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Some Agronomic Factors Affecting N-Dimethylnitrosamine Content in Cigarette Smoke*

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Some nitrosamines are reported to be environmental carcinogens (8, 12). The occurrence and carcinogenesis of various nitrosamines are under extensive study by scientists from various parts of the world (1, 9, 14). The presence of volatile nitrosamines in food (4, 5) and in tobacco smoke (2, 6, 7, 9, 12) were reported. More intensive research is needed to establish what are their sources, and how to reduce or eliminate their presence.

In tobacco production, many factors are known to affect the nitrogen absorption and metabolism in the tobacco plant (15) and thus the amount of precursors of nitrosamines in leaf tobacco. These factors may include tobacco type, variety, rate of nitrogen fertilization, cultural practice, and post-harvest handling. This study is to examine the possible effects of those factors on the amount of N-dimethylnitrosamine (DMN) content in smoke of cigarettes made of experimental tobaccos. DMN is the principal volatile nitrosamine found in tobacco smoke (6).

MATERIALS AND METHODS

The DMN content of cigarette smoke was determined at the Southwest Research Institute, following the procedure of *Rhoades* and *Johnson* (11). Labeled DMN was used as the internal standard, which improved the accuracy of the method.

Experimental cigarettes were made with leaf tobacco (Nicotiana tabacum L.) samples from four studies involving different agronomic variables. The cigarettes were 85 mm in length. They were selected to meet the tolerance in weight (0.04 g) and pressure drop (3 cm) to insure as much uniformity as possible. For purposes of this paper, the samples were divided into four groups, which represent the separate studies conducted in different years. The experimental design of each group is as follows:

Group 1 – Burley-type tobacco, grown with: [a] three rates of N fertilizer (112, 224, and 336 kg N/ha); [b] three lines from Burley 21 cultivar, including commercial or normal-alkaloid (B21), low-alkaloid (LAB21), and low-polyphenol (LPB21); and [c] three leaf positions on the stalk (lower, middle, and upper); making a total of 27 samples. The samples were composited from three replications in the field to provide a manageable workload. Leaf analyses were conducted for total N, total alkaloids, nicotine, nornicotine, total volatile nitrogenous bases, nitrate N, protein N, and α-amino N. Smoke nicotine and TPM (total particulate matter) were also measured. The data for leaf and smoke analyses will be reported in detail in separate communications, but they are used here for study of their correlation with DMN content.

Group 2 – Burley-type tobacco, grown with: [a] two lines from cultivar Burley 21 (low-nornicotine and high-nornicotine); [b] two rates of N fertilizer (112 and 448 kg N/ha); [c] two suckering practices to control axillary bud growth (hand-suckering and maleic hydrazide); and [d] three leaf positions on the stalk (lower, middle, and upper); making a total of 24 samples. Leaf analysis included total N, total alkaloids, nicotine, nornicotine, total volatile amines (dimethyl, methylethyl, diethyl, and methylpropyl), and dry-weight yield. Nicotine and TPM in smoke were also determined. Data for these leaf analyses have been reported previously (3, 13) and are used here for correlation studies.

Group 3 – Burley and bright-type tobaccos, grown with varying nitrogen fertilizer levels. This study was not a balanced factorial design, but involved two types of tobacco, burley (Burley 21) and bright-type (NC 2326), each with different nitrogen rates. For burley, N rates included 56, 112, 224, and 448 kg N/ha. Nitrogen rates for bright-type were 0, 33.6, 50.4, 67.2, and 100.8 kg N/ha. All samples were hand-suckered,

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and all stalk positions were composited. Harvested leaves were cured by means conventional to their respective types. Leaf analyses were made for total N, total alkaloids, nicotine, nornicotine, nitrate N, reducing sugars, and organic acids. Smoke nicotine and TPM were also determined. These data are used only to study their correlation with DMN in smoke.

The burley tobaccos of all groups were produced at Kentucky, while bright tobacco was produced in North Carolina. Experimental cigarettes were made by personnel of the University of Kentucky Tobacco and Health Research Institute, and leaf analyses of Groups 1, 2, and 3 were conducted by the University of Kentucky Leaf Analytical Services Laboratory. Analytical methods followed the generally accepted procedures as described by *Tso* and *Andersen* (16). The smoke analyses were made by personnel of the University of Kentucky Tobacco and Health Research Institute, following the JAOAC specifications as described by *Ogg* and *Schultz* (10).

Group 4 – Homogenized-leaf-curing (HLC) samples. These cigarette samples were made of [a] conventionally cured leaves (air or flue-cured), [b] reconstituted sheet control made from conventionally cured-leaf materials, and [c] reconstituted sheets made of HLC materials. The detailed process of HLC was described elsewhere (17). Experimental sheets and cigarettes for this study were manufactured by collaborating industrial laboratories.

Statistical analyses of the data were made where appropriate, using data within each group of samples.

Table 1. The N-dimethylnitrosamine content (ng/g of tobacco burned) in cigarette smoke of burley tobaccos varying in nitrogen nutrition and genotype (Group 1 samples).

Nitrogen rate kg/ha	Leaf position	Genotype: Commercial Burley 21 (B21)	Low-alkaloid Burley 21 (LAB21)	Low-polyphenol Burley 21 (LPB21)	Average
112	Lower	36	32	115	61.0
	Middle	33	16	50	33.0
	Upper	29	46	32	35.7
	Average	32.7	31.3	65.6	
224	Lower	68	54	36	52.7
	Middle	61	22	80	54.3
	Upper	76	43	57	58.7
	Average	68.3	39.7	57.7	
336	Lower	76	18	105	66.3
	Middle	80	29	105	71.3
	Upper	92	59	75	75.3
	Average	82.7	35.3	95.0	

Main effects

Genotype	DMN	N-rate	DMN	Leaf position	DMN
B21	61.2	112	43.2	Lower	60.0
LAB21	35.4	224	55.2	Middle	52.9
LPB21	72.8	336	71.0	Upper	56.6

RESULTS AND DISCUSSION

Data for DMN content of cigarette smoke from sample Group 1 are shown in Table 1. The DMN content varied from 16 to 115 ng/g tobacco burned. Statistical analyses showed that rates of nitrogen fertilization and cultivar had significant effects on the DMN levels in smoke, whereas differences for leaf position on the stalk were not significant.

DMN content increased as rate of nitrogen increased. The mean value of DMN for the 336 kg N/ha treatment was almost double the content for the 112 kg N rate. Values for the 224 kg rate were intermediate between those for the 112- and 336 kg rates and did not differ significantly from the other two rates at the 5 % probability level. Mean content of DMN in the low-alkaloid line of Burley 21 was about one-half that of the commercial normal-alkaloid line or low-polyphenol lines.

The DMN levels from cigarettes of Group 2 were much lower than those from Group 1 samples at comparable N fertilizer rates (Table 2). This difference may reflect the seasonal effect on leaf characteristics, which in turn affects the DMN formation in smoke. Values ranged from 5.7 to 71 ng/g and averaged more than 2½ times higher at the 448- than 112 kg N rate. Although not statistically significant, mean values for the low-nornicotine line, for the hand-suckering practice, and for the upper leaf positions were each numerically higher, respectively, than for the high-nornicotine line, the MH-30 suckering practice, and the lower leaf position.

Values for DMN contents of sample Group 3 are shown in Table 3. The DMN content varied from 1.7 to 112 ng/g. The wide difference reflects the effects of both tobacco type and N rates. In bright-type material, DMN varied between 1.7 and 7.8 ng, while in burley-type, the range was 52 to 112 ng. The burley tobacco was fertilized with much larger amounts of N fertilizer than the bright-type tobacco.

To compare the DMN results from these three studies and to identify possible significant relationships involving DMN, simple correlations for DMN and other smoke and leaf constituents within each sample group are given in Table 4. The rate of nitrogen fertilization is highly correlated with levels of DMN. Furthermore, total N, total alkaloids, nicotine, nornicotine, nitrate N, and total volatile bases are all highly correlated with DMN. Nornicotine showed the highest correlation with DMN among all variables in Sample Groups #1 and #3. It should be noted that in sample Group #2, in which high-nornicotine (low-nicotine) and low-nornicotine (high-nicotine) lines were used, no significant correlation was obtained from either nicotine or nornicotine individually with DMN. This fact can probably

Table 2. The N-dimethylnitrosamine content (ng/g of tobacco burned) in cigarette smoke of burley tobacco varying in nitrogen nutrition, genotype and suckering practice (Group 2 samples).

Nitrogen rate kg/ha	Leaf position	Low-nornicotine Burley 21		High-nornicotine Burley 21		Average
		Hand-suckered	MH-30	Hand-suckered	MH-30	
112	Lower	11.0	8.7	8.7	5.7	8.5
	Middle	8.7	10.0	26.0	8.7	13.4
	Upper	24.0	6.9	11.0	8.7	12.7
	Average	14.6	8.5	15.2	7.7	
448	Lower	18.0	30.0	53.0	11.0	28.0
	Middle	71.0	48.0	35.0	20.0	43.5
	Upper	54.0	40.0	45.0	28.0	41.2
	Average	47.6	39.3	44.3	19.7	

Main effects

Genotype	DMN	N-rate	DMN	Suckering	DMN	Leaf position	DMN
Low-nornicotine	27.5	112 kg/ha	14.4	Hand	30.4	Lower	18.3
High-nornicotine	21.7	448 kg/ha	37.6	MH-30	18.3	Middle	28.5
						Upper	27.0

be interpreted that in these two particular lines of tobacco plants, nornicotine was very significantly correlated with several variables, including nicotine ($r = 0.856$), plant variety, which identifies the special alkaloid ($r = 0.914$), and total N ($r = 0.500$), but showed no significant correlation with either total alkaloid content or total volatile bases, which are, in turn, highly related to DMN. The nicotine/nornicotine ratios from these two lines in sample Group 2 are in opposite direction and appear to cancel out the respective individual relation to DMN.

The DMN content of cigarettes made from LA Burley 21 was much lower than from commercial Burley 21 or the low-polyphenol line. LA Burley 21 leaf contains extremely low alkaloid levels as compared to the other cultivars in this test, and cultivars having high alkaloid content usually deliver a high level of DMN in smoke.

Table 3. The N-dimethylnitrosamine content (ng/g of tobacco burned) in cigarette smoke of tobaccos varying in nitrogen nutrition and type (Group 3 samples).

Nitrogen rate kg/ha	Tobacco type			Average
	Burley 21	Bright type (NC 2326) Location 1	Bright type (NC 2326) Location 2	
0	–	7.8	1.7	4.8
33.6	–	4.7	1.7	3.2
50.4	–	4.7	1.7	3.2
67.2	–	5.9	2.6	4.3
100.8	–	4.8	–	4.8
56	52	–	–	52
112	71	–	–	71
224	112	–	–	112
448	58	–	–	58
Average	73.3	5.6	1.9	

Reducing sugar in tobacco leaf is very significantly and negatively correlated with the DMN content in cigarette smoke. This may merely reflect the fact that tobacco types that have a high sugar content are usually low in nitrogenous components.

The homogenized-leaf-curing (HLC) process showed

Table 4. Correlation between DMN in smoke and certain culture, leaf, and smoke variables.

Variables	Sample Group #1	Sample Group #2	Sample Group #3
Culture variables			
N-rate	0.411*	0.722**	N.A.
Variety (or lines)	0.171	0.094	N.A.
Stalk position	−0.051	0.161	N.A.
Suckering method	N.A.	0.102	N.A.
Dry weight/plant	N.A.	0.419	N.A.
Leaf variables			
Total N	0.482**	0.630**	0.829**
Total alkaloids	0.487**	0.434*	0.808**
Nicotine	0.493**	0.184	0.826**
Nornicotine	0.524**	0.045	0.916**
Protein N	−0.171	N.A.	N.A.
Nitrate N	0.455*	0.574**	0.597*
Total vol. bases	0.407*	0.600**	N.A.
α-amino N	0.072	0.325	N.A.
Dimethylamine	N.A.	0.325	N.A.
Methylethylamine	N.A.	0.026	N.A.
Diethyl + MP amine	N.A.	0.343	N.A.
Reducing sugar	N.A.	N.A.	−0.808**
Organic acids	N.A.	N.A.	0.478
Smoke variables			
Nicotine	0.403*	0.408*	0.632*
TPM	0.229	0.214	−0.596*

* 5 % significance; ** 1 % significance; N.A. – not available.

Table 5. DMN levels in cigarette smoke of conventionally cured and homogenized cured leaf tobacco.

Samples	DMN ng/g of tobacco burned
Burley type	
Conventionally air-cured control (CAC)	97.0
Reconstituted sheet control of CAC	65.0
HLC	20.0
Bright type	
Conventionally flue-cured control (CFC)	2.6
Reconstituted sheet control of CFC	1.7
HLC	1.7

significant effects on DMN content in smoke from both burley and bright tobacco types (Table 5). The reduction of DMN is a combined effect of reconstitution and of the new curing procedure (17), which induces desired biochemical changes.

CONCLUSIONS

There are wide variations of DMN contents in cigarette smoke from these experimental tobaccos. However, under conditions of the testing procedure used in this study, the presence of DMN in the smoke from these experimental samples was established.

The overall results show that rate of N-fertilization is the most significant factor affecting DMN content in cigarette smoke. Total alkaloid content is another major factor. Varietal differences merely reflect the levels of specific alkaloid content, which again are closely associated with the level of DMN in smoke.

Despite the fact that the relationships among DMN, nitrogen rate, and alkaloid level are consistent, the measured DMN values in cigarette smoke vary from group to group produced in different years. Rainfall and other climatic conditions may influence the chemical as well as physical characteristics of the leaf, and affect the amount of DMN formed in smoke.

Under normal tobacco production practices, the rate of nitrogen fertilization for flue-cured type tobacco is between 56–78 kg/ha. In some instances only 39.2 kg/ha is applied. Even at the highest N-rate in the current test (100.8 kg/ha), the DMN in smoke of bright-type tobacco cigarettes did not exceed 7.8 ng/g cig. In burley tobacco production, the normal rate of N-fertilization is about 224 kg/ha. The range of DMN content in cigarette smoke of the burley tobaccos receiving 112 kg/ha of N varies from 6.9 to 71 ng/g and those receiving 224 kg/ha of N varies from 22 to 112 ng/g tobacco burned. These results appear to indicate that factors associated with tobacco types may influence DMN formation in smoke.

Total secondary amines in leaf tobacco showed no major effect on DMN in smoke in this study. From data of all the experimental samples involved, simple correlations of 0.32, 0.03 and 0.34 were obtained between various aliphatic secondary amines and smoke DMN. This level of correlation is below the significant level.

Reconstituted HLC has demonstrated a rather significant effect in reducing the DMN levels in smoke. This may involve two important factors. One is the redistribution of chemical components by the HLC process, and another is the physical or structural improvements through sheet reconstitution using the HLC material. The loss of certain soluble fractions during reconstitution may also contribute to the low DMN level in smoke.

These results indicate the effects of nitrogen fertilization during tobacco production on the level of DMN in smoke. The exact significance of DMN in smoke, especially its effect on human health, cannot be evaluated until the threshold hazardous level of DMN is determined by medical science. Additional studies are needed to establish in tobacco the specific nitrogenous precursors and the conditions favoring the formation of DMN in smoke. Such information may provide a basis for developing means of reducing or eliminating DMN in tobacco smoke.

SUMMARY

Experimental cigarettes from tobaccos varying in genotype, nitrogen nutrition, stalk position, suckering practice, and curing methods were used to examine the levels of N-dimethylnitrosamine (DMN) in smoke. Measurable amounts of DMN were found in all experimental samples, ranging from 1.7 to 115 ng per gram of tobacco burned.

DMN content in smoke generally increased as rate of N fertilization increased. However, there were wide seasonal, cultural, and varietal effects. Burley-type tobacco produced a much higher level of DMN than the bright-type tobacco.

DMN content in smoke was significantly and positively related to leaf total N, total alkaloids, nicotine, nornicotine, total volatile bases and nitrate N, but negatively related to reducing sugars. Reconstituted sheet tobaccos made with homogenized-leaf-curing samples produced much lower amounts of DMN than conventionally cured leaf. Additional information is needed to elucidate the primary leaf constituents that serve as precursors of DMN.

ZUSAMMENFASSUNG

Es wurde der N-Dimethylnitrosamingehalt (DMN) des Rauches von Versuchscigaretten untersucht, deren Tabake sich in folgenden Kriterien voneinander unterschieden: Genotypus, Stickstoffdüngung, Blattposition sowie Entgeizungs- und Trocknungsverfahren. In allen Versuchsgruppen wurden meßbare Mengen an N-Dimethylnitrosamin gefunden, und zwar in der Größenordnung von 1,7 bis 115 ng je g verbrannten Tabaks.

Im allgemeinen stieg der DMN-Gehalt des Rauches mit der Zunahme der Stickstoffdüngung. Es zeigten sich jedoch deutliche Auswirkungen der Faktoren Jahreszeit, Bodenbearbeitung und Tabakart. Burley-Tabak hatte einen viel größeren Gehalt an N-Dimethylnitrosamin als „Bright"-Tabak.

Der N-Dimethylnitrosamingehalt des Rauches zeigte eine signifikante und positive Relation zu dem Gehalt des Tabakblattes an Gesamtstickstoff, Gesamtalkaloiden, Nikotin, Nornikotin, gesamten flüchtigen Basen und Nitrat-N, eine negative Relation jedoch zum Gehalt an reduzierenden Zuckern. Tabakfolien aus nach dem HLC-Verfahren (homogenized leaf curing) homogenisierten und getrockneten Proben enthielten wesentlich geringere Mengen an N-Dimethylnitrosamin als in herkömmlicher Weise getrocknetes Blattgut. Zur Klärung der Frage, welche Blattinhaltsstoffe als primäre Vorläufer des N-Dimethylnitrosamin fungieren, sind weitere Informationen notwendig.

RESUME

Pour étudier la teneur en N-diméthylnitrosamine (DMN) dans la fumée, on a utilisé des cigarettes expérimentales les tabacs desquelles provenaient de différents génotypes, différents engrais azotés, différentes positions sur la tige et différentes méthodes d'ébourgeonnage et de séchage. Des quantités mesurables de DMN ont été trouvées dans tous les échantillons, variant de 1,7 à 115 ng par gramme de tabac brûlé.

La teneur en DMN augmente en général parallèlement à l'augmentation d'engraissage à l'azote. Il y a cependant de larges variations saisonnières, de cultures et de variétés. La variété Burley produit beaucoup plus de DMN que la variété Bright.

La teneur en DMN de la fumée a une relation significative et positive par rapport à l'azote total des feuilles, aux alcaloïdes totaux, à la nicotine et la nornicotine, aux bases volatiles totales et aux N-nitrates, mais une relation négative par rapport aux sucres réducteurs. Des feuilles de tabac reconstitué ayant été fabriquées à partir d'échantillons séchés d'après la methode HLC (homogenized leaf curing / séchage de feuilles homogénéisées) produisent de moindres quantités de DMN que les tabacs séchés de façon conventionelle. Un supplément d'information est nécessaire pour déterminer les constituants des feuilles formant les précurseurs primaires du DMN.

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