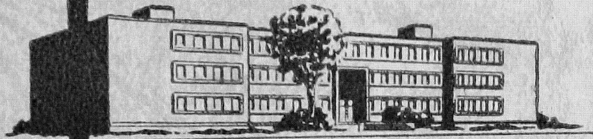


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FACTORS AFFECTING THE NICOTINE CONTENT
OF FLUE-CURED TOBACCO

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Joint Contribution from the Department of Agronomy and
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INTRODUCTION

J. A. Weybrew

There are many and varied psychological reasons why people smoke, but the physiological basis for smoking is the stimulation derived from the nicotine absorbed from tobacco smoke. Thus it is to its alkaloid nicotine that tobacco owes its place in our everyday contentment.

Along with his discovery of the West Indies, Columbus found the Indians smoking the cured aromatic leaves of the plant, now called tobacco. Thus the white man not only learned the smoking habit from the Indians but copied their forms of usage as well, in pipes, as cigars, and even snuff. The cigarette is a relatively modern innovation adopted from the Turks at the time of the Crimean Wars. Within a very few years following the close of the Civil War, a new industry for the production of "tailor-made" cigarettes in the United States was born. The first blended cigarettes were introduced about 40 years ago.

Beginning about 1920, the trend in smoker preference has been toward a milder smoking, lower nicotine cigarette. Today cigarettes and, for that matter, all other tobacco products sold on the American market, contain remarkably close to 2 per cent nicotine.

Through a combination of elaborate blending technology and stringent laboratory control, tobacco companies exercise extreme vigilance to insure the day to day and year to year constancy of their products. Vast quantities of tobaccos representing many farms in each of the several growing areas and including two or more crops are all

blended together as a means of minimizing any localized aberrant quality characteristics. Then the four types of tobacco--flue-cured, burley, Turkish, and Maryland--are blended together to make the modern domestic cigarette. Of these types, flue-cured and burley in the approximate proportion of two to one comprise about 90 per cent of the mixture.

Cigarette grades of burley tobacco usually contain about 2 to $3\frac{1}{2}$ per cent nicotine as compared with $1\frac{1}{2}$ to $2\frac{1}{2}$ per cent for the cigarette grades of most flue-cured tobaccos. It can be seen, therefore, that the nicotine contents of our tobaccos leave little latitude in the blending formulations if they are to provide a cigarette that meets with the consumers' desires.

Nicotine is synthesized in the roots of the tobacco plant. The starting materials for its production are relatively simple organic materials photosynthesized in the leaves plus simple forms of nitrogen absorbed from the soil. Once formed, nicotine is transported to the leaves and stems where it accumulates. It is to be expected therefore that a great many climatological and cultural factors, particularly those which would result in a disproportion between the relative amounts of synthesizing roots and storage tops, would likewise affect the nicotine contents of the leaves.

During the course of each year, a great many experiments with tobacco are conducted by the North Carolina Agricultural Experiment Station. All have as their broad objective the production of more tobacco of finer quality and at a lower cost. An important part of the evaluation of the outcome of these experiments is the analyses of these tobaccos for nicotine and certain other quality constituents.

Because of the intimate relationship between nicotine content and the usability of tobacco, this wealth of accumulated data has been brought together for critical study to ascertain what experimental treatments are associated with changes in the nicotine levels of tobacco. This compendium is the result.

VARIETIES

G. L. Jones and T. J. Mann

The ability to synthesize nicotine or related alkaloids is a heritable peculiarity of the genus Nicotiana. Within the genus, certain species, notably N. tabacum (tobacco), produces mostly nicotine as its alkaloid. Others, such as N. sylvestris, contain the closely similar alkaloid nornicotine almost exclusively, whereas anabasine is the predominant alkaloid of N. glauca.

The general level of alkaloid production is also genetically controlled; N. rustica often contains several times as much nicotine as do flue-cured varieties of N. tabacum. Thus, despite a close similarity in the pedigrees of most of the common flue-cured varieties, the minor variations that do exist might be expected to be reflected in small but persistent differences in the level of nicotine production.

Table 1 compares the nicotine contents^{1/} of eight varieties of flue-cured tobacco that had been grown together at several of the Branch Experiment Stations or Research Farms in the tobacco growing

^{1/} The nicotine data throughout this paper were obtained by a modification of the Garner titrimetric method on composite samples representing all harvestable leaves on the plant with their midribs removed.

areas of the State over a three year period. Even though the general level of nicotine fluctuated somewhat from year to year, the relative rank of any particular variety in relation to the others remained remarkably constant.

Table 1. Comparison of the nicotine contents of eight varieties of flue-cured tobacco.

Variety	1947 (2 locations)	1948 (4 locations)	1949 (6 locations)	Variety Average
Per Cent Nicotine				
Vesta 30	2.80	3.96	2.96	3.26
Oxford 1	2.99	3.75	2.82	3.16
Gold Dollar	2.68	3.59	2.72	3.00
402	2.40	3.34	2.64	2.83
Yellow Special	2.39	3.16	2.64	2.77
Oxford 26	2.33	3.16	2.36	2.62
Vesta 47	2.32	3.24	2.28	2.61
Bottom Special	2.32	2.74	2.33	2.46
Crop Mean	2.53	3.37	2.59	
			LSD (5%)	.23
			(1%)	.32

Several years ago, tobacco breeders intensified their efforts toward developing new varieties of tobacco which would be resistant to the then-localized but now widespread and devastating diseases, Granville wilt and black shank. Early screenings showed that the common varieties of flue-cured tobacco carry little or no resistance to these diseases. It became necessary, therefore, to cross tobacco with certain strains not of the flue-cured type in order to transfer factors for disease resistance.

Generally, when foreign introductions are made to acquire one particular trait, other characteristics may be transferred also.

Depending on their relative complexity of inheritance and ease of recognition, certain of these associated traits can be "bred-out" in subsequent generations while others may be retained.

Nicotine level is a quality index that is carefully watched in the plant breeding program. Table 2 contrasts the nicotine contents of three of the Dixie Bright varieties with five non-resistant varieties at several locations for three years. The resistant varieties average about one-half of one per cent less nicotine than do the older lines.

Table 2. Comparison of the nicotine contents of disease resistant and non-resistant varieties of flue-cured tobacco.

Variety	1949 (6 locations)	1950 (4 locations)	1951 (3 locations)	Variety Average
Non-resistant varieties:				
	Per Cent Nicotine			
Gold Dollar	2.72	2.24	2.87	2.61
402	2.64	2.13	2.67	2.49
Hicks	2.38	1.98	2.75	2.34
Va. Gold	2.31	1.96	2.58	2.27
Bottom Special	2.33	1.81	2.20	2.14
Mean	2.48	2.02	2.61	2.37
Resistant Varieties^{2/}				
Dixie Bright 101	2.11	1.75	2.08	1.99
Dixie Bright 102	2.02	1.62	2.14	1.92
Dixie Bright 27	1.86	1.67	2.03	1.84
Mean	2.00	1.68	2.08	1.92
			LSD (5%)	.17
			(1%)	.23

^{2/} Dixie Bright 27 and Dixie Bright 28 are resistant to Granville wilt only. Dixie Bright 101 and 102 are resistant to both Granville wilt and black shank.

In the preceding tables, the data from the experiments conducted at the several locations have been averaged together. In such a tabulation, the observed differences in nicotine contents from one crop to the next can thus be ascribed to climatological factors primarily, of which rainfall is of the greatest importance. In Table 3 the nicotine contents of seven varieties are contrasted at each of four experimental sites representing the four flue-cured belts of North Carolina; viz., Border Belt (Whiteville), Eastern (Rocky Mount), Middle (McCullers), and Old Belt (Rural Hall). Since these experimental locations are somewhat separated geographically, the differences in nicotine contents observed for the same variety from one experiment to the next are the combined effects of soil and weather factors.

Table 3. Nicotine contents of seven varieties of flue-cured tobaccos grown at four locations. (Average of 1949 and 1950 crops).

Variety	Experimental Sites				Variety Average
	Whiteville	Rocky Mount	McCullers	Rural Hall	
	Per Cent Nicotine				
Gold Dollar	2.10	2.13	2.26	3.51	2.50
402	2.03	2.13	2.26	3.13	2.39
Hicks	1.92	1.80	1.99	3.04	2.19
Bottom Special	1.80	1.55	1.82	3.02	2.05
Dixie Br. 101	1.97	1.57	1.60	2.55	1.92
Dixie Br. 102	1.58	1.49	1.68	2.52	1.82
Dixie Br. 27	1.30	1.53	1.65	2.25	1.68
Location Means	1.81	1.74	1.89	2.86	

Despite differences in weather conditions (crop years) and location, varieties retain their relative rankings, one with the other, in nicotine content; the inherent ability to produce nicotine supersedes climatological and soil factors.

FERTILIZATION

W. G. Woltz and T. B. Hutcheson, Jr.

As indicated in the introduction, any circumstance which would markedly alter the root-top ratio of tobacco might be expected to change the nicotine concentration in the cured leaves.

Except at critical levels of deficiency or, on the other extreme, at toxic concentration, the ordinary elements of common fertilizers, nitrogen excepted, do not greatly influence nicotine accumulation in tobacco. Nitrogen, being an integral constituent of the alkaloid molecule, might be expected to be related to nicotine elaboration.

A great many fertility experiments with tobacco have been conducted. The results, in terms of nicotine contents, are not always interpretable and are very often contradictory. Several factors complicate the evaluation of such tests. Cognizance must be taken of the native fertility of the soil or, more particularly, of any residual effects from the preceding crop. In addition, weather, especially rainfall, may seriously alter the availability of particular plant nutrients.

Over a period of three years, comprehensive experiments with Oxford 26 tobacco were conducted on the sandy loam soils at Rocky Mount and at Oxford in which varying amounts of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and chlorine were applied. Of these, only the increasing nitrogen applications were directly and

significantly accompanied by increasing nicotine concentrations in the cured tobacco. No consistent influence on nicotine content which could be attributed to variations in the phosphorus, potassium, calcium, magnesium, sulfur, or chlorine fertilization was demonstrable in these and supplementary experiments. The pertinent data are abstracted in Table 4.

Table 4. Average nicotine contents of Oxford 26 tobacco differentially fertilized with nitrogen and potash. (Sandy loam soils at Oxford and Rocky Mount, 1946-48)

Nitrogen Fertilization (Pounds Per Acre)	Potash Fertilization (lb/A)			Nitrogen Average
	30	90	150	
	Per Cent Nicotine			
0	1.41	1.31	1.28	1.33
30	----	1.89	----	1.89
60	2.98	2.80	2.79	2.86
Potash Means	2.20	2.00	2.04	
			LSD (5%)	.16
			(1%)	.21

Other experiments on a clay loam soil at Rural Hall showed a similar linear relationship between nicotine accumulation and nitrogen fertilization. These data are summarized in Table 5. Since different varieties were grown in the different years, neither varietal nor crop comparisons can validly be made.

Growth of the tobacco plant as well as its nicotine accumulation is regulated more by the nitrogen supply than any other plant nutrient. This parallelism between yield and nicotine does not continue ad infinitum. Above a certain level, depending on the soil type and other factors, further additions of nitrogen no longer increase yield where-

as nicotine production continues. Thus excessive nitrogen fertilization may result in a more than proportionate concentration of nicotine.

Table 5. Average nicotine content of tobacco, differentially fertilized with nitrogen, on a Cecil clay loam. (Rural Hall, 1948-50)

Nitrogen Fertilization (Pounds Per Acre)	Variety and Crop Year			Mean Nitrogen Effect
	400-1948	D.B. 102-1949	D.B. 101-1950	
	Per Cent Nicotine			
10	3.02	1.54	1.52	2.03
30	3.69	2.02	1.76	2.49
50	4.19	2.54	2.08	2.94

Many of the so-called minor elements are constituents in, or at least associated with, the activity of vital enzyme systems. Minor element studies with tobacco are continuing. To date, no effect on the nicotine content of the cured leaves has been exhibited in fifteen experiments with boron and four experiments involving several rates of application of copper, zinc, manganese, or molybdenum.

SOIL MANagements FOR NEMATODE CONTROL

C. J. Nusbaum

Many of the tobacco soils of North Carolina are now heavily infested with one or more species of nematodes. Certain of these parasitize the roots of tobacco and the resulting gall formation has given rise to the descriptive term "root knot". Others such as the meadow nematode, feed in root tissues and induce decay. Although nematodes seldom kill the plant, very often root damage is so severe as to impair absorption and translocation with the result that yields are greatly reduced.

Crop rotations in which at least one of the alternate crops is an uncomplimentary host for the nematodes are being practiced as a prophylactic means of discouraging population build-ups. The average nicotine contents of two recent crops of tobacco produced in several two- and three-year rotations at the McCullers Station are shown in Table 6.

Table 6. Average nicotine contents of flue-cured tobacco in various crop rotations. (McCullers, 1948 and 1951)

Alternate Crops	Per Cent Nicotine	Average Difference from Continuous Tobacco
<u>Two-Year Rotations:</u>		
Tobacco	2.48	0
Cotton	2.09	- 0.39
Peanuts	2.33	- 0.15
Corn	2.79	+ 0.31
Weeds	2.65	+ 0.17
Oats & Weeds	2.85	+ 0.37
Crotalaria & Ryegrass	3.05	+ 0.57
<u>Three-Year Rotations:</u>		
Tobacco; Tobacco	2.02	0
Peanuts; Cotton	1.95	- 0.07
Cotton; Peanuts	1.99	- 0.03
Corn; Cotton	1.99	- 0.03
Cotton; Weeds	2.22	+ 0.20
Corn; Oats & Weeds	2.27	+ 0.25
Peanuts; Oats & Weeds	2.25	+ 0.23
Weeds; Weeds	2.66	+ 0.64

The interpretation of these results is not simple. In contrasting rotations with the continuous tobacco sequence, consideration must be taken of the degree of nematode control or, conversely, of the severity of root injury, as well as the ultimate yields of the tobacco crops. Under continuous tobacco, root-knot damage is extremely severe even to the point that nicotine synthesis may be reduced. However, if growth is also restricted proportionately, no change in the nicotine content

tration will be evident.

Some degree of nematode control was effected by all of the rotations; control was quite satisfactory in the three year systems. The differences in the nicotine contents between the various rotations very probably reflect the carry-over fertility effects from the preceding crop.

Winter cover crops are often planted in tobacco fields. The nicotine contents of Dixie Bright 101 tobacco following certain winter management practices are given in Table 7. The significant increases in nicotine are invariably associated with preceding managements involving either a legume or an application of urea and hence can be explained on the basis of nitrogen fertility.

Table 7. Nicotine contents of Dixie Bright 101 tobacco following various winter cover crop treatments. (McCullers, 1951)

Winter Treatment	Per Cent Nicotine	Increase Over No Cover
No cover crop	1.80	0
Rye	1.90	+ 0.10
Rye + 75 lbs. urea/A.	2.20	+ 0.40
Rye + 100 lbs. urea/A.	2.35	+ 0.55
Rye + 150 lbs. urea/A.	2.51	+ 0.71
Rye & Vetch	2.40	+ 0.60
Vetch	2.88	+ 1.08

Certain fumigants are now available which, when properly applied to the soil, are effective as a nematode control measure. The residual effects of these chemicals on a succeeding tobacco crop are being studied. Table 8 shows no increase in nicotine attributable to the use of either dichloropropene mixture (DD) or ethylene dibromide (EDB) formulations in six experiments.

Table 8. Effect of soil fumigants on the nicotine content of tobacco (Six experiments)

Fumigation Treatment	Average Increase in Per Cent Nicotine Over Non-Fumigated Checks
<u>Dichloropropene (DD):</u>	
20 gal/acre, broadcast	+ 0.09
10 gal/acre, row application	+ 0.02
<u>Ethylene Dibromide (EDB):</u>	
Dowfume W-40, 20 gal/acre, broadcast	- 0.02
Dowfume W-40, 15 gal/acre, broadcast	- 0.09
Dowfume W-40, 10 gal/acre, row application	- 0.06
Dowfume W-40, 8 gal/acre, row application	+ 0.10
Dowfume W-85, 4½ gal/acre, plow sole	+ 0.02

PLANT POPULATIONS

W. G. Woltz and C. H. M. van Bavel

When crowded together in dense stands, the leaves of tobacco plants do not attain full expansion. In addition, competition for moisture and plant nutrients, nitrogen in particular, might be expected to alter the level of nicotine accumulation. The effect of spacing on the nicotine content of flue-cured tobacco is given in Table 9. The lower nicotine concentrations at the closer spacings would appear to reflect competition for the nitrogen supply.

Table 9. The effect of spacing on the nicotine content of flue-cured tobacco.

Spacing in Row (inches)	Plant Populations (Plants/Acre)	Per Cent Nicotine
<u>Oxford, 1948, Variety Oxford 26:</u>		
12	12450	1.52
24	6225	1.61
36	4150	1.62
<u>Whiteville, 1949, Variety 402:</u>		
14	10675	1.24
18	8295	1.38
22	6785	1.39

As shown in Table 10, under conditions of adequate moisture supply (irrigation) and when the nitrogen application is increased proportionately, the nicotine content of Dixie Bright 101 is as high or slightly higher when the stand density is increased by 55 per cent as compared with normal spacing.

Table 10. The nicotine content of different populations of Dixie Bright 101 under conditions of adequate supply of nitrogen and soil moisture. (Oxford, 1952)

Plant Populations (Plants/Acre)	Nitrogen Fertilization ^{*/}	
	Normal	Plus 1/3
	Per Cent Nicotine	
6800	1.78	1.81
10500	1.94	1.89

*/ The normal rate of fertilization in this experiment was 36 pounds of nitrogen per acre for 6800 plants; rate of application increased proportionately for the higher populations.

TOPPING AND SUCKERING

W. G. Woltz

Tobacco has a determinant growth habit in that, once the flower primordium appears, any further development is directed toward the production of a seed head or, if the flower has been broken out, to the growth of axillary suckers. Thus the new growth competes with the still-expanding upper leaves for the nitrogen and carbohydrate reserves. Topping and suckering, as commonly practiced, effectively removes this competition and permits the top leaves to attain full size. These primings necessitate, however, that all of the nicotine being synthesized by the roots is accumulated in the expanding leaves rather than being diluted out into the extra sucker growth.

This concentration of the nicotine stores is effectively demonstrated in Table 11 which shows the effect of allowing the seed head to develop as contrasted with toppings at different heights (leaf numbers), followed by complete removal of suckers, on the nicotine contents of Oxford 26 tobacco.

Table 11. Effect of height of topping on nicotine accumulation in Oxford 26 tobacco. (Oxford, 1948)

Topping Height	Per Cent Nicotine
Not topped	1.49
20 leaves	1.61
10 leaves	2.64

The additive effects of early topping followed by various degrees of sucker control on the nicotine accumulation in Dixie Bright 101 tobacco are given in Table 12. The nicotine concentration is clearly related to the severity of the pruning. Late topping of a fully developed flower head followed by a vigorous sucker development resulted in the lowest nicotine content in the harvestable leaves. Conversely, early topping with complete control of suckers, gave the highest nicotine concentration.

Table 12. Effects of topping times and various degrees of sucker control on the average nicotine concentration in Dixie Bright 101 tobacco. (Four experiments, 1950-51)

Topping Time */	Sucker Control		
	Completely Suckered	Suckered Once	Not Suckered
	Per Cent Nicotine		
Normal	2.11	1.98	1.97
Late		1.88	1.75
Not topped			1.80
		LSD (5%)	0.22
		(1%)	0.30

At Rural Hall and Oxford in 1950, and at Rocky Mount and Oxford in 1951.

*/ Normally topped high when 5 to 10 flowers showed pink; late topping two weeks later when flower was fully developed.

MOSAIC

C. J. Nusbaum

By comparison with black shank or Granville wilt which frequently results in a total loss of a crop of tobacco, mosaic, particularly in the usual situation in which the spread of the infection occurs late during the topping and suckering operations, does not appreciably decrease yields and reduces selling price only slightly. The propagating virus must, of necessity, compete for the nitrogen and carbohydrate reserves and might also interfere with the metabolism of the host plant. Table 13 shows the effect of artificial inoculations of mosaic virus on the nicotine contents of the flue-cured tobacco. The consistently lower nicotine concentrations of mosaic infected tobaccos is somewhat surprising. The lowered nicotine as the result of a systemic infection induced by early inoculation might well be explained on the basis of competition or translocative interference. The same reduction, however, resulted following late inoculation; this raises the interesting speculation that nicotine per se might be utilized in the multiplication of the virus.

Table 13. The effect of artificially induced mosaic infections on the nicotine contents of flue-cured tobacco. (McCullers, 1948, 1951)

Treatment	Average Nicotine Content (Per Cent)	Ave. Decrease from Non-infected Check
No mosaic	3.08	----
Inoculated early June	2.89	0.19
Inoculated late June	2.86	0.24
Inoculated at topping	2.88	0.20

SOIL MOISTURE

C. H. M. van Bavel

Probably the most important single factor affecting the growth of a tobacco crop and its nicotine content is soil moisture. It has been known for a long time that the nicotine level in dry-weather tobacco is considerably higher than in a crop produced under more favorable moisture conditions.

In earlier sections of this report, year to year differences in the nicotine contents of tobaccos have been noted and these have been attributed largely to differences in rainfall. Under natural conditions, where control over the volume, time, or intensity of the water application is lacking, it becomes extremely difficult to assess the specific effects of soil moisture on nicotine accumulation. Certain valuable information can, however, be gained from such experiments. The "soil moisture index" is a crude approximation of the moisture supply of a crop; it is obtained by subtracting, from the total rainfall over the $2\frac{1}{2}$ month growing season, the maximal accumulative losses from evaporation and transpiration. These moisture indices have been correlated with the nicotine contents of the tobaccos produced in variety comparisons at five locations over the six-year period, 1946-51. Regression and correlation coefficients are given in Table 14.

Varieties respond quite differently in nicotine elaboration under different soil moisture conditions. Dixie Bright 101 which is inherently low in nicotine is also influenced least by seasonal differences. On the other hand, Hicks is normally relatively high in nicotine and is markedly affected by the moisture supply. Considering all varieties, each one-inch increase in soil moisture index can be expected to be accompanied by a decrease of 0.07 per cent nicotine.

Table 14. Correlations between soil moisture indices and the nicotine contents of flue-cured tobaccos. (Five locations, 1946-51)

Variety	Regression Coefficient	Correlation Coefficient
Hicks	- 0.101	- 0.823
Oxford 1	- 0.096	- 0.666
Gold Dollar	- 0.074	- 0.653
Oxford 26	- 0.073	- 0.712
Dixie Bright 27	- 0.070	- 0.853
402	- 0.057	- 0.566
Dixie Bright 102	- 0.040	- 0.566
Dixie Bright 101	- 0.019	- 0.240
	LSD (10%)	0.040
	(5%)	0.070

Irrigation provides a means for studying directly the effects of a regulated moisture supply on the yield and quality of tobacco. The diminution of the nicotine when moisture is more plentiful is summarized in Table 15. The contrast, with respect to nicotine, between the unirrigated tobaccos and those adequately supplied with water was much greater in the 1951 experiment than in 1952 largely because of the marked difference in rainfall in the two seasons.

Table 15. The effect of irrigation on the nicotine contents of flue-cured tobacco. (Oxford)

Nitrogen Fertilization (pounds N/acre)	Applied Irrigation		
	None	Adequate	Abundant
<u>1951, Oxford 26:</u>			
	Per Cent Nicotine		
36	3.89	1.97	1.64
48	4.14	2.23	1.88
<u>1952, Dixie Bright 101:</u>			
36	2.33	1.78	
48	2.29	1.81	

SUMMARY

As demonstrated in controlled experiments, the level of nicotine accumulation in flue-cured tobacco is influenced by certain factors:

1. Varieties are inherently different in their nicotine contents. Both the level of alkaloid synthesis and the chemical nature of the alkaloid are genetically controlled. The newer disease-resistant varieties are somewhat lower in nicotine than are the older non-resistant varieties tested. Varieties retain their relative rankings in nicotine content despite differences in weather conditions and locations.
2. Nicotine accumulation is directly related to the level of nitrogen fertility.
3. The nicotine contents of flue-cured tobacco may vary in different cropping systems. Thus, when rotations are used for the control of nematodes, the observed differences may be due to differential root damage but, more probably, to the fertility residues from the previous crop.
4. Nicotine is concentrated into the remaining tissues after tobacco is topped and suckered. The degree of the accumulation is directly related to the severity of the pruning.
5. Tobacco, artificially infected with mosaic, is consistently lower in nicotine than the non-infected tobacco.
3. Moisture supply is the most important single factor in regulating the growth and nicotine accumulation of tobacco. This has been forcefully demonstrated in irrigation experiments. Varieties differ in their nicotine responses to varying moisture conditions.

Other experimentally imposed treatments appear to be without influence on nicotine elaboration:

1. Increasing the plant populations by closer spacings does not decrease the nicotine content to a degree that is not explainable on the basis of competition for the nitrogen supply.
2. Between the limits of gross deficiencies and toxic concentrations, variations in the rates of application of phosphorus, potassium, calcium, magnesium, chlorine, and sulfur do not consistently affect nicotine concentrations.
3. Fertilization with the minor elements, boron, copper, zinc, manganese, and molybdenum is not demonstrably associated with changes in nicotine.
4. The use of winter cover crops does not affect the nicotine content of the subsequent tobacco crop over and above that which can be explained on the basis of nitrogen fertility.
5. No residual effects on nicotine accumulation resulted from the use of dichloropropene mixture (DD) or ethylene dibromide (EDB) fumigants for the control of nematodes.