THE INTERACTION OF NITROGEN AND MAGNESIUM DEFICIENCIES IN CERTAIN ASPECTS OF THE PHYSIOLOGY OF THE COTTON PLANT

A Thesis

By

DEBABRATA GHOSH

Submitted to the Graduate School of the
Agricultural and Mechanical College of Texas in
partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

January 1961

Major Subject: Plant Physiology

THE INTERACTION OF NITROGEN AND MAGNESIUM DEFICIENCIES IN CERTAIN ASPECTS OF THE PHYSIOLOGY OF THE COTTON PLANT

A Thesis

By

DEBABRATA GHOSH

Approved as to style and content by:

H. E. Joham
Chairman of Committee

David W. Rosberg
Head of Department

January 1961

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. Howard E. Joham, professor of plant physiology, for his guidance, kind assistance and encouragement throughout the course of this investigation without which it could not have been completed.

Sincere thanks are due to Dr. David W. Rosberg for his helpfulness.

Appreciation is also extended to the members of the committee, Dr. Wayne C. Hall, Dr. A. G. Caldwell and Dr. C. K. Hancock for giving generously of their time in reading the manuscript.

TABLE OF CONTENTS

LIST OF TABLES

Page

THE INTERACTION OF NITROGEN AND MAGNESIUM DEFICIENCIES IN CERTAIN ASPECTS OF THE PHYSIOLOGY OF THE COTTON PLANT

INTRODUCTION

Much work has been done with single nutrient deficiencies employing both macro- and micro-nutrients. Wallace, for instance, has studied mineral deficiencies for many years and has published a series of color photographs showing particular nutrient deficiency symptoms (33). The American Society of Agronomy and the National Fertilizer Association sponsored a symposium and presented a very comprehensive treatment by various authors (2) on the subject of single mineral deficiencies in various crops. Nitrogen deficiency in cotton has been studied in some detail by Wadleigh (36) and magnesium deficiency in cotton has been studied by Cooper (6, 7) and Helmy, Joham, and Hall (11). Although our knowledge of single deficiency symptoms is rather extensive, information is essentially lacking concerning the characteristic expression of deficiency when more than one element is in short supply. Thus the first objective of this study was to observe and record the expression of deficiency symptoms resulting from multiple deficiencies of two elements. The utility of double or multiple deficiencies lies in the fact

that oftentimes poor growth of cotton may not be the result of the lack of one single nutrient, but due to lack of several nutrients together, and in agricultural practice, the recognition of such unbalance is needed.

The second objective of this investigation was to study the interaction of nitrogen and magnesium in cotton with regard to linear growth, green and dry matter production, flowering, fruitfulness, and seed cotton yield. Leibig's Law of the Minimum states that the amount of plant growth is controlled by the essential factor which is present in limiting concentration, and furthermore, growth either increases or decreases as the level of the essential factor rises or falls. Extending Leibig's idea to the situation where two essential factors are limiting growth, supplying one of them will have less effect on growth than when both are supplied together. Two such factors are said to have a positive interaction when the response of the crop to both of them simultaneously is larger than the sum of the responses to each separately. If the response to the two factors together equals the sum of the responses separately, it will mean that there exists no interaction between the two and the factors are independent of each other; whereas if the response to the two factors together is less than the sum of the responses to each of them separately, it will imply that

there exists a negative interaction. An experiment was designed to investigate the nature and degree of the interaction of nitrogen and magnesium in the growth and development of cotton.

The third and the last objective of this investigation was to study the relationship between the carbohydrate status of plants (grown under various nitrogen-magnesium treatments) and the development of anthocyanin pigments. It was presumed that nitrogen deficiency by limiting protein synthesis, and hence growth, would result in an accumulation of carbohydrates in plants which might be responsible for increasing the anthocyanin pigmentation. Frey-Wyssling and Blank (9) failed to prove any general parallel between sugar content and anthocyanin pigmentation. Theoretical considerations are weighty against the finding of Frey-Wyssling and Blank. It is believed that shikimic acid, a product of carbohydrate origin, is the precursor in the biosynthesis of various flavonoids including anthocyanins (3).

REVIEW OF LITERATURE

Interaction among Plant Nutrients other than the Nitrogen-Magnesium Interaction

In addition to the interaction of nutrients in general aspects of growth and development of plants, these interactions may result in profound and subtle physiological changes in plant metabolic systems.

The boron-calcium relationship in corn has been worked out in some detail by Marsh and Shive (19). They found that a large proportion of boron in the shoot tissue was in the soluble state and this fraction increased with increasing boron content. A direct relationship was found between substrate boron and soluble tissue calcium. Marsh and Shive concluded that there was a direct effect of boron on calcium metabolism in corn.

Richards (25) has made a thorough investigation into the effect of rubidium on the growth of the barley, using it to elucidate the function of potassium in plant metabolism. He found that the barley grown in solutions containing high concentrations of ammonium ions and low concentration of calcium ions was most sensitive to potassium deficiency. With rubidium substituted for potassium early growth was normal, but toxic symptoms soon appeared with the high

concentrations employed. Sodium, lithium, and cesium did not substitute for potassium. The effect seemed to depend in a complex way on the calcium, ammonium, and phosphorous contents of the solution. In the light of his findings, Richards outlined the effect of rubidium on barley as follows: (1) As a rule, rubidium is generally toxic. (2) Rubidium may be beneficial when phosphorous is excessive and damaging when phosphorous is deficient. Here the effect of rubidium is in contrast to that of potassium; rubidium seems to retard the rate of phosphorus intake, whereas increasing levels of potassium decrease the percentage content of phosphorus due to more vigorous growth of the plant. (3) Rubidium seems to counteract ammonium toxicity. This effect is rather doubtful, however, rubidium and potassium seemed to play a similar role.

That sodium may partially replace potassium and calcium has been investigated in some detail by Mullison and Mullison (20) and Joham (16). Mullison and Mullison found that partial substitution of potassium by sodium delayed the development of potassium deficiency symptoms. Joham working with cotton found that sodium has a significant influence on fruiting of ninety-day-old plants grown in nutrient solutions deficient in either potassium or calcium during the last forty-five days of growth. It was found that the amount of calcium

that moved out of the old tissue was almost equal to the amount of calcium accumulated in the bolls of the plants. He concludes that sodium may bring about the mobilization of calcium stored in the old tissue and its translocation to developing bolls. Joham found no influence of sodium on potassium movement indicating that the replacement of potassium by sodium in cotton nutrition cannot be attributed to the translocation of potassium from old to active tissues. However, the specific role of sodium in substitution for potassium in the cell has as yet to be identified. Evidence to date indicates that sodium does play a role in the maintenance of the tissue hydration and the ionic balance of cell sap.

A highly interesting interrelation of iron and manganese has been investigated by Shive and coworkers (28, 29). Many analyses of plants grown both in soils and in artificial nutrient media have consistently shown that the utilization by plants of one of these two elements is dependent on the other. Somers and Shive (29) studied the interrelation between the two metals under controlled conditions. They designed an experiment in which they grew soyabean plants in a basic nutrient solution to which iron and manganese were added at various rates. They found that growth responses

depended more on the relative amounts of iron and manganese rather than on their absolute concentrations in the nutrient medium. Wide variations of the two metals in the nutrient medium (0.005 ppm. iron to 0.002 ppm. manganese and 3.00 ppm. iron to 2.00 ppm. manganese) maintained good plant growth as long as the ratio of iron to manganese was nearly two. Ratios of iron to manganese above two produced a kind of chlorosis attributed to iron toxicity, whereas ratios below two resulted in chlorosis attributed to manganese toxicity. The visual symptoms ascribed to iron toxicity looked like those resulting from manganese deficiency, and conversely excess manganese gave symptoms similar to those of iron deficiency. Thus, they equated manganese toxicity with iron deficiency and vice versa. Through chemical analyses, a distinction was made between the soluble and insoluble fractions of iron and manganese. The soluble fractions of iron and manganese found in the expressed sap were considered to be physiologically active, and the insoluble fractions, found in the press cake, were considered to be physiologically inactive. Furthermore, it was found that certain ratios of soluble iron to soluble manganese were associated with good growth and freedom from chlorosis, but the ratios between total iron and total manganese showed no significant relation to plant growth. Low substrate manganese

resulted in low soluble manganese and high soluble iron in the plant. Conversely, high substrate manganese gave high soluble manganese and low soluble iron in the plant tissue. High soluble iron content in the plant always resulted in iron toxicity and a high soluble manganese in manganese toxicity.

Somers, Gilbert, and Shive (28) tried to relate the function of iron and manganese with carbon dioxide production because of their participation in cellular respiration. The maximum yield of respiratory carbon dioxide was found in plants, making normal growth, growing in a substrate with an iron to manganese ratio of about two. The absolute amounts of these metals either in the plant or in the substrate had no bearing on respiratory carbon dioxide. From all these facts, as mentioned above, Somers and Shive (29) concluded that plant cells can tolerate only a certain amount of physiologically active iron; i.e., the ferrous form. The function of manganese, they point out, is to regulate the amount of ferrous iron in the cells. When the two metals are present in a more or less balanced proportion, the excess amount of ferrous iron is oxidized by manganic manganese to the ferric form which is thus rendered physiologically inactive in some insoluble form of "ferric-phosphorous organic complex." A deficient level of manganese in the substrate produces a deficiency of manganic

manganese in the plant tissue and this causes a shift from ferric to ferrous iron resulting in iron toxicity. An excess of manganese in the substrate leads as usual to an excess of manganic manganese in the plant and shifts the ferric-ferrous equilibrium in the reverse direction leading to an excessive oxidation of ferrous iron and the resulting iron deficiency which is similar to manganese toxicity. Criticism has been put forward regarding this hypothesis of Somers and Shive which states that physiologically active iron is exclusively the ferrous iron form. We know today that iron plays a very important role in oxidation-reduction systems in plants, in which iron-porphyrin protein compounds such as various cytochromes or peroxidases participate and these systems function on the reversible oxidation-reduction states of iron, hence both the ferric and the ferrous forms are physiologically essential. It is not known whether the physiologically active iron-porphyrin enzyme system was included in the expressed sap or in the press cake fractions of Somers and Shive's work.

Employing various levels of magnesium in nutrient culture and field experiments, Truog, Goates, Gerloff, and Berger (32) investigated the interrelationships of magnesium-phosphorous in peas (seed). They found that the increasing

level of substrate magnesium, rather than the level of substrate phosphorous, was more effective in raising the phosphorous content of peas. Their results support the theory that magnesium functions as a carrier of phosphorous. They also point out the need of giving more attention to the magnesium status of agricultural soils with a view to better utilization of soil phosphorous resulting in food crops of high nutritive value.

Nitrogen-Magnesium Interaction

Although the soil-plant literature contains many examples of interactions among plant nutrients in the substrate as well as in the plant, work on interrelationships between nitrogen and magnesium in general and particularly in cotton has been rather scant. Few reports have appeared on the influence of elements other than calcium and potassium on magnesium absorption.

Walsh and Clark (35) reported that a low level of sulfur nutrition retarded the development of chlorosis due to magnesium deficiency by enhancing magnesium uptake in tomato. Obenshain (21) working with corn noted a positive correlation between substrate nitrogen and tissue magnesium; i.e., as the supply of nitrogen was increased, tissues were found to contain

more magnesium. Hoblyn (12) found that applications of nitrogen to magnesium-deficient apple trees decreased leaf-scorch. Boynton and Compton (4) observed a positive correlation between leaf nitrogen and leaf magnesium in trees differentially fertilized with nitrogen while Olson and Bledsoe (22) reported an almost constant two-fold increase of nitrogen over magnesium in all organs of the cotton plant. The foregoing review of topical literature shows how scant our knowledge is with regard to interrelationship of nitrogen and magnesium in plants.

METHODS AND MATERIALS

Cultural Technique

During the course of this investigation two experiments were conducted. The first experiment was carried out in the fall-winter of 1959-1960 inside the greenhouse and the second was conducted during the summer of 1960 outside the greenhouse. Hereafter, the first experiment will indicate the greenhouse study and the second will indicate the outside experiment.

Empire WR variety of cotton was grown in two-gallon glazed pots containing washed sand. The experimental design was a randomized block with nine treatments and four replications on a greenhouse bench and outside. For the first forty-one days of the experiments all plants were supplied with the basic nutrient solution containing the following salts:

Salt	Millimolar Concentration	Micro Element	PPM
$Ca(NO_3)_2.4H_2O$	5	Fe	2.0
KNO_3	5	Zn	0.05
$MgSO_4.7H_2O$	2	Cu	0.03
KH_2PO_4	1	Mn	0.5
		Mo	0.04

After the forty-first day, the treatments consisted of three levels of nitrogen and three levels of magnesium employed in all nine possible combinations. The other elements remained at the same levels indicated above. Nitrogen levels were (1) high, as given in the basic four-salt complete solution, (2) medium, (one-tenth of nitrogen of the basic complete solution), and (3) low, (one-fiftieth of that in the complete solution). Magnesium levels were (1) high, as in the complete solution, (2) medium, (one-fiftieth of that in the complete solution, and (3) low (no magnesium supplied except as impurities in the salts used). The nine possible combinations were: (1) HH (hereafter, the first letter indicating the nitrogen level and the second letter the magnesium level; H indicating high, M medium, and L low), (2) HM, (3) HL, (4) MH, (5) MM, (6) ML, (7) LH, (8) LM, and (9) LL.

Variations in calcium and potassium due to variations in the level of nitrogen salts were compensated by adding exact milliequivalent amounts of those elements as calcium and potassium chlorides. The changes in sulfur due to variations of magnesium as $MgSO_4$ were compensated by the use of Na_2SO_4. The pH of all the nine nutrient solutions was controlled at 6.5.

During the course of the investigations each plant

received one liter of its respective nutrient solution per day. Care was taken to prevent salt accumulation by leaching each pot with deionized water at weekly intervals.

Weekly height measurements were taken and recorded. With the onset of flowering, the plants were checked daily and the flowers were tagged and recorded. At eighty-one, and at one hundred and nine days of treatment in the case of the greenhouse experiment, and at thirty-three, and fifty-six days in the case of the outside experiment, triplicate one gram leaf samples were collected from the branches arising from the seventh node of each plant and utilized for anthocyanin, chlorophyll, and moisture determinations.

The number of bolls set per plant as well as the yield of seed cotton were recorded. As the visual symptoms developed, color photographs were taken and the progressive changes were recorded.

One hundred and fifty days after planting in the case of the greenhouse experiment, and one hundred days after planting in the case of the outside experiment, the plants were harvested and fractionated into old leaves, new leaves, stems, and roots. After recording the fresh weights the samples were dried in a force-draft oven for twenty minutes at 110°C, then transferred to a convection oven at 70°C for

forty-eight hours. Dry weights were taken and the samples were ground and stored for subsequent analysis.

Chemical Analyses

1. Anthocyanin: Anthocyanin was extracted by soaking one gram of finely chopped fresh leaf tissue in 1/10 N HCL for 12 hours. The optical density of the extract was determined at 505 mu using a Spectronic 20 colorimeter.

2. Chlorophyll: Chlorophyll was determined according to the method given by Loomis and Shull (18). One gram of fresh leaf tissue was wet with a small quantity of acetone and ground into a fine pulp in a mortar. Thirty ml. of 80 percent acetone was used to extract the acetone-soluble pigments from the sample. The chlorophylls were then recovered in 15 ml. ethyl ether by forming an ether and acetone-water partition in a separatory flask. The ether extract was diluted eight times with ethyl ether and the optical density determined at 660 mu using a Spectronic 20 colorimeter.

3. Carbohydrates: Reducing sugar, sucrose, and starch were determined according to the procedures outlined by Loomis and Shull (18) and Wildman and Hansen (37). One gram of oven dried leaf tissue was extracted overnight with 80 percent alcohol in a Soxhlet extractor. Thirty ml. of

distilled water was added to the extract and the mixture was heated on a steam bath for three hours. The extract was then treated according to the method outlined by Loomis and Shull (18) and reducing sugars and sucrose (as reducing sugars after hydrolysis with HCL) were determined by the semi-micro mehtod of Wildman and Hansen (37) using Fehling's A and B reagents.

Starch was extracted from the residue by the combined diastatic and acid hydrolysis procedure of Loomis and Shull (18) and the amount of reducing sugars formed was determined by the method of Wildman and Hansen (37).

4. Cations: Tissue magnesium, calcium, potassium, and sodium were determined according to the following method: The samples were ashed in a muffle furnace for two hours at 225°C and then the temperature was raised to 550°C for completion of the ashing. The ash was dissolved in 50 ml of 5 percent HCL. Magnesium, calcium, potassium and sodium contents were determined by the use of a Beckman DU Flame Spectrophotometer.

RESULTS

Description of Deficiency Symptoms

In the greenhouse experiment, incipient magnesium deficiency symptom as interveinal chlorosis was apparent in the HM and HL treatments sixteen days after the treatments were started. By the tenth day of treatment nitrogen deficiency became evident in the LL, LM and LH treatments.

In the outside experiment, magnesium deficiency symptom was visible in the HM and HL treatments seventeen days after the treatments were started. The symptoms exhibited in this experiment were considerably different from those observed in the greenhouse experiment. The interveinal areas appeared extra dark and eventually such areas turned deep purple in color. General chlorosis and stunted growth, typical of nitrogen deficiency, became evident in the LL, LM, LH, ML, and MH series seven days after the treatments were started. Treatment 1 HH: Growth and fruiting of these plants in both experiments appeared normal. In the outside experiment, stems and petioles showed some purple coloration which might have resulted from a greater moisture stress or other environmental conditions. No such coloration was observed in the plants of the greenhouse experiment.

Treatment 2 HM: In both experiments, growth was less vigorous in this series than in the HH treatment. In the greenhouse experiment, the leaves showed pronounced interveinal chlorosis with marked green areas around the veins. The interveinal chlorotic areas and leaf margins tended to dry up at an advanced growth stage. In the outside experiment, interveinal chlorosis was not apparent, instead a pronounced purple pigmentation appeared in the areas between the veins.

Treatment 3 HL: Growth and deficiency symptom in this treatment were similar to those observed in both experiments in the HM series.

Treatment 4 MH: The medium level of nitrogen limited growth and fruiting in both experiments. In the greenhouse experiment, the leaves developed a general chlorosis and the petioles developed a purplish coloration. In the outside experiment, the same type of chlorosis developed but the stems and petioles appeared red.

Treatment 5 MM: Growth in this series was of the same order as that observed in the MH treatment. In both experiments, the deficiency symptoms were typical of nitrogen deficiency and followed the pattern of development described for the plants in the MH treatment. In the outside experiment, the chlorosis was followed by the development of purple pigmentation.

Treatment 6 ML: In both experiments, the plants of this treatment developed deficiency symptoms typical of both nitrogen and magnesium. In the greenhouse experiment, the lower leaves developed general chlorosis typical of nitrogen deficiency while the upper leaves showed the interveinal chlorotic pattern of magnesium deficiency. In the outside experiment, the symptoms developed in a similar manner except that the chlorotic areas became purple and the stems and petioles became red.

Treatment 7 LH: Growth in this treatment was severely limited due to the low nitrogen level employed. A strong general chlorosis developed in the leaves in both experiments and was typical of severe nitrogen deficiency. Some red coloration developed in the leaves and petioles of the greenhouse plants, however, this pigmentation was much more pronounced in the plants of the outside experiment.

Treatment 8 LM: Again as in the LH treatment, poor growth was typical of the plants in this treatment. The plants in the greenhouse experiment developed symptoms typical of nitrogen deficiency with general chlorosis of older leaves and red petioles and stems. Some of the upper leaves developed the interveinal chlorosis of magnesium deficiency. Similar symptoms were noted in the outside experiment but again strong

red to purple pigmentation developed in the interveinal areas. Treatment 9 LL: Both nitrogen and magnesium deficiency symptoms were observed in plants of this series in the greenhouse experiment. Very stunted growth was characteristic of plants in this treatment with the lower leaves appearing chlorotic as typical of nitrogen deficiency. The upper leaves with chlorosis in the interveinal areas and with green margin along the veins were typical of magnesium deficiency. In the greenhouse experiment, the symptoms remained as described above but in the outside experiment the leaves developed a strong purple pigmentation as the experiment progressed and masked the symptoms of nitrogen deficiency.

Physical Data

1. Growth In Height: Both nitrogen and magnesium deficiencies restricted stem elongation. In the treatments employed, nitrogen deficiency was more limiting than the levels of magnesium used. The HL treatment (high nitrogen, low magnesium) reduced growth as compared to the control, HH treatment, in the greenhouse and outside experiments by 29.6 and 26.0 per cent respectively. The reductions in the case of the LH treatment (low nitrogen, high magnesium) in the same experiments were 48.0 and 58.0 per cent respectively (table I). Further reductions to 59.0 and

65.0 per cent were noted in the two experiments when both nitrogen and magnesium were deficient as in the LL treatment. It was evident that nitrogen and magnesium deficiencies limited stem elongation more in the outside experiment than in the greenhouse experiment.

Table II shows that the stem elongation in cotton increased by 20.0 per cent in the greenhouse experiment and by 19.0 per cent in the outside experiment due to interaction of nitrogen and magnesium.

2. Fresh Weight: Table III presents the fresh weights from both experiments. Both nitrogen and magnesium deficiencies limited fresh weight accumulation. The deficiency of nitrogen was more severe than that of magnesium (table III). The low magnesium level (treatment HL) reduced fresh weights by 58.0 and 59.0 per cent in the greenhouse and outside experiments while low nitrogen accounted for 83.0 and 92.0 per cent reductions in the same experiments. The LL treatment (i.e., the combination of two deficiencies) accounted for a small additional reduction in fresh weight. In general the fresh weight accumulation was much greater in the outside experiment.

3. Dry Weight: Table V presents the dry weight data from the two experiments. Basing the HH yield as 100 per cent, it is

evident that the low magnesium level of the HL treatment reduced dry weight by 70.0 per cent in the greenhouse experiment and by 64.0 per cent in the outside experiment. Nitrogen deficiency was more severe and accounted for 85.0 and 91.0 per cent reductions in the greenhouse and outside experiments respectively. A further reduction in dry weight was noted when the deficiencies were combined in the LL treatment in the greenhouse experiment.

From table VI it can be seen that a large positive interaction exists between nitrogen and magnesium with regard to dry matter production. By increasing the substrate level of magnesium from low to high, the dry weight increased by 5.2 per cent in the greenhouse experiment and by 1.0 per cent in the outside experiment. Changing the level of nitrogen from low to high caused increases of 20.1 and 28.1 per cent respectively in the two experiments. Increases resulting from the nitrogen-magnesium interaction in the greenhouse and the outside experiments were 64.5 per cent and 63.0 per cent respectively.

4. Tissue Hydration: The data showing the degrees of hydration of the cotton plants under various nitrogen-magnesium treatments are presented in table VII. The influence of treatments on

tissue hydration was small and of doubtful significance, however, magnesium deficiency seemed to have a greater influence in increasing hydration than did nitrogen deficiency.

5. Flowering: Table VIII presents the data showing the influence of nitrogen and magnesium on flowering. In the greenhouse experiment, the deficiencies of nitrogen and magnesium were almost equally effective in reducing the number of flowers produced. In the outside experiment, however, the deficient levels of nitrogen had a more critical influence on flowering than did the levels of magnesium employed.

6. Yield of Seed Cotton: The yield of seed cotton as influenced by substrate nitrogen and magnesium is presented in table IX. The yield data of both experiments indicate that the deficient levels of nitrogen and magnesium, both medium and low, had a critical influence on fruitfulness. The interaction between nitrogen and magnesium accounted for 88.8 and 87.3 per cent of the yield in the greenhouse and outside experiments respectively (table X). The yields obtained in the outside experiment were generally two to three-fold more than the corresponding yields in the greenhouse experiment.

The fruiting index as influenced by substrate

nitrogen and magnesium is presented in table XI. An examination of the data shows that the fruiting index was dependent on the magnesium level and independent of nitrogen. This observation fits well into the results of nitrogen-magnesium effect on the vegetative and fruiting growth as obtained under both experiments.

Chemical Data

1. Anthocyanins: Table XII presents the anthocyanin contents of leaves as obtained under various nitrogen-magnesium treatments. An examination of the data shows that in the greenhouse environment an inverse relationship existed between substrate nitrogen and the anthocyanin content of leaves while substrate magnesium had no influence on leaf anthocyanin. In the outside experiment, the anthocyanin production was much higher than in the greenhouse plants and inverse realtions were noted between the substrate level of both elements and the pigment content of leaves.

2. Chlorophylls: Table XIII presents the chlorophyll contents of leaves under various nitrogen-magnesium treatments. The data show that chlorophyll development was inhibited when both nitrogen and magnesium were deficient. A negative correlation between the anthocyanin and chlorophyll contents

of leaves was noted.

3. Carbohydrates: Carbohydrate contents (reducing sugars, sucrose and starch) of leaves as influenced by the nitrogen-magnesium treatments are reported in table XIV. Variations of substrate nitrogen and magnesium had little influence on reducing sugars and sucrose. Starch and the total carbohydrates tended to be inversely related to both substrate nitrogen and magnesium levels.

A direct association between leaf starch and leaf anthocyanin is evident from the data in table XIVa and a highly significant inverse relationship is found to exist between anthocyanin and reducing sugars.

4. Cations: The total calcium, magnesium, potassium and sodium contents of the leaves as influenced by the nine nitrogen-magnesium treatments are presented in table XV.
Calcium: Even though the substrate level of calcium of all the nine treatments was the same, wide variations in leaf calcium were obtained between treatments. No consistent influence of the treatments on the calcium contents of leaves was observed.
Magnesium: The magnesium content of leaves was inversely related to substrate nitrogen. The highest amounts of leaf

magnesium were obtained in the MH and LH treatments in which nitrogen was the limiting growth factor.

Potassium: Leaf potassium tended to be directly associated with substrate nitrogen and inversely associated with substrate magnesium.

DISCUSSION

Simultaneous Development of Nitrogen and Magnesium Deficiencies

Under the greenhouse conditions, the plants growing in the ML (medium nitrogen, low magnesium) and LL (low nitrogen, low magnesium) treatments developed nitrogen and magnesium deficiency symptoms simultaneously in the same plant. The nitrogen levels of the ML and LL treatments were maintained at 0.2 and 0.98 m.e. NO_3 nitrogen per liter respectively and magnesium was supplied only as impurities in the salts employed in both cases. The growth of these plants was very stunted with the lower leaves appearing pale and chlorotic as characteristic of nitrogen deficiency while the upper leaves showed interveinal chlorosis with green areas along the veins, typical of magnesium deficiency. In the outside experiment, such distinct double deficiency symptoms were not duplicated in the LL treatment, but instead they developed in the MM and ML treatments. The LL plants developed purple pigmentation rapidly instead of showing the interveinal chlorotic areas and this tended to mask the symptoms of nitrogen deficiency.

The pattern of double deficiency symptoms as characterized by nitrogen deficiency symptoms in the lower leaves and magnesium deficiency in the upper leaves may be

explained on the basis of the difference in mobility of the two elements within the plant. It is well known that nitrogen is largely translocated from old senescent leaves to younger tissues. Magnesium moves out of the older tissues more slowly than does nitrogen and the deficiency symptoms of magnesium progress more rapidly from the older to younger tissues. In view of the above, it is probable that the younger leaves were more deficient in magnesium than nitrogen while the reverse of this would be true for the older leaves thus expressing the deficiency symptoms in the manner they were observed. This investigation helped in characterizing nitrogen and magnesium deficiencies occurring in the same plant as developed under the experimental conditions stated above. Oftentimes poor growth of plants in the field may result from the lack of two or more nutrients and such simultaneous characterization of nitrogen and magnesium deficiencies in cotton may be helpful in the diagnosis of symptoms where both nutrients are at deficient levels.

The Interaction of Nitrogen and Magnesium

The addition of nitrogen and magnesium together resulted in large increases in vegetative and fruiting growth. When one examines the influence of these elements separately

on the two types of growth, differences in response become apparent. Nitrogen when applied alone was responsible for large increases in the vegetative production. Thus in comparing the LL (low nitrogen, low magnesium) and HL (high nitrogen, low magnesium) treatments, the addition of nitrogen was responsible for a 29.0-39.0 per cent increase in height, a 32.0-35.0 per cent increase in fresh weight and a 20.0-28.0 per cent increase in dry weight in both experiments. The effect of the addition of magnesium on vegetative growth was much more limited and a comparison of the LL (low nitrogen, low magnesium) and LH (low nitrogen, high magnesium) treatments indicates that magnesium alone accounted for a 10.0-7.0 per cent increase in stem elongation, 7.6-2.2 per cent increase in fresh weight and 5.2-1.0 per cent increase in dry weight in the greenhouse and outside experiments. Neither nitrogen nor magnesium had much influence on seed cotton yield when added alone. The response in yield due to nitrogen alone was -1.4 per cent and 3.5 per cent in the two experiments while that due to magnesium was 5.0-1.2 per cent in the greenhouse and outside experiments. When the two elements were added together, the yields increased by 89.0 and 87.0 per cent in the two experiments. Thus in these investigations, the vegetative growth response to nitrogen application was

greater than that obtained from magnesium while substantial increases in yield were not obtained until both elements were added.

Anthocyanin Pigmentation

Under the greenhouse conditions, the deficiency of substrate and tissue magnesium had very little or no effect on the anthocyanin pigmentation in the cotton leaves. On the other hand, deficiencies of substrate nitrogen were inversely related to leaf anthocyanin in the greenhouse experiment. In the outside experiment, both nitrogen and magnesium supplies were inversely related to leaf anthocyanin. The maximum relative anthocyanin content of the leaves in the greenhouse experiment was obtained in the treatment LM and produced a standard solution with an optical density of 2.9. Leaves from the same treatment but grown in the outside experiment produced a standard anthocyanin solution with an optical density of 93.3. The highest level of anthocyanin produced under the greenhouse conditions was not quite equal to the lowest anthocyanin content in the leaves of the plants grown outside. Thus it is evident that factors in addition to nutrient deficiencies contribute to anthocyanin production. The main environmental differences between the two experiments were light quality and

intensity, temperature and moisture stress. In the greenhouse experiment, the temperature varied from 23° to 30°C and the light intensity never rose above 3,000 f.c. Temperature for the outside experiment ranged from 22° to 40°C and light intensity often reached 9,000 f.c. In addition to light and temperature differences, increased moisture stress was noted in the plants of the outside experiment. From the data gathered on anthocyanin, it is apparent that two production systems were involved. The low production system operated in the greenhouse experiment and was inversely related to substrate nitrogen and independent of the magnesium supply. The high production system functioned in the plants of the outside experiment and was inversely related to magnesium supply and to a lesser extent the inverse relation also held for substrate nitrogen. Several possibilities exist for explaining the observed differences. The first possibility involves the differences observed in environmental conditions between the two experiments. Magnesium deficiency was independent of anthocyanin production until some other factor was responsible for triggering the high production system. Thus this system may be light sensitive or require high temperatures before it becomes operative. A second possibility involves the Ca/Mg ratio and the anthocyanin production. Calcium acts as a

catalyst for some enzyme systems and it has been suggested that magnesium may be inhibitory to those enzyme reactions.[1] Thus it may be that the ratio of Ca/Mg is directly associated with anthocyanin production. An examination of the data obtained from the greenhouse experiment indicates that the low anthocyanin production system is independent of the Ca/Mg ratio. The data from the outside experiments, although not complete, suggest an association between the Ca/Mg ratio and the high anthocyanin production system. A third point in this discussion is that of the association between anthocyanin production and carbohydrates. In the greenhouse experiment, highly significant positive correlations were obtained between anthocyanin and starch, and between anthocyanin and total carbohydrate. A highly significant negative correlation was found between reducing sugar and anthocyanin. In that experiment, substrate nitrogen was the controlling factor and substrate magnesium was independent of carbohydrate synthesis and therefore anthocyanin production. In the outside experiment where high anthocyanin production was in operation it may be that different results will be obtained when those tissues are analyzed for carbohydrates. The analysis of the tissues

---

[1] Enzymes by Dixon, M. and Webb, Edwin C., (Academic Press Inc. 1958)

of the outside experiment for carbohydrates was beyond the scope of this work and awaits further experimentation.

SUMMARY

Experiments were conducted to study the nitrogen-magnesium nutrition of the cotton plant. Under the greenhouse conditions, the cotton plants of the LL (low nitrogen, low magnesium) and ML (medium nitrogen, low magnesium) treatments developed nitrogen and magnesium deficiency symptoms simultaneously. The growth of these plants were stunted with the lower leaves appearing chlorotic as characteristic of nitrogen deficiency while the upper leaves showed interveinal chlorosis with marked green areas along the veins typical of magnesium deficiency. In the outside experiment, such distinct double deficiency symptoms were not duplicated in the LL (low nitrogen, low magnesium) treatment but instead they developed in the MM (medium nitrogen, medium magnesium) and ML (medium nitrogen, low magnesium) plants. The leaves of the LL plants developed purple pigmentation so rapidly as to mask both general and interveinal chlorosis. Even in the case of MM and ML plants, the transition from interveinal chlorosis to purple pigmentation was very rapid.

The addition of nitrogen and magnesium together resulted in large increases in vegetative and fruiting growth. Nitrogen was responsible for a much larger increase in

vegetative growth than was magnesium. Nitrogen or magnesium, when applied alone, had little or no influence on seed cotton yield, but the addition of two elements together accounted for as much as 87.0-89.0 per cent of the total yield.

Leaf carbohydrates and total cations were determined. An inverse relationship was noted between substrate nitrogen and leaf carbohydrates.

Under the greenhouse environment, magnesium deficiency had no effect on the anthocyanin production in the cotton leaves, but nitrogen deficiency was found to be inversely related to leaf anthocyanin. In the outside experiment, both nitrogen and magnesium deficiencies were found to be inversely related to leaf anthocyanin. The level of leaf anthocyanin produced with magnesium deficiency in the outside experiment was far greater than the amounts obtained from nitrogen deficiency either in the greenhouse or outside. The environmental factors differentiating the outside experiment from the greenhouse conditions were (I) a higher maximum and wider range of temperature, (II) higher light intensity and (III) increased moisture stress. In the light of these facts and the data obtained, it is proposed that two production systems are involved in the biosynthesis of anthocyanin in the cotton plant, (1) the low production system as that observed in the

greenhouse environment, and (2) the high production system as that functioned in the outside environment. Several possibilities are suggested for this differential production of anthocyanin in the greenhouse and outside experiments.

LITERATURE CITED

1. Arnon, D. I., Mineral Nutrition of Plants, Ann. Rev. Biochem., 12:493-528, 1943.

2. American Society of Agronomy and National Fertilizer Association, Washington, D. C., Hunger Signs in Crops, A Symposium, 1950.

3. Bogorad, L., The Biogenesis of Flavonoids, Ann. Rev. Plant Physiol., 9:417-448, 1958.

4. Boynton, D. E., Compton, O.C., The influence of differential fertilization with ammonium sulfate on the chemical composition of McIntosh apple leaves, Proc. Amer. Soc. of Hort. Sci., 45:9-17, 1944.

5. Cooper, H. P., Paden, W. R., and Garman, W. H., Some factors influencing the availability of magnesium in soil and the magnesium content of certain crop plants, Soil Sci., 63:27-41, 1947.

6. Cooper, H. P., Sand Drown or Magnesium Deficiency in Crops, S. C. Agri. Expt. Sta. Ann. Rept., 45:30-35, illus., 1932.

7. Cooper, H. P., Nutrient Deficiency Symptoms in Certain Plants, Assoc. South Agri. Workers Proc., 34:80-81, 1933.

8. Cooper, H. P., and Garman, W. H., Effect of Application of Sodium on the composition and yield of cotton at different levels of potash fertilization, Soil Sci. Soc. Amer. Proc., 7:331, 1942.

9. Frey-Wyssling, A., and Blank, F., Ber. Schweiz. Botan. Ges., 53A, 550, 1943.

10. Gregory, F. G., Mineral Nutrition of Plants, Ann. Rev. Biochem., 6:557-578, 1937.

11. Helmy, H., Joham, H. E., and Hall, W. C., Magnesium Nutrition of American Upland and Egyptian Cottons, Tex. Agri. Expt. Sta. Bul., February, 1960.

12. Hoblyn, T. N., Manurial Trials with Apple Trees at East Malling, 1920-1939. Jour. Pomol. and Hort. Sci., 18: 325-343, 1940.

13. Hunter, A. S., Toth, S. J., and Bear, F. E., Calcium-potassium ratios for alfalfa, Soil Sci., 55:61-72, 1943.

14. Joham, H. E., The nutritional status of cotton plant as indicated by tissue tests. Plant Physiol., 26:76-89, 1951.

15. Joham, H. E., Carbohydrate distribution as affected by calcium deficiency in cotton. Plant Physiol., 32:113-117, 1957.

16. Joham, H. E., The calcium and potassium nutrition of cotton as influenced by sodium. Plant Physiol., 30: 4-10, 1955.

17. Koch, F. C., and McMeekin, T. L., New direct nesslerization microkjeldahl method and a modification of the Nessler-Folin reagent for ammonia. Jour. Chem. Soc., 46:2066-2069, 1924.

18. Loomis, W. E., and Shull, C. A., Methods in plant physiol., McGraw-Hill Book Company, New York and London, 1937.

19. Marsh, R. P., and Shive, J. W., Soil Sci., 51:141-151, 1941.

20. Mullison, W. R., and Mullison, E., Plant Physiol., 17: 632-644, 1942.

21. Obenshain, S. S., The effect of different concentrations of nutrient supplies in sand cultures on the elemental composition of expressed sap and the corn plant. Va. Acad. Sci. Proc., 74:1944.

22. Olson, L. C., and Bledsoe, R. P., The chemical composition of the cotton plant and the uptake of nutrients at different stages of growth. Ga. Agri. Expt. Sta. Bul., 222, 1942.

23. Peech, Michael and Bradfield, R., The effect of lime and

magnesia on potassium, Soil Sci., 55:37-48, 1943.

24. Prince, A. L., Zimmerman, M., and Bear, F. E., The magnesium supplying powers of 20 New Jersey Soils, Soil Sci., 63:78, 1947.

25. Richards, F. J., Ann. Bot., n. s. 5, 249-61, 1941.

26. Richards, F. J., Mineral Nutrition of Plants. Ann. Rev. of Biochem., 13:611-630, 1944.

27. Russell, Sir E. J., Soil Conditions and Plant Growth, Longmans, Green and Co., London, New York and Toronto, 1950.

28. Somers, I. I., Gilbert, S. G., and Shive, J. W., Plant Physiol., 17:317-320, 1942.

29. Somers, I. I., and Shive, J. W., Plant Physiol., 17:582-602, 1942.

30. Thimann, K. V. and Edmondson, Y. H., The Biogenesis of the anthocyanins. I. General Nutritional Conditions leading to Anthocyanin Formation. Arch. Biochem., 22: 33-53, 1949.

31. Thimann, K. V., Edmondson, Y. H., and Radner, B. S., Arch. Biochem. Biophysics, 34:305, 1951.

32. Truog, E., Goates, R. J., Gerloff, G. C., and Berger, K. C., Magnesium-phosphorous relationships in plant nutrition, Soil Sci., 63:19-25, 1947.

33. Wallace, T., The diagnosis of mineral dificiencies in plants, 116 pp. (H.M. Stationery Office, London, 1943).

34. Walsh, T., O'donohoe, T. F., Magnesium Dificiency in some plants in relation to the level of potassium nutrition. Jour. Agri. Sci., 35:254-263, 1945.

35. Walsh, T., and Clark, E. J., Chlorosis of tomatoes with particular reference to potassium-magnesium relations. Proc. Irish Acad. 50B, 245-263, 1945.

36. Wadleigh, C. H., Growth Status of the cotton plant as

influenced by the supply of nitrogen. Ark. Agri. Expt. Sta. Bul. 446, 138 pp. illus., 1944.

37. Wildman, S. G., and Hansen, E., A semi-micro method for the determination of reducing sugars, Plant Physiol. 15:719, 1940.

38. Zimmerman, M., Magnesium in plants, Soil Sci., 63:1-12, 1947.

TABLE I

The heights of cotton plants as influenced by various levels of substrate nitrogen and magnesium (data given in cm.).

	Greenhouse Experiment		Outside Experiment	
	Height[1]	Per Cent Decrease basing HH as 100	Height[2]	Per Cent Decrease basing HH as 100
1 HH	98[3]		85[4]	
2 HM	78	20.5	64	24.8
3 HL	69	29.6	63	26.0
4 MH	68	30.7	48	44.0
5 MM	73	25.6	37	57.0
6 ML	71	27.6	38	55.0
7 LH	51	48.0	36	58.0
8 LM	46	53.1	36	58.0
9 LL	41	59.0	30	65.0

[1] LSD required for significance to 0.05 level = 7.96
[2] LSD required for significance to 0.05 level = 8.79
[3] Each figure is an average of 5 replications
[4] Each figure is an average of 4 replications

TABLE II

Nitrogen-magnesium interaction in linear growth of the cotton plant (data in cm.).

Greenhouse Experiment		Outside Experiment	
LH	51.0	LH	36.0
LL	41.0	LL	30.0
Response to Mg	10.0	Response to Mg	6.0
Per cent increase	10.0	Per cent increase	7.0
HL	69.0	HL	63.0
LL	41.0	LL	30.0
Response to N	28.0	Response to N	33.0
Per cent increase	29.0	Per cent increase	39.0
HH	98.0	HH	85.0
LL	41.0	LL	30.0
Simultaneous response to N amd Mg	57.0	Simultaneous response to N and Mg	55.0
Sum of the responses separately to N and Mg	38.0	Sum of the responses separately to N and Mg	39.0
N-Mg interaction	19.0	N-Mg interaction	16.0
Per cent increase	20.0	Per cent increase	19.0

TABLE III

Fresh weights in gram under the nitrogen-magnesium treatments.

	Greenhouse Experiment		Outside Experiment	
	Fresh Weight[1]	Per Cent Decrease basing HH as 100	Fresh Weight[2]	Per Cent Decrease basing HH as 100
1 HH	331.8[3]		855.8[4]	
2 HM	211.0	36	313.6	63
3 HL	140.5	58	352.0	59
4 MH	119.5	64	143.5	83
5 MM	121.0	63	100.0	88
6 ML	129.4	61	97.6	89
7 LH	60.6	83	72.3	92
8 LM	44.3	87	56.0	93
9 LL	35.2	89	52.9	94

[1] LSD required for significance to 0.05 level = 56.23
[2] LSD required for significance to 0.05 level = 67.0
[3] Each figure is an average of 5 replications
[4] Each figure is an average of 4 replications

TABLE IV

The Nitrogen-magnesium interaction in the growth of cotton (fresh weights in gram).

Greenhouse Experiment		Outside Experiment	
LH	60.6	LH	72.3
LL	35.2	LL	52.9
Response to Mg	25.4	Response to Mg	19.4
Per cent increase	7.6	Per cent increase	2.2
HL	140.5	HL	352.0
LL	35.2	LL	52.9
Response to N	105.3	Response to N	299.1
Per cent increase	31.8	Per cent increase	35.0
HH	331.8	HH	855.8
LL	35.2	LL	52.9
Simultaneous response to N and Mg	296.6	Simultaneous response to N and Mg	802.9
Sum of the responses separately to N and Mg	130.7	Sum of responses separately to N and Mg	318.5
Response due to N-Mg	165.9	Response to N-Mg	484.4
Per cent increase due to N-Mg interaction	50.2	Per cent increase due to N-Mg interaction	57.0

TABLE V

Dry weights in gram as obtained under the nine nitrogen-magnesium treatments.

	Greenhouse Experiment		Outside Experiment	
	Dry Weight [1]	Per Cent Decrease basing HH as 100	Dry Weight [2]	Per Cent Decrease basing HH as 100
1 HH	171.70[3]		286.3[4]	
2 HM	73.5	57	89.9	69
3 HL	52.1	70	102.9	64
4 MH	54.1	68	47.8	84
5 MM	44.7	74	35.4	88
6 ML	43.0	75	33.7	89
7 LH	26.4	85	25.5	91
8 LM	21.3	88	26.4	90
9 LL	17.5	90	22.6	92

[1] LSD required for significance to 0.05 level = 20.98
[2] LSD required for significance to 0.05 level = 17.33
[3] Each figure is an average of 5 replications
[4] Each figure is an average of 4 replications

TABLE VI

The nitrogen-magnesium interaction in the growth of cotton (dry weights in gram).

Greenhouse Experiment		Outside Experiment	
LH	26.4	LH	25.5
LL	17.5	LL	22.6
Response to Mg	8.9	Response to Mg	2.9
Per cent increase	5.2	Per cent increase	1.0
HL	52.1	HL	102.9
LL	17.5	LL	22.6
Response to N	34.6	Response to N	80.3
Per cent increase	20.1	Per cent increase	28.1
HH	171.7	HH	286.3
LL	17.5	LL	22.6
Simultaneous response to N and Mg	154.1	Simultaneous response to N and Mg	263.7
Sum of the responses separately to N and Mg	43.5	Sum of the responses separately to N and Mg	83.2
Response due to N-Mg interaction	110.7	Response due to N-Mg interaction	180.5
Per cent increase	64.5	Per cent increase	63.0

TABLE VII

Influence of nitrogen and magnesium on the tissue hydration of cotton.

	Greenhouse Experiment		Outside Experiment	
	Per Cent Hydration	Relative Hydration basing HM=100	Per Cent Hydration	Relative Hydration basing HM=100
1 HH	48	68	66	93
2 HM	70	100	71	100
3 HL	63	88	70	99
4 MH	50	71	66	93
5 MM	63	84	64	90
6 ML	67	93	65	92
7 LH	56	79	64	90
8 LM	52	73	53	74
9 LL	50	70	57	80

TABLE VIII

Influence of nitrogen and magnesium on flowering.

	Greenhouse Experiment		Outside Experiment	
	Number	Per Cent Decrease basing HH=100	Number	Per Cent Decrease basing HH=100
1 HH	25.0		41.2	
2 HM	6.4	76	20.5	50
3 HL	4.2	84	22.0	46
4 MH	7.2	72	8.5	79
5 MM	6.6	74	7.7	81
6 ML	5.2	79	8.3	80
7 LH	3.6	84	6.7	83
8 LM	3.2	87	6.5	85
9 LL	2.8	89	6.7	84

TABLE IX

Influence of nitrogen and magnesium on the yield of seed cotton (data presented in gram).

	Greenhouse Experiment		Outside Experiment	
	Yield[1]	Per Cent Decrease basing HH=100	Yield[2]	Per Cent Decrease basing HH=100
1 HH	42.2[3]		125.2[4]	
2 HM	5.0	88.2	17.7	85.9
3 HL	2.6	93.9	14.6	88.3
4 MH	13.2	68.7	19.4	84.5
5 MM	7.4	83.0	13.1	89.6
6 ML	3.4	90.0	12.3	90.4
7 LH	5.3	87.4	11.7	90.7
8 LM	4.8	88.0	11.0	91.0
9 LL	3.2	92.4	10.2	92.0

[1] LSD required for significance to 0.05 level = 12.06
[2] LSD required for significance to 0.05 level = 7.63
[3] Each figure is an average of 5 replications
[4] Each figure is an average of 4 replications

TABLE X

The nitrogen-magnesium interaction in the yield of seed cotton (data presented in gram).

Greenhouse Experiment		Outside Experiment	
LH	5.3	LH	11.7
LL	3.2	LL	10.2
Response to Mg	2.1	Response to Mg	1.5
Per cent increase	5.0	Per cent increase	1.2
HL	2.6	HL	14.6
LL	3.2	LL	10.2
Response to N	0.6	Response to N	4.4
Per cent increase	1.4	Per cent increase	3.5
HH	42.2	HH	125.2
LL	3.2	LL	10.2
Simultaneous response to N and Mg	39.0	Simultaneous response to N and Mg	115.0
Sum of the responses separately to N and Mg	1.5	Sum of the responses separately to N and Mg	5.9
N-Mg interaction	37.5	N-Mg interaction	109.1
Per cent increase	88.8	Per cent increase	87.3

TABLE XI

The influence of nitrogen and magnesium on the fruiting index[1] of cotton.

	Greenhouse Experiment	Outside Experiment
1 HH	0.25	0.46
2 HM	0.06	0.20
3 HL	0.05	0.15
4 MH	0.24	0.43
5 MM	0.15	0.39
6 ML	0.09	0.38
7 LH	0.20	0.49
8 LM	0.21	0.44
9 LL	0.18	0.48

[1] Fruiting index = $\frac{\text{Dry weight of seed cotton}}{\text{Dry weight of stems and leaves}}$

TABLE XII

Influence of nitrogen and magnesium on anthocyanin pigmentation in the cotton leaf. (Figures represent relative densities of extracted anthocyanin).

	Greenhouse Experiment		Outside Experiment	
	First[1] Harvest	Second[2] Harvest	First[3] Harvest	Second[4] Harvest
1 HH	0.11	0.11	2.57	3.23
2 HM	1.30	0.12	6.19	63.10
3 HL	1.26	0.12	8.03	79.43
4 MH	1.60	1.40	4.12	4.36
5 MM	1.62	1.94	7.71	14.13
6 ML	1.49	1.68	5.27	89.13
7 LH	2.04	2.07	9.44	13.34
8 LM	2.23	2.90	28.18	93.30
9 LL	2.22	2.23	56.23	100.00

[1] Sampled from seventh node on one hundred and twenty-second day of growth (February 26, 1960); i.e., eighty-one days after starting treatments on December 7, 1959.

[2] Sampled from the tenth node on one hundred and forty-ninth day of growth (March 24, 1960); i.e., one hundred and nine days after treatment.

[3] Sampled from the seventh node on seventy-sixth day of growth (August 7, 1960); i.e., thirty-third day after starting treatment on July 5, 1960.

[4] Sampled from the tenth node on ninety-ninth day of growth (August 30, 1960), fifty-six days after treatment.

TABLE XIII

Relative amounts of chlorophyll and anthocyanin in composite, duplicate samples. (Figures in relative densities).

	Greenhouse Experiment		Outside Experiment			
			First Harvest		Second Harvest	
	Anthocyanin	Chlorophyll	Anthocyanin	Chlorophyll	Anthocyanin	Chlorophyll
1 HH	0.11	6.48	2.57	5.01	3.23	5.51
2 HM	1.30	1.35	6.19	1.45	63.10	1.32
3 HL	1.26	1.29	8.03	1.31	79.43	1.28
4 MH	1.60	1.32	4.12	1.31	4.36	1.55
5 MM	1.62	0.12	7.71	1.31	14.13	1.27
6 ML	1.49	1.27	5.27	1.27	89.13	1.15
7 LH	2.04	0.11	9.44	1.22	13.34	1.24
8 LM	2.23	0.10	28.18	1.21	93.33	1.16
9 LL	2.22	0.11	56.23	1.17	100.00	1.10

TABLE XIIIa

Correlation coefficient, r, of anthocyanin and chlorophyll pigments.

Experiment	r
First	-0.977
Second	
First harvest	-0.287
Second harvest	-0.489

TABLE XIV

Leaf carbohydrates (in per cent dry weight) and anthocyanin under various nitrogen-magnesium treatments.

	Reducing sugar	Sucrose	Soluble sugars	Starch	Total Carbo-hydrates	Antho-cyanin
1 HH	.61	.60	1.21	8.43	9.64	0.11
2 HM	.66	--	.66	9.57	10.23	0.12
3 HL	.56	--	.56	12.30	12.86	0.12
4 MH	.30	.13	.43	13.10	13.53	1.40
5 MM	.27	.45	.72	14.70	15.42	1.94
6 ML	.56	.22	.78	18.84	19.62	1.68
7 LH	.30	.27	.57	18.80	19.37	2.07
8 LM	.24	.27	.51	20.44	20.95	2.87
9 LL	.43	.83	1.26	20.44	20.70	2.23

TABLE XIVa

Correlation coefficient (r) at 8 d.f. between anthocyanin and various carbohydrate fractions.

	r
Total carbohydrate	+0.864*
Starch	+0.906*
Reducing sugar	-0.813**
Sucrose	+0.359
Soluble sugars	-0.1076

*Highly significant, significant at and below one per cent.

**Highly significant negative correlation at and below one per cent.

TABLE XV

Distribution of cations (greenhouse experiment) in leaf tissue at 150 days of growth. (Figures in m.e. per 100 gram of dry weight).

	Ca	Mg	K	Na	Total	$\frac{Ca+Mg}{K+Na}$	$\frac{Ca}{Mg}$
1 HH	206.0	46.6	127.8	16.5	396.9	1.75	4.42
2 HM	166.5	7.9	159.5	21.3	355.2	0.96	21.00
3 HL	170.2	4.2	179.0	16.8	370.2	0.89	40.52
4 MH	151.0	104.1	135.0	22.6	412.8	1.62	1.45
5 MM	127.0	15.8	140.9	15.6	299.3	0.91	8.03
6 ML	75.0	10.4	147.0	16.3	248.7	0.52	7.21
7 LH	143.7	83.3	102.3	16.6	345.9	1.90	1.72
8 LM	194.0	18.3	126.8	22.6	361.7	1.42	10.60
9 LL	175.0	13.7	104.3	12.1	305.2	1.62	12.77