Nitrification Inhibition by Metalaxyl as Influenced by pH, Temperature, and Moisture Content in Three Soils

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(ABSTRACT)

Metalaxyl, [N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester], is used extensively in tobacco (Nicotiana tabacum L.) production for prevention of black shank (Phytophthora parasitica Dast. var. nicotianae), blue mold (Peronospora tabacina Adam), and damping-off (Pythium spp.). Metalaxyl is also patented as a nitrification inhibitor, although not marketed for that purpose. Proper maturity and ripening of flue-cured tobacco depends on an adequate supply of N through the time of removal of the inflorescence, with a declining supply of N from that point. Use of a chemical which might prolong the availability of N in tobacco could delay maturity and reduce the quality of the cured leaf. These studies were conducted to determine whether metalaxyl might inhibit nitrification under a broad range of soil physical and environmental conditions prevalent in the tobacco producing areas of Virginia. The influence of soil type, soil pH, soil temperature,

and soil moisture on inhibition of nitrification by metalaxyl (1 mg kg^{-1}) were investigated in three soils used extensively for tobacco production. Soils used in the study were Cecil sandy loam (clayey, kaolinitic, thermic Typic Hapludult), Appomattox fine sandy loam (clayey, mixed, thermic Typic Kandhapludult), and Mattoponi sandy loam (clayey, mixed, thermic Typic Hapludult). Metalaxyl did not inhibit nitrification under any of the conditions studied. However, NO_2^- accumulation with metalaxyl was sometimes greater than the control, especially at high pH (7.0) in the Cecil and Appomattox soils, and at 10 and 20°C. Nitrite and NO_3^- accumulations from four rates of metalaxyl (1, 5, 25, and 125 mg kg^{-1}) were compared with those of an untreated control and a nitrapyrin standard over a seven week soil incubation period in further studies using the same soils and adjusted pH levels. Significant NO_2^- accumulation occurred during the first week after treatment at high pH in all soil types, with 5, 25, and 125 mg kg^{-1} metalaxyl. Only the 125 mg kg^{-1} metalaxyl treatment caused NO_2^- accumulation at the high pH in all soils beyond the second week after treatment, with the peak occurring in most cases between weeks three and four. Nitrate accumulation proceeded normally in all soil types and pH levels except with treatments of 25 and 125 mg kg^{-1}. Nitrate accumulations with 25 mg kg^{-1} were similar to those for nitrapyrin. The 125 mg kg^{-1} rate was consistent in causing near total inhibition of NO_3^- accumulation at all pH levels in all soils. Nitrate accumulation tended to be lower at lower soil pH levels compared to the highest

pH for all soils. Little difference in nitrification due to soil appears to be evident. Use of metalaxyl at recommended rates of 0.25 to 1.5 mg kg^{-1} would not be expected to inhibit nitrification.

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CHAPTER I

INTRODUCTION

Metalaxyl has been widely used by Virginia tobacco (Nicotiana tabacum L.) producers as a systemic fungicide for control of blue mold (Peronospora tabacina Adam) and black shank (Phytophthora parasitica Dast var. Nicotianae) since 1981. As much as 60 to 80 percent of flue-cured tobacco and 80 to 90 percent of burley tobacco in Virginia was treated with metalaxyl in 1982 (Arnett, 1982; C. S. Johnson, 1988, personal communication).

Metalaxyl can inhibit the activity of nitrifying bacteria in the soil in addition to fungicidal activity (Bashore and Lander, 1981 ; Ciba-Geigy, 1984). Olin Corporation holds a U. S. patent on metalaxyl as a nitrification inhibitor for soil or fertilizer application (Bashore and Lander, 1981). Personnel from Ciba-Geigy (1984) reported 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).

Nitrification is a biological process in soils by which chemoautotrophic bacteria oxidize NH_4^+ to NO_3^-. This is a two step process in which NH_4^+ is first oxidized to NO_2^- by nitrifying

bacteria, usually Nitrosomonas, but often one of five genera of soil bacteria. The oxidizing bacteria responsible for the conversion of NO_2^- to NO_3^- are considered to be of the genus Nitrobacter (Schmidt and Belser, 1982).

The nitrification process may be inhibited by chemicals which interfere with the oxidation of NH_4^+ to NO_2^- or with the oxidation of NO_2^- to NO_3^- (Hauck, 1980). Numerous pesticides commonly used in the production of tobacco can inhibit nitrification (Hauck, 1982; Goring and Laskowski, 1982). Many soil fumigants used to control disease and insect pests can inhibit nitrification by 25% or more, at or below recommended rates. In order for most other pesticides to cause 25% inhibition, their rates must be elevated to at least the highest recommended rate (Goring and Laskowski, 1982).

The main factors which limit nitrification in soil are substrate NH_4^+, O_2, CO_2, pH, and temperature. Nitrifying bacteria in cold and wet soils are essentially inactive. The optimum temperature for nitrification appears to vary widely among U. S. soils, generally from 20° to 40° C. Soil O_2 is supplied by the liquid phase of the soil system. Depletion of soil O_2 is favored by (i) high soil moisture content, in which water fills soil pores and restricts recharge of O_2 from the gaseous phase; (ii) high soil temperatures, which reduce the solubility of O_2 and increase O_2 demand by heterotrophic microorganisms; and (iii) high oxidizable organic

matter, which also increases heterotrophic O_2 demand (Schmidt, 1982).

Nitrification proceeds at soil pH levels far below the limits observed for nitrifying bacteria in pure culture. A lower limit for nitrification of pH 4.0 is suggested by most previous observations, with obvious nitrification occurring in the pH 4 to 6 range, and pH independent nitrification proceeding in the range 6 to 8 (Schmidt, 1982). Several chemicals are commercially marketed to inhibit nitrification. Of these chemicals, nitrapyrin has been the most widely researched and used in the southeastern United States (Touchton and Boswell, 1978).

Some of the same physical, chemical, and biological soil factors that affect nitrification also affect the efficacy of nitrapyrin as a nitrification inhibitor. Nitrapyrin is less effective on light than heavy textured soils. Nitrapyrin is also more effective at a soil temperature of 15° C than at a soil temperature of 30° C. Nitrification was inhibited by 87 to 89 percent at a soil temperature of 15° C after 28 d of incubation (Bundy and Bremner, 1978).

Commercial nitrification inhibitors provide a means of increasing the efficiency of N fertilizer use by maintaining the N in the NH_4^+ form for a longer period of time. The potential for denitrification and leaching are reduced by use of a nitrification inhibitor (Hawkins, 1976). While inhibition of nitrification may be

beneficial for crops which may continue to utilize N throughout the season, in tobacco production prolonged retention of the N as NH_4^+ is a disadvantage. Tobacco producers prefer to control the N available to the plant such that N becomes depleted in the soil shortly after topping. This allows tobacco to begin the ripening process shortly after the desired leaf number is produced.

The presence of a large quantity of NH_4^+ ions in the soil can be detrimental to the growth and quality of flue-cured tobacco (McCants and Woltz, 1967). Normally, tobacco fertilizer contains no more than 50 percent of the total N in the NH_4^+ form. Use of metalaxyl has been shown to reduce the quality of flue-cured tobacco under environmental conditions which might be unfavorable for nitrifying bacteria (Rideout, 1986). The objective of this study was to determine the influence of soil moisture, temperature, pH and metalaxyl on nitrification in three soil types commonly used for tobacco production in Virginia.

CHAPTER II

LITERATURE REVIEW

Nitrification

Nitrification is the process by which NH_4^+ is oxidized to NO_2^- and/or NO_3^- by nitrifying bacteria. The nitrification process is carried out in two steps: one group of nitrifying bacteria oxidizes NH_4^+ to NO_2^-; another group oxidizes the NO_2^- to NO_3^-. Nitrite, the intermediate in the process, rarely accumulates to detectable levels in soil. All known nitrifying bacteria are capable of strictly autotrophic growth in the laboratory on inorganic nutrients. These are known as chemoautotrophic nitrifying bacteria because they obtain energy by oxidizing NH_4^+ via several intermediates to NO_3^-. This process of nitrification goes on in any oxic terrestrial or aquatic environment (Grant and Long, 1981). Energy is derived from oxidation of the appropriate specific substrate, either NH_4^+ or NO_2^-, and all C requirements can be met from assimilation of CO_2 (Schmidt and Belser, 1982).

Chemoautotrophic nitrifying bacteria are grouped in the family Nitrobacteriaceae. Nitrosomonas europeae and Nitrobacter winogradskyi are the nitrifiers most commonly isolated from soils,

sewage and the freshwater environment (Grant and Long, 1981). Ammonium oxidizing bacteria carry out a six-electron oxidation of NH_4^+ to NO_3^-. Recent work has shown that genera other than Nitrosomonas may contribute more than once thought to the oxidation of NH_4^+ (Schmidt, 1982). Ammonium oxidizers commonly isolated from soil include Nitrosomonas europeae, Nitrosospira briensis, Nitrosolobus multiformis, Nitrosovibrio tenuis, and Nitrosococcus nitrosus (Grant and Long, 1981). Nitrosomonas, however carries the primary responsibility for oxidation of NH_4^+ in soil.

The oxidation of NH_4^+ to NO_2^- by Nitrosomonas europeae proceeds according to the following equation:

$$NH_4^+ + 1.5O_2 \xrightarrow{6e^-} NO_2^- + H_2O + 2H^+;\ F\ -65\ kcal.$$

This is a three step reaction which proceeds with the addition of two electrons at each step. Ammonium is oxidized to NH_2OH in an energy requiring step by the enzyme oxygenase. Hydroxylamine is then oxidized to NO_2- by the enzyme hydroxylamine oxidoreductase in a two step process in the presence of a suitable electron acceptor. The intermediate product is thought to be NOH, which is very unstable. ATP is created as a result of the oxidation of NH_2OH.

NO_2^- oxidizing bacteria oxidize NO_2^- to NO_3^- by a two-electron oxidation process. The oxidation of NO_2^- to NO_3^- by Nitrobacter proceeds as follows:

$$NO_2^- + 0.5O_2 \xrightarrow{2e^-} NO_3^-;\ F\ -17.8\ kcal.$$

Only a two electron shift in oxidation state from +3 to +5 is required for the oxidation of NO_2^-. The reaction is carried out by a NO_2^- oxidase system, with electrons carried to O_2 via cytochromes, leading to the generation of ATP. The energy yield of this reaction is low and an extensive cytomembrane system with a large number of NO_2^- oxidizing sites is present. Carbon dioxide is fixed by the Calvin cycle.

The biochemistry of nitrification remains relatively obscure. During the six-electron oxidation catalyzed by the NH_4^+ oxidizers, hydroxylamine (NH_2OH) is almost certainly an intermediate and traces of nitric oxide (NO) and nitrous oxide (N_2O) are evolved. However, N_2O and NO do not seem to be utilized by NH_4 oxidizers, and are probably produced as a byproduct of some as yet undescribed intermediate, perhaps nitroxyl (HNO). The process may proceed in the following manner NH_4^+ -> NH_2OH -> HNO -> NO_2^-. The process is poorly understood and the existence of HNO, which is very unstable, has never been proven. The first enzyme involved in the oxidation of NH_4^+ to NH_2OH is an oxygenase which has a requirement for reducing power. Oxygen is directly incorporated into the substrate, and the reaction may be analogous to the oxygenase-catalyzed oxidation of CH_4 by methylotrophs. N-Serve operates by inhibition of this particular enzymic step. A subsequent cytochrome-linked

oxidation of NH_2OH to NO_2 occurs without detectable intermediates. The oxidation of NO_2^- is much better understood and appears to be a cytochrome-linked, single-step hydrolytic oxidation (Grant and Long, 1981).

Nitrification is much more rapid at high than at low pH and oxygen tensions. Little or no nitrification takes place in dry or extremely wet soils. Optimum moisture is between one-half and two-thirds moisture-holding capacity. From 2 to 35°C, rates may increase as much as 50 to 100-fold (Goring and Laskowski, 1982).

Factors Influencing Nitrification

The main factors which limit nitrification in soil are substrate NH_4^+, O_2, CO_2, pH, and temperature (Schmidt, 1982). Gilmour (1984) found nitrification to obey zero-order kinetics, with the nitrification rate constant being a function of temperature, moisture, and pH. The absolute zero-order rate was also a function of initial NH_4^+ concentration. The rate constant increased linearly as temperature increased over the range of 16 to 28°C. The relative influence of an inhibitor on the rate constant decreased with increasing temperature. A linear decline in rate was observed as soil moisture decreased from 0.20 to 0.12 g g^{-1} (-40 to -140 J kg^{-1}). This decline was greater in untreated soil than in soil amended

with etradiazole. Nitrification increased linearly with soil pH over the range 4.9 to 7.2. Inhibition by etradiazole decreased as organic C content increased above 13 g kg^{-1} with the extent of the decrease being modified by the amount of clay in the soil. As etradiazole rate increased, inhibition was enhanced by a constant amount, suggesting that the relationships described above remained in effect (Gilmour, 1984).

Justice and Smith (1962) conducted soil incubation experiments to consider some of the interactions involved in nitrification of NH_4^+ fertilizers at various levels of soil moisture, soil temperature, and applied N. Initiation of nitrification was delayed at moisture tensions of 700, 1000, and 1500 J kg^{-1} when incubated at favorable temperatures. No NO_3^- was produced after 8 weeks at 11 500 J kg^{-1} moisture tension. No NO_3^- was produced at 2°C, but NH_4^+ was converted to NO_2- after 10 weeks of incubation. Nitrite accumulated when conditions were made unfavorable for nitrification (higher additions of NH_4^+, lowered temperatures, lowered moistures, and increased temperature above 25°C).

Carbon Dioxide

Carbon dioxide is a product of both aerobic and anaerobic metabolism and can have a direct influence on microbial growth. The

sole C source for chemoautotrophs and photoautotrophs is CO_2. Heterotrophs also require CO_2 in addition to organic C (Alexander, 1977). While CO_2 is rarely limiting in soil due to continual evolution from plant roots and from organic matter decomposition, excess CO_2 may be toxic to some soil fungi (Alexander, 1977).

More rapid nitrification was found to occur in the range 0.6 to 2.9% CO_2 than at higher and lower CO_2 concentrations (Clark,1968). Measurements of CO_2 in the atmospheres of field soils and animal feedlots have given values ranging from ambient air (0.3% CO_2) to as high as 100% CO_2, although values below 20% CO_2 are more commonly observed. However, these are bulk soil atmospheres and microsite CO_2 concentrations will probably be higher than those in large voids (Keeney et al., 1985).

Keeney et al. (1985) conducted a study of the effects of different CO_2 concentrations on nitrification, denitrification and N_2O production and reduction associated with a silt loam soil. Ten g dry wt samples of soil were added to a 125 ml Erlenmeyer flasks fitted with rubber septa and incubated at 25°C for 4 and 7 d following the addition of no additional N, 95 ug N g^{-1} as NO_3^-, or 90 ug N g^{-1} as NH_4^+. Carbon dioxide levels did not increase markedly during incubation. Nitrous oxide production tended to increase with CO_2 concentration in the unamended and NO_3^- amended soils to 50% CO_2, increased dramatically at 73% CO_2, and declined to nil at 100% CO_2 with the nonamended soil. Results indicate that a CO_2 increase in

soils, particularly arable soils containing high amounts of nitrifiable N, could result in increased N_2O production during nitrification in the unusual instances when CO_2 comprises >50% of the soil atmosphere.

Miller and Johnson (1964) found that CO_2 increased during the first d of incubation, from a minimum in air-dry soil to a maximum at soil moisture tensions of from 50 to 15 J kg^{-1}, and then decreased with further increases in soil moisture. However, there was little difference in the CO_2 evolved in the soil moisture tension range from zero to 15 J kg^{-1} at the end of the 14 d incubation period.

Moisture

The O_2 and HCO_3^- needed for nitrification are provided by the liquid phase of the soil system. Therefore, the amount and composition of the soil water influence nitrification. Depletion of O_2 in the soil water is favored by: (i) high soil moisture content, which fills soil pores and restricts recharge of O_2 from the gaseous phase; (ii) high soil temperatures, which reduce the solubility of O_2 and increase O_2 demand by heterotrophic microorganisms; and (iii) oxidizable organic matter, which also increases heterotrophic O_2 demand. Optimum moisture is between one-half and two-thirds water-

holding capacity (Goring and Laskowski, 1982). Moderately high soil moisture levels (pF 1.0-2.0) enhance nitrification in most soils, so long as aeration is adequate. Nitrification in dried soils varies with soil texture and those properties affecting osmotic pressure (Schmidt,1982).

Bacterial metabolism effectively ceases in soils at moisture potential values below -500 J kg^{-1} as a consequence of reduced mobility. However, most common soil bacteria have the ability to grow in osmotically stressed media at much lower solute potential values (Grant and Long, 1981). Nitrification was found to be minimal (1.2 to 1.3 ug N g^{-1}) at -1500 J kg^{-1} soil moisture potential for three Alberta soils, agreeing with results of Miller and Johnson (1964) and Dubey (1968). Maximum nitrification rates for three Alberta soils at -33 J kg^{-1} agreed with the findings of Justice and Smith (1962). Nitrification did not occur at 0 J kg^{-1} in the Alberta soils (Malhi and McGill, 1982). Miller and Johnson (1964) had similar results with soils at 0 J kg^{-1}. However, Dubey (1968) reported appreciable nitrification at 0 J kg^{-1}.

Sabey (1969) reported on a study involving two limed silt loam loessial derived, Illinois soils to which a non-limiting source of NH_4^+ was added and in which samples were subjected to 0, 10, 33, 100, 500, and 1500 J kg^{-1} of soil moisture. Nitrate accumulation was greatest at 10 J kg^{-1} tension for both soils in the study, with nitrate accumulation decreasing as soil moisture tension increased

or decreased. Others have also found NO_3^- accumulation to be highest at 10 J kg^{-1} tension, with less accumulation at higher and lower tensions (Parker and Larson, 1962; Miller and Johnson, 1964; and Sabey and Johnson, 1971).

pH

Nitrification proceeds in soil at pH levels far below the limits observed for nitrifying bacteria in pure culture. Most observations indicate an arbitrary lower limit for nitrification of pH 4.0, obvious nitrification in the pH 4 to 6 range, and pH-independent nitrification in the range 6 to 8. A soil sample having a pH of 5.5 as based on the overall measurement may contain microhabitats of pH 7.0 or higher and microhabitats of pH 4.5 or lower causing considerable variability in the nitrification rates of the various microhabitats (Schmidt, 1982).

Aleem and Alexander (1960) demonstrated that NH_4^+ inhibits the activity of Nitrobacter agilis as the culture media becomes more alkaline. Soil conditions that favor the occurrence of NH_3, such as high pH and low CEC, restrict nitrification because of the toxicity of free NH_3. The NO_2^- oxidation stage is most susceptible to inhibition by molecular NH_3, so that applications of urea or

anhydrous NH_3 to calcareous soils have resulted in NO_2^- accumulation (Schmidt, 1982).

Nitrite usually occurs only in trace amounts (Cornfield, 1952; Morrill and Dawson, 1967; Weber and Gainey, 1962; and Schmidt, 1982). Exceptions to this statement occur under unusual circumstances of high applications of nitrogenous fertilizers or with high soil pH (Duisberg and Buehrer, 1960; and Morrill and Dawson, 1967). Such conditions lead to NO_2^- accumulations in some soils but not in others. Nitrite oxidizers are apparently more efficient than NH_4^+ oxidizers in acid soils, indicating that Nitrosomonas spp. limit nitrification rates in acid soils (Dancer et al., 1973). Nitrite accumulation is of concern since this anion is mobile and highly toxic to many microorganisms. Nitrate is mobile also, but is less reactive than NO_2^- and commonly accumulates in soils in the range of a few to several hundred ug g^{-1} (Schmidt, 1982).

Stojanovic and Alexander (1958), using kinetics of the oxidation process, showed that there was no effect of NH_4^+ concentration on the rate of NH_4^+ oxidation to nitrites. High NH_4^+ concentrations, however, caused accumulation of nitrites in soils of high pH by virtue of the specific effects on the Nitrobacter-catalyzed oxidation of nitrite. Justice and Smith (1962) supported the premise that the second step of nitrification is also more sensitive to lowered temperature.

Nitrates are produced in some soils at pH values of 4.5. Nitrification has also been reported in pasture soil with a pH of 3.8 (Tisdale and Nelson, 1975). Naftel (1931) observed that nitrification varied considerably in different soils at similar pH levels and that nitrification increased with the percentage base and Ca saturation.

Gilmour (1984) found that nitrification increased linearly with soil pH over the range of 4.9 to 7.2 in a Crowley silt loam from Arkansas. Weier and Gilliam (1986) also found a significant linear correlation between soil pH value and nitrification rate for six Histosols of the Tidewater region of the Atlantic Coastal Plain in North Carolina. Each soil pH was adjusted with reagent grade $CaCO_3$ to provide four pH levels ranging from 3.7 to 7.6 for all soils. Three of the six soils had appreciable nitrification of added $(NH_4)_2SO_4$ occurring at pH 4.5 and 4.6 during 3-weeks incubation. However, nitrification was very slow in three soils with similarly low pH values before adjustment. Variation in soil organic matter could explain some of the differences in nitrification rates of the six soils (Weier and Gilliam, 1986).

Roseberg et al. (1986) found that nitrification rate increased significantly in silty clay loam and silt loam Oregon soils as soil pH increased (5.5 to 6.7 and 4.9 to 6.2). Dancer et al. (1973) studied the effect of soil pH on nitrification rates in soil samples ranging in pH from 4.7 to 6.6 taken from an established lime and N

fertility experiment. Nitrification rates and production of NO_3^- were similar for soil pH values of 5.3, 6.0, 6.3, and 6.6 with a different pattern at pH 4.7.

Morrill and Dawson (1967) used percolation techniques to study nitrification in 116 soils of the U.S. ranging in pH from 4.4 to 8.8. Four different patterns of nitrification were related directly to soil pH and are as follows: (i) NH_4^+ oxidized rapidly to NO_2^- which accumulated for extended periods of time before being oxidized to NO_3^-; associated with alkaline soils (pH 6.9 to 7.9), (ii) NH_4^+ and NO_2^- are oxidized rapidly to NO_3^-; associated with soils of pH values 5.0 to 6.4, (iii) NH_4^+ oxidized slowly to NO_3^- without NO_2^- appearing, and (iv) accumulation of NH_4^+ with very little oxidation to NO_2^- and NO_3^-. Patterns iii and iv were observed in acid soils ($pH \leq 5.4$). Morrill and Dawson measured the population of Nitrosomonas spp. and Nitrobacter spp. in soil cultures and concluded that numbers and proliferation rates were responsible for the four different nitrification patterns. Type patterns ii and iii could be changed to type pattern i by adding $CaCO_3$.

Martin et al. (1942) had earlier reported nitrification patterns similar to those of Morrill and Dawson (1967). Martin et al. studied nitrification in six typical Arizona desert soils and suggested a pH threshold (maximum) value of 7.7 for nitrification to approach completion. Appreciable amounts of nitrite accumulated for up to two weeks during these studies, depending upon the magnitude

of the pH above the threshold. Nitrite also accumulated in considerable amounts in well-aerated soils under favorable conditions of temperature and moisture, but decreased to trace quantities almost immediately after NO_3^- began to form (Martin et al., 1942).

Soil Texture

Soil texture directly influences a number of factors which control nitrification. Coarse-textured soils which are highly permeable and better drained warm-up quicker in the spring. These soils provide more suitable temperatures for nitrification than heavier textured soils. Generally, coarse-textured soils have lower cation exchange capacities, resulting in low retention of substrate NH_4^+ for nitrification. Some heavier textured soils with higher clay content may trap NH_4^+, also limiting availability to nitrifying bacteria (Touchton and Boswell, 1980).

In certain clay mineral soils, added K appears to interfere with nitrification of NH_4^+ by blocking the release of fixed NH_4^+. Laboratory studies with Wyoming bentonite, Grundite illite, and three vermiculites were conducted to determine the effect of added K on the release and subsequent nitrification of fixed NH_4^+ (Welch and Scott, 1960). Added K (100 mg kg^{-1}) did not block nitrification of

fixed NH_4^+ in the vermiculites. However, greater than 50% of the fixed NH_4^+ in the bentonite and the illite samples was available at even the 0 mg kg^{-1} level of added K. The percent of fixed NH_4^+ that was nitrified in the vermiculite and bentonite soils was decreased by increasing rates of added K (Welch and Scott, 1960).

Temperature

Temperature and redox are also important factors affecting nitrification. Nitrifiers are obligately aerobic. Nitrification does not occur in significant amounts at redox values lower than +200 mV, although NO_2^- oxidation is more sensitive than NH_4^+ oxidation. Nitrite tends to accumulate at low temperature (<6°C) (Grant and Long, 1981).

The optimum temperature for nitrification appears to vary widely among soils. Cold and wet soils are essentially inactive with respect to nitrification. Such limitations prevail until the soil warms to approximately 4 or 5°C (Grant and Long, 1981). The temperature-activity relationship of nitrification appears to depend on climate, with lower optimum temperature requirements for nitrification in cool climates as compared to warmer climatic regions (Frederick, 1956; Sabey et al., 1959; Justice and Smith, 1962; Mahendrappa et al., 1966; Thiagalingam and Kanehiro, 1973;

Myers, 1975; Malhi and McGill, 1982). Maximum nitrification rates were found at 20 to 25°C for a group of soils from the northwestern United States and at 30 to 40°C for soils of the southwestern United States. Forty degrees C was found to be the maximum temperature for nitrification in the midwestern United States, while a tropical Australian soil was found to support nitrification at temperatures up to 60°C (Schmidt, 1982). The optimum temperature for nitrification in three Alberta soils examined by Malhi and McGill (1982) was 20°C. Although the influence of temperature on actual maximum rates of nitrification differ greatly among soils, increasing soil temperature generally results in increased maximum nitrification rates (Anderson and Purvis, 1955; Frederick, 1956; Sabey et al., 1956; Sabey et al., 1959). Gilmour (1984) found that as temperature decreased there was an increase in the efficacy of etradiazole, a nitrification inhibiting fungicide, agreeing with work by McClung et al. (1983).

Nitrification Inhibitors

Added NH_4^+ will be nitrified within a few weeks (Broadbent and Tyler, 1957; Justice and Smith, 1962) in most agricultural soils. Under favorable conditions, the use of a nitrification inhibitor can spread this process out over 10 weeks or more (Goring, 1962a;

Rathsack, 1978). Nitrification inhibitors interfere with the conversion of NH_4^+ to NO_3^- and are sometimes used with NH_4^+-N fertilizers to reduce losses of NO_3^-. Loss of NO_3^- may occur during periods of high soil moisture where leaching and/or denitrification occur (Tanji, 1982). Loss of applied N may result in reduced yields or additional expense to replace lost N. High soil NO_3^- levels may promote eutrophication when leached into waterways and may be toxic (Grant and Long, 1981).

Numerous compounds have been identified as nitrification inhibitors in soils under laboratory conditions. A few of the more notable nitrification inhibitors are: dicyandiamide (DCD)(Cowie,1919), nitrapyrin (Goring, 1962b), potassium azide (Hughes and Welch, 1970), 4-amino-1,2,4-triazole (ATC)(Bundy and Bremner, 1973), 2-amino-4-chloro-6-methylpyrimidine (AM)(Hauck, 1972), sulfathiazole (Hauck, 1972), etridiazole (Turner and MacGregor, 1978) and NH_4^+ thiosulfate (Goos, 1985). Nitrapyrin and DCD, under the trade names N-Serve and Didin, respectively, are the only compounds currently marketed as nitrification inhibitors in the United States. Etridiazole (trade name Dwell) is EPA-approved, but is no longer marketed (Scharf and Alley, 1988).

Nitrapyrin has been the most widely tested nitrification inhibitor and appears to effectively inhibit nitrification in most field situations (Hughes and Welch, 1970; Cochran et al., 1973; Chancy and Kamprath, 1982) and from many sources of ammoniacal N

(Swezey and Turner, 1962; Liu et al., 1984). However, no yield response is observed in many cases where nitrification is inhibited (Boswell et al., 1976; Hendrickson et al., 1978a, 1978b; Townsend and McRae, 1980). In these cases, either the amount of fertilizer that was lost in the absence of an inhibitor was small, the reduction in loss due to the inhibitor was not large enough to measurably affect crop yield, or N availability was not a yield-limiting factor (Scharf and Alley, 1988). Nitrapyrin inhibits the cytochrome oxidase involved in ammonia oxidation by Nitrosomonas. The inhibition can be completely reversed by addition of Cu^{+2} (Hauck, 1980).

The efficacy of nitrification inhibitors has been related to inhibitor properties such as volatility and solubility, and soil properties such as organic matter content, temperature, moisture, pH, and texture (Goring, 1962a; Kai and Harada, 1969; Prasad, 1971; Bundy and Bremner, 1973; Keeney, 1980; Gilmour, 1984). Numerous laboratory and field studies have shown nitrapyrin to be less effective as soil pH increases (Goring, 1962a; Bundy and Bremner, 1973; Laskowski and Bidlack, 1977; and Hendrickson et al., 1978b). However, using a bioassay, Hendrickson and Keeney (1979) found that the nitrifier population appeared to be more susceptible to nitrapyrin as pH increased from 4.7 to 7.4. Guthrie and Bomke (1980) reported longer persistence of nitrapyrin and ATC (4-amino-1,2,4-triazole) in silt (86 d) than in loamy sand (63 d). Both

inhibitors were equally effective nitrification inhibitors when coated on urea prills and banded in the silt, but ATC was more effective than nitrapyrin in loamy sand, probably due to more rapid volatilization of nitrapyrin in the coarse-textured soil.

The effectiveness of N-Serve and other inhibitors of soil nitrification depends greatly upon the soil studied. These compounds are most effective with light-textured soils (Bundy and Bremner, 1973). Bundy and Bremner (1973) showed that the effects of 11 nitrification inhibitors were much greater in a sand-amended soil than the effects observed with the unamended soil (Harps soil, 24% sand, 34% clay). Goring (1962a) showed that the effectiveness of N-Serve varied markedly with the soil studied. As little as 0.2 mg kg^{-1} of N-Serve added to a sandy loam soil (65% sand) treated with 200 mg kg^{-1} of NH_4^+ caused more than 75% inhibition of nitrification when this soil was incubated at 21°C for 4 weeks. Mean relative rates of NO_3^- accumulation were found to be slightly lower for silty clay loam than for silt loam at soil moisture tensions ranging from 10 to 500 J kg^{-1} (Sabey and Johnson, 1971).

Inhibition of nitrification has other significant agronomic impacts beyond the ability to reduce the losses of N from leaching and denitrification. Nutrient uptake and plant composition may be altered. Plant disease may be enhanced or reduced. All of these agronomic effects need to be considered when pesticides are used at rates known to inhibit nitrification (Goring and Laskowski, 1982).

Pesticides as Nitrification Inhibitors

Fumigants and biocides (such as allyl alcohol, carbon disulfide, chloropicrin, dazomet, DBCP, DD, ethylene dibromide, metham-sodium, and methyl bromide) inhibit nitrification at or below recommended rates. The dithiocarbamate fungicides (ferbam, maneb, nabam, zineb, and ziram) appear to inhibit nitrification at rates near the upper end of recommended rates, probably because they release carbon disulfide when they decompose in soil. Carbon disulfide is particularly active at inhibiting nitrification at rates far below recommended rates (Goring and Laskowski, 1982).

McCants et al. (1959) found methyl bromide and DD significantly reduced nitrification. N-Serve, Telone, and Vorlex inhibited nitrification of $(NH_4)_2SO_4$ after four weeks at 28°C (Tu, 1973). Elliot et al. (1974) found that the levels of NH_4^+ in the soil tended to be higher, and NO_3^- lower, following treatment with DD. Treatment with the soil fumigants Telone, Telone C, and Vorlex resulted in higher NH_4^+ than in nontreated soil or soil treated with the non-fumigant nematicides oxamyl (Vydate) and triazophos (Elliot, et al. 1977). Elliot et al. (1974) indicated that ethoprop (Mocap) had no apparent effect on soil levels of NH_4^+ and only inconsistent effects on soil levels of NO_3^-. However, Tu (1973) did observe inhibition of nitrification by Mocap.

Some herbicides, such as barban, methabenzthiazuron, metobromuron, monolinuron, and simazine, appear to inhibit conversion of NO_2^- to NO_3^- at rates normally recommended for weed control. Much higher rates were required to inhibit conversion of NH_4^+ to NO_2^-. (Goring and Laskowski, 1982). Phenyl mercuric acetate has long been recognized as a highly active antimicrobial compound and has been shown by Bundy and Bremner (1973) to be a moderately active nitrification inhibitor. The fungicide etridiazole is a potent inhibitor similar in activity to nitrapyrin (Huber, 1980).

Nitrogen Source

Form of N utilized for the fertilization of flue-cured tobacco can be a major factor in the production of an acceptable crop from the standpoint of yield and acre value. Because of seasonal effects, NH_4^+ and NO_3^- should be included in preplant N applications to obtain highest yields of good quality tobacco (Gous et al., 1971).

A number of crops, including tomatoes (Lycopersicon esculentum Mill.), potatoes (Solanum tuberosum L.), and tobacco (Nicotiana tabacum L.), are highly sensitive to excessive NH_4^+ nutrition (Goring and Laskowski, 1982). Ammonium toxicity in tobacco is characterized by leaf characteristics similar to symptoms of excess

chlorine (greener than normal color; thick, leathery textured, fleshy leaves with a pronounced upward cupping of the leaf margins). These characteristics are observed in field situations following fumigation with halogenated hydrocarbons and fertilization with NH_4^+ (McCants and Woltz, 1967).

Under normal cultural practices, essentially all the N absorbed by tobacco is in the NH_4^+ and/or the NO_3^- form. Nutrient uptake, N and carbohydrate metabolism, and growth of the tobacco plant are markedly different depending on the dominant form absorbed. Relative uptake of NH_4^+ and NO_3^- vary considerably depending on the composition and acidity of the solution and the stage of plant development (McCants and Woltz, 1967). Nitrate was utilized more effectively from an acid medium, with pH 5, while maximal growth occurred with NH_4^+ source supplied at pH 8 (McEvoy, 1957). Young tobacco seedlings (39 d after seeding) absorbed N primarily as NH_4^+. Older plants (70 d after seeding) absorbed more NO_3^- than NH_4^+ in experiments by Jackson and Volk (1966) (from McCants and Woltz, 1967).

The relative response of field-grown tobacco to the NH_4^+ and the NO_3^- form of N is also closely related to the extent to which nitrification occurs. Differences in response to NH_4^+ and NO_3^- applied under field conditions are small and generally non-significant when conditions are favorable for rapid nitrification. However, the value per acre was numerically higher in 13 out of 15

experiments with flue-cured tobacco where the fertilizer contained at least 50 percent of the N in the NO_3^- form and no fumigation treatments were included.

McCants et al. (1959) found that the yield and quality index of tobacco from DD and methyl bromide fumigation treatments increased with increasing percentage of the total N applied in the NO_3^- form. Response to NO_3^- relative to NH_4^+ was greater in fumigated soils since the fumigants used inhibited the nitrification process. Lower yields from NH_4^+ fertilizer sources may be attributed to soil conditions, including high acidity and low temperatures, which may reduce nitrification and favor the absorption of NH_4^+ and a reduction in growth (McCants and Woltz, 1967).

Tobacco plants absorb fewer cations when fertilized with NH_4^+ than with NO_3^-, while anion absorption is reduced by predominantly NO_3^-. Reduced cation absorption with NH_4^+ may result in deficiencies of K and Mg. Potassium deficiencies in the leaves may result from a decrease in the amount of K absorbed by the roots in an NH_4^+ containing media, as well as a reduction in the amount of K translocated in the plant (McCants and Woltz, 1967).

Traditional fertilization practices for tobacco have included obtaining a portion of the fertilizer N from natural organic materials such as oil seed meals, tankage, fish scrap, or urea-formaldehyde compounds. Use of these natural organic materials is based on the belief that the N from these materials is released at a

rate which will meet plant requirements but is slow enough that no major quantities of N are lost from the root zone by leaching. However, these materials have not been shown to give results superior to those of the standard inorganic sources of fertilizer N under either normal rainfall conditions or intensive leaching. Only 20 to 60% of the N applied in the form of natural organics or urea-formaldehyde compounds is transformed into forms that are available to the plant during the normal tobacco growing season. Conversion of N from the natural organic form to the inorganic form is usually quite rapid, with 85% or more of this conversion occurring within the first three weeks after the materials are incorporated into the soil. The rapidity of the conversion of NH_4^+ to NO_3^- limits the effectiveness of natural organics to reduce the probability of leaching losses (McCants and Woltz, 1967).

The maturity of all tobacco types is significantly influenced by the supply of available N in the latter stages of development. Proper maturity and ripening requires the rate of N absorption decrease rather rapidly during the latter portion of the growth period. The readily available supply of N in the soil should be essentially exhausted by the time the plant has attained maximal leaf area (McCants and Woltz, 1967).

Excessive N delayed the maturity of flue-cured tobacco, preventing normal development of the leaves and resulting in a lack of the desired chemical and physical properties. Tobacco leaves

grown under the influence of excessive N generally appear dark brown to black in color and dry and chaffy. A deficiency of N results in the premature yellowing of the leaves causing the cured leaves to be pale in color and to lack the desired textural properties associated with high quality tobacco (McCants and Woltz,1967).

Nitrogen is also considered to be a dominant factor influencing the strength of tobacco smoke. Nitrogen is an integral constituent of the nicotine molecule. Thus N is an important factor in nicotine synthesis. Nicotine content in field-grown tobacco plants increases with increases in the amount available (up to the point where excesses result in physiological breakdown of the leaves). The concentration of N in the tissue is positively correlated with nicotine levels, and negatively related to the sugar content of the leaf. The relationship of sugar to nicotine, or the sugar:nicotine ratio, is one of the important quality characteristics of flue-cured tobacco for use in cigarettes. The sugar:nicotine ratio is greater the sooner N deficiency occurs after transplanting. Nicotine synthesis is severely reduced shortly after the roots are deprived of adequate quantities of external N. Even temporary N deficiency has been shown to result in a low quality leaf physically and chemically. The most acceptable performance of tobacco may be expected from N fertilization practices in which a high percentage of the total available N is present during early stages of plant growth and rapidly diminishes during the later phase (McCants and

Woltz, 1967). The nicotine content of plants grown at high NH_4^+ levels has been shown to be increased in tobacco leaves in the early stages of growth. However, the differences soon disappeared and nicotine concentrations were similar to those of plants the same age grown on NO_3^-.

Metalaxyl

Metalaxyl, [N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester], the active ingredient in Ridomil, Apron, and Subdue, is an acylalanine fungicide specific for the control of fungi in the order Peronosporales. Metalaxyl has high postinfection eradicative activity. Current labeling provides for the use of metalaxyl in various formulations as a seed treatment, soil application, or foliar spray depending on the specific crop and disease to be treated. The Ridomil formulation is recommended for the control of black shank (Phytophthora parasitica Dast var. nicotianae), blue mold (Peronospora tabacina Adam), and damping-off (Pythium spp.) in tobacco (Ciba-Giegy, 1984).

Metalaxyl is marketed for use with tobacco as a 2E emulsifiable concentrate formulation. Labeled rates provide for applications of 2.34 to 4.68 L ha^- for blue mold control and 2.34 to 14 L ha^{-1} for black shank control as a preventative soil-incorporated treatment.

Laboratory studies using radiolabeled metalaxyl and a biologically active loamy sand showed relatively rapid degradation of metalaxyl under aerobic conditions. The half-life of metalaxyl under the laboratory conditions employed in this study was 41 d. In other bioassay studies where large numbers of soils were used, the half-life of metalaxyl in soil was 27 to 97 d (Ciba-Giegy, 1984). Olin Corporation holds a U.S. patent on metalaxyl as a nitrification inhibitor for soil or fertilizer application (Bashore and Lander, 1981). However, Olin indicated in patent documents that metalaxyl provided an average of 11% nitrification inhibition, compared to 37% for etradiozle, and 44% for another chemically related fungicide, following a 28 d soil incubation study.

Metalaxyl is readily taken up by plant roots and translocated through the plant when applied to soil. Movement of metalaxyl in the plant is apoplastic and is gradual and continuous as new plant growth occurs. Absorption of metalaxyl may also occur through the leaves and stems of plants, providing for one to two percent of the applied chemical to be transported symplastically to other stems or to the roots (Ciba-Giegy, 1984).

In a soil incubation study, Ercegovich et al. (unpublished data) did not find metalaxyl to kill the nitrifying bacteria in either of two soil types at 5, 25, or 125 mg kg^{-1} metalaxyl. Metalaxyl at 125 mg kg^{-1} significantly retarded nitrification in a Hagerstown silt loam for up to four weeks, after which time there was no significant

difference between the control and any of the three rates of application in this soil. A longer induction period than usual was required for nitrification to start in the Morrison soil under laboratory conditions. However, metalaxyl did not have an inhibitory effect on NO_3^- formation in the Morrison soil. Nitrification appeared to have been stimulated at 5 and 125 mg kg^{-1} of metalaxyl after 6 weeks of incubation. Schmitt (1983) found no significant differences in NH_4^+ concentrations in the NH_4^+ retention zone for nitrapyrin (0.56 kg ha^{-1}), metalaxyl (0.56 and 1.12 kg ha^{-1}), and a control after 3 weeks.

Personnel with Ciba-Geigy (1984) reported that in laboratory studies, the nitrification process in soil was significantly retarded with 125 mg kg^{-1} of metalaxyl initially but recovered within 8 weeks. Other field and laboratory studies have demonstrated that metalaxyl inhibits the nitrification process in soil, but is not as effective as nitrapyrin. Two of 10 bacterial species tested showed significant growth rate reductions at 125 mg kg^{-1} and 4 species showed significant growth rate increases at 5 to 125 mg kg^{-1} (Ciba-Geigy, 1984).

Rideout (1986) found reductions in tobacco quality index and in the percentage of mature and ripe grades of tobacco in field experiments where metalaxyl was used in 1984, but not in 1985. Rideout and Jones (1987) reported reductions in NO_3^- accumulation with metalaxyl (1.12 kg ha^{-1}) in a soil incubation study in which

there were no differences in NH_4^+ disappearance and no differences in pH. Ammonium oxidizer populations were reduced following application of metalaxyl, but not as drastically as those receiving nitrapyrin.

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Chapter III

Influence of Soil Type, pH, Temperature, and Moisture on Inhibition of Nitrification by Metalaxyl

by

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ABSTRACT

Metalaxyl, [N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester], is used extensively in tobacco (Nicotiana tabacum L.) production for prevention of black shank (Phytophthora parasitica Dast. var. nicotianae), blue mold (Peronospora tabacina Adam), and damping-off (Pythium spp.). Metalaxyl is also patented as a nitrification inhibitor, although not marketed for that purpose. Proper maturity and ripening of flue-cured tobacco depends on an adequate supply of N through the time of removal of the inflorescence, with a declining supply of N from that point. Use of a chemical which might prolong the availability of N in tobacco could delay maturity and reduce the quality of the cured leaf. These studies were conducted to determine whether metalaxyl might inhibit nitrification under a broad range of soil physical and environmental conditions prevalent in the tobacco producing areas of Virginia. The influence of soil type, soil pH, soil temperature, and soil moisture on inhibition of nitrification by metalaxyl (1 mg kg^{-1}) were investigated in three soils used extensively for tobacco production. Soils used in the study were Cecil sandy loam (clayey, kaolinitic, thermic Typic Hapludult), Appomattox fine sandy loam (clayey, mixed, thermic Typic Kandhapludult), and Mattoponi sandy

loam (clayey, mixed, thermic Typic Hapludult). Metalaxyl did not inhibit nitrification under any of the conditions studied. However, NO_2^- accumulation with metalaxyl was sometimes greater than the control, especially at high pH (7.0) in the Cecil and Appomattox soils, and at 10 and 20°C.

INTRODUCTION

Metalaxyl has been widely used by Virginia tobacco producers as a systemic fungicide for control of blue mold (Peronospora tabacina Adam) and black shank (Phytophthora parasitica Dast. var. nicotianae) since 1981. As much as 60 to 80 percent of flue-cured tobacco and 80 to 90 percent of burley tobacco in Virginia have been treated yearly with metalaxyl (Arnett, 1982; C.S. Johnson, 1988, personal communication).

Metalaxyl can inhibit the activity of nitrifying bacteria in the soil in addition to fungicidal activity (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) personnel reported 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).

Nitrification in soils is a biological process by which chemoautotrophic bacteria oxidize NH_4^+ to NO_3^-. This is a two step process in which NH_4^+ is oxidized to NO_3^- by nitrifying bacteria, usually Nitrosomonas, but often from one or more of five genera of

soil bacteria. The oxidizing bacteria responsible for the conversion of NO_2^- to NO_3^- are in the genus Nitrobacter (Schmidt and Belser, 1982).

The nitrification process may be inhibited by chemicals which interfere with the oxidation of NH_4^+ to NO_2^- or with the oxidation of NO_2^- to NO_3^- (Hauck, 1980). The main factors which limit nitrification in soil are substrate NH_4^+, O_2, CO_2, pH, and temperature. Nitrifying bacteria are essentially inactive in cold and wet soils. The optimum temperature for nitrification appears to vary widely among U. S. soils, from 20° to 40° C.

Nitrification proceeds in soil at pH levels far below the pH limits observed for nitrifying bacteria in pure culture. A lower limit for nitrification of pH 4.0 is suggested by most previous observations, with obvious nitrification occurring in the pH 4 to 6 range and with pH-independent nitrification in the range 6 to 8 (Schmidt, 1982). Several chemicals are commercially marketed to inhibit nitrification. Of these chemicals, nitrapyrin has been the most widely researched and used in the southeastern United States (Touchton and Boswell, 1980). Some of the same physical, chemical, and biological soil factors that affect nitrification also affect the efficacy of nitrapyrin. The effectiveness of nitrapyrin is lower on light textured soils compared to heavier soils. Nitrapyrin is also more effective at a soil temperature of 15°C than at a soil temperature of 30°C. Nitrification was inhibited by 87 to 89% at a

soil temperature of 15°C after 28 d of incubation (Bundy and Bremner, 1978).

Commercial nitrification inhibitors provide a means of increasing the efficiency of N fertilizer use by maintaining the N in the NH_4^+ form for a longer period of time. The potential for denitrification and leaching are reduced by use of a nitrification inhibitor (Hawkins, 1976). Prolonged availability of N may be a disadvantage in tobacco production. Depletion of soil N shortly after topping allows tobacco to mature and begin the ripening process.

The presence of a large quantity of NH_4^+ ions in the soil has been reported to be detrimental to the growth and quality of flue-cured tobacco (McCants and Woltz, 1967). Normally, tobacco fertilizer contains no more than 50 percent of the total N as NH_4^+. This NH_4^+ is rapidly oxidized to NO_3^- and usually poses no problem for the tobacco. Use of metalaxyl has been shown to reduce the quality of flue-cured tobacco under environmental conditions which might be unfavorable for nitrifying bacteria (Rideout, 1986). The objective of this study was to determine the influence of soil moisture, temperature, pH and metalaxyl on nitrification in three soil types commonly used for tobacco production in Virginia.

MATERIALS AND METHODS

Soil Collection, pH Adjustment, and Storage

The soils used (Table 1) were surface (0 to 15 cm) samples representative of Cecil sandy loam (clayey, kaolinitic, thermic Typic Hapludult), Appomattox fine sandy loam (clayey, mixed, thermic Typic Kandhapludult), and Mattoponi sandy loam (clayey, mixed, thermic Typic Hapludult) used extensively for flue-cured tobacco production in the Piedmont region of Virginia. The soil samples were passed through a 1 cm screen and stored at ambient temperature in plastic buckets until needed. Each soil was divided into three equal quantities. Soil pH of one portion was adjusted up approximately one pH unit by gradually adding reagent grade $CaCO_3$ as the soil was mixed in a rotary cylinder mixer. A second portion was adjusted down approximately one pH unit by gradually adding a dilute solution of H_3PO_4 as the soil was mixed (Tennessee Valley Authority, 1976). The third portion of soil was also homogenized in the mixer, but the pH was not adjusted from the original field level. Following mixing, the three portions of each soil type were spread on plastic in a greenhouse and wetted. Each portion was air-dried

Table 1. Selected characteristics[1] of Cecil, Appomattox, and Mattoponi soils studied.

	Soil Characteristics									
Depth	Ca^{2+}	Mg^{2+}	K^{+}	CEC	BS	pH	OM	H^{+}	Al^{3+}	ECEC
cm	--- $cmol_c kg^{-1}$ Soil ---				%		%	- $cmol_c kg^{-1}$ Soil -		
					Cecil					
0-25	1.36	0.49	0.25	3.3	39.62	6.0	0.97	3.2	0.15	2.25
25-76	1.88	0.84	0.27	9.1	31.18	5.8	0.19	6.6	0.35	3.34
76-120	0.94	0.64	0.16	7.1	25.07	5.7	0.06	5.2	0.85	2.59
120-162	0.47	0.39	0.10	7.5	15.09	5.6	0.03	5.4	1.45	2.41
162-185	0.26	0.23	0.08	5.9	10.61	4.9	0.06	4.8	1.45	2.02
					Appomattox					
0-38	0.70	0.26	0.20	2.1	1.26	5.6	0.61	2.6	0.10	1.26
38-89	1.49	0.99	0.40	10.9	4.73	5.1	0.09	8.0	1.85	4.73
89-137	0.26	0.46	0.12	11.7	4.89	5.0	0.03	12.0	4.05	4.89
137-185	0.09	0.18	0.04	10.1	4.86	5.1	0.03	18.2	4.55	4.86
					Mattoponi					
0-25	0.95	0.46	0.20	2.5	40.15	5.7	0.90	2.4	0.15	1.76
25-38	0.51	0.33	0.13	2.1	30.60	5.8	0.35	2.2	0.15	1.12
38-89	0.47	0.44	0.12	8.1	9.01	5.3	0.12	10.4	3.25	5.40
89-114	0.39	0.49	0.18	12.7	8.65	5.1	0.06	11.2	4.25	5.31
114-152	0.30	0.27	0.11	7.1	8.85	4.8	0.03	7.0	3.50	4.18
152-183	0.24	0.19	0.10	8.90	5.94	4.8	0.06	8.4	4.15	4.68

[1] - NH_4OAc (pH 7) extractable bases, cation- exchange capacity (CEC), base saturation (BS) by sum of cations, pH, Organic Matter (OM), exchangeable acidity (H^{+}), KCl extractable Al^{3+}, and effective-cation-exchange capacity (ECEC).

for three d, mixed, and wetted again. After three additional d of drying, the soil was mixed and stored in plastic buckets at ambient temperature until the experiments were initiated.

Nitrification Study I (Controlled Chambers)

Influence of Soil Temperature, Soil Type, and Soil pH on Inhibition of Nitrification by Metalaxyl

Nitrification, as influenced by metalaxyl and nitrapyrin was determined in the three soils (Cecil, Appomattox, Mattoponi). Selected properties and initial ionic N concentrations of adjusted soils prior to their use are presented in Table 2. The moist equivalent of 100 g of oven dry soil was placed in 250 mL glass bottles. Aqueous solutions of $(NH_4)_2SO_4$ and metalaxyl, or $(NH_4)_2SO_4$ and nitrapyrin were added to each soil sample in sufficient quantity to provide rates of 100 mg N kg^{-1}, 0 and 1 mg metalaxyl kg^{-1}, or 0 and 1 mg nitrapyrin kg^{-1}, respectively, at 60% of soil water-holding capacity (WHC). Bottles were capped to retain the moisture and vented every 3 d to maintain an aerobic environment. Treated samples were incubated in thermostatically-controlled chambers at 10, 20, and

Table 2. Selected properties and initial ionic nitrogen concentrations of adjusted soils prior to Nitrification Study I.

Soil			19 d Incubation				26 d Incubation			
Series	Texture	WHC[1]	pH	NH_4+	NO_2^-	NO_3^-	pH	NH_4^+	NO_2^-	NO_3^-
		g kg^{-1}		-- mg kg^{-1} ---				-- mg kg^{-1} --		
Cecil	sl	248	7.0	2.1	0	7.1	6.5	1.7	0	24.8
		248	5.4	1.9	0	2.9	5.3	1.7	0	18.0
		248	4.7	2.3	0	4.6	4.9	2.5	0	13.3
Appomattox	fsl	241	6.8	2.3	0	6.5	7.0	2.0	0	11.2
		241	5.5	2.1	0	3.2	5.8	1.9	0	10.0
		241	5.0	2.0	0	5.2	5.0	6.7	0	7.1
Mattoponi	sl	225	6.5	3.2	0	15.1	6.6	2.1	0	17.7
		225	4.9	2.2	0	3.3	5.5	3.5	0	11.7
		225	4.2	6.0	0	1.2	4.8	8.1	0	8.0

[1]WHC = Water-Holding Capacity.

30°C. The study was conducted in two runs with the first run incubated for 19 d and the second run for 26 d. Differences in incubation times resulted from power outages caused by electrical storms.

Nitrite and NO_3^- were determined for each experimental unit using an autoanalyzer system (Technicon Industrial Systems, 1977). The studies were considered as three-factor factorial experiments with two replications of each treatment combination. Each of the three temperature regimes was treated as a separate experiment. Soil N levels were normalized prior to analyses by subtracting previously existing levels from those determined following the studies.

Nitrification Study II (Controlled Chambers)

Influence of Moisture on Inhibition by Metalaxyl

The influence of metalaxyl and nitrapyrin on nitrification was studied at three temperatures (10, 20, 30°C) and three moisture levels (40, 60, 80% Water-Holding Capacity (WHC)) in two runs of two experiments. Each experiment contained two repetitions of each treatment. Cecil soil adjusted to high or low pH, as in Run II of Table 2, provided the basic material for the two experiments.

Methodology and rates of $(NH_4)_2SO_4$ and the inhibitors were the same as in the experiments described previously.

Amendments were added uniformly to the soil in sufficient quantity of water to establish moisture levels of 40, 60, or 80% of WHC. Treated soil was incubated for 16 and 24 d for the high pH, and 24 d for the low pH. Differences in incubation times resulted from an electrical storm which caused damage to a transformer and resulted in an extended power outage.

Nitrite and NO_3^- were determined for each experimental unit using an autoanalyzer system (Technicon Industrial Systems, 1977). The studies were considered as three series of three-factor factorial experiments with two replications.

Nitrification Study III (Greenhouse)

Influence of Soil Type, and Soil pH on Inhibition of Nitrification by Metalaxyl

A greenhouse experiment was conducted at the Southern Piedmont Agricultural Experiment Station, Blackstone, Virginia to determine the influence of metalaxyl on nitrification in three soils (Cecil, Appomattox, and Mattoponi) used extensively for flue-cured tobacco

production in the state of Virginia. Selected properties and initial ionic N concentrations of adjusted soils prior to their use are presented in Table 2. The experiment was conducted in two runs and repeated four times in each run.

Six week-old tobacco transplants (cv 'K 326') were transplanted into 10-cm diameter clay pots containing 600 g of dry soil. Treatments consisted of three soil types, three soil pH levels, and three nitrification inhibitors (as previously described) arranged in factorial combinations.

Nutrient rates were equivalent to 73 kg N, 88 kg P, and 116 kg K ha_{-1}. Phosphorus and K were supplied by KH_2PO_4. Metalaxyl and nitrapyrin rates were equivalent to 2.24 kg ha^{-1}. Nutrients, metalaxyl, or nitrapyrin were applied to the soil surface in 10 mL aqueous solution and distributed throughout the pot along with addition of 100 mL water. Four pots, each containing the same soil type-pH combination and a single plant, were placed in a 23cm X 30cm pan and considered an experimental unit. Water was added to the pans as needed to keep the plants from wilting. Soil samples were removed from each pot with a standard soil sampling tube 1, 2, and 4 weeks after treatment. Samples for each experimental unit were composited and stored in a freezer for later determination of NO_3^-.

A 10 g soil sample was taken from the composite of each experimental unit four weeks after the initiation of the experiment for quantification of NH_4^+ and NO_2^- oxidizing bacteria. The samples were added to 95 mL sterile 1 mM phosphate buffer and the bottles

shaken for 30 s. The extract was serially diluted to 10^{-8} with sterile buffer. One milliliter aliquots of the 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions were transferred to culture tubes containing 4 mL of the NH_4^+ or NO_3^- oxidizer medias described by Schmidt and Belser (1982). Five tubes from each dilution were inoculated. The culture tubes were incubated in the dark at 30°C for 13 weeks.

Tubes containing the NH_4^+ oxidizer media were checked weekly for acid production after the first three weeks of incubation. 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. The total number of positive tubes was recorded after 13 weeks when no additional tubes continued to change color from week to week.

Tubes containing the NO_2^- oxidizer media were checked after 13 weeks of incubation using the spot test method (Schmidt and Belser, 1982). Tubes that did not change color indicated the oxidation of NO_2^-, and were recorded as positive for the presence of NO_2^- oxidizing bacteria. Estimates of bacterial NH_4^+ and NO_2 oxidizer populations were calculated using the most probable number (MPN) technique of Alexander (1982).

Analyses

Soil samples were frozen within 30 min of collection and stored frozen to prevent nitrification. Samples were allowed to warm to room temperature prior to analysis. Nitrite and NO_3^- were determined from 8 g samples of composites of each experimental unit following a three week incubation period. Procedures used for soil N are based on those outlined by Keeney and Nelson (1982). Nitrite and NO_3^- were determined with an autoanalyzer system (Technicon Industrial Systems, 1977) from 8 g samples after extraction with 80 mL 2N KCl shaken for 1 h. Analyses of variance were performed on the data using Statistical Analysis Systems (SAS Institute Inc., 1985). Interaction means are presented when the level of probability was ≤ 0.05.

RESULTS AND DISCUSSION

Nitrification Study I (Controlled Chambers)

Influence of Soil Temperature, Soil Type, and Soil pH on Inhibition of Nitrification by Metalaxyl

The two runs of the study were not combined due to the magnitude of error variances when compared using the Bartlett's test for homogeneity (Gomez and Gomez, 1984) and because of differences in incubation times between the two. The first run was incubated for 19 d and the second run incubated for 26 d. Results are presented by run within temperature. The significance of the F-test for main effects and interactions are summarized in Table 3. Numerous significant soil X inhibitor and pH X inhibitor interactions prevented the drawing of statistical conclusions regarding the influence of soil type, pH, and inhibitor treatments on nitrification. Interaction means are presented for NO_2^- and NO_3^- as graphs in Fig. 1 thru 4.

Table 3. Significance of the F-test of main effects and interactions for NO_2^- and NO_3^- accumulations after 19 and 26 d incubations. Nitrification Study I.

	Days of incubation			
	19		26	
Source	NO_2^-	NO_3^-	NO_2^-	NO_3^-
		10°C		
Soil(S)	**	***	*	NS
pH	***	***	***	***
S X pH	**	**	**	***
Inhibitor(I)	***	***	*	***
S X I	NS	NS	NS	NS
pH X I	***	*	*	***
S X pH X I	NS	NS	NS	NS
		20°C		
Soil	NS	***	***	NS
pH	*	***	***	***
S X pH	NS	***	***	NS
Inhibitor	NS	***	NS	***
S X I	NS	***	NS	NS
pH X I	*	***	NS	***
S X pH X I	NS	***	*	NS
		30°C		
Soil	NS	***	***	**
pH	NS	***	NS	***
S X pH	NS	***	***	***
Inhibitor	NS	***	*	***
S X I	NS	***	NS	***
pH X I	NS	***	NS	***
S X pH X I	NS	***	NS	***

*,**, *** - Significant at 0.05, 0.01, and 0.001 respectively.
NS - Not Significant at the 0.05 level.

Soil Type X Inhibitor

The interaction of soil type X inhibitor was not significant for NO_2^- or NO_3^- at 10°C in either run (Table 3). Nitrite accumulated primarily in the control and metalaxyl treated samples (Fig. 1). All soil types contained similarly low NO_2^- levels when incubated at 10°C. However, relatively larger NO_2^- accumulations are apparent with metalaxyl in Cecil and Appomattox soils after incubation at 20°C for 19 d than for any other incubation temperature or for any combination of treatments following a 26 d incubation. While nitrification varies with the soil studied (Goring, 1962), higher accumulations of NO_2^- in the Cecil and Appomattox soils may relate to the relatively higher pH determined for each of the pH regimes for these soils compared to the Mattoponi (Table 2). Work by Duisberg and Buehrer (1960) and Morill and Dawson (1967) indicated that NO_2^-, which usually only occurs in trace amounts may occur in higher concentrations at high soil pH. Nitrite would only be expected to accumulate substantially at temperatures less than 6°C (Grant and Long, 1981). The occurrence of NO_2^- with metalaxyl only after 19 d might be partially explained if metalaxyl is an inhibitor prior to the oxidation of NO_2^-. Nitrite accumulation did not seem to differ after 26 days for any of the inhibitors or for soil treatments at 20°C (Fig. 1). Accumulated NO_2^-, present after 19 d incubation, appears to have been further oxidized before samples drawn after 26 d

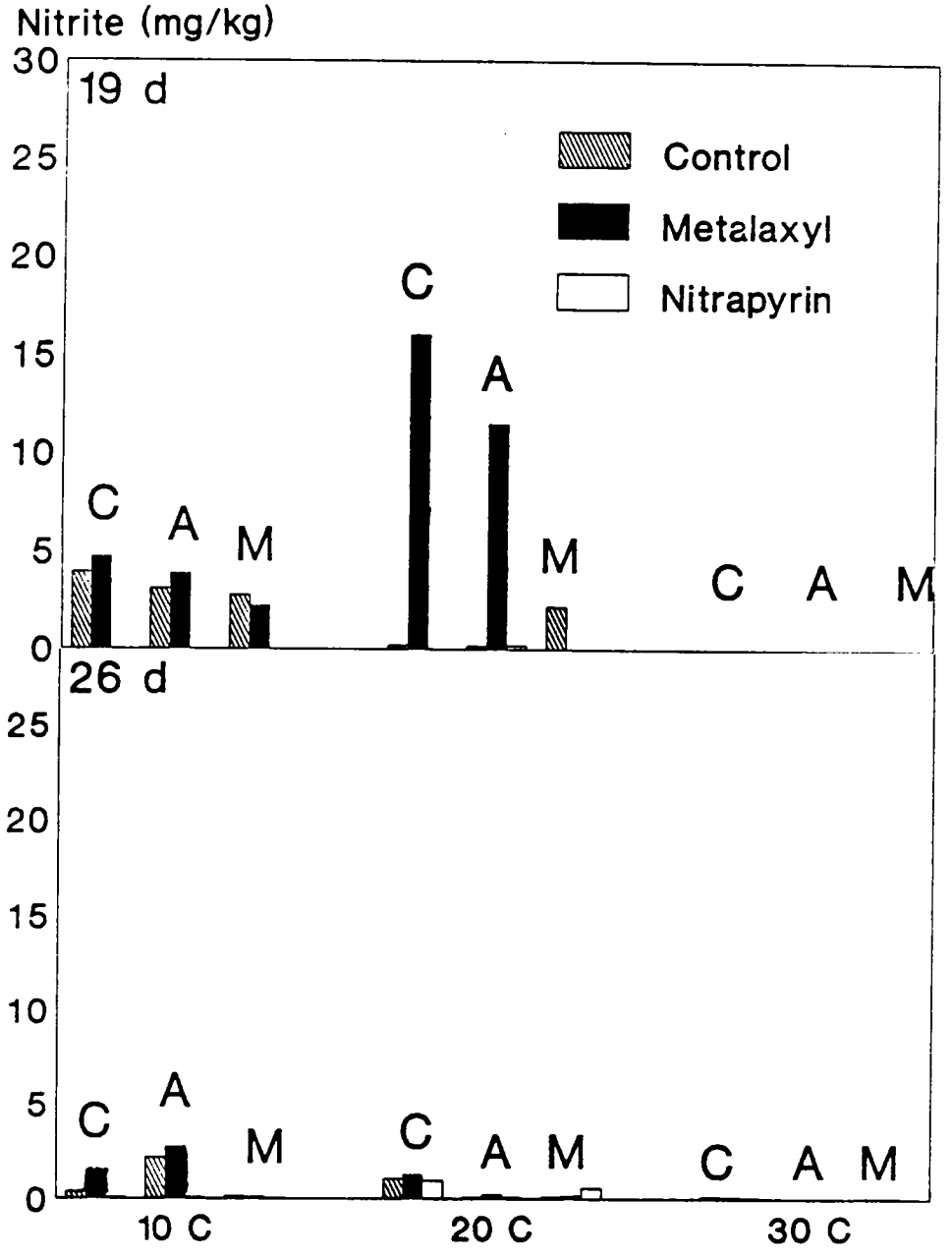

Fig. 1. Nitrite accumulation (mg kg^{-1}) as influenced by the interaction of soil type X inhibitor after incubation for 19 and 26 d. Nitrification Study I. C-Cecil, A-Appomattox, M-Mattoponi.

(Fig. 1). The control and metalaxyl treatments resulted in similar NO_3^- accumulations at 10, 20, and 30°C and low, medium, and high pH levels after 19 and 26 d (Fig. 2 and 4). Nitrapyrin inhibited NO_3^- accumulation significantly compared to the control and metalaxyl treatments at all soil temperatures and pH treatments.

pH X Inhibitor

The interaction of pH X inhibitor was significant at 10°C for NO_2^- and NO_3^- in both runs (Table 3). Nitrite accumulated primarily at high pH at 10°C (Fig. 3). Review of pH values for each soil adjusted to the high pH level (Table 2) reveals these values to be in the range of 6.5 to 7.0. Nitrite would be expected to accumulate under these conditions (Duisberg and Buehrer, 1960; and Morrill and Dawson, 1967). Nitrate accumulation decreased with decreasing pH with similar accumulation for the control and metalaxyl at all pH levels and incubation times (Fig. 4). Nitrapyrin inhibited NO_3^- accumulation compared to either metalaxyl or the control.

The interaction of pH X inhibitor was significant at 20°C for NO_2^- and NO_3^- after 19 d incubation and for NO_3^- only after 26 d (Table 3). Nitrite accumulation was substantially greater than the untreated control at high pH at 20°C following 19 d incubation with metalaxyl. The interaction of pH X inhibitor was significant at 30°C for NO_3^- accumulation after both the 19 and 26 d incubation periods

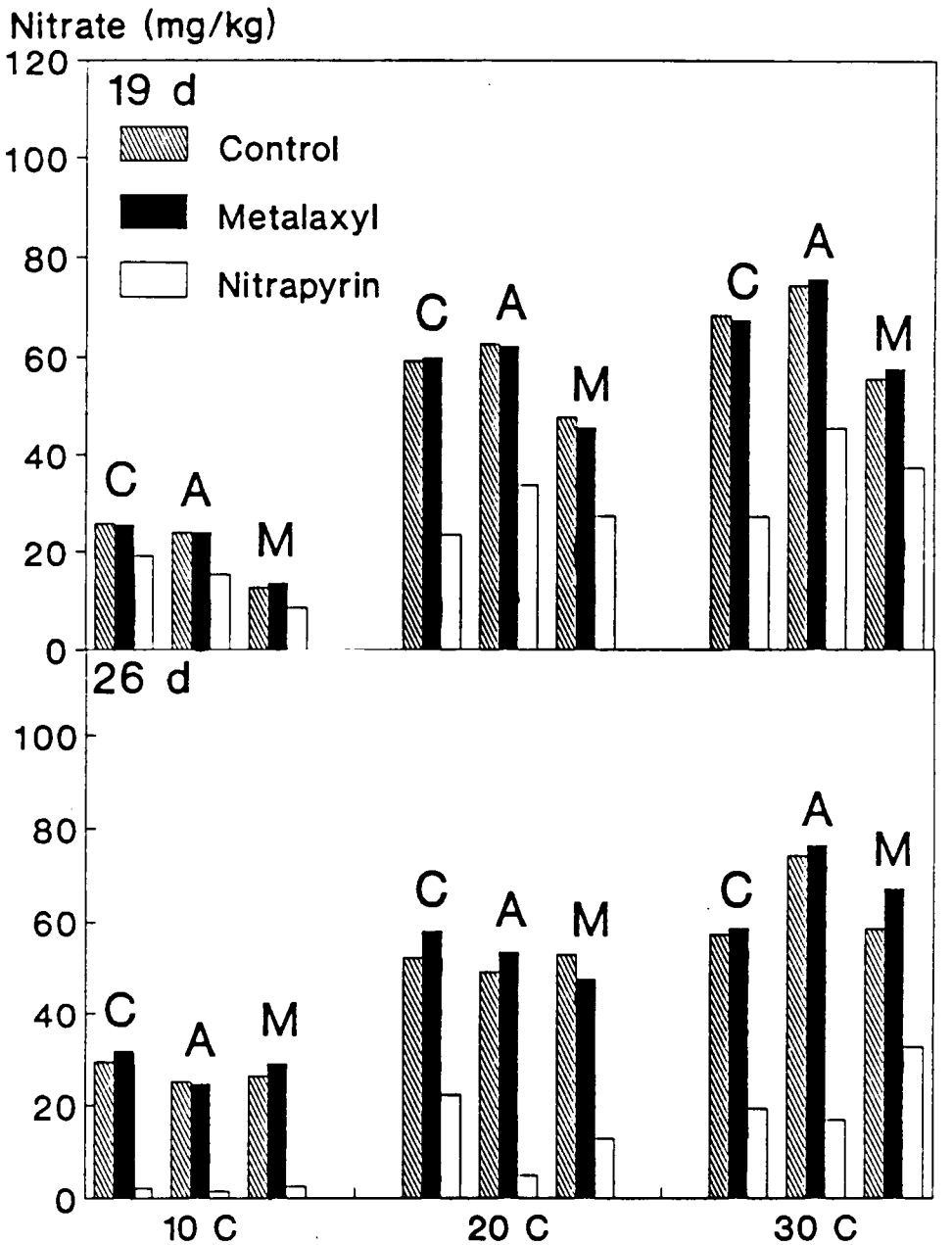

Fig. 2. Nitrate accumulation (mg kg^{-1}) as influenced by the interaction of soil type X inhibitor after incubation for 19 and 26 d. Nitrification Study I. C-Cecil, A-Appomattox, M-Mattoponi.

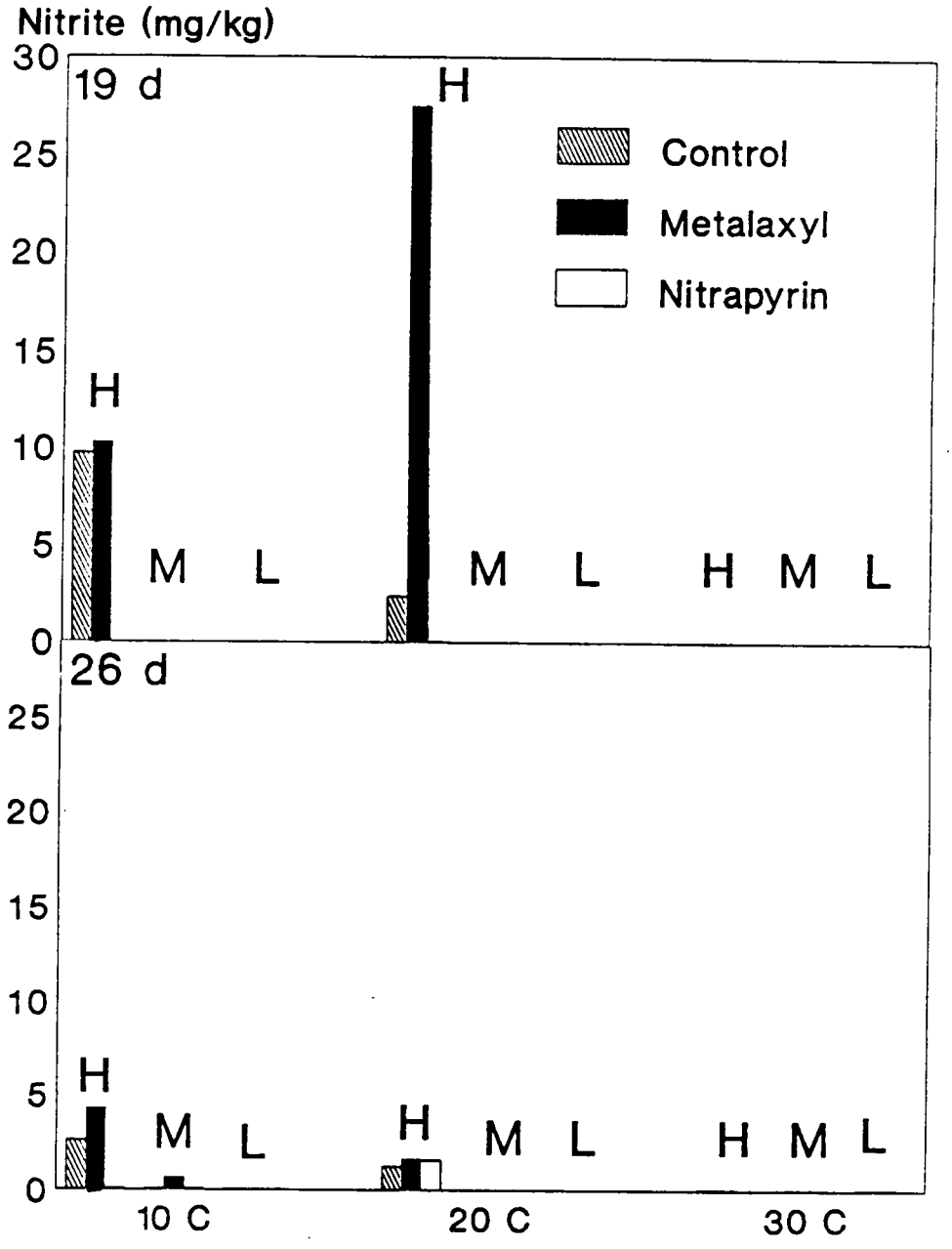

Fig. 3. Nitrite accumluation (mg kg^{-1}) as influenced by the interaction of pH X inhibitor after incubation for 19 and 26 d. Nitrification Study I. H - High pH, M - Medium pH, L - Low pH.

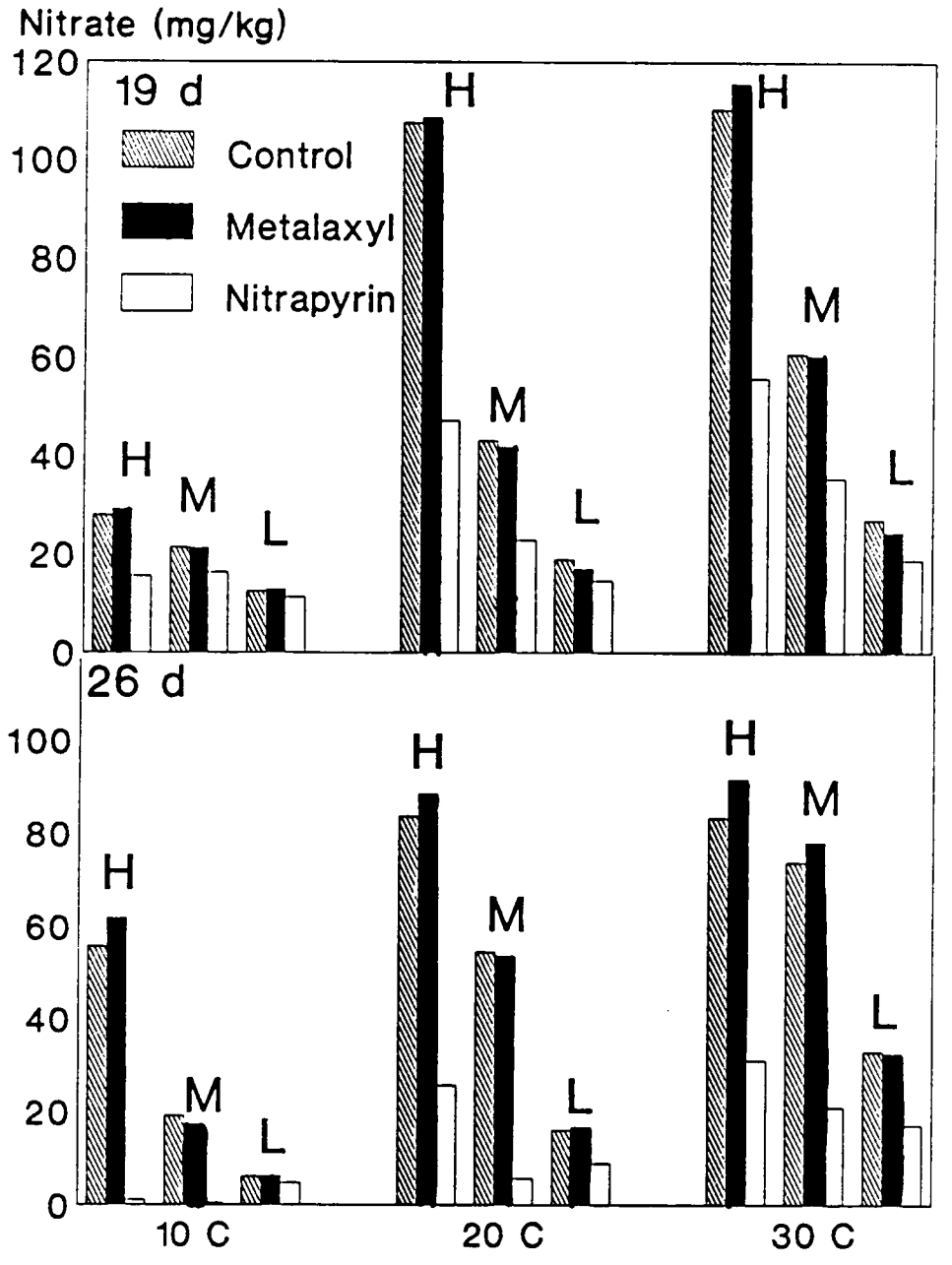

Fig. 4. Nitrate accumluation (mg kg^{-1}) as influenced by the interaction of pH X inhibitor after incubation for 19 and 26 d. Nitrification Study I. H - High pH, M - Medium pH, L - Low pH.

(Table 3 and Fig. 4). This interaction was due to significant differences in NO_3^- between nitrapyrin and other treatments at high and medium pH levels, but not at low pH. Nitrite did not accumulate at any pH at 30°C (Fig. 3).

pH X Inhibitor for Each Soil Type and pH

Nitrate accumulation as influenced by pH and inhibitor treatments for each soil type at 10, 20, and 30°C are presented in Tables 4 through 6. Data for NO_2^- accumulation are not presented because of the small number of instances of NO_2^- occurrence. Significance of the pH X inhibitor interaction are presented after analyses of data for each soil type individually. Nitrate accumulations were averaged over all pH levels when the pH X inhibitor interactions were not significant at the 0.05 level.

The interaction of pH X inhibitor was significant in the overall ANOVA for NO_3^- accumulation after 19 d at 10°C (Table 3). The interaction of pH X inhibitor was not significant at the 0.05 level at 10°C after 19 d incubation for NO_3^- accumulation in any of the three soils studied when data for each soil type were analyzed individually (Table 4). This difference in significance is apparently due to lower degrees of freedom in the analysis by soil type. Nitrapyrin resulted in significantly lower NO_3^- accumulation

Table 4. Nitrate accumulation as influenced by soil pH and inhibitor treatments for each soil type at 10°C. Nitrification Study I.

Soil		Inhibitor[1]				Inhibitor			
Type	pH	Cntrl.	Metal.	Nitra.	Avg.[2]	Cntrl.	Metal.	Nitra.	Avg.
		Days of incubation							
		19				26			
		mg kg^{-1}							
Cecil	High	32.2	34.0	19.4	28.5a	52.9a*	57.7a	-5.5b	--
	Medium	21.6	23.8	18.9	21.4b	21.6a	23.3a	-1.5b	--
	Low	20.9	19.0	19.2	19.7b	13.7a	14.2a	13.5a	--
Avg.[2]		24.9a	25.6a	19.1b		--	--	--	
pH X Inhibitor		P = 0.1445				P = 0.0001			
		SE = 3.84				SE = 4.08			
Appomattox	High	31.5	29.9	17.1	26.2a	57.0a	57.2a	4.1b	--
	Medium	31.8	30.2	20.8	27.6a	15.5a	13.5a	0.0b	--
	Low	8.6	11.8	7.9	9.4b	2.8a	3.0a	0.4b	--
Avg.		24.0a	24.0a	15.3b		--	--	--	
pH X Inhibitor		P = 0.5124				P = 0.0049			
		SE = 5.45				SE = 7.61			
Mattoponi	High	19.8	23.4	9.7	17.6a	57.3b	70.7a	4.7c	--
	Medium	10.5	9.8	9.1	9.8b	20.4a	15.3a	2.9b	--
	Low	7.6	7.4	6.7	7.2b	1.6a	1.3a	0.2a	--
Avg.		12.6a	13.5a	8.5a		--	--	--	
pH X Inhibitor		P = 0.3211				P = 0.0001			
		SE = 4.68				SE = 2.31			

* Means in the same row of the same run followed by the same letter do not differ at the 0.05 level.

[1] Inhibitors = Control(Cntrl), Metalaxyl(Metal), Nitrapyrin (Nitra).

[2] Average (Avg.) Inhibitor and soil pH means may be compared within the same row and column, respectively, for each soil within each run.

than the control or metalaxyl in the Cecil and the Appomattox soils. Inhibitor treatments did not differ in the NO_3^- accumulated in the Mattoponi soil after 19 d (Table 4). The Mattoponi soil was lower in clay content and a lighter textured soil. Lower NO_3^- accumulations for the Mattoponi soil with all treatments compared to the other soils indicate lower nitrifying capabilities for the Mattoponi soil. Already low levels of nitrification may not have been significantly affected by nitrapyrin. Based on the significance of the interaction of pH X inhibitor in the overall ANOVA (Table 3) and observation of the means after 19 d of incubation (Table 4) there appears to be a trend for greater reductions in NO_3^- accumulation with nitrapyrin at high pH than at either medium or low pH. Greater NO_3^- accumulation with increasing pH indicates an improved environment for nitrifying bacteria and increased nitrification. The magnitude of NO_3^- accumulation at high pH appears to differ based on soil type and may be more related to the actual pH of the soil than the soil type. In all cases NO_3^- accumulation due to metalaxyl does not appear to differ from that of the control.

The interaction of pH X inhibitor was highly significant for all soil types at 10°C after 26 d incubation (Table 4). Nitrate accumulation with the metalaxyl treatment differed from the control only for the high pH in the Mattoponi soil where NO_3^- accumulation was greater with the metalaxyl treatment (Table 4). No reason for this increased rate of nitrification was apparent. Nitrapyrin

resulted in lower NO_3^- accumulation than the control or metalaxyl in all soils at high pH and in Cecil and Mattoponi at medium pH. The pH of Cecil and Mattoponi soils in the medium range were 0.5 and 0.3 pH units, respectively, lower than Appomattox. Hendrickson and Keeney (1979) found that the nitrifier population was more susceptible to nitrapyrin at lower pH. Appomattox was the only soil to have lower NO_3^- accumulation with nitrapyrin than the control and metalaxyl at low pH.

The interaction of pH X inhibitor was highly significant at 20°C for all soil types after incubation for 19 d (Table 5). Nitrate accumulation after application of metalaxyl was similar to that of the control at each pH in each of the soils at 10°C (Table 5). Nitrapyrin resulted in lower NO_3^- accumulation than the control and metalaxyl at high and medium pH levels in all soils except Mattoponi at medium pH, where NO_3^- accumulation with nitrapyrin was less than with the control, but not less than with metalaxyl.

The interaction of pH X inhibitor was not significant for Cecil soil after 26 days incubation at 20°C (Table 5). Nitrate accumulation in the Cecil soil at 20°C was lower with nitrapyrin than with the control and metalaxyl (Table 5). Nitrapyrin caused lower accumulations of NO_3^- than the control and metalaxyl after incubation at 20°C at high and medium pH in the Appomattox and the Mattoponi soils, but not at low pH. Nitrate accumulation did not differ from the control when metalaxyl was used at all pH levels in

Table 5. Nitrate accumulation as influenced by soil pH and inhibitor treatments for each soil type at 20°C. Nitrification Study I.

Soil		Inhibitor[1]				Inhibitor			
Type	pH	Cntrl.	Metal.	Nitra.	Avg.[2]	Cntrl.	Metal.	Nitra.	Avg.
		Days of incubation							
		19				26			
		mg kg^{-1}							
Cecil	High	110.5b	116.0a	26.4c		75.0	84.3	38.5	65.9a
	Medium	44.5a*	41.4a	26.1b	--	57.2	61.1	6.1	41.4b
	Low	22.4a	22.2a	18.1a	--	24.7	28.5	22.5	25.2b
Avg.[2]		--	--	--		52.3a	57.9a	22.4b	
pH X Inhibitor		P = 0.0001				P = 0.3232			
		SE = 2.01				SE = 18.02			
Appomattox	High	109.5a	109.5a	59.4b	--	88.8a	93.3a	11.0b	--
	Medium	57.2a	59.7a	27.9b	--	47.1a	54.9a	2.2b	--
	Low	21.1a	17.0a	14.0a	--	11.6a	12.4a	1.8a	--
Avg.		--	--	--		--	--	--	
pH X Inhibitor		P = 0.0001				P = 0.0002			
		SE = 3.93				SE = 6.58			
Mattoponi	High	102.3a	100.6a	55.9b	--	87.3a	88.1a	27.8b	--
	Medium	27.7a	24.4ab	14.7b	--	59.6a	45.2a	8.7b	--
	Low	13.0a	11.6a	11.6a	--	12.0a	9.6a	2.4a	--
Avg.		--	--	--		--	--	--	
pH X Inhibitor		P = 0.0005				P = 0.0038			
		SE = 4.91				SE = 7.56			

* Means in the same row of the same run followed by the same letter do not differ at the 0.05 level.
[1] Inhibitors = Control(Cntrl), Metalaxyl(Metal), Nitrapyrin (Nitra).
[2] Average (Avg.) inhibitor and soil pH means may be compared within the same row and column, respectively, for each soil within each run.

all soils at 20°C. The interaction of pH X inhibitor was highly significant for all three soil types after 19 d incubation and for Cecil and Mattoponi after 26 d incubation at 30°C (Table 6). Nitrate accumulation for the control and metalaxyl did not differ for any soil at any pH (Table 6). The pH X inhibitor interaction was significant after 26 days incubation at 30°C in Cecil and Mattoponi soils, but not in the Appomattox soil (Table 6). The significant interaction of pH X inhibitor appears to result from the magnitude of differences among treatments at the various pH levels (Fig. 4). Nitrate was lower in the Appomattox soil when nitrapyrin was used or when the pH was low (Table 6).

Nitrate accumulation following application of metalaxyl was not lower than the control in any case in this experiment. In almost every case, nitrapyrin significantly reduced NO_3^- accumulation to levels below that of the control and metalaxyl. Metalaxyl did not appear to inhibit nitrification as compared to a non-treated check or to nitrapyrin under the majority of conditions studied. Apparent numerical differences were not always significantly different due to the variability of the samples analyzed. Based on the low NO_3^- values at low pH for all soils with all inhibitor treatments, the nitrification process was already proceeding at a low rate. Although a trend toward lower NO_3^- accumulation following application of nitrapyrin was observed at low pH, these apparent numerical differences were not always significant, as might be

Table 6. Nitrate accumulation as influenced by soil pH and inhibitor treatments for each soil type at 30°C. Nitrification Study I.

Soil Type	pH	Inhibitor[1] Cntrl.	Metal.	Nitra.	Avg.	Inhibitor Cntrl.	Metal.	Nitra.	Avg.[2]
		Days of incubation							
		19				26			
		mg kg^{-1}							
Cecil	High	117.0a*	120.0a	26.5b	--	79.5a	79.1a	-2.1b	--
	Medium	57.3a	55.4a	34.6b	--	53.3a	59.1a	22.8b	--
	Low	30.5a	26.8a	20.7b	--	39.2a	38.0a	37.9a	--
Avg.		--	--	--		--	--	--	
pH X Inhibitor		P = 0.0001				P = 0.0004			
		SE = 2.52				SE = 8.36			
Appomattox	High	108.5a	115.0a	72.0b	--	94.5	97.4	30.5	74.1a
	Medium	82.0a	83.3a	44.2b	--	88.6	91.4	13.9	64.6a
	Low	32.3a	28.2a	20.0b	--	39.9	41.3	6.6	29.3b
Avg.[2]		--	--	--		74.3a	76.7a	17.0b	
pH X Inhibitor		P = 0.0003				P = 0.72			
		SE = 3.39				SE = 10.12			
Mattoponi	High	105.4a	112.0a	68.6b	--	76.1b	98.9a	65.2b	--
	Medium	42.7a	42.2a	27.6b	--	79.7a	84.1a	26.1b	--
	Low	18.3a	18.3a	15.7a	--	20.1a	18.8a	6.9a	--
Avg.		--	--	--		--	--	--	
pH X Inhibitor		P = 0.0002				P = 0.0017			
		SE = 3.55				SE = 6.21			

* Means in the same row of the same run followed by the same letter do not differ at the 0.05 level.

[1] Inhibitors = Control(Cntrl), Metalaxyl(Metal), Nitrapyrin (Nitra).

[2] Average (Avg.) inhibitor and soil pH means may be compared within the same row and column, respectively, for each soil within each run.

expected based on the work of Hendrickson and Keeney (1979). Low temperature (10°C) also appeared to contribute to the inherently low NO_3^- accumulation at low pH supporting the work of a number of workers (Anderson and Purvis, 1955; Frederick, 1956; Sabey et al., 1956; and Sabey et al., 1959) Nitrification was inhibited by nitrapyrin at high and medium pH at all temperatures studied. However, low pH and/or low temperature (10°C) appear to inhibit nitrification sufficiently that any additional inhibition by nitrapyrin was not significant at the 0.05 level.

Nitrification Study II (Controlled Chambers)

Influence of Soil Moisture on Inhibition of Nitrification by Metalaxyl

Cecil Soil - High pH

The significance of the F-tests for main effects and interactions for NO_2^- and NO_3^- for Nitrification Study II are shown by run and incubation temperature in Table 7. Both runs will be considered together by temperature with major emphasis on NO_3^- accumulation as an indicator of nitrification.

Nitrate accumulation did not differ as a result of water-holding capacity (WHC) at 10°C, at 20°C and low pH, or at 20°C, high pH, and 16 versus 24 d of incubation (Table 7). Nitrate accumulation was lower with nitrapyrin at all temperatures, incubations, and WHC's (Table 8). Nitrite accumulated at 10°C after 16 d following the control and metalaxyl treatments at all WHC levels. Little NO_2^- accumulation was noted for nitrapyrin at 10°C because nitrapyrin inhibits nitrification prior to the production of NO_2^- (Hauck, 1980). Ammonium oxidation in the control and metalaxyl treatments appears to have been proceeding at a more rapid rate than NO_2^- oxidation. Decreased oxidation rate of NO_2^- in the metalaxyl

Table 7. Significance of the F-test of main effects and interactions for NO_2^- and NO_3^- in Cecil soil at two pH levels in two runs (16 or 24 d of incubation, respectively) at three temperatures. Nitrification Study II.

	High pH				Low pH			
	Days of incubation							
	16		24		24		24	
Source	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-
				10°C				
Moisture (M)	***	NS	*	NS	NS	NS	NS	NS
Inhibitors (I)	***	***	***	***	NS	***	NS	***
M X I	***	NS	NS	NS	NS	NS	NS	NS
				20°C				
Moisture	***	NS	NS	***	NS	NS	NS	NS
Inhibitor	NS	***	NS	***	NS	**	NS	***
M X I	NS	NS	NS	**	NS	NS	NS	NS
				30°C				
Moisture	NS	***	NS	*	NS	***	**	***
Inhibitor	NS	***	NS	***	NS	***	***	***
M X I	NS	***	NS	NS	NS	***	**	NS

*, **, *** - Significant at 0.05, 0.01, and 0.001 respectively.
NS - Not Significant at the 0.05 level.

Table 8. Nitrite and NO_3^- accumulations resulting from three inhibitor treatments incubated at three temperatures, and three moisture levels for 16 and 24 d in Cecil soil at high pH. Nitrification Study II.

	10°C					
	16 d				24 d	
	% WHC[1]					
Inhibitor	40	60	80	NO_3^-	NO_2^-	NO_3^-
	-- mg NO_2^- kg^{-1} --			---------- mg kg^{-1} -------		
Control	12.4a*	11.1b	14.2a	56.8a	14.8a	48.9a
Metalaxyl	11.9a	16.9a	14.5a	61.5a	14.3a	50.4a
Nitrapyrin	0.0b	0.0c	0.0b	18.9b	0.0b	18.6b

WHC X Inhibitor P = 0.001

	20°C					
	16 d			24 d		
				% WHC		
	NO_2^-	NO_3^-	NO_2^-	40	60	80
	-------- mg kg^{-1}--------			------- mg NO_3^- kg^{-1} -----		
Control	3.9a	108.9a	0.0a	106.7b	100.6a	95.1a
Metalaxyl	3.4a	107.8a	0.0a	116.6a	103.3a	92.7a
Nitrapyrin	3.1a	26.7b	0.0a	22.4c	21.9b	24.9b

WHC X Inhibitor P = 0.0017

	30°C					
	16 d				24 d	
		% WHC				
	NO_2^-	40	60	80	NO_2^-	NO_3^-
	mg kg^{-1}	--- mg NO_3^- kg^{-1} ----			--- mg kg^{-1} ----	
Control	0.0a	111.2a	91.6b	88.2b	0.0a	107.2a
Metalaxyl	0.0a	109.3a	103.6a	104.2a	0.0a	109.2a
Nitrapyrin	0.0a	28.0b	32.2c	23.4c	0.0a	32.3b

WHC X Inhibitor P = 0.008

* Means in the same column followed by the same letter do not differ at the 0.05 level according to Duncan's Multiple Range Test.
[1] WHC = Water-Holding Capacity.

treatment may be explained by the reduced activity of Nitrobacter at high pH levels (Duisberg and Buehrer, 1960; Morrill and Dawson, 1967) such as those in this soil (pH = 7.0). After 24 d of incubation at 20°C, the interaction of moisture X inhibitor was highly significant for NO_3^- (Table 7). This significant interaction resulted from a significantly higher NO_3^- accumulation with metalaxyl at 40% WHC and would not indicate inhibition due to metalaxyl. Nitrapyrin resulted in the lowest nitrate levels of all treatments at 20°C at all moisture levels after 24 d incubation.

The interaction of moisture X inhibitor was also highly significant for NO_3^- accumulation after the 16 d incubation at high pH, and in the first of two runs at low pH at 30°C (Table 7). Accumulation of NO_3^- at 30°C was greater as a result of the metalaxyl treatment than for the control at 60 and 80% WHC. Nitrate accumulation does not appear to have been inhibited by metalaxyl after 16 d at 30°C at any of the tested moisture levels (Table 8). Following 24 d of incubation at 30°C, NO_3^- accumulation was significantly lower with nitrapyrin than either the control or metalaxyl treatments (Table 8).

Cecil Soil - Low pH

Nitrate accumulation was similar for the control and the metalaxyl treatments at all temperatures and after both 24 d incubation periods (Table 9). The nitrapyrin treatment reduced NO_3^- levels compared to the control and the metalaxyl treatments at all temperatures after both incubation periods. Nitrite accumulation in the control and metalaxyl treatments occurred only at 30°C following the second 24 d incubation study (Table 9). No nitrite was detected after treatment with nitrapyrin at any temperature or moisture level. The interaction of moisture X inhibitor was significant for NO_3^- in soil incubated at 30°C after the first 24 d incubation study and for NO_2^- after the second incubation study (Table 7). Nitrate accumulation after the first study, and NO_2^- accumulation after the second study at 30°C were similar for the control and metalaxyl treatments at all moisture levels with lower accumulations with nitrapyrin (Table 9).

Metalaxyl did not inhibit nitrification compared to the control under any of the conditions studied. Nitrapyrin reduced nitrification in Cecil soil at both high and low pH levels and at 10, 20, and 30°C.

Table 9. Nitrite and NO_3^- accumulations resulting from two runs of three inhibitor treatments incubated at three temperatures, three moisture levels and for 24 d in Cecil soil at low pH.

	10°C			
	24 d		24 d	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-
	-- mg kg^{-1} --		--- mg kg^{-1} ---	
Control	0.0a	25.8a	0.0a	20.2a
Metalaxyl	0.0a	24.7a	0.0a	20.2a
Nitrapyrin	0.0a	20.7b	0.0a	15.5b

	20°C			
	24 d		24 d	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-
	-- mg kg^{-1} --		--- mg kg^{-1} ---	
Control	0.0a	39.2a	0.0a	34.5a
Metalaxyl	0.0a	36.5a	0.0a	33.8a
Nitrapyrin	0.0a	28.0b	0.0a	20.2b

	30°C							
	24 d				24 d			
		% WHC			% WHC			
	NO_2^-	40	60	80	40	60	80	NO_3^-
	mg kg^{-1}	-- mg NO_3^- kg^{-1} --			-- mg NO_2^- kg^{-1} --			mg kg^{-1}
Control	0.0a	42.3a	53.0a	55.3a	0.4a	0.6a	0.5a	41.5a
Metalaxyl	0.0a	43.9a	49.2a	46.9a	0.4a	0.6a	0.3a	42.6a
Nitrapyrin	0.0a	33.5b	37.1b	27.1b	0.0b	0.0b	0.0b	33.3b

* Means in the same column followed by the same letter do not differ at the 0.05 level according to Duncan's Multiple Range Test.
[1] WHC = Water-Holding Capacity.

Nitrification Study III (Greenhouse)

Influence of Soil Type and Soil pH on Inhibition of Nitrification by Metalaxyl

The significance of the F-test of main effects and interactions for NO_2^- and NO_3^- from soil samples for Nitrification Study III are shown in Table 10. Runs were not combined due to differences in variances and will be discussed separately. Values reported for NO_3^- are very low. Plants growing in these studies became chlorotic and exhibited typical nitrogen starvation symptoms after two to three weeks. Additional studies indicated that the clay pots may have accumulated very high amounts of NH_4^+ and NO_3^- as a result of the constant evaporation of moisture from the exterior pot surface. In addition, the N application rate included in the studies was determined to have been too low for the pot work conducted (Tennesse Valley Authority, 1976). Even so, differences in final NO_3^- were determined as a result of the inhibitor treatments. Nitrate accumulation as affected by soil type, pH, and inhibitor in two different runs of Nitrification Study III are summarized in Table 11.

Table 10. Significance of the F-test of main effects and interactions for NO_2^- and NO_3^-. Nitrification Study III.

	Run I		Run II	
Source	NO_2^-	NO_3^-	NO_2^-	NO_3^-
	Week I			
Soil(S)	***	***	NS	***
pH	***	***	NS	***
S X pH	***	NS	NS	NS
Inhibitor(I)	*	***	NS	*
S X I	**	NS	NS	NS
pH X I	**	**	NS	*
S X pH X I	***	NS	NS	NS
	Week II			
Soil	NS	***	NS	**
pH	NS	NS	NS	NS
S X pH	NS	NS	NS	NS
Inhibitor	NS	*	NS	NS
S X I	NS	NS	NS	NS
pH X I	NS	NS	NS	NS
S X pH X I	NS	NS	NS	NS
	Week IV			
Soil	NS	NS	NS	***
pH	NS	NS	NS	NS
S X pH	NS	NS	NS	NS
Inhibitor	NS	*	NS	NS
S X I	NS	NS	NS	NS
pH X I	NS	NS	NS	NS
S X pH X I	NS	NS	NS	NS

*, **, *** - Significant at 0.05, 0.01, and 0.001 respectively.
NS - Not Significant at the 0.05 level.

Table 11. Nitrate accumulation as affected by soil type, pH, and inhibitor in two runs. Nitrification Study III.

	Week I					
	Run I			Run II		
	Soil pH			Soil pH		
Inhibitor	High	Medium	Low	High	Medium	Low
	-- mg NO_3^- kg^{-1} --			----- mg NO_3^- kg^{-1} -----		
Control	7.5a	2.7a	3.6a	2.0a	1.4a	1.1a
Metalaxyl	6.1a	1.7a	3.4a	2.8a	0.8a	1.5a
Nitrapyrin	1.8b	1.3a	1.8a	1.1a	1.1a	0.9a
pH X Inhibitor	P = 0.0056			P = 0.0385		
	SE = 2.25			SE = 1.04		

	Week II			
	Run I		Run II	
Inhibitor	NO_3^-	Soil	NO_3^-	Soil	NO_3^-
[1]	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}
Control	1.9a	Cecil	2.3a	Cecil	2.8a
Metalaxyl	1.2ab	Appomattox	0.5b	Appomattox	1.3b
Nitrapyrin	0.8b	Mattoponi	1.1b	Mattoponi	2.5a

	Week IV		
	Run I	Run II	
Inhibitor	NO_3^-	Soil	NO_3^-
	mg kg^{-1}		mg kg^{-1}
Control	2.6a	Cecil	3.1a
Metalaxyl	2.0ab	Appomattox	0.5b
Nitrapyrin	1.5b	Mattoponi	0.7b

* Means in the same column followed by the same letter do not differ at the 0.05 level according to Duncan's Multiple Range Test.
[1] WHC = Water-Holding Capacity.

One Week

Significant interactions of pH X inhibitor occurred for NO_3^- accumulation in samples taken the first week after treatment in both runs (Table 10). Graphs of the interaction means are presented in Fig. 5. Nitrate accumulation decreased for both the control and metalaxyl as initial pH decreased in Run I.

Two Weeks

Nitrapyrin significantly reduced NO_3^- accumulation compared to the control in Run I, but not for Run II, two weeks after treatment (Table 10). Accumulated NO_3^- for the metalaxyl treatment did not differ from that for the control or nitrapyrin (Table 11). Cecil soil had greater NO_3^- accumulation than either Appomattox or Mattoponi in Run I (Table 11). Accumulated NO_3^- was higher in Mattoponi and Cecil soils compared to Appomattox in Run II.

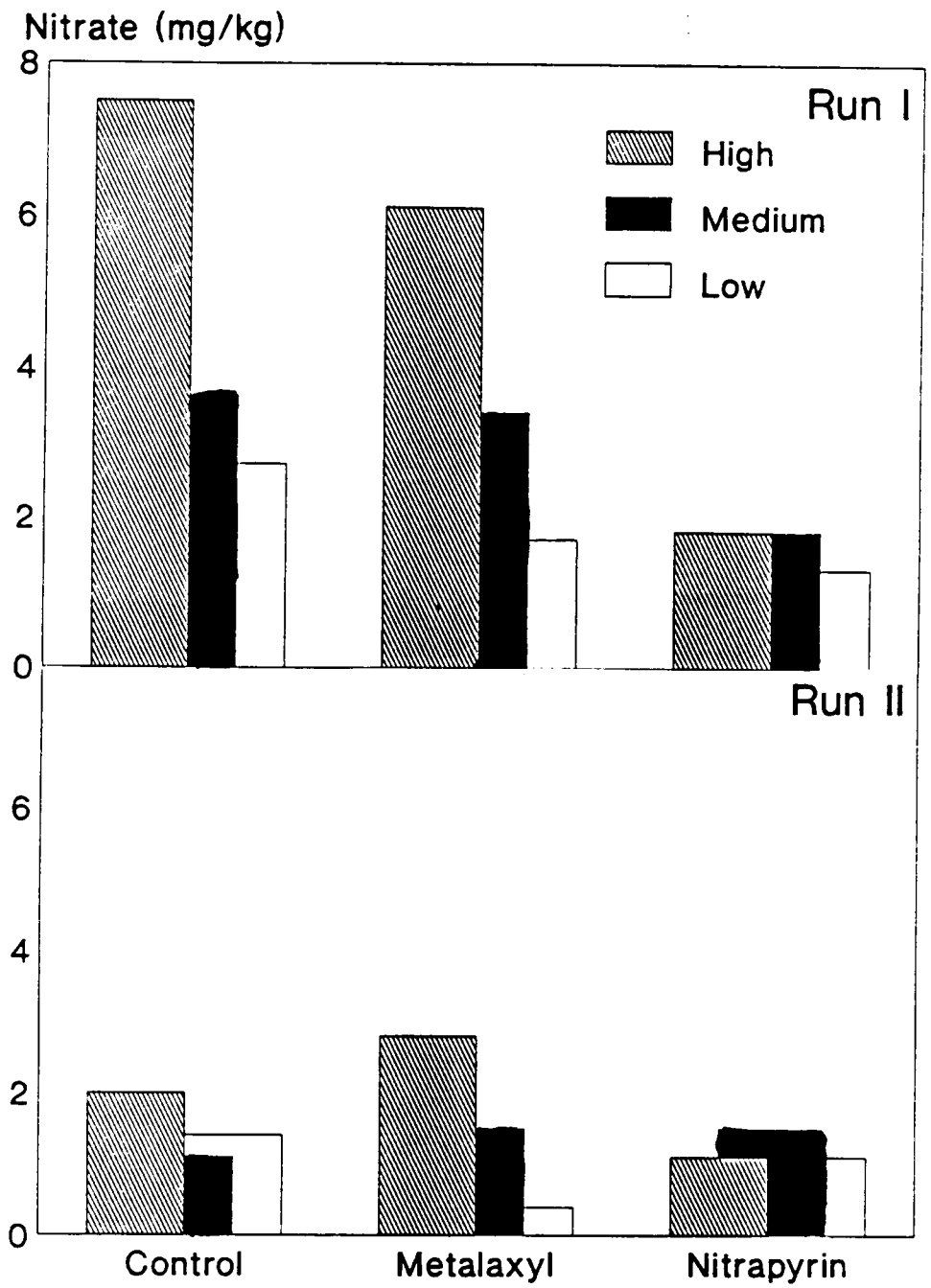

Fig. 5. Nitrate accumulation (mg kg^{-1}) as influenced by the interaction of pH X inhibitor for Run I and Run II of Nitrification Study III.

Four Weeks

Inhibitor treatments resulted in differences in NO_3^- accumulation four weeks after treatment in Run I but not in Run II (Table 10). Nitrate accumulation for the metalaxyl treatment did not differ from the control or nitrapyrin in Run I, which were significantly different from each other (Table 11). Nitrate values differed due to soil type in Run II after four weeks (Table 10). Greater NO_3^- accumulation was observed in the Cecil soil after four weeks than in the Appomattox or Mattoponi soils (Table 10 and 11). Differences in the results of Run I and Run II do not appear to have been linked to any difference in the conduct of these studies. All conditions and management were as similar as possible.

Nitrifier Quantification

Most probable number estimates of soil nitrifier populations after four weeks in the greenhouse did not differ as a result of the inhibitor treatment in either of the two runs of the study (Table 12). Significant differences in NH_4^+ oxidizers due to pH occurred in both runs, and for NO_2^- oxidizers in Run I. Reductions in populations would not be expected unless an inhibitor killed nitrifying bacteria as a means of inhibition.

Table 12. Significance of F-test of main effects and interactions for Most Probable Number estimation of NH_4^+ and NO_2^- oxidizers four weeks after treatment in the greenhouse. Nitrification Study III.

	Oxidizers		Oxidizers	
Source	NH_4^+	NO_2^-	NH_4^+	NO_2^-
	Run I		Run II	
Soil(S)	**	NS	NS	*
pH	*	*	***	NS
S X pH	NS	**	NS	NS
Inhibitor(I)	NS	NS	NS	NS
S X I	NS	NS	NS	NS
pH X I	NS	NS	NS	NS
S X pH X I	NS	NS	NS	NS

*, **, *** - Significant at 0.05, 0.01, and 0.001 respectively.
NS - Not Significant at the 0.05 level.

SUMMARY

Metalaxyl would not be expected to cause harmful reductions in the nitrification process at the application rate studied, in any of the soils studied, under any of the varying conditions of pH, temperature, or soil moisture studied. Nitrite values determined for the greenhouse experiments were excessively low indicating no apparent inhibition after formation of NO_2^-. Metalaxyl did not cause reductions in nitrification compared to the control based on NO_3^- accumulation. Nitrapyrin significantly reduced nitrification as compared to the control for up to four weeks providing an example of the effects of a marketed nitrificaion inhibitor.

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CHAPTER IV

Influence of Soil Type, Soil pH, and Metalaxyl Rate on Nitrification

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ABSTRACT

Metalaxyl, [N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester], is used extensively in tobacco (Nicotiana tabacum L.) production for prevention of black shank (Phytophthora parasitica Dast. var. nicotianae), blue mold (Peronospora tabacina Adam), and damping-off (Pythium spp.). Metalaxyl is also patented as a nitrification inhibitor, although not marketed for that purpose. Proper maturity and ripening of flue-cured tobacco depends on a declining supply of N after removal of the inflorescence. Use of a chemical which might prolong the availability of N after removal of tobacco inflorescence could delay maturity and reduce the quality of the cured leaf. These studies were conducted to determine whether metalaxyl might inhibit nitrification under a broad range of soil physical and environmental conditions prevalent in the tobacco producing areas of Virginia. The influence of soil type, soil pH, and metalaxyl rate on inhibition of nitrification were investigated in a Cecil sandy loam (clayey, kaolinitic, thermic Typic Hapludult), Appomattox fine sandy loam (clayey, mixed, thermic Typic Kandhapludult), and Mattoponi sandy loam (clayey, mixed, thermic Typic Hapludult) from the southern piedmont region of Virginia.

Nitrite and NO_3^- accumulations from four rates of metalaxyl (1, 5, 25, and 125 mg kg^{-1}) were compared with those of an untreated control and a nitrapyrin standard over a seven week soil incubation period in further studies using the same soils and adjusted pH levels. Significant NO_2^- accumulation occurred during the first week after treatment at high pH in all soil types, with 5, 25, and 125 mg kg^{-1} metalaxyl. Only the 125 mg kg^{-1} metalaxyl treatment caused NO_2^- accumulation at the high pH in all soils beyond the second week after treatment, with the peak occurring in most cases between weeks three and four. Nitrate accumulation proceeded normally in all soil types and pH levels except with metalaxyl treatments of 25 and 125 mg kg^{-1}. Nitrate accumulations with 25 mg kg^{-1} metalaxyl were similar to those for nitrapyrin. The 125 mg kg^{-1} rate was consistent in causing near total inhibition of NO_3^- accumulation at all pH levels in all soils. Nitrate accumulation tended to be lower at lower soil pH levels compared to the highest pH for all soils. Little difference due to soil appears to be evident. Use of metalaxyl at recommended rates of 0.25 to 1.5 mg kg^{-1} would not be expected to inhibit nitrification.

INTRODUCTION

Metalaxyl, [N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester], is an acylalanine fungicide specific for the control of fungi in the order Peronosporales. Current labeling provides for the use of metalaxyl in various formulations as a seed treatment, soil application, or foliar spray depending on the specific crop and disease to be treated. The Ridomil 2E formulation is recommended for the control of black shank (Phytophthora parasitica Dast var. nicotianae), blue mold (Peronospora tabacina Adam), and damping-off (Pythium spp.) in tobacco (Ciba-Giegy, 1984). Labeled rates provide for applications of 2.34 to 4.68 L $ha^{-1}$for blue mold control and 2.34 to 14 L ha^{-1} for black shank control as a preventative soil-incorporated treatment.

Laboratory studies using radiolabeled metalaxyl and a biologically active loamy sand showed relatively rapid degradation of metalaxyl under aerobic conditions. The half-life of metalaxyl under the laboratory conditions employed in this study was 41 d. In other bioassay studies where large numbers of soils were used, the half-life of metalaxyl in soil was 27 to 97 d (Ciba-Giegy, 1984).

Olin Corporation holds a U.S. patent on metalaxyl as a

nitrification inhibitor for soil or fertilizer application (Bashore and Lander, 1981). However, Olin indicated in patent documents that metalaxyl provided an average of 11% nitrification inhibition, compared to 37% for etradiozle, and 44% for another chemically related fungicide, following a 28 d soil incubation study.

The facts that metalaxyl is patented as a nitrification inhibitor and that metalaxyl is so widely used by tobacco producers have caused concern in the tobacco industry. Industry concerns relate to the level of inhibition expected from metalaxyl and the inhibition expected from the various rates of metalaxyl used. In a soil incubation study conducted at Penn State University, Ercegovich, et al. (unpublished data) found no nitrificidal effects of metalaxyl in either of two soil types at 5, 25, or 125 mg kg^{-1} metalaxyl. Metalaxyl at 125 mg kg^{-1} significantly retarded nitrification in a Hagerstown silt loam for up to four weeks, after which time there was no significant difference between the control and any of the three rates of application in this soil. A longer induction period than usual was required for nitrification to start in the Morrison soil under laboratory conditions. However, metalaxyl did not have an inhibitory effect on NO_3^- formation in the Morrison soil. Nitrification appeared to have been stimulated at 5 and 125 mg kg^{-1} of metalaxyl after 6 weeks of incubation. Personnel with Ciba-Geigy (1984) reported that in laboratory studies, the nitrification process in soil was significantly

retarded with 125 mg kg^{-1} of metalaxyl initially but recovered within 8 weeks. Other field and laboratory studies have demonstrated that metalaxyl inhibits the nitrification process in soil, but is not as effective as nitrapyrin. Two of 10 bacterial species tested showed significant growth rate reductions at 125 mg kg^{-1} and 4 species showed significant growth rate increases at 5 to 125 mg kg^{-1} (Ciba-Geigy, 1984).

Rideout (1986) found reductions in tobacco quality index and in the percentage of mature and ripe grades of tobacco in field experiments where metalaxyl was used in 1984, but not in 1985. Rideout and Jones (1987) reported reductions in NO_3^- accumulation with metalaxyl (1.12 kg ha^{-1}) in a soil incubation study in which there were no differences in NH_4^+ disappearance and no differences in pH. Ammonium oxidizer populations were reduced following application of metalaxyl, but not as drastically as those receiving nitrapyrin.

This study was conducted to determine the determine the influence of soil type, soil pH, and metalaxyl rate on nitrification.

MATERIALS AND METHODS

Soil Collection, pH Adjustment, and Storage

Surface (0 to 15 cm) soil samples representative of Cecil sandy loam (clayey, kaolinitic, thermic Typic Hapludult), Appomattox fine sandy loam (clayey, mixed, thermic Typic Kandhapludult), and Mattoponi sandy loam (clayey, mixed, thermic Typic Hapludult) used extensively for flue-cured tobacco production were collected from the Southern Piedmont region of Virginia. Soil samples were passed thru a 1 cm screen and stored at ambient temperature in plastic buckets until needed.

Each soil was divided in triplicate. Soil pH of one portion was adjusted up approximately one pH unit by gradually adding reagent grade $CaCO_3$ as the soil was mixed in a rotary cylinder mixer. A second portion was adjusted down approximately one pH unit by gradually adding a dilute solution of H_3PO_4 as the soil was mixed (Tennessee Valley Authority, 1976). The third portion of soil was also homogenized in the mixer, but the pH was not adjusted from the original field level. Following mixing, the three portions of each soil type were spread on plastic in a greenhouse and wetted. Each

portion was air-dried for three d, mixed, and wetted again. After three additional d of drying the soil was mixed and stored in plastic buckets at ambient temperature until the experiments were initiated.

Nitrification, as influenced by metalaxyl rate and nitrapyrin was determined in three soils (Cecil, Appomattox, Mattoponi). The moist equivalent of 100 g of oven dry soil at each pH was placed in 250 mL glass bottles. Aqueous solutions of $(NH_4)_2SO_4$ and metalaxyl or nitrapyrin were added to each soil sample to achieve rates of 100 mg N kg^{-1} soil; 0, 1, 5, 25, and 125 mg metalaxyl kg^{-1} soil; or 1 mg nitrapyrin kg^{-1} soil, respectively, and 60% of soil water-holding capacity (WHC). Bottles were capped to retain moisture and vented every 3 days to maintain an aerobic environment. Treated samples were incubated in thermostatically controlled chambers at 30°C for 50 days. Nitrite and NO_3^- were determined on 8 g samples drawn weekly from each experimental unit using an autoanalyzer system (Technicon Industrial Systems, 1977). The study was considered as a three-factor factorial experiment with two replications of each treatment combination. Selected properties and initial ionic nitrogen concentrations of adjusted soils prior to their use are presented in Table 1.

Analyses

Soil samples were frozen within 30 min of collection and stored frozen until analysis to prevent nitrification. Samples were allowed to warm to room temperature prior to analysis. Procedures used for determining soil N were outlined by Keeney and Nelson (1982). Nitrite and NO_3^- were determined with an autoanalyzer system (Technicon Industrial Systems, 1977) from 8 g samples after extraction with 80 mL 2N KCl shaken for 1 h.

Analyses of variance were performed on the data using the Statistical Analysis System (SAS Institute Inc., 1985). Least Significant Differences were determined for inhibitor rates at each weekly sampling. Regression and multivariate analyses were considered, but not used because of the primary interest in comparison of inhibitor rates at each weekly interval.

RESULTS AND DISCUSSION

Soil type X inhibitor and pH X inhibitor interactions were highly significant for NO_2^- and NO_3^- in almost every week when all soil types and pH levels were included in the analyses (Table 13). Significant pH X inhibitor interations were still present after analyses by soil type (Table 14). Nitrate values are plotted by pH for each soil type in Fig. 6, 7, and 8. Metalaxyl did not reduce NO_3^- accumulation at 1 or 5 mg kg^{-1} at any pH in any soil at any time during the study (Fig. 6, 7, and 8). Nitrate accumulation was very similar with 25 mg kg^{-1} metalaxyl to that with 1 mg kg^{-1} nitrapyrin in all soils at low and medium pH. Nitrate accumulation was reduced for one week by 25 mg kg^{-1} metalaxyl in all soils at high pH (Fig. 6, 7, and 8). However, NO_3^- levels in subsequent samples from high pH soils treated with 25 mg kg^{-1} metalaxyl were similar to the untreated control. Nitrate accumulation was totally inhibited by the 125 mg kg^{-1} treatment for seven weeks in all soil types at low and medium pH. The 125 mg kg^{-1} metalaxyl treatment suppressed NO_3^- accumulation in all soils at high pH until week 5.

Table 13. Significance of the F-test for main effects and interactions for three soils, three pH levels, and three inhibitor treatments by week.

Source	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-
Soil (S)	***	***	NS	*	***	*	***	**	*	***	NS	***	NS	***
pH	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Soil X pH	***	***	NS	**	***	***	***	***	***	***	*	***	NS	***
Inhibitor (I)	***	***	***	***	***	***	***	***	***	***	***	***	***	***
S X I	***	NS	NS	NS	***	***	***	NS	***	***	**	***	NS	***
pH X I	***	***	***	***	***	***	***	**	***	***	***	***	***	***
S X pH X I	***	***	NS	NS	***	*	***	NS	***	NS	**	*	NS	***

*, **, *** - Significant at the 0.05, 0.01, and 0.001 level, respectively.
NS - Not Significant at the 0.05 level.

Table 14. Significance of the F-test for main effects and interactions for three soils, three pH levels, and three inhibitor treatments by soil and week.

Source	Cecil		Appomattox		Mattoponi	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-
Week 1						
pH	***	***	***	***	***	***
Inhibitor (I)	***	***	***	***	***	***
pH X I	***	***	***	***	***	***
Week 2						
pH	NS	***	***	***	***	***
Inhibitor	NS	***	***	***	***	***
pH X I	NS	***	***	***	***	***
Week 3						
pH	***	***	***	***	***	***
Inhibitor	***	***	***	***	***	***
pH X I	***	***	***	***	***	***
Week 4						
pH	***	***	***	***	***	***
Inhibitor	***	***	***	***	***	***
pH X Inhibitor	***	NS	***	NS	***	***
Week 5						
pH	***	***	***	***	***	***
Inhibitor	***	***	***	***	***	***
pH X Inhibitor	***	*	***	NS	***	**
Week 6						
pH	***	***	NS	***	***	***
Inhibitor	***	***	NS	***	***	***
pH X Inhibitor	***	NS	NS	**	***	***
Week 7						
pH	NS	***	NS	***	***	***
Inhibitor	NS	***	NS	***	***	***
pH X Inhibitor	NS	NS	NS	***	***	***

*, **, *** - Significant at the 0.05, 0.01, and 0.001 level, respectively.
NS - Not Significant at the 0.05 level.

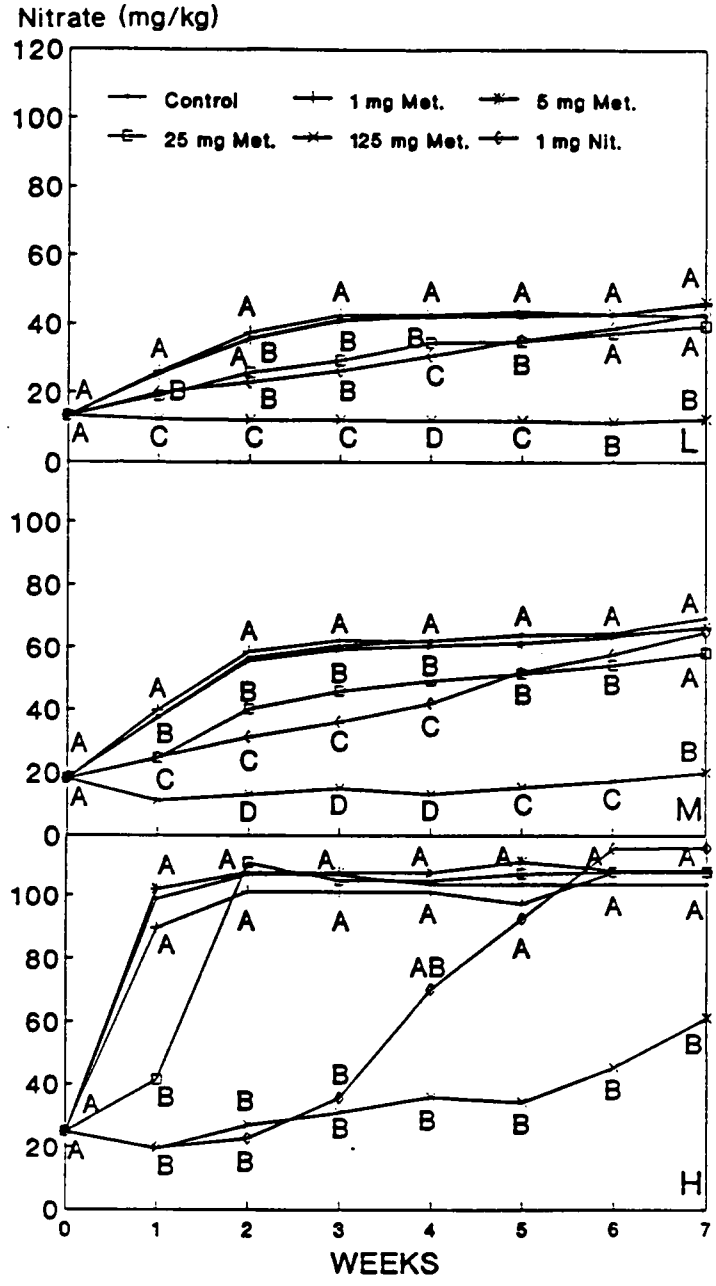

Fig. 6. Nitrate accumulation following applications of metalaxyl and nitrapyrin to Cecil soil at Low (L), Medium (M), and High (H) pH levels. Data points for the same week followed by the same letter do not differ by $LSD_{0.05}$.

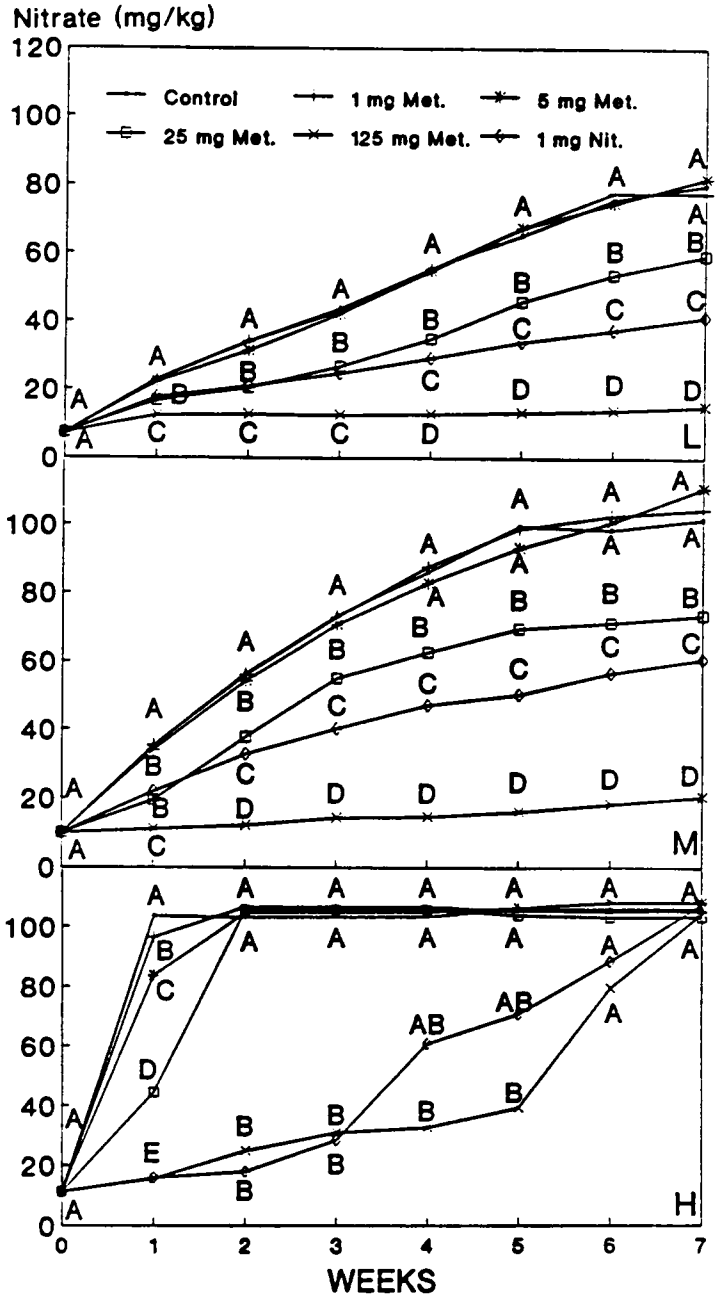

Fig. 7. Nitrate accumulation following applications of metalaxyl and nitrapyrin to Appomattox soil at Low (L), Medium (M), and High (H) pH levels. Data points for the same week followed by the same letter do not differ by $LSD_{0.05}$.

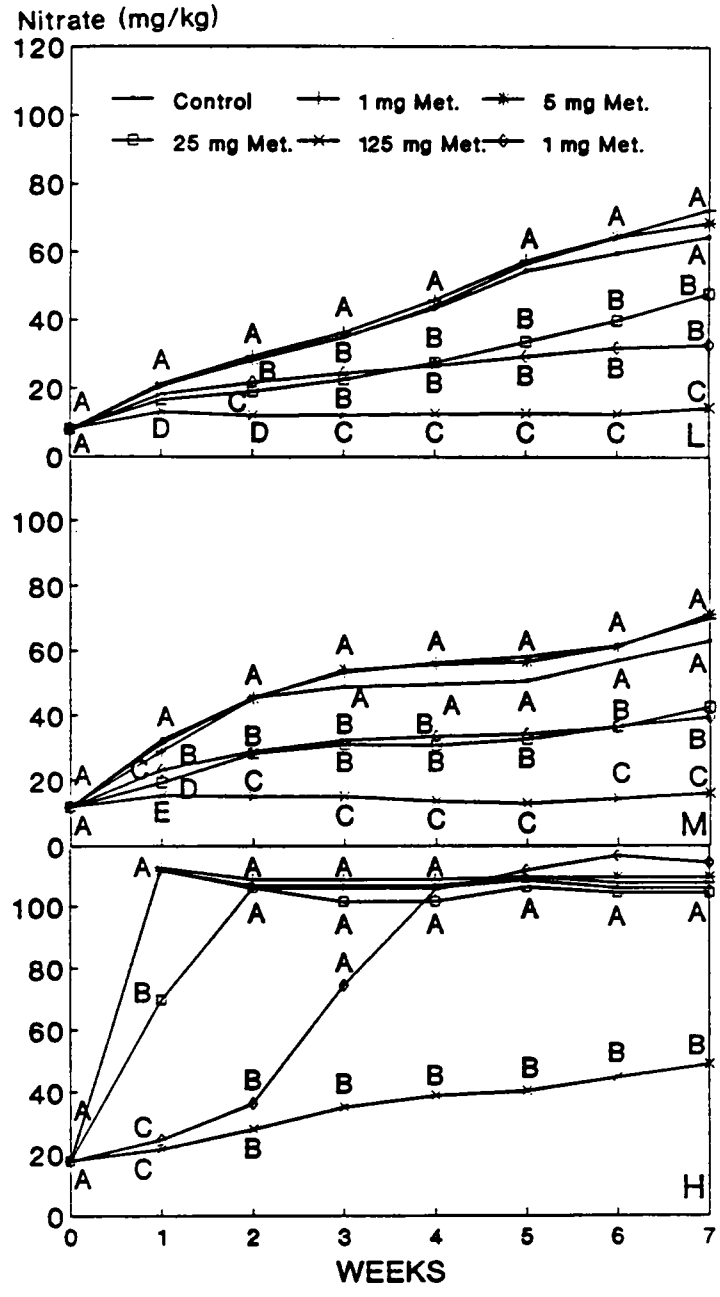

Fig. 8. Nitrate accumulation following applications of metalaxyl and nitrapyrin to Mattoponi soil at Low (L), Medium (M), and High (H) pH levels. Data points for the same week followed by the same letter do not differ by $LSD_{0.05}$.

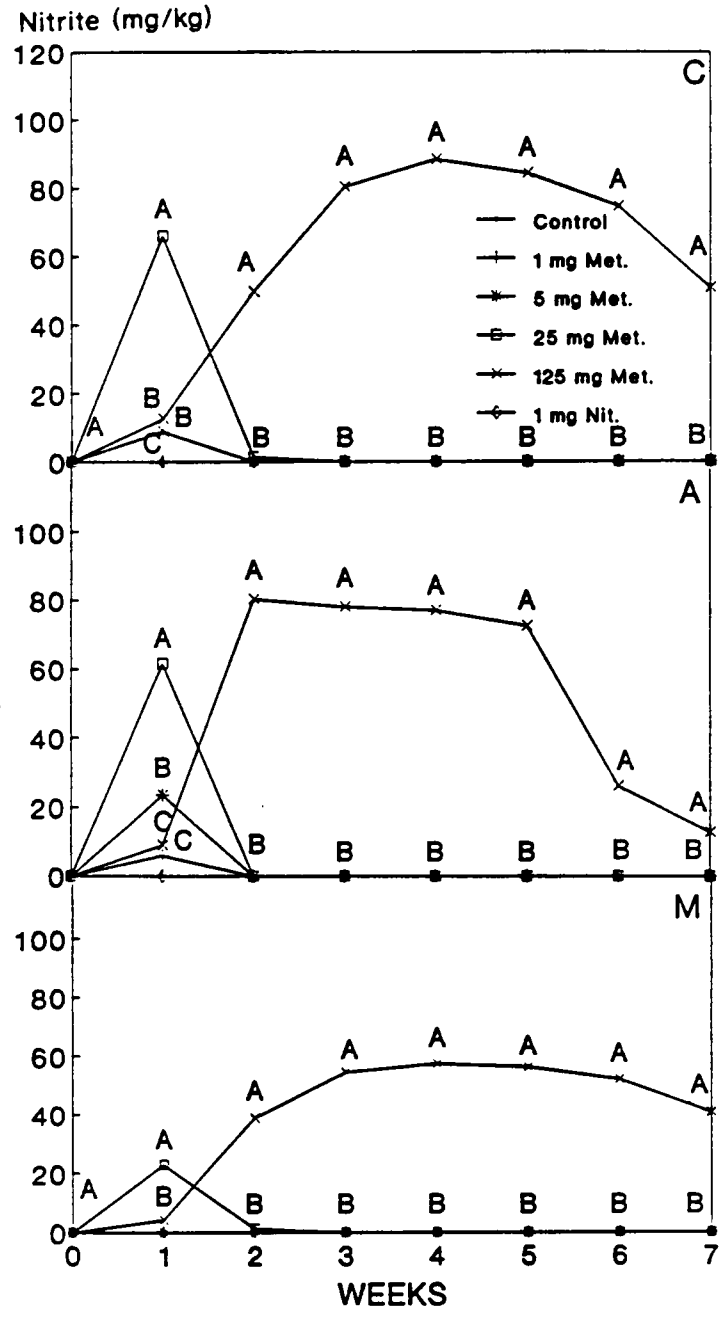

Fig. 9. Nitrite accumulation following applications of metalaxyl and nitrapyrin to Cecil (C), Appomattox (A), Mattoponi (M) soil at high pH. Data points for the same week followed by the same letter do not differ by $LSD_{0.05}$.

After two weeks, NO_2^- accumulantion was sonly significant following treatment with 125 mg kg^{-1} metalaxyl in all soils at high pH. After five weeks, NO_2^- accumulation was reduced as NO_3^- accumulation increased. Little or no accumulated NO_2^- was detected after 2 weeks of incubation with 5 and 25 mg kg^{-1} metalaxyl in Cecil and Appomattox soils at high pH or with 25 mg kg^{-1} metalaxyl in Mattoponi soil at high pH (Fig. 9). After three weeks of incubation, NO_3^- was only detected with 125 mg kg^{-1} metalaxyl.

Rates of 1 and 5 mg kg^{-1} metalaxyl were generally not effective in inhibiting nitrification. Short term inhibition of nitrification by metalaxyl occurred at high pH and 5 or 25 mg kg^{-1}. Inhibition was most likely occurring prior to the oxidation of NO_2^-. Longer term and more significant inhibition occurred at 25 and 125 mg kg^{-1} at low and medium pH in the three soils included in these studies. Metalaxyl should not have a negative impact on tobacco producers as a result of the chemical's somewhat limited abilities to inhibit nitrification. Maximum labeled rates would be equivalent only to 1.5 mg kg^{-1}, making application of enough metalaxyl to cause inhibition illegal. Application of enough metalaxyl to cause inhibition would not be economical from the producer's viewpoint as the cost for 1 mg kg^{-1} is approximately $130.00.

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