrates in the xylem sap during periods of partial oxygen deficiency.

It was also interesting to note the changes in amino acid composition of the sap in these two treatments. From Table II it can be seen that almost all the amino acids made significant increases in concentration under partial anaerobiosis except asparagine which disappeared entirely. The amino acids which were found in much higher concentration than under aerobic conditions were alanine, γ-aminobutyric acid, and glutamine.

Dubinina (12) showed that under conditions of oxygen starvation, the over-all organic acid content in pumpkin roots was increased. She demonstrated that a considerable accumulation of ketoacids occurred in the roots, especially pyruvic, α-ketoglutaric, and, to some extent, glyoxalic acid. The accumulation of pyruvic acid indicates an increase of glycolysis by oxygen deficiency (Pasteur Effect). The orgin of α-ketoglutaric acid may be secondary as the initial stages of the Krebs Cycle would be slowed by anaerobic conditions. Dubinina feels that the excess of pyruvate formed under oxygen deficiency would remove NH_2 groups from glutamic acid and glutamine in the process of

transamination, causing an accumulation of alanine and free α-ketoglutaric acid.

If the nitrogen metabolism of pumpkin roots and cotton roots are similar, Dubinina's study can be used to explain some of the results of this experiment. The build-up of pyruvic acid under anaerobic conditions could lead to a build-up of alanine, since pyruvate is a precursor of alanine.

The accumulation of γ-aminobutyric acid, ornithine, glutamic acid, and glutamine probably can be directly related to an increased formation of α-ketoglutaric acid in oxygen-deficient roots. The amination of ketoacids proceeds quite efficiently under oxygen deficiency (12). However, their subsequent utilization in roots is probably weakened, and instead their translocation with the sap to the aerial portions may be increased. An explanation for this might be that less ATP would be available under anaerobic conditions for amino acid activation for protein synthesis and for other synthetic reactions in root growth. Thus, these amino acids and organic acids would be available for translocation to the aerial portions of the plant.

It is interesting to note that there is a correlation in the data of this experiment and recent studies made on root excretions. It has been found that plants under anaerobic conditions excrete more γ-aminobutyric acid, alanine, glutamic acid, and threonine into the nutrient media than aerated plants (16). A build-up of these amino acids in the sap and the nutrient media may be due to an increase of the permeability of root membranes.

No explanation can be offered for the decrease of serine under anaerobic conditions. Theoretically, it should have increased the same

as the other amino acids.

Another interesting result of this experiment was the complete disappearance of asparagine. Work has been done which has shown that 3.5 kcal/mole is necessary for amide bond formation and that ATP is indispensable in the biosynthetic reaction (8). The overall synthesis of asparagine, catalyzed by asparagine synthetase, may be summarized as:

$$\text{L-aspartate} + NH_3 + \text{ATP} \xrightarrow[\text{enzyme}]{Mg^{++}} \text{L-asparagine} + \text{ADP} + P_i$$

Thus, if oxygen is not present, terminal oxidation would not occur and no ATP would be produced. Therefore, there would be no energy to drive the above reaction. This could account for the disappearance of asparagine under anaerobic conditions. However, this would contradict the proposed mechanism for the utilization of nitrate under oxygen deficiency. It may be that the above explanation is correct for the disappearance of asparagine and that nitrate may not play a prominent role as a terminal hydrogen acceptor in cotton roots. It may be possible that the reduced concentration of nitrates under anaerobic conditions can be simply explained by the fact that energy in the form of ATP is not available for active absorption.

It is interesting to note that glutamine is synthesized by essentially the same reaction as asparagine but glutamine greatly increased in concentration under anaerobic conditions. Glutamic acid, which is the precursor of glutamine, increased appreciably during anaerobiosis. On the other hand, aspartic acid which is the precursor of asparagine changed very little during the two treatments. This difference in precursor concentration may aid in explaining the curious difference in amide concentration during oxygen deficiency.

Thus, precursor availability may contribute to the difference in amide concentration.

According to Hageman (19), corn grown under high nitrogen fertility accumulates appreciable quantities of amides. Bonner (8) quotes Prianischnikov as saying that amide formation may be a mechanism for detoxification of ammonia. If this is so, it may be another explanation for the build-up of glutamine in the present study. However, it would be in direct conflict with the disappearance of asparagine.

With intensified aeration of roots, the overall content of free amino acids was reduced. A possible explanation may lie in the fact that with a sufficient supply of oxygen, the growth of the roots could be intensified and that their use of amino acids for protein synthesis could be increased (12). This could be connected with the fact that activation of amino acids is required for protein synthesis.

It may be concluded that the aerobic metabolism in the roots of cotton is disturbed at low levels of oxygen, probably in the root-growth zone. There is also good evidence to assume that changes in oxygen metabolism can affect the absorptive activity of roots. The oxygen availability to the roots should, therefore, be considered as an important environmental factor governing the normal growth and development of a plant as a whole. Root aeration should be considered when any form of nitrogen analysis is attempted.

Experiment 3. The effect of root temperature on the nitrogen composition of xylem sap.

This experiment was set up to investigate the temperature dependence of nitrogen metabolism in cotton roots. Since the tissue

nitrate test is a common testing criterion to establish the nutritional status of a plant, it was thought to be important to establish environmental parameters which may affect results of such a test. Hageman has done much work in the area of the inducibility of nitrate reductase in leaf and stem tissue of cereal crops. He has shown that the nitrate reductase activity of the stem and leaf tissue shows a high degree of dependence on the concentration and content of the xylem sap (19, 20, 29). Other workers have also shown this dependence (1, 35). It is, therefore, very important to be able to interpret the results of the tissue nitrate test in relation to the environmental conditions preceding and during the time of testing.

The results of this experiment are found in Figure 4 and 5. Figure 4 demonstrates the gradual increase of nitrate nitrogen as the temperature increased from 20°C to 30°C. However, the increase from 129 mM of nitrate nitrogen at 20°C to 132 mM at 30°C was not statistically significant.

Figure 5 shows the change in the reduced nitrogen content of the xylem sap of cotton in relation to changes in root temperature. It can be seen that very little variation in the reduced nitrogen content occurred as a result of root temperature. The changes that did occur at 20, 25, and 30°C were found not to be statistically significant.

Table III illustrates those amino acids which were identified at the three different root temperature treatments. The observed quantitative differences correlated with the changes in reduced nitrogen.

In the survey of the literature, no information was found pertaining to root temperature and its effect on nitrate reductase. Most of

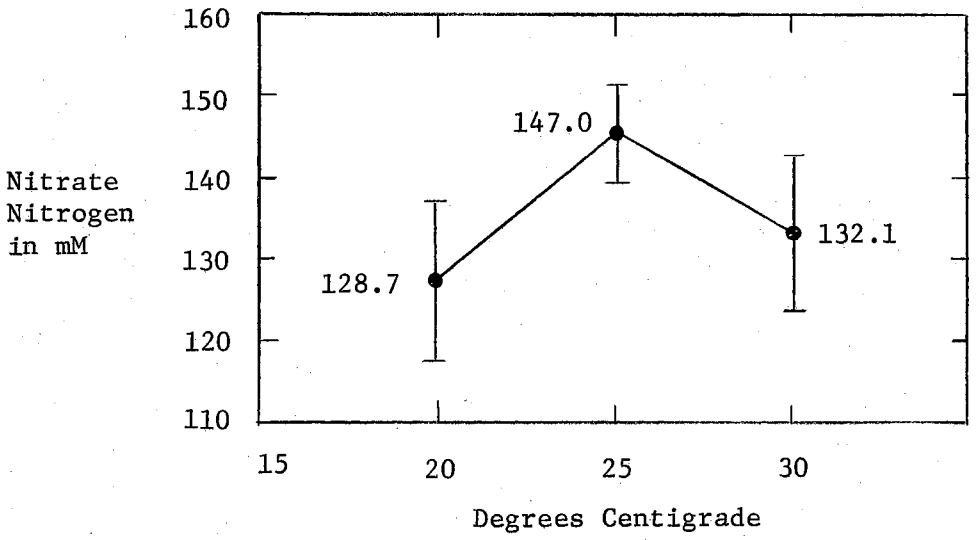

Figure 4. The behavior of the nitrate nitrogen content of the xylem sap of cotton in response to increases of root temperature. The vertical lines represent the standard error of the treatment mean.

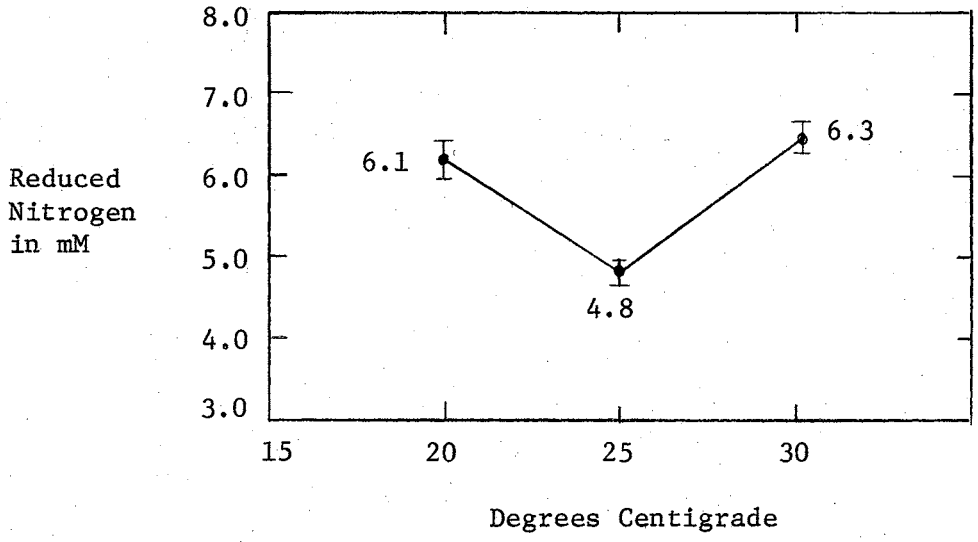

Figure 5. The reduced nitrogen content of the xylem sap of cotton in relation to increase in root temperature. The vertical lines represent the standard error of the treatment mean.

TABLE III

THE EFFECT OF 20°, 25°, AND 30°C ROOT TEMPERATURES ON THE AMINO ACID COMPOSITION OF THE XYLEM SAP OF COTTON

20°C (Treatment 1)	25°C (Treatment 2)	30°C (Treatment 3)
Alanine	Alanine^{-}	Alanine
Asparagine	Asparagine^{-}	Asparagine
Aspartic Acid	Aspartic Acid^{-}	Aspartic Acid
γ-Aminobutyric Acid	γ-Aminobutyric Acid^{--}	γ-Aminobutyric Acid
Cystein	----------	Cystein
Glutamic Acid	Glutamic Acid^{-}	Glutamic Acid
Glutamine	Glutamine^{-}	Glutamine
Glycine	----------	Glycine
Ornithine	Ornithine^{-}	Ornithine
Serine	Serine^{-}	Serine
Threonine	Threonine	Threonine
Isoleucine	----------	Isoleucine
Valine	----------	Valine
----------	----------	Histidine

$^{-}$ Decrease in quantity

$^{--}$ Extreme decrease in quantity

the work has been done on the aerial portions of the plant. Such a work was done by Beevers et al. (5). They found that induction of nitrate reductase is temperature dependent, and a part of this dependence is based on the increase in nitrate content of the tissue with increases in temperature during induction. Maximum induction temperatures were 31°C and 38°C for radish cotyledons and corn seedlings respectively.

The data from this experiment indicate that soil temperature should be condsidered as a minor variable in evaluating tissue nitrate tests.

Experiment 4. The effect of photoperiod on nitrogen composition of xylem sap.

Quality, quantity, and periodicity of light have exhibited marked effects on nitrate reductase activity in leaves. However, little work has been done with the photoperiod and its effect on nitrate reductase activity in the roots. Much of the work done with light quality and light quantity has explored an indirect link between nitrogen and carbohydrate metabolism and CO_2 fixation (20). This experiment was an attempt to determine effects of day-length on nitrate content and amino acid composition of xylem sap.

The data for the photoperiod experiment are shown in Table IV. As the day-length was increased from 8 hours to 16 hours, the nitrate content of the sap decreased from 116.6 mM to 79.5 mM. The reduced nitrogen also showed a corresponding decrease with increasing day-length. The reduced nitrogen content of the sap decreased from 3.0 mM during the 8 hour day-length to 1.2 mM during the 16 hour

TABLE IV

THE EFFECT OF THE PHOTOPERIOD ON THE TRANSPORT FORM OF NITROGEN IN THE XYLEM SAP OF COTTON

Composition	8 Hour Day (Treatment 1)	16 Hour Day (Treatment 2)
Nitrate Nitrogen	116.6 mM	79.5 mM
Reduced Nitrogen	3.0 mM	1.2 mM
Amino Acids	Alanine	----------
	Asparagine	Asparagine^{-}
	Aspartic Acid	Aspartic Acid
	γ-Aminobutyric Acid	γ-Aminobutyric Acid^{--}
	Glutamic Acid	Glutamic Acid^{--}
	Glutamine	----------
	Isoleucine	----------
	Ornithine	----------
	Serine	----------
	Threonine	Threonine^{--}
	Valine	----------

\- Decrease in quantity

-- Extreme decrease in quantity

day-length. It can also be seen that the amino acids showed a corresponding decrease with increasing day-length. All differences due to day-length were found to be statistically significant at the 5% level.

These findings were partially substantiated by Harper and Paulsen (21) in their work with light influences on nitrogen reduction and assimilation in wheat. These workers found that more nitrate accumulated in blade and root tissue of plants grown under 8 hour light periods than those grown under 16 hour light periods. This is in agreement with the findings of this study. However, they found that nitrate reductase activity in root tissue did not differ between photoperiods. They found no relationship between photperiod and protein content in the root tissue of wheat. My results, on the other hand, exhibited a very distinct decrease in reduced nitrogen content of the sap with an increase of the photoperiod. Thus, indirectly via protein synthesis, I may have demonstrated a link between photoperiod and potential protein content in the root tissue of cotton. Low concentrations of amino acids could very well be a result of very active protein synthesis.

Harper and Paulsen (21) found a decreased amount of root growth under short-day conditions. This observation was made in the present study also. It would seem that if the reduced nitrogen produced in the roots of plants grown under short photoperiods is not being utilized in root growth, it would be transported to the aerial portions or excreted into the nutrient media.

The reason why Harper and Paulson's data differed from my data may be the fact that the source of nitrogen supplied in their study

was ammonia, while nitrate was the nitrogen source supplied in this experiment. Beside the possibility of there being a difference in root activity in cotton and wheat, there may be a definite difference in the physiological reaction of roots to different nitrogen sources. I feel that nitrate reductase activity would be hard to detect if all the nitrogen supplied were already reduced.

Harper and Paulsen (21) postulated that the accumulation of nitrate in the roots of plants grown under an 8 hour light period, indicated that light was more closely associated with nitrate translocation than with absorption. In the present study, it was found that there is also an increase of nitrates in the xylem sap of cotton under an 8 hour light period. Perhaps short days decreased the amount of sugar transported to the roots. This could have a number of effects. First, there would be less NADH and NADPH available for NO_3 and NO_2 reduction. This would certainly cause an accumulation in the roots and possibly an increase in transport of nitrates. Also, there would be less ATP available for amino acid activation for protein synthesis and for other synthetic reactions for root growth. This could account for the increased reduced nitrogen content of the sap. In any event, it is clear that the photoperiod is a variable which must be considered when making any analysis of plant nitrogen metabolism.

CHAPTER IV

CONCLUSIONS AND SUMMARY

The purpose of this investigation was to study the effects of plant age, root temperature, root aeration, and day-length on root nitrogen metabolism as exemplified by the ratio of reduced to nitrate nitrogen found in the exuded sap of excised cotton roots.

Nitrates decreased with age, while the reduced nitrogen fluctuated with age. The ratios of reduced nitrogen to nitrate nitrogen at 35, 50, and 90 days were 1:38, 1:85, and 1:23 respectively. These data suggest that, although the ratio of nitrate to reduced nitrogen varies with age, most of the nitrogen in the xylem sap is in the form of nitrate regardless of age.

Root aeration proved to be an important external factor. The nitrate content decreased, while the reduced nitrogen increased with root anaerobiosis. The ratio of reduced nitrogen to nitrate nitrogen was 1:35 under aerobic conditions but dropped to 1:7 under anaerobiosis. Changes in amino acid composition were also detected. Almost all the identified amino acids increased during anaerobiosis except asparagine, which disappeared completely. The availability of oxygen for root metabolism is obviously a very important environmental consideration when dealing with tissue nitrate tests.

Root temperature over a range of 20°C to 30°C proved to be of minor importance. The ratios of reduced nitrogen to nitrate nitrogen

remained fairly constant; 1:21 at 20°C, 1:31 at 25°C, and 1:21 at 30°C. It was concluded, therefore, that cotton roots have a wide range of temperature tolerance before significant changes in the ratio of nitrate to reduced nitrogen in xylem sap occur.

Photoperiod also had marked effects on root nitrogen metabolism. Nitrate and reduced nitrogen decreased with increasing day-length. There appeared to be some effect on nitrate absorption which was reflected in the amount of reduced nitrogen recovered. The ratios of reduced nitrogen to nitrate nitrogen during short-day conditions was 1:39. Under long-day conditions the ratio was 1:66. It would appear that, when working with plant nitrogen metabolism, the photoperiod should be considered an important environmental variable.

The variation in nitrate and reduced nitrogen content of sap in the four experiments must be explained by the variation in age, photoperiods, and in intervals between nutrient renewal and collection. Of particular importance is the variation in the interval of nutrient renewal and collection time among the four different experiments. Tserling et al. (32) demonstrated this in their work on oats. They noted differences in protein synthesis according to the period in which new nutrient was applied. Evidently, age of the plant has an important effect on the synthetic activity of plant tissues; the younger the plant, the more intensive the formation of complex protein molecules. They noted these differences in three different exposures: at 4 hours, 24 hours, and 100 hours after renewing the nutrient solution.

Unfortunately these data were not called to the attention of this investigator until after the collection procedures had been completed. The variation among the different experiments can be attributed to the

unequal intervals between the time of collection and nutrient renewal. The intervals varied from 12 to 24 hours. The variation in age and photoperiods must also have contributed to the lack of correlation among the four different experiments, though to a minor extent. It can be seen from this observation that time of fertilization is yet another variable to be considered when dealing with plant nitrogen metabolism.

In conclusion, it can be seen that the nitrogen metabolism of cotten roots is extremely sensitive to changes in external and internal factors. The ratio of nitrate to reduced nitrogen varied greatly with photoperiod, root aeration, and day-length; but it varied much less with root temperature. Despite these variations, nitrate was found in all cases to be the major form of nitrogen in xylem sap, thus indicating that nitrate is the dominant form of nitrogen transported from roots to leaves in the cotton plant.

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