68-17,584

ARMSTRONG, George Michael, 1938-

EFFECTS OF POTASSIUM, MAGNESIUM, AND NITROGEN DEFICIENCIES ON THE CONCENTRATION OF CHLOROGENIC ACID AND SCOPOLIN IN TOBACCO.

The University of Oklahoma, Ph.D., 1968
Botany

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

EFFECTS OF POTASSIUM, MAGNESIUM, AND NITROGEN DEFICIENCIES ON THE CONCENTRATION OF CHLOROGENIC ACID AND SCOPOLIN IN TOBACCO

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

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NORMAN, OKLAHOMA

1968

EFFECTS OF POTASSIUM, MAGNESIUM, AND NITROGEN DEFICIENCIES ON THE CONCENTRATION OF CHLOROGENIC ACID AND SCOPOLIN IN TOBACCO

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ACKNOWLEDGMENT

I wish to express my thanks to Dr. L. M. Rohrbaugh for his advice in formulating this problem, and his continued interest in my work; to Dr. E. L. Rice and Dr. S. H. Wender for their advice and concern which allowed me to complete this study; and to Dr. N. H. Boke for a critical reading of the text. I would like to thank the National Science Foundation for the N. S. F. Traineeship which made my studies possible.

I would like to thank my fellow graduate students, especially David E. Koeppe and Hsien-Chi Li, for their advice, cooperation, and friendship. I also thank Mr. R. J. Welty for the preparation of the figures in this paper.

Finally, I wish to express my thanks to my wife, Lorna, and to our children, Mike, Phil, and Lori, for without their love, understanding, encouragement, and patience, the following would not have been possible.

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CHAPTER I

INTRODUCTION

Widespread occurrence of chlorogenic acid and scopolin among higher plants was reported in recent comprehensive studies (58,76). Quantitative studies of their distribution within a particular plant under varying environmental conditions have been limited. Best (5) identified the blue-fluorescing scopoletin (6-methoxy-7-hydroxycoumarin), the aglycone of scopolin, in virus infected tobacco. He later reported on its distribution in tobacco (76), and its distribution in Avena roots was reported by Goodwin and Pollock (20).

Monophenols, including scopoletin and scopolin, have been considered several times in relation to inhibitory and stimulatory effects on growth and IAAO (indoleacetic acid oxidase) activity (2,3,4,14,21,43,53,68,74). Interpretation of the various studies was complicated by possible hydrolysis to the aglycone, and the carrying out of some studies on roots where natural concentrations of IAA may have already been above the optimum for growth (66).

Chlorogenic acid has received considerable and continued attention since its discovery in 1837 by Robiquet and Boutron (58).

Its biosynthesis, translocation, and distribution within plants (13,37,41,46,47,79), and its distribution among plants (57,58) have been studied. Its relation to the IAAO system has been a major area of study (24,67,68,77), but chlorogenic acid was also found to be a likely precursor of lignin (19,64,65).

The effects of light quantity and quality on the phenol content including chlorogenic acid content of plants have been studied, especially as related to IAAO activity (17,25,32,33,49,72,78,80). Physiological roles in relation to IAA and to lignin biosynthesis are of critical importance to plant growth and development, and further studies under varying environmental conditions are important.

The amounts of scopolin and scopoletin in tobacco and sunflower plants changed when they were treated with 2,4-dichlorophenoxyacetic acid, and changes in these compounds occurred also in tobacco treated with maleic hydrazide (9,10,76). Recently, UV light was found to have a pronounced effect on the scopolin content of tobacco and sunflower (32).

Only a few quantitative studies of effects of mineral deficiencies on phenolic compounds have been reported. Increases in scopolin were found in boron deficient tobacco and sunflower leaves, while possible decreases in chlorogenic acid were reported (70,71). Chouteau and Loche (7,38) found increases in scopolin and decreases in chlorogenic acid in magnesium, calcium, and phosphorus deficient tobacco leaves. They found a decreased chlorogenic acid concentration in potassium deficient tobacco leaves, and an increased concentration in nitrogen deficient tobacco leaves. Tso et al. (69) reported increases

in chlorogenic acid and scopolin in the leaves of three varieties of tobacco with increases in nitrogen fertilization. The results for a fourth variety, in which chlorogenic acid and scopolin concentrations were inversely related to the levels of nitrogen fertilization, correlated with the work of Chouteau and Loche.

Previous work clearly indicated that various stress conditions affect the amount of chlorogenic acid and scopolin in leaves. I decided, therefore, to study mineral deficiency further as a stress condition and to analyze roots and stems in addition to leaves. Nitrogen, magnesium, and potassium deficiency symptoms became apparent in tobacco leaves in the order listed. Selection of these mineral nutrients for study was made in hopes that the appearance of deficiency symptoms might be correlated with changes in chlorogenic acid and scopolin concentrations.

CHAPTER II

MATERIALS AND METHODS

Tobacco plants (Nicotiana tabacum, One-Sucker variety) were grown in pure quartz sand in 4-inch glazed pots in Percival growth chambers. A daily cycle with a 16 hr light period at 28.8 C and an 8 hr dark period at 16.6 C was maintained throughout the study. Plants were watered with Fe-EDTA double strength Hoagland's nutrient solution (26) until treatment began.

Approximately 70 days after germination, plants were selected for uniformity, and the pots were leached thoroughly with distilled water. Control plants were watered thereafter with the double strength nutrient solution, whereas the treated plants were made deficient for potassium, magnesium, or nitrogen by watering with similar nutrient solutions made deficient for these minerals by a procedure similar to that outlined by Machlis and Torrey (39). Soil jars were leached every 7 days with 3000 ml of distilled water.

Illumination in Experiment 1 was 1000 ft-c and in Experiment 2 was 1300 ft-c. Deficient plants were raised on blocks so that apices of deficient and control plants were kept at the same level.

A control plant and a plant from each treatment were harvested at the end of 1,3, and 5 weeks. Leaves at various developmental stages were harvested from each plant, with sampling

kept uniform. The top 1 cm of the plant was removed before the stem was harvested, while the entire root system was harvested. These separate harvests were weighed and fixed in boiling isopropyl azeotrope (88% isopropanol, 12% water). After grinding thoroughly in a blender, the fixed plant matter was transferred to a soxhlet extraction thimble and washed with boiling isopropanol: water (1:1, v/v), boiling IBMW (isopropanol: benzene: methanol: water, 2:1:1:1, v/v/v/v), and boiling isopropyl azeotrope, 4,5, and 4 times the wet weight of sample respectively. The thimble containing the washed plant matter was placed in a soxhlet extractor and extracted with isopropyl azeotrope for 24 hr and then with isopropanol for 24 hr. The fixing, washing, and extracting solvents were combined and evaporated to dryness in vacuo, and the residue brought to a known volume in a ratio of approximately 3:1 (g wet weight of sample: ml IBMW).

Air dry weights of samples were obtained by addition of the air dry weight of soluble material remaining in the extraction thimble and the air dry weight of a 1 ml aliquot of the sample extract multiplied by the volume of the sample (ml).

Scopolin and the three chlorogenic acids, CGA (chlorogenic acid), B510 (Band 510), neo CGA (neochlorogenic acid) were quantitated with the method developed by Koeppe (32). The method utilized one dimensional, descending paper chromatography on Whatman #1 paper ($9\frac{1}{2}$ X 22 in) which had been washed with approximately 50 ml methanol: water (5:95, v/v). An aliquot of the sample extract was streaked along a 6 in. strip on the dried paper. Development,

using KFW (methylisobutyl ketone: formic acid: water, 14:3:2, v/v/v) as the solvent system, for 22 hr in a non-equilibrated small glass chomatocab, yielded separation of the compounds into 4 distinct bands. As reported by Koeppe, the bands, observed under UV light, were readily distinguished and separated from other fluorescent compounds.

Positive identification of compounds was made by cochromatography in various solvent systems. Sample compounds ran exactly with authentic compounds in every system. Absorption spectra of authentic and sample compounds were identical.

The sample bands were cut out and eluted from the paper by allowing 5% methanol to descend through the paper from above. After an elution period of at least 12 hr, the eluates were brought to a known volume and read against blanks carried through the same procedure. Chlorogenic acids were read on a Beckman DB-G spectrophotometer at a wavelength of 324 mμ. Scopolin was read on a model 110 Turner fluorometer using pyrex cuvettes and a high sensitivity attachment at a setting of 1X. The filters used were a primary filter #7-60, and a secondary filter #2A plus #48 (Kodak Wratten filter). Standard reference curves (Fig. 1,2) were prepared using known quantities of authentic compounds which were carried through the same procedure as the unknowns. Quantitation of the three chlorogenic acids was made with the CGA standard reference curve since the absorption spectra and the extinction coefficients of the three are the same (80). The average percent recovery obtained for chlorogenic acid was 85% and for scopolin was 79%.

The data reported are based on averages of at least two determinations except where changes in the sample extracts with time prohibited such replication (32).

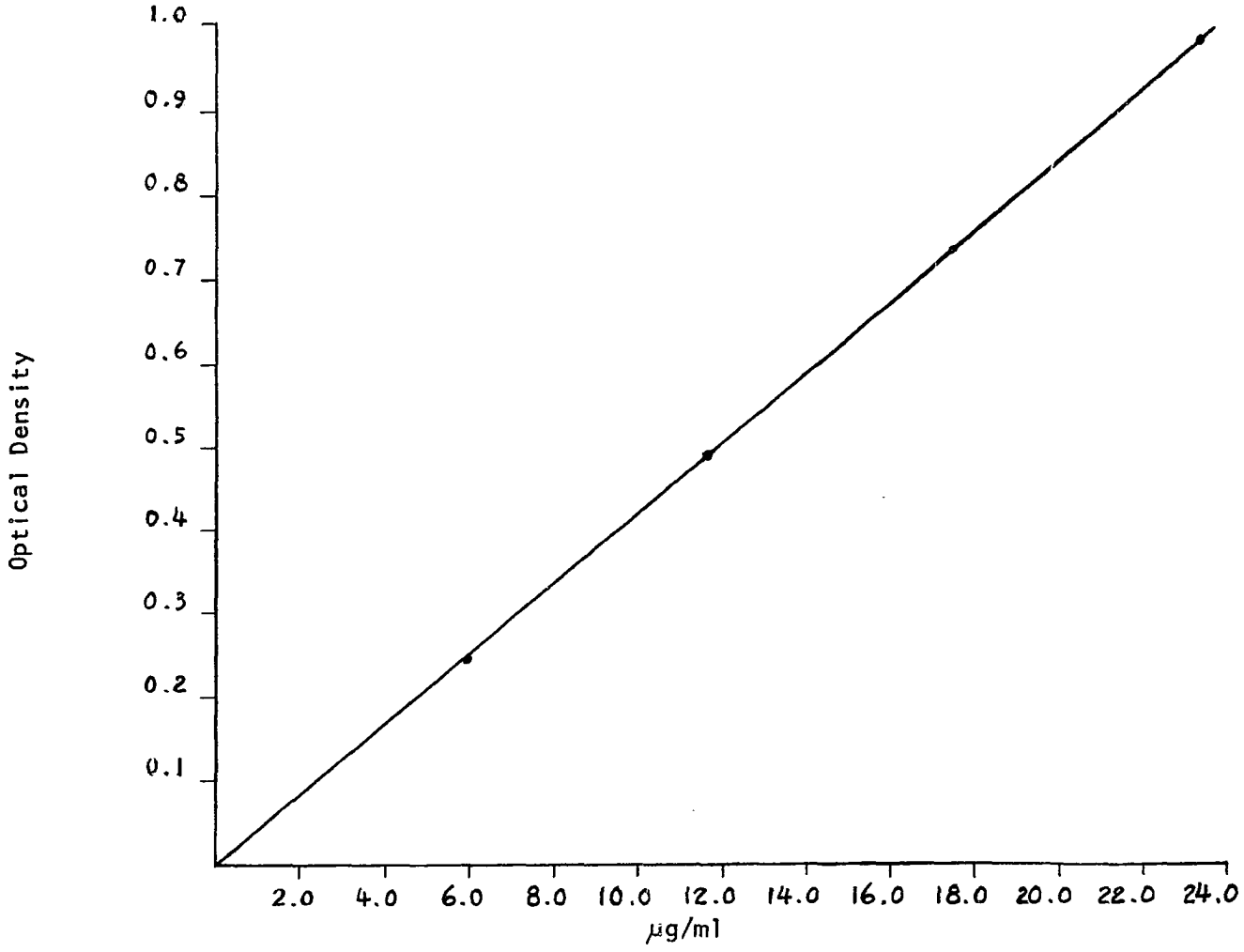

Figure 1. Relationship of optical density to chlorogenic acid concentration.

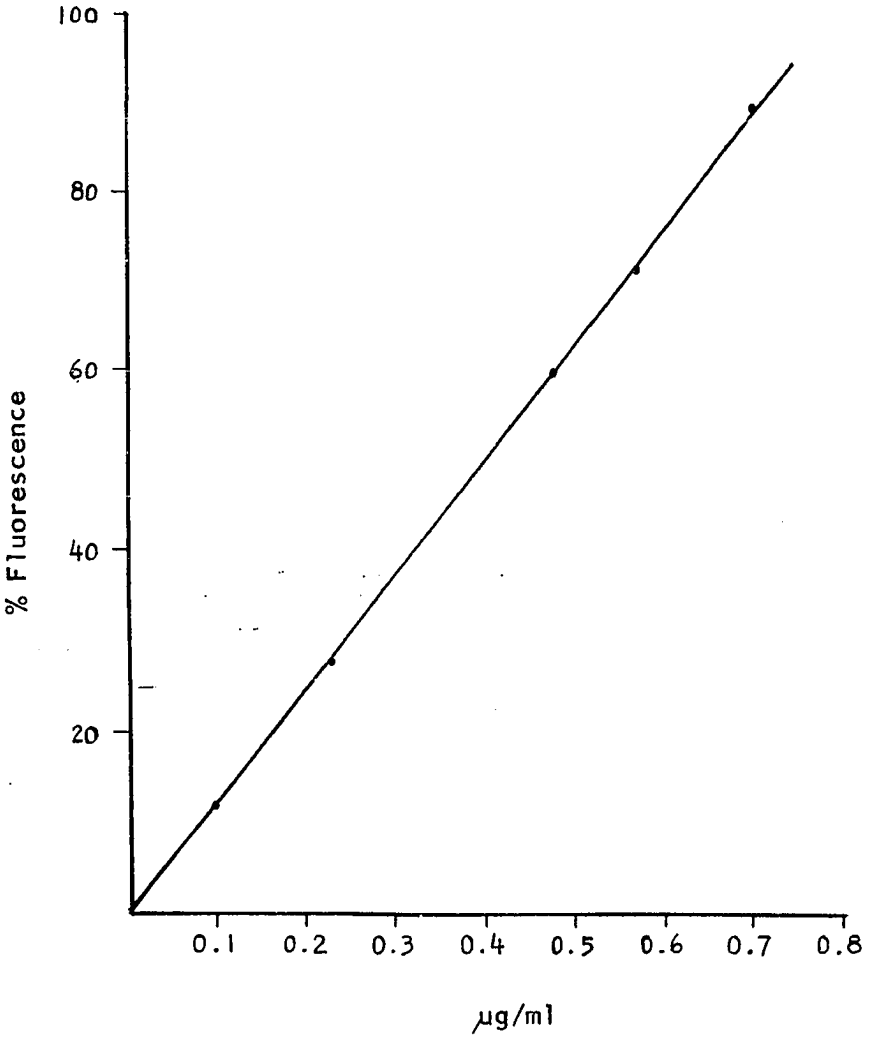

Figure 2. Relationship of per cent fluorescence to scopolin concentration.

CHAPTER III

RESULTS

In control plants the concentrations of the chlorogenic acids and scopolin increased with age in leaves (Fig. 3a,3b,9a,9b) and roots (Fig. 8a,8b,11a,11b), but decreased with age in stems (Fig. 7a,7b,10a,10b).

Control plants had greater concentrations of the chlorogenic acids and scopolin in their leaves at 1300 ft-c than at 1000 ft-c (Fig. 3a,3b,9a,9b). Stems of these plants had lower concentrations of CGA (Fig. 7a,7b), and higher concentrations of scopolin (Fig. 10a,10b) at the higher light intensity. Light intensity had little effect in the roots of these plants (Fig. 8a,8b,11a,11b).

Chlorosis and stunting were evident in nitrogen deficient plants within 1 week after the start of treatment. Little growth was apparent after 1 week of deficiency. Stunting and interveinal chlorosis of magnesium deficiency became evident after 2 weeks on treatment. It was not until nearly the end of the 5 week treatment period, that the potassium deficiency symptoms of chlorosis and "cupping under" of leaves became evident. Slight stunting was observed somewhat earlier.

Effects of Mineral Deficiencies on Chlorogenic Acid

Slightly greater concentrations of chlorogenic acids were found in leaves of potassium deficient plants than in control leaves in 1000 ft-c of light (Fig. 3a). After 5 weeks at 1300 ft-c, however, considerably less of the chlorogenic acids was found in the leaves of potassium deficient plants than in controls (Fig. 3b). Less of the chlorogenic acids was found in magnesium deficient leaves than in controls after 5 weeks on treatment (Fig. 4a, 4b). Pronounced accumulations of CGA were observed in nitrogen deficient leaves (Fig. 5a,5b). The total concentration was more than 2 times that of the controls by week 1 and had increased to nearly 5 times the control in 1000 ft-c of light, and to more than 3 times the control in 1300 ft-c, after 5 weeks. These increases were due mainly to increases in CGA (3-O-caffeoylquinic acid). B510 (4-O-caffeoylquinic acid) and neo CGA (5-O-caffeoylquinic acid) had both decreased in concentration by week 5 in 1300 ft-c of light. CGA, expressed as a percent of the total of the chlorogenic acids, was about 50% throughout leaf analysis, except under nitrogen deficiency, where it steadily increased to about 90% in week 5.

In stems, CGA was the only isomer present in quantitatable amounts. Potassium deficient stems decreased in CGA content as did the controls through week 3, but increased slightly subsequently (Fig. 7a,7b). The decrease in CGA with age was carried to a lower level in magnesium deficient stems than in controls. The CGA concentration in nitrogen deficient stems increased with age and time on treatment at 1300 ft-c.

CGA was the only isomer found in roots (Fig. 8a,8b) and it was slightly lower under potassium deficiency than in the controls. While increases in CGA were found to occur with age in control roots, under magnesium deficiency a decrease in CGA occurred by week 5 in roots. Nitrogen deficient roots were found to have greater concentrations of CGA than controls throughout the treatment period.

Effects of Mineral Deficiencies on Scopolin

Scopolin concentrations were generally greater in the leaves of mineral deficient plants than in controls (Fig. 9a,9b). These increases approximated the time of appearance of deficiency symptoms. The increases were evident after 1 week on nitrogen deficiency, after 3 weeks on magnesium deficiency, and after 5 weeks on potassium deficiency.

Levels of scopolin in potassium deficient stems were similar to controls, generally showing a decrease with age (Fig. 10a,10b). Magnesium deficient stems contained lower concentrations than control stems by week 5 in both experiments, but had reached this level by increases in concentrations with age at 1300 ft-c of light (Fig. 10a,10b). Scopolin concentrations in nitrogen deficient stems generally increased with age, reaching a level about six times that of the controls by week 5 (Fig. 10a,10b).

Scopolin increased with age in potassium deficient roots as in control roots (Fig. 11a,11b). The scopolin concentration attained in potassium deficient roots in 1000 ft-c of light was somewhat less than in the control, whereas it was slightly greater than the control in

1300 ft-c. Magnesium deficient roots contained considerably less scopolin than controls by week 5 (Fig. 11a,11b). Scopolin increased with age in nitrogen deficient roots and reached a level approximately twice that of controls (Fig. 11a,11b).

Basically the same trends were found when results were based on dry weights as on wet weights (Fig. 5b,6). Increases in the compounds with age were lessened when based on dry weight, due to decreasing wet weight/dry weight ratios. Where decreases were noted in the compounds with age based on wet weight, the decreases were greater when based on dry weight. The greatest differences in wet weight/dry weight ratios as compared with controls were found in nitrogen deficient leaves.

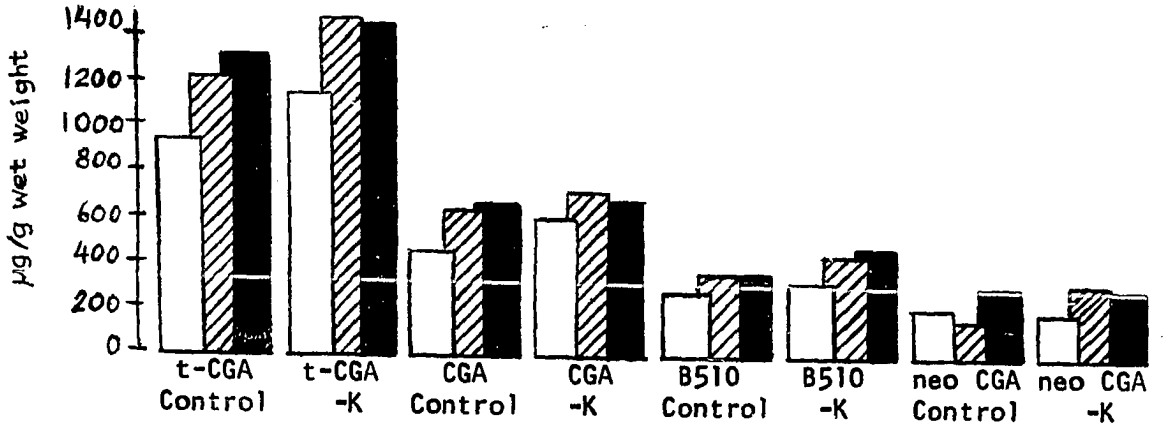

Figure 3a. Concentrations of chlorogenic acids in control and potassium deficient leaves at 1000 ft-c of light in Experiment 1. Symbols: t-CGA, total of the isomers; open bar, after 1 week; slashed bar, after 3 weeks; shaded bar, after 5 weeks.

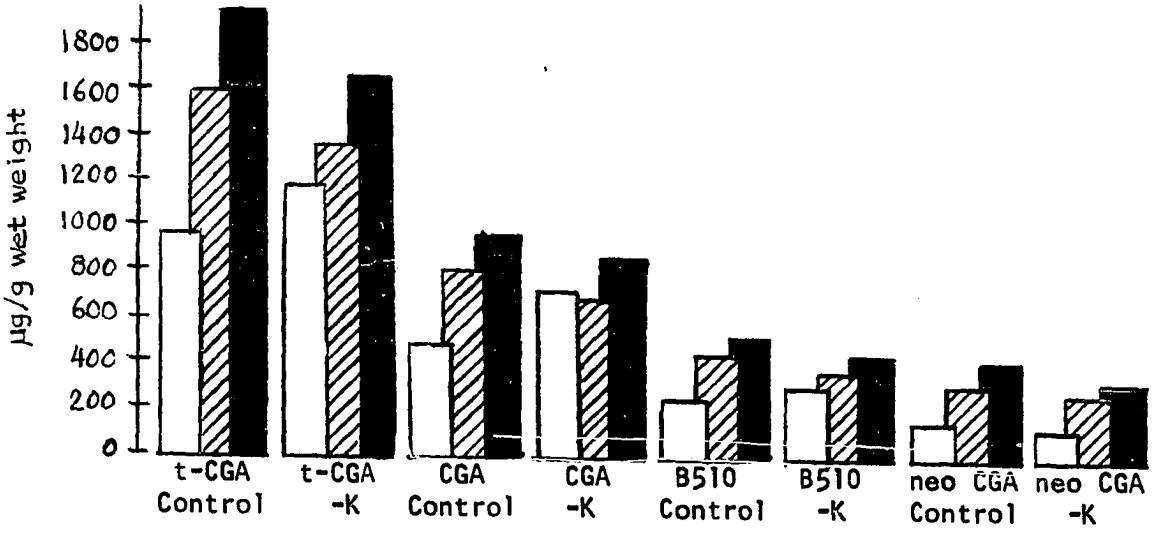

Figure 3b. Concentrations of chlorogenic acids in control and potassium deficient leaves at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

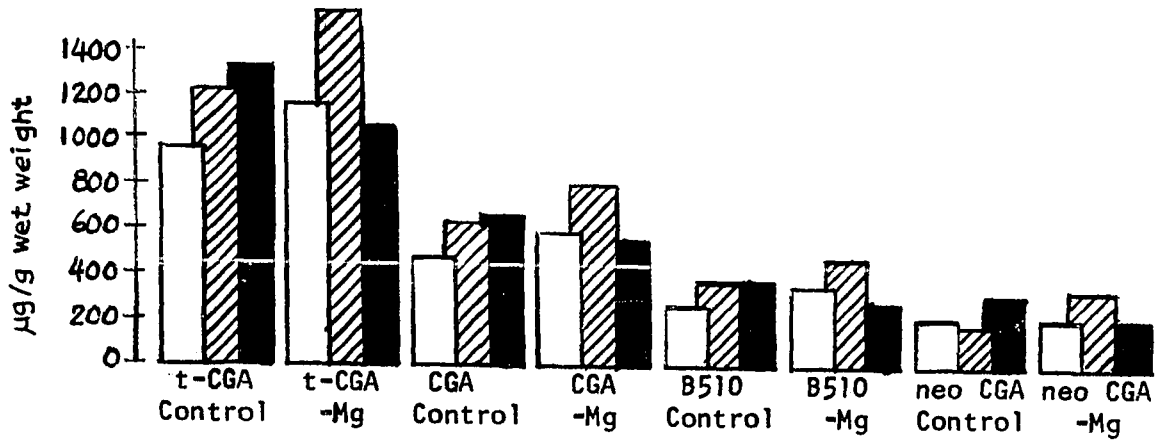

Figure 4a. Concentrations of chlorogenic acids in control and magnesium deficient leaves at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

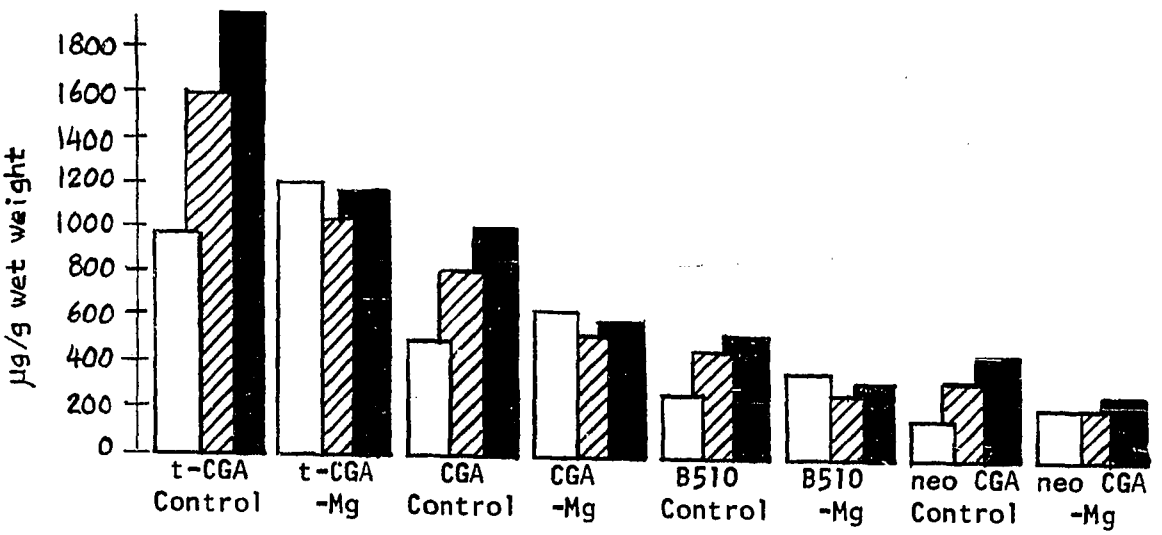

Figure 4b. Concentrations of chlorogenic acids in control and magnesium deficient leaves at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

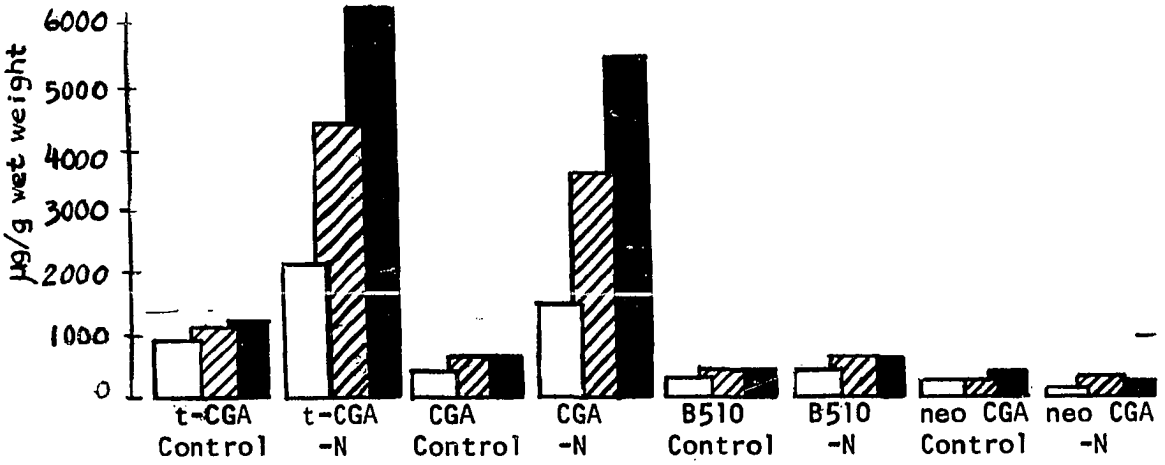

Figure 5a. Concentrations of chlorogenic acids in control and nitrogen deficient leaves at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

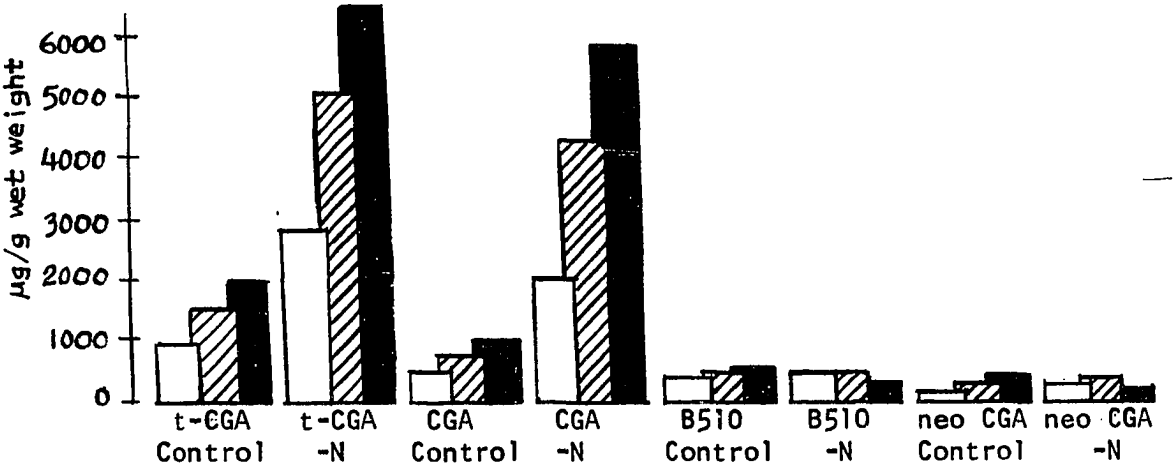

Figure 5b. Concentrations of chlorogenic acids in control and nitrogen deficient leaves at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

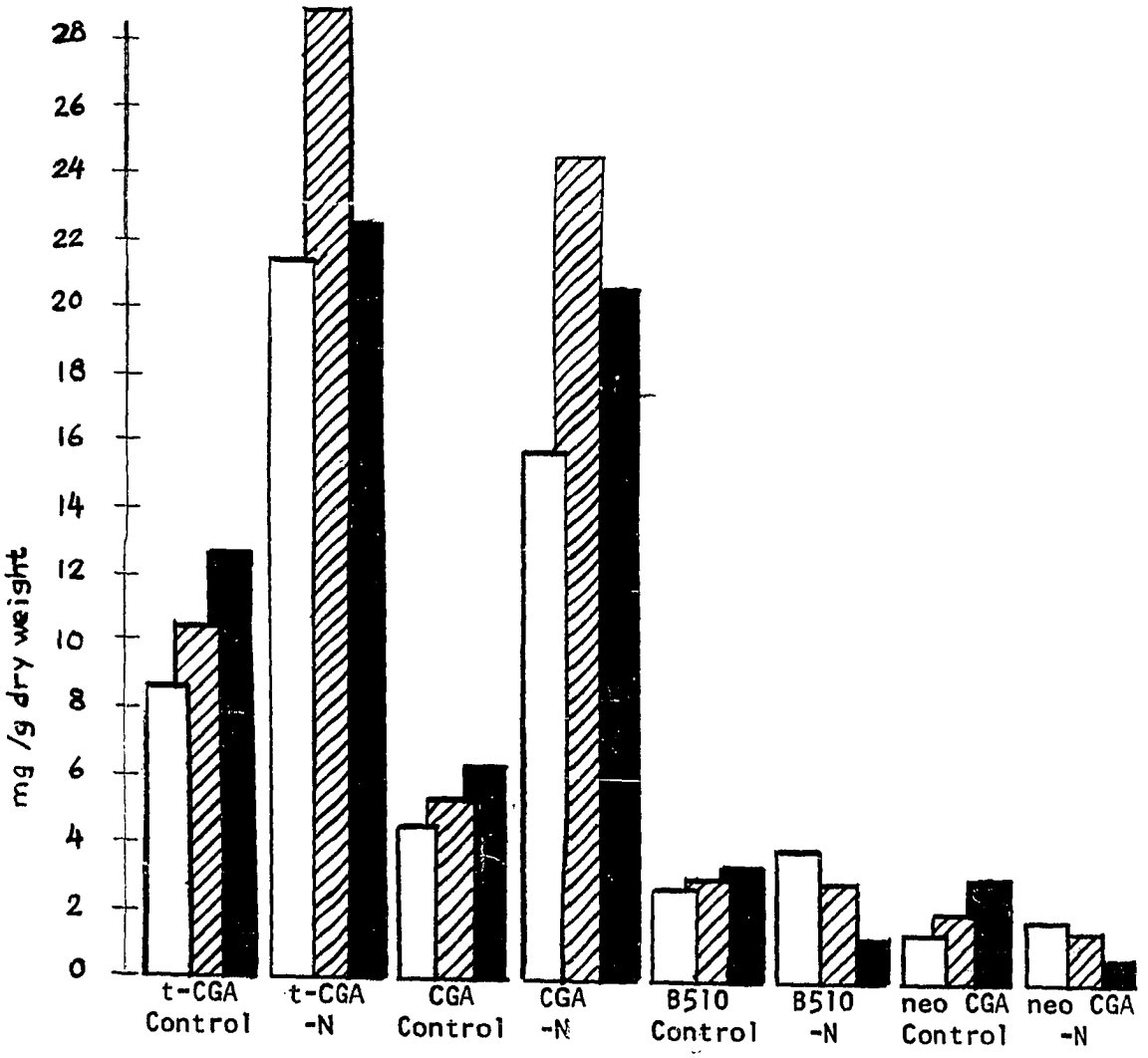

Figure 6. Concentrations of chlorogenic acids, based on dry weights, in control and nitrogen deficient leaves at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

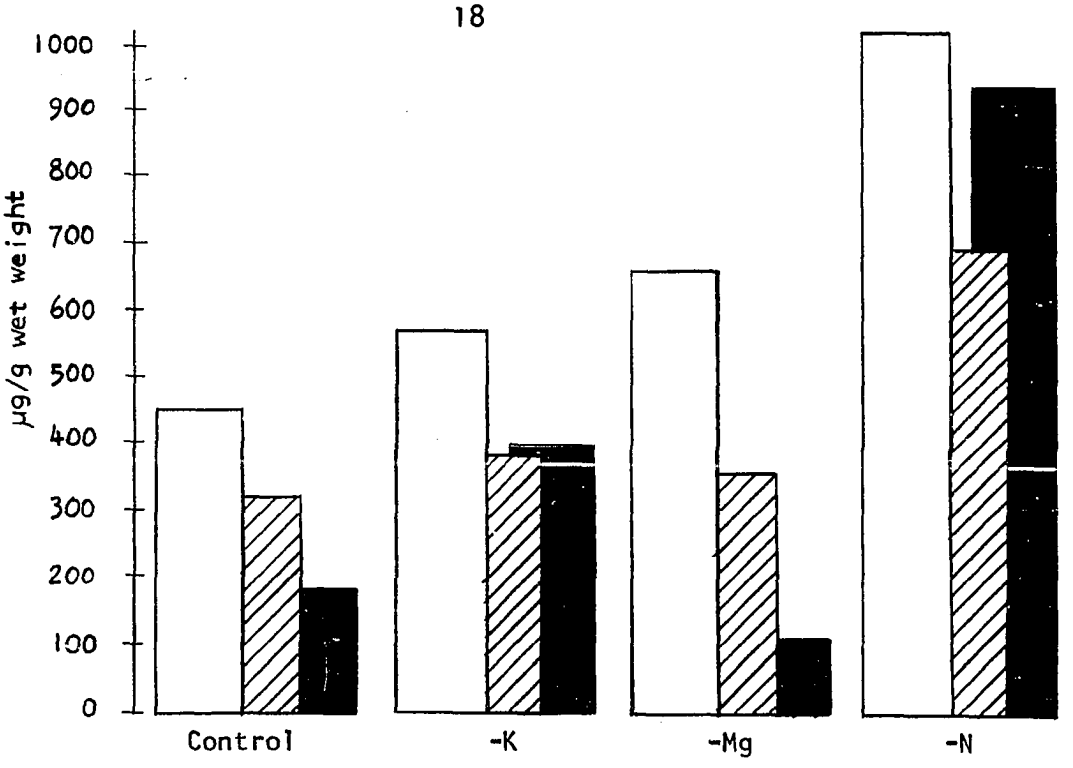

Figure 7a. Chlorogenic acid concentrations in stems at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

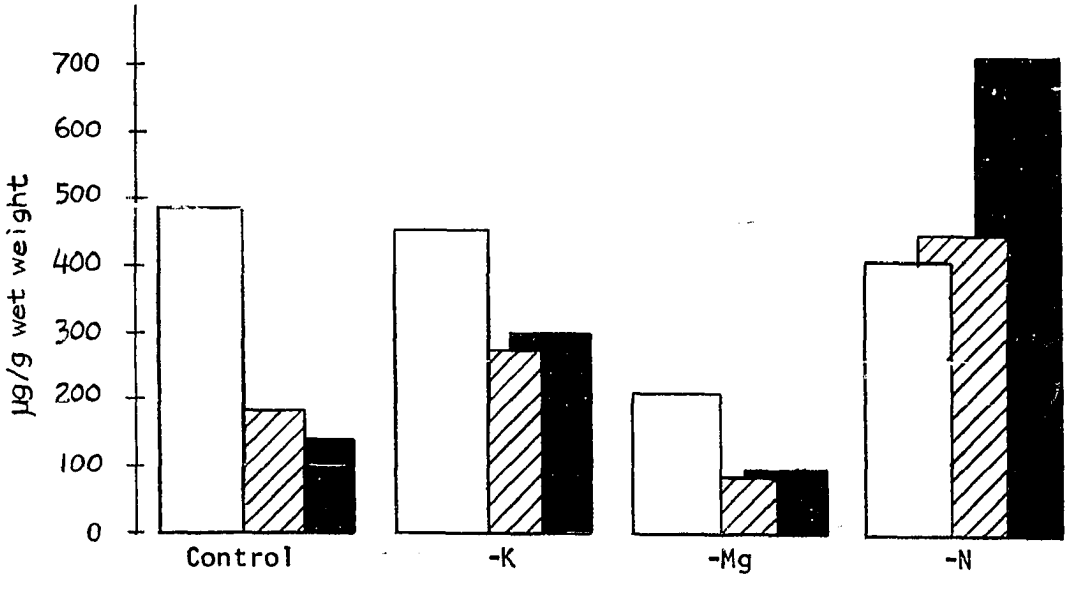

Figure 7b. Chlorogenic acid concentrations in stems at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

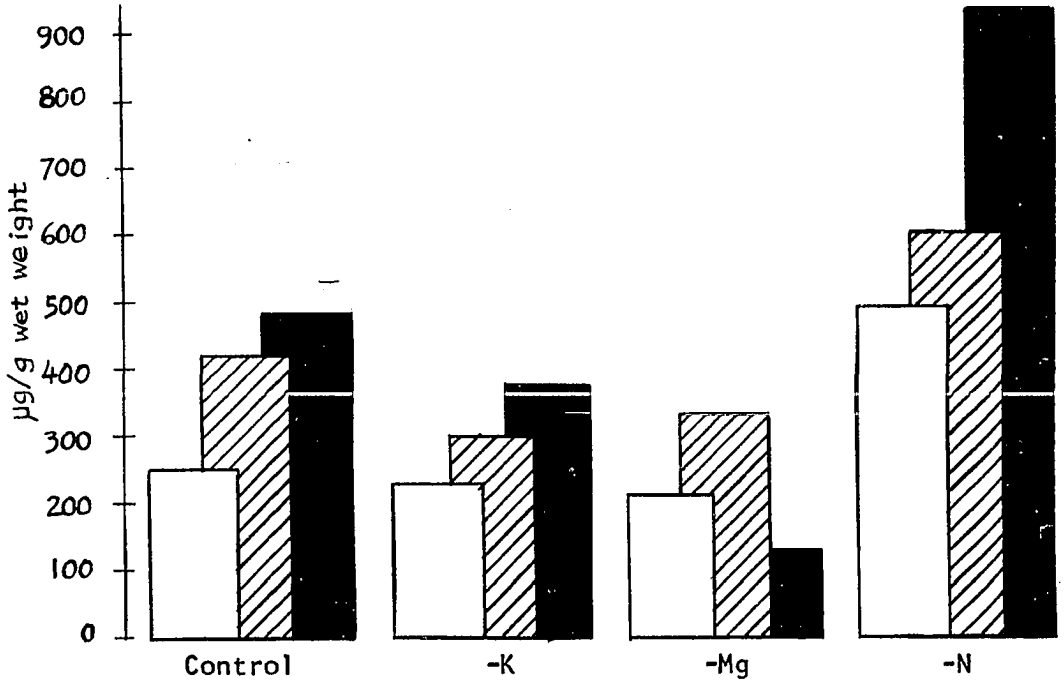

Figure 8a. Chlorogenic acid concentrations in roots at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

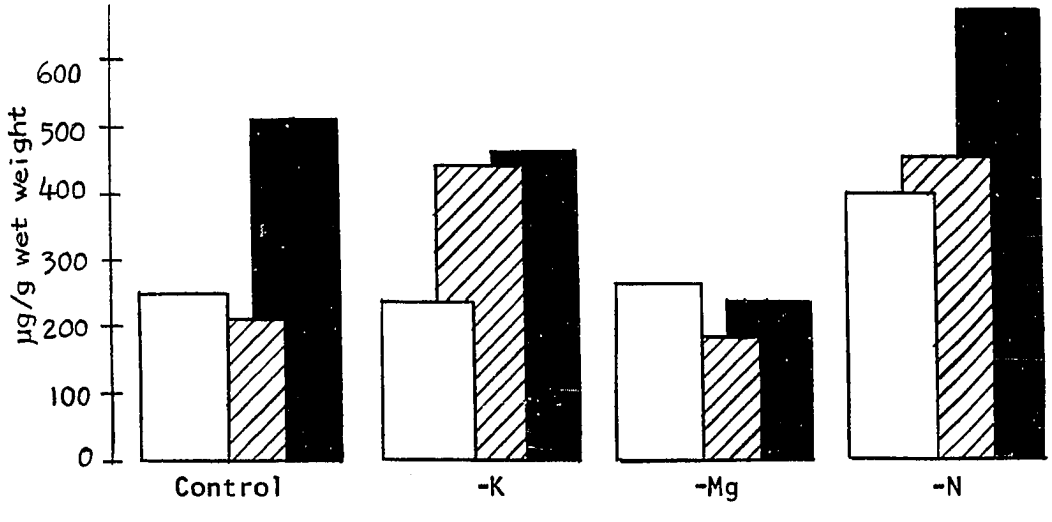

Figure 8b. Chlorogenic acid concentrations in roots at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

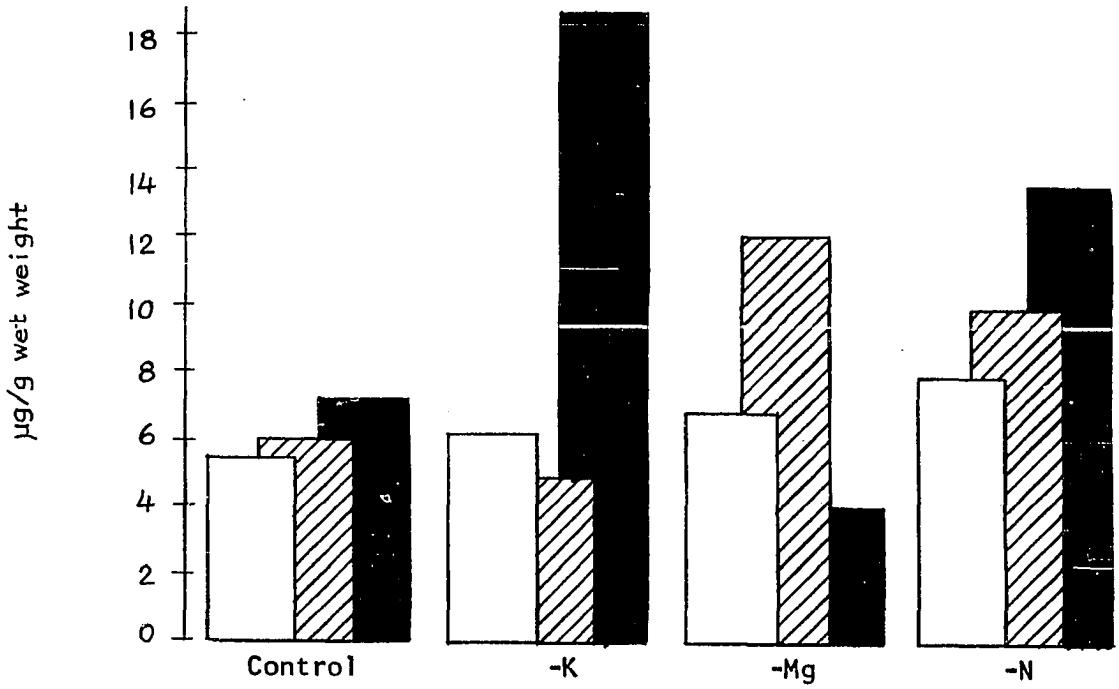

Figure 9a. Scopolin concentrations in leaves at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

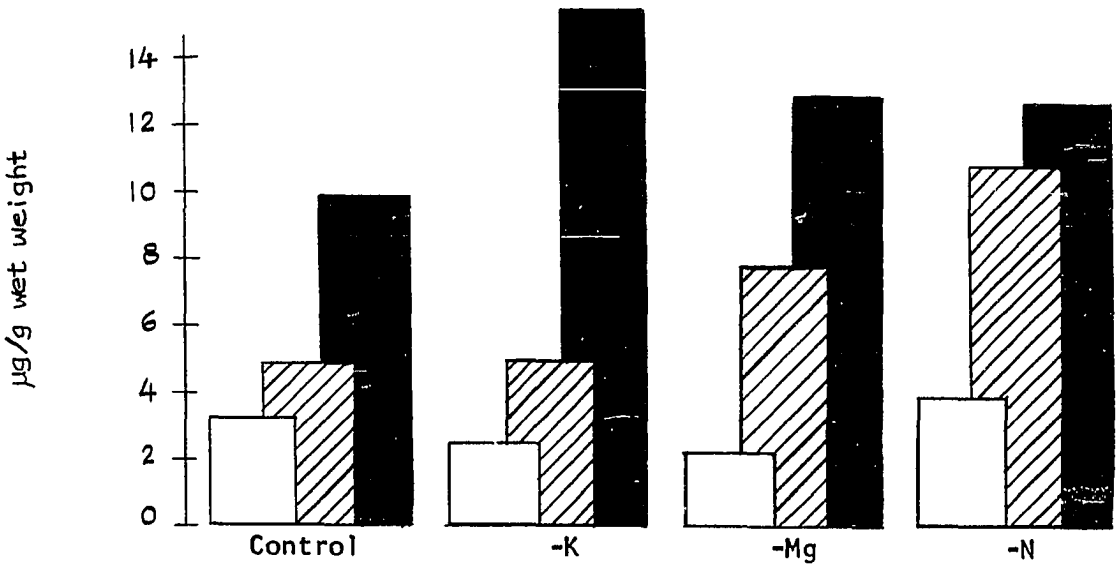

Figure 9b. Scopolin concentrations in leaves at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

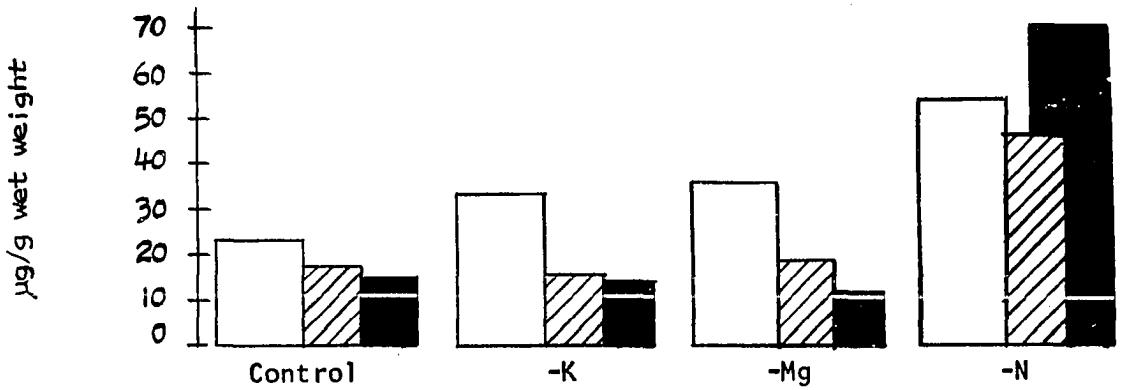

Figure 10a. Scopolin concentrations in stems at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

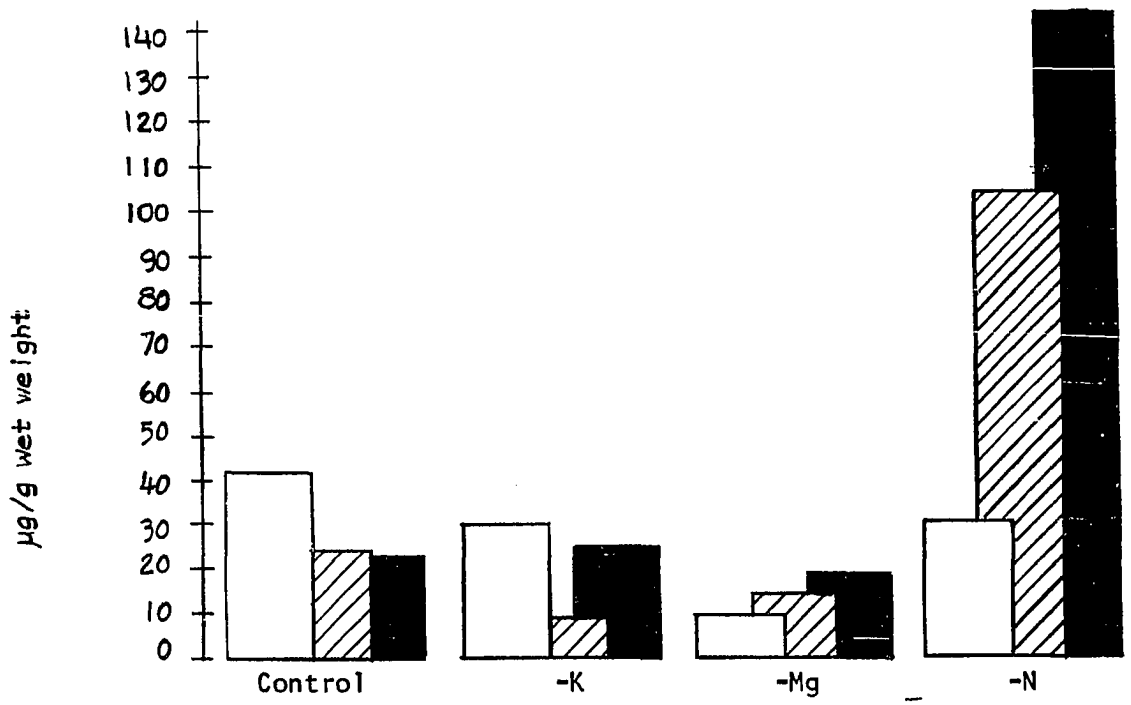

Figure 10b. Scopolin concentrations in stems at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

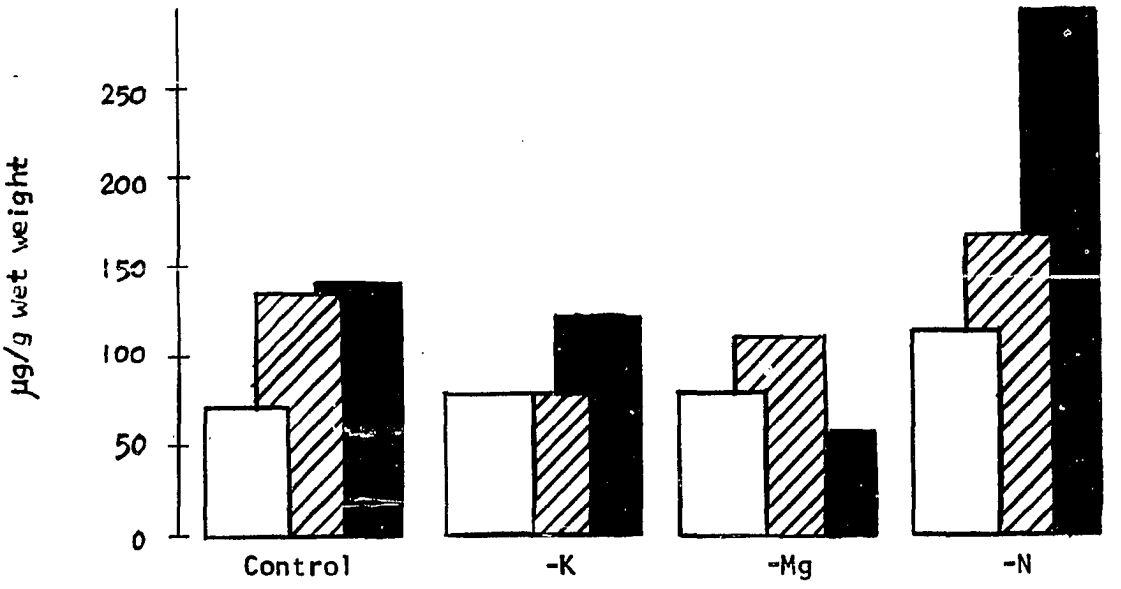

Figure 11a. Scopolin concentrations in roots at 1000 ft-c of light in Experiment 1. See Fig. 3a for symbols.

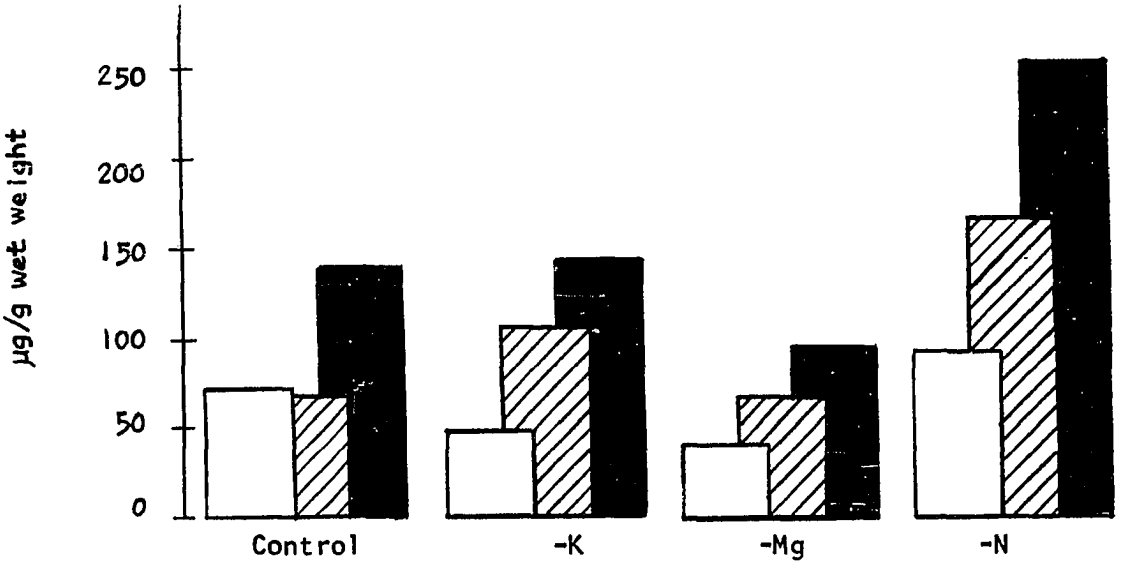

Figure 11b. Scopolin concentrations in roots at 1300 ft-c of light in Experiment 2. See Fig. 3a for symbols.

CHAPTER IV

DISCUSSION

Effects of Age

A decreasing gradient of chlorogenic acid concentrations in tobacco plants has been reported to occur from young to old leaves on a given plant (32,54). Similar findings were reported in Coffea arabica, Theobroma cacao, and in cotton (13,23,42). A similar distinct gradient was even found from leaf tip to leaf base in tobacco (79). I found that the concentration of the chlorogenic acids increased with age in tobacco leaves when the criterion used was age of the entire plant instead of position of the leaf on the plant. These results must not be considered as being contradictory to the above reports, since as pointed out by Morgan (42) the concentration in a particular leaf may well increase with age but still remain lower than in a younger leaf on the same plant.

Basipetal decreases in CGA in stems of Coffea arabica, peas, and tobacco have been reported (13,15,32). CGA concentration decreased with age in stems harvested as entire units. This fits the pattern of decreasing CGA concentration with increasing distance from the meristematic region, since in successive stem samples a greater ratio of "old" stem to "young" stem would be present. Morgan (42) reported basipetal decreases in phenol activity in cotton stems. He found that

the basipetal decrease of inhibitor correlated with a basipetal increase in IAAO activity. Such basipetal increases in IAAO activity were reported in pea stems by Galston and Dalberg (18) and in tobacco stems by Kerstetter and Keitt (29). It is generally considered that IAA concentrations decrease basipetally (36), and Scott and Briggs (52) reported such a decrease in pea stems.

The correlation between chlorogenic acid and other polyphenols and IAAO activity led Tomaszewski and Thimann (68) to suggest that polyphenols synergize IAA, and thus stimulate growth by counteracting the decarboxylation of IAA. This idea has received considerable support from other workers in the field, including Gortner and Kent (22), Henderson and Nitsch (24), Rabin and Klein (44), Sagi and Garay (49), Sondheimer and Griffin (59), and Witham and Gentile (77).

The role of scopolin in the IAAO system is not clear. Tomaszewski and Thimann (68) suggested monophenols stimulate the decarboxylation of IAA and depress growth. Furuya, Galston and Stowe (14) and Waygood, Oaks, and MacLachlan (73) suggested the same role for monophenols in relation to IAAO. However, several reports indicated that scopoletin, a monophenol, inhibited IAAO (2,3,53,74). Root growth has been inhibited by scopoletin (4,21,43), but it is difficult to explain such results on the basis of increases or decreases in IAA. Since Thimann (66) proposed that the natural IAA content in roots is above optimum for growth, it could be interpreted that scopoletin was inhibiting IAAO, and decreasing growth by increasing IAA concentration to a level inhibitory to growth. Interpretation is further complicated, since early reports on

scopoletin may have been incorrect due to hydrolysis of scopolin in the procedure. Sargent and Skoog (50,51) and Skoog and Montaldi (56) indicated that the equilibrium between scopoletin and scopolin may be important in tobacco tissue culture. Recent studies in this laboratory indicated that the amount of scopoletin in tobacco tissue was minute. This does not negate the possible importance of an equilibrium. Although there is no direct evidence that scopolin stimulates IAAO, the increases in scopolin concentration in leaves with age could have been related to a stimulation of IAA degradation counterbalancing to some degree the inhibition of IAAO by chlorogenic acid. The decreases in scopolin in stems with increased age, as was noted for the CGA decrease, was at least in part due to the decreasing proportion of young tissue in the sample with increased age. Koeppe (32) reported that the scopolin concentration was higher in the lower stem sections of tobacco than in the upper parts of the stem, possibly due to translocation into the lower stem regions from the roots where it was found in its highest concentration. The scopolin in the lower regions of the stem may be augmenting the low CGA concentrations by stimulating IAA decarboxylation. The increasing concentrations of scopolin in the roots could have been counterbalancing the increasing inhibition of IAAO due to increased CGA. Since the highest concentrations of scopolin were found in the roots, it could be possible that these high concentrations may function in keeping IAA at a low enough level that it will not completely inhibit root growth.

Another role of these compounds which would be affected by age is lignin biosynthesis (56,64,65). Koukol and Conn (34) identified the enzyme phenylalanine deaminase for the conversion of phenylalanine to cinnamic acid. This reaction coupled with the $^{14}CO_2$ findings of El-Basyouni and Neish (12) yields the following sequence:

phenylalanine ⟶ cinnamic acid ⟶ p-coumaric acid ⟶ caffeic acid ⟶ ferulic acid.

Steck (62) using $^{14}CO_2$ found that ferulic acid could be incorporated into fiber components through a feruloylglucose intermediate or into scopolin through an ester of ferulic acid glucoside in tobacco. Gamborg (19) proposed a pathway for chlorogenic acid synthesis starting with glucose, through shikimic acid to phenylalanine, and on to chlorogenic acid, the final step being esterification of caffeic acid with quinic acid. He proposed a side branch from caffeic acid through ferulic acid to lignin. Levy and Zucker (37) suggested esterification of quinic acid with cinnamic acid followed by conversion to p-coumarylquinic acid and then to chlorogenic acid. Taylor (64) used $^{14}CO_2$ and found that chlorogenic acid was converted into insoluble polymers, presumably lignin, in Xanthium. A possible relationship between chlorogenic acid and scopolin is considered in the following proposed scheme for lignin biosynthesis (Fig. 12).

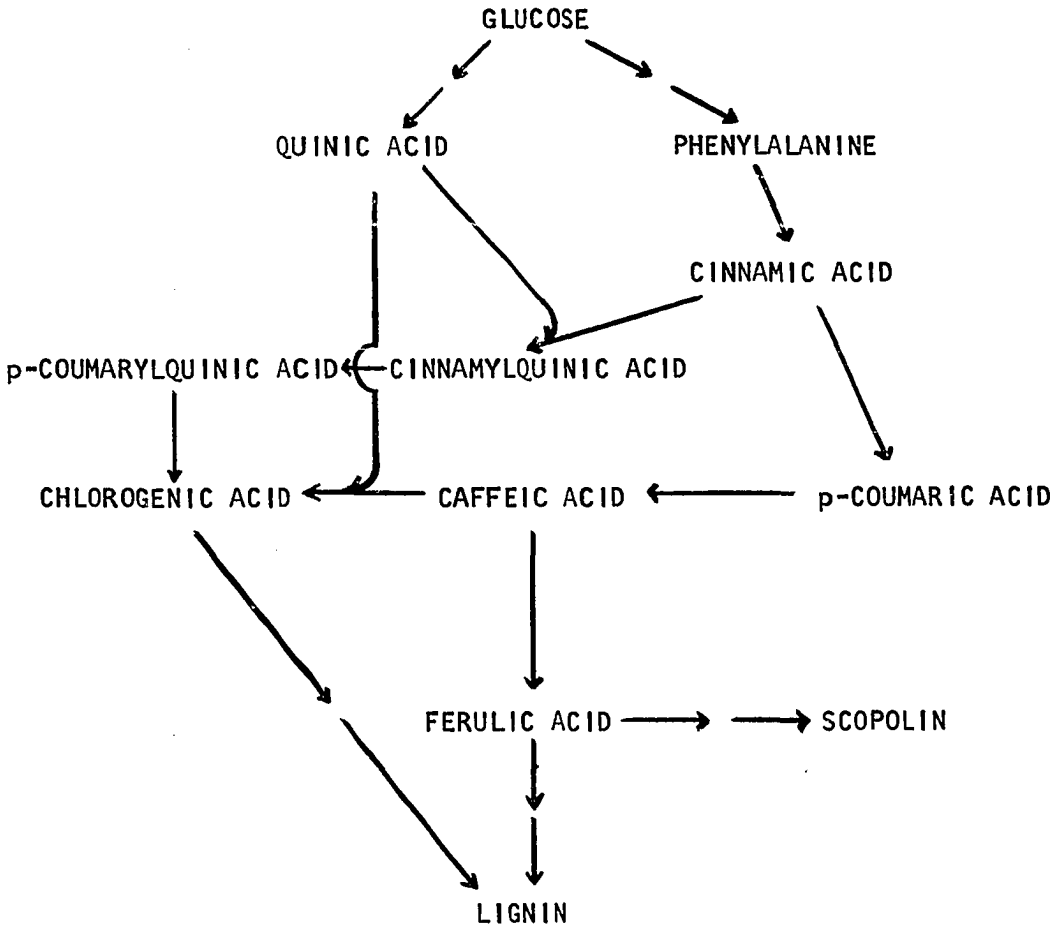

Figure 12. A proposed scheme for lignin biosynthesis.

According to the proposed scheme, increased synthesis of chlorogenic acid, scopolin, or lignin could alter the level of precursor compounds by more rapidly removing them from the scheme and this might alter the production of the other compounds.

Therefore, a decrease in CGA synthesis may have been the cause of CGA decreases noted in stems, and might have caused an increase in lignification, as would be expected in the older portions of the stem. Stafford (60,61) reported that ferulic acid induced lignin accumulation in Phleum pratense and in Sorghum vulgare. A decrease in CGA synthesis could cause increases in ferulic acid. The reverse of this could also have occurred, since an increase in lignin production could have caused a decrease in caffeic acid available for incorporation into CGA. Lignin production may also cause decreases in CGA through the incorporation of the CGA into lignin. The higher level of scopolin in the lower regions of tobacco stems reported by Koeppe (32), was considered to be caused possibly by translocation from the roots. In view of the proposed scheme (Fig. 12), this could have been due to an increase in scopolin synthesis, which through utilization of caffeic acid decreased the synthesis of CGA. However, this would have caused a decrease in lignin synthesis also. In vivo and in vitro studies in Phleum pratense by Stafford (60) and in celery by Siegel, Frost and Porto (55) indicated that IAA inhibited lignification. Considering the roles suggested for CGA and scopolin in relation to IAAO activity, both the increase in scopolin and the decrease in CGA would decrease IAA and thereby stimulate lignification. Balances are evident in this scheme, since the increased lignification would

tend to keep the IAA level up, which would tend to slow down lignification and tend to keep the CGA level up. It is suggested that the proposed roles of scopolin and chlorogenic acid in the IAAO system and in lignification are interrelated.

Effects of Light Intensity

Konishi and Galston (33) reported that phenolic compounds change quantitatively and qualitatively on exposure to light. Zucker (78) reported that both increased light intensity and duration stimulated the production of chlorogenic acid in potato tuber tissue. Similar studies by Tang and Bonner (63), Watanabe and Stutz (72) and Sagi and Garay (49) have correlated increase in inhibitor (of IAAO) level and decreases in IAAO activity with increased light intensity. The results of the leaf analyses of the present study support these findings. The CGA levels in the stems were considerably lower, however, and only the scopolin level was increased. Galston and Baker (16) reported that the naturally occurring inhibition of IAAO from peas was reversed upon exposure to light. Both the decrease in CGA and the increase in scopolin, in the stems under the higher light intensity, would tend to stimulate IAAO activity, according to their proposed roles. Leopold (36) indicated that inhibitions of growth with increased light intensity are common. The stimulation of IAAO and the consequent reduction in IAA in the higher light intensity would tend to inhibit growth.

Effects of Mineral Deficiency

Loche and Chouteau (38) found that magnesium deficient leaves of tobacco contained about 1/3 less chlorogenic acid than controls after 72 days on treatment. They also reported higher concentrations of scopolin in the deficient leaves. In a later study they found that concentrations of chlorogenic acid in tobacco leaves increased with decreasing levels of nitrogen nutrition, while decreases in potassium appeared to yield decreases in chlorogenic acid (7). They did not report on roots or stems in either study. Tso and coworkers (69) studied 4 varieties of flue-cured and air-cured tobacco leaves at different levels of nitrogen fertilization. They found a direct relationship between level of nitrogen fertilization and concentrations of chlorogenic acid and scopolin in 3 varieties, but found an inverse relationship in the fourth variety. Results of the present study support the finding reported by Chouteau and Loche, and coupled with the findings of Tso and coworkers, indicate that different tobacco varieties respond differently to similar environmental conditions.

The proposed roles of chlorogenic acid and scopolin in relation to IAAO are rather difficult to interpret in mineral deficient plants. Observed stunting could be due to the deficiency itself, or to decreases in IAA, or to both. High levels of IAA could be overshadowed by stunting due to other effects of the deficiency.

The high chlorogenic acid concentrations in nitrogen deficient plants were not balanced by comparable increases in scopolin, as the chlorogenic acid to scopolin ratios were about twice as high in nitrogen deficient plants as in controls. A net decrease in IAA

in the leaves could have occurred because of the requirement for nitrogen in IAA and in the enzymes responsible for its synthesis, even though IAAO activity was markedly inhibited.

Chlorogenic acid and scopolin concentrations were high throughout the samples from nitrogen deficient plants. If these two compounds have opposite effects upon IAA concentrations, the resultant effect upon the IAA level would depend on their levels of effectiveness. It is logical, however, to consider the IAA level to be low in nitrogen deficient plants since IAA and the enzymes responsible for its synthesis contain nitrogen. Nitrogen deficient stems were most difficult to grind, and this could be related to increased lignin content. Siegel et al. (55) reported that organic nitrogen compounds, including IAA, inhibit lignification. Such compounds would be low in nitrogen deficient plants, and stimulation of lignification could have occurred. If the reaction equilibria were upset by the high levels of chlorogenic acid and scopolin, caffeic acid and ferulic acid could have been available for lignin synthesis. The large quantities of chlorogenic acid could have increased lignification, since Taylor (64) found it was a precursor of lignin. The fact that wind-lodging is more evident in plants grown on rich supplies of nitrogen may be related to these factors in lignification.

Although Dutta and McIlrath (11) reported a decrease in lignification in boron deficient sunflower tissue cultures, McIlrath and Skok (40) reported increases in lignification in boron deficient sunflower and tobacco stems. Boron deficient tissues were shown to be high in scopolin (70,71), and possibly low in chlorogenic acid (71). The decreased chlorogenic acid and increased

scopolin could have yielded low levels of IAA, based on their proposed roles, and caused an increase in lignification. These findings indicate that studies of lignification in the potassium, magnesium, and nitrogen deficient tissues will shed light on the roles of chlorogenic acid and scopolin. Such studies are in progress.

Mineral deficiencies developed in this study were induced by deficiencies in the nutrient solution. Mineral deficiencies are not always due to low mineral levels in the medium or the soil, but often are related to the ability of the plant to take up available minerals. Low temperature is a factor that can limit mineral uptake (6,48). In studies carried out in this laboratory by Koeppe (not published), tobacco plants grown on full nutrients were exposed to low temperature. These plants developed visual symptoms nearly identical with nitrogen deficiency symptoms. Quantitative analyses of these plants showed levels of the chlorogenic acids and scopolin very similar to those resulting from nitrogen deficiency. it is likely that mineral uptake is a critical factor in relation to cold treatment, and nitrogen is probably of special importance since its deficiency is so rapidly attained.

Increases in phenolic compounds with nitrogen deficiency has been considered important in the resistance of higher plants to pathogen infection. Kiraly (30) reported that wheat rust resistance was enhanced, when phenol content increased, at low levels of nitrogen fertilization. Resistant varieties of wheat tend to accumulate phenolic compounds, after infection, more rapidly than non-resistant varieties (31). These and other workers have suggested

that phenolic compounds, including chlorogenic acid, may be part of a protective mechanism in the resistance of many plants (27,28,35,53).

Chlorogenic acid and scopolin may also be important ecologically, since Abdul-Wahab and Rice (1) and Rice (45) found that chlorogenic acid along with other phenolic compounds had significant phytotoxic effects, and this was reported for scopolin by Wilson (75). The study of environmental conditions which induce quantitative or qualitative changes in these compounds may be very significant, therefore. This study is of particular significance since chlorogenic acid and scopolin increased in nitrogen deficient plants, and nitrogen has been found deficient in abandoned fields (8) where allelopathic effects have been shown to be important in succession (1,45,75).

CHAPTER V

SUMMARY

Concentrations of chlorogenic acid and scopolin increased with age in roots and leaves of tobacco plants, but decreased with age in stems. An increase in light intensity caused little change in these compounds in the roots, but increased the concentrations in the leaves. Higher light intensity increased the scopolin concentrations and decreased the chlorogenic acid concentrations in stems.

Potassium, magnesium, and nitrogen deficiencies increased the scopolin concentrations in leaves. The increases were approximately correlated with the time of first observable deficiency symptoms. Nitrogen deficiency symptoms became apparent early in the five week treatment period, magnesium in the middle, and potassium very late.

Pronounced increases in chlorogenic acid concentrations were found in nitrogen deficient leaves, while decreases were noted in magnesium deficient leaves. Potassium deficient leaves showed a slight increase in chlorogenic acid concentration in a light intensity of 1000 ft-c, but a considerable decrease in 1300 ft-c. Chlorogenic acid and scopolin concentrations increased with age in stems of nitrogen deficient plants, while in controls the concentrations decreased with age. Increases in these compounds with age in the

roots of nitrogen deficient plants were more pronounced than in controls.

Magnesium deficient stems and roots had lower concentrations of chlorogenic acid and scopolin than controls. The scopolin concentrations in potassium deficient roots and stems did not vary greatly from the controls, and the concentrations of chlorogenic acid in the roots were similar to those in the controls. Chlorogenic acid decreased with age in potassium deficient stems in the early stages of treatment but increased later.

A composite scheme for lignin synthesis was proposed, including chlorogenic acid and scopolin. In relation to IAA, chlorogenic acid was considered as an inhibitor of IAAO, and the suggestion was also made that scopolin stimulates IAAO. The effects of age, light intensity, and mineral deficiencies on the chlorogenic acid and scopolin concentrations were shown to fit the proposed scheme for lignin biosynthesis and the roles of the compounds in relation to IAAO, when IAA and other nitrogen-containing organic compounds act as inhibitors of lignification.

It seems likely that allelopathic effects of certain species which are important in old-field succession may be accentuated due to the increases in concentrations of scopolin and chlorogenic acid resulting from the very low levels of nitrogen in such areas.

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