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AND NITROGEN AVAILABILITY AND BOLL SHEDDING  
IN GOSSYPIMUM.

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INTER-RELATIONSHIPS BETWEEN CARBOHYDRATE AND NITROGEN  
AVAILABILITY AND BOLL SHEDDING IN GOSSYPIMUM

by  
Daniel W. Vomhof

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I hereby recommend that this dissertation prepared under my  
direction by Daniel W. Vomhof

entitled Inter-relationships Between Carbohydrate and Nitrogen  
Availability and Boll Shedding in *Gossypium*

be accepted as fulfilling the dissertation requirement of the  
degree of Doctor of Philosophy

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Daniel W. Vornhof

## PREFACE

The work reported in this dissertation is part of an investigation supported by a grant from the Cotton Producers Institute of the National Cotton Council of America.

The purpose of the project was to investigate the roles nitrogen, carbohydrates and growth regulators played in flower initiation and abundance in cotton. Much of the work was done under field conditions at the University's Cotton Research Center, Tempe, and the Experiment Station at Yuma. These experiments, extending over a three year period, provided the background and preliminary information necessary for the execution of the present work.

The author is indebted to Sue Hibbs, Silver Darmer, Lewis Dewberry, and Richard Dupont for the technical assistance they provided; and to his wife, Joan, and son, Dan, for their continued help, encouragement and understanding.

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## ABSTRACT

Nitrogen fractions in roots, leaves, stems, fruiting branches and squares were followed quantitatively through several stages of development from seedling through early shedding. Free sugars were observed semi-quantitatively in some plant parts using thin-layer chromatography.

It was found that the soluble reduced nitrogen concentration declined significantly prior to first flower. Several qualitative changes in the amino acid pools of the stem and squares occurred at this time. This decline in soluble nitrogen could be related to boll abscission later in the fruiting period. The changes observed in free sugar concentrations were not considered significant in this regard.

These results were then used to explain several diverse findings reported in the literature for growth, boll abscission, and senescence in cotton.

## INTRODUCTION

The phenomenon of fruit abscission has been the object of concern and study for many years. In many plants the lack of fruit abscission results in a reduction of both quality and quantity of mature fruit, presumably through the inability of the plant to supply the developing fruit with the quantity of nutrients necessary for optimum growth. Costly methods of artificially thinning the plant are required if a profitable yield is to be obtained. On the other hand, there is an apparent decrease in yield of many economic plants as the result of the spontaneous abscission of many of the floral buds and young fruit. Thus, a knowledge of the physiological events terminating in the abscission of the immature fruit could be of considerable value. In the first case, a knowledge of the metabolic reactions would enable the development of more specific and selective chemicals for use in thinning the fruit at the most profitable time. In the second case, another class of chemicals could be developed which could block the biochemical reactions leading to abscission and thus increase the yield obtained per plant. In both cases, plant breeders would know what specific physiological traits should be incorporated or eliminated in order to improve a line.

The majority of studies on abscission have been involved with the biochemical reactions and physiological changes which produce the actual abscission layer. Relatively few studies have been concerned

with the question of why one fruit develops normally while another does not develop and is therefore shed.

Since under normal conditions fruit abscission generally occurs after the plant has already set a relatively large number of fruit, the most widely accepted explanation of the primary cause has been that the plant has become depleted of either carbohydrates or nitrogenous compounds. The organs which absciss, then, are those which receive an insufficient supply of nutrients to develop normally. Few experiments have been conducted to test this hypothesis and the results have been inconclusive at best. The cotton plant has been studied the most in this regard.

The present study has attempted to:

1. Follow various nitrogen and carbohydrate compounds through several stages of plant development in various tissues.
2. Determine if there are any apparent correlations between possible changes in nitrogen or carbohydrate compounds and fruit shedding at the same or a different stage of plant development.
3. Compare the results of previous studies, as reported in the literature, with the results obtained in this investigation.
4. Develop a hypothesis which can explain why some fruits are more likely to shed than others on the same plant, and which is compatible with data in the literature.

## LITERATURE REVIEW

### A. Growth and Development

Two species of cultivated cotton are of economic importance in the United States. Of these the American Upland species (Gossypium hirsutum) accounts for over 90 percent of the annual crop and in 1965 ten major varieties accounted for 89 percent of the total Upland production (61, 55). The other species of importance is American-Egyptian (G. barbadense) which is restricted to a four state area of the Southwest (31).

The United States Cotton Belt can be divided into three general regions: the warm, humid Southeast; the cooler, shorter season of the High Plains; and the hot, dry West. Despite the many varieties of Upland cotton which have been developed over the years for optimum production in the specific growing regions, the growth and development habits have remained surprisingly stable. The remainder of this discussion pertains primarily to G. hirsutum although much of the information is applicable to at least some of the varieties of G. barbadense such as the Sea Island cottons.

#### 1. Growth Characteristics

The following summary of the growth of the cotton plant is taken largely from the reviews of Eaton (19) and Tharp (61).

Every node of the main stem above the cotyledons bears a single true leaf and at least two axillary buds (47). The leaves are arranged in a three-eighths spiral which may be either right- or left-handed. Normally, the first axillary bud develops into a branch which may be either vegetative or fruiting. The vegetative branches develop in a manner almost identical to that of the main stem; each develops continuously from an apex. The vegetative branches usually occur in a definite zone near the base of the plant. They may arise at upper nodes when environmental factors restrict development of fruiting branches.

In most Upland varieties the first fruiting branch appears no lower than the fifth or sixth node. New fruiting branches are produced at the sub-apical region of the main stem about every three days during the period of active growth. The flowering and fruiting habit of the cultivated cottons is day-length neutral.

Whereas the development of the vegetative branch is quite similar to that of the main stem, the development of the fruiting branch is quite different. The fruiting branch is smaller in diameter and more nearly horizontal in attitude than the vegetative branch. Rather than a single growing point which continuously produces new plant parts, the growing point of the fruiting branch is terminated by each flower.

The first part of the fruiting branch to become visible as the branch develops from the axillary bud is the first floral bud, or square. The bud is carried away from its original position by the lengthening of

the first internode. A leaf develops adjacent to the square but remains small until four to seven days after the square becomes visible. As this leaf then enlarges, its first axillary bud grows out with its floral bud to form the second internode and square of the fruiting branch. This continues for each fruiting branch throughout its growth period. Fruiting branches which arise from a vegetative branch develop in exactly the same way as those from the main stem.

## 2. Rate of Development

The cotton plant follows an orderly pattern of development. The time required to complete each phase varies within narrow limits for different varieties, cultural practices and environmental conditions.

The general time schedule is as follows (61):

Emergence - one to two weeks from date of planting.

First floral bud - the first floral bud, or square, is observed about 40 days after emergence. The time and node are quite dependent on temperature (46).

Floral bud to bloom - about 25 days elapse from the time the square is first visible until it blooms. This phase of development is much less dependent on temperature than are the first two.

Blooms after the first - the interval between blooms at the same node on the next higher fruiting branch and the just

opened flower is 3 days; between adjacent nodes on the same fruiting branch is 6 days.

Bloom to boll maturation - the time from bloom until mature boll is 50 to 70 days. The bolls set in the hot part of the season develop more rapidly than those set later in the cool part of the season. The boll reaches its maximum diameter about 18 days after fertilization.

Seed development - the fertilized ovule attains its final length in about 18 days, and final volume about 25 days, after anthesis. It does not attain its final weight, however, until just a few days before the boll opens.

Development of the embryo proceeds quite slowly for the first 5 to 10 days after fertilization, and then quite rapidly from the 10th to the 22nd day. Full volume is reached by about the 32nd day.

Oil accumulation begins about the 15th day and is slow until the 25th day; from the 25th to the 42nd day accumulation is rapid. This is also the period of most rapid protein production.

Fiber development - the lint fiber reaches its maximum length within 18 days after anthesis, after which time thickening occurs by deposition of cellulose on its inner surface.

Shedding - squares can be shed at any stage of their development but most are shed well before blooming. Boll shedding occurs between 4 and 10 days after anthesis. Bolls older than 10 days seldom absciss unless they have suffered severe insect, chemical or mechanical damage.

### 3. Occurrence of shedding

Shedding of buds and young fruit can occur for many reasons including extremes of temperature, drought, mechanical injury such as from wind or hail, or damage by insects. When all of these causes have been eliminated the plant still sheds a large percentage of its bolls and squares. Data is scarce on the number of floral buds shed during a growing season but "normal" fruit shed ranges from 30 to 70 percent of the flowers which bloom (13, 28, 29). The percentage varies with species, variety, climate, nutritional status of the soil, and elevation.

Shedding of young bolls can occur anytime during the season after the first flower blooms. Most commonly, the number of bolls shed increases from the third to the seventh week of the fruiting period. The peak flower production usually occurs four to six weeks after first bloom. Under some conditions flowering is fairly constant over the first six or seven weeks of the season and a definite peak is not obtained. In this case, the number of bolls shed per week is more uniform over the entire flowering period. This seems to be atypical, however. Flower and boll

data are given by Lloyd (42), Crowther (13), Eaton (17), Hancock (33), Mason (45), and Gardner (28, 29).

Square shed normally does not occur until after blooming has commenced. Like boll shed, square abscission increases as the fruiting season progresses. Late in the season, about eight to nine weeks after first bloom, the plant essentially quits flowering and enters a period of "cut-out." At this time most of the young squares are shed (29). Mason (45) reports that the age at which the squares shed decreases as the season progresses and that the majority of the squares are shed when very young.

It has often been stated that heavy boll shedding ( and probably square shedding ) is commonly coincidental with growth inhibition ( 19, 61 ).

The zonal fruiting behavior of the cotton plant has been depicted schematically by McNamara, et al. (48). This is reproduced in Figure 1. Often in the case of early, heavy insect infestation zones A and B are absent and only zones C and D occur.

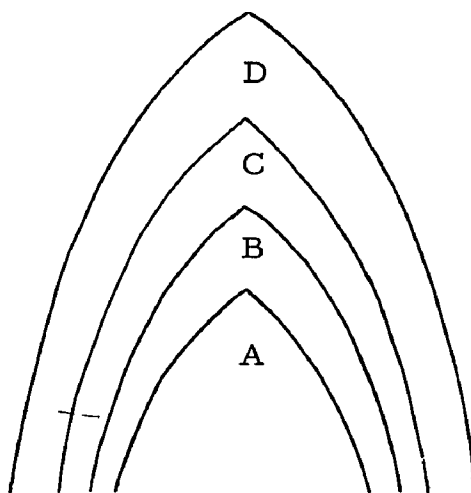


Figure 1. Schematic diagram of a cotton plant showing successive zones of fruiting and physiological defruiting. (A) Normal fruit zone; (B) small-boll shedding zone; (C) small-square shedding zone; (D) late-season growth and fruiting. (After McNamara, et al.)

## B. Theories of Boll Shedding

Boll shedding in cotton has been a subject of study for several years, as it has been for other plants of economic interest such as apple and citrus. Several theories have been suggested over the years as being fundamental causes of normal fruit shed. Those pertaining to cotton directly will be discussed in the order of historical occurrence.

### 1. Environmental

Probably the earliest experimental work pertaining to fruit shed in cotton was carried out by Balls in Egypt. He recognized two causes of shedding: constitutional and environmental. The former is seen by the coincidence of maximum shedding with maximum flowering. As to

environmental causes, Balls believed that, while not exclusively due to soil water conditions, the position of the water table has a major influence in the irrigated regions of Egypt. Both excesses and deficiencies of water in the root zone are correlated with waves of boll shedding ( 7 ).

Ewing compared shedding rates with soil moisture and evaporation through two seasons. He concluded the relationship was questionable ( 26 ).

Lloyd concluded that late forenoon and early afternoon rains caused a high degree of shedding of bolls through its destruction of pollen - although this did not account for square shedding. More fundamental causes were concluded to be the depletion of soil water late in the season and a competition between developing bolls for the internal water of the plant ( 42 ).

Mason ( 45 ) noted that during the later stages of plant development augmented shedding rates followed dark, humid days accompanied by much rain. This did not account for shedding at other times, however.

Recently there has been some thought given to the relation between high night temperature and augmented shedding rates ( 27 ).

As Eaton points out, the same "causes" which were associated with shedding late in the summer were usually ineffectual early in the flowering period ( 19 ).

## 2. Nutritional Balance

The nutritional theory has probably been the most widely

accepted explanation of the basic cause for the initiation and regulation of fruit shed. It is also one of the oldest.

As early as 1892 Atkinson felt boll shedding was a "purely physiological trouble" often induced by extreme changes in climatic conditions. The proximate cause of shedding, however, was the interference "with the supply of nutrient materials or moisture - a partial withholding of the customary daily supply of tissue forming material just at a very critical period in the life of the young forms" (6). (Forms include both square and young bolls.)

Mason is generally given credit for formulating the nutritional theory of boll shed in spite of Atkinson's statements of thirty years earlier. In 1922 Mason reported on some field observations of the shedding habit of Sea Island cotton. He stated:

The general conclusion was drawn that the proportion of shedding over any given period was the resultant of two opposing factors, the rate at which food was synthesized by the plant and the rate at which it was utilized in the maturation of the fruit; and that any check in the former augmented the rate of shedding (45).

Hawkins, et al., (34) were probably the first to study changes in carbohydrates and nitrogen compounds which occurred during the season in different plant parts. They found a correlation between the osmotic pressure of the bolls and the shedding rate in Acala cotton.

Crowther studied the effect of nitrogen and water regime on the growth and yield of cotton in the Sudan as well as the changes in nitrogen

content of many plant parts with time. He concluded that the function of nitrogen was the stimulation of meristematic activity; and nitrogen, as well as carbohydrates, was a limiting factor resulting in the shedding of bolls later in the season.

Ergle (24, 25) followed changes in some carbohydrate fractions in root and top parts of field grown plants for two seasons. He found the soluble sugars steadily increased in the tops and remained high in the roots throughout the season.

Wadleigh reported on a study of the influence of nitrogen on the growth of cotton plants. From carbohydrate and nitrogen analyses of plants grown on four levels of nitrogen he concluded that boll shedding was the result of insufficient carbohydrate supply when nitrogen was adequate. Nitrogen was the principal cause when it was deficient. There were, however, no differences in carbohydrate levels between his treatments (69).

Eaton and Joham studied the relation of sugar accumulations to mineral uptake in cotton roots. They concluded, "It appears that much of the decline in mineral uptake with heavy fruiting can be attributed to the reduced movement of carbohydrate to the roots" (22).

Eaton and Rigler studied the effects of light intensity and nitrogen supply on carbohydrate levels in relation to shedding. They found the average carbohydrate level was 2.7 times higher for high light than low

light intensity growth. Nitrogen levels were about the same in the roots of plants grown under the two light conditions. Although still assuming that carbohydrate levels should influence the number of bolls retained, the authors, in summarizing their data, raised the question as to why the carbohydrates under high light were not reduced to the low light intensity levels before shedding occurred (23).

In 1945 Eaton and Ergle repeated Ergle's earlier studies on carbohydrate levels at different times during the growing season. These studies were done under carefully controlled conditions of moisture. Their results supported Ergle's previous findings (20).

Eaton and Ergle (21) measured the carbohydrate and nitrogen levels in leaves, stems, and 13-day old bolls of early, medium, and late plantings of Acala and Stoneville cottons at Shafter, California. The plant parts for analyses were taken from the middle third of the plant. When last sampled in mid-August there were no appreciable differences in the carbohydrate and nitrogen concentrations between the first plantings which were shedding most of their new bolls and the late plantings which were retaining their bolls. In light of the previous literature and their experimental data, they concluded, "...the view often held that the cotton plant sets only as many bolls as it can nourish (or more specifically in the present paper, as many as it can supply with carbohydrates and nitrogen) requires new or other evidence for its support." However, "...it still follows that the highest yields result under the nutritional conditions that give the largest plants."

### 3. Hormonal Control

Hormonal controls of various aspects of plant growth and development have been recognized for 40 years. In 1926 Went (73) in Holland and Kurosawa in Japan (37) showed that materials extracted from living plant tissue enhanced the growth of seedlings. The former had isolated auxin, while the latter had separated one of the gibberellins. A third class of hormones was recognized in coconut milk by van Overbeek (64) in 1941. These are the cytokinins. A good introduction to the action of these classes of compounds, as well as several inhibitors of their action, is given in Leopold's recent text (38). The current knowledge of the mode of physiological action of the plant hormones has been reviewed by van Overbeek (65). Crane (12) has reviewed the current information on the chemical control of fruiting.

In 1944 Wadleigh (69) suggested that the older bolls produced a hormone which initiated the formation of the abscission layer in younger bolls. The potency of the hormone, then, may be dependent on the nutritional status of the plant.

Eaton and Ergle (21), after deciding there was insufficient evidence to support the nutritional hypothesis as a cause of abscission, suggested that leaves produce an auxin and older fruit an anti-auxin. The abscission of young fruit is then regulated by the interaction and balance of these compounds in the fruiting branches.

Walhood (70, 71) and Millhollen (49) have shown that gibberellic acid sprayed on the flower at anthesis often improves the percent set of the plant. In no instance, however, was 100 percent set achieved.

Walhood has also shown that the bolls which shed have fewer than a minimum number of seeds while those which mature have more than this minimum number. This is in keeping with the observation of Crane (12) that "...fruits which absciss prematurely are usually multi-seeded ones with a lower seed content than those which do not absciss." These findings could, at least in part, explain the lower levels of auxin found in aborted bolls.

None of the above conclusions, however, explain why squares absciss or why the rate of abscission increases later in the season for both squares and bolls.

Carns, et al., (10, 11) and Addicott and co-workers (4) have isolated two abscission accelerating compounds from carpels of bolls. The latter group has identified their compound as Abscisin II, a sesquiterpene. The concentration of Abscisin II increases to a maximum in 5- to 10-day old bolls, the age of greatest abscission of young cotton fruit. This compound also has general growth inhibiting and senescence inducing properties (60). Searle has recently reviewed the evidence for other compounds being present in the plant which inhibit the development of floral buds after differentiation has occurred (58).

Much of the research on abscission has been reviewed in five recent articles (1, 2, 3, 9, 36). The majority of the work has been conducted on leaf petiole explants and has been aimed primarily at elucidating the role of indole-3-acetic acid (IAA) in the formation of the abscission layer. The fundamental causes for the initiation of physiological changes has been given much less consideration. Osborne and Moss (54) and Leopold and co-workers (57) have recently come to the conclusion that the abscission process is one of the final steps in senescence. The decrease in auxin in the organ may trigger the abscission mechanism but it is a result of previous senescence processes.

#### 4. Senescence

There has been much reported through the years on various correlation effects occurring during flowering and fruiting in plants. The reader is referred to an excellent review of the subject by Leonard (41) for further information.

The most notable effect observed has been that the stem essentially ceases to grow sometime after floral buds have begun to be initiated. The time interval is dependent on plant species and nutrient status. Nitrogen seems to be the controlling nutrient. Murneek (52, 53) was probably the first to show this effect of fruiting with tomatoes. Eaton and co-workers (17, 18, 22, 23) and others (14, 45, 30) have demonstrated the restriction of growth with fruiting in cotton on several occasions.

The usual interpretation of the data has been that fruit load has drained the plant of nutrients to a level too low to support vegetative growth.

Recently a different interpretation has been placed on these and similar experiments. Leopold, et al., (40) have shown that removal of the inflorescence before or soon after anthesis retards the onset of senescence and concurrent retardation in growth. Leopold (39) has recently reviewed the relationships between reproduction and senescence in plants, and Varner (66) has discussed some of the biochemical changes observed in senescing tissues.

The conclusions based on the above data as related to abscission are:

- a. The developing flowers or fruit in some way cause other plant parts to senesce.
- b. This senescence results in a breakdown of many compounds within a particular plant tissue or organ. These breakdown products are capable of being translocated out of the aging tissue to be reused by growing tissue. Developing flowers and fruit act as strong mobilizing centers which direct this flow of compounds from the senescent tissue to themselves (in a manner not yet completely understood).
- c. When the level of certain compounds within the tissue reaches a predetermined minimum, either because of translocation out of the organ, or because of decreased synthesis, the tissue abscises (9).

This last point, especially, is as applicable to boll shed as it is to leaf abscission. However, this hypothesis still does not explain why some fruit or floral buds absciss and others do not.

## MATERIALS AND METHODS

### A. Experimental

An experiment was conducted in a plastic-covered greenhouse in the spring of 1966. The greenhouse is located at the University of Arizona's Campbell Avenue Farm in Tucson.

The plants were grown in crocks containing approximately 30 pounds of Mojave sandy loam. Plants for two experiments had been grown on this soil previously. This soil provides adequate quantities of all essential elements required for growth of cotton except nitrogen.

The Upland variety used in this experiment was Gregg 35. This variety has been adapted for the short growing season of the High Plains region of the Cotton Belt.

Sufficient crocks were planted so that at maturity two plants could be taken at each sampling time for each replication. Two replications were analyzed. Seeds were planted April 4, 1966. All crocks had seedlings emerged by April 9. The plants were thinned to four per crock at the one true leaf stage. Nitrogen was added as  $\text{NH}_4\text{NO}_3$  solution. A total of 2.5 grams of N was added to each crock by the five-leaf stage of growth. Distilled water was added to each crock as needed. The minimum nightly temperature was about 72°F and the maximum daytime temperature was about 100°F.

Plants were sampled at various stages of development rather than at different chronological ages. Plants were carefully selected so that all plants were at the same stage of development for a given sampling.

The samples were rinsed in distilled water, sectioned, plant parts placed in labelled bags, and frozen in dry ice. Samples were always taken between 1:00 and 3:00 p.m. The samples were stored in a deep-freeze until analyzed.

The plants were sectioned longitudinally as depicted in Figure 2. Each organ of each section was analyzed separately. The plant parts were labelled as follows: S - stem, L - leaf, P - petiole, R - root, FB - fruiting branch, and F - squares.

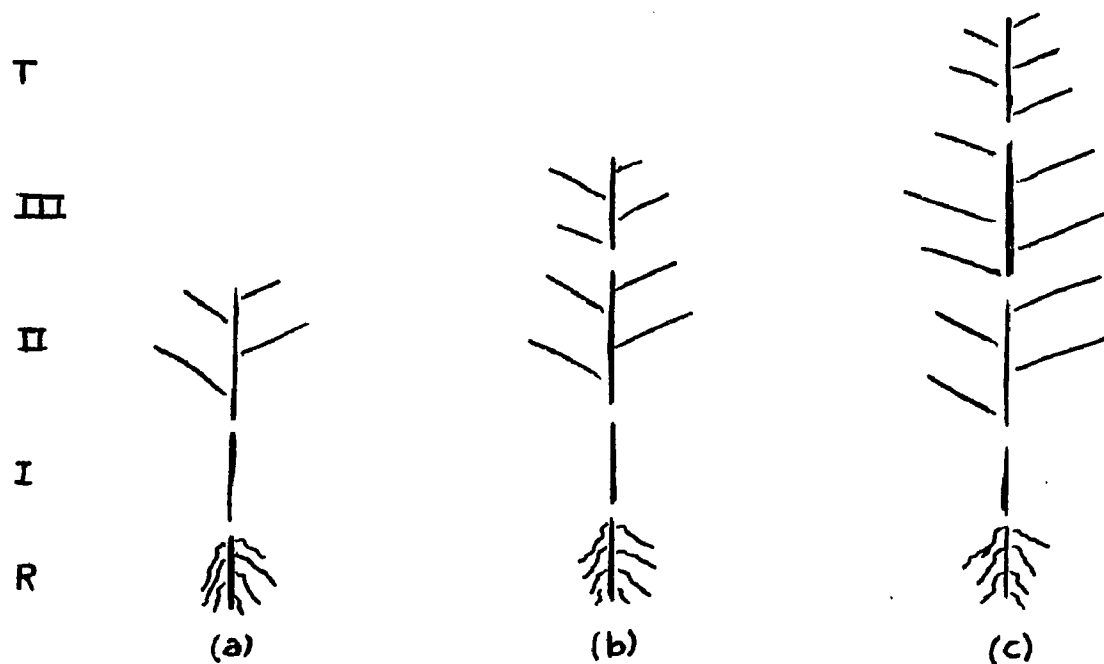


Figure 2. System of sectioning plants at different stages of growth. (a) Vegetative stage; (b) Square stages; (c) Fruiting stages.

R - tap root; I - stem to first true leaf node; II - vegetative branch section, 4 nodes maximum; III - first fruiting region, 5 nodes maximum; T - top region of plant, 6 nodes maximum at stage (c). The top region of stage (a) becomes region II of stages (b) and (c).

## B. Sample Preparation

The samples were prepared for analyses as follows:

1. Frozen samples were lyophilized for three days at 0.050 mm Hg.
2. The dry sample was ground to pass a 40-mesh screen in a Wiley mill.

3. A portion of the ground sample was extracted with 80 percent ethanol for 24 hours in a Soxhlet extractor.
4. The alcoholic extract was concentrated at 40°C at reduced pressure (water aspirator).
5. The concentrate was made to 10 ml with 10 percent isopropanol.
6. The liquid sample was stored in a refrigerator until analyzed.
7. The extracted residue was dried in a forced-air oven at 70°C.

The dried residue was stored in a plastic vial in the laboratory.

### C. Chemical Analyses

#### 1. Nitrogen

The extract was analyzed for nitrate, ammonium,  $\alpha$ -amino acid nitrogen, total reduced alcohol-soluble nitrogen. The residue was analyzed for total reduced nitrogen which was designated "protein N." These determinations were made using the semi-micro steam distillation methods of Bremner (8).

Changes in individual amino acids were observed semi-quantitatively using the thin-layer chromatographic procedure of Turner and Redgwell (63) and the detection system of Moffat and Lytle (51).

#### 2. Carbohydrates

Free sugars were observed semi-quantitatively using the

thin-layer chromatographic system of Vomhof and Tucker (67, 68).

In the semi-quantitative thin-layer technique used, the quantities of extract applied to a thin-layer plate were adjusted so that the same dry weight of plant material was represented in each spot. Any major change in final spot size reflected a significant change in the quantity of a particular compound with a change in the stage of development of the plant.

## RESULTS

Preliminary experiments had shown that the desired information could only be obtained by sampling plants at successive stages of development rather than by chronological time from planting. These experiments also indicated that each organ should be analyzed separately in order to obtain a more complete picture of the nitrogen status in relation to development. Gregg 35 was the variety chosen for this study since previous experiments had shown it was quite uniform in its growth habit and fruiting characters.

Data on the stage of development of the plants at sampling are given in Table 1.

The plants were separated into root, stem sections, fruiting branches, petioles, and leaves from the appropriate stem sections, as well as squares from the two fruiting sections as shown in Figure 2. The fruiting stage samples were taken when the boll in question was four days old, i. e., at 2 Boll stage the second boll was four days past anthesis. Thus there were actually one or two additional, younger, bolls on the plant than the particular stage would indicate.

### 1. Total Soluble Reduced and Protein Nitrogen

The means of the Soluble and Protein nitrogen determination for the two replications are presented in Tables 2 and 3. The changes

Table 1. Stage of Development of Plants at Sampling.

Stage of Development	Average Number of Squares per Plant	Average Number of Bolls per Plant	Comments
5 Leaf	0	0	Fifth leaf about 1/2 inch long. No squares initiated.
Early Square	2	0	Squares about 2 mm in diameter.
6 Square	6	0	Four nodes on S-II, five on S-III.
Late Square	10 S-III 7 S-T	0	About 18 hours before first bloom.
1 Boll	10 S-III 25 S-T	3 S-III	First boll 4 days past bloom.
2 Boll	8 S-III 16 S-T	3 S-III	
4 Boll	6 S-III 11 S-T	7 S-III 1 S-T	One almost open flower on one plant of each replication.
6 Boll	10 S-III 13 S-T	7 S-III 1 S-T	2/3 of the squares in each section were very small at each of the last three samplings.

S-III - Fruiting branches of stem section III.

S-T - Fruiting branches of stem section Top.

Table 2. Total Soluble Reduced Nitrogen Concentration at Different Stages of Growth.

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
S-T	a	18.67	23.31	9.74	8.04	7.57	6.82	9.87
	b	(1.86)	(7.14)	(0.02)	(0.40)	(0.38)	(2.00)	(0.62)
S-III				8.42	8.47	8.53	8.17	8.36
				(1.28)	(1.82)	(3.14)	(0.18)	(0.88)
S-II	18.16	12.50	13.65	8.59	7.58	8.73	7.82	11.35
	(7.72)	(2.72)	(1.22)	(0.54)	(3.28)	(0.82)	(0.36)	(0.94)
S-I	7.21	7.94	13.00	5.77	14.54	7.60	7.69	8.67
	(1.22)	(3.40)	(5.36)	(1.10)	(11.45)	(2.52)	(1.26)	(0.26)
R	15.40	18.71	15.56	8.87	11.39	7.86	6.99	9.22
	(4.56)	(4.94)	(1.04)	(2.18)	(2.26)	(0.44)	(2.18)	(0.00)
L-T		7.88	11.24	6.00	5.66	8.51	7.18	4.95
		(3.24)	(0.52)	(2.08)	(2.62)	(5.22)	(0.24)	(0.54)
L-III				7.44	4.00	9.21	6.08	6.78
				(4.12)	(2.26)	(3.14)	(0.16)	(0.52)
L-II	7.48	8.27	8.73	4.73	5.08	7.60	5.36	6.01
	(0.00)	(1.18)	(2.78)	(0.10)	(0.28)	(4.44)	(0.68)	(2.26)
P-T		14.98	11.58	7.12	9.43	4.70	9.48	8.96
		(7.08)	(0.16)	(0.00)	(0.78)	(0.68)	(2.60)	(5.76)
P-III				8.64	9.65	10.92	11.63	9.60
				(3.04)	(5.38)	(3.08)	(1.34)	(7.40)
P-II	10.88	13.01	10.53	7.69	6.64	14.83	14.63	11.46
	(0.52)	(5.10)	(2.26)	(0.78)	(3.40)	(2.90)	(3.66)	(3.92)
F-T				8.67	7.91	10.00	10.47	7.40
				(5.71)	(3.66)	(1.22)	(0.62)	(0.16)

Table 2--Continued

Plant Part	Stage of Development					
	Early 5 Leaf Square	Late 6 Square Square	1 Boll	2 Boll	4 Boll	6 Boll
	(mg N/gm dry tissue)					
F-III	10.00 (0.08)	11.22 (1.15)	10.65 (6.18)	11.08 (0.60)	10.48 (0.44)	9.56 (1.20)
FB-T		8.92 (0.00)	6.86 (0.12)	9.30 (2.44)	13.44 (0.44)	9.87 (4.42)
FB-III	26.98 (3.60)	13.64 (0.04)	16.08 (2.96)	20.92 (1.12)	13.45 (0.26)	12.78 (1.92)

a - Mean ( $\bar{X}$ )

b - (Range) (R)

$$M = \frac{\bar{X}_1 - \bar{X}_2}{R_1 + R_2}$$

$$M_{.90} = 1.161$$

Ref: E. L. Bauer, A Statistical Manual for Chemists, Academic Press  
(1960).

Table 3. Protein Nitrogen Concentration at Different Stages of Growth

Plant Part	Stage of Development							
	Early				Late			
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
S-T		45.33 (7.39)	26.67 (1.71)	18.73 (1.91)	17.62 (0.71)	14.04 (0.45)	12.50 (0.09)	14.19 (1.55)
S-III				12.67 (2.39)	11.28 (2.87)	11.71 (0.78)	12.58 (0.08)	9.57 (0.34)
S-II	15.12 (3.93)	18.93 (2.13)	18.38 (1.31)	11.91 (3.35)	11.32 (3.14)	9.67 (2.61)	12.41 (3.57)	8.71 (0.52)
S-I	12.94 (0.90)	9.89 (1.17)	12.04 (0.46)	10.49 (1.16)	9.32 (2.27)	8.47 (0.65)	6.79 (0.87)	7.91 (0.50)
R	10.62 (0.00)	10.04 (0.70)	10.54 (3.17)	8.49 (0.23)	9.36 (1.06)	8.95 (1.02)	8.68 (4.65)	7.03 (0.49)
L-T		46.45 (6.62)	51.04 (8.52)	43.49 (0.61)	47.01 (0.17)	51.31 (0.22)	44.57 (1.27)	47.12 (0.15)
L-III				42.78 (7.37)	39.83 (14.35)	41.57 (11.06)	38.18 (5.13)	38.61 (0.96)
L-II	44.36 (1.04)	42.13 (4.98)	34.12 (4.06)	30.08 (4.79)	26.29 (11.32)	27.11 (5.81)	23.73 (5.50)	25.91 (7.90)
P-T		18.48 (5.76)	14.95 (2.36)	17.24 (3.66)	15.75 (0.00)	13.25 (0.29)	13.51 (2.83)	12.71 (0.34)
P-III				11.10 (0.26)	8.86 (2.82)	10.36 (0.18)	12.59 (0.98)	9.93 (0.53)
P-II	14.75 (0.78)	12.96 (3.11)	11.03 (0.39)	9.39 (0.13)	11.84 (1.74)	13.55 (1.48)	12.19 (0.31)	10.27 (0.36)
F-T				33.80 (1.23)	34.28 (6.93)	33.42 (2.00)	29.74 (2.69)	30.76 (1.80)
F-III			33.06 (6.96)	29.98 (0.62)	28.43 (4.79)	33.40 (1.64)	31.08 (0.52)	30.36 (3.57)
FB-T				24.65 (2.21)	24.94 (4.81)	21.17 (1.33)	18.93 (0.67)	18.18 (0.20)

Table 3--Continued

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
FB-III			36.14	18.63	15.18	15.34	14.50	15.69
			(10.13)	(1.38)	(3.67)	(3.49)	(2.16)	(3.18)

occurring in these nitrogen fractions with progressive stages of development of the various organs are depicted graphically in Figures 3 through 6. All graphs use the means reported in the appropriate Tables.

It can be readily seen that significant decreases in the concentration of total soluble reduced and protein nitrogen occurred between the first third of the squaring period and first bloom. These decreases are most pronounced in the various organs of the two fruiting sections of the plant. The concentration of these fractions remains essentially constant at the lower level from first bloom through the 6 Boll stage of development which was just prior to the start of abscission of new bolls. It is interesting to note that the fruiting branches of region III (FB-III) contain a significantly higher concentration of total soluble reduced nitrogen than do the top fruiting branches (FB-T) during the period from Late Square through 2 Boll stages of development. This is probably due to developing bolls present in the lower fruiting region.

This large decline in the concentration of nitrogen in these fractions is not primarily due to dilution by secondary cell wall formation and lignification of the different tissues. The fruiting regions (III and T) of the plant had leaves, petioles, fruiting branches and squares, as well as the main stem, in various stages of maturity. However, the decline in concentration occurred simultaneously in all of these organs. This decline, then, must reflect a general change in the metabolism of the plant which occurs prior to first bloom.

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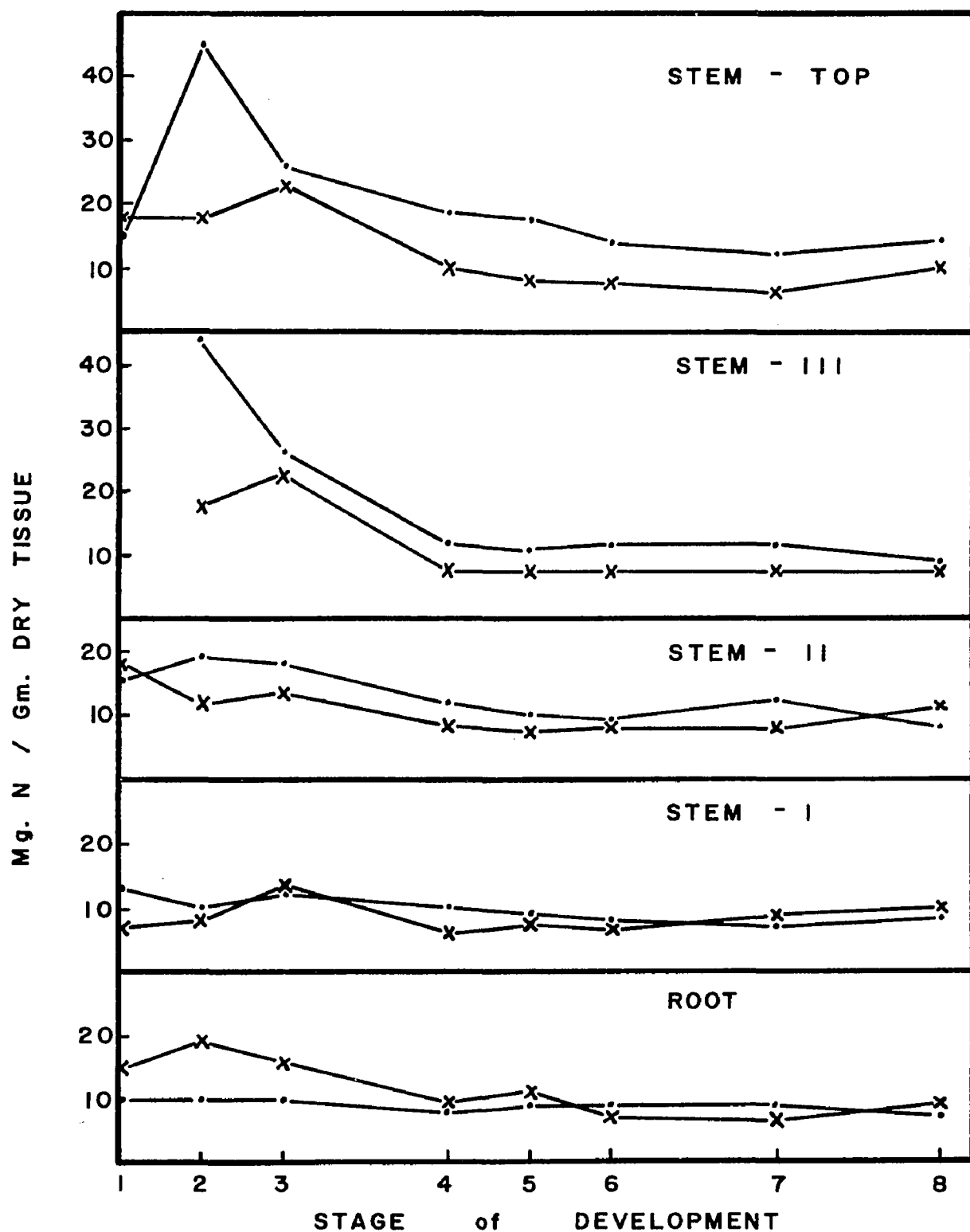


FIGURE 3. TOTAL SOLUBLE REDUCED AND PROTEIN NITROGEN -  
ROOT AND STEM

x—Soluble

•—Protein

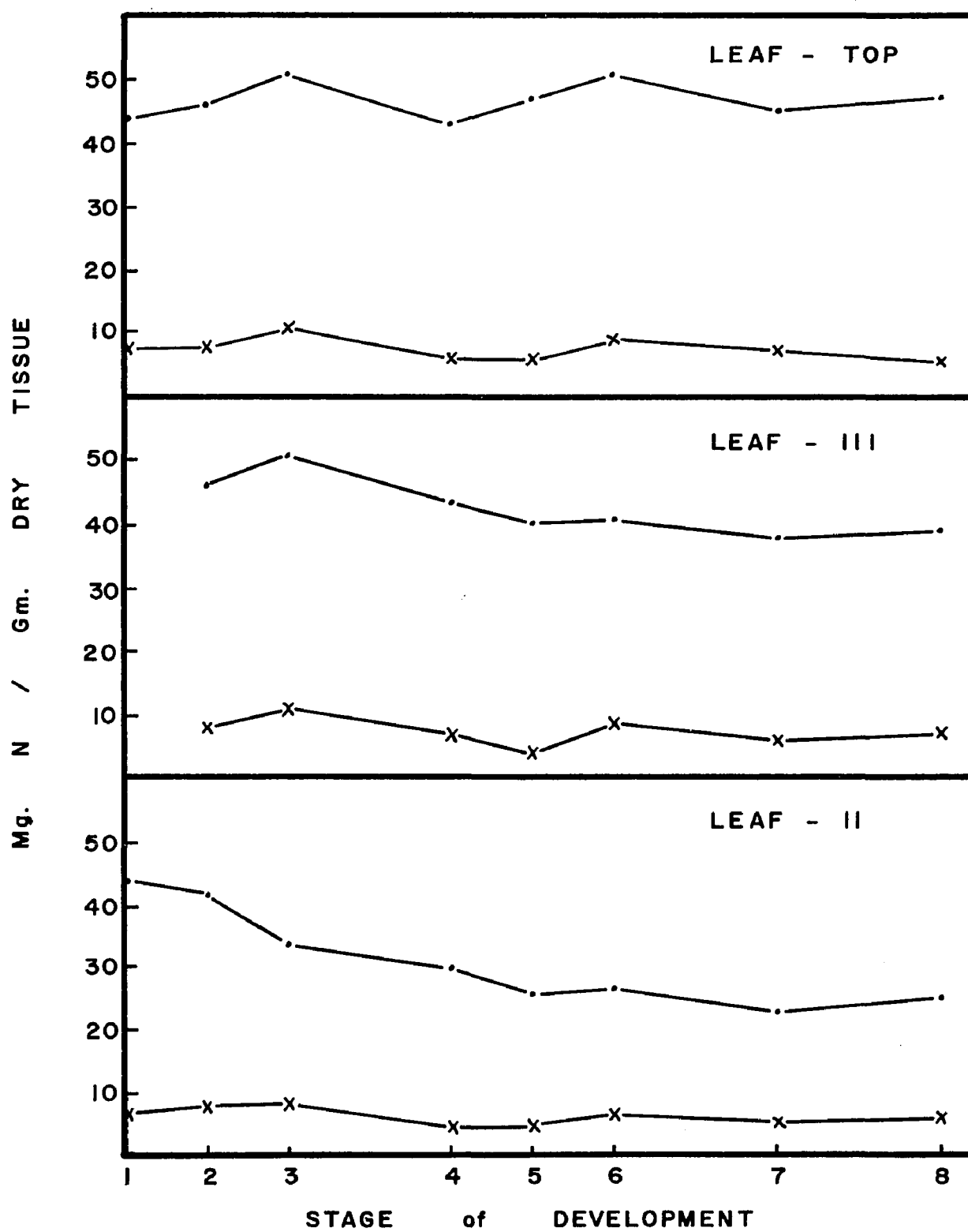


FIGURE 4. TOTAL SOLUBLE REDUCED AND PROTEIN NITROGEN - LEAF

x—Soluble

.—Protein

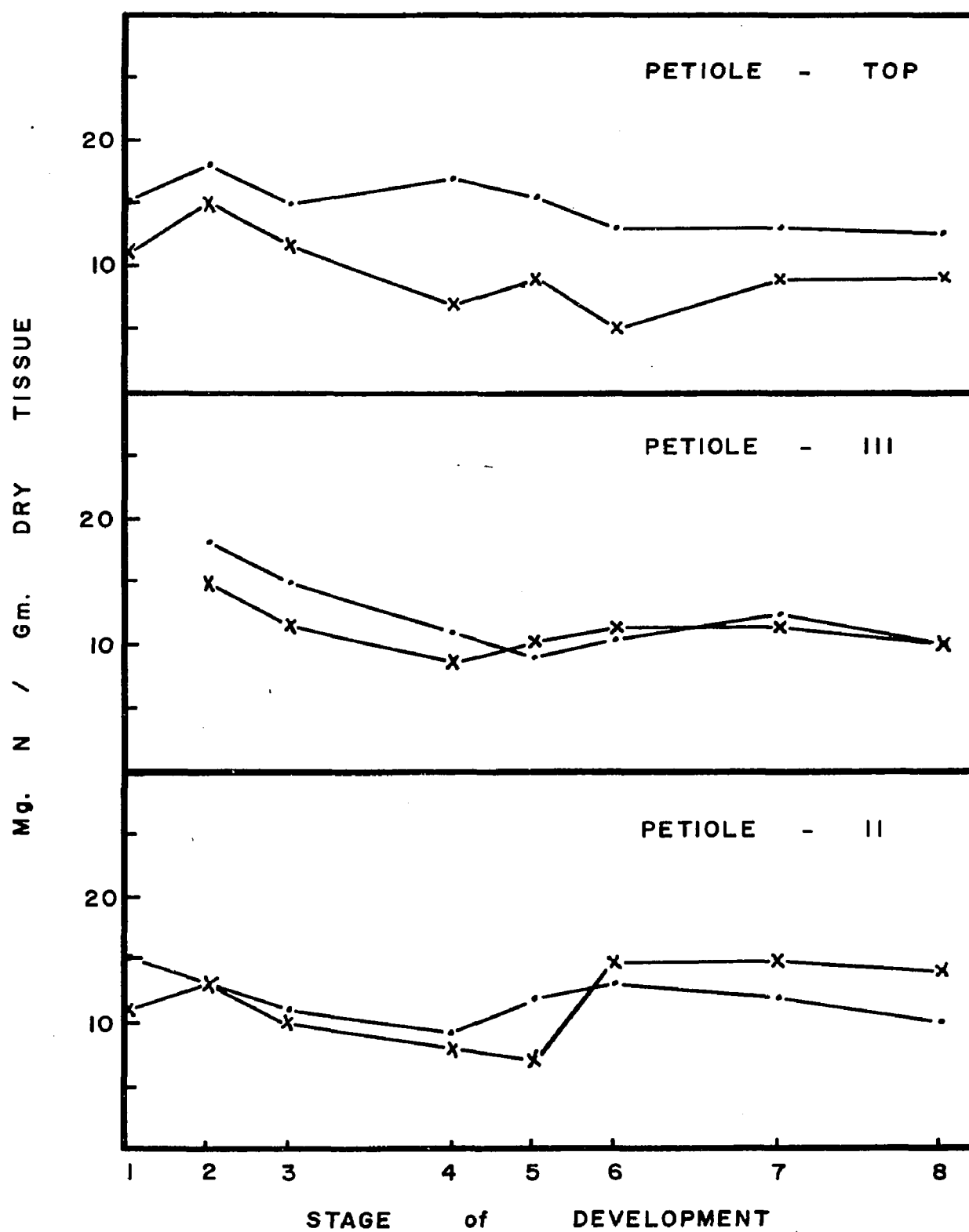


FIGURE 5. TOTAL SOLUBLE REDUCED AND PROTEIN NITROGEN - PETIOLE

x—Soluble

.—Protein

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## 2. Soluble Reduced Nitrogen Components

The principal nitrogen compounds soluble in 80 percent ethanol are ammonium, amino acids, and nitrate. The nitrogen is in the oxidized form in the latter compound and as such is not a component of the Total Soluble Reduced Nitrogen fraction. It will be considered later.

The changes occurring in the concentrations of ammonium and amino acids, as a-amino nitrogen, with progressive stages of development of the plant are depicted graphically in Figures 7, 8, and 9. The means of the chemical analyses for these fractions are presented in Tables 4 and 5.

Although there are few statistically significant differences in the change in concentration of ammonium with time for a given organ, the evident trend is for a lower ammonium concentration after first bloom. The very high concentration of ammonium in the top stem section (S-T) at Late Square stage of development cannot be explained. It is undoubtedly related to the general change in metabolism which occurs at this time.

The a-amino nitrogen concentration fluctuates in much the same manner as the total soluble reduced nitrogen fraction. This is to be expected since the free amino acid pool is a major constituent of the soluble nitrogen. The decline in the concentration of free amino acid nitrogen can be taken as further indication that a metabolic shift occurs between the 6 Square and Late Square stages of development.

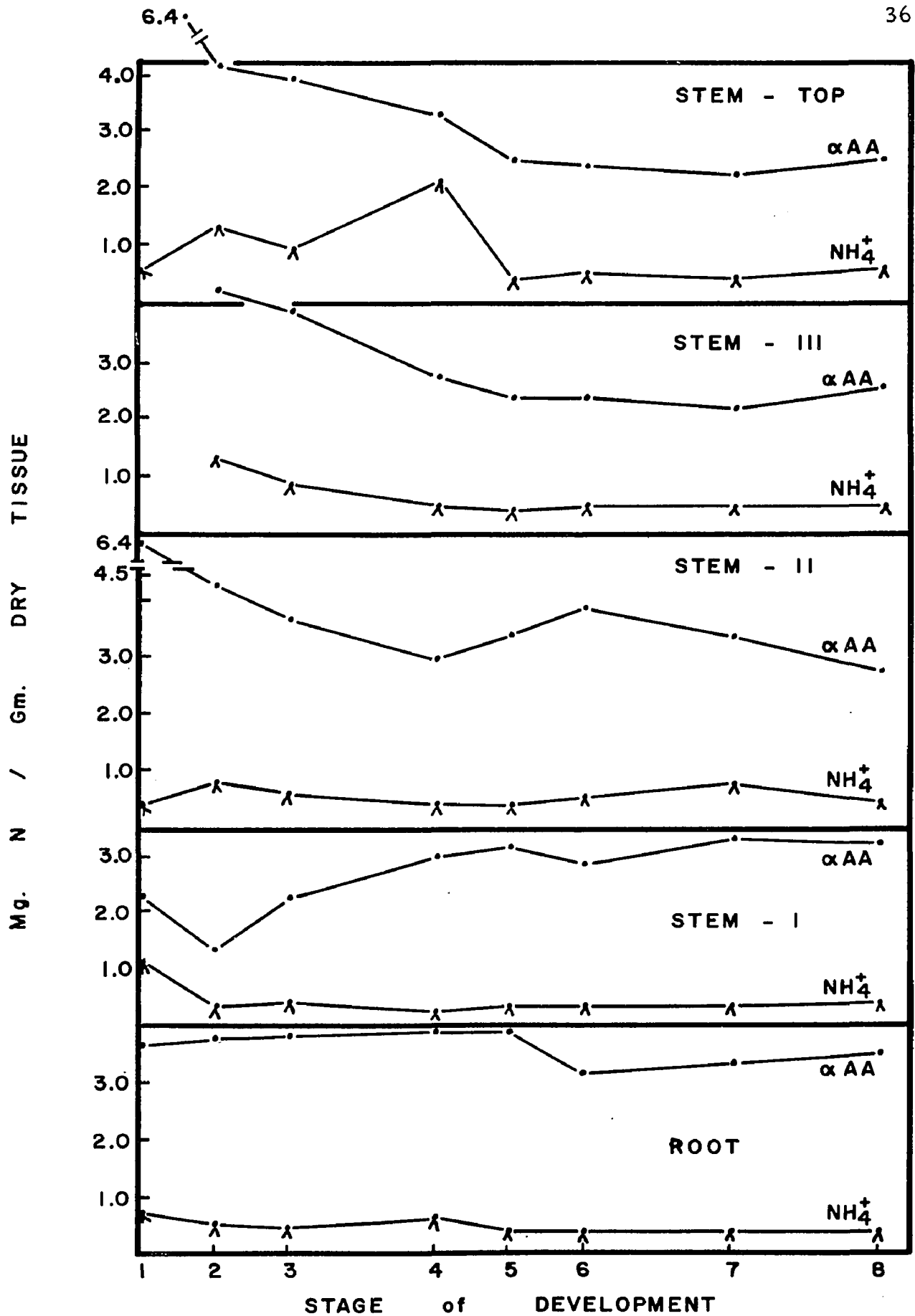


FIGURE 7. α-AMINO AND AMMONIUM NITROGEN - STEM AND ROOT

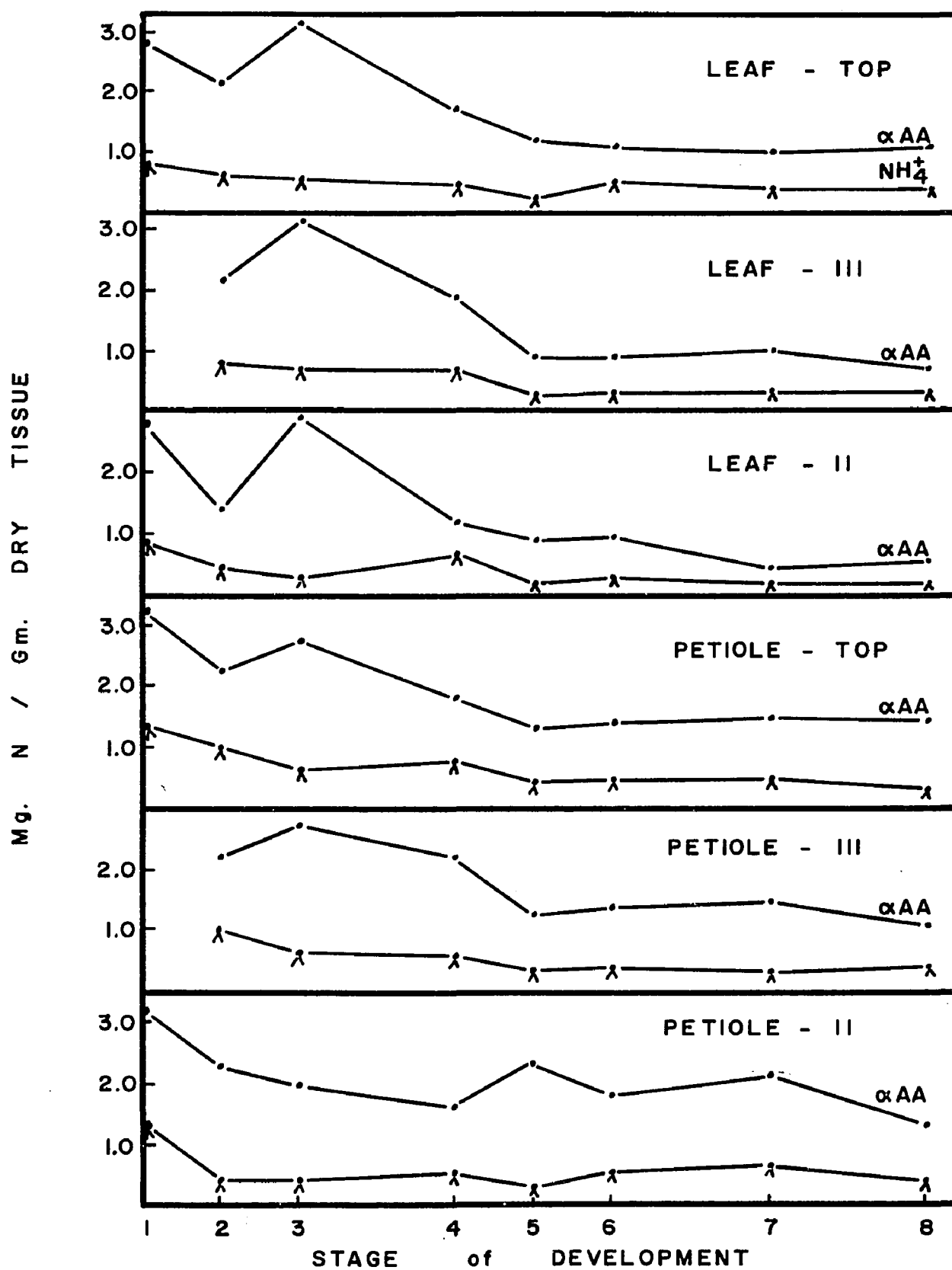


FIGURE 8. α-AMINO AND AMMONIUM NITROGEN - LEAF AND PETIOLE

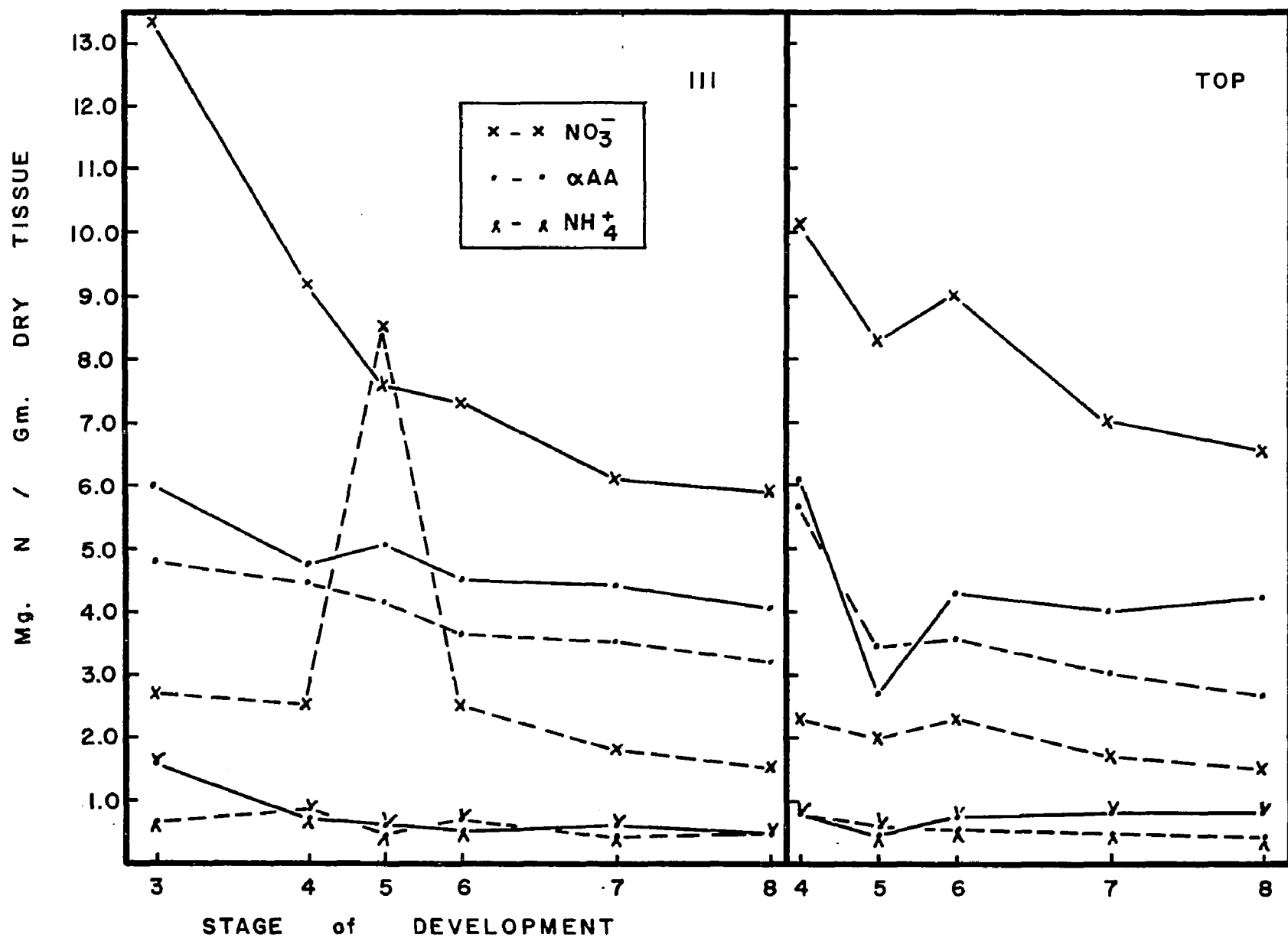


FIGURE 9. NITRATE,  $\alpha$ -AMINO AND AMMONIUM NITROGEN - SQUARES AND FRUITING BRANCHES

----- Squares

—— Fruiting Branch

Table 4. Ammonium Nitrogen Concentration at Different Stages of Growth.

Plant Part	Stage of Development							
	5 Leaf	Early Square	6 Square	Late Square	1 Boll	2 Boll	4 Boll	6 Boll
	(mg N/gm dry tissue)							
S-T		1.28 (0.47)	0.93 (0.10)	2.12 (0.21)	0.36 (0.05)	0.48 (0.07)	0.44 (0.10)	0.59 (0.09)
S-III				0.49 (0.09)	0.39 (0.15)	0.48 (0.01)	0.47 (0.14)	0.48 (0.12)
S-II	0.45 (0.08)	0.83 (0.02)	0.65 (0.21)	0.43 (0.10)	0.42 (0.09)	0.58 (0.22)	0.80 (0.07)	0.57 (0.15)
S-I	1.18 (0.17)	0.37 (0.00)	0.49 (0.24)	0.34 (0.06)	0.38 (0.09)	0.42 (0.07)	0.39 (0.24)	0.46 (0.03)
R	0.74 (0.33)	0.55 (0.36)	0.52 (0.41)	0.71 (0.39)	0.42 (0.10)	0.39 (0.28)	0.40 (0.15)	0.41 (0.02)
L-T		0.78 (0.03)	0.74 (0.14)	0.62 (0.01)	0.35 (0.30)	0.57 (0.12)	0.47 (0.05)	0.51 (0.10)
L-III				0.69 (0.02)	0.25 (0.10)	0.29 (0.05)	0.32 (0.01)	0.32 (0.09)
L-II	0.85 (0.22)	0.43 (0.21)	0.31 (0.16)	0.68 (0.18)	0.18 (0.05)	0.30 (0.00)	0.19 (0.00)	0.22 (0.05)
P-T		1.01 (0.41)	0.66 (0.32)	0.78 (0.19)	0.38 (0.15)	0.50 (0.18)	0.49 (0.10)	0.28 (0.02)
P-III				0.62 (0.29)	0.34 (0.07)	0.41 (0.04)	0.28 (0.04)	0.44 (0.46)
P-II	1.38 (0.08)	0.46 (0.05)	0.46 (0.01)	0.54 (0.08)	0.28 (0.04)	0.52 (0.02)	0.60 (0.24)	0.38 (0.09)
F-T				0.84 (0.17)	0.62 (0.12)	0.61 (0.10)	0.50 (0.07)	0.48 (0.17)

Table 4--Continued

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
	(mg N/gm dry tissue)							
F-III			0.70 (0.18)	0.82 (0.03)	0.56 (0.11)	0.66 (0.06)	0.49 (0.04)	0.56 (0.10)
FB-T				0.92 (0.15)	0.50 (0.01)	0.75 (0.00)	0.80 (0.19)	0.80 (0.43)
FB-III			1.55 (0.30)	0.75 (0.09)	0.61 (0.01)	0.57 (0.23)	0.58 (0.10)	0.52 (0.01)

Table 5. a-Amino Acid Nitrogen Concentration at Different Stages of Growth.

Plant Part	Stage of Development							
	Early		Late		1 Boll	2 Boll	4 Boll	6 Boll
	5 Leaf	Square	6 Square	Square				
	(mg N/gm dry tissue)							
S-T		4.25 (1.02)	3.94 (1.10)	3.28 (0.20)	2.48 (0.17)	2.42 (0.19)	2.25 (0.53)	2.46 (0.27)
S-III				2.76 (0.96)	2.38 (0.29)	2.42 (1.78)	2.16 (0.90)	2.58 (0.21)
S-II	6.39 (1.51)	4.32 (2.37)	3.73 (0.77)	3.09 (0.16)	3.38 (0.10)	3.43 (0.90)	3.39 (0.61)	2.83 (0.21)
S-I	2.38 (0.73)	1.41 (0.42)	2.30 (0.84)	3.05 (0.53)	3.16 (0.68)	2.89 (0.94)	3.39 (0.75)	3.28 (0.07)
R	3.70 (0.56)	3.80 (0.12)	3.86 (0.95)	3.04 (0.59)	4.06 (0.12)	3.18 (0.97)	3.37 (0.07)	3.56 (0.49)
L-T		2.14 (0.73)	3.22 (0.00)	1.70 (0.19)	1.18 (0.01)	1.14 (0.02)	1.02 (0.16)	1.07 (0.33)
L-III				1.90 (0.05)	0.94 (0.28)	0.92 (0.16)	0.98 (0.25)	0.73 (0.10)
L-II	2.86 (1.25)	1.41 (0.98)	3.00 (0.75)	1.33 (1.24)	0.89 (0.38)	0.95 (0.08)	0.47 (0.34)	0.57 (0.33)
P-T		2.25 (0.45)	2.76 (1.31)	1.84 (0.75)	1.32 (0.29)	1.37 (0.02)	1.49 (0.19)	1.49 (0.87)
P-III				2.22 (0.40)	1.28 (0.22)	1.40 (0.03)	1.49 (0.08)	1.09 (0.40)
P-II	3.26 (0.33)	2.30 (0.17)	1.97 (0.78)	1.59 (0.59)	2.34 (0.18)	1.79 (0.08)	2.10 (0.23)	1.28 (0.51)
F-T				5.70 (1.45)	3.43 (0.10)	3.57 (0.61)	3.07 (0.68)	2.68 (0.84)

**Table 5. a-Amino Acid Nitrogen Concentration at Different Stages of Growth.**

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
	(mg N/gm dry tissue)							
S-T		4.25 (1.02)	3.94 (1.10)	3.28 (0.20)	2.48 (0.17)	2.42 (0.19)	2.25 (0.53)	2.46 (0.27)
S-III				2.76 (0.96)	2.38 (0.29)	2.42 (1.78)	2.16 (0.90)	2.58 (0.21)
S-II	6.39 (1.51)	4.32 (2.37)	3.73 (0.77)	3.09 (0.16)	3.38 (0.10)	3.43 (0.90)	3.39 (0.61)	2.83 (0.21)
S-I	2.38 (0.73)	1.41 (0.42)	2.30 (0.84)	3.05 (0.53)	3.16 (0.68)	2.89 (0.94)	3.39 (0.75)	3.28 (0.07)
R	3.70 (0.56)	3.80 (0.12)	3.86 (0.95)	3.04 (0.59)	4.06 (0.12)	3.18 (0.97)	3.37 (0.07)	3.56 (0.49)
L-T		2.14 (0.73)	3.22 (0.00)	1.70 (0.19)	1.18 (0.01)	1.14 (0.02)	1.02 (0.16)	1.07 (0.33)
L-III				1.90 (0.05)	0.94 (0.28)	0.92 (0.16)	0.98 (0.25)	0.73 (0.10)
L-II	2.86 (1.25)	1.41 (0.98)	3.00 (0.75)	1.33 (1.24)	0.89 (0.38)	0.95 (0.08)	0.47 (0.34)	0.57 (0.33)
P-T		2.25 (0.45)	2.76 (1.31)	1.84 (0.75)	1.32 (0.29)	1.37 (0.02)	1.49 (0.19)	1.49 (0.87)
P-III				2.22 (0.40)	1.28 (0.22)	1.40 (0.03)	1.49 (0.08)	1.09 (0.40)
P-II	3.26 (0.33)	2.30 (0.17)	1.97 (0.78)	1.59 (0.59)	2.34 (0.18)	1.79 (0.08)	2.10 (0.23)	1.28 (0.51)
F-T				5.70 (1.45)	3.43 (0.10)	3.57 (0.61)	3.07 (0.68)	2.68 (0.84)

Table 5--Continued

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
F-III			4.80 (0.70)	4.48 (0.07)	4.15 (0.03)	3.63 (0.21)	3.47 (0.53)	3.14 (0.54)
FB-T				6.12 (1.06)	2.71 (1.42)	4.34 (0.68)	4.08 (0.89)	4.20 (1.26)
FB-III			6.00 (0.95)	4.73 (0.48)	5.04 (1.52)	4.50 (1.16)	4.41 (0.89)	4.06 (0.59)

Individual amino acids were observed semi-quantitatively in top stem and squares at the 6 Square and 1 Boll stages of development, i. e., before and after the general decrease in the soluble nitrogen concentration occurred. As can be seen from Figures 10 through 14, this decrease in  $\alpha$ -amino N was accompanied by qualitative changes in individual acids as well as quantitative changes. These changes can be taken as further evidence of a change in plant metabolism with respect to nitrogen after the initiation of squares had commenced. The major changes occurring in the individual amino acids between these two stages of development are listed in Table 6.

### 3. Nitrate Nitrogen

The changes in the concentration of nitrate in the various organs with stage of development are depicted graphically in Figures 9, 15, and 16. The means of the chemical analyses of the two replications are given in Table 7.

The decline in nitrate within the plant with stage of development is in agreement with the work of Gardner (28). It can be seen that the decline in nitrate nitrogen precedes heavy fruit load rather than being a result of heavy fruiting.

### 4. Correlations Between Nitrogen Fractions

Partial correlations between several nitrogen fractions for the different stages of development are given in Table 8.

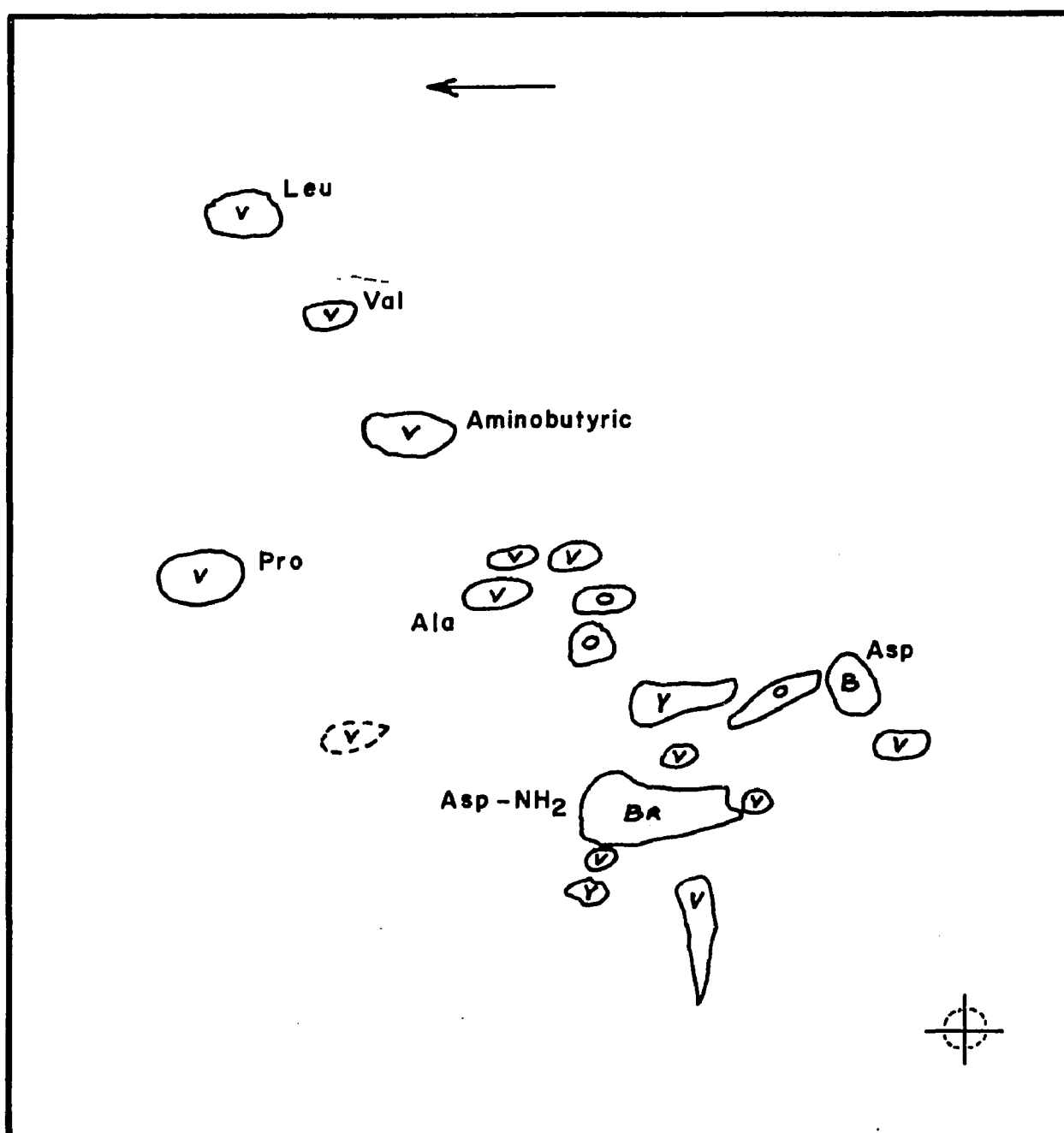


FIGURE 10. AMINO ACIDS - STEM, TOP SECTION, 6 SQUARE STAGE

The arrow denotes the direction of flow of the first solvent.  
 Spot colors: v - violet, y - yellow, o - orange, B - blue,  
 Br - brown.

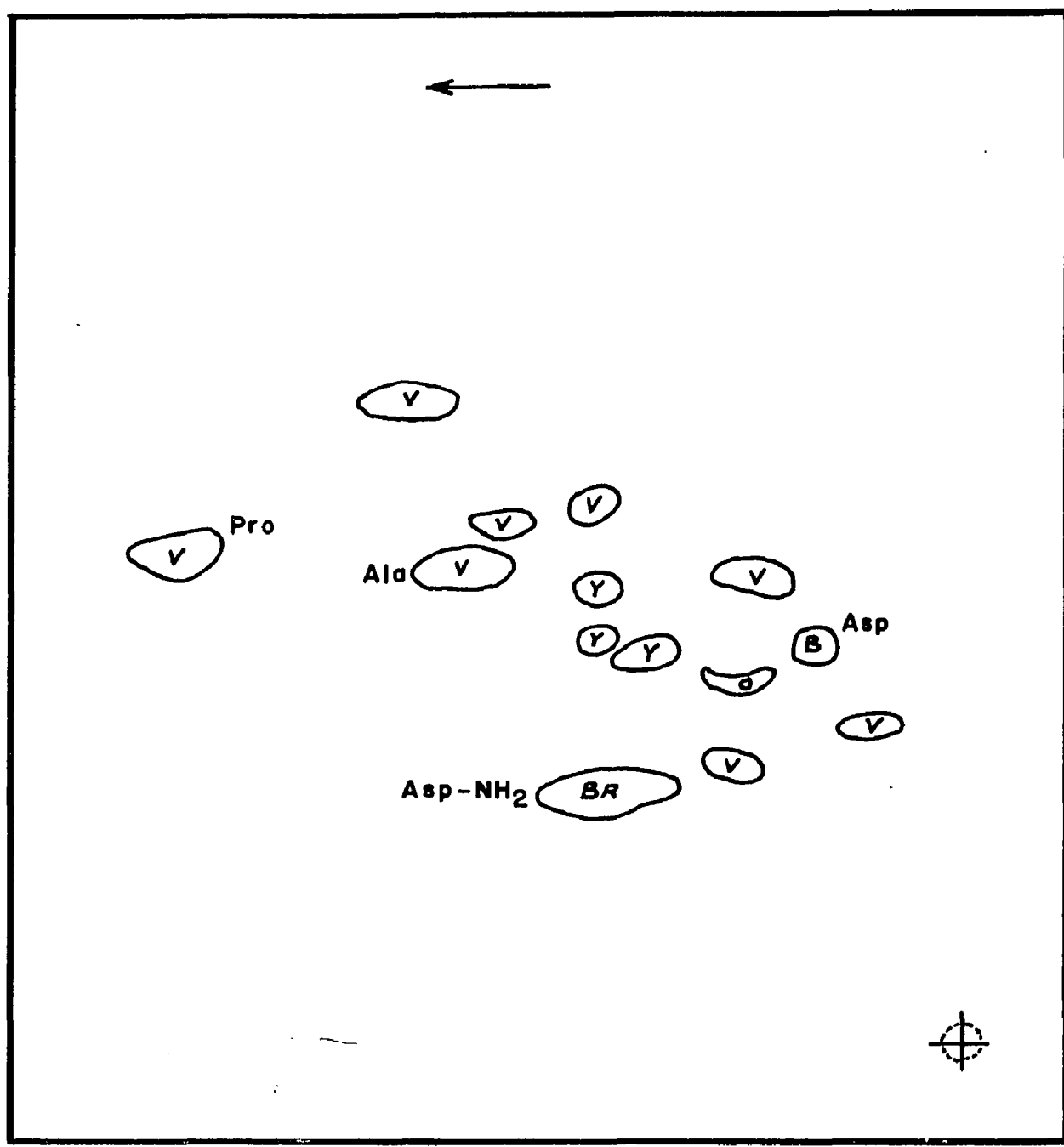


FIGURE 11. AMINO ACIDS - STEM, TOP SECTION, 1 BOLL STAGE

The arrow denotes the direction of flow of the first solvent.  
 Spot colors: v - violet, y - yellow, o - orange, B - blue,  
 Br - brown.



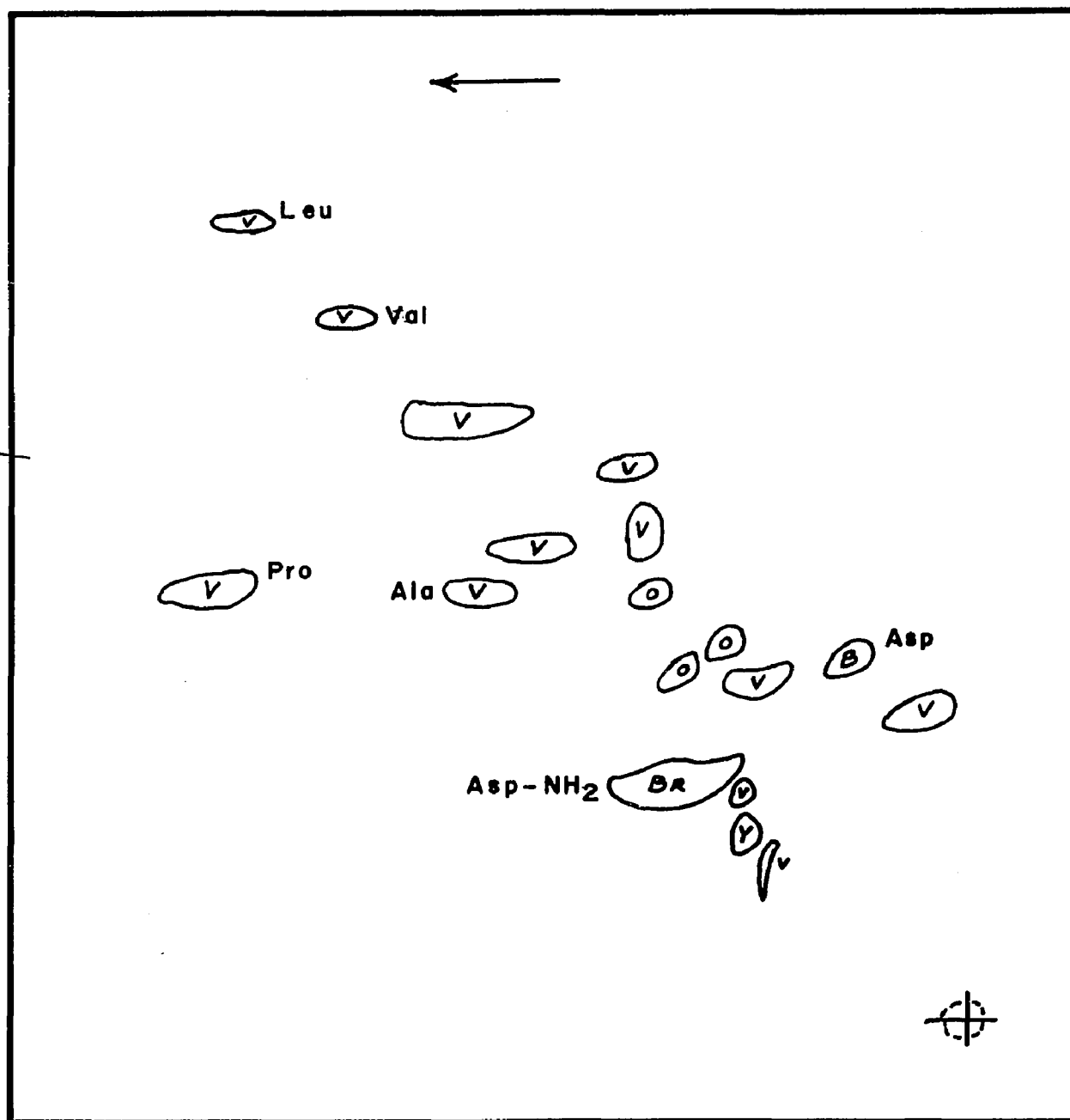


FIGURE 13. AMINO ACIDS - SQUARES, SECTION III, 1 BOLL STAGE

The arrow denotes the direction of flow of the first solvent.  
 Spot colors: v - violet, y - yellow, o - orange, B - blue,  
 Br - brown.

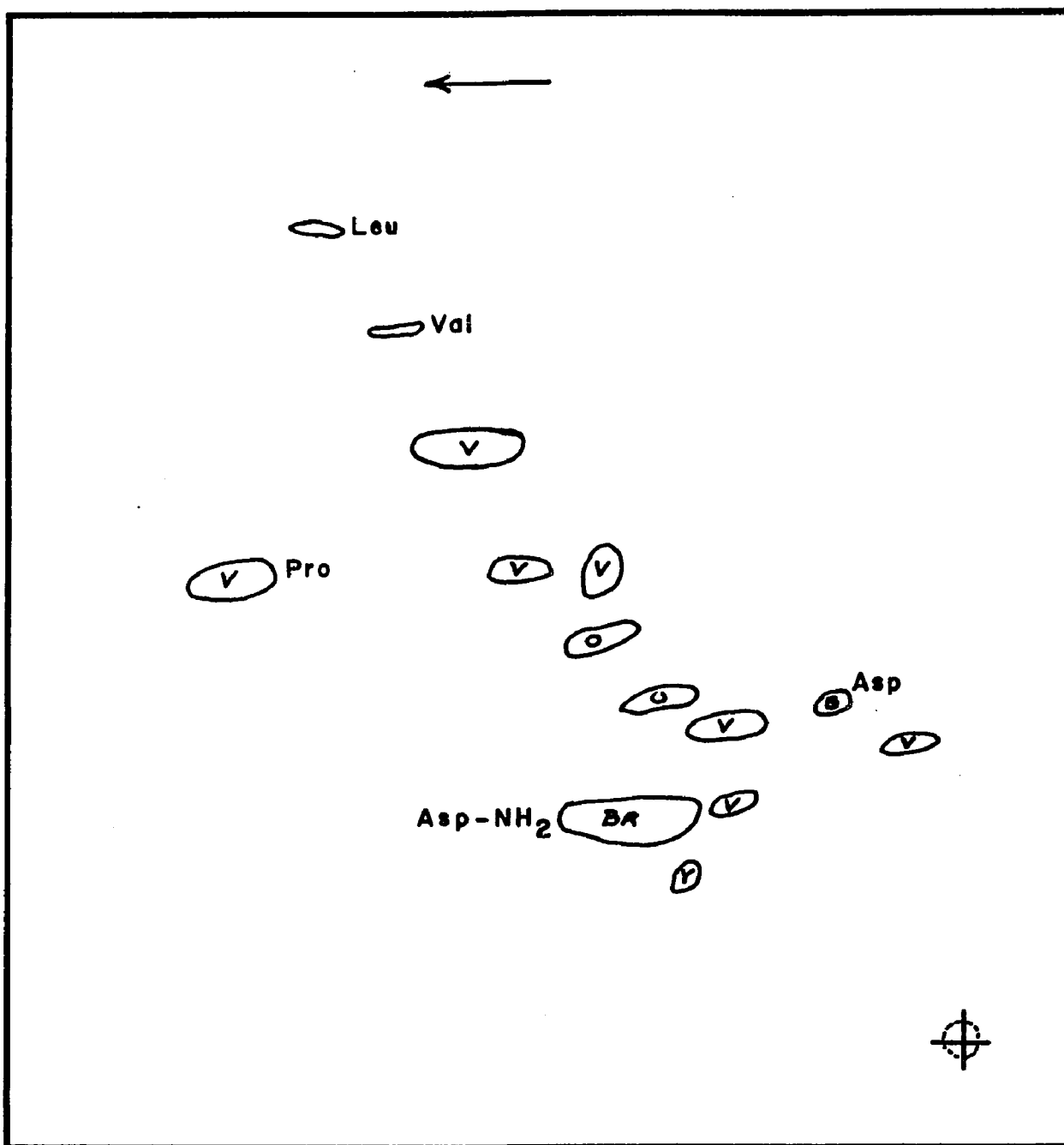


FIGURE 14. AMINO ACIDS - SQUARES, TOP SECTION, 1 BOLL STAGE

The arrow denotes the direction of flow of the first solvent.  
 Spot colors: v - violet, y - yellow, o - orange, B - blue,  
 Br - brown.

Table 6. Changes Occurring in Individual Amino Acids with Stage of Development.

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Acids present at the 6 Square stage but absent at the 1 Boll stage in the top stem (S-T):

Leucine/isoleucine	Histidine	Arginine	Valine	two unknown spots
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Other changes:

Serine increased in concentration at 1 Boll stage.

Aspartic acid and Asparagine decreased considerably.

Alanine more than tripled in quantity;

Glutamic acid was detectable at 1 Boll stage but not at 6 Square stage.

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Acids present at the 6 Square stage but absent at 1 Boll stage in the squares of the two fruiting sections (F-T and F-III):

<u>Section III</u>		<u>Top Section</u>	
Glutamine	Lysine	Glutamine	Lysine
Alanine	Arginine	Alanine	Arginine
two unknown spots		three unknown spots	

Other Changes in These Sections:

Three unknown spots appear at the 1 Boll Stage.

Aspartic acid decreases considerably.

Leucine and Valine decrease about three times from the amount present at the 6 Square stage.

One unknown, possibly Threonine, appears at the 1 Boll stage.

Less Leucine and Valine are present in this section than in Section III at the 1 Boll stage.

Aspartic acid decreased from that in section III at the 1 Boll stage.

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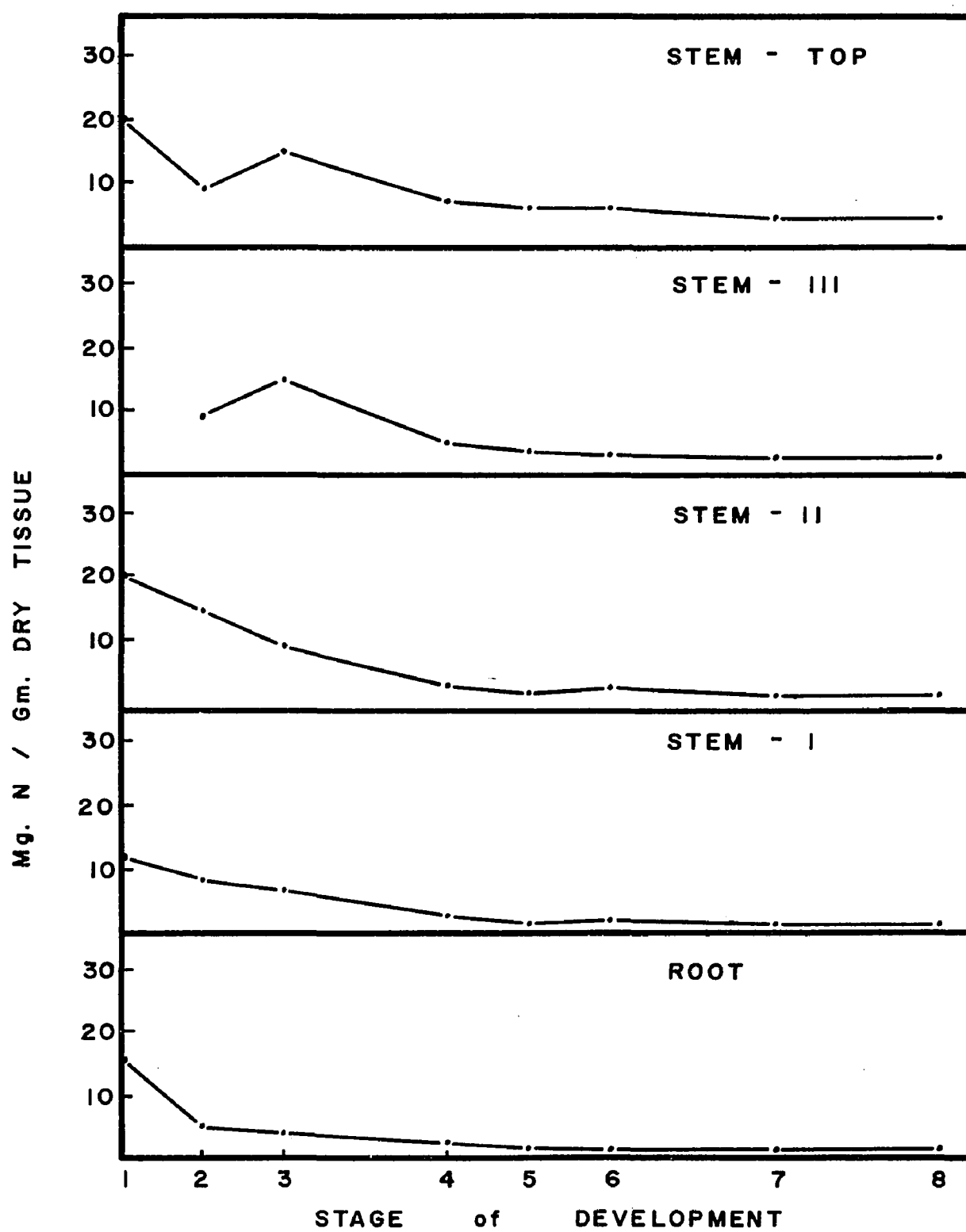


FIGURE 15. NITRATE NITROGEN - STEM AND ROOT

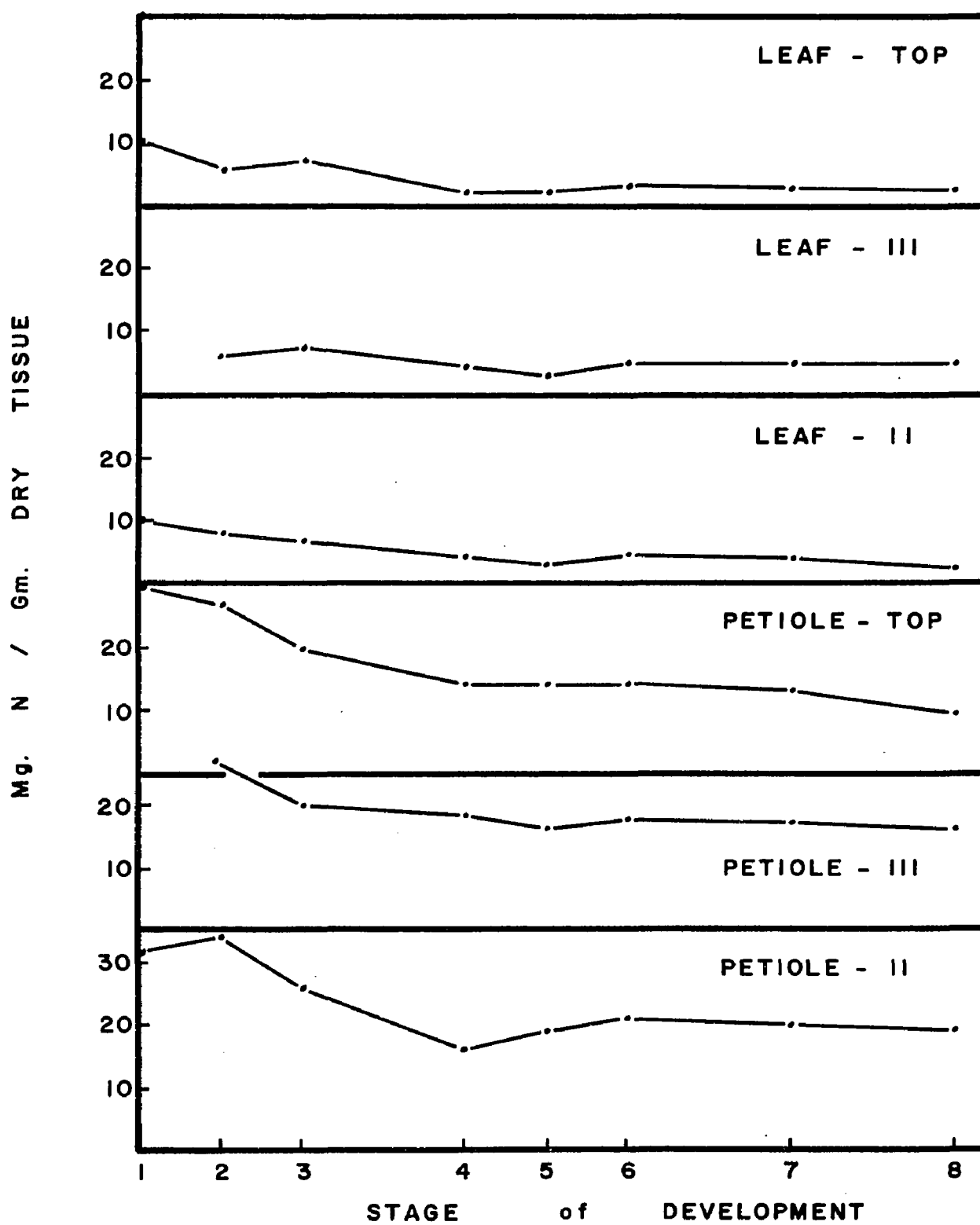


FIGURE 16. NITRATE NITROGEN - LEAF AND PETIOLE

Table 7. Nitrate Nitrogen Concentration at Different Stages of Growth.

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
S-T		9.07 (0.27)	14.84 (0.56)	7.37 (1.96)	6.25 (1.08)	5.77 (0.62)	3.87 (0.09)	4.27 (0.69)
S-III				4.79 (0.64)	3.72 (1.70)	3.32 (0.07)	2.46 (0.49)	2.51 (0.49)
S-II	20.30 (2.23)	14.29 (4.82)	8.93 (0.27)	3.55 (0.14)	2.69 (0.35)	2.91 (0.84)	1.95 (0.21)	1.87 (0.55)
R	16.15 (0.32)	5.48 (0.71)	4.36 (0.28)	2.56 (0.87)	1.10 (0.45)	1.30 (0.36)	0.98 (0.46)	1.02 (0.55)
S-I	11.97 (0.94)	7.75 (0.40)	6.44 (0.43)	2.77 (0.39)	1.26 (0.35)	1.52 (0.23)	0.99 (0.12)	1.05 (0.32)
L-T		5.78 (0.18)	6.68 (0.41)	1.72 (0.74)	1.60 (0.50)	3.16 (1.13)	2.85 (0.42)	1.98 (0.33)
L-III				3.87 (0.49)	3.00 (0.00)	4.59 (1.17)	4.36 (0.47)	4.49 (0.35)
L-II	10.04 (1.52)	8.33 (0.88)	7.07 (0.01)	4.44 (0.44)	2.84 (1.08)	4.70 (2.48)	4.29 (0.26)	2.56 (0.60)
P-T		26.34 (0.88)	19.76 (7.41)	14.06 (0.11)	14.40 (0.20)	13.89 (2.56)	13.24 (0.82)	9.62 (2.19)
P-III				17.95 (1.00)	15.65 (0.90)	17.61 (1.87)	17.33 (0.98)	11.03 (8.97)
P-II	31.28 (0.87)	34.12 (2.18)	26.16 (2.52)	15.96 (6.39)	19.08 (0.64)	20.75 (1.89)	19.78 (4.55)	19.15 (1.81)
F-T				2.32 (0.21)	2.00 (0.05)	2.30 (0.35)	1.66 (0.00)	1.50 (0.41)

Table 7--Continued

Plant Part	Stage of Development							
	Early		Late					
	5 Leaf	Square	6 Square	Square	1 Boll	2 Boll	4 Boll	6 Boll
(mg N/gm dry tissue)								
F-III			2.68 (0.59)	2.44 (0.48)	8.49 (0.42)	2.44 (0.23)	1.79 (0.02)	1.50 (0.40)
FB-T				10.09 (0.97)	8.23 (0.67)	8.97 (1.58)	7.04 (0.30)	6.51 (1.25)
FB-III			13.38 (1.72)	9.21 (0.09)	7.58 (0.65)	7.33 (0.55)	6.08 (0.32)	5.77 (1.45)

Table 8. Partial Correlations of Some Nitrogen Fractions.

N Frac- tions	Stage of Development							
	5 Leaf	Early Square	6 Square	Late Square	1 Boll	2 Boll	4 Boll	6 Boll
Sol. x	1.958	1.990	4.029	1.557	1.516	1.006	1.885	2.490*
NH <sub>4</sub>	0.659	0.515	0.721	0.303	0.290	0.197	0.353	0.446**
Sol. x	2.243	2.779	3.876	2.573	3.897	2.009	2.601	2.945
a-AA	0.708	0.642	0.707	0.465	0.615	0.373	0.462	0.508
a-AA x	2.257	2.126	3.985	1.114	4.703	3.544	4.031	4.256
NH <sub>4</sub>	0.710	0.540	0.717	0.222	0.685	0.578	0.606	0.648
Prot.		1.389	1.363	3.602	1.746	1.541	1.315	1.450
x NO <sub>3</sub>		0.386	0.332	0.592	0.330	0.295	0.254	0.278
a-AA x			1.687		1.721	1.490	2.012	1.946
Prot.			0.399		0.326	0.286	0.373	0.363
Sol. x				2.625	2.883			3.227
Prot.				0.472	0.500			0.542
Sol. x						1.385	3.798	2.702
NO <sub>3</sub>						0.267	0.605	0.475
a-AA x						1.564	1.442	1.540
NO <sub>3</sub>						0.299	0.277	0.294
df	5	11	15	-----25-----				
t <sub>.80</sub>	1.476	1.363	1.341			1.316		
t <sub>.90</sub>	2.015	1.796	1.753			1.708		
t <sub>.95</sub>	2.571	2.201	2.131			2.060		

\* t-value

\*\* coefficient of partial correlation

Sol. - Total Soluble Reduced N

NO<sub>3</sub> - Nitrate NNH<sub>4</sub> - Ammonium N

Prot. - Protein N

a-AA - a-Amino acid N

The high degree of correlation between the total soluble and the  $\alpha$ -amino acid nitrogen fractions is to be expected for the reasons discussed above.

The majority of the correlations between the various nitrogen fractions are to be expected on the bases of the accepted metabolic pathways of nitrogen. Likewise, it is to be expected that most of these nitrogen fractions should show a high degree of correlation once boll development had begun. At this time growth of the plant has essentially ceased, the various organs and tissues have reached maturity, and the various metabolic reactions are in a state of dynamic equilibrium.

Two rather surprising features of this statistical analysis are (1) the lack of correlation between ammonium and nitrate, especially after boll development has begun; and (2) the degree of correlation between the protein and nitrate fractions throughout the growth of the plant. Neither of these observations can be explained from the present study.

#### 5. Fruiting Pattern of Gregg 35

Four plants from this study were selected at random and were allowed to grow until all of the bolls which were set had matured. A composite fruiting pattern from these four plants is depicted schematically in Figure 17. This fruiting pattern is quite similar to the generalized pattern of McNamara, et al., (49).

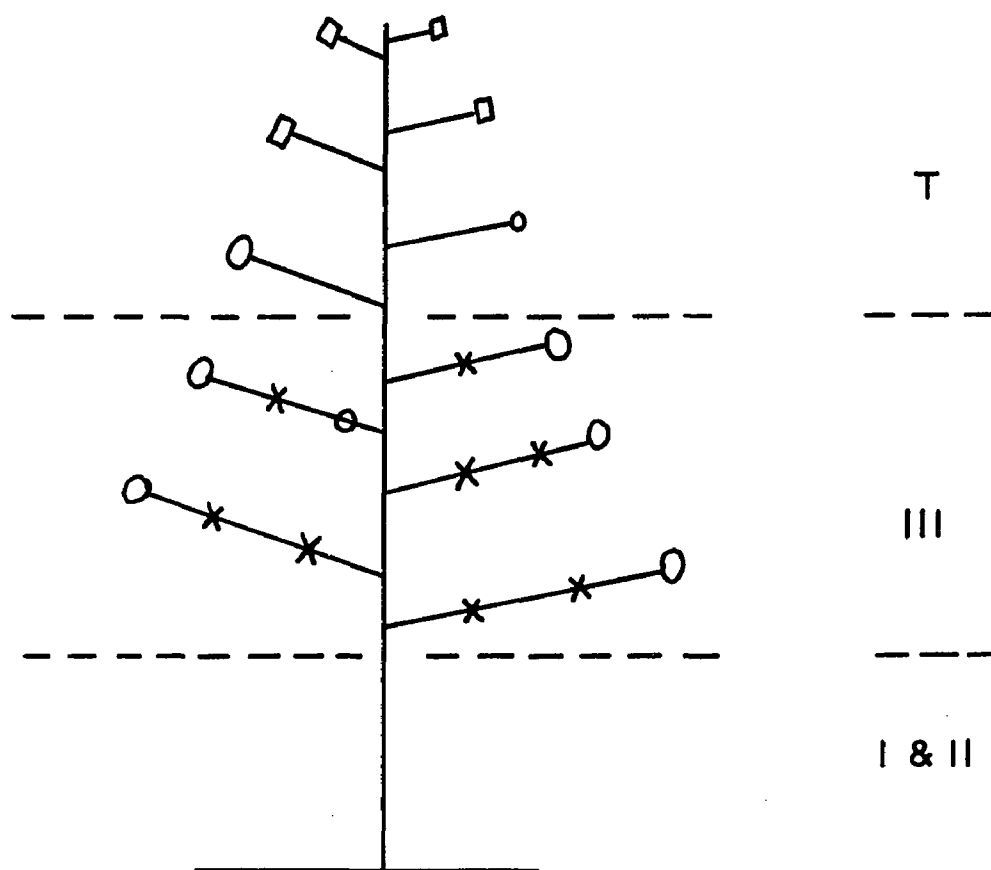


FIGURE 17. FRUITING MAP OF GREGG 35.

X = FLOWER WHICH MATURED A BOLL.

O = YOUNG BOLL ABSCISSED.

□ = SQUARE ABSCISSED.

## 6. Free Sugars

The free sugars - fructose, glucose, and sucrose - were observed semi-quantitatively in several plant parts. Although there were some fluctuations in the quantities of these sugars with the stage of development, it was apparent that there were adequate quantities of these sugars available in the various organs at all stages of development. The only qualitative change was the absence of sucrose at Late Square stage in the top stem section. This may be related to the high level of ammonium which occurs in this section at this time as discussed above.

One observation of interest was the fact that no sucrose was detected in the squares at any time. This indicates a high rate of metabolism is occurring at all stages of their development.

Reproductions of the thin-layer chromatograms are presented in Figures 18 through 20.

FIGURE 18. FREE SUGARS - STEM, SECTIONS III and TOP

A = origin, S = sucrose, G = glucose, F = fructose

1 = 5 Leaf stage of development

2 = Early Square stage of development

3 = 6 Square stage of development

4 = Late Square stage of development

5 = 1 Boll stage of development

6 = 4 Boll stage of development

7 = 6 Boll stage of development

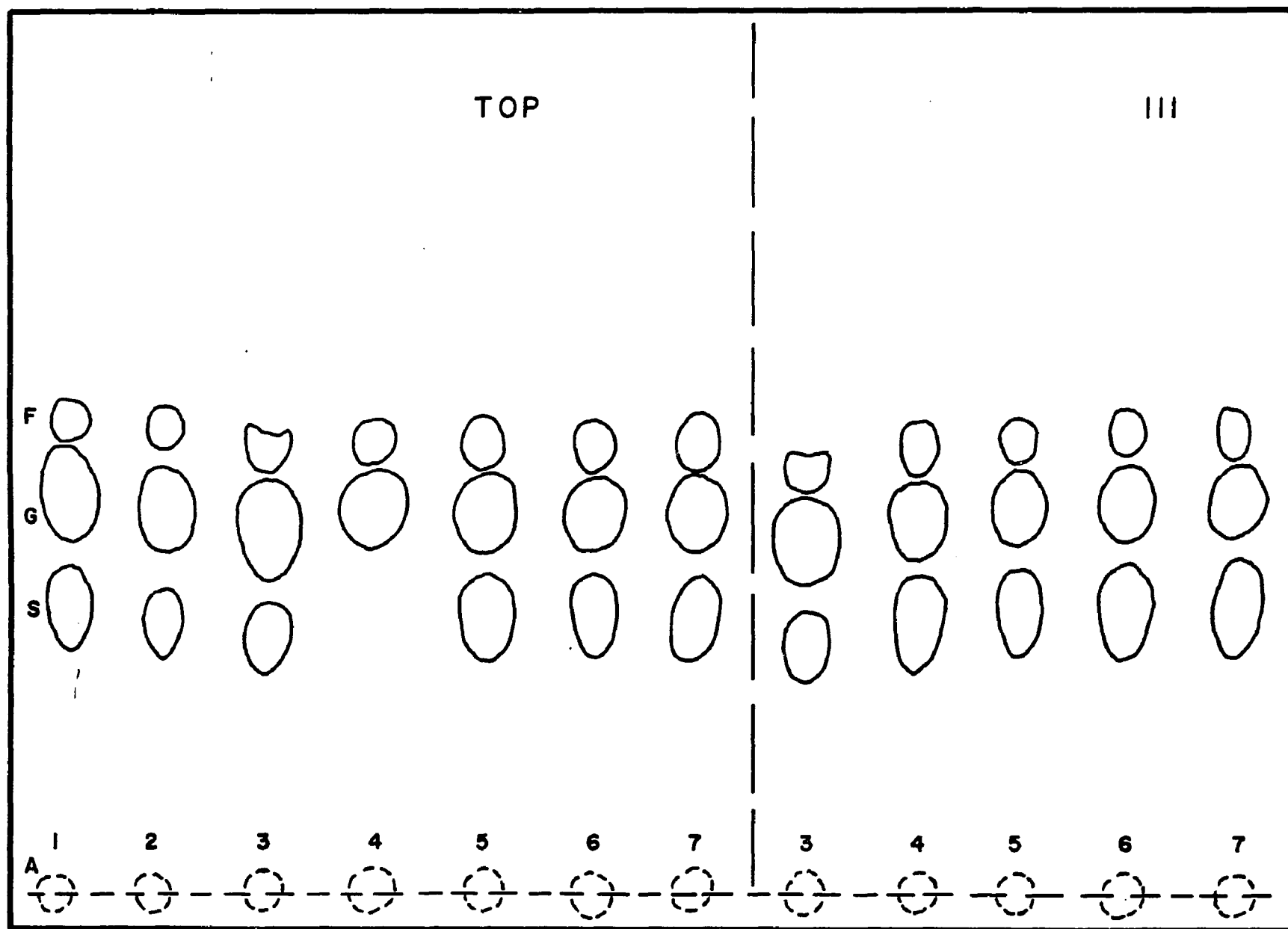


FIGURE 18.

FIGURE 19. FREE SUGARS - FRUITING BRANCH, SECTIONS III  
and TOP

A = origin, S = sucrose, G = glucose, F - fructose  
1 = 6 Square stage of development  
2 = Late Square stage of development  
3 = 1 Boll stage of development  
4 = 4 Boll stage of development  
5 = 5 Boll stage of development

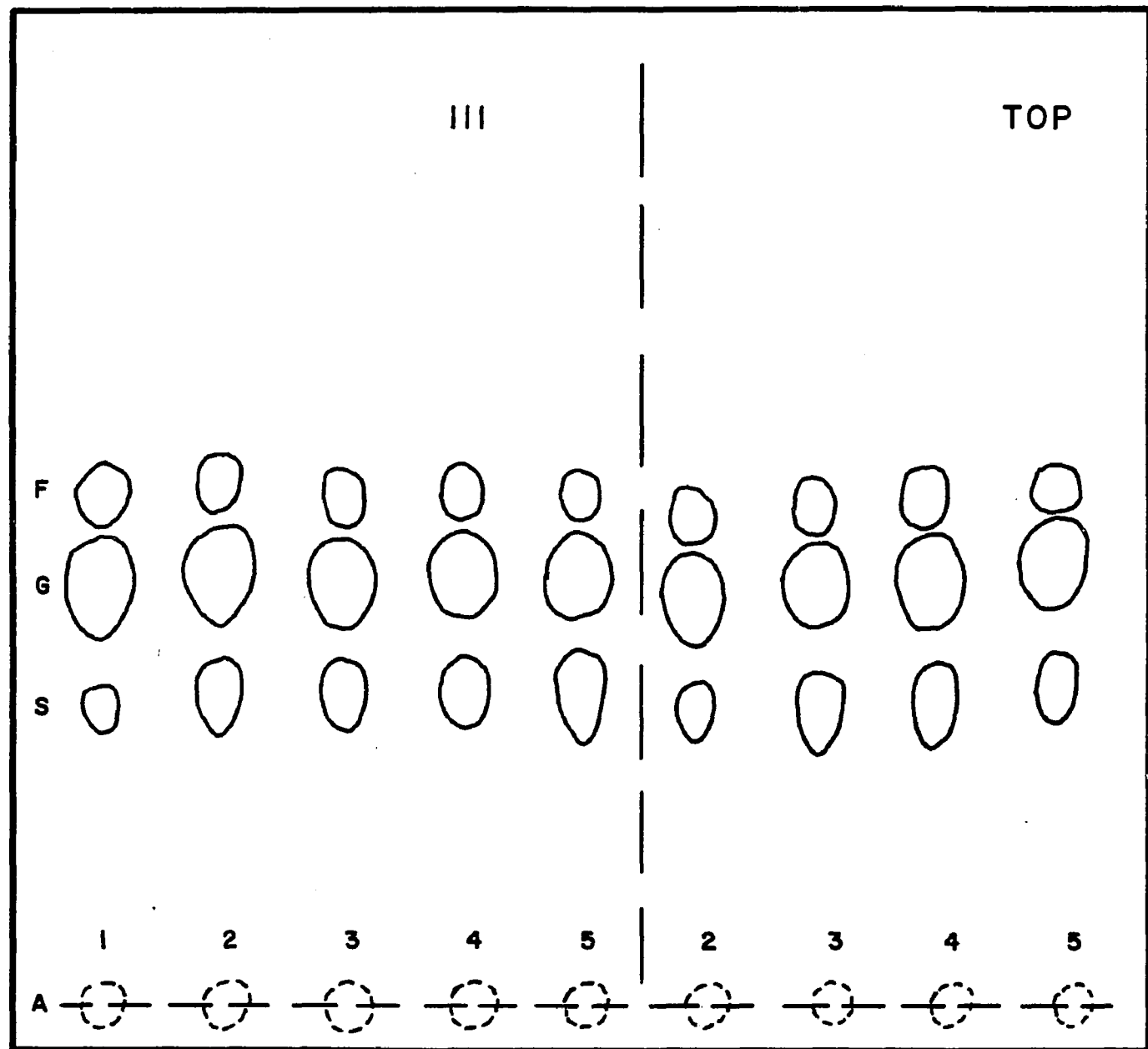


FIGURE 19.

FIGURE 20. FREE SUGARS - SQUARES, SECTIONS III and TOP

A = origin, G = glucose, F = fructose  
1 = 6 Square stage of development  
2 = Late Square stage of development  
3 = 1 Boll stage of development  
4 = 4 Boll stage of development  
5 = 6 Boll stage of development

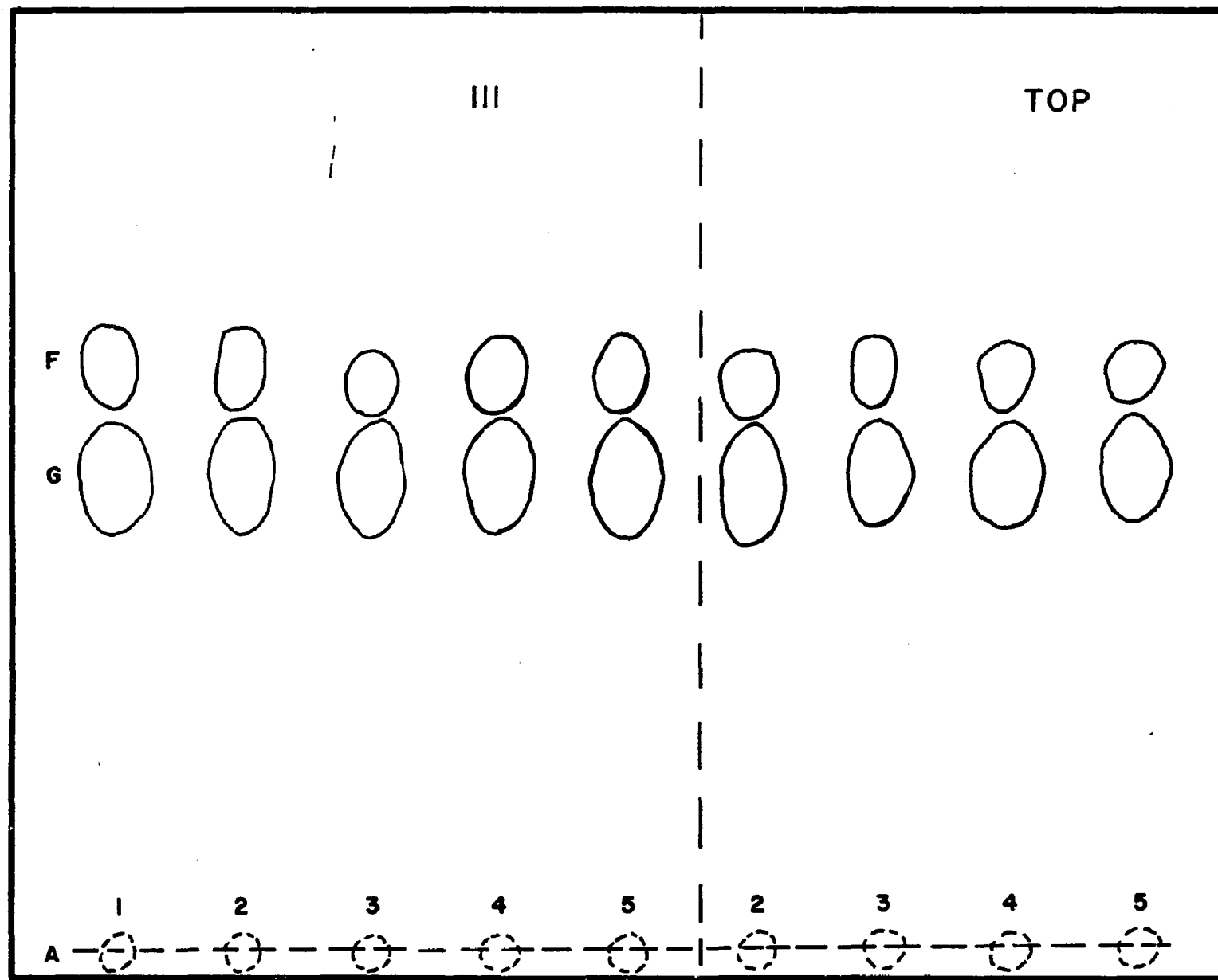


FIGURE 20.

## DISCUSSION

### A. Lack of Fit of Previous Data to the Occurrence of Boll Shedding

Although it is well known that plants adapt themselves to their environment and can adjust rapidly to extreme changes in climatic conditions, it is doubtful that the environmental factors (with the possible exception of high night temperatures) play an important role in fruit abscission. Certainly climatic conditions are not the principal factor causing abscission.

That the nutritional theory has much in its favor can be seen from the amount of study devoted to it for over forty years by many workers using many kinds of plants. There are many discrepancies in the cotton literature, however, between the data reported and the conclusions which have been drawn regarding the nutritional theory. A few of these will be discussed in some detail in this section.

#### 1. Morphological Observations

The statement has often been made that heavy boll shedding is commonly coincidental with growth inhibition (19, 61). The inference is that the developing bolls exert such a drain on nutrients that there is an insufficient amount to support the growth of either new bolls or additional vegetative tissue.

Mason's work on Sea Island cotton (G. barbadense) showed that the growth rate declined steadily and rapidly from the onset of flowering. Growth had essentially stopped two weeks before peak flowering and about 25 days before the heaviest shedding began (45). He also states that in other studies it was found that the growth-rate began to decline as soon as boll-development was initiated.

Crowther's data shows that growth had essentially ceased at least 8 days before peak flowering. How long before this the growth-rate began to decline cannot be determined since data is only presented for two dates.

Wadleigh showed that the maximum rate of growth occurs during the period of square formation. The rate of growth is reduced within one to two weeks after first bloom (69). The length of time after bloom and the degree of reduction of the growth-rate are determined in a large part by the nutritional status (especially nitrogen) of the plant. Regardless of the nutritional status, however, the rate of growth is reduced before peak flowering is reached.

Hancock reported on three years' experiments with five varieties of G. hirsutum grown near Knoxville, Kentucky (33). The rate of plant growth consistently began to decline at least two weeks before peak flowering.

Hamilton, et al., reported no additional nodes were added to the main stem after blooming began at Yuma, Arizona (32). In another

study conducted at Yuma, Martin observed the plant ceased growth at least 11 days prior to peak flowering (44).

Gardner, in studies conducted at two elevations in Arizona, has also reported the decline in growth-rate precedes peak flowering (28).

From these studies it can be concluded that the decline in growth-rate is more closely related to the onset of blooming than it is to the flower and fruit load of the plant. The extent of the decline in growth-rate for a given variety is influenced by the nutritional status - especially the nitrogen status - prior to blooming rather than by the demand for nutrients exerted by a heavy boll load late in the season.

## 2. Nutrient Supply

Basic to the nutritional theory of fruiting is the supposition that the developing bolls drain the plant of nutrients taken up from the soil and stored during the early stages of growth. The plant then has an insufficient supply of nutrients (primarily nitrogen) to produce additional vegetative growth or bolls during peak flowering. The result, then, is that after a certain number of bolls are set, any additional fertilized ovules must absciss because of a lack of building material.

Data of Crowther (13), Olson and Bledsoe (53), Dastur and Ahad (15), and Malinkin and Protasov (43) are at variance with this supposition. These data show that regardless of variety, season, or location at least 80 percent of the nutrients (N, P, K, Ca, and Mg)

are taken up after the plant has begun blooming. Based on flowering curves of Mason (45), Crowther (13), and Gardner (28, 29) most of this 80 percent is taken up in the interval from peak flowering to the end of the normal season. This period, from peak flowering until cut-out, is also the interval of heaviest shedding. Thus it does not appear that nitrogen or other elements are limiting boll-set when they are present in adequate amounts in the soil throughout the season.

### 3. Carbohydrates

One of the earliest assumptions of a cause of fruit abscission has been that the carbohydrates become limiting when fruit load is heavy. This has been cited without substantiation by many authors (22, 41, 45, 52) to explain decreased growth with the onset of fruiting as well as fruit abscission in many kinds of plants. It has been claimed that the fruit drains the roots of carbohydrates to the extent that there is an insufficient amount left to provide the energy for nutrient uptake from the soil (22). The data on nutrient uptake cited in the preceding sections fail to support this view.

The studies of Ergle (24, 25) show that the soluble carbohydrate level in the tops increases during the summer. Dastur and Bhatt (16) show reducing sugars in the leaf and reducing and non-reducing sugars in the stem decline from square stage to flowering and remain at a constant level throughout the flowering period. Starch declines briefly during heavy flowering but regains the former level rapidly thereafter.

These data strongly suggest that since soluble sugar levels remain essentially constant, or increase slightly during the fruiting period, and since there still is some reserve starch even during heavy shedding ( 16, 22 ) carbohydrates are not the factor causing abscission through limiting supply.

#### B. Interpretation of the Present Data

The first observations to be noted are morphological. Firstly, at the time of first flower the plant already has initiated and is developing more squares than the total number of bolls which will be set by that plant. Secondly, the first squares initiated are the ones most likely to produce mature bolls. Thus, the lower fruiting section contains most of the bolls while the top region sheds the majority of its squares and young bolls. These facts, by themselves, would seem to support the view that the first bolls set have first call on nutrients with the results that there is an insufficient supply to enable additional bolls to develop.

However, the decided drop in soluble nitrogen in the various plant parts between the middle of the square period and Late Square stage indicates some other phenomenon is the principal cause of fruit abscission. The number of squares which had been initiated before the drop in nitrogen occurred is the number of bolls which matured. Their positions on the plant also coincide.

Another observation which invalidates the hypothesis of boll-load causing the drain on nutrients is that this drop in soluble nitrogen occurs about, or before, first bloom depending on the variety. Furthermore, if the developing bolls created a sink for soluble nitrogen one would expect to find a continuous decrease in soluble nitrogen inversely proportional to the number of bolls developing. This is decidedly not the case. The soluble nitrogen concentration remains essentially constant in the various plant parts at the low level regardless of the number of bolls developing.

The squares generally do not reflect the same pattern for nitrogen as do the other plant parts. Since the squares in each section were not subdivided into stages of development one would expect the concentration of nitrogen to remain constant throughout the growth cycle for a given fruiting section. However, if the developing bolls in region III were draining the nutrients away from the top fruiting region one would expect the squares of the top section to contain less soluble nitrogen than those of region III. This is not the case.

The qualitative differences in amino acids further indicate that the decrease in the soluble nitrogen content reflects a change in the over-all metabolism of the plant. It is outside the scope of this investigation to determine exactly which biochemical reactions and metabolic pathways have been adversely affected by these amino acid changes. The decrease in the quantity of asparagine is to be expected since its role as

the predominate organic transporter of nitrogen would require a decrease in this compound coincidental with a decrease in the total soluble reduced nitrogen fraction (72).

The absence of certain amino acids in squares which are shed, or which produce bolls that are shed, could indicate a lack of certain key enzyme systems necessary for the proper development of a viable flower and eventual mature boll. This could also be interpreted as the cause of the absence of these enzyme systems. If one of these amino acids serves as an effector of an inducible enzyme system of the type demonstrated by Jacob and Monod (35), then its absence would result in the repression of this enzyme system. It is also possible that both explanations are applicable.

The results of this present study, as well as those results of other investigators discussed previously, indicate that soluble carbohydrates are not limiting at any stage of growth under normal conditions. It is doubtful, then, that a deficiency of carbohydrates is a cause for square and boll shed.

One further observation concerning flower pigmentation supports the thesis that the squares which are initiated after the decrease in soluble nitrogen occurs do not develop normally. It has been observed for both Acala 4-42 and Gregg 35 under controlled greenhouse conditions that the flowers whose young bolls shed do not develop the usual red

pigmentation until at least 24 hours after the flower opens compared with about six hours for flowers whose bolls mature. The specific causes of this difference are not known. It is evidence, however, that the metabolism of the later flowers is different than that of the first blooms.

Based on the results of this study, it is suggested that a change in nitrogen metabolism occurring prior to flowering is a fundamental cause of later boll and square abscission. It is hypothesized that these phenomena are related in the following manner. The normal cotton plant contains a high concentration of soluble nitrogen during its early stages of growth. A high concentration of soluble nitrogen is required for proper square development. The developing squares produce a growth-regulating compound which, when present in sufficient amounts, causes a change in the nitrogen metabolism of the plant which results in a general decrease in the soluble nitrogen concentration through an accelerated utilization rate, as well as in the qualitative change of specific compounds such as certain amino acids. Squares initiated after this occurs do not develop normally and cannot, therefore, produce viable bolls. Bolls produced from these later squares are shed. Growth is also affected, probably as a result of these qualitative changes in soluble compounds. Normally developing bolls also produce a soluble growth regulator (Abscisin II ? ) which, when present in sufficient amounts, causes the

cessation of square initiation, i. e., cut-out. The difference between varieties in flowering and boll-set patterns are probably due to differences in threshold values of susceptibility to the regulator produced by the squares.

### C. Agreement Between the Present Hypothesis and Other Studies

A necessary test of any hypothesis is its agreement with data reported in the literature previously. Some of these findings will be considered in this section.

#### 1. Morphological

As has been discussed above, the cotton plant's main stem elongation rate is greatly retarded about the time of first bloom. The same is true for the tap root (56). The exact physiological stage at which this occurs depends on variety, nitrogen status, and environment. Close examination of the literature, however, reveals that growth is inhibited much before peak flowering and boll-set.

The present data indicate that the major decline in soluble nitrogen precedes the decline in growth by a few days. It has also been shown that certain amino acids disappear from the soluble nitrogen pool at this time, reflecting a general change in the nitrogen metabolism of the plant. It is this metabolic shift, then, which results in cessation of growth rather than boll load.

Several investigations of the effect of removal of fruit on growth have been conducted. The work by Dale (14) will be considered here since it is more complete than most. Dale found that by weekly removing all squares whose bracts measured larger than 5mm the plants continued to grow and initiate squares. The plant would soon cease growth and square initiation when debudding was terminated. The controls ceased growth between 7 and 10 weeks after first square, i.e., 3 to 6 weeks after first bloom. It can be estimated from his data that the controls resumed growth after cut-out about 14 weeks after first square, or about the time the first samples were taken for chemical analyses. The debudded plants ceased growth within three weeks after normal fruiting was allowed. This would indicate it was the square load rather than boll load which regulated growth in some manner.

Dale reported further that at 23 weeks after planting, soluble nitrogen was slightly higher, although not significantly so at the 0.95 probability level, in the debudded plants than in the controls. Perhaps one reason the difference was not significant may be that the controls were nearing the end of the cut-out period and beginning new growth, hence the level of soluble nitrogen was rising again. The controls had a significantly greater percentage of both total and reducing sugars in the stem at the 23 week sampling period and total sugars in the stem at the end of the experiment (35 weeks after planting) than did the debudded

plants. Thus carbohydrates were not limiting fruiting or growth.

## 2. Nitrogen

Crowther (13) reports a continual increase in square production through the first six weeks of the squaring-fruiting period for adequate nitrogen, but a constant decrease in the nitrogen concentration of the squares throughout this period. The majority (over 80 percent) of the seed cotton was produced from squares containing more than 3 percent nitrogen. Peak boll production occurred after the nitrogen in the squares fell below this level. Again the evidence indicates the change in nitrogen metabolism which occurred by the time of first flower rather than boll load regulates boll abscission. Similarly, Armstrong and Albert (5) reported on the basis of three years' experiments the nitrogen concentration in the stem began to decline several days before the first flowers bloomed.

Eaton and co-workers (20, 22, 23) consistently found a higher nitrogen level in debudded plants than in normally fruiting controls. Since their control plants were still fruiting at the time of sampling it would appear that by removing flower buds the shift in nitrogen metabolism is prevented and the plant is maintained in the immature state. The nitrogen shift occurred prior to peak boll load as evidenced by the low nitrogen concentration of the still fruiting plants.

Wadleigh found a decrease in nitrogen concentration between his two sampling dates and concluded the loss of nitrogen was due to boll load (69). In view of other evidence presented above, it is likely that he would have found the same decrease if his samples had been taken two weeks apart. His first sample was taken just before first flower.

### 3. Seed Number

Walhood (70, 71) has reported that the bolls which are shed have fewer seeds than those which mature. He concluded: "...there was sufficient pollination but fertilization was not complete" (70). This is in agreement with the hypothesis that the decrease in soluble nitrogen adversely affects the development of the squares initiated after the shift in metabolism occurs.

### 4. Earliness, Percent Set, and Relative Fruitfulness

Earliness in cotton is generally regarded as the percent of total yield obtained on first picking. This topic has recently been reviewed by Tucker and Tucker (62). Where detailed records are available, it can be seen that between 70 and 90 percent of total yield is produced before peak flowering occurs (28, 29, 44). With a long season the second flowering peak, or "top crop," may contribute significantly to the total yield. This is true, however, only when adequate nitrogen is available (29).

"Percent Set" reflects the same phenomenon; high percent set occurs early in the fruiting period and low percent set is found from about peak flowering until cut-out. In keeping with the present hypothesis, the squares initiated before the nitrogen shift occurs produce most of the mature bolls with the resulting high percent set. The squares initiated after the shift in nitrogen seldom develop a mature boll. These flowers are responsible for a small part of the total yield and result in lowering the percent set.

The relative fruitfulness concept of Eaton can be explained similarly. Each variety has its own early growth rate. Each undergoes the nitrogen shift at slightly different physiological stages. The more squares which can be initiated before the nitrogen shift occurs, the greater will be the relative fruitfulness value.

## 5. Growth Hormones and Senescence

The role of growth hormones in abscission has been extensively studied since 1933. The majority of the work has dealt with excised petioles and leaf explants. Although much has been learned about changes within the abscission zone proper, little can be deduced of the physiological events within the whole plant which initiate the abscission process. Also, although the occurrences in the abscission zone may be similar, the physiological changes preceding activation of the abscission zone may be quite different in the fruit than in a leaf.

The distal-proximal gradient of IAA seems to be involved in the actual triggering of the abscission process (3, 9). Evidence is accumulating, however, which supports Jacob's view (36) that "auxin is primarily causing growth and only indirectly affecting abscission." Thus it would seem that the physiology of the organ is affected adversely before IAA declines and triggers the abscission process.

The multi-faceted growth inhibitor Abscisin II has been isolated from young cotton bolls. This compound has been shown to accelerate the abscission process, partially inhibit the action of auxins, gibberellins, and cytokinins, and promote senescence (9, 59). It seems doubtful that such a powerful compound can be active in bolls which mature as well as in bolls which are shed. Smith has reported preliminary findings of an abscission retarding substance, not an auxin or gibberellin, which is also present in young bolls (58, 59). The literature does not indicate from which stage of the fruiting period the bolls were taken for extraction of these compounds, however.

These findings indicate that bolls which mature contain different enzyme systems than do those which absciss. This view is also supported by the findings of Walhood that even with adequate pollination, fertilization is partially prevented in flowers whose bolls are shed.

The work of Leopold and co-workers (56) supports the view that the abscission phenomenon is the result of senescence. Thus the tissue

has ceased to grow and has begun to degenerate before the abscission zone is activated.

None of these studies, however, have attempted to explain why bolls at one stage of the fruiting period mature while those of another stage absciss. The present hypothesis is in agreement with the above facts and offers an explanation of why some squares and bolls have a different physiological chemistry than others. Those squares which are initiated during the period of high soluble nitrogen concentration and "complete" amino acid pool possess the enzyme systems necessary for boll maturation and abscission inhibition. Those initiated after the decrease in soluble nitrogen concentration and depletion of the amino acid pool do not possess the necessary enzymes for continued growth. Hence, shortly after anthesis senescence activity commences, terminating with the abscission of the boll.

Leopold, et al., (40) have shown that the developing reproductive organs initiate the senescence process in annuals. Leopold (39) has reviewed other investigations which show the same phenomenon. Cotton is similar to the tomato in that it is a weakly senescing species of the progressively senescing type. Murneek's results with tomatoes (51, 52) have been reproduced in cotton by several workers (for example, Dale). The results with cotton indicate that a compound or group of compounds - growth inhibitors - are produced by the developing squares. The effect

of the inhibitors is cumulative. When a sufficient number of squares produce the necessary concentration of the inhibitor(s) the senescence process is initiated. Some of the effects of the senescence process are the decrease in concentration of soluble nitrogen, phosphorus and potassium, and changes in the amino acids of the free amino acid pool. Only after this process has been initiated do the older vegetative leaves yellow and fall off. Also after this process has begun, an increase in the number of aborted bolls occurs.

The process is reversible since on removal of developing bolls and squares the soluble nitrogen level increases and a rapid growth rate resumes. When nitrogen is adequate in the soil, the vegetative leaves are retained.

#### D. Areas for Future Investigation

One of the most critical areas requiring immediate investigation is the developmental physiology of the flower bud. Essentially nothing is known of this aspect of plant development for any plant. Until detailed information on the types of compounds present and their qualitative and quantitative changes during development is obtained, little can be learned of the inter-relationships between the whole plant and the reproductive organs regarding senescence and abscission.

The qualitative and quantitative aspects of the soluble nitrogen fraction should be studied in greater detail within the squares and with

regard to the inter-relationships with other organs. Changes in RNA and non-nitrogenous compounds such as the phenolics and steroids should be investigated in relation to shifts in soluble nitrogen and square and boll development. The compounds in the squares which induce the metabolic shift must be isolated and identified. Little can be done to increase fruit set until this has been accomplished. The occurrence of Abscisin II and the anti-inhibitors should be determined in the flower buds as well as in bolls taken from different stages of the fruiting period. The occurrence should be considered in relation to the concentration and components of the soluble nitrogen pool.

These studies should involve several different varieties of a species. When possible, different species within a genus should be investigated simultaneously. If this is not done, it is difficult to develop theories having general applicability. Several stages of development should be studied to reveal relatively long range cause-effect relationships. Much of the research on cotton has been wasted effort because the investigators studied either a few plant parts at a single stage of development or a single plant part at only a few stages of development. The total plant must be considered to reveal correlations between the various organs and tissues. These relationships cannot be observed when only explants are used.

## SUMMARY

The role of nitrogen in fruiting abundance of cotton has been investigated. The study involved a greenhouse experiment using Gregg 35. Several nitrogen fractions were followed in various plant parts through progressive stages of development. Free sugars were also observed semi-quantitatively.

It was found that a decrease in soluble nitrogen accompanied by changes in some free amino acids shortly before first bloom could be correlated with boll abscission later in the fruiting period. There was no apparent correlation between free sugar content and boll abscission.

These results were compared with data reported by others. It was concluded that the present results could explain the cause of many diverse phenomena observed by others.

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