EFFECT OF SOIL TYPE ON TOBACCO SEEDLINGS AS INFLUENCED BY SOIL FUMIGATION, TEMPERATURE AND NITROGEN SOURCES[1]

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Tobacco transplant seedlings were grown for 30 days in fumigated and non-fumigated Cecil and Appling soil material in growth chambers at either 13, 21 or 29 C temperatures. The plants were fertilized with either KNO_3, $(NH_4)_2SO_4$, or NH_4NO_3. No abnormal growth characteristics were observed on the non-fumigated soils with any of the nitrogen sources used. Stunted plants with leaf abnormalities developed on the fumigated soils when ammonium was the nitrogen source; the abnormalities were not observed when nitrate was the nitrogen source and only slight abnormalities were observed when ammonium nitrate was used. Plants recovered more quickly from growth inhibition and leaf abnormalities at the higher temperatures. Greater total nitrogen uptake occurred on the Cecil soil and more nitrogen uptake by the plants occurred on the fumigated treatments.

INTRODUCTION

Soil-borne nematodes, parasitic to plants, are often controlled by preplant soil applications of volatile chemicals such as DD (mixture of 1,3-dichloropropene, 1,2-dichloropropane, and related chlorinated C_3 hydrocarbons) (20, 23). However, treatment of soils with such chemicals may cause changes in the composition, yield, and quality of crops (12, 20). In fumigated soils, changes in nutrient availability (16), production of possible toxic substance (19), and changes in microbiological balance resulting in accumulation of ammonium (20, 21) may be responsible for the adverse effects on plants. However, others (1, 3) have found a stimulation of plant growth after DD application which can be related to control of nematodes and increases in availability of certain nutrients.

Detection of extracted DD from fumigated soils failed when the extraction was delayed for 24 hours or more (15). Incubation studies (1) have shown that release of chloride in DD fumigated soils occurs rather slowly during the first ten days indicating the possibility of intermediate chloride containing products (5, 11) which may be phytotoxic (24).

Soil fumigation effectively alters the microbiological balance in favor of certain actinomycetes and fungi (28) while the nitrifiers are severely reduced in numbers (17, 28); this could lead to an accumulation of ammonium (20, 21). Accumulation of ammonium in the growth medium has been shown to adversely affect the growth and development of certain plants such as tobacco (14, 20, 22).

Due to the high sensitivity of *Nitrobactor* to DD (28) the possibility exists that nitrite may accumulate in fumigated soils. However, soils used in flue-cured tobacco production are usually acid and it has been shown that nitrite seldom accumulates in acid soils (28). Nitrite produced on nitrification will give rise to the formation of undissociated nitrous acid (HNO_2) when the concentration of hydrogen ions is high. Reaction of nitrous acid with soil organic matter (6, 13, 14, 25) or chemical decomposition (2, 3, 10) will result in gaseous loss of nitrogen. Although nitrous acid is more unstable the lower the soil pH, it has also been shown that in soils with lower pH values smaller nitrite concentrations are required to affect plant growth detrimentally (9).

The depression effect of soil fumigation with DD on the nitrification rate has been shown to last from 4-8 weeks under field conditions (30). This becomes of special importance in production of flue-cured tobacco where NH_4-N may be applied. However, the degree and length of nitrification inhibition varies widely with soil type, moisture content, temperature, rate of fumigation and other factors (4, 18, 28). The need thus arises to study the interactions of soil fumigation, soil type, and form of available nitrogen on plant growth under controlled conditions for short periods of time.

MATERIALS AND METHODS

Soil samples of the Ap horizons of two important tobacco producing soils (Appling and Cecil) in Virginia were air-dried, and crushed to a fineness of 4 mm or less prior to use in the growth chambers. Soils were thoroughly mixed and subsamples taken for fumigation. A fumigation treatment equivalent to 187 liters of DD per hectare was used with the untreated soil as check. Soils were fumigated at a moisture content of 75 percent of field capacity, stored for seven days at room temperature and then air-dried for a period of 21 days. Chemical analyses (according to Rich, 27) of the two soils are shown in **Table 1.**

Fifteen hundred grams of air-dried soil were used per pot and $CaHPO_4$ and K_2SO_4 applied at the equivalent of 220 milligrams P and 415 milligrams K per pot. Nitrogen as all nitrate (KNO_3), all ammonium ($(NH_4)_2SO_4$), or a 1:1 ratio of nitrate and ammonium

Table 1. Chemical analysis of Appling sandy loam and Cecil fine sandy loam

	Cecil fsl	Appling Sl
pH*	6.2	5.2
Organic Matter (%)	1.4	1.4
Total N (%)	0.080	0.073
	kg/ha	
Ca	1978	590
Mg	312	70
K	170	154
P	54	60

**1:1 soil-water ratio*

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Table 2. Effect of soil temperature, fumigation, form of applied nitrogen, and soil type on yield of young tobacco

Form of applied N	Soil type	Temperature and fumigation treatments 13C None	13C DD	21C None	21C DD	29C None	29C DD
				dry wt (g)			
NO_3	Cecil	0.62	0.45	1.67	1.82	2.44	2.86
	Appling	0.51	0.43	1.44	1.54	2.18	2.63
$1/2NO_3$-$1/2NH_4$	Cecil	0.61	0.48	1.63	2.10	2.39	2.85
	Appling	0.53	0.28	1.40	1.26	2.05	2.58
NH_4	Cecil	0.59	0.59	1.75	1.86	2.28	2.69
	Appling	0.45	0.26	1.27	1.67	1.94	2.32
Means for temperature†		0.48c		1.62b		2.43a	
Means for fumigation							
None		0.55a‡		1.53b		2.21b	
DD		0.41b		1.71a		2.65a	
Means for form of applied N							
NO_3		0.50a		1.62a		2.53a	
$1/2NO_3$-$1/2NH_4$		0.47a		1.60a		2.47a	
NH_4		0.47a		1.64a		2.31b	
Means for soil type							
Cecil		0.56a		1.80a		2.59a	
Appling		0.41b		1.43b		2.28b	

†*Means for temperatures within the row or*
‡*Means for other variables within columns not followed by common letters differ significantly (p = 0.05, Duncan's Multiple Range test).*

Table 3. Effect of soil temperature, fumigation, form of applied nitrogen, and soil type on total nitrogen content of young tobacco

Form of applied N	Soil type	Temperature and fumigation treatments 13C None	13C DD	21C None	21C DD	29C None	29C DD
				% N*			
NO_3	Cecil	1.62	2.20	1.34	1.57	0.96	1.18
	Appling	2.04	1.85	1.36	1.36	0.87	0.97
$1/2NO_3$-$1/2NH_4$	Cecil	1.70	1.90	1.22	1.43	0.94	1.11
	Appling	2.06	2.15	1.30	1.25	0.92	0.93
NH_4	Cecil	1.67	1.66	1.12	1.28	0.97	1.15
	Appling	1.91	1.74	1.18	1.53	1.11	1.04
Means for temperature†		1.88		1.33a		1.01b	
Means for fumigation							
None		1.83a‡		1.25b		0.96b	
DD		1.92a		1.40a		1.06a	
Means for form of applied N							
NO_3		1.93a		1.41a		1.00a	
$1/2NO_3$-$1/2NH_4$		1.95a		1.30a		0.98a	
NH_4		1.75a		1.28a		1.07a	
Means for soil type							
Cecil		1.79a		1.33a		1.05a	
Appling		1.96a		1.33a		0.97a	

**Average of four replications except with data obtained at 13 C since replications were composited before nitrogen analysis.*
†*Means for temperatures within the row or*
‡*Means for other variables within columns not followed by common letters differ significantly (p = 0.05, Duncan's Multiple Range test).*

(NH_4NO_3) was applied directly to each pot as a liquid. Nitrogen was applied at a rate equivalent to 37 kilograms nitrogen per hectare (185 mgN/pot). All fertilizer materials were mixed thoroughly with all soil in each pot and applied on the day of transplanting. Treatments were replicated four times in a completely randomized design (within temperatures).

Tobacco seedlings of the variety Coker 319 were grown in vermiculite until the two leaf stage with NH_4NO_3 as nitrogen source. The application of nitrogen was discontinued seven days before transplanting. Plants of uniform size were selected and one plant transplanted per pot. Soils were kept near moisture equivalent for the total growing period.

The seedlings were grown in three separate growth chambers with constant day and night temperatures and with a constant 16 hours of light (about 2500 foot-candles) per 24-hour period. Temperatures were 13, 21 and 29 C, respectively.

Top growth was harvested 30 days after transplanting. The harvesting date was chosen at a stage when plants in the 21 C chamber started to show nitrogen deficiency symptoms. Plant material was frozen, freeze-dried, weighed, ground to pass a 20 mesh screen, and analyzed for total nitrogen by Kjeldahl method using a digestion mixture as suggested by Peterson and Chesters (26). Plants grown at 21 C were also analyzed for chloride on an Aminco Cotlove chloride titrator.

The soil from each pot, passing a 2 mm sieve, was thoroughly mixed and subsampled for determination of inorganic nitrogen. Potassium chloride extractable ammonium, nitrate, and nitrite were determined (on the moist samples stored at −10 C) by an extraction-distillation method (7).

To investigate the effect of soil fumigation on added nitrite, 92μg NO_2-N per gram of dry soil was added to 10 gram samples of Appling (pH = 6.0) and the Cecil soil (pH = 6.6). Soils were fumigated with the equivalent of 925 liters DD per hectare at 70 percent of the field moisture capacity and stored for seven days before application of $NaNO_2$.

RESULTS AND DISCUSSION

Yield:

Dry weight yields of tobacco are presented in **Table 2.** Significant yield increases were obtained at higher temperatures. No significant differences in yields were found when plants were fertilized with either nitrate or half nitrate-half ammonium at lower temperatures but ammoninum fertilization resulted in signfiicantly lower yields at 29 C.

Higher yields were obtained from the Cecil soil at all three temperatures. Chemical analysis of soils before transplanting showed no differences in organic matter or inorganic nitrogen. However, the concentration of divalent bases was lower in the Appling soil leading to a lower soil pH. This might have had a direct effect on plant growth due to factors such as phosphorus fixation and higher solubility of sesquioxides. Indirect effects on nitrification due to lower soil pH might also have been involved.

Initial growth was more rapid with plants on non-fumigated soil than with the ones on fumigated soil. Plants on the fumigated soil fertilized with ammonium showed leaf abnormalites and dark green color. Recovery seemed to occur more rapidly on the Cecil soil than on the Appling. At harvest time higher yields were obtained from the plants on fumigated soil.

Analysis of soil before transplanting of tobacco showed increases in ammonium nitrogen and total inorganic nitrogen after fumigation. With nitrogen as a limiting factor in this experiment, higher yields were obtained on the fumigated soil which might have indicated the existence of higher level of available nitrogen.

Nitrogen Content of Plants:

Significantly lower nitrogen concentrations were found in the plants grown at 29 C than at 21 C (**Table 3**). Since nitrogen was limiting at the time of harvest this effect is directly related to the higher yields obtained at 29 C. Fumigation of soils led to higher nitrogen contents of plants grown at 21 C. At 13 C nitrogen was not limiting at time of harvest and a significant effect was found. It seems logical that fumigation might have led to more available nitrogen for plant growth since both yield and nitrogen content were higher on the fumigated soils.

A significant soil type X fumigation interaction was found on the nitrogen content of plants grown at 29 C. Although both soils contained the same percentage organic material, higher nitrogen concentrations were found in plants grown on the fumigated Cecil soil than on the Appling, while values were the same on the non-fumigated soils. The higher pH of the Cecil soil could have led to more nitrogen mineralization by the recovery microbial population after fumiga-

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tion (4). This nitrogen would then subsequently become available for plant growth on reduction of microbial numbers when food supply became limiting.

Nitrogen Uptake:

The effect of temperature, soil type, fumigation, and form of applied nitrogen on the amount of nitrogen taken up were significant (**Table** 4). Due to the limiting supply of nitrogen a very close relationship was found between yield and nitrogen uptake per pot.

Higher temperatures led to increased yield and nitrogen uptake at 21 C and 29 C, and significantly lower uptake at 13 C on the fumigated soils. The amounts of nitrogen removed by plants were lower when ammonium nitrogen was used as fertilizer than when nitrate nitrogen or ammonium nitrate was used at the 21 C temperature. No significant differences were found at other temperatures. The yield, however, showed only significant differences due to form of nitrogen applied at 29 C. Higher amounts of nitrogen were taken up at all three temperatures by plants grown on the Cecil soil. Yields showed the same general trend. The higher soil pH and fertility status seemed to enhance growth and thus nitrogen removal although amounts of fertilizer nitrogen were the same. This might have been due to a soil effect on nitrogen mineralization processes in the soil. A significant soil type X fumigation interaction was also found at all three temperatures (**Table** 5). On the non-fumigated soil nearly equal uptakes were recorded for both soils but on the fumigated soils constantly higher values were obtained for the Cecil soil. No significant interaction was found for yield between soil type and fumigation, while nitrogen content gave only significant differences at 29 C. This inidcates that although growth was not significantly restricted on Appling soil, the uptake of nitrogen was detrimentally affected by fumigation.

The enrichment of the fumigated soils with highly proteinaceous materials resulting from the killing of large numbers of microorganisms on fumigation is hypothesized to be responsible for the higher nitrogen uptake and yield on the fumigated soils. Only at 13 C where nitrogen was not limiting and a lower yield was obtained on the fumigated soil due to poor growth, did the nitrogen uptake on the non-fumigated soil exceed that on the fumigated. The effect of nematode injury of plants on the non-fumigated soil can be ruled out since nematode essays at the end of the growing period showed populations to be very small in the fumigated as well as non-fumigated soil.

Uptake of nitrogen on the fumigated Cecil was higher than on the fumigated Appling soil while uptake on the non-fumigated soils was nearly equal. This indicates that higher amounts of mineral nitrogen became available in the Cecil soil after fumigation and/or that nitrogen losses following fumigation were larger on the more acid Appling soil. Since nitrite accumulation is not a prerequisite for volatilization of nitrogen in acid soils (13) such losses might have involved reactions between traces of nitrite present in the fumigated acid soil and organic matter or ammonium released on ammonification. Volatilization of nitrogen would also explain why less nitrogen was taken up by plants grown at 21 C when NH_4-N was applied (**Table** 4). The absence of such an effect at 29 C while a lower yield was simultaneously obtained with the ammonium treatments needs further investigation.

The results of the nitrite incubation study are presented in **Table 6**. The rate of NO_2-N disappearance was very rapid and occurred essentially at the same rate in both fumigated and non-fumigated soil although a faster rate of disappearance was observed in the non-fumigated Appling soil beginning 12 hours after application. In accord with other workers (27) very little conversion of NO_2 to NO_3 occurred in either the fumigated or non-fumigated soils. However, the rate of NO_2 disappearance was faster in both the fumigated and non-fumigated Appling soil than in the Cecil with a higher pH. This indicates that NO_2 conversion in acid soils may involve non-biological reactions with the loss of NO_2-N as gaseous products, presumably as N_2, NO_2, and N_2O (8, 10, 25). Higher rates of NO_2 conversion were obtained when non-fumigated soils were incubated at 29 C indicating that initial nitrifier population might have been too low in both soils to be effective over such a short period and low incubation temperature (21 C).

Table 4. Effect of soil temperature, fumigation, form of applied nitrogen, and soil type on nitrogen uptake from soil by young tobacco

Form of applied N	Soil type	Temperature and fumigation treatments 13C None	13C DD	21C None	21C DD	29C None	29C DD
		N uptake (mg)					
NO_3	Cecil	10.0	9.9	22.1	28.0	23.3	33.7
	Appling	10.4	8.0	19.6	19.9	19.1	25.4
$1/2NO_3$-$1/2NH_4$	Cecil	10.4	9.1	19.5	29.9	22.1	31.6
	Appling	10.9	6.0	17.9	20.7	18.3	24.1
NH_4	Cecil	9.9	9.8	19.1	24.2	21.8	30.5
	Appling	8.6	4.5	14.9	18.6	21.4	23.8
Means for temperature†		9.0		21.2b		24.6a	
Means for fumigation							
None		10.0a‡		18.8b		21.0b	
DD		7.9b		23.6a		28.2a	
Means for form of applied N							
NO_3		9.6a		22.4a		25.4a	
$1/2NO_3$-$1/2NH_4$		9.1a		22.0a		24.0a	
NH_4		8.2a		19.2b		24.4a	
Means for soil type							
Cecil		9.9a		23.8a		27.2a	
Appling		8.1b		18.6b		22.0b	

†*Means for temperatures within the row or*
‡*Means for other variables within columns not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test).*

Table 5. Soil type X fumigation interactions* illustrated by nitrogen uptake

Soil temperature	Fumigation treatment	Soil type Cecil fsl	Appling sl
		N uptake/pot (mg)	
13 C	None	10.1	10.0
	DD	9.6	6.2
21 C	None	20.2	17.5
	DD	27.4	19.7
29 C	None	22.4	19.6
	DD	31.9	24.4

**Interactions were significant ($p = 0.05$) at all three temperatures.*

Table 6. Effect of soil fumigation on recovery and conversion of nitrite nitrogein added to two acid soils incubated at 21 C

Time (hours)	Fumigated NO_2-N (ppm)	Fumigated NO_3 + NH_4-N (ppm)	Non-fumigated NO_2-N (ppm)	Non-fumigated NO_3 + NH_4-N (ppm)
		Appling sandy loam		
Initial	<1*	26	<1	28
0	75	38	81	41
3	78	31	77	31
6	70	27	68	29
12	62	39	57	43
24	61	36	45	40
48	52	36	36	39
96	16	41	15	38
		Cecil fine sandy loam		
Initial	<1	22	<1	17
0	84	28	85	22
3	82	25	83	24
6	73	29	76	25
12	70	31	69	31
24	67	26	68	26
48	58	31	60	27
96	32	29	39	33

**All values represent the average of at least two determinations.*

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SUMMARY

A growth chamber experiment was conducted to determine the effect of soil fumigation on the relative efficiency of different forms of nitrogen on the growth and nitrogen uptake by young tobacco grown on different soils and at different temperatures. Examination of the results showed:

(1) that plants grown on fumigated soil fertilized with ammonium nitrogen were stunted and developed leaf abnormalities within a period of one week after transplanting;

(2) that plants fertilized with nitrate nitrogen were normal in appearance while only slight leaf abnormalities could be observed when ammonium nitrate was applied;

(3) that normal plant growth was obtained on non-fumigated soils independently of the form of applied nitrogen;

(4) that higher temperatures led to shorter periods of growth inhibition and a quicker disappearance of leaf abnormalities;

(5) that higher amounts of nitrogen were absorbed from the fumigated soils;

(6) that soil type influenced nitrogen uptake but uptake was also dependent upon the fumigation treatment.

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