To the Graduate Council:

I am submitting herewith a thesis written by Thomas Charles Corbin entitled "Variation Among Genotypes for Aluminum and Manganese Accumulation in Burley Tobacco." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

Vernon H. Reich, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Thomas Parlin

Date 8-12-85

VARIATION AMONG GENOTYPES FOR ALUMINUM AND MANGANESE ACCUMULATION IN BURLEY TOBACCO

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Thomas Charles Corbin

August 1985

ACKNOWLEDGMENTS

The author wishes to express his gratitude to all those who gave encouragement, support and assistance during this course of study. Special appreciation is expressed to the following:

The Plant and Soil Science Department, Dr. Lloyd Seatz, Head, for providing financial assistance, the necessary materials and equipment.

R. J. Reynolds Tobacco Company for providing funding for this study.

Dr. Creighton Gupton for his guidance and assistance throughout the course of this study.

Dr. Vernon H. Reich for his guidance and assistance throughout the course of this study and in preparation of this manuscript.

Drs. R. J. Lewis, R. D. Miller and Mr. B. C. Nichols who gave their advice and constructive criticisms during the preparation of this manuscript.

Mr. Uel D. Wilhoit of the Tobacco Experiment Station Greeneville, Tennessee, for his labor and assistance.

Dr. and Mrs. Charles Corbin, Jr. for their support and encouragement throughout this course of study.

Sherry and Anne Corbin for their patience and support throughout this course of study.

Fellow graduate students for their encouragement, assistance, and fellowship throughout graduate work.

ABSTRACT

Fifteen burley tobacco (Nicotiana tabacum L.) genotypes were transplanted into unlimed (pH 4.5) and limed (pH 5.3) soil blocks to study Mn and Al toxicities over two years. The interactions of the burley tobacco genotypes and the pH, Mn, and Al of the soil were investigated for possible differential tolerance. Correlations were made between the concentrations of Mn and Al in the soil with the amount of these metals in the laminae of the tobacco.

In 1978 there was a highly significant reduction of Mn in the soil accompanied by a highly significant reduction in the laminae from the unlimed to the limed soil blocks. Correlation coefficients were high indicating that correlation between soil and laminae Mn are reliable and support the analyses of variance. No differential tolerance to toxic levels were found, but it was concluded that liming is effective in preventing Mn toxicity problems.

In 1979 the results of six genotypes transplanted into the same soil conditions supported the previous years conclusions. When the pH passed the critical point, Mn was not as available to the plant and the correlations between the laminae and soil Mn were not as strong.

Aluminum in the laminae was reduced, nonsignificantly, by liming. The correlation between Al in the soil and laminae was very weak. There was no indication that Al in the laminae was of any value in studying Al in the tobacco plant.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Manganese and aluminum toxicity in burley tobacco (Nicotiana tabacum L.) is a production problem for many growers. The problem stems from a low pH soil condition which allows Mn and Al to become more available in the soil solution. High annual rates of acid forming fertilizers, necessary for tobacco production, contribute to the acid soil condition.

Manganese toxicity occurs at a soil pH of 5.5 or below. There is a 95% decrease of exchangeable Mn when the soil pH increases from 4.6 to 6.5 (11). Manganese toxicity symptoms begin as chlorosis in the tips of the leaves then spreading over the entire leaf. This chlorosis between the small veins gives the leaves a mottled appearance; in more severe cases the chlorosis advances to necrotic leasions, thus reducing leaf quality. Very high concentrations of Mn are necessary for reduced growth of the tobacco plant to be observed.

Aluminum toxicity usually occurs at a soil pH of 5.0 or below. In soils of pH greater than 5.5 aluminum is not excessive in the soil solution. The concentration of Al increases sharply as the pH drops from 5.0 to 4.5 and below (32). Aluminum toxicity does not result in clearly identifiable above ground plant symptoms as does Mn toxicity, but it does reduce the growth of tobacco.

When tobacco is grown on low pH soils, Al and Mn toxicity both affect the crop. Aluminum reduces growth and Mn reduces leaf quality

(15). Basically, this problem can be controlled by proper soil pH management. However, a plant breeder can utilize the information on the genetic variation associated with burley tobacco cultivars and Mn and Al toxicities on low pH soils to incorporate tolerance in new cultivars.

The objectives of this research were: (1) to study the interaction between burley tobacco genotypes and Mn and Al toxicities on low pH soils, and (2) to correlate Mn and Al accumulation in the laminae of the different genotypes with soil pH and measures of Mn and Al availability.

CHAPTER II

LITERATURE REVIEW

Tobacco

Manganese toxicity was investigated a few years before aluminum toxicity, perhaps because the above ground Mn toxicity symptoms are more readily noticeable than is the reduced growth resulting from Al toxicity. As early as 1929 Jacobson and Swanback (30) described an abnormality in tobacco, mainly mottled chlorosis, and sought to confirm the cause as Mn toxicity. They obtained identical symptoms by growing tobacco in water culture containing 1 mg L^{-1} of manganese sulfate, and in sand culture with 80 mg L^{-1} manganese sulfate. They continued their investigations using several Connecticut soils. By 1932 Jacobson and Swanback (31) concluded that high amounts of active Mn in the soil were toxic to tobacco. In addition they reported that increased soil acidity greatly increased water-soluble Mn in the leachates and was accompanied by increased uptake of Mn by tobacco plants.

Similar work was done on Turkish tobacco by Bortner (4), in 1934. Using nutrient solutions and acid soils from Kentucky, he investigated Mn and Al as causes for chlorotic mottling of the leaves. Aluminum in water cultures and in soil cultures was found to produce shorter, thicker roots than in control cultures. However, the damage was less extensive in the soil cultures. Aluminum reduced top growth but did not produce any chlorosis in the leaves. Manganese concentrations of 15 mg L^{-1} in nutrient solution produced chlorosis in the

leaves which increased in severity as the concentration of Mn increased. These symptoms were concluded to be the same as those observed in the field. The results of the soil cultures showed a general correlation between the extent of the leaf injury, amount of Mn in the plant and the amount of Mn in the soil leachates. Bortner reported finding no toxicity symptoms on tobacco grown in the greenhouse using limed Kentucky soils. However, he did find the addition of phosphorus slightly reduced the chlorosis in the acid soil.

Anderson et al. (2) reported that growers in Connecticut had problems with "black" shade leaf tobacco. This was a low quality, dull, dark cured leaf that had a muddy colored ash. In a few fields, spots of black tobacco were grown in the same field that produced mostly light-colored leaves. Investigating the source of the problem, they sampled hands of black and light leaves of shade, Havana seed and broadleaf cultivars and compared chemical constituents among these samples. In addition they collected soil samples for analyses from the same fields. All of the black tobacco analyzed had higher amounts of Mn and Fe than did the light colored leaves. A gradation from light to darker leaf color was accompanied by an increase of Mn and Fe. The soils associated with black tobacco were acid and low in Ca and P.

Manganese toxicity in burley tobacco was investigated by Hiatt and Ragland (27) in 1963. They used nutrient solutions to grow Burley 21 to determine the Mn concentration in the solution and plant that resulted in the appearance of toxicity symptoms. They also varied the Fe and Al concentrations in some of the solutions to

determine their effect upon the expression of the toxicity symptoms in tobacco. They found that symptoms of chlorosis and necrotic spotting of the leaves were due to excess Mn alone. Aluminum was more injurious to the plant growth but did not produce the chlorosis characteristic of Mn toxicity. They reported the tissue concentration of Mn to be 3000 mg kg^{-1} before symptoms appeared and that as much as 5000 mg kg^{-1} of Mn in the tissue did not reduce plant growth. The addition of Al and Fe to the solution decreased the amount of Mn in the tobacco.

Link (36) in 1979 reported on the critical soil pH for expression of Mn toxicity in burley tobacco. After surveying burley tobacco fields in southwest Virginia, two farm locations were selected for further study. The fields were limed using different rates from 0 to 9.0 mt ha^{-1}. Tobacco was grown in these fields for the next two successive seasons. Manganese toxicity symptoms on tobacco grown on soils of 40 to 200 mg kg^{-1} Mn did not occur until the pH dropped below 4.8. The addition of small amounts of lime reduced the visual symptoms of Mn toxicity but did not reduce the tissue Mn levels. Tobacco with visual toxicity symptoms had variable amounts of Mn in the leaf tissue (1710 to 5410 mg kg^{-1}). Some normal leaves contained more Mn than chlorotic leaves from other years, which suggested an environmental effect.

Nutrient solution soil drenches and growth chambers were used to manipulate the Mn concentration and the environment to study their effects on flue-cured tobacco by Rufty et al. (42). They found that

plants grown at higher temperatures expressed less severe toxicity symptoms and less reduction in growth despite generally higher concentrations of leaf Mn. In their studies, tolerance to high levels of Mn increased as the temperature increased even though Mn concentration in the leaf did not decrease. Sometimes the Mn toxicity symptoms in the leaves diminished completely as the temperature increased and as the leaves matured, but the concentration of Mn in the tissue was unchanged. They also reported that an enhanced growth rate had the effect of decreasing Mn toxicity sensitivity. Rufty et al. concluded that the critical concentration of Mn can change with changes in the environment, especially temperature.

Other Crops

Heritability of tolerance to Mn toxicity in alfalfa (Medicago sativa L.) was reported by Dessureaux (12) in 1959. Selfed and crossed progenies of four parents were evaluated for tolerance to high and low levels of Mn applied as nutrient solution to sand media. Manganese content and percent reduction of dry weight were two of the four criteria, for measuring the effects of Mn toxicity, that suggested high heritability of Mn tolerance. The parent progeny regression coefficient was close to one with a low standard deviation in the two cases indicating high heritability of tolerance to Mn toxicity.

Buss et al. (9) found essentially the same thing as Dessureaux in the response of alfalfa to a high concentration of Al in the growing media. Acid Tatum soil was used to find the significant interaction between soil pH and clones. Layers of soil differing in pH were used

to approximate acid subsoil horizons. Alfalfa was concluded to have sufficient genetic variation to acid soil high in Al to respond to selection pressure. There was evidence that the tolerance was more narrow than in other crops such as wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.). This study as well as an earlier one by Dessureaux (13) were in agreement that dry weight was the best indicator of differential tolerance to Al in alfalfa seedlings.

Selection for tolerance to Al toxicity in alfalfa was done by Devine et al. (14). Selections were made from six cultivars grown on Al-toxic Bladen soil at a pH of 4.1 to 4.3. The tolerant and susceptible plants were intercrossed within each class to form two populations. Each population was subjected to two cycles of recurrent phenotypic selection. The strains were then planted in Al-toxic Tatum soil and evaluated for top growth vigor and degree of root development. The top-growth vigor was strongly correlated with root scores. They concluded that the heritable differences in alfalfa for tolerance to Al toxicity could be used in a recurrent selection program to develop tolerant strains.

Neenan (38) in 1960 reported on the differential response of wheat and barley cultivars to excess Mn and Al, on the acid soils of Ireland. He conducted a series of experiments to characterize the effects of acid soils on cereals. The field experiments were grown with different cultivars of wheat, barley and oats (Avena sativa L.) under different pH levels and with several concentrations of added Mn and Al. Sand culture was used to find differential responses of

wheat, barley and oats to five concentrations of Al and six of Mn in the nutrient solutions. The oat cultivars showed no changes in most of the tests. A general response was observed in the barley cultivars, but they did not provide a differential response and four cultivars of wheat were then taken into the greenhouse and grown in acid soils using different rates of time. Among the wheat cultivars selected, April Red was tolerant to both Al and Mn. Altae and Karn were both sensitive to Mn and Al toxicities. Progress was tolerant of high concentrations of Mn but was very sensitive to excess Al. An interesting observation was that the plants grown at intermediate levels of Mn contained different amounts of Mn and the plants grown at high levels generally contained the same amount of Mn.

To find the range of Al tolerance available in wheat and barley cultivars, Foy et al. (16) screened 27 wheat and 15 barley cultivars on Al-toxic Bladen soil. The wheat was grown on unlimed soil only, while the barley was grown both on soil receiving no lime and 375 mg kg^{-1} $CaCO_3$. After screening the cultivars, six wheat and six barley cultivars were selected to confirm the differential Al tolerance on Al-toxic Tatum soil at different lime levels.

The wheat cultivars developed in Brazil had the greatest Al tolerance, twentyfold that of the Al sensitive cultivars. Cultivars, in general, from the southeastern U.S. were more tolerant to high Al in the soils than cultivars from the plains and western states. The range in barley cultivars was wide. The tolerant cultivars had about a ninefold increase in yield over the Al sensitive cultivars. The

southeastern cultivars generally were more tolerant of excess Al than the cultivars from the plains and western states.

The wheat cultivars, Atlas 66 and Monon were grown in nutrient solutions and on acid Bladen soil by Foy et al. (18) to confirm their differential tolerance to Al and to associate this tolerance with plant-induced pH changes around the roots. The experiment to confirm the differential Al tolerance was grown in the acid Bladen soil involving a high and low lime application. Atlas 66 yields were not as depressed on the low lime treatment as were Monon's yields. This supported the original hypothesis that Atlas 66 was Al tolerant and Monon was sensitive to high levels of Al in the soil. From additional soil and nutrient solution experiments they concluded that the differential Al tolerance was in part due to the pH changes produced in the thin layer around the roots.

In 1967, Foy et al. (20) reported on a series of experiments designed to characterize the differential Al tolerance between two cultivars each of wheat and barley. An Al tolerant and an Al sensitive cultivar were selected for each species. The Al sensitive wheat and barley cultivars both adjusted the pH of nutrient solutions to lower levels than did the Al tolerant cultivars. The Al sensitive cultivars had a higher root cation exchange capacity than did the Al tolerant cultivars. These cultivars had a higher Al and P concentration in the roots with less top growth and a lower concentration of Ca. This occurred when the cultivars were grown in nutrient solutions with sensitive and tolerant together and separately, suggesting that the

thin zone of pH change was still associated with the root or that the sensitive cultivars accumulated more Al at the same pH. Differential Al tolerance of cultivars was not closely related to the differences of Al or P content of plant tops.

Evidence of genetic resistance to Al toxicity in wheat was reported by Kerridge and Kronstad (33) in 1968. Two parental cultivars, Druchamp Al tolerant and Brevor Al sensitive, were grown together with the F_1 and F_2 generations in nutrient solutions containing two levels of Al. The parental cultivars were distinctly different in their response to added Al, especially in root length. The F_1 generation was uniform for root development. The F_2 generation segregated into two distinct visible groupings of short and long roots. The ratio of tolerant to sensitive was 3:1, suggesting a single gene model for resistance to Al toxicity. This hypothesis was supported when Reid (41) reported there was some genetic evidence pointing to one major gene controlling Al tolerance in barley.

Tolerance to Al in a given genotype grown on an acid soil does not necessarily mean tolerance to another toxic element. Foy et al. (22) in 1973 used acid soils and nutrient solution culture to show that Atlas 66, an Al tolerant wheat cultivar, was less tolerant to excess Mn than Al sensitive Monon wheat. Al-toxic Bladen soil and Mn-toxic Zanesville soil produced opposite differential tolerances to acid soil conditions in these two cultivars. The Mn tolerance of Monon was apparently an internal tolerance to high amounts of Mn in the tops and roots of the plant and not due to reduced Mn uptake. Atlas 66

expressed more severe toxicity symptoms and reduced top and root yield at a lower concentration of plant Mn than did Monon.

Monon wheat was developed in Indiana where Mn would be a growth limiting factor; whereas Atlas 66 was developed in North Carolina which would have Al as a growth limiting factors. These cultivars were probably unknowingly selected for the tolerance most needed in their given acid soil toxicity.

In 1974, Foy et al. (24) continued to relate Al tolerance to the region of development. The studies were conducted with 27 cultivars and 19 experimental lines from Ohio to Indiana. The wheat developed in Indiana was generally more sensitive to Al than those developed in Ohio. They concluded that the older cultivars from Ohio were unknowingly selected for Al tolerance and the newer Ohio lines were selected on soils that had been limed to a higher pH so that Al toxicity was not a problem. The Indiana cultivars were selected on soil that was low in Al.

Differential tolerance to Mn and Al toxicities have been reported for soybean (Glycine max (L.) Merrill) cultivars. Armiger et al. (3) in 1968 reported on the differential tolerance in 48 soybean cultivars from 10 maturity groups to acid soil high in exchangeable Al. All 48 cultivars were grown in the greenhouse on unlimed Al-toxic Bladen subsoil (pH 4.8) for screening. Wide differences in the cultivars were seen in the weight of the tops and roots.

Ten of these cultivars and five not screened were then tested on Bladen surface soil at different $CaCO_3$ levels to obtain a more precise

ranking of Al tolerance. The differential response in this experiment agreed with the ranking of the first screening. Two factors led to the conclusion that the cultivars differed in their tolerance to Al: (1) the cultivar differences were minimized and often eliminated by liming the soil to a pH of 5.5, and (2) acid Bladen soil contains a high level of KCl extractable Al.

Variation in response to Mn toxicity by soybean genotypes was reported by Carter et al. (10) in 1975. Thirty soybean genotypes were grown in nutrient solutions containing six treatments from 0.1 mg L^{-1} to 20 mg L^{-1} Mn. The visual ratings of leaf wrinkle, chlorosis and necrosis indicated a wide range in tolerance among genotypes. All of the cultivars grown in 0.1 mg L^{-1} of Mn were normal. The plants showed a varied response in the 20 mg L^{-1} added Mn from a normal appearance in Lee to extreme leaf curl in Bragg.

Similar results were reported by Heenan and Carter (26) in 1976 involving four of these cultivars. In addition, the scions of each cultivar Lee and Bragg were grafted onto their own rootstocks and the rootstocks of the other cultivar. It was concluded that the expression of Mn toxicity symptoms was dependent on the plant tops and was not affected by the rootstock. The Mn tolerance appeared to be the ability of the plant top to tolerant toxic levels of internal Mn.

In a study by Brown and Jones (5) 1977, ten soybean cultivars were grown on several different soils with varying nutritional problems associated with each soil. They concluded that for acid soils, Mn toxicity varied more than Al toxicity. This study with soils agreed

with the nutrient solution experiment which found Lee to be Mn tolerant and Forrest and Bragg Mn intolerant. None of the ten cultivars showed any Al toxicity symptoms on acid Bladen soil high in Al. Another report by Brown and Jones (8) 1977 used Mn tolerant, Lee and T203, and Mn intolerant Forrest and Bragg soybean combinations with approach grafts of all of these cultivars. Manganese tolerance was concluded to be controlled by the top of the soybean. When the grafted plants were exposed to excess Mn, plants in the culture solution having Forrest and Bragg scions developed Mn toxicity symptoms regardless of the rootstock. The amount of Mn was about the same in all plant tops regardless of the rootstock which supported the theory that Mn tolerance is the ability of a genotype to tolerate high levels of internal Mn in the plant top.

Ohki et al. (40) 1980 did some work with differential responses of soybean cultivars to Mn toxicity and deficiency. Seven different cultivars were grown in nutrient solution to induce Mn toxicity or deficiency. There were differential responses in both cases. The cultivars that were the most sensitive to severe Mn deficiency were Davis, Lee 74 and Bragg. Davis and Lee 74 were the most sensitive to sever Mn toxicity. Lee was reported earlier as being Mn tolerant. Although Lee 74 was a selection out of Lee, the two genotypes are slightly different. The authors of this study concluded that in the development of Lee 74 the Mn tolerance was lost implying a genetic base for Mn tolerance in soybeans.

Differential tolerance of cotton (Gossypium hirsutum L.) cultivars to Al and Mn toxicities was reported by Foy et al. (19), (17) and

Brown and Jones (6). Foy et al. (17) 1967 took 14 cultivars of cotton and grew then on Al-toxic, acid Bladen soil treated with different amounts of $CaCo_3$. The cultivars differed greatly in their tolerance to the Al-toxic Bladen soil. The addition of lime to the soil increasing pH to 5.4 eliminated most of the yield differences of the sensitive and tolerant cultivars. The tolerance or susceptibility of certain cultivars did not follow specific patterns of origin as did wheat. The most tolerant cultivars were western in origin and adaptation, Pima S-2, Acala 4-42 and Acala 44-10. The cultivars originating in the south-east, where soil was high in Al, would be expected to be more Al tolerant. The sensitive cultivars were developed in the easter, delta and western regions. The most sensitive cultivar was Coker 100A developed in South Carolina's coastal plains. The coastal plains soils are expected to be low in soluble Al. Foy et al. (19) 1969 reported that cotton cultivars differed significantly in their tolerance to excess Mn when grown in nutrient solutions. The most tolerant cultivars included Rex Smooth Leaf, Acala 1517D and Gregg from the western region. Acala 4-42 and Coker 100A from the western and eastern regions, respectively, were the least tolerant cultivars tested. Acala 4-42 was tolerant to high levels of Al but sensitive to excess Mn which points out that tolerance to one acid soil characteristic does not mean tolerance to another. Coker 100A on the other hand, is very sensitive to both Al and Mn levels.

Brown and Jones (6) 1977 grew 15 cotton cultivars on seven soils with different nutrition problems including toxic levels of Al and Mn.

The growth on the Richland soil high in Mn had the best yields over all soils. None of the cultivars showed any deficiencies or toxicities in this soil. The cotton cultivars differed in response to the unlimed Bladen soil; although the yield was the lowest in this soil, two cultivars were significantly lower in yield. This was concluded to be a differential tolerance to Al toxicity.

Differential tolerance to Mn and Al toxicity has been reported for ryegrass (Lolium perenne L. and L. multiflorum Lam.) (44) and investigated for sorghum (Sorghum bicolor (L.) Moench) (7).

A classical study of Mn and Al toxicity using ryegrass was reported by Vose and Randall (44). Ryegrass cultivars had a wide variation in resistance to Al toxicity. Some individual plants that showed an unusual resistance to Al were selected for increase resistance. After the fourth generation of selection, a higher concentration of Al in the nutrient solution was needed to induce toxicity symptoms. Among the ryegrass cultivars grown some, were resistant and others were susceptible to Mn toxicity. They concluded that ryegrass cultivars resistant to both Al and Mn toxicities had low cation-exchange capacity roots.

Fifteen sorghum cultivars were tested on seven different soils for nutritional tolerances by Brown and Jones (7) in 1977. Sorghum cultivars responded differently to Al toxicity on Bladen soil. Five cultivars contained a higher concentration of Al and lower Fe and P concentrations. None of the sorghum cultivars developed persistent Mn toxicity symptoms when grown on Richland soil.

Aluminum sensitivity has been the subject of inquiry for the species snapbeans (Phaseolus vulgaris L.) (21), rice (Oryza sativa L.) (28), sugarbeet (Beta vulgaris L.) (34), fescue (Festuca rubra L.) (39), tomato (Lycopersicon esculentum Mill) (23), sunflowers (Helianthus annuus L.) (25), and pea (Pisum sativum L.) (35).

In a nutrient solution study with two snapbean cultivars, Foy et al. (21) demonstrated the Bladen soil tolerant Dade cultivar was more tolerant to Al than Bladen sensitive Romano. The tolerance was not related to a differential change in the pH of the nutrient solution or to Al concentrations of plant tops or roots. Reduced Ca uptake does provide a good indicator of possible Al sensitivity to Al toxicity in snapbean.

Foy et al. (23) found similar results in tomato cultivars. Aluminum tolerant and sensitive cultivars were screened on Bladen soil and confirmed by using nutrient solutions containing varying amounts of Al. The tomato cultivars that showed the greatest tolerance to Al in the soil tended to contain less Al, Ca, and P in the tops of the plant. In nutrient solutions the Al, Ca, and P in the tops were similar in all cultivars. However, the roots of the Al tolerant cultivars tended to contain less of these three elements.

Sunflowers, Foy et al. (25), were also grown on Bladen soil and in nutrient solutions with different Al concentrations. Sunflower genotypes did exhibit a differential Al tolerance in both growing media and no differences were detected in the chemical constituents of the tops or roots.

Howeler and Cadavid (28) developed a screening technique for Al toxicity in rice cultivars. The upland rice cultivars have been noted for their tolerance to Al in soils with low pH compared to the semi-dwarf cultivars. Many rice cultivars were screened for tolerance to Al toxicity in nutrient solutions with high and low Al concentrations. The differential Al tolerance showed up well using relative root lengths of the high and low Al concentration solutions. The relative root lengths correlated with the yield data taken in the field screening for differential Al tolerance.

Klimashevskii and Berczovskii (35) reported differential tolerance to Al in pea. An experiment was conducted in nutrient solution with rates ranging from 0-10 mg L^{-1} of Al at a pH of 4.5. The two cultivars of pea had a differential tolerance to toxic Al. They concluded that the difference was attributed to the unequal cation and anion-exchange capacities and to the increased pH in the solution by the roots of the resistant cultivar.

Sugarbeets were investigated for tolerance to low pH and high levels of soluble Al. Kesser et al. (34) used accumulated aluminum phosphate and growth patterns in the meristematic zones of primary and lateral roots as criteria for tolerance. All cultivars grown in the nutrient solution with no Al added showed normal anatomical and morphological development. The nutrient solution with 4 mg L^{-1} of added Al, pH 4.8, showed some cultivars had accumulated large amounts of aluminum phosphate. There were variations for aluminum phosphate both among and within cultivars. The examination of the

root meristematic regions also showed the same type among and within cultivar variation. They concluded that there is differential tolerance at an early stage to Al under low pH conditions in sugarbeet cultivars and variation within most cultivars.

An effort was made by Nittler and Kenny (39) to use differential Al tolerance of fescue cultivars to test for cultivar purity. The differential Al tolerance was great enought to distinguish contrasting cultivars. This tolerance would not allow classification of cultivars but can be used to certify cultivar purity when compared to a sample of known purity.

Burley tobacco as well as other crops do respond to high levels of Mn and Al. Toxicities to both of these metals have been observed for many of these crops. Differential response has been reported for some crops but not for burley tobacco.

CHAPTER III

MATERIALS AND METHODS

1978

In 1978 an experiment was designed to determine the accumulation of Al and Mn in the laminae of burley tobacco grown on limed and unlimed soil. The 15 genotypes used and their agronomic characteristics are shown in Table 1.

The experiment was located at The University of Tennessee, Plateau Experiment Station, Crossville, Tennessee. In April, 1976, a low pH (pH 4.5) field was selected with a soil classification of a Muskingum rocky fine sandy loam with 5-12% slope. Eight blocks were established in a randomized complete block design with four of the blocks limed to increase the pH. Liming was to increase the pH to 5.5 in order to decrease the availability of Mn and Al. In 1978 the different blocks were still well defined by soil pH.

The 15 burley tobacco genotypes were arranged in a split-block design. Each genotype was assigned a single row in a randomized complete block design to form strips across the two blocks. One subplot of each genotype appeared in a limed block and one subplot appeared in an unlimed block for each of four replications.

Plant beds were sown at the Plateau Experiment Station in March, 1978. Prior to transplanting, 2,240 kg ha^{-1} of 5-10-15 fertilizer was applied to the field. In early June the tobacco was transplanted in rows of 26 plants per subplot spaced 107 cm between rows and 41 cm

Table 1. Tobacco genotypes and their characteristics used in the 1978 study.

Genotype	Maturity	Yield Potential	Quality	Disease Reaction	
				Black[a] Shank	Black[b] Root Rot
Ky 10	medium	good	high	S	R
Ky 14	medium	good	high	S	R
Ky 15	medium	good	high	S	R
Ky 17	medium	low	good	R-0,1	R
Bu 21	medium	good	high	S	S
Bu 49	medium	low	good	R-0,1	R
Bu 64	late	low	good	R-0,1	R
VA 509	medium	good	good	R-0,1	S
Ms Ky 14 x L8	early	high	high	R-0	R
Ms Bu 37 x L8	early	good	good	R-0 MR-1	R
Ms Bu 21 x L8	early	good	high	R-0	R
Ms Bu 21 x Ky 10	medium	high	high	R-0	LR
Gr 96A	medium	good	good	R-0,1	R
Gr 97A	medium	good	good	R-0,1	R
Gr 102A	medium	good	good	R-0,1	R

[a]S--susceptible races 0 and 1; R--resistant race following; MR--moderate resistance race following.

[b]S--susceptible; LR--low resistance; R--resistant.

within rows using a mechanical transplanter. Sidedress fertilization consisted of 336 kg ha^{-1} of NH_4NO_3 applied with the first cultivation after transplanting. Recommended cultural practices were followed throughout the growing season.

Soil samples, 15 to 20 cm in depth, were taken on July 5 and kept in a freezer (-18°C) until pH, water soluble Mn (w-Mn), exchangeable Mn (e-Mn), easily reducible Mn (r-Mn), and exchangeable Al (e-Al) were determined. The soil samples were passed through a 10 mesh screen prior to analyses to eliminate large particles. Soil pH was determined by the method used by the Tennessee soil testing laboratory, 10 ml of deionized H_2O was added to 10 g of soil and allowed to stand for 30 minutes (43). The pH was measured using a potentiometer with glass electrodes. Manganese extraction was done in three phases: (1) the w-Mn was extracted with deionized H_2O, (2) e-Mn was extracted with neutral normal ammonium acetate, and (3) r-Mn, a measure of total Mn in the soil, was extracted with neutral normal ammonium acetate with 0.2% hydroquinone as a reducing agent (1). Aluminum was extracted by leaching the soil with 1N KCl solution (37). The only modification of these extraction procedures was to use field moist soil and then adjust the results for soil moisture. The same 10 g of soil was used for w-Mn and e-Mn. Available Mn was calculated by adding w-Mn and e-Mn for each sample.

Manganese and Al were analyzed by atomic absorption spectrophotometry (Perkin and Elmer models 372 and 5000). Manganese atomization was accomplished with an air-acetylene flame and Al electrothermal atomization was accomplished with a heated graphite furnace.

Five plants for each subplot were stripped of all the laminae at harvest time. The laminae were dried in a forced air oven at 40°C for 48 hours before being weighed and ground in a Wiley Mill to pass through a 100 mesh screen.

Chemical analyses of the laminae were done by ashing a 0.5 g ground sample and using HCl to bring the Mn and Al into solution (29). The color of the ash was noted when the HCl was added to the porcelain crucibles. Aluminum and Mn analyses were done with flame atomic absorption spectrophotometry.

Analyses of variance for a randomized complete block were calculated on the soil determination for pH, w-Mn, e-Mn, a-Mn, r-Mn, and e-Al. Analyses of variance for a split-block design were calculated on Mn and Al laminae determinations. When differences were indicated by an F test at the 5% level of probability, means were subjected to Duncan's New Multiple Range Tests using a 5% probability level.

Correlations were calculated to examine the relationship between the plant Mn and Al and soil pH, w-Mn, e-Mn, a-Mn, r-Mn, and e-Al.

1979

In 1979 three field studies were conducted. The first experiment was in the same field as the previous experiment at The University of Tennessee, Plateau Experiment Station, Crossville, Tennessee. Six burley tobacco genotypes were laid out as the strip treatments in a split-block design. One subplot of each genotype appeared in a limed

block and one subplot appeared in an unlimed block for each replication. Due to the extremely wet spring in 1979 only three replications were harvestable.

The burley tobacco genotypes planted were Bu 64, Ky 17, Va 509 and F_1 hybrids male sterile (Ms) Ky 14 x L8, and Ms Bu 37 x L8. The plant beds were sown on the Plateau Experiment Station in mid-March. The field had a pretransplant application of 2,240 kg ha^{-1} of 5-10-15 fertilizer. Plants were set using a mechanical transplanter. Due to the wet weather, cultivation of this field was made impossible for more than a month after transplanting and the planned amonium nitrate sidedressing was not applied. Recommended cultural practices were followed for the remainder of the growing season.

At harvest time, soil samples were taken from each of the six blocks, 15 to 20 cm in depth and kept in a freezer (-18°C) until extraction.

Five plants from each subplot were cut, speared on sticks and hung in a burley barn for air curing. After curing, the leaves were stripped from the five stalks per subplot, bundled up and saved for chemical analyses.

The same six burley tobacco genotypes were treatments in two other locations in 1979. These survey sample locations were chosen to give additional information over a wider range of pH and soil Mn and Al concentrations.

One field was located on The University of Tennessee, Plateau Experiment Station, Crossville, Tennessee. The test was conducted on a soil originally mapped as a Hartsells loam with a 2-5% slope.

The other field was located at The University of Tennessee Middle Tennessee Experiment Station, Spring Hill, Tennessee. The test was conducted on Maury silt loam soil. Both locations were laid out in a randomized complete block design with three replications each.

Plant beds were sown at each location in March, 1979. Prior to transplanting, 2,240 kg ha^{-1} of 5-10-15 fertilizer was applied to each field. In early June the six tobacco genotypes were transplanted with a mechanical transplanter in rows of 35 plants per plot spaced 107 cm between rows and 41 cm within rows. Sidedress fertilizer consisted of 336 kg ha^{-1} of NH_4NO_3 applied with the first cultivation. Recommended cultural practices were followed throughout the growing season.

Soil samples were taken to the depth of 15 to 20 cm at harvest time and kept in a freezer (-18°C) until extraction. Soil samples from all three tests were analyzed together.

The soil was analyzed for pH, a-Mn, and e-Al. The pH was determined in water with a glass potentiometer and Al determinations were made as previously described. The w-Mn and e-Mn were extracted together in one neutral normal ammonium acetate extraction for a-Mn. Manganese and Al were analyzed by atomic absorption spectrophotometry (Perkin and Elmer models 372 and 5000). Manganese atomization was accomplished with an air-acetylene flame and Al electrothermal atomization was accomplished with a heated graphite furnace.

Five plants from each subplot were cut, speared on sticks and hung in a burley barn for air curing. After curing, the leaves were

stripped from the five stalks per plot, bundled up and saved for chemical analyses. The leaves were cleansed of soil and debris by dipping them in distilled water. The laminae were separated from the stem, dried in a forced air drier at 40°C for 48 hours, and ground in a Wiley Mill to pass through a 100 mesh screen. Chemical analyses of the laminae were done by ashing a 0.5 g ground sample and using HCl to bring the Mn and Al into solution as described earlier. The color of the ash was noted when the HCl was added to the porcelain crucibles. Aluminum and Mn were analyzed by flame atomic absorption spectrophotometry.

Analyses of variance for a randomized complete block were calculated on the soil determination for pH, a-Mn, and e-Al. Analyses of variance for a split-block design were calculated on Mn and Al laminae determinations. When differences were indicated by an F test at the 5% level of probability, means were subjected to Duncan's New Multiple Range Tests using a 5% probability level.

Correlations were calculated to examine the relationship between the plant Mn and Al and soil pH, a-Mn, and e-Al.

CHAPTER IV

RESULTS AND DISCUSSION

Soil Properties 1978

The establishment of the soil pH blocks was necessary to create the proper environments for growing the different burley tobacco genotypes. Increasing the soil pH to about 5.5, to decrease the availability of Mn and Al, on four of the eight blocks was the goal of liming. Means for the 1978 soil determinations are given in Table 2. The mean hydrogen ion activity of the unlimed blocks was 29.9×10^{-6} (pH 4.5), close to the original hydrogen ion activity before cultivation. The decrease in hydrogen ion activity in the limed blocks was highly significant. The mean hydrogen ion activity of the limed blocks was 4.5×10^{-6} (pH 5.3). The means in Table 2 show that there were significant or highly significant differences in the limed and unlimed blocks for w-Mn, e-Mn, a-Mn and e-Al.

Easily reducible Mn is somewhat, a measure of how much total Mn is in the soil. There was not a significant difference in the concentration of Mn in either the high or low hydrogen ion activity blocks. Means for these blocks were 2,281 and 2,125 $\mu mol\ kg^{-1}$, respectively, Table 2. Easily reducible Mn was not considered to be available to the plants.

The water soluble and e-Mn together are better than r-Mn as a measurement of the Mn concentration that may be taken up and accumulated by the plant. The w-Mn, which is the most mobile and

Table 2. Means and coefficients of variation for H^+ activity, water soluble Mn, exchangeable Mn, available Mn, easily reducible Mn, and exchangeable Al--1978.

Soil Blocks	H^+	w-Mn	e-Mn	a-Mn	r-Mn	e-Al
		----------	----------	$\mu mol\ kg^{-1}$	----------	----------
Unlimed	29.9×10^{-6}**	68**	204*	272*	2281 NS	5236**
Limed	4.5×10^{-6}	7	88	95	2125	485
CV (%)	6.5	18.9	30.0	26.0	26.5	18.2

*,**F-test for differences between unlimed and limed soil blocks is significant at the 0.05 and 0.01 levels of probability, respectively.

NS--F-test for differences is not significant at the 0.05 level of probability.

available fraction of Mn had the greatest comparative difference in concentration with a mean of 7 μmol kg^{-1} for the limed plots of 68 μmol kg^{-1} for the unlimed plots. The e-Mn had concentrations of 88 μmol kg^{-1} for the limed block and 204 μmol kg^{-1} for the unlimed soil block. The a-Mn, exchangeable plus water soluble, had a 65% decrease in Mn concentration in the soil from the unlimed blocks. Values of 272 μmol kg^{-1} for the unlimed blocks as compared to a mean of 95 μmol kg^{-1} Mn in the limed blocks. The means for e-Al in the soil blocks were markedly different with 485 μmol kg^{-1} for the limed and 5,236 μmol kg^{-1} for the unlimed, a 91% decrease in Al concentration.

The analyses showed that there were measurable and significant differences created by the increase in soil pH or the decrease in hydrogen ion activity. The availability of Mn and Al decreased with the addition of lime and associated the decrease in hydrogen ion activity. Liming did not change the total amount of Mn in the soil. Easily reducible Mn was not different between the limed and unlimed soil blocks.

Leaf Determinations 1978

The color of the ash from the laminae of the leaf was observed when the HCl was added to the crucibles to bring the Mn and Al into solution. Samples that came from the unlimed blocks where the hydrogen ion activity was still high had a dull, brown, muddy color. This agrees with Anderson et al. from the studies done in Connecticut on the black shade leaf tobacco in 1941 (2). None of the laminae samples from the limed blocks produced a muddy colored ash.

Analyses of variance were calculated for Mn, Al and yield. Mean squares and error mean squares are shown in Table 3. For the variables yield and Al, there were no significant differences for genotypes, soil pH or their interaction. There was not a significant difference among genotypes and genotypes x pH interaction for Mn. There was a highly significant difference between soil pH blocks for Mn. Means were 11 mmol kg^{-1} for the unlimed blocks as compared to the 3 mmol kg^{-1} of laminae for the limed blocks, Table 4. The decrease of Mn in the laminae of the genotypes on the limed blocks was a result of the decreased Mn availability when the hydrogen ion activity was decreased in the soil solution. Manganese toxicity symptoms appeared in the leaves; this was was where Mn was expected to accumulate.

There was not a significant decrease for Al concentrations in the laminae. Values were 37 mmol kg^{-1} and 28 mmol kg^{-1} for the unlimed blocks and limed blocks, respectively. There was a 91% decrease in the availability of Al from the unlimed to the limed blocks in the soil extract but this did not result in a difference in the Al in the laminae. Aluminum toxicity symptoms are expressed in the roots of the tobacco plant (4). If roots are the site of accumulation of excess Al as the symptoms would suggest, then the nonsignificant differences in the laminae would be explained.

The mean increase of yield from 37 to 44 g $plant^{-1}$ in the limed over the unlimed blocks is probably a function of the greater availability of most of the essential nutrients. The plants on the unlimed

Table 3. Mean squares for genotype, soil pH, genotype x soil pH, and errors for Mn and Al in the laminae (mmol kg^{-1}) and yield (g $plant^{-1}$)--1978.

Source	Mean Squares		
	Mn	Al	Yield
Genotypes (G)	8 NS	297 NS	256 NS
Error	6	236	223
Soil pH (pH)	1652 **	2345 NS	1372 NS
Error	19	548	783
G x pH	3 NS	134 NS	74 NS
Error	2	140	61

**F-test for differences betweeen soil pH blocks is significant at the 0.01 level of probability.

NS--F-test for differences is not significant at the 0.05 level of probability.

Table 4. Laminae means of 15 genotypes for Mn, Al concentrations and yield--1978.

Soil Blocks	Mn	Al	Yield
	---- mmol kg^{-1} -----		g $plant^{-1}$
Unlimed	11 **	37 NS	37 NS
Limed	3	28	44

**F-test for differences between soil blocks is significant at the 0.01 level of probability.

NS--F-test for differences is not significant at the 0.05 level of probability.

soil were observed to be smaller. There were no notable differences in the Mn toxicity symptoms across these replications other than size of plants which may be attributed to Al toxicity although the Al concentration in the leaves was not significantly different.

Correlation coefficients between Mn, Al and yield of laminae using the mean of all 15 genotypes with hydrogen ion activity, w-Mn, e-Mn, a-Mn, and e-Al in the soil are given in Table 5. Hydrogen ion activity was highly significantly correlated, .99, with Mn in the laminae. Aluminum and yield of the laminae correlations with hydrogen ion activity were nonsignificant. These were expected from the analyses of variance and means. Manganese was highly significant for soil pH blocks and correlated well with hydrogen ion activity indicating that the Mn in the laminae does change as the hydrogen ion activity increases. Aluminum or yield was not significant in either analyses of variance or correlations. Aluminum was positively and yield was negatively correlated with hydrogen ion activity.

Water soluble Mn and a-Mn were highly significantly and e-Mn significantly correlated with Mn in the laminae with coefficients of .96, .88, and .80, respectively. This agrees with the analyses of variance and correlations can be made between the Mn in the soil with Mn in the laminae.

The r-Mn in the soil was not significantly correlated with Mn in the plant. This was expected because all of the Mn in the soil is not available to the plant.

Aluminum in the laminae was not significantly correlated with hydrogen ion activity or with e-Al and was not significant in the

Table 5. Correlation coefficients between genotypic means for Mn, Al, and yield of laminae and H^+ activity, water soluble Mn, exchangeable Mn, available Mn, easily reducible Mn, and exchangeable Al in the soil--1978.

Laminae	H^+	w-Mn	e-Mn	a-Mn	r-Mn	e-Al	Yield
Mn	.99**	.96**	.80*	.88**	.12	.90**	-.62
Al	.61	.54	.25	.36	-.08	.34	-.74*
Yield	-.54	-.48	-.23	-.32	.03	-.37	1.00

*,**Coefficients are significantly different from zero at the 0.05 and 0.01 level of probability, respectively.

analyses of variance indicating the laminae is not the appropriate plant part for studying Al.

Yield was negatively correlated with Mn and Al in the laminae of the leaf. These correlation coefficients were -.62 and -.74, respectively. Although, the correlation between yield and Al was significant, the analysis of variance was not. The associations between yield with Mn and Al in the laminae are too weak to predict genotype sensitivity to Mn and Al.

Soil Properties 1979

Three fields were sampled in 1979. The same field as the one used in 1978 was again used for the pH test. A production field at the Plateau Experiment Station (PES) and another production field at the Middle Tennessee Experiment Station (MTES), Spring Hill, Tennessee were also sampled. Means for hydrogen ion activity, a-Mn, and e-Al determinations and mean separations are given in Table 6. Hydrogen ion activity was 19.0×10^{-6} (pH 4.7) for the unlimed which was significantly different from the limed soil treatment's hydrogen ion activity of 2.8×10^{-6} (pH 5.6). This was a reduction in hydrogen ion activity from the previous year in both limed and unlimed soil blocks. There are two possible explanations for this reduction. The ammonium nitrate sidedressing was not applied in 1979. This is an acid forming fertilizer and probably increased the hydrogen ion activity in 1978 and did not in 1979. Secondly, the sidedressing was applied in June 1978 shortly before the field was sampled in early July and the effects of the fertilizer were high. In 1979 the soil sampling was not done until

Table 6. Means and coefficients of variation for H^+ activity, available Mn, and exchangeable Al for four pH environments--1979.

pH Environment	H^+	a-Mn	e-Al
		--- µmol kg^{-1} ---	
Unlimed pH test	19.0×10^{-6} a[a]	300 a[a]	2934 a[a]
Limed pH test	2.8×10^{-6} b	2 b	539 b
CV 1 (%)[b]	22.0	27.2	16.3
MTES sample	1.7×10^{-6} b	34 b	71 c
PES sample	0.7×10^{-6} b	14 b	21 c
CV 2 (%)[b]	29.9	30.5	24.9

[a]Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's New Multiple Range Test.

[b]CV 1, based on the unlimed and limed treatments for the 1979 soil pH test. CV 2, based on all four pH environments.

September; this gave the soil more time to equilibrate after the application of the fertilizer and to decrease the hydrogen ion activity.

Production fields had mean hydrogen ion activity, above the critical pH 5.5, of 1.7×10^{-6} (pH 5.8) and 0.7×10^{-6} (pH 6.2) for MTES and PES, respectively. These were significantly different from the unlimed soil blocks, but not the limed soil blocks in the pH test or each other.

Available Mn, in the pH test, was decreased significantly from 300 μmol kg^{-1} for the unlimed soil to 2 μmol kg^{-1} for the limed treatment. This was a 99% drop in a-Mn. Apparently the decrease in hydrogen ion activity was enough to shift most of the Mn to the unavailable state. The MTES and PES fields had a-Mn means of 34 μmol kg^{-1} and 14 μmol kg^{-1}, respectively. These levels were not significantly different from each other or from the limed treatment in the pH test, but were significantly different from the unlimed soil blocks. Although a-Mn means were higher in the production test than they were in the limed soil treatment in the pH test, the hydrogen ion activity was lower. A decrease or close to the same level in a-Mn was expected. The soil types were different which could account for the small nonsignificant differences or the application of sidedress fertilizer to the production tests could have affected the availability of soil Mn.

Exchangeable Al means were significantly different in the pH test. The unlimed soil blocks had a mean of 2,934 μmol kg^{-1} and the limed blocks had a mean of 539 μmol kg^{-1}, this represents an 82% decrease in e-Al. A significant decrease in e-Al was calculated between the

limed soil blocks in the pH test and both production tests. Means for MTES and PES were not significantly different from each other with values of 71 μmol kg^{-1} and 21 μmol kg^{-1}, respectively. The reduction in hydrogen ion activity was enough to cause a significant reduction in e-Al from the unlimed soil blocks to the limed soil blocks in the pH test, a reduction for MTES and further to a nonsignificant reduction for PES.

Leaf Determinations 1979

Analyses of variance were calculated for Mn and Al in the laminae for the six genotypes in the pH test. Table 7 shows the mean squares for genotypes, soil pH, their interactions, and errors. Soil pH blocks for laminae Mn was the only source of variation that was significant. This difference was expected because, with the addition of HCl to the crucibles to dissolve the Mn and Al a dull brown, muddy colored ash for all of the samples from the unlimed soil blocks was observed. The means for laminae Mn concentration are in Table 8. The unlimed or the high hydrogen ion activity soil blocks produced laminae with 16 mmol kg^{-1}, the highest Mn concentration in all of the environments tests in 1979. Where liming reduced the hydrogen ion activity, Mn in the laminae was reduced significantly to 3 mmol kg^{-1} in the laminae. This large significant difference indicates that when the hydrogen ion activity decreased from 19.0×10^{-6} to 2.8×10^{-6} it passed the critical hydrogen ion activity for the availability of Mn and the plant did not take up and accumulate as much Mn in the laminae.

Table 7. Mean squares for genotype, soil pH, genotype x soil pH, and errors for Mn and Al in the laminae (mmol kg^{-1})--1979.

Source	Mean Squares	
	Mn	Al
Genotypes (G)	21 NS	6 NS
Error	18	5
Soil pH (pH)	1643 *	87 NS
Error	30	33
G x pH	17 NS	3 NS
Error	17	4

*F-test for differences between soil pH blocks is significant at the 0.05 level of probability.

NS--F-test for differences is not significant at the 0.05 level of probability.

Table 8. Mean laminae Mn and Al concentrations by environment--1979.

Environment	Mn	Al
	mmol kg^{-1}	
Unlimed	16 a[a]	7 b[a]
Limed	3 b	4 b
MTES	3 b	13 a
PES	2 b	7 b

[a]Means followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's New Multiple Range Test.

Analyses of variance were calculated for Mn and Al in the laminae using all four environments in 1979; their levels of significance are indicated in Table 8. The two production fields also had concentrations of Mn in the laminae close to and were not significantly different from that of the limed soil blocks of the pH test. Mean concentrations of 3 mmol kg^{-1} and 2 mmol kg^{-1} were determined for the MTES and PES experiments, respectively.

The laminae Mn concentration agreed with the soil Mn concentration and the hydrogen ion activity in the soil. Soil blocks with the greatest hydrogen ion activity (19.0×10^{-6}) had the most a-Mn in the soil with levels of 300 μmol kg^{-1}. This soil treatment was significantly different from the limed, MTES and PES soil samples for hydrogen ion activity and a-Mn; the three treatments were not significantly different from each other for hydrogen ion activity or a-Mn. Manganese in the laminae reflected these soil trends and did indicate when the critical pH was passed (11). The highest laminae Mn determined was the 16 mmol kg^{-1} on the unlimed portion of the pH test; in the other environments, the limed, MTES and PES had 3 or 2 mmol kg^{-1}. The 2.8×10^{-6} hydrogen ion activity (pH 5.6) is just over the critical point for a-Mn in the soil. Manganese in the soil becomes more available to plants as the hydrogen ion concentration increases above this point and is taken up and accumulated in the laminae. Below this critical point there is no further significant reduction of a-Mn in the soil and Mn is not accumulated in the laminae as well.

Mean squares in Table 7 indicated that there were no significant differences for the Al in the laminae of the leaf in the pH test in 1979.

Means for Al concentration in the laminae over all of the environments showed a different response than the Mn in 1979. Table 8 shows the mean separations for laminae Al for all four environments. The unlimed, limed and PES environments were not significantly different from each other for Al concentrations which were 7 mmol kg^{-1}, 4 mmol kg^{-1}, and 7 mmol kg^{-1}, respectively. These three means were significantly different from MTES which had a value of 13 mmol kg^{-1} of Al in the laminae. As noted earlier in Table 6 the unlimed and limed environments in the pH test were significantly higher in e-Al in the soil than was MTES. The PES test had 21 μmol kg^{-1} of e-Al in the soil which was not significantly different from that of MTES. However, there was a significant increase in laminae Al at the MTES location shown in Table 8, (13 mmol kg^{-1}). There was much less e-Al in the soil at MTES than in the pH test and slightly more than the PES location.

The reason for the increased Al concentration in the laminae for MTES could be that although the soils from the limed and unlimed treatments of the pH test had higher amounts of e-Al, their hydrogen ion activity was high enough for the Al to be toxic to the roots. With the slightly lower hydrogen ion activity, 1.7×10^{-6}, (pH 5.8), the critical pH was passed where Al is no longer toxic to the roots. Above this hydrogen ion activity Al is toxic to the roots and accumulation in the laminae is affected. Below that hydrogen ion activity Al is taken up and accumulated in the laminae more efficiently than when it is toxic to the roots. When the hydrogen ion activity was lowered

further, a nonsignificant decrease in e-Al at the PES resulted in a corresponding decrease in laminae Al. As Al became less available and it was accumulated less in the laminae.

Correlations were calculated between the soil hydrogen ion activity, a-Mn, and e-Al and the laminae Mn and Al for the six genotypes used in common in the 1978 pH test, the 1979 pH test, and the 1979 MTES and PES production tests. Correlation coefficients are shown in Table 9. A highly significant correlation coefficient of .87 and .99, in the 1978 and 1979 pH tests, between a-Mn and laminae Mn indicated that the difference between the hydrogen ion activity of limed and unlimed soil treatments had a significant effect on the accumulation of Mn.

The production fields in 1979 had a much lower correlation between a-Mn in the soil and Mn accumulated in the laminae. Both of these locations had pH's above the critical point and the a-Mn in the soil was low. They did not show any indication of observable Mn toxicity symptoms.

Hydrogen ion activity correlated well with laminae Mn in all environments. This indicates that the pH does have an affect upon accumulation of Mn in the laminae. Analyses of variance for laminae Mn support this finding.

Table 9 also shows the laminae Al correlations with soil hydrogen ion activity and e-Al. The correlation coefficients for genotypic means were not high until hydrogen ion activity decreased passed the critical point. The 1979 sample had a significant correlation coefficient of .85

Table 9. Correlation coefficients between genotypic means of laminae Mn and Al concentration and H^+ activity, available Mn, and exchangeable Al in the soil.

	78 pH			79 pH			79 sample		
Laminae	H^+	a-Mn	e-Al	H^+	a-Mn	e-Al	H^+	a-Mn	e-Al
Mn	.97**	.87**		.92*	.99**		.91*	.73	
Al	.76*		.52	.73		.63	.85*		.91*

*,**Coefficients are significantly different from zero at the 0.05 and 0.01 level of probability, respectively.

for hydrogen ion activity and .91 for e-Al, it was above that critical point. There was still no clear-cut correlation between Al accumulated in the leaf and e-Al.

Combined Analyses--pH Test

Analyses of variance were calculated for the 1978 and 1979 pH tests for all measured variables. Caution must be used in the interpretations of the combined analyses because of several different factors in these two years. The 1978 soil samples were taken early in the growing season, the first week in July, and shortly after the sidedressed application of the ammonium nitrate. In 1979 the sidedressing was not applied and the soil was sampled in September at harvest time. In 1979 there were 3 replications compared to 4 in 1978. Two blocks of tobacco drowned and were not usable, so the limed block of one replication was combined with the unlimed block of another replication to create the third replication in 1979. The errors associated with 1978 and 1979 were not homogeneous when Hartley's test for heterogeneity was calculated. Because of the lack of homogeneity and the differences in cultural practices for the two years it seemed appropriate not to use the combined analyses.

Further Research

Two separate studies would be appropriate in order to get an indication on whether there are differential tolerances to Mn and Al in burley tobacco genotypes. Each metal should be studied independently, Foy et al. (22) found that tolerance to one metal did not mean tolerance to another. Environmental control would play a very

important part in either study. Rufty et al. (42) has shown that temperature and moisture may change the expression of Mn toxicity symptom at the same level of Mn concentration. Environmental factors may also change the critical pH point at which the toxicity symptoms appear. Precise control of the pH levels would be very important. The soil should have a pH range of approximately 5.0 to 6.5 with a minimum of four levels. This should guarantee that we pass the critical point at which Mn is no longer available in toxic amounts. Toxicity symptoms on the leaves should be rated and used as an indicator of the degree of Mn toxicity to the plant. The expression of Mn toxicity is dependent on the top and not the root (8, 19, 26). Using ten genotypes and four replications should be sufficient to assure adequate degrees of freedom to analyze for each source of variation in the data analyses.

A similar experimental design for Al toxicity could be utilized. Because data from the present study indicated that Al may be toxic at a higher pH than Mn and the range of pH should be adjusted. Aluminum toxicity symptoms on the roots should be rated and used as an indicator of differential tolerance to higher e-Al in the soil. Foy et al. (23) concluded that plant tops were not the place to study Al toxicity; Kesser et al. (41) found that roots were useful in Al toxicity studies. Correlating root Al with hydrogen ion activity and e-Al in the soil may indicate differential tolerance to Al in the soil, or may indicate that liming will take care of toxicity problems. Results from these studies should indicate if there is differential tolerance in burley genotypes to toxic levels of Mn and Al.

CHAPTER V

SUMMARY AND CONCLUSIONS

Burley tobacco genotypes were grown for two years, 1978 and 1979, on soils that were approximately pH 4.5 and 5.5 in an effort to correlate hydrogen ion activity, Mn, and Al in the soil with Mn and Al in the laminae of the tobacco in an attempt to determine if a differential genotypic tolerance exists to high levels of Mn and Al. Results for 1978 were inconclusive. Aluminum in the plant did not correlate well with hydrogen ion activity or e-Al in the soil. The correlation coefficients were small and when the Al in the laminae was analyzed there were no significant differences in genotypes or genotype x soil pH interaction. Correlation coefficients for Al did increase considerably in 1979 but they never became strong enough, until the pH went above the critical point, to place much confidence in them. This supports the hypothesis that to obtain an indication of toxicity symptoms Al should not be measured in the laminae of the leaf, but the roots may be a better indicator of Al toxicity.

Correlations between Mn in the laminae and hydrogen ion activity and Mn in the soil were stronger than those of Al. Analysis of variance calculated on the Mn in the laminae indicated there were no differences among genotypes or interaction between genotypes and the different pH soils.

In 1979, fewer genotypes were used on soil blocks of different pH levels to evaluate for differential tolerance to Mn and Al toxicity. The

results for Mn were similar to those obtained in 1978. Hydrogen ion activity and available Mn in the soil did correlate with Mn in the laminae. In the production fields where the pH was well above the point where Mn was not toxic to the plants, correlation coefficients were lower. Because of the differences in cultural practices and heterogeneous errors the data from the two years could not be combined for analyses. Correlations and analyses of variance did point out that increasing the pH does affect the accumulation of Mn in the laminae. Liming would be an effective way to prevent Mn toxicity problems. There was not any indication, from the studies described here, as to whether or not there is differential tolerance to Mn toxicity.

Environmental control plays a very important part in assessment of Mn toxicity. A study with four pH levels, beginning at approximately 5.0 and ending at approximately 6.5, and ten genotypes using toxicity symptoms as an indicator might afford an indication of differential tolerance to Mn toxicity.

Hydrogen ion activity and e-Al in the soil did not correlate well with Al in the laminae in either year. This would indicate that further studies should use the roots of the plant to determine Al toxicity levels. A study set up similarly to that described for the Mn could be utilized using Al toxicity symptoms in the roots as the indicator. There was not any indication, from the studies described here, as to whether or not there is differential tolerance to Al toxicity.

LIST OF REFERENCES

LIST OF REFERENCES

1. Adams, F. 1965. Manganese. pp. 1011-1018. In C. A. Black (ed.) Methods of soil analysis: Part 2 Chemical and microbiological properties. Agronomy 9: Am. Soc. Agron. Madison, Wis.

2. Anderson, P. J., T. R. Swanback and S. B. LeCompte, Jr. 1941. Tobacco substation at Windsor report for 1940 studies on black tobacco. Connecticut Agr. Exp. Sta. Bul. 44:270-278.

3. Armiger, W. H., C. D. Foy, A. L. Fleming and B. E. Caldwell. 1968. Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminum. Agron. J. 60:67-70.

4. Bortner, C. E. 1935. Toxicity of manganese to turkish tobacco in acid Kentucky soils. Soil Sci. 39:15-33.

5. Brown, J. C., and W. E. Jones. 1977. Fitting plants nutritionally to soils. I. Soybeans. Agron. J. 69:399-404.

6. Brown, J. C., and W. E. Jones. 1977. Fitting plants nutritionally to soils. II. Cotton. Agron. J. 69:405-409.

7. Brown, J. C., and W. E. Jones. 1977. Fitting plants nutritionally to soils. III. Sorghum. Agron. J. 69:410-414.

8. Brown, J. C., and W. E. Jones. 1977. Manganese and iron toxicities dependent on soybean variety. Commun. in Soil Sic. and Plant Analysis 8:1-15.

9. Buss, G. R., J. A. Lutz, Jr. and G. W. Hawkins. 1975. Yield response of alfalfa cultivars and clones to several pH levels in Tatum subsoil. Agron. J. 67:331-334.

10. Carter, O. G., I. A. Rose and P. F. Reading. 1975. Variation in susceptibility to manganese toxicity in 30 soybean genotypes. Crop Sci. 15:730-732.

11. Christensen, P. D., S. J. Toth and F. E. Bear. 1950. The status of soil manganese as influenced by moisture, organic matter, and pH. Soil Sci. Soc. Am. Pro. 15:279-283.

12. Dessureaux, L. 1959. Heritability of tolerance to manganese toxicity in lucerne. Euphytica 8:260-265.

13. Dessureaux, L. 1969. Effect of aluminum on alfalfa seedlings. Plant and Soil 30:93-98.

14. Devine, T. E., C. D. Foy, A. L. Fleming, C. H. Hanson, T. A. Campbell, J. E. McMurtrey III, and J. W. Schwartz. 1976. Development of alfalfa strains with differential tolerance to aluminum toxicity. Plant and Soil 44:73-79.

15. Foy, C. D. 1978. Tailoring plants for greater tolerance to mineral toxicities and deficiencies on hill land soils. In Proceeding of the 1976 Int. Symp. on Hill Lands--Their Effective Use and the Future, Morgantown, W. Va. West Virginia Univ. Press.

16. Foy, C. D., W. H. Armiger, L. W. Briggle and D. A. Reid. 1965. Differential aluminum tolerance of wheat and barley varieties in acid soils. Argon. J. 57:413-417.

17. Foy, C. D., W. H. Armiger, A. L. Fleming, and C. F. Lewis. 1967. Differential tolerance of cotton varieties to an acid soil high in exchangeable aluminum. Agron. J. 59:415-418.

18. Foy, C. D., G. R. Burns, J. C. Brown and A. L. Fleming. 1965. Differential aluminum tolerance of two wheat varieties associated with plant-induced pH changes around their roots. Soil Sci. Soc. Am. Proc. 29:64-67.

19. Foy, C. D., A. L. Fleming and W. H. Armiger. 1969. Differential tolerance of cotton varieties to excess manganese. Agron. J. 61:690-694.

20. Foy, C. D., A. L. Fleming, G. R. Burns and W. H. Armiger. 1967. Characterization of differential aluminum tolerance among varieties of wheat and barley. Soil Sci. Soc. Am. Proc. 31:513-521.

21. Foy, C. D., A. L. Fleming, and G. C. Gerloff. 1972. Differential aluminum tolerance in two snapbean varieties. Agron. J. 64:815-818.

22. Foy, C. D., A. L. Fleming, and J. W. Schwartz. 1973. Opposite aluminum and manganese tolerances of two wheat varieties. Agron. J. 65:123-126.

23. Foy, C. D., G. C. Gerloff and W. H. Gabelman. 1973. Differential effects of aluminum on the vegetative growth of tomato cultivars in acid soil and nutrient solution. J. Am. Soc. Hort. Sci. 98:427-432.

24. Foy, C. D., H. N. Lafever, J. W. Schwartz and A. L. Fleming. 1974. Aluminum tolerance of wheat cultivars related to region of origin. Agron. J. 66:751-758.

25. Foy, C. D., R. G. Orellana, J. W. Schwartz and A. L. Fleming. 1974. Responses of sunflower genotypes to aluminum in acid soil and nutrient solution. Agron. J. 66:293-296.

26. Heenan, D. P., and O. G. Carter. 1976. Tolerance of soybean cultivars to manganese toxicity. Crop Sci. 16:389-391.

27. Hiatt, A. J., and J. L. Ragland. 1963. Manganese toxicity of burley tobacco. Agron. J. 55:47-49.

28. Howeler, R. H., and L. F. Cadavid. 1976. Screening of rice cultivars for tolerance to Al-toxicity in nutrient solutions as compared with a field screening method. Agron. J. 68:551-555.

29. Jackson, M. L. 1958. Plant Tissue Analysis-Mineral Constituents. pp. 326-338. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.

30. Jacobson, H. G. M., and T. R. Swanback. 1929. Manganese toxicity in tobacco. Sci. 70:283-284.

31. Jacobson, H. G. M., and T. R. Swanback. 1932. Manganese content of certain Connecticut soils and its relation to the growth of tobacco. J. Am. Soc. Agron. 24:237-245.

32. Kamprath, E. J., and C. D. Foy. 1971. Lime-fertilizer-plant interactions in acids. pp. 105-151. In R. A. Olson, T. J. Army, J. J. Hanway and V. J. Kilmer (eds.) Fertilizer technology and use. Soil Sci. Soc. Am., Madison, Wis.

33. Kerridge, P. C., and W. E. Kronstad. 1968. Evidence of genetic resistance to aluminum toxicity in wheat (Triticum aestivum; Vill., Host). Agron. J. 60:710-711.

34. Keser, M., B. F. Neubauer, F. E. Hutchinson and D. B. Verrill. 1977. Differential aluminum tolerance of sugarbeet cultivars, as evidenced by anatomical structure. Agron. J. 69:347-350.

35. Klimashevskii, E. L., and K. K. Berezovskii. 1973. Genetic resistance of plants to ionic toxicity in the root zone. Soviet Plant Physiol. 20:51-54.

36. Link, L. A. 1979. Critical pH for the expression of manganese toxicity on burley tobacco and the effect of liming on growth. Tob. Sci. 23:100-102.

37. McLean, E. O. 1965. Aluminum. pp. 979-998. In C. A. Black (ed.) Methods of soil analysis: Part 2 Chemical and microbiological properties. Agronomy 9: Am. Soc. Agron., Madison, Wis.

38. Neenan, M. 1960. The effects of soil acidity on the growth of cereals with particular reference to the differential reaction of varieties thereto. Plant and Soil 12:324-338.

39. Nittler, L. W., and T. J. Kenny. 1980. Cultivar response of Festuca rubra seedlings to aluminum. Agron. J. 72:766-769.

40. Ohki, K., D. O. Wilson and O. E. Anderson. 1980. Manganese deficiency and toxicity sensitivities of soybean cultivars. Agron. J. 72:713-716.

41. Reid, D. A. 1975. Genetic control of aluminum response in barley. Agron. Abstracts p. 63.

42. Rufty, T. W., G. S. Miner and C. D. Raper, Jr. 1979. Temperature effects on growth and manganese tolerance in tobacco. Agron. J. 71:638-644.

43. Sabbe, W. E. and H. L. Breland. 1974. Procedures used by state soil testing laboratories in the southern region of the United States. Southern Cooperative Series Bul. 190.

44. Vose, P. B., and P. J. Randall. 1962. Resistance to aluminum manganese toxicities in plant related to variety and cation-exchange capacity. Nature 196:85-86.

VITA

Thomas Charles Corbin was born on June 3, 1949 in Oklahoma City, Oklahoma. He is the son of Dr. and Mrs. Charles Corbin, Jr. of Franklin, Tennessee. He graduated from Father Ryan High School, Nashville, Tennessee, in June 1967. He attended St. Louis University where he received his B.A. degree in biology in January 1975. Shortly after his B.A. degree program he entered The University of Tennessee, Knoxville, and received a B.S. degree in plant and soil science in June of 1976. His Master of Science program in plant breeding began in winter of 1977 at The University of Tennessee, Knoxville. Interim to his degree program he has worked as a research technician from April 1977 to September 1978 and for another brief period from April of 1980 to January 1981 as a senior research technician in tobacco breeding at the Greeneville Experiment Station. He now plans to receive said degree in August of 1985.

He is a member of Gamma Sigma Delta, the American Society of Agronomy, and the Crop Science Society of America. He began his work toward the Doctor of Philosophy degree in January of 1981 at North Carolina State University on a predoctoral research assistantship with the Crop Science Department.