THE RELATIONSHIP BETWEEN TIME AND RATE OF NITROGEN FERTILIZATION AND NITRATE-NITROGEN ACCUMULATION IN COTTON PETIOLES

By

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I. INTRODUCTION

Much of the time and effort of those engaged in soil fertility and plant nutrition research are directed toward the development and improvement of diagnostic techniques. Originally, researchers emphasized chemical analyses of the soil as a means of diagnosing nutrient deficiencies. However, in the past few decades interest has been generated in the use of different types of plant analyses as a supplement and complement to soil analyses.

The study was initiated to determine the feasibility of using petiole analyses as a means of determining the nitrogen fertilization requirements of cotton. Cotton is non-determinate in its fruiting habit and presents a rather unique problem when grown in areas conducive to large amounts of vegetative growth. A relatively large amount of nitrogen is required by the plants during the peak period of growth, prior to boll-setting, while a much lower level is desirable during the latter part of the growing season. Unnecessary or excessive applications of nitrogen in mid- or late-season may cause rank growth and lodging. An accurate plant analysis (petiole) should enable one to determine the nitrogen status of the plant throughout the growing season.

Nitrate-nitrogen accumulates in various parts of the cotton plant. The amount accumulated is related to the stage of growth of the plant and the amount of nitrogen being supplied to the plant. The objective of the study was to determine whether or not a relationship exists between the level of nitrates found in the plant at a given stage of development and the yield of that plant. If a relationship does exist, then the nitrate level of the cotton petiole at various stages of growth can be used as an index to the nitrogen fertilization needs. The results of this research are presented in this thesis.

II. LITERATURE REVIEW

Plant analyses is becoming increasingly popular among agriculturalists. Until recently, nutrient deficiencies were determined primarily by the chemical analyses of the soil. However, during the past few decades interest has increased in the use of plant analysis as a means of diagnosing nutrient deficiencies.

In 1804, De Saussure (13)[1] found that the composition of the ash of a plant varied with the soil upon which the plant grew, with the age of the plant, and with the part of the plant analyzed. Liebig (10) stated that nutrients removed from the soil by plants should be replaced through fertilization. Perhaps Hall (4) was the first to envision plant analyses as a means of diagnosing nutrient deficiencies.

Hoffer (6) discovered the chemical "quick test" of the inner cornstalk as an aid in diagnosing nitrogen and potassium deficiencies. Thorton et al. (17), Carolus (1), Hester (5), Scarseth (14) and Krantz (9) further developed this "quick test". They made it much more precise and quantitative. Simultaneous development of more accurate laboratory tests by Macy (12), Thorton (17), Ulrich (19) and several others further enhanced the interest in plant analyses. These more accurate laboratory tests led to the study of the interrelationships of the nutrient supplying power of the soil, the nutrient content of

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[1] Figures in parenthesis refer to Literature Cited.

the plant, and yields. Ulrich (20) stated that the successful use of plant analyses depends on the reliability of the critical nutrient levels established by the study of these relationships.

Work toward the development of plant analyses was very much intensified between 1930 and 1940. Many researchers were attempting to determine the "critical nutrient level" for various crops and nutrients. Several were interested in determining the nitrogen status of crops by the use of plant analyses. Nitrate-nitrogen accumulates in many plants. The amount accumulated is related to the amount supplied to the plant by the growth medium. In 1934, Emmert (2) found that the nitrogen status of the tomato plant could be determined by testing the plant tissue. Later he established that tomato plants should contain a minimum of 1,000 ppm. nitrogen, but not over 1,500 ppm. nitrogen during the early growing season. He also reported that as the season progressed, the nitrogen content should decrease to approximately 500 ppm. He stated that higher levels of nitrogen in the tissue are likely to cause vegetative tendencies which will be harmful to yield while lower levels of nitrogen in the tissue indicate nitrogen deficiencies which will also reduce the yields (3).

As the result of an extensive study, the possibility of using tissue tests on cotton was proposed by Joham (7) in 1950. He found the main stem petiole near the apex of the plant was best suited for analyses. These petioles were highest in nitrate-nitrogen throughout the season and were influenced to a greater degree than other plant parts by the amount of nitrogen supplied to the plant. Joham stated that the amount of nitrate-nitrogen accumulated is also influenced by the level of other nutrients in the soil since an interrelationship of nutrients exists. For instance, a high nitrate level in the plant tissue might be due to a deficiency of phosphorus. Joham also reported that the "critical concentration" of nitrate-nitrogen in cotton is 0.03% (based on fresh weight) for the samples collected at the 90 day growth stage.

From 1954-1960, MacKenzie and his associates (11) conducted studies of the seasonal nitrate-nitrogen content of cotton petioles as affected by nitrogen application and its relationship to yield of irrigated cotton. Results from these studies indicated that the nitrates were highest during the early stages of development. Levels of up to 18,000 ppm. were found in the early stages of development. During the latter part of the growing season, the nitrate level declined to less than 2,000 ppm. The levels never declined to less than 1,000 ppm. They also stated that a pronounced increase in the nitrate-nitrogen content of petioles occurred when nitrogen was applied to nitrogen deficient plants. The nitrate level of petioles was affected by varieties and soil moisture, but the effect was slight compared to the effect brought about by a change in the nitrogen supplied to the plant. MacKenzie et al. concluded that the concentration of nitrate in the cotton petioles during the growing season was directly related to the rate of nitrogen supplied to the plants. They further reported that under the conditions in California, petiole nitrate-nitrogen levels of 16,000, 8,000, and 2,000 ppm. during the early-, mid-, and late-bloom stages of growth, respectively, were adequate for a high level of production. Yield reductions were related to the length of time petiole nitrate-nitrogen fell below minimum levels of 2,000 ppm.

Tucker et al. (18) concluded that petiole analysis could be used successfully to supplement the soil test for determining the nitrogen needs of cotton grown in Arizona. According to these researchers, petioles should be collected at two-week intervals starting at the first-square stage of growth. Other conclusions of their studies were similar to those of MacKenzie et al. The desirable levels of nitrate-nitrogen were 15,000 to 18,000 ppm. at the first-square stage, 12,000 to 14,000 ppm. at the first-flower stage, 6,000 to 10,000 ppm. at the first-boll stage and 4,000 ppm. when the bolls began to open. Excessive amounts of nitrate-nitrogen induced excessive vegetative growth, delayed fruiting, caused lodging, and reduced yields.

III. METHODS AND MATERIALS

Field Plots

Field plots were located at the Irrigation Research Station, Altus, Oklahoma and the Cotton Research Station, Chickasha, Oklahoma. The respective soil types on which experiments were located are the Hollister and Tillman clay loam complex[2] and the McLain silt loam[3].

The statistical design used was a randomized complete block with three replications. Plots were six rows wide and 60 feet long. Three rates of nitrogen (40, 80 and 160 pounds per acre) were applied at two different stages of growth. These treatments were compared to a check plot which received no nitrogen. During the 1963 growing season, nitrogen sidedressing applications were made at the 8-leaf stage of growth (June 17) and two weeks following the appearance of the first squares (July 10). During the 1964 growing season, nitrogen applications were made at planting time (May 8) and at the 8-leaf stage of growth (June 23) at Altus while at Chickasha applications were made at the 4-leaf stage (May 25) and the 8-leaf stage of growth (June 22).

Urea was the source of nitrogen used at Altus while ammonium nitrate was the source used at Chickasha. Austin was the variety of

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[2] Oklahoma Agricultural Experiment Station Process Series P-454.

[3] Oklahoma Agricultural Experiment Station Process Series P-314.

cotton used at Altus and Deltapine Smoothleaf was used at Chickasha. The cotton was cultivated and irrigated as the need occurred at both locations. Adequate phosphorus and potassium were available to the plants. Insects were controlled with frequent applications of insecticides as the need occurred.

Sampling

Petioles were collected at two week intervals starting at the 8-leaf stage of growth (June 17) during 1963 and at the first square stage (July 15 at Altus and July 6 at Chickasha) during 1964. Approximately 25 petioles were chosen at random from the second and fifth row of each plot giving a total of two samples per plot for each sampling date. The most recent, fully matured petioles were selected for analyses. Cotton lint yields were measured by hand harvesting 50 foot of the third and fourth rows of each plot.

Analyses of Plant Material

The petioles were dried at 80 degrees centigrade for 24 hours immediately after sampling. Samples were then ground in a Wiley mill to pass through a 40-mesh sieve. The nitrate-nitrogen content of the petioles was determined by the phenoldisulfonic acid method described by Johnson and Ulrich (8).

Analyses of Data

An analysis of variance and coefficient of variation were made for all data (yield and nitrate-nitrogen content of petioles), using procedures suggested by Steele and Torrie (15).

IV. RESULTS AND DISCUSSION

Altus - 1963

Yield of lint - Yield increases from nitrogen applications were unusually large at Altus during 1963 (Table I). Nitrogen applied at the 8-leaf stage of growth (June 17) was apparently more efficiently utilized than nitrogen applied at the mid-square stage of growth (July 10). The yield of lint was increased from 570 pounds per acre on the check plots to 1163 pounds per acre on the plots which received 160 pounds of nitrogen per acre at the 8-leaf stage. A yield of only 864 pounds per acre was obtained when the 160 pounds of nitrogen was applied at the mid-square stage of growth.

Nitrate-nitrogen level - The nitrate-nitrogen content of the cotton petioles was affected by the rates and times of nitrogen application as well as by the stages of growth sampled. In general, the nitrate-nitrogen (nitrogen in the nitrate form) level increased with increasing rates of nitrogen applied. However, the level of nitrates decreased for any given rate of nitrogen as the season progressed (Figure 1).

Petiole samples were first collected on July 2, at approximately the time the first squares were appearing. This was two weeks after the nitrogen was applied. The nitrate levels were not significantly different at the 5% level at this stage of growth. The nitrate-nitrogen

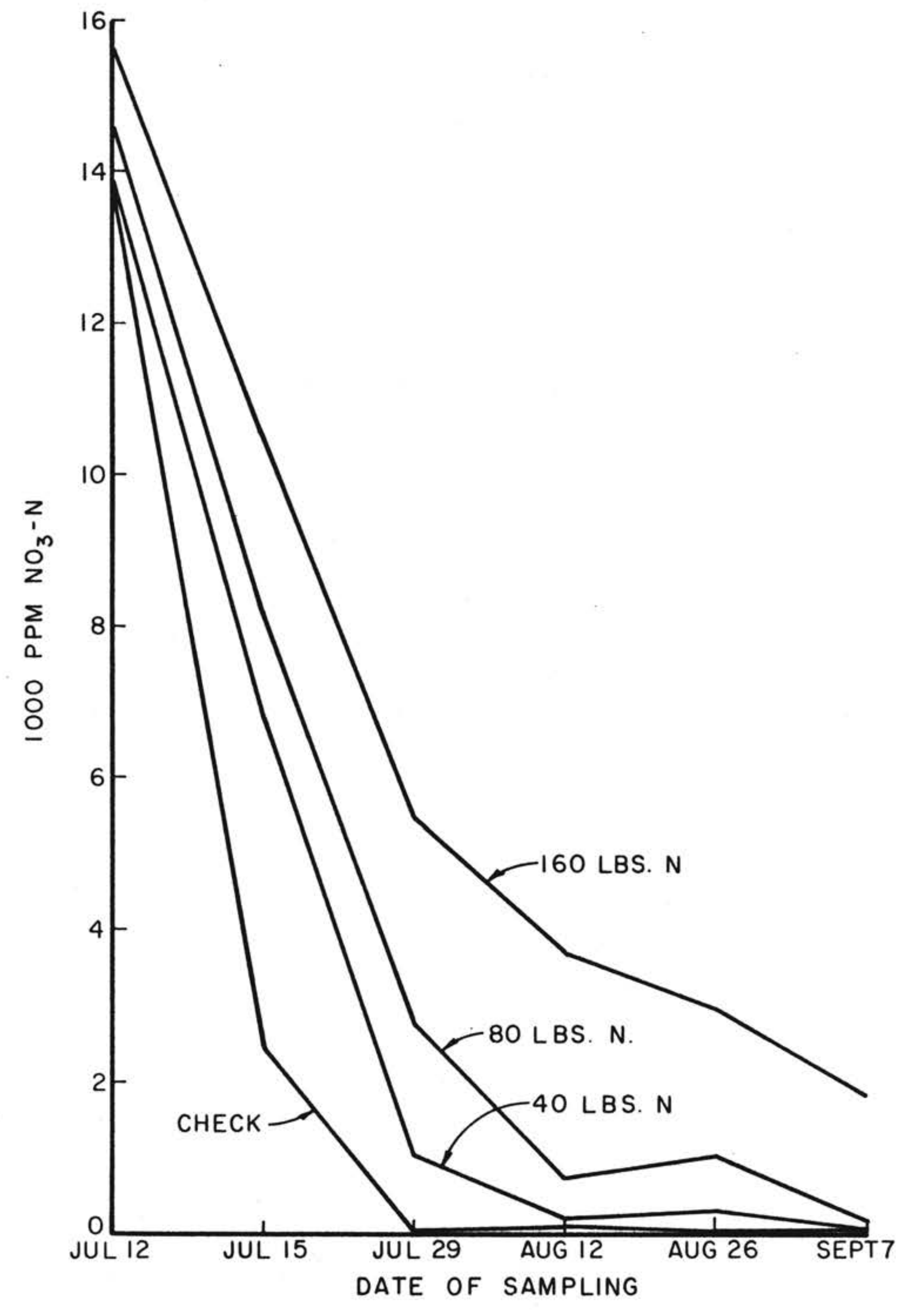

Figure 1. The Effect of Nitrogen Applied at the 8-Leaf Stage of Development (June 17) on the Nitrate-Nitrogen Content of Cotton Petioles. Altus, 1963.

TABLE I

THE EFFECT OF RATE AND TIME OF APPLICATION OF NITROGEN ON THE YIELD OF COTTON ALTUS, OKLAHOMA, 1963

Rate of Nitrogen Applied (Pounds per Acre)	Yield (Pounds of Lint per Acre)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	570	570
40	718	780
80	843	826
160	1163	864

Calculated F Value = 47.63

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 5.48%

levels (Appendix Table V and Figure 1) ranged from 13,570 ppm. (micrograms of nitrate-nitrogen per gram of dry plant material) on the check plots to 15,678 ppm. with the application of 160 pounds of nitrogen applied at the 8-leaf stage of growth. Since the nitrate-nitrogen levels of the petioles were not materially affected by the rates of applied nitrogen, the relationship between yields of cotton and the nitrate-nitrogen content of petioles was not established at this stage of growth (Figure 2). Thus, the optimum nitrogen rate could not be determined at this stage of growth.

Petiole samples were collected the second time on July 15 at approximately the mid-square stage of growth. The effects of nitrogen on the nitrate level of the petioles was large at this stage of growth

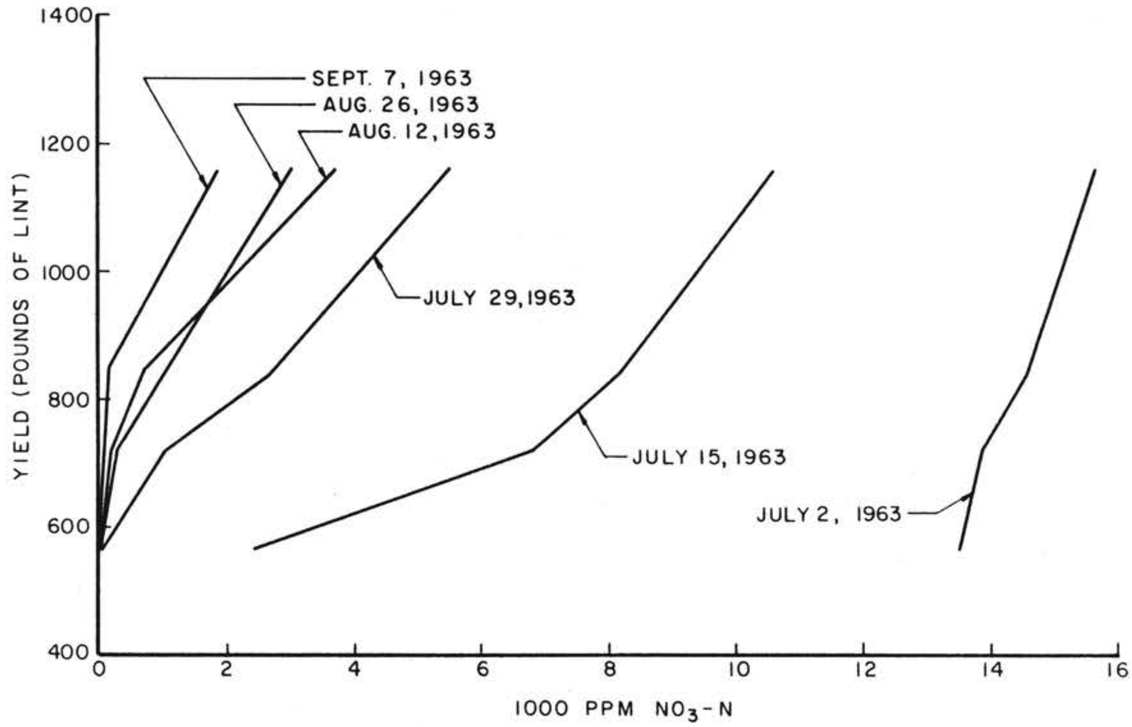

Figure 2. Yields of Lint Versus Nitrate-Nitrogen Levels as Affected by Nitrogen Applied at the 8-Leaf Stage of Development. Altus, 1963.

(Appendix Table V and Figure 1). The level of nitrates increased from 2,453 ppm. on the check plots to 10,850 ppm. for the treatment of 160 pounds of nitrogen applied at the 8-leaf stage of growth. The fact that both yields and nitrate accumulations were materially affected by nitrogen would indicate that a relationship exists between these two variables. The slope of the line representing the response of yield of cotton to rates of nitrogen is very nearly the same as the slope representing the response of nitrate accumulation to rates of nitrogen (Figure 3). Therefore, as the nitrate level of the petioles increased, the yield of cotton also increased (Figure 4).

Petiole samples were again collected July 29. The nitrate levels were somewhat less than at the above stage of growth for any given rate of nitrogen application. However, the level accumulated was greatly influenced by the rate of nitrogen applied. The level of nitrate-nitrogen increased from 48 ppm. on check plots to 5,493 parts per million on plots which received 160 pounds of nitrogen at the 8-leaf stage of growth (Appendix Table V and Figure 1). The same relationship existed at this date of sampling that existed at the previous sampling date. The magnitude of nitrate-nitrogen response was slightly less than at the previous sampling, but sufficiently large for diagnostic purposes.

On July 29 samples were collected for the first time from those plots which received their nitrogen at the mid-square stage of growth. The nitrate content of the petioles collected from these plots was relatively low. The check plots had 48 ppm. nitrate-nitrogen while those which received 160 pounds of nitrogen per acre had 1,110 ppm. (Appendix Table V and Figure 4).

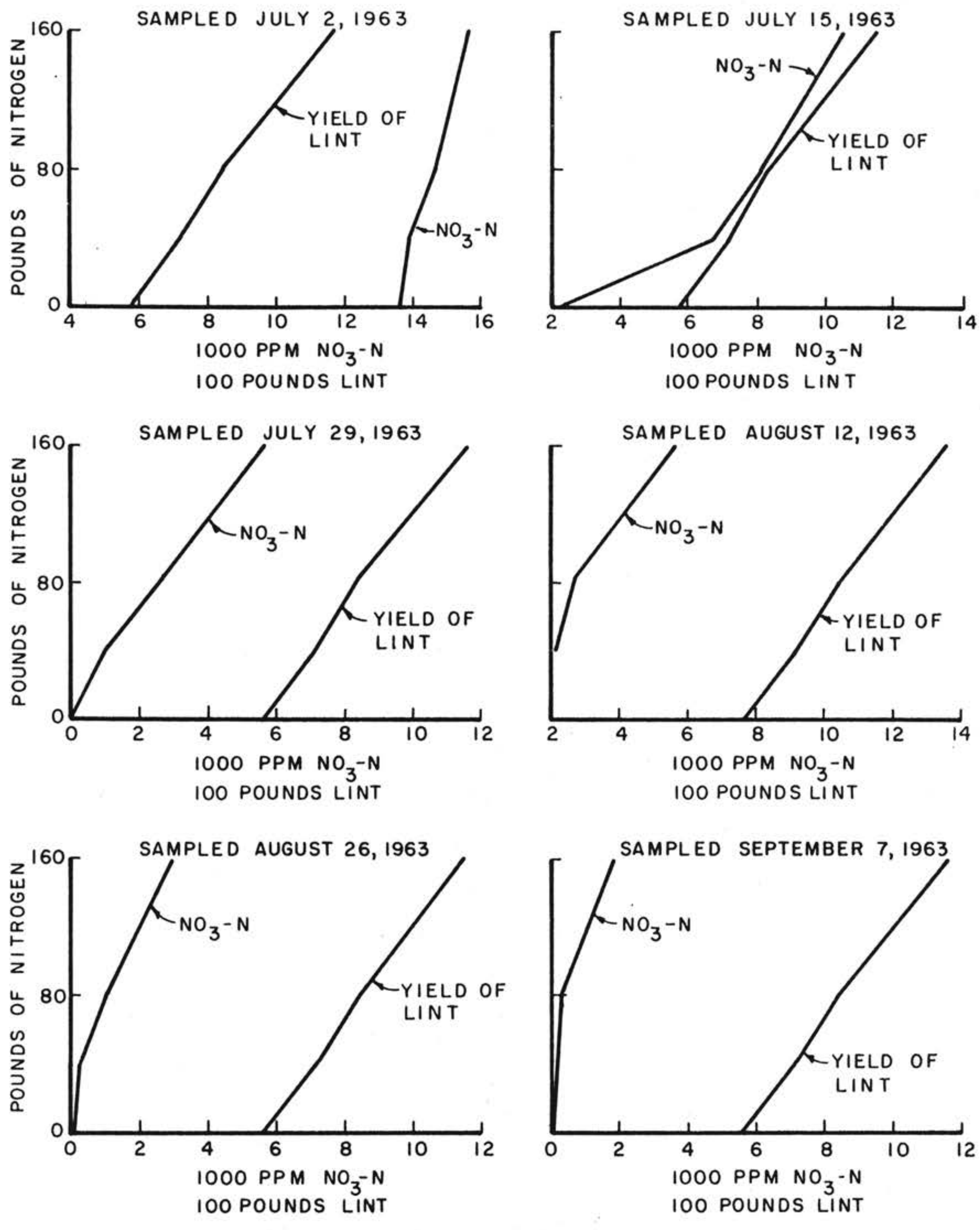

Figure 3. The Relationship Between the Nitrate-Nitrogen Response Curves and the Yield of Lint Response Curves to Nitrogen Applied at the 8-Leaf Stage of Development at Different Sampling Dates. Altus, 1963.

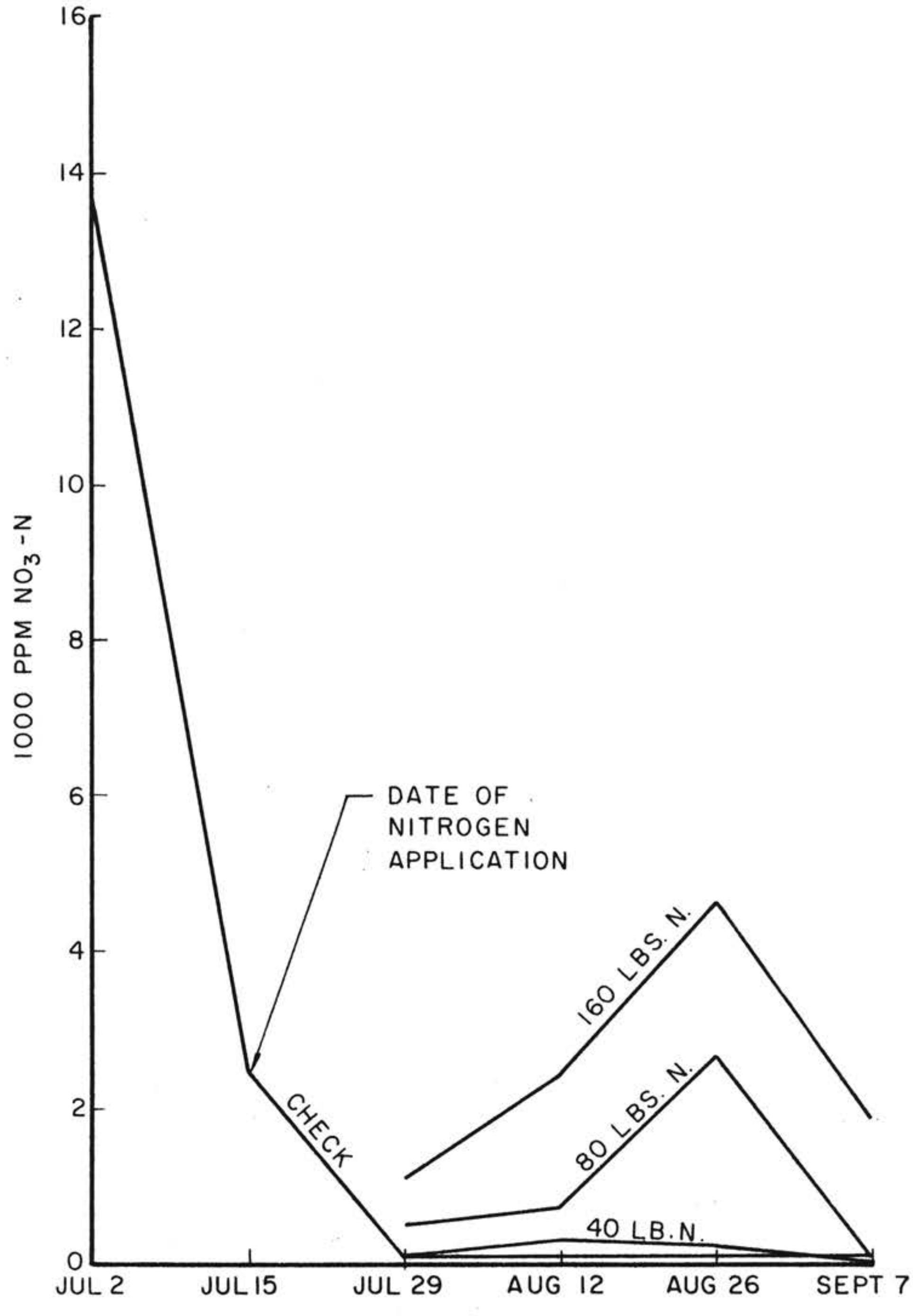

Figure 4. The Effect of Nitrogen Applied at the Mid-Square Stage of Development (July 10) on the Nitrate-Nitrogen Content of Cotton Petioles. Altus, 1963.

Samples were collected August 12, August 26, and September 7 in addition to the above sampling dates. The level of nitrate accumulation (Appendix Table V and Figures 1 and 4) was much lower at these dates than at previous sampling dates for any given rate of nitrogen applied at the 8-leaf stage of growth. However, the nitrate level increased for any given rate of nitrogen applied at the mid-square stage of growth (Figure 4). The nitrate level increased as the rate of nitrogen applied increased regardless of the time of application.

The highest levels of nitrates accumulated were found in the samples collected July 2, July 15, and July 29. These values decreased rapidly as the season further progressed. The degree of response was also greatest at the July 15 and July 29 dates of sampling. In general, the coefficient of variation increased as the season progressed. The sampling dates best suited for diagnoses were July 15 and July 29. These dates represented the early-square and mid-square stages of growth. The relationship between yields and the level of nitrates accumulated at the various stages of growth are illustrated in Figures 2 and 3.

Chickasha - 1963

Yield of lint - The yield of lint was not substantially affected by rates of nitrogen on the experimental sites at Chickasha during 1963 (Table II). The lack of a nitrogen response was probably due to the high nitrogen supplying power of the soil. The lowest yield, 1292 pounds per acre, was obtained from plots which received 160 pounds of nitrogen at the 8-leaf stage of growth (June 17). The highest yield,

TABLE II

THE EFFECT OF RATE AND TIME OF APPLICATION OF NITROGEN ON THE YIELD OF COTTON. CHICKASHA, OKLAHOMA, 1963.

Rate of Nitrogen Applied (Pounds per Acre)	Yield (Pounds of Lint per Acre)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	1356	1356
40	1319	1354
80	1330	1395
160	1292	1298

Calculated F Value = 0.41

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 7.3%

1,395 pounds of lint per acre, was obtained from 80 pounds of nitrogen applied at the mid-square stage of development (July 10). Yields were not significantly different at the 5% level.

Nitrate-nitrogen level - Contrary to the effects on lint yields, nitrate-nitrogen concentrations were affected by nitrogen at all but the first date of sampling. The same trends that were observed at Altus during 1963 were also found at Chickasha. However, the magnitude of nitrate concentration was much larger at Chickasha.

Petiole samples collected on July 3 were very high in their nitrate content. Petioles on check plots contained 17,058 ppm. while the petioles of plants which received 160 pounds of nitrogen at the 8-leaf stage of growth had 19,051 ppm. of nitrate-nitrogen. The influence of nitrogen applied on nitrate accumulation are reported in Appendix Table VI and Figure 5. Since the yields of lint were not materially affected by nitrogen, the relationship between nitrate concentrations and yields of lint were not established.

Petiole samples were again collected at the early- to mid-square stage of growth (July 16). Concentration of nitrates was slightly less at this sampling date than at the previous date of sampling, however, the effects of nitrogen applications on the nitrate content was still slight (Appendix Table VI). Check treatments had 13,820 ppm. nitrate-nitrogen and the 160 pound nitrogen treatments contained 16,953 ppm. nitrate-nitrogen.

The nitrate level of plants had further decreased at the next sampling date, July 30. Samples were also collected for the first time from plots fertilized at the mid-square stage of development (July 30). Results are reported in Appendix Table VI and Figures 5 and 6. The yields of lint and the nitrate-nitrogen content of the petioles were similar for a given rate of nitrogen at both dates of nitrogen application. The nitrate concentration increased from 7,812 ppm. on check treatments to 13,942 ppm. on 160 pound nitrogen treatments applied at the 8-leaf stage. A concentration 14,930 ppm. was found on the plots which received 160 pounds of nitrogen applied at the mid-square stage of growth.

The last three dates of sampling were August 13, August 27, and September 8, 1963. Results obtained from these dates of sampling are also reported in Appendix Table VI and Figures 5 and 6. The nitrate-nitrogen concentration differences were much larger at these latter

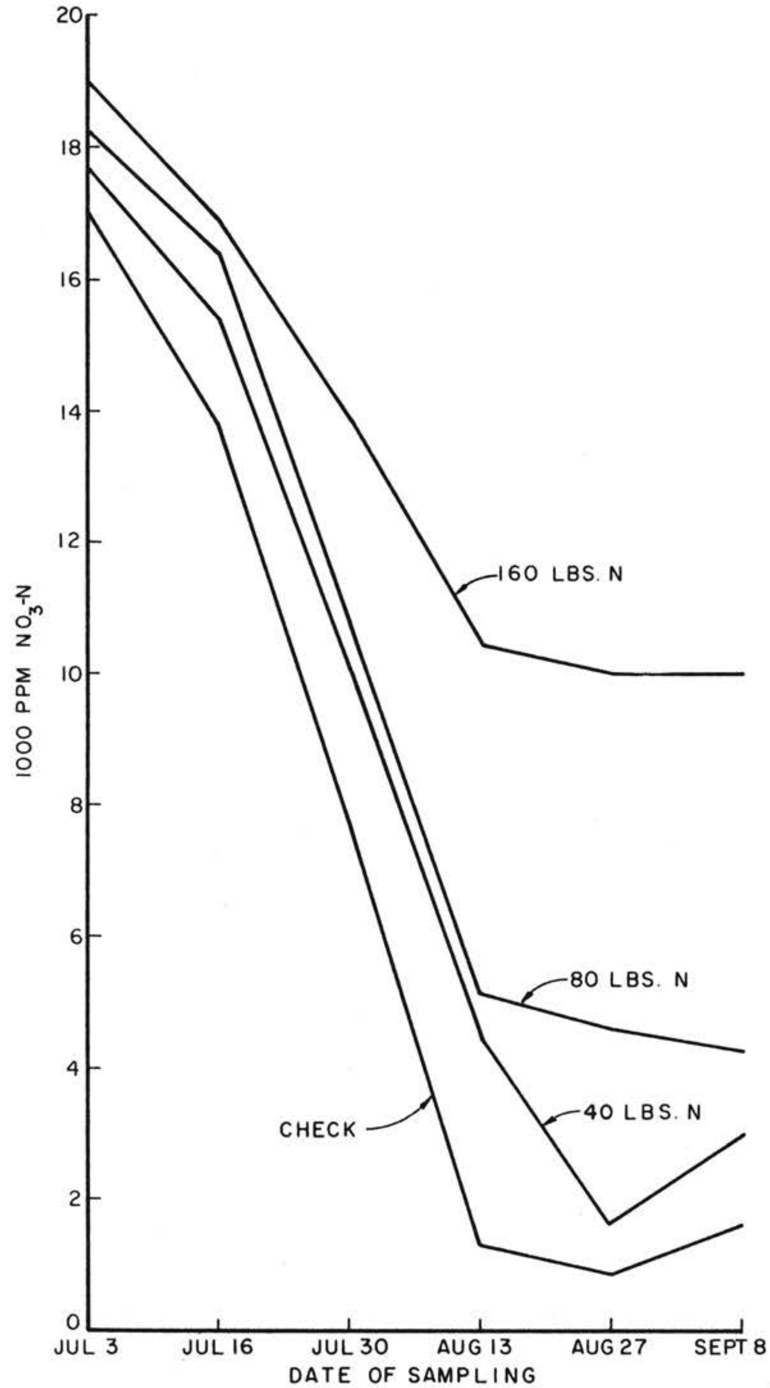

Figure 5. The Effect of Nitrogen Applied at the 8-Leaf Stage of Development (June 17) on the Nitrate-Nitrogen Content of Cotton Petioles. Chickasha, 1963.

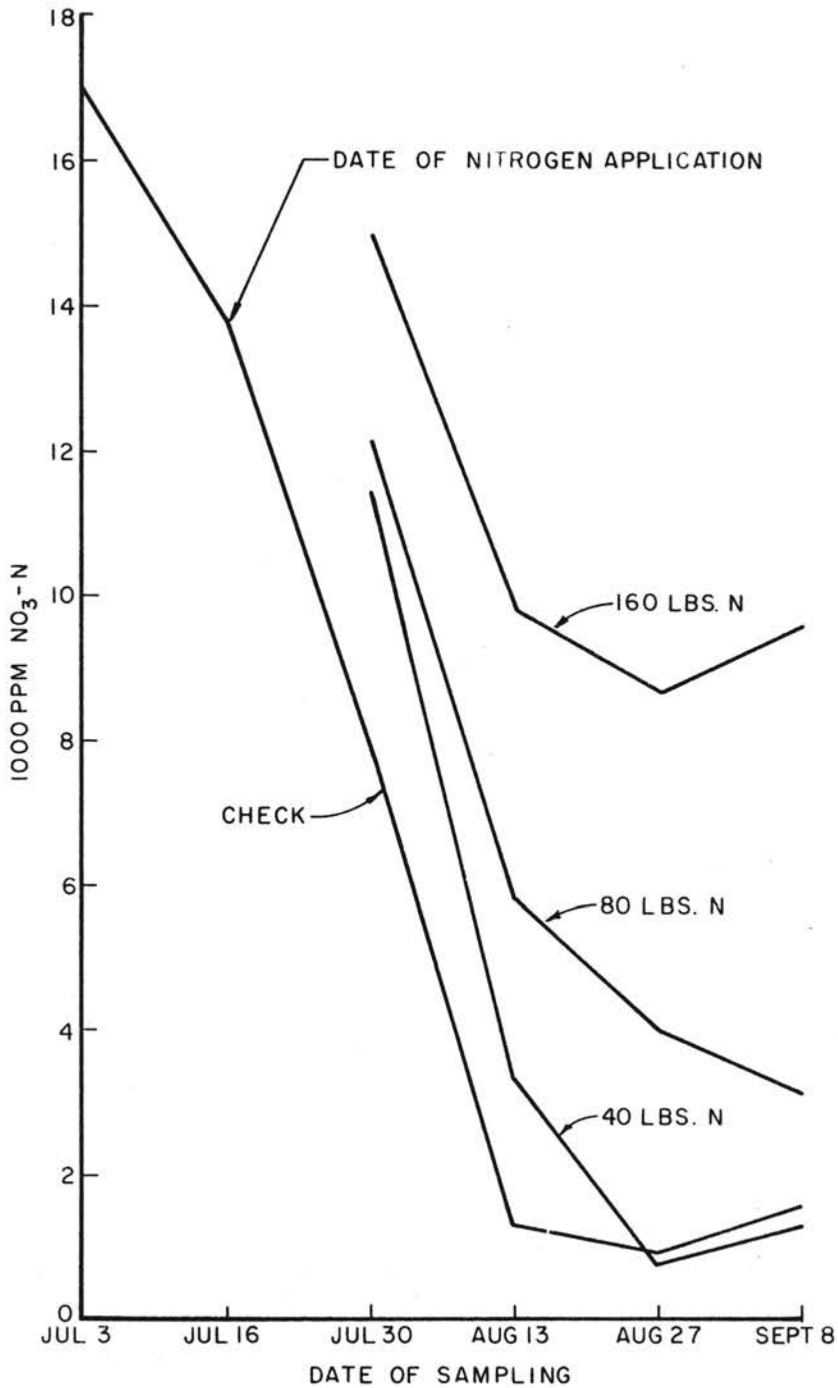

Figure 6. The Effect of Nitrogen Applied at the Mid-Square Stage of Development (July 10) on the Nitrate-Nitrogen Content of Cotton Petioles. Chickasha, 1963.

dates of samplings than at previous sampling dates. However, the total amount of nitrates was much lower than at earlier stages.

The effect of nitrogen applied at the 8-leaf stage on the nitrate-nitrogen content of the cotton petioles at the various stages of growth for both the Altus and Chickasha locations is represented graphically in Figure 7. The curve representing the check treatment at Chickasha lies just above the curve representing the 160 pound nitrogen application at Altus. Since the 160 pound rate was optimum at Altus and the check treatment was optimum at Chickasha, it may be assumed that the nitrate levels obtained from these two treatments approximate the optimum nitrate levels at the different stages of growth. Factors other than nitrogen which affect nitrate accumulation are disregarded for the above assumptions.

1964 Results

A change was made in the time of nitrogen fertilization. Instead of the mid-square nitrogen application used in 1963, which was found to be less efficient than the earlier application, an application was made at planting time at Altus and at the 4-leaf stage at Chickasha. The application at the 8-leaf stage was unchanged.

During 1964, plant samples were collected at only those stages of growth indicated most feasible by data from the previous year.

Altus - 1964

Yield of lint - The yields of lint were much lower than those obtained in 1963 for any given nitrogen treatment. Evidently, factors

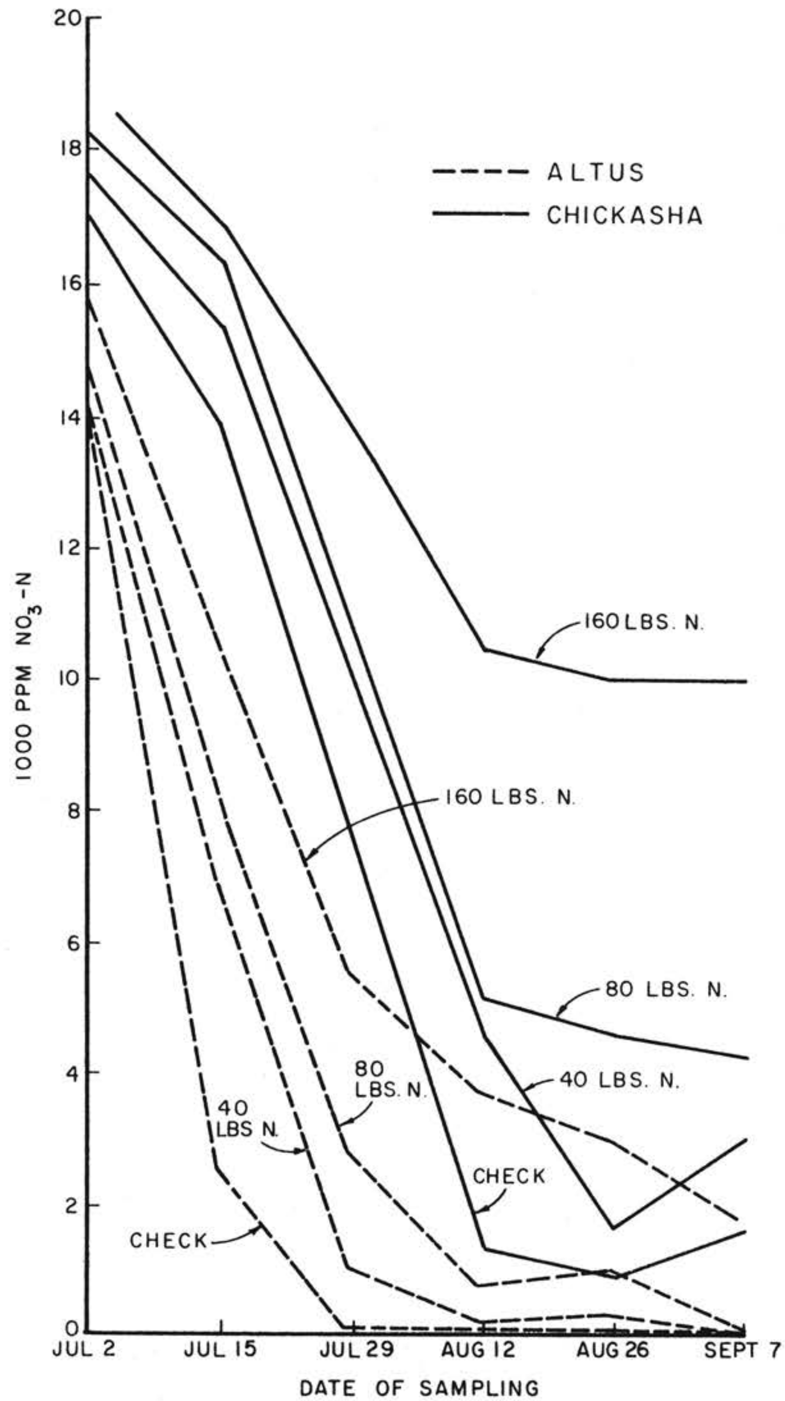

Figure 7. The Effect of Nitrogen Applied at the 8-Leaf Stage of Development (June 17) on the Nitrate-Nitrogen Content of Cotton Petioles. Altus and Chickasha, 1963.

other than nitrogen limited yields to these low levels because the response to nitrogen was not significant at the 5% level. The lowest yield obtained was 440 pounds per acre while the highest obtained was 556 pounds per acre (Table III). Though yields varied, the effect of nitrogen applications was not consistent. Since yields of lint were not materially increased with nitrogen additions, it is assumed that the soil supplied ample nitrogen for the yield levels obtained.

TABLE III

THE EFFECT OF RATE AND TIME OF APPLICATION OF NITROGEN ON THE YIELD OF COTTON. ALTUS, OKLAHOMA, 1964.

Rate of Nitrogen Applied (Pounds per Acre)	Yield (Pounds of Lint per Acre)	
	Fertilized at planting	Fertilized at 8-leaf stage
0	440	440
40	566	478
80	504	508
160	519	531

Calculated F Value = 1.54

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 11.0%

<u>Nitrate-nitrogen level</u> - Petiole samples were collected July 15, July 30, and August 20 at the first-square, mid-square, and first-boll stages of growth, respectively.

The nitrate levels varied little as a result of the time of nitrogen application. Apparently the applied nitrogen is utilized with equal efficiency when applied at or prior to the 8-leaf stage of growth. However, applications made as late as the mid-square stage may result in a reduction in efficiency as evidenced by the data from the previous year.

The nitrate levels in the cotton petioles were very similar to those obtained in 1963. The nitrate levels are listed by treatment and stage of growth in Appendix Table VII and illustrated in Figure 8. When graphic representations of the two years data are superimposed, it is found that the nitrate-nitrogen response curves almost coincide for any given rate of nitrogen applied at the 8-leaf stage of growth. The effect of the stage of growth at the time of sampling was also very similar during both 1963 and 1964. Due to the similarity of results, comments made for 1963 are applicable to 1964 and are not repeated. However, since the yield of lint was unaffected by nitrogen, the relationship between the yield of lint and nitrate-nitrogen levels was not as predictable as it was in 1963.

Chickasha - 1964

Yield of lint - The yield of lint was not consistently affected by any rate of nitrogen. The effects of treatments on yields were not statistically different at the 5% level. Yields ranged from 1,312 pounds per acre on the check treatment to 1,570 pounds per acre on the 80 pound nitrogen treatment applied at the 4-leaf stage of growth (Table IV). These yields were substantially higher than ordinarily obtained at this location. Yet, nitrogen still did not greatly enhance production of lint.

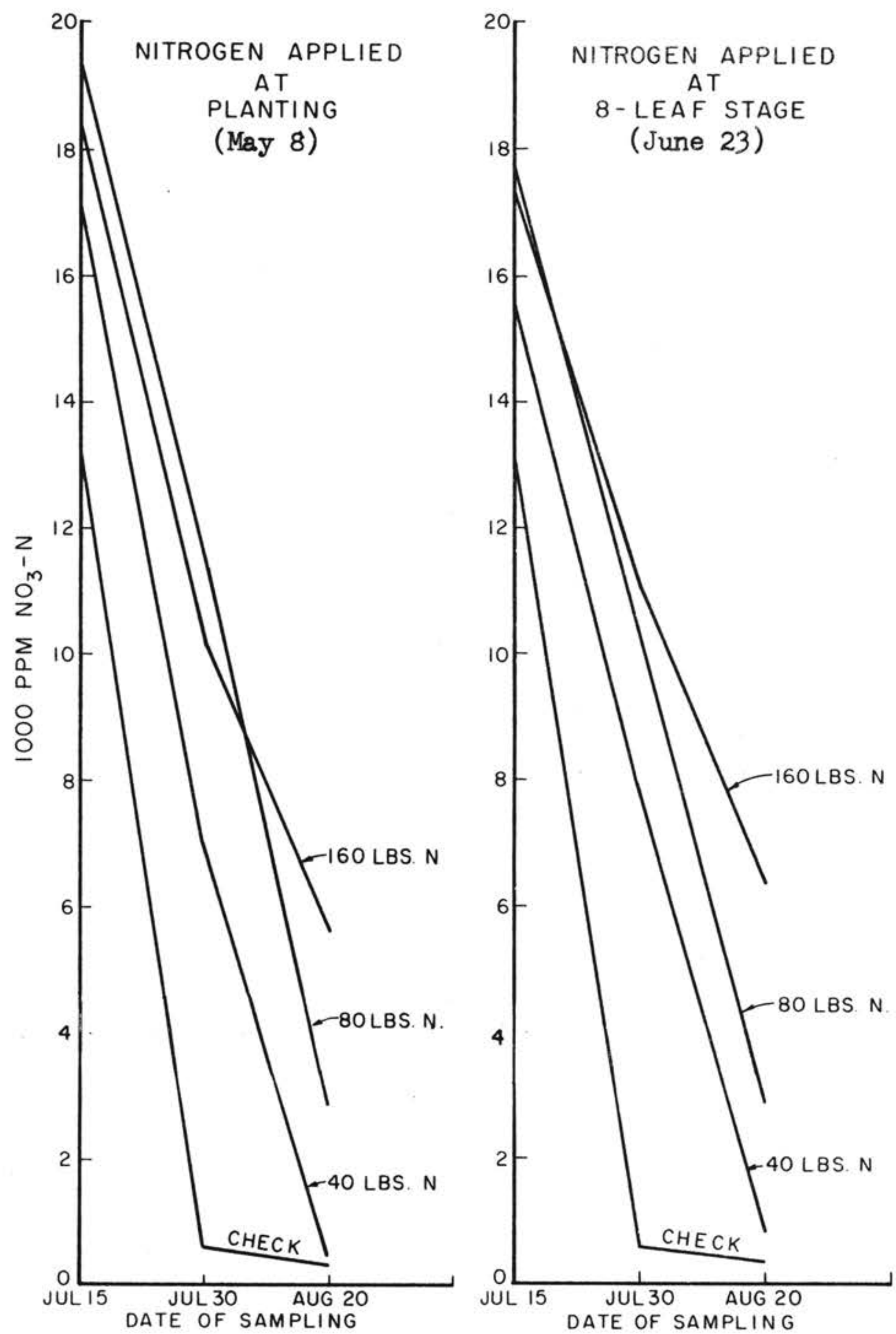

Figure 8. The Effect of Nitrogen Applications on the Nitrate-Nitrogen Content of Cotton Petioles. Altus, 1964.

TABLE IV

THE EFFECT OF RATE AND TIME OF APPLICATION OF NITROGEN ON THE YIELD OF COTTON. CHICKASHA, OKLAHOMA, 1964.

Rate of Nitrogen Applied (Pounds per Acre)	Yield (Pounds of Lint per Acre)	
	Fertilized at 4-leaf stage	Fertilized at 8-leaf stage
0	1,312	1,312
40	1,523	1,479
80	1,570	1,418
160	1,457	1,529

Calculated F Value = 1.34

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 8.7%

Nitrate-nitrogen level - Petiole samples were collected at 4 stages of growth. The dates of collection were July 6, July 20, August 4, and August 20. The respective stages of growth were the 12-leaf stage, first-square stage, mid-square stage, and the first-boll stage. Again, those samples collected at the mid-square and first-boll stages of growth were affected to the largest degree by the soil nitrogen level regardless of when the nitrogen was applied. The response magnitude on July 20 was much larger than at the previous sampling dates. Samples collected from check plots contained 11,939 ppm. nitrate-nitrogen and those taken from plots which received 160 pounds of nitrogen at the 4-leaf stage had 16,765 ppm. However, plants collected at the mid-square stage of development (August 4) contained 3,126 ppm. on the check treatments and 12,627 ppm. when treated with 160 pounds of nitrogen applied at the 4-leaf stage. Plants collected at the last sampling date, the first-boll stage had only 1,604 ppm. on the check plots and 10,414 ppm. on those which received 160 pounds of nitrogen at the 4-leaf stage. Results are reported in Appendix Table VIII and Figure 9. The date of nitrogen application had little effect on the nitrate concentrations accumulated in the cotton petioles.

Other trends obtained from the 1964 data were very similar to those observed in 1963. Therefore, those statements made about 1963 data are applicable to the 1964 Chickasha data and are not repeated.

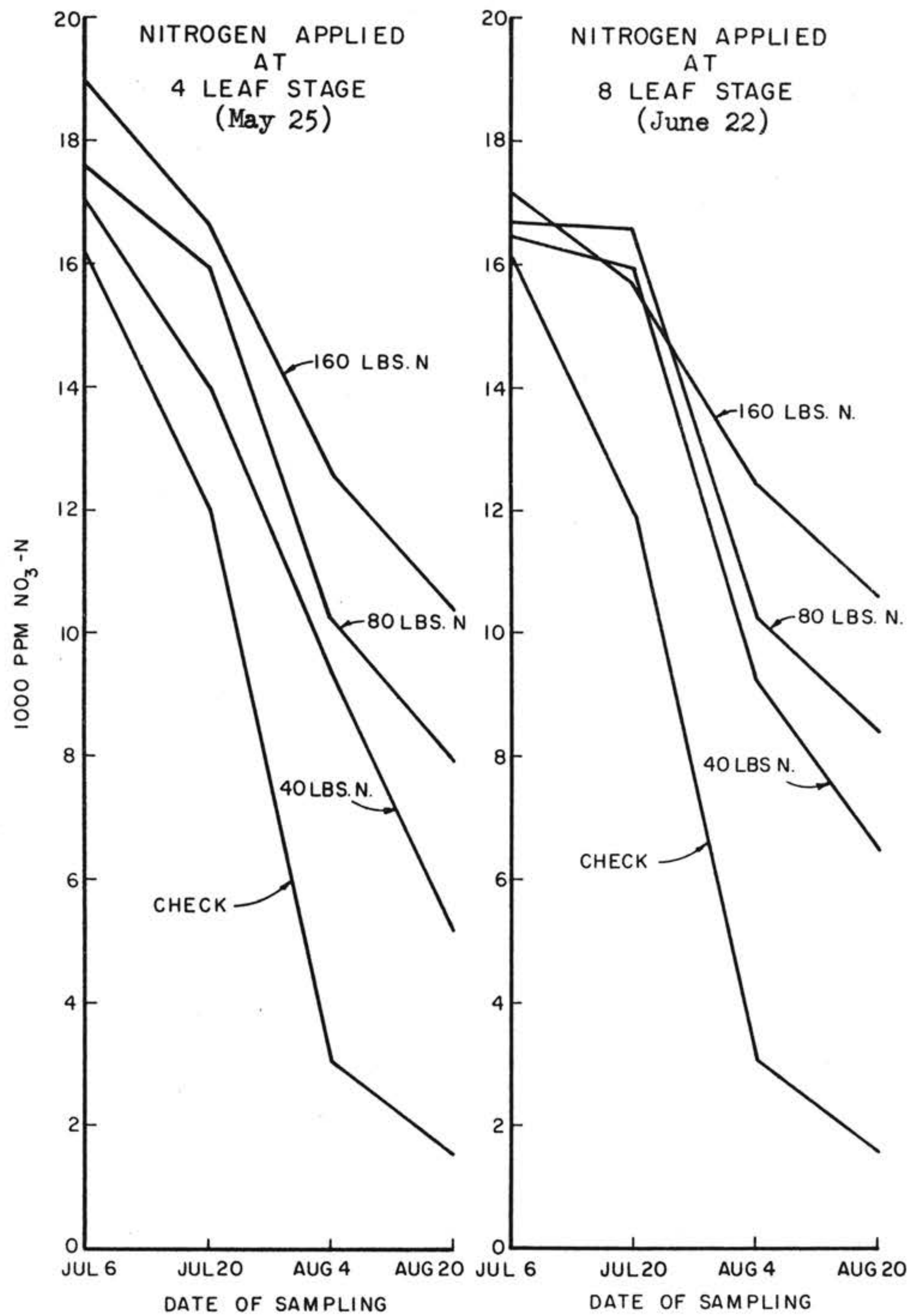

Figure 9. The Effect of Nitrogen Applications on the Nitrate-Nitrogen Content of Cotton Petioles. Chickasha, 1964.

V. SUMMARY AND CONCLUSIONS

A study of the feasibility of using the nitrate-nitrogen content of cotton petioles as an index to the nitrogen fertilization needs of cotton was made. Experiments were conducted on irrigated cotton at Chickasha and Altus in 1963 and 1964. The youngest fully-matured petiole was collected from several plants to compose a sample. Samples were collected at regular intervals throughout the growing season. These samples were analyzed for water-soluble nitrates in the laboratory.

The data consistently indicated that the concentration of nitrates in the petioles, at a given date, was directly related to the level of nitrogen applied to the soil. The nitrate levels also declined as the growing season progressed. The magnitude of nitrate-nitrogen response was greatest at the mid-square to first-bloom stages of growth. A relationship between yield of lint and nitrate concentrations was found to exist where yield was influenced by nitrogen. This relationship was most predictable with samples collected at the mid-square to first-bloom stages of growth (July 15 and July 29 at Altus, 1963) because the magnitude of treatment effects were greatest at these stages. Data suggest that nitrogen may be assumed to be adequate if nitrate-nitrogen levels of 4,000 to 6,000 ppm. were found in petioles at these stages of growth and does not fall below 1,500 to 2,000 ppm. at any time during the growing season (based on data collected from Altus, 1963).

The effects of stage of growth on the concentration of nitrates accumulated were much larger than the effects of the level of soil nitrogen. Therefore, it is extremely important, for the proper interpretation of the analyses, that the exact stage of growth at the time of sampling be accurately defined. Also, the stages of growth that best depicted the nitrogen status of the crop occurred rather late in the growing season (mid-square to first-bloom stages). Nitrogen is utilized more efficiently on cotton if it is applied prior to the 8-leaf stage of growth. Therefore, it was impossible to diagnose a deficiency on the basis of petiole analysis in time to apply nitrogen which would be used with the highest efficiency. If the physiological stage of growth can be defined accurately within narrow limits, however, petiole analyses would be useful in determining the rate of nitrogen that should be applied to a subsequent crop of cotton.

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APPENDIX

TABLE V

THE EFFECT OF RATES AND TIME OF APPLICATION OF NITROGEN ON THE NITRATE-NITROGEN CONTENT OF COTTON PETIOLES. ALTUS, OKLAHOMA, 1963.

Date Sampled - July 2

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	13,570	Samples were not collected
40	13,877	from these plots until
80	14,643	after the nitrogen was
160	15,678	applied.

Calculated F Value = 1.05

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 11.0%

Date Sampled - July 15

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	2,453	Samples were not collected
40	6,804	from these plots until
80	8,240	after the nitrogen was
160	10,580	applied.

Calculated F Value = 9.18

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 31.3%

Table V Continued

Date Sampled - July 29

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	48	48
40	1,032	62
80	2,772	526
160	5,493	1,110

Calculated F Value = 8.04

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 75.8%

Date Sampled - August 12

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	100	100
40	201	311
80	710	670
160	3,744	2,433

Calculated F Value = 2.85

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 122%

Table V Continued

Date Sampled - August 26

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	41	41
40	279	220
80	995	2,687
160	2,992	4,749

Calculated F Value = 6.10

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 74%

Date Sampled - September 7

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	56	56
40	38	9
80	143	210
160	1,806	1,877

Calculated F Value = 4.70

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 116%

TABLE VI

THE EFFECT OF RATES AND TIME OF APPLICATION OF NITROGEN ON THE NITRATE-NITROGEN CONTENT OF COTTON PETIOLES. CHICKASHA, OKLAHOMA, 1963.

Date Sampled - July 3

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	17,058	Samples were not collected
40	17,786	from these plots until
80	18,361	after the nitrogen was
160	19,051	applied.

Calculated F Value = 1.47

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 6.7%

Date Sampled - July 16

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million)	
	Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	13,820	Samples were not collected
40	15,456	from these plots until
80	16,406	after the nitrogen was
160	16,953	applied.

Calculated F Value = 6.96

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 5.8%

Table VI Continued

Date Sampled - July 30

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	7,812	7,812
40	10,084	11,429
80	10,720	12,221
160	13,942	14,930

Calculated F Value = 1.46

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 30%

Date Sampled - August 13

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	1,343	1,343
40	4,474	3,434
80	5,166	5,802
160	10,431	9,883

Calculated F Value = 16.22

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 25%

Table VI Continued

Date Sampled - August 27

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-square stage
0	917	917
40	1,662	802
80	4,629	4,026
160	10,009	8,749

Calculated F Value = 34.87

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 25%

Date Sampled - September 8

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 8-leaf stage	Fertilized at mid-stage stage
0	1,607	1,607
40	3,050	1,295
80	4,250	3,156
160	10,047	9,698

Calculated F Value = 18.29

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 32%

TABLE VII

THE EFFECT OF RATES AND TIME OF APPLICATION OF NITROGEN ON THE NITRATE-NITROGEN CONTENT OF COTTON PETIOLES. ALTUS, OKLAHOMA, 1964.

Date Sampled - July 15

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at planting	Fertilized at 8-leaf stage
0	13,071	13,071
40	16,918	15,610
80	19,245	17,692
160	18,376	17,427

Calculated F Value = 3.91

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 11%

Date Sampled - July 30

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at planting	Fertilized at 8-leaf stage
0	643	643
40	6,899	7,084
80	11,442	10,271
160	10,160	11,011

Calculated F Value = 18.02

Tabulated $F_{(.05)}$ Value = 4.76

Coefficient of Variation = 18.9%

Table VII Continued

Date Sampled - August 20

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at planting	Fertilized at 8-leaf stage
0	391	391
40	520	910
80	2,809	2,876
160	5,702	6.349

Calculated F Value = 3.74

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 25%

TABLE VIII

THE EFFECT OF RATES AND TIME OF APPLICATION OF NITROGEN ON THE NITRATE-NITROGEN CONTENT OF COTTON PETIOLES. CHICKASHA, OKLAHOMA, 1964.

Date Sampled - July 6

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 4-leaf stage	Fertilized at 8-leaf stage
0	16,203	16,203
40	16,928	16,490
80	17,664	16,767
160	18,988	17,215

Calculated F Value = 1.88

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 6.8%

Date Sampled - July 20

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 4-leaf stage	Fertilized at 8-leaf stage
0	11,939	11,939
40	13,959	15,917
80	16,004	16,606
160	16,765	15,844

Calculated F Value - 2.75

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 11.9%

Table VIII Continued

Date Sampled - August 4

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 4-leaf stage	Fertilized at 8-leaf stage
0	3,126	3,126
40	7,564	9,382
80	10,237	10,335
160	12,627	12,627

Calculated F Value = 18.10

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 14.2%

Date Sampled - August 20

Rate of Nitrogen Applied (Pounds per Acre)	Nitrate-Nitrogen (Parts per Million) Fertilized at 4-leaf stage	Fertilized at 8-leaf stage
0	1,604	1,604
40	5,267	6,545
80	7,931	8,439
160	10,414	10,661

Calculated F Value = 26.46

Tabulated $F_{(.05)}$ Value = 3.00

Coefficient of Variation = 14.6%

VITA

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Candidate for the Degree

of

Master of Science

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