INFLUENCE OF NITROGEN SOURCE AND METALAXYL ON NITRIFICATION IN SOILS AND THE YIELD AND QUALITY OF FLUE-CURED TOBACCO

by

James W. Rideout

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
Agronomy

APPROVED:

Dr. James L. Jones, Chairman

Dr. Mark J. King

Dr. Dale D. Wolf

July 1986

Blacksburg, Virginia

ABSTRACT

Numerous investigators have reported that NH_4^+ uptake reduces the yield and quality of flue-cured tobacco (Nicotiana tabacum L.). Metalaxyl, a fungicide commonly used in the production of flue-cured tobacco, has been patented as a nitrification inhibitor.

The objectives of this study were to 1) determine the influence of metalaxyl at commonly applied rates on the nitrification of NH_4^+ from various sources; 2) study the influence of soil pH on inhibition of nitrification by metalaxyl; 3) study the influence of N source and metalaxyl on N accumulation in the plant; and 4) evaluate the influence of N source and metalaxyl on the yield, quality, and chemical composition of flue-cured tobacco leaf. Field, greenhouse, and laboratory experiments were conducted in the Southern Piedmont region of Virginia in 1984 and 1985 to carry out these objectives.

Metalaxyl was found to reduce the population of NH_4^+ oxidizers in soil and inhibit nitrification at applications of 0.56, 1.12, and 3.36 kg ha^{-1}. The inhibitory effects of metalaxyl were much weaker than nitrapyrin. Nitrification was inhibited by metalaxyl only in soils where nitrification was slowed by low pH and wet conditions. In soils of high nitrifying capacity, metalaxyl did not inhibit nitrification.

Nitrogen uptake was enhanced by high NO_3^- concentrations in the soil, except where metalaxyl reduced NO_3^- leaching. Cured leaf concentrations of N were not affected by N source but were slightly reduced by metalaxyl. Nitrogen source and metalaxyl did not affect yield, total alkaloids, or reducing sugars. Quality index was reduced by decreased soil NO_3^- from both N source and metalaxyl.

ACKNOWLEDGEMENTS

The author extends appreciation to advisory committee chairman Dr. James L. Jones for his guidance and assistance through this period of graduate study. Appreciation is also extended to advisory committee members Dr. Mark J. King and Dr. Dale D. Wolf for their helpful inputs into this investigation. Gratude is expressed to Dr. James L. Tramel for his guidance and for making the excellent facilities of the Southern Piedmont Center available for this study.

The financial support of Philip Morris, USA is greatly appreciated. Without this support, this program would not have been possible. Appreciation is also extended to Ciba-Geigy, Agricultural Chemical Division, and Dow Chemical Company for providing the metalaxyl and nitrapyrin used in this investigation.

Finally, appreciation is extended to the following personel at Southern Piedmont Center for their invaluable assistance: Mac Tilson (laboratory assistance), Bill Wilkerson (Farm Manager), Gilly Owen (field assistance), and Dixie Watts (laboratory and field assistance). Gratitude is also expressed to Betty Lou Mayton and Terri Redford for their assistance in the preparation of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

The source of nitrogen has been demonstrated to affect the yield and quality of flue-cured tobacco (Nicotiana tabacum L.) (McCants and Woltz, 1967; Tso, 1972). In general, nitrogen obtained from uptake of the nitrate ion produces superior yield and quality. The response to nitrate nitrogen is the greatest in soils which have been treated with a multi-purpose fumigant or contain factors which otherwise inhibit nitrification. Any substance applied to the soil which inhibits nitrification has the potential to reduce the yield and quality of flue-cured tobacco.

Since 1981, metalaxyl, a soil applied systemic fungicide, has been widely used in the production of flue-cured tobacco in Virginia. Metalaxyl was used on over 80 percent of Virginia's flue-cured tobacco acreage in 1983 (Arnett, 1983). Metalaxyl provides effective control of blue mold (Peronospora tabicina), a foliar disease of tobacco. In 1979, losses to the Virginia flue-cured and burley tobacco crop caused by blue mold were estimated at 10.5 million dollars. A similar epidemic in 1980 caused an estimated 7 million dollars damage. Since 1981, metalaxyl use has been widespread and blue mold losses have occurred only in isolated fields. In most of the cases where losses occurred, metalaxyl was not used.

In addition to control of blue mold, metalaxyl is active against the soil-borne diseases black shank (Phytophthora parisitica var. nicotiane) and damping off (Pythium spp.). Through control of these three important diseases, metalaxyl is a important tool for the production of flue-cured tobacco.

Metalaxyl is also patented as a nitrification inhibitor. While the fungicidal properties of metalaxyl are well documented, little is known about the compound's nitrification inhibiting activity. Metalaxyl is a weak nitrification inhibitor, and higher than normal field application rates have been needed to observe a significant effect on nitrification. Most of the limited research on the nitrification inhibitory actions of metalaxyl has been conducted in the laboratory under controlled conditions and at rates much higher than those used for tobacco production.

Tobacco manufacturers and growers have been concerned with a trend toward less mature, lower quality flue-cured tobacco in recent years. By inhibiting nitrification, metalaxyl may be partly responsible for this trend. The quality of U.S. tobacco must be maintained at a high level for the leaf to be competitve in the world market. Given the disease loss potential, elimination of metalaxyl from flue-cured tobacco production is not feasible. This investigation was conducted to quantify the extent of nitrification inhibition caused by metalaxyl in flue-cured tobacco production and to determine if the inhibition has any effect on yield and quality.

The objectives of this investigation were as follows:

(i). to determine and quantify the influence of metalaxyl at commonly applied rates on the nitrification of ammonium from various nitrogen sources;

(ii). to study the influence of soil pH on the inhibition of nitrification by metalaxyl;

(iii). to study the influence of nitrogen source and metalaxyl on green leaf total nitrogen and nitrate concentrations of flue-cured tobacco at various times in the growing season; and

(iv). to evaluate the influence of nitrogen source and metalaxyl on the yield, quality, and certain chemical constituents of cured tobacco leaf.

LITERATURE REVIEW

Metalaxyl

Metalaxyl [*N*-(2,6-Dimethylphenol)-*N*-(Methoxyacetyl)-alanine methyl ester], commercially registered as Ridomil®, Apron®, and Subdue®, is an acylalanine fungicide specific for fungal organisms of the order Peronosporales. The Ridomil formulation is suggested for the control of blue mold (*Peronospora tabicina*), black shank (*Phytophthora parisitica* var. *nicotianae*) and damping off (*Pythium* spp.) in tobacco. The chemical is formulated as an emulsifiable concentrate containing 240 grams of metalaxyl per liter of concentrate (Ciba-Geigy, 1984).

Blue mold reached epidemic proportions on field grown tobacco in the U.S. in 1979. In 1979 and 1980, production losses from blue mold on Virginia flue-cured tobacco were estimated at 4.98 and 4.0 million dollars, respectively (Arnett, 1983). Widespread use of metalaxyl began in 1981, and since that time only a few isolated occurrences of blue mold have been reported on flue-cured tobacco in the U.S. (Arnett, 1983). In 1983, metalaxyl was applied to approximately 80 percent of the Virginia flue-cured tobacco crop (Arnett, 1983).

Metalaxyl provides effective protection against disease. In addition to protective activity, metalaxyl also has post-infection eradicative activity. It may be applied as a seed treatment, soil treatment, or foliar spray, depending on the formulation, disease and crop. Pre-plant soil incorporation application is the only usage method approved by the EPA for tobacco grown in the United States (Ciba-Geigy, 1984).

Kucharek, et al. (1983) reported that pre-plant incorporated or immediate post-transplant soil application resulted in better control of black shank than later post-transplant or transplant water treatment.

Metalaxyl is readily absorbed by plant roots and then transported upward in the plant via the apoplastic pathway. Translocation of the chemical is a slow, continuous process, providing disease protection to new growth (Ciba-Geigy, 1984). Metalaxyl is also absorbed through the leaves and stems of tobacco plants. Phloem transport occurs in limited amounts, and only one to two percent of the metalaxyl absorbed by the foliage can be detected in the phloem (Ciba-Geigy, 1984).

In addition to activity against peronosporales, metalaxyl may also inhibit the activity of ammonium oxidizing bacteria in the soil (Ciba-Geigy, 1984; Bashore and Lander, 1981). Olin Corporation holds a U.S. patent on metalaxyl as a nitrification inhibitor for soil or fertilizer application (Bashore and Lander, 1981). Ciba-Geigy (1984) reports that under laboratory conditions 125 mg kg^{-1} of metalaxyl in soil retards nitrification for up to eight weeks. In other studies, metalaxyl was not as effective as nitrapyrin (N-Serve®) in inhibiting nitrification (Ciba-Geigy, 1984). They do not consider metalaxyl active enough to be commericaly useful as a nitrification inhibitor (L.D.

Houseworth, 1984, personal communication).

Nitrification Inhibitors

Nitrification is the microbiological process in which soil ammonium (NH_4^+) is converted to nitrate (NO_3^-.) The conversion is a stepwise biological oxidation. In the first step, NH_4^+ is oxidized to NO_2^-. This step is commonly carried out in soil by the bacteria Nitrosomonas europaea, but also may be carried out to a lesser extent by Nitrosospira briensis, Nitrosolobus multiformis, Nitrosovibrio tenuis, and Nitrosococcus nitrosus. All of these organisms are obligate chemolithotropths and carry out a 6 electron oxidation of NH_4^+ (Grant and Long, 1981).

The overall NH_4^+ oxidation reaction carried out by N. europaea is given below:

$$NH_4^+ + 1\ 1/2\ O_2 \xrightarrow{6\ e^-} NO_2^- + H_2O + 2H^+ \text{ ;F, -65 kcal.}$$

This reaction proceeds in three steps, with 2 electrons added in each step. The first step is the conversion of NH_4^+ to NH_2OH by the enzyme oxygenase in an energy consuming step. The last two steps of the process are catalyzed by hydroxylamine oxidoreductase and involves oxidation of NH_2OH to NO_2^-. The intermediate in this process is most likely the unstable compound NOH. Production of ATP is coupled to the oxidation of NH_2OH. ATP produced by this reaction is the cell's only source of energy and is used for CO_2 fixation via the Calvin cycle. The cell does not require organic C (Schmidt, 1982).

Oxidation of NO_2^- to NO_3^- in soil is carried out by _Nitrobacter winogradkyi_ (Schmidt, 1982). No other genus of NO_2^- oxidizing bacteria has been identified in soil. Like the NH_4^+ oxidizing bacteria, _N. winogradkyi_ is an obligate chemolithotroph. The oxidation reaction carried out by _N. winogradkyi_ is given below:

$$NO_2^- + 1/2\ O_2 \xrightarrow{2\ e^-} NO_3^- \ ;F,\ -17.8\ \text{kcal.}$$

This reaction proceeds in a single step, two electron process by a NO_2^- oxidase system. ATP production is coupled to this enzyme system. Since the energy yield of this reaction is low, an extensive cytomembrane system with a large number of NO_2^- oxidizing sites is present. CO_2 is fixed via the Calvin cycle and no organic C is required (Schmidt, 1982).

The main soil environmental factors which affect the rate of nitrification are the amount of O_2 in the soil solution and the temperature and pH of the soil microsites in which the nitrifiers exist (Schmidt, 1982).

Nitrifying bacteria require O_2 from the soil solution for their metabolism. Rates of NH_4^+ and NO_2^- oxidation are dependent on the supply of available O_2. Nitrification is slowed by conditions which reduce the concentration of O_2 in the soil solution. Such conditions include excessive soil moisture, temperature higher than 40 °C, and large amounts of oxidizable organic C (Schmidt, 1982). Excessive soil moisture reduces the amount of soil air, which in turn reduces O_2 recharge of the soil solution. High soil temperature limits the solubility of O_2 in water and increases O_2 demand by heterotropic microorganisms in the soil. A high amount of oxidizable C also increases the demand for O_2 by increasing the population of soil microbes (Schmidt, 1982).

Nitrifiers are much more sensitive to wet soil conditions than to dry conditions. Nitrate production ceases when the soil water potential approaches 0 cm H_2O (0 kPa). The rate of NO_3^- production rises rapidly as the moisture tension increases from 0 to 40 cm H_2O (0 to 3.9 kPa). Maximum NO_3^- production occurs at 40 cm H_2O (3.9 kPa). The rate of NO_3^- production does not decrease from limited water until the soil moisture tension reaches 275 cm H_2O (26.9 kPa) (Follett, et al., 1981).

Nitrifiers are able to metabolize over a broad temperature range. Nitrification has been reported at temperatures as low as 5° C and as high as 60° C, although 40° C is the maximum reported for U.S. soils (Schmidt, 1982). Maximum nitrification rates occur in U.S. soils between 30° C and 35° C (Follett, et al., 1981).

Nitrifying organisms are sensitive to low soil pH. The rate of NO_3^- production is very slow at soil solution pH values less than 5.0. There is a sharp increase in the nitrification rate as the pH increases from 5.0 to 6.0. At pH values higher than 6.0, the nitrification rate continues to increase, but at a slower rate. The maximum rate is reached at pH 8.3 to 8.5 (Follett, et al., 1981).

Many soil fumigants [vapam, vorlex, chloropicrin, telone, DD (a mixture of dichloropropane and dichloropropene), ethylene dibromide, and methyl bromide] are potent inhibitors of nitrification when applied at normal rates (Hauck, 1980; Goring and Laskowski, 1982). The non-fumigant nematicides ethoprophos (mocap), oxamyl (vydate), and fanamiphos (nemacur) are not inhibitory to nitrification when applied at normal rates (Elliot, et al., 1974; Elliot et al.,1977; Goring and Laskowski, 1982).

Certain fungicides have been reported to inhibit nitrification. The dithiocarbamate fungicides (ferbam, maneb, nabam, zineb, and ziram) will inhibit nitrification by release of carbon disulfide upon decomposition in the soil (Goring and Laskowski, 1982). A single soil application at the maximum labeled rate or above is required to obtain a 25 percent decrease in nitrification (Goring and Lawkowski, 1982). Normal, low level foliar applications do not usually cause nitrification inhibition (Goring and Laskowski, 1982).

Several chemicals designed specifically to inhibit nitrification are commercially available. Of these chemicals, nitrapyrin has been the most widely researched and used in the southeastern United States (Touchton and Boswell, 1980).

Nitrapyrin [2-chloro-6 (trichloromethyl) pyridine] inhibits oxidation of NH_4^+ to NO_2^- by interfering with the _Nitrosomonas_ cytochrome oxidase system that oxidizes NH_4^+ to NH_2OH. Nitrapyrin does not interfere with the oxidation of NH_2OH or NO_2^-. Inhibition of nitrification by nitrapyrin can be reversed by addition of Cu^{2+}, which is chelated by nitrapyrin (Hauck, 1980).

Several of the same physical, chemical, and biological soil factors that affect nitrification also influence the efficacy of nitrapyrin. This inhibitor is more effective on coarse textured soils than on light textured soils. Nitrapyrin is also more effective at a soil temperature of 15° C than at a soil temperature of 30° C. At the 15° C soil temperature, nitrification was inhibited 87 to 89 percent after 28 days of incubation (Bundy and Bremner, 1973).

Commercial nitrification inhibitors provide a potential means of increasing the efficiency of N fertilizers by retaining N in the NH_4^+ form for a longer period of time. By reducing the percentage of total N in the NO_3^- form in the soil, losses from denitrification and leaching are reduced (Touchton and Boswell, 1980).

The effectiveness of any nitrification inhibitor depends on the soils ability to support a large nitrifier population. The larger the population, the less the effect that a given chemical will have on NO_3^- production (Dubey, 1969; Dubey and Rodriguez, 1970; Parr, 1976). This principle is most evident with weak nitrification inhibitors (Parr, 1976). In experiments with the herbicides diuron and picloram, Dubey (1969) and Dubey and Rodriguez (1970) found that the inherent nitrifying capacity of the soil influenced the effects of these herbicides on nitrification. Both herbicides inhibited nitrification in soils of low nitrifying capacity but had little effect on nitrification in soils of high nitrifying capacity.

Nitrogen Source

Nitrogen has a greater effect on the growth and development of flue-cured tobacco than any of the other required mineral elements. Increasing the amount of N has many effects including delayed maturity, increased leaf size, reduced leaf thickness, increased nicotine content, and reduced sugar content. For proper maturity, the N supply should be exhausted by the time the plant reaches maximum leaf area (Hawks and Collins, 1983). Quality is lowered by deficient or excessive N. Excessive N may promote leaf drop, excessive axillary bud growth, increased insect populations and development of leaf diseases. Deficient or highly excessive N will reduce yield. The level of soil N should be maintained in the narrow range that promotes high leaf yield and quality (Hawks and Collins, 1983).

The influence of the source of N on tobacco is well documented (McCants and Woltz, 1967). Reviews of N nutrition of tobacco (McCants and Woltz, 1967; Tso, 1972; Hawks and Collins, 1983) indicate that NO_3-N is more favorably utilized than NH_4-N in tobacco fertilization.

In earlier times, tobacco was fertilized with N derived partially or fully from organic sources such as oil seed meal, tankage, and fish scrap (Tisdale et al., 1952; McCants and Woltz, 1963; McCants and Woltz, 1967). In most cases the organic N sources were more expensive than mineral N (Tisdale, et al., 1952).

Experiments conducted with flue-cured tobacco in Georgia from 1922 to 1932 showed that yield with inorganic N sources, $NaNO_3$ and $(NH_4)_2SO_4$, was slightly higher than with organic N. Further experiments in Virginia, North Carolina, and Georgia did not show any superiority of organic N over mineral N (Tisdale et al., 1952).

In early work with inorganic sources of N, McEvoy (1946) reported that the fresh weight of two burley tobacco varieties after three months in sand culture was highest when the N was supplied by solutions containing 66 to 100 percent NO_3-N with the remainder of the N supplied as NH_4-N. Solutions containing over 33 percent NH_4-N resulted in significantly lower fresh weights after three months. The roots of plants grown on high NH_4-N solutions were observed to have a brown discoloration, and the foliage exhibited symptoms of Mg deficiency (interveinal chlorosis of the lower leaves).

Skogley and McCants (1963) conducted experiments in which tobacco seedlings were grown in steam sterilized soil for 32 days. N was supplied as either all NO_3-N or NH_4-N in combination with various rates of Cl. Dry weight yields from the NO_3-N plants were twice as high as the NH_4-N plants. Leaves of plants grown with NH_4-N were abnormally thick, with the margins rolled upward. The severity of this abnormality was greater with higher rates of Cl. No leaf abnormality was observed in the NO_3-N plants, regardless of the rate of applied Cl. Tissue concentrations of Cl were similar for both the NO_3-N and NH_4-N plants, indicating that the concentration of Cl alone was not responsible for the observed leaf abnormality. Increasing the application rate of NH_4-N with no increase in Cl did not increase the severity of the leaf abnormality. The authors concluded that the severity of the leaf abnormality was controlled by an interaction between NH_4^+ and Cl^-.

In a review of N source field experiments, McCants and Woltz (1967) stated that in 13 of 15 experiments, the value per acre of flue-cured tobacco was generally higher when the N fertilizer contained at least 50 percent NO_3-N. In these experiments, no fumigants were used and nitrification was assumed to proceed at a rapid rate.

In fumigated soils the favorable response to NO_3-N was greater (McCants et al., 1959; McCants and Woltz, 1963). They concluded that the yield and quality response of tobacco to high NO_3-N in fumigated soil was due to a reduction in the rate of nitrification. Also, soil conditions which limit nitrification, such as low pH and temperature, may contribute to the favorable response to increased NO_3-N in the fertilizer under non-fumigated conditions (McCants and Woltz, 1967).

Results from experiments in Virginia indicated that the early growth of tobacco was slower when the fertilizer contained all NH_4-N (Gous, et al., 1971). In this study, soil fumigation did not affect the response of tobacco to NH_4-N, indicating that the population of nitrifying organisms may not have been greatly affected by fumigation. The ammonium toxicity symptoms described by Skogley and McCants (1963) were not observed in any of the fumigant and N source combinations (Gous, et al., 1971).

Yield was reduced by NH_4-N in the first year of the experiment, but was not affected in the second year (Gous, et al.,1971). NH_4-N did not reduce leaf quality index either year. During the second year, where heavy rains fell nine weeks after transplanting, NH_4-N resulted in higher leaf quality than NO_3-N. A combination of 50 percent NH_4-N and 50 percent NO_3-N produced the most consistent yield and quality over the two years of the study (Gous et al., 1971).

Rhoads (1972) reported that NH_4NO_3 was an acceptable source of N for production of cigar wrapper tobacco. Nitrapyrin was used on all treatments to prevent nitrification from changing the ratio of NH_4^+ to NO_3^- in the soil. Tobacco yield and quality decreased when the NO_3-N content was reduced below 50 percent.

Jones (1981) conducted 10 on-farm N source experiments in

Virginia during 1978 and 1979. The objective of this study was to determine if urea could be substituted for the more expensive N sources $NaNO_3$ and NH_4NO_3. Combined results from the 10 test locations did not indicate any significant difference in yield, value per acre, or price per cwt among N sources.

Use of urea as a N source for flue-cured tobacco has also been evaluated in North Carolina. A series of eight experiments to compare urea to $NaNO_3$ was conducted by Williams and Miner (1982) at three locations from 1976 to 1978. Mixtures of $NaNO_3$ and urea were formulated to supply 100, 66, 50, 33, and 0 percent NO_3-N. A fumigant factor was also included in the experiments. In seven of the eight experiments, fumigants did not have a significant effect on yield or leaf quality index.

The effect of urea on yield differed during the three years of the study (Williams and Miner, 1982). Urea reduced yields under dry conditions, increased yields under wet conditions, and did not affect yields under normal rainfall conditions (Williams and Miner, 1982). Urea did not reduce quality in any year of the study. However quality was increased by urea under the wet conditions of 1978 (Williams and Miner, 1982).

Isobutylidene diurea (IBDU), a slow-release N carrier, has been evaluated as a possible N source for flue-cured tobacco. Miner, et al. (1978) reported that IBDU did not release N fast enough to supply the uptake demand of the tobacco. Plants fertilized with IBDU exhibited N deficiency symptoms throughout the growing season, and gave significantly lower yield than plants fertilized with $NaNO_3$ or urea.

Root uptake of the NO_3^- ion is coupled with the release of an HCO_3^- ion to maintain electrical neutrality across the soil-root interface. The HCO_3^- ion that is released will increase the soil solution pH in the area of the root. On the other hand, plant uptake of the NH_4^+ ion is coupled with the release of an H^+ ion. The H^+ ion decreases the pH of the soil around the root (Nye, 1984).

Nitrification of applied NH_4-N is also a source of acidity; the conversion of one mole NH_4^+ to NO_3^- results in the production of two moles of H^+ (Reneau, et al.,1968; Schmidt, 1982). In addition, inorganic, NH_4^+ containing fertilizers exhibit a salt effect which decreases the soil pH (Reneau, et al., 1968). Depression of pH by NH_4-N fertilizers is more severe in a concentrated fertilizer band than when the NH_4-N is broadcast (Sims, et al., 1984).

Urea is rapidly hydrolyzed to $(NH_4)_2CO_3$ by the soil enzyme urease. Accompanying the hydrolysis is a rapid increase in the soil pH in the vicinity of the urea granule (Bremner and Douglas, 1971; Bundy and Bremner, 1974; Petrie and Jackson, 1984).

Hydrolysis of urea takes place very rapidly. Hargrove and Kissel (1979) reported that 30 to 57 percent of applied urea is hydrolyzed within 24 hours. In this experiment urea hydrolysis was complete within 10 days.

Petrie and Jackson (1984) measured soil pH 7, 14, and 28 days after application of urea and NH_4Cl. After 7 days, soil pH where urea was applied was higher than the initial pH, whereas, NH_4Cl had decreased the pH. At 14 and 28 days, the urea soil pH was lower than the initial pH, but was significantly higher than the pH of soil that had received NH_4Cl.

Gaseous loss of N as NH_3 is a problem associated with the soil application of urea (Bremner and Douglas, 1971; Bundy and Bremner, 1974; Hargrove and Kissel, 1979). Hargrove and Kissel (1979) measured gaseous losses from prilled urea for two weeks after surface application. During the two week period, 31 percent of the applied N (56 kg ha^{-1})was lost as NH_3 gas.

Urea may also lead to an accumulation of NO_2^- in the soil. The combination of high NH_3 levels and the high soil pH that result from urea hydrolysis may inhibit bacterial oxidation of NO_2^-, followed by possible plant root injury. High levels of NO_2^- may also lead to N loss through chemical decomposition of NO_2^- (Bundy and Bremner, 1974).

The form of N taken up by tobacco plants affects the accumulation of halogens in the leaf (McCants et al., 1959; Williams and Miner, 1982). McCants et al. (1959) reported that tobacco plants fertilized with NH_4-N contained higher leaf halogen concentrations than plants fertilized with NO_3-N. The difference was greatest when sampled 21 days after transplanting. Fertilization with NO_3-N prevented excessively high leaf halogen content in treatments where halogenated (Cl or Br) hydrocarbon fumigants were applied.

A similar interaction of NH_4^+ and Cl^- has been reported by Williams and Miner (1982). They showed that leaf Cl concentration increased as the percentage of urea in the N fertilizer increased. The highest Cl concentrations were recorded 4 to 6 weeks after transplanting.

Uptake of NH_4^+ has been reported to reduce the concentration of K^+, Mg^{++}, and Ca^{++} in tobacco leaves (Skogley and McCants, 1963; McCants and Woltz, 1967). In some instances, cation uptake is depressed to the point where Mg and K deficiency symptoms become visible (McCants and Woltz, 1967). Although NO_3-N enhances plant uptake of K^+, Mg^{++}, and Ca^{++}, leaf concentrations of P and S are lower than in tobacco supplied with NH_4-N (Skogley and McCants, 1963).

The N source supplied to tobacco plants has also been demonstrated to affect other biochemical and physiological properties of the plant. Plants fertilized with NH_4-N have a much higher leaf water content than plants supplied with NO_3-N (Tso, 1972). Citric and malic acid concentrations are decreased by NH_4-N, while total organic acid and total sugar concentrations are increased by NO_3-N (Tso, 1972). Nicotine and reducing sugar concentrations are usually unaffected by N source (McCants and Woltz, 1963).

MATERIALS AND METHODS

Soil Incubation Experiment

A soil incubation experiment was conducted to determine the effects of metalaxyl on NH_4^+ oxidizing bacteria in a controlled environment. The experiment consisted of two phases: soil incubation and bacterial quantification. Two repetitions of the experiment were conducted under identical conditions using soil from the same source. Treatments were replicated three times in a randomized complete block design in each repetition.

Untreated soil (Durham sandy loam) was collected from an area adjacent to the 1985 Southern Piedmont field experiment. The pre-treatment NO_3-N and NH_4-N concentrations of the soil were 6 and 2 mg kg^{-1}, respectively. The pH of the soil was 5.5, and the water content 0.11 kg kg^{-1}. The soil was tightly sealed in plastic bags and kept in the dark until use.

One hundred grams (moist weight) of soil was placed into sterile 250 ml disposible beakers, which were covered by Al foil. The weight of the beaker, soil, and foil were recorded so that water content of the soil could be maintained during the experiment. The untreated soil was allowed to equilibrate for one week in an incubator set to provide a 25° C dark incubation.

A 0.2 M $(NH_4)_2SO_4$ stock solution was formulated. Metalaxyl or nitrapyrin was added to the stock solution as required to provide treatments of no inhibitor, 0.56 kg ha^{-1} metalaxyl, 1.12

kg ha^{-1} metalaxyl, and 6.72 kg ha^{-1} nitrapyrin. Treatments were applied by adding 2 ml of the solutions to the soil surface. The N rate for all inhibitor treatments was 123.2 kg ha^{-1}.

The beakers were returned to the incubator for 3 weeks. Beakers were weighed weekly, and water content was maintained by addition of sterile, distilled water. At the end of the incubation, the soil was analyzed for pH, NO_3-N, and NH_4-N.

A 10 g soil sample was taken from each of the beakers at the end of the soil incubation experiment for quantification of NH_4^+ oxidizers. The soil samples were placed in 95 ml sterile 1 mM phosphate buffer and blended for 60 s. This extract was serially diluted to 10^{-7} with sterile buffer. One milliliter aliquots of the 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions were transferred to culture tubes containing 4 ml of the NH_4^+ oxidizer media described by Schmidt and Belser (1982). Five tubes from each dilution were inoculated. The culture tubes were incubated in the dark at 25° C for six weeks.

After the first three weeks of incubation, the tubes were checked weekly for acid production. Bromothymol blue in the media changed from blue to yellow as a result of acid produced by oxidation of NH_4^+ (Schmidt and Belser, 1982). Tubes that had changed color were recorded as positive. At the end of the incubation period, the total number of positive tubes was recorded. Estimates of bacterial populations were calculated using the most probable number technique of Alexander (1982).

Southern Piedmont Field Experiment

Field experiments were conducted at the Va. Tech Southern Piedmont Agricultural Experiment Station (SP), Blackstone, Va. on a Durham sandy loam soil (Typic Hapludult, fine-loamy, siliceous, thermic) in 1984 and 1985. A randomized complete block design in a split-plot arrangement replicated four times was used with N sources as main-plots and metalaxyl treatments as sub-plots. Each sub-plot consisted of a single row containing 24 plants spaced 51 cm apart within the row and 122 cm between rows (1/665 ha), with common border rows separating the experimental units. Two border plants at the ends of each plot row were eliminated from the harvest.

Nitrogen sources used in 1984 were $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$. Urea ($H_2N\text{-}CO\text{-}NH_2$) was added as a main plot treatment in 1985. In 1984, metalaxyl treatments consisted of no metalaxyl (control), 1.12 kg ha^{-1} at transplanting, and 1.12 kg ha^{-1} at transplanting plus 0.56 kg ha^{-1} at the last (layby) cultivation. The experiment was expanded to include 0.56 kg ha^{-1} at transplanting and 0.56 kg ha^{-1} at transplanting plus 0.56 kg ha^{-1} at layby in 1985. Nitrapyrin, at 6.72 kg ha^{-1}, was also added in 1985 as an indicator of the strength of metalaxyl's nitrification inhibitor properties.

Conventionally produced tobacco seedlings of the cultivar

'VA 182' were transplanted onto raised beds on 10 May and 8 May in 1984 and 1985, respectively. The plants were allowed to recover from transplant shock for approximately 7 days before treatments were applied. Fertilizer blends were formulated from the different N sources, triple super phosphate, and K_2SO_4 to supply 73 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 140 kg ha^{-1} K_2O. Elemental S was added to the $NaNO_3$, NH_4NO_3, and urea blends to equalize S levels among the treatments. Hutcheson et al. (1959) reported that Na did not exhibit an effect on tobacco growth and development except in the case of severe K deficiency. Therefore, Na levels were not adjusted.

A shallow furrow was opened on both sides of the bed approximately 10 cm from the center of the row. Fertilizer for each plot and border row was distributed into these furrows. Metalaxyl treatments were sprayed in a 90 cm band over the row using a CO_2 powered backpack sprayer calibrated to deliver 234 L ha^{-1}. The sprayer was fitted with a 60 cm drop nozzle boom containing two 8004 spray nozzles. Boom pressure was maintained at 0.2 MPa. After spraying, the fertilizer and metalaxyl were incorporated into the soil by cultivation. The layby metalaxyl treatment was applied immediately before the last normal cultivation (15 June and 11 June in 1984 and 1985, respectively) using the sprayer described above, and was incorporated by cultivation.

Soil samples were taken on 20 June in 1984, and on 29 May, 12 June, and 27 June in 1985. Green leaf tissue samples were taken on 20 June and 20 July in 1984, and on 12 June, 27 June, and 11 July in 1985. Composite soil samples consisting of 10 cores taken to a depth of 15 cm from the side of the bed in the area of the fertilizer band were collected from each plot. The soil samples were then frozen for later determination of NH_4-N, NO_3-N, and pH. Whole leaf tissue samples were collected from the fourth leaf from the top of five consecutive plants, starting with the fourth plant in the row, for total N and NO_3-N determinations. The tissue was dried for 48 h at 70° C in a forced air oven, then ground using a UD Cyclone mill.

Other than fertilizer and metalaxyl application, conventional cultural practices recommended by the Virginia Cooperative Extension Service were followed. No soil fumigants were applied. The tobacco was harvested five times in 1984 and four times in 1985 and cured in a conventional flue-cured tobacco barn. Cured leaf of each plot was weighed by harvest, assigned an official U.S. Government grade by a U.S.D.A. Marketing Service tobacco inspector, and plot yields and grade indicies were computed (Wernsman and Price, 1975). Composite samples consisting of approximately 30 g cured leaf were collected from each harvest for total N, total alkaloids, and reducing sugar determinations (Horwitz, 1980; Davis, 1976).

On-Farm Experiment

Various rates of metalaxyl applied at transplanting and/or the layby cultivation were evaluated at three farm locations in 1985. NH_4NO_3 was used as the source of N in each of these tests. Metalaxyl treatments were as follows: 0, 0.56, 1.12, and 3.36 kg ha^{-1} at transplanting, and 0.56 or 1.12 kg ha^{-1} at transplanting plus 0.56 kg ha^{-1} at layby.

Preplant fertilizer (6-12-18 analysis) was placed in a single band under a raised bed before metalaxyl application at all locations. Fertilizer rates and application dates are presented in Table 1.

Transplant metalaxyl treatments were sprayed in a 60 cm band over the raised bed immediately after fertilizer application. A single 8004 nozzle attached to a CO_2 powered backpack sprayer operated at 0.2 MPa pressure was used to apply the metalaxyl. The rows were re-bedded to incorporate the metalaxyl.

Tobacco seedlings were transplanted onto the beds within two weeks of treatment. Soil conditions were dry at this time, and irrigation was applied as needed to supplement rainfall throughout the season. General management practices and variety selection were those of individual grower preference, but were considered acceptable for the production of flue-cured tobacco in Virginia (Table 1).

Table 1. Test locations, varieties, fertilizer rates, and dates of treatment application. Three on-farm locations, 1985.

Location	Variety	Trans-plant date	Metalaxyl pre-plant	Metalaxyl layby	Total applied nutrients N	Total applied nutrients P_2O_5	Total applied nutrients K_2O	Side-dress date
			---- date ----		---kg ha^{-1}---			
May	C-176	5/4	5/4	6/14	74	61	101	5/17
Hawthorne	K-326	5/10	5/6	6/17	75	61	101	5/20
Smith	K-326	5/20	5/6	6/26	68	74	111	5/29

Additional NH_4NO_3 was applied approximately two weeks after transplanting as a sidedress fertilizer. Layby metalaxyl treatments were applied just before the last normal cultivation with the drop nozzle sprayer described above. Soil and tissue samples were taken as in the 1985 on-station test.

Plot size at all locations was 1/30 ha. Plots consisted of four rows at two locations (May and Smith), and three rows at the other (Hawthorne). Data were collected from the center two rows of the four row plots and from the center row of the three row plots.

The tobacco was harvested and cured by the farmer cooperators. The cured leaf from each plot was weighed, and assigned an official U.S. Government grade by a U.S.D.A. Marketing Service tobacco inspector. Plot yields and grade indices were computed (Wernsman and Price, 1975). A representive cured-leaf sample was taken from each plot for total N, total alkaloids, and reducing sugars determination (Horwitz, 1980; Davis, 1976).

Greenhouse Experiments

Two greenhouse experiments were conducted at Southern Piedmont, Blackstone, Va. Each greenhouse experiment was conducted twice. Seeds of cultivar 'VA 182' were sown into pans containing vermiculite on 12 Aug. 1985 and 27 Nov. 1985. Nutrient solution, formulated by addition of 75 g Peters Hydro-Sol 5-11-26 (Peters Fertilizer Products, W.R. Grace & Co., Fogelsville PA) and 50 g $CaNO_3$ to 75.6 L H_2O, was applied weekly.

Seedlings were transferred to Speedling styrofoam containers with 5 cm square cells containing vermiculite on 4 Sept. and 30 Dec. 1985. Water was applied daily and nutrient solution applied weekly until the plants reached a height of approximately 10 cm, at which time the plants were transferred to 10 cm diameter pots containing 500 g soil (Durham sandy loam). Transplanting to the pots occurred 30 Sept. 1985 and 22 Jan. 1986. Treatments were replicated four times in a randomized complete block design. The experimental unit was one pot containing a single plant. After potting, the plants were watered with deionized water as needed to prevent wilting.

In both experiments the plants were removed from the pots two weeks after treatment and separated into shoot and root tissue. The shoot tissue was dried for 48 h at 70^{o} C for dry weight determination. The root tissue was discarded. Soil samples were taken from each pot for NH_4-N, NO_3-N, and pH determination.

Metalaxyl Rate Experiment.

This experiment was designed to determine the effects of metalaxyl rates on the nitrification of N from various sources. Treatments consisted of three N sources in factorial combination with four metalaxyl rates. N was supplied by $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$. Nutrient rates were equivalent to 73 kg ha^{-1} N, 204 kg ha^{-1} P_2O_5, and 140 kg ha^{-1} K_2O. Phosphorus and K were supplied by KH_2PO_4. Metalaxyl rates were equilvant to 0, 1.12, 2.24, and 3.36 kg ha^{-1}. The nutrients and metalaxyl were applied to the soil surface in 10 ml H_2O and distributed throughout the pot by application of 100 ml deionized H_2O.

pH Adjustment Experiment.

This experiment was designed to determine the effect of raising soil pH on inhibition of nitrification by metalaxyl. The nutrient rates for this experiment were the same as in the metalaxyl rate experiment. N was supplied by $(NH_4)_2SO_4$ and P and K were supplied by KH_2PO_4. Metalaxyl rates were equlivalent to 0, 0.56, and 1.12 kg ha^{-1}. The fourth inhibitor treatment was nitrapyrin at 6.72 kg ha^{-1}. The nutrients and metalaxyl were applied to the soil surface in 10 ml distilled H_2O.

The pH adjustment was provided by applying 10 ml 0.1 M NaOH or distilled H_2O in factorial combination with the inhibitor treatments. The nutrients, inhibitor and NaOH were distributed in the pot by application of 100 ml deionized H_2O to the soil surface.

Soil Analysis Procedures

All soil samples were frozen or packed in dry ice within 30 minutes of collection. The samples were stored frozen to prevent the occurrence of nitrification before analysis. Immediately before extraction, the samples were removed from the freezer and allowed to warm to room temperature.

Ion-selective electrodes (ISE) were used for all soil analyses. In all instances, the appropriate ISE was connected to an Orion 407A specific ion meter calibrated for direct solution concentration readings. All procedures used for soil N are based on those outlined by Keeney and Nelson (1982).

Soil NO_3-N was determined on an 8 g subsample of the original sample. An extracting solution with a high background SO_4^{2-} concentration was prepared by addition of 20 ml 2 M $(NH_4)_2SO_4$ ionic strength adjuster (ISA) to 1 L distilled H_2O. Forty milliliters of the extracting solution were added to the soil subsample in a 100 ml plastic centrifuge tube to provide a 5:1 dilution. The tubes were stoppered and shaken for 30 minutes. The soil extract was allowed to stand for 15 minutes, after which approximately 30 ml was decanted for NO_3-N determination with an Orion 93-07 NO_3^- electrode. The outer chamber of the reference electrode was filled with the extracting solution to match the background ionic strength of the samples. The meter was standardized with 1, 10, and 100 mg L^{-1} standards prepared daily from 1400 mg L^{-1} NO_3-N stock solution (100 mM $NaNO_3$).

The procedure used for the extraction of soil NH_4-N was identical to the NO_3-N procedures except than 2 M KCl was used in place of the $(NH_4)_2SO_4$ extracting solution. One milliliter 10 M NaOH was added to each sample and standard to convert the NH_4^+ to NH_3. NH_4-N was determined immediately with an Orion 95-12 NH_3 electrode. 1, 10 and 100 mg L^{-1} standards were prepared daily from 1000 mg L^{-1} NH_4-N stock solution (72 mM NH_4Cl).

The soil pH was determined on a 40 g subsample to which 40 ml distilled H_2O was added. The soil-water suspension was stirred and allowed to sit for 40 minutes. The suspension was again stirred and pH determined by a standard pH electrode, standardized with pH 4 and 7 buffers.

Tissue Analysis Procedures

Tissue NO_3-N was extracted by shaking 500 mg dried, ground green leaf tissue with 100 ml 0.05 M KCl. Approximately 15 ml of the extract was filtered through Whatman #4 filter paper.

Nitrate was determined by the hydrazine reduction autoanalyzer method as described by Markus, et al. (1985). The procedure was modified for tobacco leaf tissue by replacing the resample loop with a 15 cm dialyzer. All reagent flow rates were increased by a factor of two to reduce contamination from the previous sample. To compensate for the increased flow rate, delay coils for the reduction and color development steps were increased to 20 turns. A 37° C, 7.7 ml Technicon heating bath was used for the reduction step. The sampler was operated at 40 samples per hour at a 1.5 to 1 sample to wash ratio. The calibration curve was determined with 40, 30, 20, 10, and 5 mg NO_3-N l^{-1} standards prepared from stock 7.2 mM KNO_3. The stock solution and working standards were prepared in 0.05 M KCl.

Samples were analyzed for total N by the Kjeldahl method (Horwitz, 1980). Semi-micro digestion was carried out using a Technicon 40 tube block digestor (Technicon Industrial Systems, Tarytown, NY). A Technicon Autoanalyzer II was used for N determination on the digested sample.

Cured leaf samples were analyzed for total alkaloids and reducing sugars by the autoanalyzer procedure of Davis (1976). Sulfanilic acid was substituted for buffered analine in the total alkaloids procedure.

RESULTS AND DISCUSSION

Soil Incubation Experiment

Error variances of the two repetitions of the study were found to be homogeneous using Bartlett's test (Little and Hills, 1978). The data were combined for analysis of variance.

Metalaxyl at 1.12 kg ha^{-1} significantly decreased the production of NO_3-N compared to the control and 0.56 kg ha^{-1} treatment (Table 2). Soil NH_4-N concentration and pH were not affected by metalaxyl. The population of NH_4^+ oxidizing bacteria was significantly reduced by both rates of metalaxyl (Table 2).

Nitrapyrin significantly decreased NO_3-N as compared to the control and metalaxyl treatments (Table 2). The bacterial population was reduced 100 fold by nitrapyrin, while soil pH and NH_4-N were significantly increased. Of the initial NH_4-N (107 mg kg^{-1}), 80 percent was recovered from the nitrapyrin treated soil after three weeks, compared with only 42 percent in the check and metalaxyl treatments (Table 2).

Metalaxyl inhibited production of NO_3^- without inhibiting disappearance of NH_4^+ as was observed in the nitrapyrin treatment. These results suggest that the modes of action of nitrapyrin and metalaxyl in inhibiting NO_3^- production are different. Nitrapyrin inhibits nitrification by interfering with the

Table 2. Effect of metalaxyl and nitrapyrin on form of soil N, pH, and nitrifyer populations under laboratory conditions.

Inhibitor		N Form			NH_4^+ Oxidizer
Chemical	Rate	NO_3^-	NH_4^+	pH	Population
	kg ha^{-1}	---- mg kg^{-1} ----			Cells kg^{-1} X 10^6
None	--	76.2 a[†]	45.1 b	4.6 b	550 a
Metalaxyl	0.56	73.6 a	49.9 b	4.5 b	297 b
Metalaxyl	1.12	67.7 b	45.7 b	4.6 b	305 b
Nitrapyrin	6.72	29.5 c	85.5 a	5.6 a	6 c

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

cytochrome oxidase system that oxidizes NH_4^+ to NH_2OH (Hauck, 1980). Metalaxyl may allow the oxidation of NH_4^+ to NH_2OH described by Schmidt (1982), but inhibits subsequent oxidation to NO_3^-. Since metalaxyl lowered the population of NH_4^+ oxidizers, the inhibition is most likely in the conversion of NH_2OH to NO_2^-. This oxidation is a two-step process, catalyzed by hydroxylamine oxidoreductase, that proceeds via an unstable intermediate postulated to be NOH (Schmidt, 1982). Metalaxyl may inhibit one or both steps of this oxidation. Several compounds, including CN, CO, and H_2O_2, have been reported to inhibit NH_2OH oxidation with little effect on the production of NH_2OH. The NH_2OH is often oxidized to NO and N_2O, which is lost from the soil system (Hauck, 1980).

Hydrogen ion production by the bacteria results from the dehydrogenation of NH_2OH during oxidation to NO_2^- (Schmidt, 1982). The technique used to estimate the bacterial populations is dependent on H^+ production to indicate the presence of bacteria in a particular dilution (Schmidt and Belser, 1982). Bacteria capable of oxidizing NH_4^+ to NH_2OH, but not capable of the subsequent oxidation of NH_2OH to NO_2^-, would not be included in the population estimate. The occurrence of NH_4^+ oxidation without production of NO_3^- in the metalaxyl treatments is indicated by the decrease in NO_3^- without an increase in NH_4^+ in this experiment. The N which was not recovered as NO_3^- or NH_4^+ in the metalaxyl treatments may have been lost from the soil system as NO or N_2O gas as described by Hauck (1980).

Nitrification inhibition by metalaxyl was not as great as would be expected from the population reduction observed in the experiment. Nitrification of the control treatment was limited by a factor other than low bacterial population. Since soil moisture content and temperature were maintained at the range reported favorable (Follett et al., 1981) for nitrification, soil pH may have been the limiting factor. Eno and Blue (1957) observed that soil pH decreases rapidly after application of $(NH_4)_2SO_4$ to the soil, resulting in less nitrification than where lime was applied with the $(NH_4)_2SO_4$. Final soil pH was between 4.4 and 4.5 in the control and metalaxyl treatments. Sarathchandia (1978) reported that in many soils the nitrifying bacteria population and nitrifying activity were not related. In particular, soils with low pH exhibited little nitrification, despite high populations of nitrifying bacteria. The high population, low pH conditions that Sarathchandia (1978) described are similar to the final population and pH observed in this experiment. In both cases, low pH may have prevented nitrification at the population's full potential.

Nitrapyrin was much more effective in inhibiting nitrification than metalaxyl. Both the population and NO_3-N concentration were reduced to levels far below those for the metalaxyl treatments. The reduction in pH associated with nitrification of $(NH_4)_2SO_4$ did not occur in the nitrapyrin treatment.

This experiment shows that metalaxyl has the potential to reduce nitrification at both the 0.56 and 1.12 kg ha^{-1} rates. Both rates reduced the population to the same level, but only 1.12 kg ha^{-1} inhibited production of NO_3^- in the soil. While the exact mode of inhibition can not be identified from these data, inhibition of the NH_2OH oxidation pathway seems to be involved. As a nitrification inhibitor, metalaxyl is relatively weak and much less effective than nitrapyrin. Unlike nitrapyrin, the nitrification inhibition provided by metalaxyl is of little practical use, since N may be lost from the soil system.

Southern Piedmont Field Experiment

Differences in environmental conditions in 1984 and 1985 contributed to a significant ($P=.10$) year X metalaxyl treatment interaction. In addition, additional N source and metalaxyl treatments were added in 1985, as well as additional sampling dates. Therefore, each year's data were analyzed separately. In the absence of significant N source X metalaxyl interactions, means for N sources are averaged over metalaxyl treatments and means for metalaxyl treatments are averaged over N sources.

Nitrogen source significantly affected soil NO_3-N, NH_4-N, and pH on all sampling dates in 1984 and 1985 (Tables 3 and 4) . $NaNO_3$ produced significantly higher NO_3-N concentrations than

Table 3. Effect of N source and metalaxyl application on the form of N in soil and pH when sampled 5 weeks after treatment. Southern Piedmont field experiment, 1984.

Treatment		Nitrogen Form		pH
		NO_3^-	NH_4^+	
		------- mg kg^{-1} ------		
N Source[‡]				
$NaNO_3$		46.8 a[†]	2.3 c	5.4 a
NH_4NO_3		29.8 b	9.7 b	5.0 b
$(NH_4)_2SO_4$		14.8 c	23.1 a	4.8 b
Metalaxyl Application[§]				
Trans-plant	Layby			
-- kg ha^{-1} --				
0.00	0.00	32.1 a	11.7 a	5.1 a
1.12	0.00	33.6 a	11.3 a	5.0 a
1.12	0.56	25.8 b	12.0 a	5.1 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

Table 4. Effect of N source and metalaxyl application on the form of N in soil and pH when sampled at two, four, and six weeks after treatment. Southern Piedmont field experiment, 1985.

Treatment		Two Weeks			Four Weeks			Six Weeks		
		N Form			N Form			N Form		
		NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH
		-- mg kg^{-1} --			-- mg kg^{-1} --			-- mg kg^{-1} --		
<u>N Source</u>[‡]										
$NaNO_3$		53.7 a[†]	2.5 c	5.4 a	43.6 a	0.3 c	5.5 a	25.5 ab	0.7 b	5.7 a
NH_4NO_3		30.5 b	13.2 b	5.2 b	34.6 b	3.3 b	5.2 b	27.4 ab	2.5 b	5.2 b
$(NH_4)_2SO_4$		16.8 c	33.8 a	5.0 c	29.5 b	7.1 a	5.1 bc	20.6 b	6.0 a	5.2 b
Urea		22.0 c	17.0 b	4.8 d	36.7 ab	3.0 b	4.9 c	31.0 a	2.4 b	5.0 c
<u>Metalaxyl Application</u>[§]										
Trans-<u>plant</u>	<u>Layby</u>									
-- kg ha^{-1} --										
0.00	0.00	31.7 a	12.6 a	5.2 a	37.0 a	4.4 a	5.2 ab	25.9 a	4.2 a	5.3 a
0.56	0.00	28.9 a	19.3 a	5.1 a	36.2 a	3.8 a	5.1 b	21.7 a	2.0 b	5.2 a
1.12	0.00	29.9 a	13.3 a	5.2 a	36.0 a	1.4 b	5.2 a	32.8 a	2.1 b	5.3 a
0.56	0.56	30.2 a	16.9 a	5.1 a	35.7 a	3.4 a	5.1 b	23.7 a	1.9 b	5.3 a
1.12	0.56	31.4 a	19.5 a	5.1 a	36.3 a	2.9 ab	5.2 ab	27.0 a	3.8 ab	5.3 a
Nitrapyrin		32.3 a	18.3 a	5.1 a	35.3 a	4.9 a	5.2 ab	25.8 a	3.5 ab	5.3 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

NH_4NO_3 and $(NH_4)_2SO_4$ on all sampling dates except at six weeks after transplanting in 1985 (Tables 3 and 4). The lowest NO_3-N concentrations on all sampling dates in both years were produced by $(NH_4)_2SO_4$. NH_4NO_3 resulted in NO_3-N concentrations intermediate between $NaNO_3$ and $(NH_4)_2SO_4$ in 1984 and on the first two sampling dates in 1985. Urea resulted in NO_3-N concentrations intermediate between NH_4NO_3 and $(NH_4)_2SO_4$ on the first two sampling dates, and the highest NO_3-N at the final sampling date. A reduction in pH was associated with increased fertilizer NH_4-N on all sampling dates in both years.

The ratio of NO_3-N to NH_4-N in the soil was reported to affect the growth and development of flue-cured tobacco (McCants and Woltz, 1967; Gous et al., 1971). Five weeks after treatment, in 1984, the $(NH_4)_2SO_4$ had not nitrified sufficiently to provide at least 50 percent of the N in the NO_3-N form (Table 3). In 1985, nitrification was faster for all NH_4^+ containing treatments, and $(NH_4)_2SO_4$ nitrified sufficiently between the two weeks and four weeks after treatment sampling dates to provide over 50 percent NO_3-N (Table 4). In addition, the initial soil pH was higher in 1985 than in 1984 and was not depressed below 5.0 by $(NH_4)_2SO_4$ during the experiment. The soil pH was depressed to 4.8 in 1984 by $(NH_4)_2SO_4$. Nitrification potential declines rapidly at soil pH values under 5.0 (Follett et al., 1981), which may be the cause of slow $(NH_4)_2SO_4$ nitrification in 1984. Eno and Blue (1957) reported that application of $(NH_4)_2SO_4$

reduces soil pH and subsequently reduces nitrification. None of the NH_4^+ containing fertilizers nitrified fully within four or five weeks in 1984 or 1985, respectively. Six weeks after treatment in 1985 all NH_4^+ containing fertilizers, with the exception of $(NH_4)_2SO_4$, had fully nitrified.

Metalaxyl at 1.12 kg ha^{-1} at transplant plus 0.56 kg ha^{-1} at layby reduced soil NO_3-N concentration when measured one week after layby in 1984 (Table 3). Metalaxyl did not affect NO_3-N concentration of the soil when measured 2 weeks after transplanting, at the time of the layby treatment or 2 weeks after layby in 1985 (Table 4). In addition, no nitrification inhibition due to nitrapyrin was measured in 1985 (Table 4). Metalaxyl did not have any effect on soil NH_4-N concentrations in 1984 or the first two samples of 1985. Metalaxyl significantly lowered the NH_4-N concentration measured at four and six weeks after treatment in 1985. This influence of metalaxyl could not be explained by these data. No soil pH effect from metalaxyl was observed in 1984. Although a statistically significant ($P=.10$) effect of metalaxyl on pH was observed at layby in 1985, the differences are small and do not appear to follow a meaningful trend.

Since the $NaNO_3$ treatment did not contain NH_4^+ and metalaxyl is a nitrification inhibitor, a significant N source X metalaxyl interaction was expected but did not occur in either year. In 1984, inhibition of nitrification by metalaxyl was both weak and erratic in the N sources which contained NH_4^+. Neither metalaxyl nor nitrapyrin inhibited nitrification in 1985. Coefficients of variation (CV) were high in both years. The high CV, coupled with weak and erratic effects, prevented observance of a statistically significant N source X metalaxyl interaction.

Environmental conditions at the Southern Piedmont Agricultural Experiment Station differed greatly in 1984 and 1985. Initial soil pH was 5.4 and 6.1 in 1984 and 1985, respectively. Nitrification rates are pH dependant, with the greatest sensitivity between pH 5.0 and 6.0 (Follett, et al., 1981). Rainfall distribution in the five week period after transplanting differed in the two years of the study. Total rainfall was 10.3 and 8.8 cm in 1984 and 1985, respectively; however, 7.5 cm of the 1984 rainfall fell during the third week after transplanting, creating wet soil conditions, which may have slowed nitrification. Slower nitrification in 1984 is indicated by the amounts of NH_4-N (Table 3) remaining in the soil compared to 1985 (Table 4).

The nitrification inhibitory actions of many compounds are reduced by high nitrification rates (Dubey, 1969; Dubey and Rodriguez, 1970). In soils of high inherent nitrifying capacity, weak nitrification inhibitors do not exhibit nitrification inhibitory action (Parr, 1974). The differences in the nitrification potential in 1984 and 1985 may explain why metalaxyl inhibited nitrification only in 1984.

Green leaf N content was significantly higher for the NH_4NO_3 and $(NH_4)_2SO_4$ treatments than the $NaNO_3$ treatment five weeks after treatment in 1984 (Table 5). At the time of bloom removal (topping), green leaf N content increased as the percentage of NO_3-N in the fertilizer increased. Green leaf N content was not affected by N source in 1985 (Table 6).

Although not significant, NH_4NO_3 tended to increased green leaf NO_3-N content at the first sampling date in 1984 (Table 5). The lack of significance is most likely due to the reduced main plot error degrees of freedom (6 df) inherent in the split plot design (Little and Hills, 1978). The NO_3-N content declined greatly at topping, with $NaNO_3$ slightly higher. Green leaf NO_3-N levels were significantly higher with NH_4NO_3 at layby in 1985 (Table 6). At two weeks after layby $NaNO_3$ and NH_4NO_3 were significantly higher. Sodium nitrate produced significantly higher NO_3-N levels at topping.

Ammonium nitrate consistently produced high green leaf NO_3-N concentrations in both years of the experiment. Nitrate concentration at layby was highest where NH_4NO_3 was applied. Concentrations where NH_4NO_3 was applied were unexpectedly higher than the $NaNO_3$ at layby in both years. Early season movement of NO_3^- below the root zone may have occurred, followed by rapid nitrification of NH_4^+ from NH_4NO_3. The NH_4NO_3 was nitrifyed more rapidly than the urea or $(NH_4)_2SO_4$ because pH conditions were more favorable. As the roots grew deeper into the soil

Table 5. Influence of N source and metalaxyl application on green leaf N and NO_3-N when sampled 5 weeks after treatment and at topping. Southern Piedmont field experiment, 1984.

Treatment		5 Weeks		Topping	
		N	NO_3-N	N	NO_3-N
		$g\ kg^{-1}$			
N Source[‡]					
$NaNO_3$		43.1 b[†]	4.1 a	32.9 a	0.9 a
NH_4NO_3		45.6 a	4.6 a	30.7 ab	0.5 a
$(NH_4)_2SO_4$		45.5 b	3.9 a	30.0 b	0.4 a
Metalaxyl Application[§]					
Transplant	Layby				
-- $kg\ ha^{-1}$ --					
0.00	0.00	44.9 ab	4.2 a	30.8 b	0.6 a
1.12	0.00	44.0 b	4.2 a	32.5 a	0.6 a
1.12	0.56	45.3 a	4.0 a	30.6 b	0.5 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

Table 6. Effect of N source and metalaxyl application on green leaf N and NO_3-N when sampled at four and six weeks after treatment, and at topping. Southern Piedmont field experiment, 1985.

Treatment		4 Weeks		6 weeks		Topping	
		N	NO_3-N	N	NO_3-N	N	NO_3-N
		g kg^{-1}					
N Source[‡]							
$NaNO_3$		49.5 a[†]	3.8 b	52.6 a	3.9 a	42.3 a	2.6 a
NH_4NO_3		50.1 a	4.1 a	53.4 a	3.8 a	40.6 a	2.0 b
$(NH_4)_2SO_4$		49.8 a	3.7 b	51.9 a	3.5 b	37.5 b	1.9 b
Urea		49.5 a	3.6 b	52.5 a	3.5 b	41.1 a	2.1 b
Metalaxyl Application[§]							
Transplant	Layby						
-- kg ha^{-1} --							
0.00	0.00	48.6 b	3.7 a	52.5 abc	3.8 a	38.5 a	2.3 a
0.56	0.00	50.9 a	3.8 a	53.7 a	3.6 a	37.9 a	2.4 a
1.12	0.00	50.6 a	3.8 a	51.9 c	3.7 a	40.4 a	1.6 a
0.56	0.56	49.2 ab	4.0 a	52.6 abc	3.7 a	41.2 a	2.3 a
1.12	0.56	49.9 ab	3.6 a	53.3 ab	3.7 a	42.9 a	2.0 a
Nitrapyrin		49.1 ab	3.8 a	52.1 bc	3.7 a	41.3 a	2.3 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

after layby, the NO_3^- became available to the plant.

Metalaxyl did not affect green leaf N content on the first sampling date in 1984 (Table 5). At topping, in 1984, the transplant only treatment was higher than the control and transplant plus layby treatment. The N content for all treatments at topping was below the sufficiency range established at bloom for Virginia conditions by Donahue and Hawkins (1979), but was in the range in 1985 (Table 6).

All metalaxyl treatments exhibited N values slightly higher than the control on the first sampling date in 1985 (Table 6). Two weeks later, the 1.12 kg ha^{-1} at transplanting treatment was lowest, although none of the metalaxyl treatments were significantly different from the control. A metalaxyl effect on N was not observed at topping. Although direct evidence is lacking, early season increases in N observed in metalaxyl plots may be the result of increased root system growth. Slight leaching of NO_3^- may have moved the N to greater depths where it could be utilized by the larger root system. Also, early season inhibition of nitrification may have reduced N leaching in the metalaxyl treatments.

Green leaf NO_3-N was not affected by metalaxyl on any of the sampling dates in 1984 or 1985. The small differences in soil NO_3-N in 1984 (Table 3) would not be expected to cause a significant change in tissue NO_3-N (Table 5). The tissue NO_3-N tended to be lower in the 1.12 kg ha^{-1} + 0.56 kg ha^{-1} treatment five weeks after treatment in 1984 (Table 5). Soil NO_3-N was significantly lower in this treatment (Table 3).

In general, N source did not significantly affect yield in 1984 (Table 7) or 1985 (Table 8), with the exception of lower yields produced by urea in 1985. There are numerous reports in the literature (McEvoy, 1946; Skogley and McCants, 1963; McCants and Woltz, 1967; Gous et al., 1971) that yield may be reduced by NH_4-N containing fertilizers; however, there are also reports (Jones, 1981; Williams and Miner, 1982) that yield is not affected by N source. Although not significantly different, yield was lower for the $(NH_4)_2SO_4$ treatment in both years. Lack of statistical significance may be the result of reduced main plot treatment sensitivity inherent in the split-plot experimental design (Little and Hills, 1978).

Reduced yields obtained from urea have been documented when dry soil conditions occur early in the growing season (Williams and Miner, 1982). Soil moisture was low at the time of urea application and remained low until the second week after treatment (Appendix Table 4). There is no evidence that significant amounts of N were lost by volatilization, since soil NH_4-N and NO_3-N levels were similar to the other N sources at four and six weeks after treatment. Green leaf N, measured at layby, two weeks after layby, and topping (Table 6), and cured leaf N (Table 8) were not lowered by urea. The yield reduction can not be explained by NH_4^+ uptake, since the

Table 7. Effect of N source and metalaxyl on yield, quality, and cured leaf chemical composition. Southern Piedmont field experiment, 1984.

Treatment		Yield	Quality Index	Mature and Ripe Grades	Total Alkaloids	Reducing Sugar	N
		Kg ha^{-1}		%	-----------%-----------		g kg^{-1}
N Source[‡]							
N_aNO_3		3623 a[†]	61.6 a	46.2 a	1.69 a	18.8 a	16.7 a
NH_4NO_3		3670 a	59.5 ab	45.7 a	1.62 a	18.3 a	16.2 a
$(NH_4)_2SO_4$		3512 a	58.0 b	40.1 b	1.70 a	20.1 a	16.5 a
Metalaxyl Application[§]							
Transplant	Layby						
-------Kg ha^{-1}-------							
0.00	0.00	3661 a	61.9 a	46.0 a	1.70 a	18.5 a	16.8 a
1.12	0.00	3584 a	58.0 b	41.5 b	1.66 a	19.7 a	16.1 b
1.12	0.56	3560 a	59.3 b	44.4 ab	1.65 a	19.0 a	16.5 ab

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

Table 8. Effect of N source and metalaxyl on yield, quality, and cured leaf chemical composition. Southern Piedmont field experiment, 1985.

Treatment		Yield	Quality Index	Mature and Ripe Grades	Total Alkaloids	Reducing Sugar	N
		Kg ha^{-1}		%	------------%-----------		g kg^{-1}
N Source[‡]							
$NaNO_3$		3097 a[†]	75.5 a	97.2 a	2.66 a	18.4 a	18.9 a
NH_4NO_3		3081 a	74.0 ab	93.2 a	2.67 a	19.2 a	18.5 a
$(NH_4)_2SO_4$		3044 ab	72.8 b	90.6 a	2.65 a	18.6 a	18.4 a
Urea		2848 b	73.9 ab	95.4 a	2.83 a	18.8 a	18.9 a
Metalaxyl Application[§]							
Transplant	Layby						
-------Kg ha^{-1}-------							
0.00	0.00	2984 a	73.7 a	94.3 a	2.73 a	18.4 a	18.8 a
0.56	0.00	2993 a	73.9 a	96.4 a	2.67 a	19.5 a	18.3 a
1.12	0.00	3058 a	74.1 a	91.7 a	2.64 a	19.0 a	18.4 a
0.56	0.56	2989 a	73.9 a	94.0 a	2.75 a	18.4 a	19.0 a
1.12	0.56	3044 a	74.1 a	92.8 a	2.73 a	18.6 a	18.8 a
Nitrapyrin		3039 a	73.6 a	95.5 a	2.71 a	18.7 a	18.8 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl applications.

[§] Means averaged over N sources.

$(NH_4)_2SO_4$ treatment produced both higher soil NH_4-N concentrations and higher yields.

Nitrogen source significantly affected quality index in both years of the study (Table 7 and 8). As in previous reports (McCants and Woltz, 1967; Hawks and Collins, 1983), quality was decreased by NH_4^+ containing fertilizers. In both years, highest quality was obtained by fertilizing with $NaNO_3$, although the differences from NH_4NO_3 and urea were not significant in either year. Quality with $(NH_4)_2SO_4$ was significantly lower than $NaNO_3$ in both years.

Associated with the quality decrease in 1984 was a decrease in the percentage of the cured leaf receiving a mature or ripe grade (Table 7). The 1985 growing season favored rapid maturity, and no effect due to N sources was observed (Table 8).

The yield and quality performance of NH_4NO_3 was acceptable under the conditions at Southern Piedmont Agricultural Experiment Station. The small increase in quality obtained from $NaNO_3$ would not offset the increased cost of the $NaNO_3$. Gous et al. (1971) and Jones (1981) found NH_4NO_3 to be an acceptable N source for flue-cured tobacco production at several locations in Virginia. Although $(NH_4)_2SO_4$ did not reduce yield, decreases in quality and maturity limit its use as an N source for flue-cured tobacco.

Metalaxyl did not affect yield in either year of the study (Table 7 and 8). Since the N source treatments did not affect yield, a response to the small change in the ratio of NO_3-N to

NH_4-N caused by metalaxyl was not expected. Metalaxyl would be expected to increase yields in the presence of blue mold or black shank. No symptoms of either disease were observed during the experiment, making disease control related yield increases unlikely.

Quality index was reduced at all combinations of metalaxyl in 1984 (Table 7). Associated with the decrease in quality was a decrease in the percent mature and ripe grades, which represents the occurrence of L, F, and V color symbols in the leaf grades, with a subsequent increase in KL, KF, and KM color symbols. The L, F, and V symbols indicate tobacco which is mature or ripe at harvest, while the KL, KF, and KM symbols indicate immature or unripe tobacco (USDA, 1984).

Metalaxyl did not affect quality index or the percentage of mature and ripe grades in 1985 (Table 8). Combined analysis of variance of the treatments common to the 1984 and 1985 study indicated a significant (P=0.10) year X metalaxyl interaction for quality index.

Growing conditions differed in 1984 and 1985. Rainfall and temperature in 1985 was more favorable than in 1984 for production of high quality tobacco. The average quality index across all treatments was 59.7 and 74.1 in 1984 and 1985, respectively. Similarly, the percentage of tobacco receiving mature or ripe grades increased from 44.1 percent in 1984 to 94.1 percent in 1985.

In addition to increased quality potential in 1985, conditions for nitrification were more favorable. Rapid nitrification increases the quality potential (McCants and Woltz, 1967) and prevents nitrification inhibition by weak nitrification inhibitors (Parr, 1974). Metalaxyl inhibited nitrification in 1984 but did not in 1985. The lack of an effect on quantities of NO_3^- available to the plant combined with the excellent growing season prevented a quality response to metalaxyl in 1985.

Nitrogen source did not affect cured leaf content of total alkaloids, reducing sugars, or N in 1984 (Table 7) or 1985 (Table 8). These observations support the work of McCants and Woltz (1963). Nitrate determinations on cured leaf samples indicated that NO_3-N content of the tissue was below the detection limit (5 mg l^{-1} in the extract) of the autoanalyzer. In light of these low levels, NO_3-N was not reported on the cured leaf samples.

Metalaxyl did not affect total alkaloids or reducing sugars in 1984 (Table 7) or 1985 (Table 8). Cured leaf N was decreased by metalaxyl applied at transplanting only compared to the control in 1984 (Table 7). Cured leaf N was not affected by metalaxyl in 1985 (Table 8).

These studies indicate that N source has an effect on the growth and development of flue-cured tobacco in non-fumigated soils. Much of the early work (McCants et al., 1959; McCants and Woltz, 1963; McCants and Woltz, 1967) with N source was conducted in soils where the nitrifying potential was reduced by fumigation. Under these conditions, delayed nitrification increased early season NH_4^+ uptake, with subsequent reductions in yield and quality. Later studies were conducted either without fumigants (Jones, 1981) or with non-fumigated treatments (Gous, et al. 1971; Williams and Miner, 1982). In these studies, a favorable response to NO_3-N was recorded at certain locations in certain years.

A favorable quality response to NO_3-N was obtained in both years of this experiment. $NaNO_3$ and NH_4NO_3 consistently produced higher quality leaf than $(NH_4)_2SO_4$, even under the rapidly nitrifying soil conditions of 1985. Urea produced quality equal to $NaNO_3$ and NH_4NO_3 in 1985, but yield was lower. Application of $(NH_4)_2SO_4$ causes a rapid decrease in soil pH, which limits nitrification. Perhaps the usefulness of $(NH_4)_2SO_4$ as on N source for flue-cured tobacco could be increased by application of lime in the fertilizer band to limit the decrease in pH.

Although N and NO_3-N content of green leaves was affected by N source, cured leaf content of N, NO_3-N, total alkaloids, and reducing sugars were not affected. Interactions of these factors contribute to smoke flavor and aroma (Tso, 1972), and often determine the usability of the leaf by tobacco manufacturers. The lack of a significant effect of N source on these parameters is important in that chemical quality of the leaf is not changed.

The decrease in maturity at harvest by NH_4-N is of concern to both the grower and manufacturer. Immature leaf is of lower quality, and is worth less at the market. In addition, immature tobacco has poor burning characteristics (Tso, 1972).

Metalaxyl has the ability to reduce the concentration of NO_3-N in the soil under conditions where nitrification is limited by environmental factors, such as in 1984. The most significant implication of this is decreased quality and profits for the grower in some growing seasons. Metalaxyl is a very weak and erratic nitrification inhibitor, capable of reducing soil NO_3^- concentration only under conditions which are not conducive for rapid nitrification. Given the destructive actions of blue mold and black shank, the increased quality where metalaxyl is not used is far outweighed by the benefits of the fungicidal properties of metalaxyl.

When metalaxyl affects cured leaf N, the flavor characteristics of the leaf may be changed. Decreasing the total N to nicotine ratio to 1.0 or less improves the smoke quality of the leaf (Tso, 1972). In this respect, transplant applications of metalaxyl improved the chemical quality of the leaf.

On-Farm Experiment

Error variances across locations were tested by Bartlett's test (Little and Hills, 1978) and were found to be homogeneous for all parameters measured. Analyses of variance combined over the three locations did not indicate that the location X treatment interaction was significant for any of the parameters. Since the interaction was not significant, only the combined data are presented here. The treatment means for each individual location are presented in the appendix.

Metalaxyl at the 3.36 kg ha^{-1} rate significantly increased the soil NH_4-N concentration as compared to the control at two weeks after treatment (Table 9). The 0.56 and 1.12 kg ha^{-1} treatments were intermediate between the control and 3.36 kg ha^{-1} treatments. Metalaxyl did not significantly affect NO_3-N or pH at the two week sampling, although NO_3-N for the control is slightly lower. Metalaxyl did not affect NO_3-N, NH_4-N, and pH at the four and six weeks after treatment sampling date.

Green leaf NO_3-N was not affected by metalaxyl at any sampling date (Table 10). N in the green leaf samples was significantly lower in the control treatment at layby (Table 10). An effect of metalaxyl on N was not observed at the other sampling dates.

The increase in soil NH_4-N in metalaxyl plots after two weeks indicates short-term inhibition of nitrification occurred. The decrease in NH_4-N in the control should have been accompanied by an increase in NO_3-N. It is likely that the NO_3-N, which is more easily leached than NH_4-N (Terry and McCants, 1973), was leached out of the sampling zone before the first sampling date. Soil moisture was low at all locations at the time of treatment, and all locations received 2.5 cm of irrigation within one week of

Table 9. Effect of metalaxyl on form of N in soil and pH when sampled two, four, and six weeks after treatment. Three on-farm field experiments, 1985.

Metalaxyl		2 Weeks			4 Weeks			6 Weeks		
		N Form			N Form			N Form		
Transplant	Layby	NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH
-- kg ha^{-1} --		-- mg kg^{-1} --			--- mg kg^{-1} ---			--- mg kg^{-1} ---		
0.00	0.00	15.2 a[†]	12.3 b	5.0 a	46.2 a	15.6 a	5.0 a	37.2 a	5.2 a	5.3 a
0.56	0.00	28.4 a	13.0 b	5.2 a	46.7 a	8.5 a	5.3 a	32.5 a	4.8 a	5.4 a
1.12	0.00	30.1 a	29.2 ab	5.2 a	34.8 a	8.9 a	5.3 a	46.0 a	4.7 a	5.4 a
3.36	0.00	33.1 a	33.1 a	5.2 a	54.1 a	15.8 a	5.2 a	35.5 a	8.5 a	5.4 a
0.56	0.56	26.6 a	27.4 ab	5.1 a	48.4 a	11.3 a	5.1 a	37.0 a	6.5 a	5.4 a
1.12	0.56	34.3 a	24.0 ab	5.1 a	40.1 a	8.0 a	5.2 a	30.1 a	6.1 a	5.4 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

Table 10. Effect of N source and metalaxyl on N in green leaf tissue when sampled at layby, two weeks after layby and at topping. Averaged over three on-farm field experiments, 1985.

Metalaxyl Application		Four Weeks		Six Weeks		Topping	
Transplant	Layby	N	NO_3-N	N	NO_3-N	N	NO_3-N
-- kg ha^{-1} --		g kg^{-1}					
0.00	0.00	51.0 c[†]	4.6 a	59.5 a	4.8 a	41.1 a	1.2 a
0.56	0.00	58.4 ab	5.4 a	61.5 a	4.5 a	42.2 a	1.2 a
1.12	0.00	56.1 ab	4.4 a	60.7 a	4.7 a	41.9 a	1.7 a
3.36	0.00	55.1 b	4.5 a	59.5 a	4.3 a	42.2 a	1.6 a
0.56	0.56	58.5 a	4.9 a	59.0 a	4.6 a	42.1 a	1.4 a
1.12	0.56	57.3 ab	4.9 a	62.4 a	5.0 a	42.6 a	1.9 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

treatment. The irrigation probably caused NO_3^- leaching. Since the control nitrified rapidly, NO_3^- leaching was greater than in the metalaxyl treatments. Downward movement of NO_3^- in the control is also evidenced by the lower green leaf N at layby. The NO_3^- was reached by the roots between layby and topping, as indicated by N concentrations at topping.

Metalaxyl did not affect cured leaf yield (Table 11). Blue mold did not occur at any location. Black shank occurred on scattered plants in the control treatments at the Hawthorne location, but less than one percent of the plants were affected. As in the Southern Piedmont experiment, disease incidence was not a factor affecting yield.

Although not significant, quality index tended to be lower in the treatments receiving metalaxyl (Table 11). Raper and McCants (1970) reported that delays in N availability may reduce tobacco quality. The delay in N uptake observed in the control treatment may have lowered the quality sufficiently to prevent detectable improvement in quality from increased nitrification. Maturity at harvest was not affected (Table 11).

Cured leaf content of total alkaloids, reducing sugars and N (Table 11) was not affected by metalaxyl. The lack of effect on total alkaloids and reducing sugars by metalaxyl is consistent with the data from the Southern Piedmont field experiment.

This experiment shows metalaxyl inhibits nitrification under field conditions in conventionally produced flue-cured tobacco.

Table 11. Effect of metalaxyl on yield, quality, and cured leaf chemical composition. Three on-farm field experiments, 1985.

Metalaxyl Application							
Transplant	Layby	Yield	Quality Index	Mature and Ripe Grades	Total Alkaloids	Reducing Sugar	N
-------Kg ha^{-1}-------		Kg ha^{-1}		%	-----------%----------		g kg^{-1}
0.00	0.00	2946 a[†]	54.1 a	63.3 a	3.25 a	12.6 a	24.2 a
0.56	0.00	3154 a	52.7 a	62.6 a	3.49 a	11.0 a	26.0 a
1.12	0.00	3071 a	52.1 a	65.2 a	3.37 a	12.1 a	25.2 a
3.36	0.00	3004 a	50.8 a	59.8 a	3.39 a	11.0 a	25.3 a
0.56	0.56	3005 a	51.7 a	59.3 a	3.36 a	10.7 a	25.9 a
1.12	0.56	3063 a	52.2 a	61.8 a	3.37 a	12.1 a	25.4 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

The inhibition is very short-term and relatively milder than the inhibition reported for nitrapyrin (Touchton and Boswell, 1980). The only effect of metalaxyl on early N uptake appeared to be related to reduced leaching of NO_3^-. Metalaxyl was not detrimental to quality in this experiment, although slight reductions occurred. The components of chemical leaf quality that were measured were not affected by metalaxyl.

Greenhouse Experiments

Error variances were homogeneous for all parameters measured in both greenhouse experiments. All repetition X main effect interactions were not significant at the 10 percent level of probability. The data from the two runs were combined for analysis of variance. Metalaxyl did not interact with N source (Metalaxyl Rate Experiment) or pH (pH Adjustment Experiment). Therefore, N source, pH, and metalaxyl means were averaged over the other main effects.

Soil N concentrations were low at the termination of the greenhouse experiments. Presumably, rapid plant uptake depleted the N in the small volume of soil used in the experiments. Tissue analyses for N and NO_3-N were precluded by the small dry weight yield obtained.

Metalaxyl Rate Experiment

Nitrogen source affected soil NO_3-N, NH_4-N and pH (Table 12). The $NaNO_3$ treatments resulted in the highest NO_3-N concentration and pH, and the lowest NH_4-N concentrations. Ammonium sulfate resulted in the lowest NO_3-N concentrations and pH and the highest NH_4-N concentrations. Ammonium nitrate resulted in NO_3-N, NH_4-N and pH values intermediate between $NaNO_3$ and $(NH_4)_2SO_4$. Plant dry weight was not affected by N source.

As in the 1984 and 1985 Southern Piedmont field experiments, the effects of N source on soil NO_3-N, NH_4-N and pH are related to the nitrifying characteristics of various N sources. The high pH associated with $NaNO_3$ is the result of lack of substrate for nitrification, which produces H^+ (Schmidt, 1982). The only nitrification taking place in the $NaNO_3$ treatment is nitrification of residual soil NH_4^+. The NH_4NO_3 and $(NH_4)_2SO_4$ treatments contained NH_4^+, so nitrification and thus H+ production took place. The NH_4^+ from these two sources did not nitrify fully during the course of the experiment, causing the differences in soil NH_4-N and NO_3-N.

Metalaxyl reduced the soil NO_3-N concentrations at the 1.12 kg ha^{-1} rate, but did not reduce NO_3-N at 2.24 or 3.36 kg ha^{-1} (Table 12). Soil NH_4-N, pH and plant dry weight were not affected by metalaxyl (Table 12).

These data confirm field observations that metalaxyl

Table 12. Effect of N source and metalaxyl on soil N form, pH and plant dry weight. Metalaxyl rate greenhouse experiment, 1985.

Treatment	N Form NO_3^-	 NH_4^+	pH	Plant Dry Weight
	---- mg kg^{-1}	----		g
N Source[‡]				
$NaNO_3$	2.8 a[†]	1.1 c	6.5 a	0.65 a
NH_4NO_3	2.2 b	2.0 b	6.1 b	0.71 a
$(NH_4)_2SO_4$	1.9 b	3.8 a	6.0 c	0.65 a
Metalaxyl[§] kg ha^{-1}				
0.00	2.6 a	2.2 a	6.2 a	0.68 a
1.12	2.0 b	2.5 a	6.2 a	0.66 a
2.24	2.2 ab	2.1 a	6.3 a	0.68 a
3.36	2.5 a	2.5 a	6.2 a	0.65 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over metalaxyl treatments.

[§] Means averaged over N sources.

performs erratically as a nitrification inhibitor. The inhibition was strongest at the lowest metalaxyl rate, and weakest at the highest rate. In contrast with the on-farm experiment, inhibition did not seem to increase under increased metalaxyl rate. The high pH (6.2 to 6.3) at the end of the experiment may have limited inhibition of nitrification by metalaxyl as in the 1985 Southern Piedmont field experiment.

Weak nitrification inhibitors would not be expected to consistently inhibit nitrification under these pH conditions (Dubey, 1969; Dubey and Rodriquez, 1969; Parr, 1974). Also, disproportionate plant uptake of NO_3^- and NH_4^+ may have prevented finding significant differences at NO_3^- and NH_4^+ in the soil. The NH_4^+ toxicity symptoms and dry weight decreases reported by Skogely and McCants (1963) were not observed for any of the test plants.

pH Adjustment Experiment

Increasing the soil pH from 5.7 to 6.0 (measured at the termination of the experiment) significantly increased production of NO_3-N (Table 13). Soil NH_4-N and plant dry weight were not affected by the pH variable.

The great sensitivity of nitrification of pH in the 5.0 to 6.0 range reported by Follett, et al. (1978) is supported by this experiment. The lack of an effect on NH_4-N may be the result of organic matter oxidation by the NaOH used to provide the pH increase. Soil organic matter was observed to be blackened in

Table 13. Effect of pH adjustment and metalaxyl on soil N form, pH and plant dry weight. PH adjustment greenhouse experiment, 1985.

Treatment		N Form		pH	Plant Dry Weight
		NO_3^-	NH_4^+		
		---- kg ha^{-1} ----			g
pH[‡]					
adjusted		2.7 a[†]	3.1 a	6.0 a	0.71 a
not adjusted		2.0 b	2.7 a	5.7 b	0.69 a
Inhibitor[§]					
Chemical	Rate kg ha^{-1}				
None	0.00	2.5 a	3.2 ab	5.7 b	0.73 a
Metalaxyl	0.56	2.4 a	2.2 c	5.8 b	0.70 a
Metalaxyl	1.12	2.3 a	2.5 bc	5.8 b	0.65 a
Nitrapyrin	6.72	2.4 a	3.7 a	6.0 a	0.73 a

[†] Means followed by the same letter are not significantly different at the 10 percent level of probability by Duncan's Multiple Range test.

[‡] Means averaged over inhibitor treatments.

[§] Means averaged over pH treatments.

the NaOH treatments.

Metalaxyl did not affect soil NO_3-N or pH. The 0.56 kg ha^{-1} rate of metalaxyl unexpectedly decreased soil NH_4-N concentration below that of the control. Nitrapyrin increased soil pH, but did not affect NO_3-N or NH_4-N concentrations. Plant dry weight was not affected by any of the inhibitor treatments.

Neither metalaxyl nor nitrapyrin was effective as a nitrification inhibitor in this experiment. As in the metalaxyl rate experiment, high pH may have caused the reduced effectiveness of the inhibitors. Nitrapyrin did appear to slightly inhibit nitrification, as indicated by increased soil pH. As in the metalaxyl rate experiment, plant uptake of NO_3^- and NH_4^+ may have made detecting any soil N differences impossible.

SUMMARY

Field, greenhouse, and laboratory experiments were conducted to determine the effects of metalaxyl on nitrification of several N sources, and to determine if inhibition of nitrification by metalaxyl caused any subsequent effects on the yield, quality, and certain chemical constituents of flue-cured tobacco. Field experiments were conducted at the Virginia Tech Southern Piedmont Agricultural Experiment Station, Blackstone, Va. and at three farm locations in the Southern Piedmont region of Virginia.

Consistent with earlier investigations, increasing the percentage of NO_3-N in the preplant fertilizer increased the quality of flue-cured tobacco; however, the yield increase reported in the literature for NO_3-N was not observed. Soil NO_3-N and NH_4-N measurements indicated that nitrification of applied NH_4^+ was not completed until at least six weeks after application. The nitrification of the NH_4^+ seemed to be limited by depression of the soil pH caused by H^+ production by the nitrifying bacteria. Wet soil conditions at Southern Piedmont in 1984 may have also reduced the nitrification rate.

Nitrogen source affected green leaf N and NO_3-N. In 1984, increased NH_4-N in the fertilizer increased early N uptake, but resulted in lower N at topping. Most likely, the increase in N early in the season was caused by NO_3^- movement to just below the root zone. This NO_3^- was recovered by the plant later in the season as the roots expanded downward. Nitrogen source did not affect N in 1985. Green leaf concentrations of NO_3-N were increased by heavy NO_3^- uptake in the $NaNO_3$ and NH_4NO_3 plots in both years. Cured leaf concentrations of N, total alkaloids, and reducing sugars were not affected by N source.

Metalaxyl has the potential to inhibit nitrification at rates commonly applied to flue-cured tobacco. The laboratory data indicate significant reductions in NH_4^+ oxidizer populations at rates as low as 0.56 kg ha^{-1}, which is the minimum rate recommended for flue-cured tobacco. Inhibition of nitrification by metalaxyl was weak and erratic, and only occurred in situations where nitrification was limited by environmental factors. Wet soil conditions and low pH seem to promote inhibition of nitrification by metalaxyl. In situations of high nitrifying potential, such as in the greenhouse experiments and at Southern Piedmont in 1985, metalaxyl did not inhibit nitrification. The exact mode of action of metalaxyl as a nitrification inhibitor was not determined, but inhibition of the NH_2OH oxidation pathway seemed to be involved.

Metalaxyl did not affect green leaf NO_3-N at any test location. In the farm tests, metalaxyl increased green leaf N early in the season. This increase can be attributed to the nitrification inhibitory action of metalaxyl reducing early season NO_3-N leaching. The influence of metalaxyl on green leaf N at Southern Piedmont are less clear, but the metalaxyl treated plants were generally higher in N.

In the instances where metalaxyl inhibited nitrification, cured leaf quality was reduced. Quality reductions were not detected at Southern Piedmont in 1985. Since the effects on quality are slight and do not always occur, the advantages of metalaxyl's fungicidal action far outweigh the negative effects on quality.

Metalaxyl did not affect cured leaf concentration of total alkaloids and reducing sugars in any of the field experiments. Cured leaf N was slightly decreased at Southern Piedmont by the transplant only applications in both years, although the decrease was not significantly different from the control in 1985. Metalaxyl did not affect cured leaf N at any of the farm locations.

This investigation suggests that the negative effect of metalaxyl on nitrification, and thus quality, may be reduced or eliminated by adopting cultural practices which promote high soil nitrification potential. Since metalaxyl does not inhibit nitrification at high pH, liming to maintain a root zone pH of above 5.5 during the early part of the growing season may reduce the effect of metalaxyl on nitrification. In addition, avoiding excessive early season irrigation may also reduce nitrification inhibition by metalaxyl. Further research is needed to determine if use of these cultural practices does indeed reduce the potential detrimental effects of metalaxyl while maintaining acceptable disease protection.

LITERATURE CITED

Alexander, M. 1982. Most probable number method for microbial populations. In A.L. Page (ed.) Methods of soil analysis, part 2 - Chemical and microbiological properties. Agronomy 9:815-820.

Arnett, J.D. (ed.) 1983. Tobacco disease loss estimates. Tobacco disease loss evaluation committee report, 1983.

Bashore, S.L. and F.H. Lander. 1981. Use of selected N-(2,6 Dimethyl phenyl)-Alanine methyl ester compounds as ammonium nitrification compounds. U.S. Patent 4 294 604. Date issued: 13 Oct.

Bremner, J.M. and L.A. Douglas. 1971. Inhibition of urease activity in soils. Soil Biol. Biochem. 3:297-307.

Bundy, L.G. and J.M. Bremner. 1973. Inhibition of nitrification in soils. Soil Sci. Soc. Am. Proc. 37:396-398.

Bundy, L.G. and J.M. Bremner. 1974. Effects of nitrification inhibitors on transformations of urea nitrogen in soils. Soil Biol. Biochem. 6:369-376.

Ciba-Geigy Corporation. 1984. Metalaxyl fungicide. Technical Bull. CGA 630-00100-B.

Davis, R.E. 1976. A combined automated procedure for the determination of reducing sugars and nicotine alkaloids using a new reducing sugar method. Tob. Sci. 20:139-144.

Donohue, S.J. and G.W. Hawkins. 1979. Sampling instructions and nutrient sufficiency ranges for plant tissue analysis. Va. Coop. Ext. Serv. MA-211.

Dubey, H.D. 1969. Effect of picloram, diuron, ametryne, and prometryne on nitrification and ammonification, and their degradation in tropical soils. Soil Sci. Soc. Am. Proc. 33:893-896.

Dubey, H.D. and R.L. Rodriguez. 1970. Effect of dyrene and maneb on nitrification and ammonification, and their degradation in tropical soils. Soil Sci. Soc. Am. Proc. 34:435-439.

Elliot, J.M., C.F. Marks, and C.M. Tu. 1974. Effects of the nematicides DD and mocap on soil nitrogen, soil microflora, populations of Pratylenchus penetrans, and flue-cured tobacco. Can. J. Plant Sci. 54:801-809.

Elliot, J.M., C.F. Marks, and C.M. Tu. 1977. Effects of certain nematicides on soil nitrogen, soil nitrifiers, and populations of Pratylenchus penetrans in flue-cured tobacco. Can. J. Plant Sci. 57:143-154.

Eno, C.F. and W.G. Blue. 1957. The comparative rate of nitrification of anhydrous ammonia, urea, and ammonium sulfate in sandy soils. Soil Sci. Soc. Am. Proc. 21:392-396.

Follett, R.H., L.S. Murphy, and R.L. Donahue. 1981. Fertilizers and soil amendments. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Goring, C.A.I. and D.A. Laskowski. 1982. The effects of pesticides on nitrogen transformations in soils. In F.J. Stevenson (ed.) Nitrogen in agricultural soils. Agromony 22:689-720.

Gous, P.J., T.R. Terrill, and W. Kroontje. 1971. Effects of soil fumigation and the form of nitrogen on the growth, yield, and value of tobacco grown on two soil types. Agron. J. 63:221-224.

Grant, W.D. and P.E. Long. 1981. Environmental microbiology. Halsted Press, New York, NY.

Hargrove, W.L. and D.E. Kissell. 1979. Ammonia volatilization from surface applications of urea in the field and laboratory. Soil Sci. Soc. Am. J. 43:359-363.

Hauck, R.D. 1980. Mode of action of nitrification inhibitors. p. 19-32. In M. Stelly (ed.) Nitrification inhibitors-potentials and limitations. Spec. Pub. 38. American Society of Agronomy, Madison, WI.

Hawks, S.N., Jr. and W.K. Collins. 1983. Principles of flue-cured tobacco production. S.N. Hawks, Jr. and W.K. Collins. N.C. State Univ., Raleigh, NC.

Horwitz, W. (ed.). 1980. Official methods of analysis, 13th ed. AOAC, Washington, DC.

Hutcheson, T.B., W.G. Woltz, and S.B. McCaleb. 1954. Potassium-Sodium interrelationships I: Effects of various rates and combinations of K and Na on yield, value, and physical and chemical properties of flue-cured tobacco grown in field and greenhouse. Soil Sci. 87:28-36.

Jones, J.L. 1981. Summary of agronomic on-farm test demonstrations in flue-cured tobacco. Va. Coop. Ext. Sev. SP-38.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - Inorganic forms. In A.L. Page (ed.) Methods of soil analysis, part 2 - Chemical and microbiological properties. Agronomy 9:643-693.

Kucharek, T., E.B. Whitty, S. Johnson, and J. Taylor. 1983. Effects of application techniques with metalaxyl fungicide on black shank of tobacco. Proc. of Soil and Crop Sci. Soc. of Fla. 42:136-139.

Little, T.M. and F.J. Hills. 1978. Agricultural experimentation - Design and analysis. John Wiley and Sons, New York, NY.

Markus, D.K., J.P. McKinnon, and A.F. Buccafuri. 1985. Automated analysis of nitrite, nitrate, and ammonium nitrogen in soils. Soil Sci. Soc. Am. J. 49:1208-1215.

McCants, C.B., E.O. Skogley, and W.G. Woltz. 1959. Influence of certain soil fumigation treatments on the response of tobacco to ammonium and nitrate forms of nitrogen. Soil Sci. Soc. Am. Proc. 466-469.

McCants, C.B. and W.G. Woltz. 1963. Relationships between forms of nitrogen and yield and quality components of flue-cured tobacco. Proc. Third World Tobacco Scientific Congress, Salisbury, 1963.

McCants, C.B. and W.G. Woltz. 1967. Growth and mineral nutrition of tobacco. Adv. Agron. 19:211-265.

McEvoy, E.T. 1946. Response of burley tobacco varieties to ionic forms of nitrogen. Sci. Agric. 26:640-653.

Miner, G.S., J. P. Lilly, and D.L. Terry. 1978. Nitrogen release characteristics of isobutylidene diurea and its effectiveness as a source of N for flue-cured tobacco. Agron. J. 70:434-438.

Nye, P.H. 1984. pH changes and phosphate solubilization near roots: an example of coupled diffusion processes. p. 89-100. In S.A. Barber and D.R. Bouldin (ed.) Roots, nutrient and water influx and plant growth. Spec. Pub. 49. American Society of Agronomy, Madison, WI.

Parr, J.F. 1974. Effects of pesticides on microorganisms in soil and water. p. 315-337. In W.D. Guenzi (ed.) Pesticides in soil and water. Soil Science Society of Americia, Madison, WI.

Petrie, S.E. and T.L. Jackson. 1984. Effects of fertilization on soil solution pH and manganese concentration. Soil Sci. Soc. Am. J. 48:315-318.

Raper, C.D. and C.B. McCants. 1970. Performance of flue-cured tobacco on selected soil nitrogen availability regimes. Tob. Sci. 14:22-25.

Reneau, R.B., J.L. Ragland, and W.O. Atkinson. 1968. Effects of ammonium nitrate and the growth of burley tobacco plants on soil pH. Tob. Sci. 12:50-53.

Rhoads, F.M. 1972. A comparison of ammonium and nitrate nitrogen for cigar wrapper tobacco. Agron. J. 64:209-210.

Sarathchandra, S.U. 1978. Nitrification activities of some New Zealand soils and the effect of some clay types on nitrification. New Zealand J. Agric. Res. 21:615-621.

Schmidt, E.L. 1982. Nitrification in soil. In F.J. Stevenson, et al. (ed.) Nitrogen in agricultural soils. Agronomy 22:253-283.

Schmidt, E.L. and L.W. Belser. 1982. Nitrifying bacteria. In A.L. Page, et al. (ed.) Methods of soil analysis, part 2 - chemical and microbiological properties. Agronomy 9:1027-1042.

Sims, J.L., M. Casey, and K.L. Wells. 1984. Fertilizer placement effects on growth, yield, and chemical composition of burley tobacco. Agron. J. 76:183-188.

Skogley, E.O. and C.B. McCants. 1963. Ammonium and chloride influences on growth characteristics of flue-cured tobacco. Soil Sci. Soc. Am. Proc. 27:391-394.

Terry, D.L. and C.B. McCants. 1973. Estimating the leaching of ammonium and nitrate nitrogen, potassium, and magnesium in certain North Carolina soils. North Carolina Agric. Exp. Sta. Tech. Bul. 221.

Tisdale, S.L., W.G. Woltz, and J.M. Carr. 1952. Sources of nitrogen for flue-cured tobacco production. In S.L. Tisdale, et al. (ed.) Sources of nitrogen in crop production. North Carolina Ag. Exp. Stn. Tech. Bull. 96.

Touchton, J.T. and F.C. Boswell. 1980. Performance of nitrification inhibitors in the southeast. p. 63-74. In M. Stelly (ed.) Nitrification inhibitors - potentials and limitations. Spec. Pub. 38. American Society of Agronomy, Madison, WI.

Tso, T.C. 1972. Physiology and biochemistry of tobacco plants. Dowden, Hutchenson, and Ross, Stroudsburg, PA.

United States Department of Agriculture. 1984. Official standard grades for flue-cured tobacco. USDA Agric. Marketing Service, Tobacco division.

Wernsman, E.A. and E.L. Price. 1975. North Carolina grade index for flue-cured tobacco. Tob. Sci. 19:111.

Williams, L.M. and G.S. Miner. 1982. Effect of urea on yield and quality of flue-cured tobacco. Agron. J. 74:457-462.

Appendix Table 1 - Chemical and physical properties of N-sources[†].

N-source	Chemical formula	% N	Acidity[‡] equivalent	Alkalinity[§] equivalent
Sodium Nitrate	$NaNO_3$	16	-	1.8
Ammonium Nitrate	NH_4NO_3	34	0.9	-
Ammonium Sulfate	$(NH_4)_2SO_4$	20	5.4	-
Urea	$NH_2 \cdot CO \cdot NH_2$	45	1.8	-

[†] compiled from Follet, et al., 1981.

[‡] kg pure ($CaCO_3$ required to neutralize residual acidity from 1 kg N.

[§] kg pure $CaCO_3$ equivalent added to soil per 1 kg N.

Appendix Table 2. Initial soil test results. Southern Piedmont Agricultural Experiment Station, 1984 and 1985.

Year	P	K	Ca	Mg	NO_3-N	pH
	---------------	---------------	mg kg^{-1}	---------------	---------------	
1984	16	15	150	23	9	5.4
1985	10	31	192	36	3	6.1

Appendix Table 3. Initial soil test results for off-station locations, 1985.

Location	P	K	Ca	Mg	NO_3-N	$NH4^+N$	pH
	------	------	------mg	kg^{-1}------	------	------	
May	60	82	264	39	8	1	6.0
Hawthorne	60	82	324	35	16	2	5.6
Smith	47	36	228	36	6	2	5.9

Appendix Table 4. Weekly rainfall at Southern Piedmont Agricultural Experiment Station, Blackstone, Virginia, 1984 and 1985.

1984		1985	
Week	Precip.	Week	Precip.
	--cm--		--cm--
Apr 29 - May 5	0.13	Apr 28 - May 4	1.91
May 6 - 12	2.41	May 5 - 11	0.00
13 - 19	0.00	12 - 18	1.91
20 - 26	1.27	19 - 25	5.20
27 - Jun 2	7.49	26 - Jun 1	0.00
Jun 3 - 9	0.00	Jun 2 - 8	2.54
10 - 16	1.52	9 - 15	1.27
17 - 23	0.13	10 - 22	0.89
24 - 30	5.21	23 - 29	0.76
Jul 1 - 7	1.65	30 - Jul 6	2.54
8 - 14	7.62	Jul 7 - 13	5.72
15 - 21	1.52	14 - 20	0.00
22 - 28	9.02	21 - 27	7.11
28 - Aug 4	0.76	28 - Aug 3	1.40
Aug 5 - 11	5.46	Aug 4 - 10	0.00
12 - 18	0.89	11 - 17	0.89
19 - 25	0.00	18 - 24	7.11
26 - Sep 1	2.54	25 - 31	1.02
Sep 2 - 8	1.78	Sep 1 - 7	0.00
9 - 15	0.13	8 - 14	0.00

Appendix Table 5. Effect of metalaxyl on form of N in soil and pH when sampled 2, 4, and 6 weeks after treatment. Three on-farm locations, 1985.

Metalaxyl		2 Weeks			4 Weeks			6 Weeks		
Trans-		N Form			N Form			N Form		
plant	Layby	NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH	NO_3^-	NH_4^+	pH
-- kg ha^{-1} --										
May Location										
0.00	0.00	7.3	8.0	5.3	38.3	14.0	5.4	21.3	3.3	5.6
0.56	0.00	13.0	14.7	5.5	23.3	6.3	5.3	15.0	3.0	5.6
1.12	0.00	10.7	15.0	5.4	23.3	4.3	5.4	20.4	2.8	5.5
3.36	0.00	25.0	19.3	5.2	30.0	24.0	5.2	21.8	3.0	5.3
0.56	0.56	10.0	33.3	5.1	23.3	5.0	5.3	13.4	3.1	5.5
1.12	0.56	32.0	22.3	5.5	40.0	5.3	5.5	19.3	3.1	5.6
Hawthorne Location										
0.00	0.00	21.7	14.3	4.8	37.7	3.3	4.8	50.1	2.8	5.1
0.56	0.00	33.7	11.0	5.0	49.0	1.3	5.2	46.4	2.1	5.4
1.12	0.00	39.3	23.7	5.0	28.7	1.0	5.3	60.9	2.6	5.3
3.36	0.00	30.3	10.0	5.2	58.0	3.7	5.2	42.4	1.9	5.5
0.56	0.56	30.3	10.3	5.1	52.3	2.0	5.1	48.7	2.3	5.3
1.12	0.56	34.3	13.0	5.0	18.0	1.7	5.0	32.1	2.1	5.4
Smith Location										
0.00	0.00	16.7	14.7	5.0	62.6	29.5	4.9	40.3	19.8	5.2
0.56	0.00	38.7	13.3	5.0	67.8	17.7	5.2	36.2	9.3	5.3
1.12	0.00	40.3	49.0	5.1	52.5	21.5	5.1	56.6	21.7	5.5
3.36	0.00	42.0	70.0	5.2	73.9	19.6	5.3	42.3	20.7	5.4
0.56	0.56	39.3	38.7	5.1	69.4	27.0	4.9	48.8	14.0	5.4
1.12	0.56	36.7	36.7	4.9	62.4	17.0	5.0	38.9	13.2	5.2

Appendix Table 6. Effect of metalaxyl on yield and quality for each of three on-farm locations, 1985.

Metalaxyl				
Transplant	Layby	Yield	Quality Index	Mature and Ripe Grades
-- kg ha^{-1} --		kg ha^{-1}		%
			<u>May Location</u>	
0.00	0.00	3152	43.3	24.0
0.56	0.00	3089	45.3	39.7
1.12	0.00	3185	42.7	32.0
3.36	0.00	3106	43.1	27.7
0.56	0.56	3020	41.7	25.7
1.12	0.56	3211	41.3	21.7
			<u>Hawthorne Location</u>	
0.00	0.00	2977	61.7	97.3
0.56	0.00	3285	57.0	87.3
1.12	0.00	3114	60.3	97.0
3.36	0.00	3097	54.3	83.3
0.56	0.56	3272	55.0	77.7
1.12	0.56	3127	58.9	93.0
			<u>Smith Location</u>	
0.00	0.00	2708	57.3	68.7
0.56	0.00	3088	55.7	60.7
1.12	0.00	2915	53.3	66.7
3.36	0.00	2808	56.7	68.3
0.56	0.56	2725	58.3	70.7
1.12	0.56	2852	56.7	74.7

Appendix Table 7. Effect of metalaxyl on cured leaf chemical composition on each of three on-farm locations, 1985.

Metalaxyl Trans-plant	Metalaxyl Layby	Total Alkaloids	Reducing Sugar	TKN
-- kg ha^{-1} --		-------- % --------		g kg^{-1}
		May Location		
0.00	0.00	2.67	16.4	19.5
0.56	0.00	3.01	15.2	21.9
1.12	0.00	3.05	15.8	21.9
3.36	0.00	3.02	14.0	22.2
0.56	0.56	3.25	13.0	24.9
1.12	0.56	2.89	16.5	20.5
		Hawthorne Location		
0.00	0.00	3.72	11.7	23.7
0.56	0.00	3.92	8.0	26.3
1.12	0.00	3.82	10.2	25.9
3.36	0.00	3.84	8.8	26.3
0.56	0.56	3.65	10.5	24.5
1.12	0.56	3.91	9.9	26.1
		Smith Location		
0.00	0.00	3.36	9.8	29.4
0.56	0.00	3.53	10.0	29.8
1.12	0.00	3.25	10.3	27.7
3.36	0.00	3.31	10.3	27.4
0.56	0.56	3.16	8.6	28.1
1.12	0.56	3.30	9.8	29.6

Appendix Table 8. Effect of N source and metalaxyl on N in green leaf tissue when sampled at layby, two weeks after layby and at topping by location. Three on-farm locations, 1985.

Metalaxyl							
Transplant	Layby	Layby		Layby ± 2 Weeks		Topping	
		TKN	NO_3-N	TKN	NO_3-N	TKN	NO_3-N
-- kg ha^{-1} --		-------------------- g kg^{-1} --------------------					
		May Location					
0.00	0.00	62.5	3.4	55.8	4.4	44.7	0.8
0.56	0.00	62.9	4.7	62.2	3.8	43.8	0.6
1.12	0.00	64.9	3.4	59.4	4.0	43.5	1.0
3.36	0.00	65.9	3.1	58.1	3.3	45.1	0.8
0.56	0.56	61.2	4.2	54.5	4.7	46.0	1.0
1.12	0.56	65.4	4.0	65.1	4.7	45.5	1.4
		Hawthorne Location					
0.00	0.00	62.5	4.7	65.3	5.2	38.4	1.4
0.56	0.00	62.9	5.9	64.3	5.4	43.3	1.3
1.12	0.00	64.9	4.3	66.7	4.5	42.9	2.0
3.36	0.00	65.9	4.9	67.8	5.3	41.4	2.3
0.56	0.56	61.1	5.4	64.7	4.7	40.7	1.2
1.12	0.56	65.4	5.3	66.2	5.7	39.8	1.3
		Smith Location					
0.00	0.00	55.6	5.7	57.5	4.7	40.1	1.5
0.56	0.00	57.9	5.7	57.9	4.2	39.4	1.9
1.12	0.00	56.9	5.6	56.1	5.7	39.4	2.0
3.36	0.00	55.6	5.5	52.7	4.3	40.2	1.6
0.56	0.56	57.9	5.1	57.7	4.4	39.6	2.1
1.12	0.56	57.9	5.4	56.0	4.6	42.3	3.1