NITROGEN FERTILIZATION AND GENOTYPE EFFECTS ON SELECTED CONSTITUENTS OF SMOKE FROM ALL-BURLEY CIGARETTES[1]

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A field experiment was conducted in 1972 at Lexington, Kentucky, on Maury silt loam soil (Typic paleudalf) to determine the effect of N fertilization (ammonium nitrate) and burley tobacco (**Nicotiana tabacum L.**) genotype on selected smoke constituents from all-burley cigarettes. Two commercial cultivars (Ky 14 and Burley 21) and three burley breeding lines (low-alkaloid Burley 21, low-intermediate-alkaloid Burley 21, and high-intermediate-alkaloid Burley 21 were used.

Generally, adding N fertilizer at the rate of 224 kg N/ha produced small increases in average levels of total particulate matter (TPM), nicotine, tar (TPM-nicotine-water), NO, total phenols, o-cresol, and m + p-cresol in cigarette smoke over amounts obtained from the 112-kg rate. Further additions of N to either 336 or 448 kg N/ha greatly increased values for NO, decreased TPM, tar, total phenols, and o-cresol, and had little effect on nicotine or m + p-cresol. The data suggest that the decreases in TPM, tar, and phenols at the high rates of N fertilizer may be attributed to high levels of leaf nitrate. Cigarette smoke from the reduced alkaloid genotypes contained smaller amounts of all constituents measured than Ky 14 and Burley 21. Among the reduced alkaloid lines, low- alkaloid Burley 21 contained lowest amounts of all contituents, and the amount of each smoke constituent was related positively to concentration of total alkaloids in leaves.

INTRODUCTION

Wide differences in the characteristics of cured tobacco (*Nicotiana tabacum L.*) leaf can be brought about by altering genotype, fertilization and other cultural practices, and curing methods (1, 2, 4, 6, 9, 11, 14, 15, 16, 17, 20). Although much is known about factors influencing the composition of tobacco leaves, few studies have reported the effects of agronomic factors on components of cigarette smoke.

Benner, Burton, and Burdick (3) determined levels of certain chemical constituents in smoke of cigarettes made from two lots of burley tobacco containing a 17-fold difference in naturally occurring nitrate. The smoke from high-nitrate cigarettes contained more nicotine but slightly lower levels of benzo (a) pyrene (BaP) and m- and p-cresols than smoke from low-nitrate cigarettes. Little difference was found in contents of total phenols or total particulate matter (TPM) among the two lots of tobacco. Tso (19) reported nicotine and TPM in smoke to vary with cultivar, N fertilizer rate, and suckering practice. Nicotine in smoke was related to leaf nicotine and was greatest in tobaccos grown under high rates of N fertilization. Tso *et al.* (21) noted that both TPM and BaP in cigarette smoke was higher when cigarettes were made of flue-cured leaves from upper, rather than lower, stalk positions. Increasing N fertilizer from 0 to 67 kg N/ha increased the total alkaloids and pH but had no apparent effect on TPM (dry), CO, or CO_2 of smoke from all-flue cured cigarettes (5). Leggett *et al.* (10) reported TPM, nicotine, and gas phase hydrogen cyanide delivered from all-burley cigarettes, respectively, were decreased by 24, 7, and 50% from the control when plots were supplied with 448 kg K/ha. However, gas phase aldehyde content of the smoke was increased by K application.

The purpose of the present investigation was to determine the effect of N fertilization and genotype on selected constituents of smoke from all-burley cigarettes.

METHODS

Field Procedures: A field experiment was conducted in 1972 at the University of Kentucky Agricultural Experiment Station at

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Table 1. Effects of N fertilization on dry total particulate matter (TPM), nicotine, tar, total oxides of N (NO), total phenols, o-cresol, and m + p-cresols of smoke from burley tobacco cigarettes.

Smoke Constituent	Nitrogen Rate, kg/ha 112	224	336	448
TPM, mg/cig.	22.8ab*	23.9a	22.2b	21.5b
Nicotine, mg/cig.	1.81b*	2.14a	2.00ab	1.97ab
Tar, mg/cig.	21.0ab*	21.8a	20.2b	19.6b
NO, mg/cig.	0.31d**	0.69c	1.22b	1.72a
Total phenols, μg/cig.	48.6ab*	51.4a	48.9ab	45.7b
o-cresol, μg/cig.	6.1ab*	6.5a	5.5b	5.2b
m + p-cresol, μg/cig.	19.9a*	22.3a	20.1a	20.6a

** and **Means in this line that are not followed by the same letter or letters are significantly different at the .05 and .01 probability levels, respectively, by Duncan's multiple range test.*

Lexington, on Maury silt loam soil (Typic Paleudalf). Certain chemical characteristics of soil as well as data for yield and nitrogenous constituents of cured leaf were reported previously (2).

Two commercial burley cultivars and three burley breeding lines were used. These were Burley 21 (B 21) and Kentucky 14 (Ky 14), low-alkaloid Burley 21 (LA B 21), low-intermediate-alkaloid Burley 21 (LIA B 21), and high-intermediate-alkaloid Burley 21 (HIA B 21). The low-alkaloid burley gentoypes were selected by plant breeders to provide a range in alkaloid concentration of about 0.3 to 3.0% (9). Commercial burley varieties usually range from 3.5 to 5.0% alklaoids, depending on culture and rainfall. Ammonium nitrate was used to supply 112, 224, 336, and 448 kg N/ha. A split-plot design was used, and treatments were replicated three times. The main plots were N fertilization and the sub-plots were cultivars or breeding lines. Each sub-plot consisted of two rows 1 m apart and 18 m long, with plants spaced 46 cm apart within rows. All of the N fertilizer and sulfate of potash to provide 336 kg K/ha was broadcast and disked into the soil prior to transplanting. The tobacco was cultured, harvested, and air-cured in a manner conventional for burley.

Cigarette Manufacture and Smoke Analysis: The tobacco from each treatment was humidified to 20% moisture and stemmed, slowly dried to 16% moisture and shredded (12.6 cuts/cm), slowly dried to 13% moisture and made into cigarettes with a Huani-Baby cigarette making machine. All cigarettes discussed in this work were 85 mm long, 25 mm in circumference and made to approximate the resistance to draw of the reference cigarette 1R1, approximately 7.2 cm of water. Within each treatment a mean cigarette weight was determined by weighing several 100-cigarette batches and the individual cigarettes were selected by weighing to a ±20 mg center cut. This latter group of cigarettes was used for all analytical determinations.

Total particulate matter (TPM) and nicotine determinations were carried out using the method described by Ogg and Schultz (13). The values reported for TPM and nicotine are all averages of at least 20 ports of five cigarettes each. All TPM values were corrected for water, i.e., dry particulate matter values. Values for tar are defined as TPM (dry)-nicotine. Total N oxides, analyzed as NO_2 and reported as NO, were determined by a modification of the procedure described by Newsome *et al.* (12). In this work the smoke from a single median puff (at least five replications) was analyzed. The level of total phenols and the cresols were determined by an adaptation of the GLC method described by Spears (18). The sum of phenol, the cresols, ethyl phenols and dimethyl phenols is designated as "total phenols" in this work.

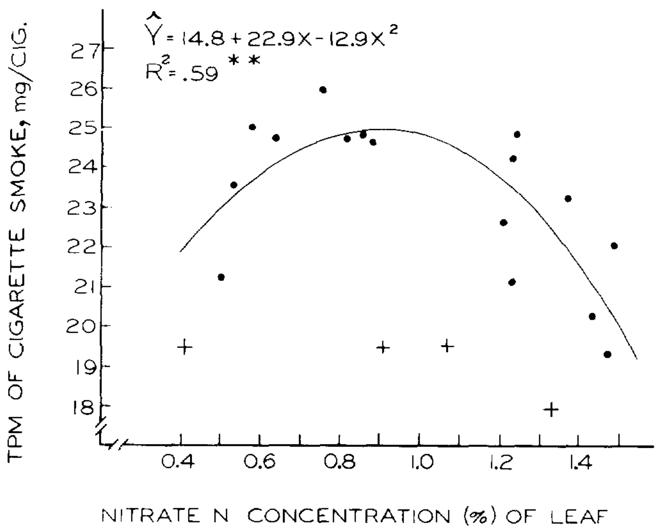

Figure 2. The relationship of dry total particulate matter (TPM) of burley cigarette smoke and nitrate N concentration of tobacco leaves. Data points marked with crossed lines (+) are for low-alkaloid B 21 breeding line and were not included in the regression.

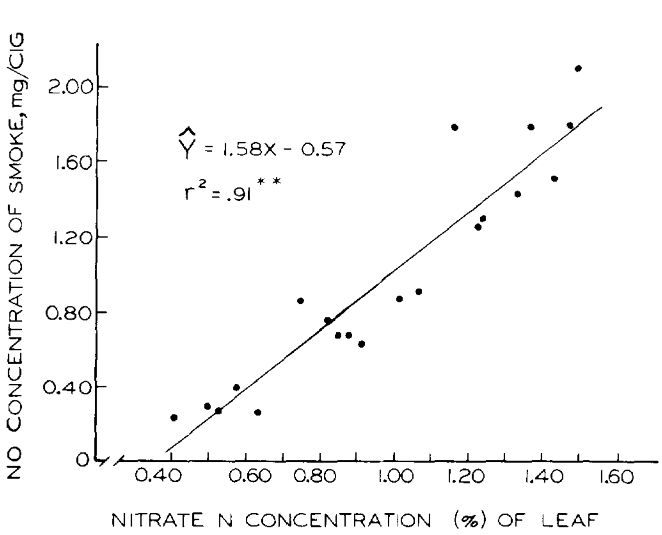

Figure 1. The relationship of total oxides of N (NO) of burley cigarette smoke and nitrate N concentration of tobacco leaves.

RESULTS AND DISCUSSION

Nitrogen Effects: Generally, adding N fertilizer at 224 kg N/ha increased average levels of TPM, nicotine, tar, NO, total phenols, o-cresol, and m + p-cresol in smoke from all-burley cigarettes over amounts obtained from the 112-kg N rate **(Table 1).** However, differences between the 112- and 224-kg N rates were statistically significant ($p \leq .05$) for only nicotine and NO. Further additions of N above the 224-kg rate increased values for NO, decreased values for TPM, tar, total phenols, and o-cresol, and had little if any effect on nicotine or m + p-cresol. A trend existed for the latter two constituents to decrease at the highest rates of N.

A close positive linear relationship existed between nitrate N concentration (%) of leaf and total oxides of N (NO) of smoke **(Fig. 1).** The nitrate data of leaf used in the regressions for **Figs. 1** and **2** were taken from the study reported previously (2). The relationship of Fig. 1 explains why NO of smoke increased as rate of N fertilizer increased **(Table 1)** and confirms results reported previously by Broaddus *et al.* (4). A curvilinear relationship existed between TPM of smoke and nitrate N concentration of leaf when data for all cultivars and breeding lines were used except that for LA B 21 **(Fig. 2).** Deletion of the data for LA B 21 improved the regression considerably. Reasons why LA B 21 differed from the other genotypes isn't known with certainty but

Table 2. Effects of genotype on dry total particulate matter (TPM), nicotine, tar, total oxides of N (NO), total phenols, o-cresol, and m + p-cresols of smoke from burley tobacco cigarettes.

Smoke Constituent	Genotype				
	LA B 21	LIA B 21	HIA B 21	B 21	Ky 14
TPM, mg/cig.	19.1c**	22.4b	22.0b	24.7a	24.9a
Nicotine, mg/cig.	0.53c**	1.60b	1.42b	3.23a	3.10a
Tar, mg/cig.	18.6b**	20.8ab	20.6ab	21.4a	21.8a
NO, mg/cig.	0.80b*	0.96ab	0.90ab	1.19a	1.16ab
Total phenols, μg/cig.	39.9c**	48.5b	44.9bc	49.9b	60.0a
o-cresol, μg/cig.	4.9b**	5.7ab	5.5ab	6.4ab	6.5a
m + p-cresol, μg/cig.	18.4b*	20.9ab	20.1ab	21.2ab	23.0a

** and **Means in this line that are not followed by the same letter or letters are significantly different at the .05 and .01 probability levels, respectively, by Duncan's multiple range test.*

may be because LA B 21 had lower concentrations of nitrate, higher concentrations of protein and carbohydrates, and generally responded less to rates of N fertilization (2, 14, 17).

The data of **Fig. 2** reveal that TPM increased from a minimum nitrate N concentration of 0.4%, reached a maximum at about 0.9% nitrate N, then decreased with further increases in nitrate. These data may partially explain why contents of TPM, tar, total phenols, and o-cresol in cigarette smoke were decreased by high rates of N fertilizer **(Table 1),** and why no consistent effects of N fertilizer on TPM of smoke were observed by others (3, 5, 19). Selective reductions of certain smoke constituents when either sodium or potassium nitrate salts were added to cigarette tobaccos or when the tobaccos contained high levels of naturally occurring nitrates have been reported previously (3, 7, 8).

Genotype Effects: Generally, cigarette smoke from the reduced alkaloid genotypes delivered smaller amounts of all smoke constituents measured than the commercial cultivars B 21 and Ky 14 **(Table 2).** There was little difference between B 21 and Ky 14, with the exception that Ky 14 contained greater amounts of total phenols than B 21. Among the reduced alkaloid lines, LA B 21 contained lower amounts of TPM, nicotine, tar, NO, and total phenols of smoke than the parent cultivar B 21, and equal amounts of the cresols. Values of the intermediate-alkaloid lines, LIA B 21 and HIA B 21, did not differ for any smoke constituent and were intermediate between those of LA B 21 and B 21.

The results of this study indicate that many of the constituents of cigarette smoke that are of interest may be altered by both genetics and N fertilization. The effect of genotype on nicotine was relatively large but quite small for the other parameters measured. Similarly, the effect of N fertilization on NO of smoke was large and small for the other constituents. Such information should aid the development and management of tobacco cultivars with improved smoke quality characteristics.

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