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Distribution of Tobacco Constituents in Tobacco Leaf Tissue. 1. Tobacco-Specific Nitrosamines, Nitrate, Nitrite, and Alkaloids

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Leaves from a dark air-cured tobacco variety (Ky 171) were divided into 41 defined leaf segments. All samples were analyzed for nitrate nitrogen, nitrite nitrogen, nicotine, myosmine, nornicotine, anabasine, anatabine, *N'*-nitrosonornicotine (NNN), *N'*-nitrosoanatabine (NAT), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Nitrite concentration was highest at the base of the leaf and decreased toward the tip of the leaf. Concentration of alkaloids was lowest at the base and tip of the leaf and greatest at the periphery of the leaf and decreased toward the tip of the leaf. Individual nitrosamines were lowest at the tip and the periphery of the leaf. Midvein contained lowest concentrations of NNN, NNK, and NAT at two-thirds length of the leaf and was highest at the base of the leaf. There appeared to be a better relationship between nitrite nitrogen and tobacco-specific nitrosamines than there was with alkaloid content in the leaf.

INTRODUCTION

The accumulation of tobacco-specific nitrosamines (TSNAs) during air-curing has been of considerable interest for the past several years (Burton et al., 1989a,b; Peng, 1990; Djordjevic et al., 1989). This has been a result of the report that several TSNAs induce malignant tumors in mice, rats, and hamsters (Hoffman and Hecht, 1985). The TSNAs are derived via nitrosation of secondary and tertiary alkaloid amines which are present in tobacco. Even though there have been numerous reported studies concerned with the accumulation of nitrosamines in tobacco, there sometimes have been conflicting conclusions. Brunnemann et al. (1983) reported there was a direct relationship between nitrate concentrations and TSNAs in tobacco. Djordjevic et al. (1989) reported there was a significant, positive correlation between alkaloid content in tobacco and TSNA accumulation; however, they found no relationship between nitrate and TSNA content. Recent studies by Burton et al. (1989a,b, 1990) have shown there was a direct relationship between TSNA levels and nitrite concentration in tobacco. They also reported there was no relationship between alkaloid or nitrate content and nitrosamine concentration. Analyses of lamina and midrib tissue showed that midrib contained higher TSNA levels than lamina. Also, the midrib contained significantly higher concentrations of nitrite nitrogen. Djordjevic (1989) reported midribs contained lower TSNA concentration in comparison to lamina.

Because of the confusion generated by published data, it was decided to investigate nitrosamine accumulation in greater detail. The previous studies involved the analysis of lamina and veinal tissue of the whole tobacco leaf. It was proposed that TSNAs may accumulate in higher or lower concentration in certain locations of the leaf on the basis of the reports that nicotine has been shown to accumulate in higher concentrations on the periphery of the lamina tissue (Andreadis et al., 1939; Gay et al., 1984). Neurath and Emke (1964) reported nitrate accumulation was highest in the midvein and lowest at the tip of green tobacco leaf. Also, Jenkins (1986) reported that ions, e.g., Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, and Br^{-}, were nonuniformly distributed throughout the laminar tissue. The purpose of this study was to segment lamina and midvein tissue and to determine if there was nonuniform distribution of TSNAs, nitrate, and nitrite, and alkaloids in these tissues and to determine if there were significant correlations between levels of these constituents. These data would aid in determining which constituents have greatest influence on the accumulation of TSNAs.

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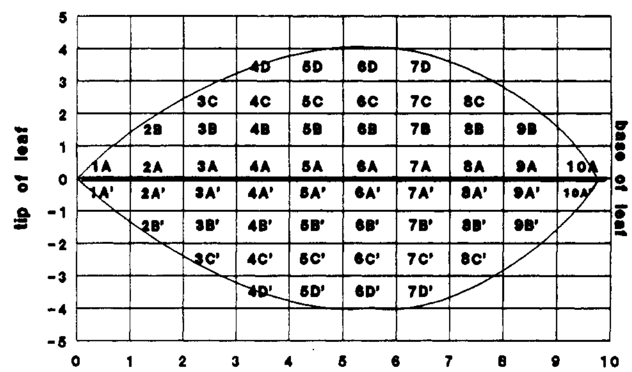

Figure 1. Grid used for taking leaf segments.

EXPERIMENTAL PROCEDURES

A dark tobacco type (Ky 171), which is used primarily for smokeless tobacco, was grown at the University of Kentucky Agricultural Experiment Station Farm in 1990 using standard cultural practices for the production of dark tobacco. The tobacco was stalk-cut and air-cured in a conventional curing barn. After curing, four leaves were removed from the top third of five plants. The leaves were then placed on a grid and sectioned into 7 cm long × 4 cm wide segments along the length of the leaf (Figure 1). There were 10 segments along the length of the leaf, and there was a maximum of 8 segments across the width of the leaf at its widest point. The midrib was segmented in 7-cm lengths. Lateral veins were separated from the lamina segments and identified by the corresponding lamina segment number. To reduce the number of samples and have sufficient quantities of tobacco for analyses, the lamina segments were combined with their mirror image segments (Figure 1). The lateral veins were combined in the same manner. Weights for all leaf segments were determined after drying. After the samples were ground in a small laboratory mill, the samples were stored at −20 °C until analyses.

Nitrate was analyzed using a Technicon autoanalyzer and Technicon procedure (Technicon Industrial Method 100-70W/B). Nitrite concentrations were determined using a procedure

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Table I. Distribution of Nitrate, Nitrite, and Alkaloids in Leaf

leaf segment	tissue[a]	wt, g	NO_3^- N, μg g^{-1}	NO_2^- N, μg g^{-1}	nicotine, mg g^{-1}	myosmine, mg g^{-1}	nornicotine, mg g^{-1}	anabasine, mg g^{-1}	anatabine, mg g^{-1}	total alkaloids, mg g^{-1}
1A	L	10.1	380	1.58	33.06	0.37	0.37	0.078	0.49	34.35
1B	L	0.5	–[b]	–	–	–	–	–	–	–
2A	L	12.4	730	4.46	35.99	0.16	0.36	0.066	0.44	37.02
2B	L	10.0	230	1.29	45.42	0.22	0.42	0.080	0.57	46.71
2C	L	1.4	220	1.34	48.82	0.29	0.49	0.095	0.61	50.30
3A	L	11.7	1070	1.65	42.96	0.13	0.46	0.081	0.52	44.15
3B	L	13.3	290	1.56	44.28	0.20	0.44	0.083	0.50	45.50
3C	L	8.2	190	1.53	50.36	0.16	0.43	0.089	0.56	51.59
3D	L	0.7	–	–	–	–	–	–	–	–
4A	L	11.6	1500	1.80	47.25	0.12	0.47	0.087	0.52	48.45
4B	L	13.7	520	1.66	47.26	0.14	0.43	0.089	0.51	48.42
4C	L	12.9	310	1.67	54.56	0.15	0.48	0.093	0.59	55.87
4D	L	3.4	250	1.55	66.91	0.20	0.55	0.108	0.66	68.43
5A	L	11.1	1610	1.84	52.69	0.11	0.48	0.090	0.56	53.93
5B	L	13.4	650	1.90	54.02	0.14	0.47	0.094	0.57	55.29
5C	L	12.5	410	1.69	56.67	0.15	0.49	0.101	0.63	58.04
5D	L	5.8	470	1.61	67.19	0.18	0.54	0.109	0.75	68.77
6A	L	10.9	2340	1.50	57.01	0.12	0.53	0.100	0.70	58.45
6B	L	11.9	650	1.12	57.40	0.22	0.53	0.109	0.65	58.90
6C	L	12.5	490	1.20	63.14	0.24	0.52	0.108	0.65	64.66
6D	L	5.6	420	1.30	68.74	0.17	0.59	0.109	0.68	70.28
7A	L	10.0	1570	1.51	57.23	0.12	0.59	0.108	0.68	58.73
7B	L	10.0	670	1.40	66.80	0.20	0.60	0.122	0.73	68.45
7C	L	10.9	540	1.42	66.40	0.19	0.60	0.117	0.68	68.00
7D	L	2.8	510	1.55	75.06	0.18	0.71	0.122	0.79	76.86
8A	L	9.7	1140	1.58	58.29	0.14	0.53	0.103	0.62	59.68
8B	L	6.2	710	1.87	65.31	0.18	0.60	0.114	0.82	67.03
8C	L	2.3	520	1.68	76.10	0.16	0.76	0.125	0.76	77.90
9A	L	7.5	1340	1.79	46.86	0.14	0.47	0.094	0.63	48.19
9B	L	0.7	–	–	–	–	–	–	–	–
10A	L	1.7	1730	1.58	36.99	0.17	0.38	0.075	0.41	38.03
1	M	0.5	5450	1.19	13.81	0.06	0.20	ND[c]	0.14	14.21
2	M	1.9	5450	1.19	13.81	0.06	0.20	ND	0.14	14.21
3	M	3.5	7150	1.14	10.29	ND	0.15	ND	0.10	10.53
4	M	6.0	8210	1.06	8.17	ND	0.12	ND	0.07	8.36
5	M	8.7	9250	1.12	7.64	ND	0.11	ND	0.07	7.81
6	M	10.4	8350	1.09	7.80	ND	0.11	ND	0.09	8.00
7	M	13.5	7720	1.25	6.73	ND	0.09	ND	0.07	6.89
8	M	16.4	7480	2.37	7.12	ND	0.08	ND	0.08	7.28
9	M	19.7	5430	3.05	5.63	0.04	0.06	ND	0.04	5.76
10	M	7.1	5430	3.05	5.08	0.04	0.07	ND	ND	5.19
2A	V	1.4	960	1.37	24.61	0.13	0.31	0.042	0.25	25.34
3A	V	1.9	1950	1.56	20.14	0.06	0.26	0.034	0.23	20.72
3B	V	1.1	–	–	–	–	–	–	–	–
4A	V	2.7	2970	1.74	17.05	0.04	0.23	ND	0.17	17.49
4B	V	2.0	1440	1.85	23.75	0.08	0.30	0.041	0.30	24.47
4C	V	0.6	–	–	–	–	–	–	–	–
5A	V	3.2	3650	1.40	16.58	ND	0.23	ND	0.17	16.98
5B	V	2.7	1980	1.58	26.04	0.07	0.31	0.044	0.25	26.71
5C	V	1.2	–	–	28.68	0.19	0.37	0.058	0.31	29.60
6A	V	4.0	4580	1.14	14.75	ND	0.23	ND	0.17	15.15
6B	V	2.6	2530	1.82	29.42	0.06	0.35	0.050	0.29	30.17
6C,D	V	1.2	–	–	27.75	0.15	0.39	0.058	0.27	28.61
7A	V	3.3	4590	1.89	14.77	0.22	0.58	0.146	0.63	16.35
7B	V	1.9	2530	2.10	21.67	0.31	0.83	0.201	0.91	23.93
7C,D	V	0.6	–	–	–	–	–	–	–	–
8A	V	2.0	3500	2.19	16.51	0.24	0.59	0.097	0.23	17.66
8B,C	V	0.7	–	–	–	–	–	–	–	–
9A,B	V	0.5	–	–	–	–	–	–	–	–

[a] L, lamina; M, midvein; V, lateral vein. [b] –, insufficient sample for analysis. [c] ND, not detected.

described by Crutchfield and Burton (1989). Extraction of tobacco for analyses of TSNAs employed a procedure reported by Burton et al. (1988) for the analyses of acylated nornicotine. Because of the potential carcinogenis of the TSNAs, all extractions and concentrations of the extracts were carried out in laboratory hoods. Individual TSNAs from the extract were quantified on a Thermedics Inc. TEA Model 543 analyzer coupled to a Hewlett-Packard Model 5890 gas chromatograph equipped with a 50 M × 0.25 mm capillary column coated with a 0.25-μm DB-5 liquid phase. The chromatographic conditions and calculation of response factors have previously been reported (Peng, 1990). Three-dimensional graphical presentation of data was obtained using the Surfer program purchased from Golden Software Inc. All graphical data are presented on a 65 × 73 grid.

RESULTS AND DISCUSSION

Data from leaf segments for the weight, nitrate and nitrite, and individual alkaloids are presented in Table I. The leaf segments correspond to those shown in Figure 1. The lowest number was from the tip of the leaf. Increasing letters A–D were from the midvein to the outer edge of the leaf. Because of the insufficient weight of the lateral vein, leaf segments were combined within Arabic numeral. For example, lateral veins from leaf segments 1A, 1B, 2A, 2B, and 2C were combined to obtain sufficient quantities of material for subsequent analysis of individual alkaloids, nitrate N, nitrite N, and individual TSNAs. Even then

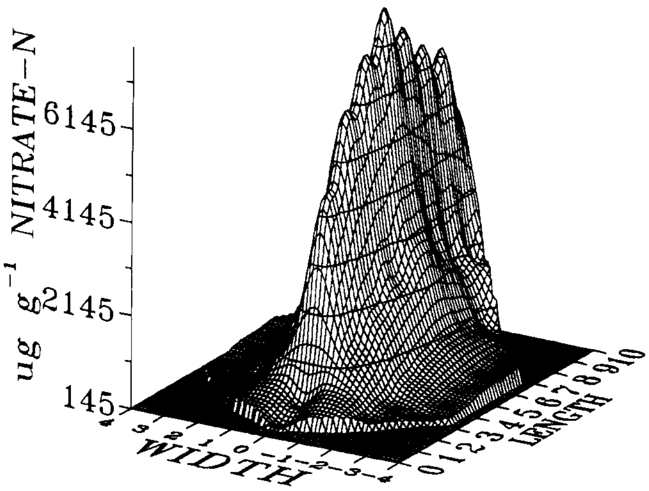

Figure 2. Distribution of nitrate nitrogen in leaf.

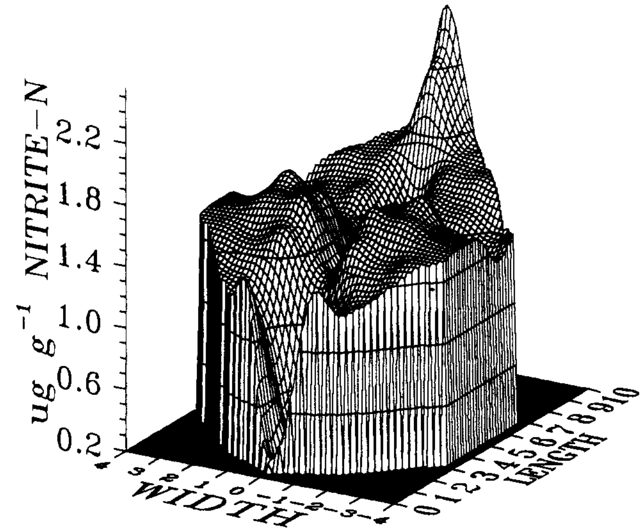

Figure 3. Distribution of nitrite nitrogen in leaf.

there was sufficient sample in several combined segments for only analyses of TSNAs. In general, the weights of the lamina segments increase from the base of the leaf to the tip of the leaf and from the midrib toward the outer edge of the leaf. The outer edge of the leaf segment contains lower net weight, but this was because a full 28-cm^2 section was not obtained due to the geometry of the leaf. The weight of the midvein segments decreased from the base to the tip of the leaf. Approximately 50% of midrib was contained in segments 8–10, which is only 30% of the total length of the midvein.

Nitrate N and Nitrite N Concentrations in Leaf. In lamina (Table I), nitrate nitrogen is highest in the middle (segment 6) of the leaf, whereas, it is lower at the tip and base of the leaf. The tip of the leaf contains the lowest concentration of nitrate N. Nitrate levels are highest next to the midvein and lowest at the outer edges of the lamina. The highest concentration of NO_3^- N is in the sixth segment of the leaf. This was not predicted, since it was expected to decrease from the base to the tip of the leaf. This was based on the premise that since nitrate concentration is always highest in the midvein of the leaf (Burton et al., 1983, 1989b) and that through movement of nitrate from the root to the lamina, a differential concentration of nitrate along the length of the leaf would be expected. Data for midvein also show that nitrate concentration is highest in the middle (Table I) and not at the base of the midvein. It is interesting to note nitrate is highest in the sixth segment and is also highest in the sixth of the lateral veinal tissue, indicating the highest concentration of nitrate in the midsection of the total leaf. To obtain the best overview of differences in the levels of nitrate in the leaf, data were graphically presented as a surface map. The data for nitrate using a surface map are shown in Figure 2. The X,Y dimensions are the length and width of the leaf, respectively, and the Z dimension is the concentration for nitrate (micrograms per gram) generated from the data for the leaf segment values including both lamina and midvein. Fitting these data points gave the approximation of the concentration for nitrate in the lamina. Because grid plots used a rectangular grid and the leaf geometry was oval, we forced the values outside the leaf boundary to 0. This plot clearly showed the tip of the leaf (segment 1) and the periphery contained the lowest level of nitrate, whereas the midrib (0) contained the highest concentration of nitrate N.

The concentrations of nitrite N did not parallel the concentration of nitrate in the cured lamina (Table I and Figure 3). Nitrite N ranged from 1 to 4 $\mu g\ g^{-1}$, which was as much as 2000 times lower than nitrate N. It was apparent only a small amount of nitrate was converted to nitrite under normal air-curing conditions. Concentration of nitrite was highest at the base of the leaf and lowest at the tip of the leaf (Figure 3). The concentration of nitrite in the midrib (segments 1–6) was lower than in the surrounding lamina segments, even though the midrib contained the highest levels of nitrate. This indicates that factors other than nitrate concentration influenced nitrite accumulation. The greater accumulation of nitrite at the base of the leaf can be explained by the observation that dry-down of lamina is slower at the base of the leaf. Maintenance of higher moisture content during death of the cells should support greater leaf and microbial metabolism, which can result in enhanced reduction of nitrate to nitrite (Burton et al., 1988, 1989b). At present, it would seem that nitrate reductase is coming from microbial metabolism. However, this cannot be shown by the data from this study. From Figure 3 it is apparent that the midrib from the base of the leaf contains the highest concentration of nitrite.

Distribution of Alkaloids in Leaf. Data of the individual alkaloids in general show lamina contains the highest concentration of individual alkaloids, followed by the lateral veins and then midvein (Table I). It has been well documented that midvein tissue contains the lowest levels of alkaloid (Burton et al., 1983, 1988; Djordjevic et al., 1989; Gay et al., 1984; Andreadis et al., 1939). The accumulation of alkaloids increases from the center of the leaf to the outer edges. This is in agreement with data reported by Gay et al. (1984) using a single leaf of a greenhouse-grown burley tobacco variety. The data can be best visualized by using a surface plot of the analytical data. The nicotine values in Figure 4 are weighted averages of lamina plus lateral veinal tissue. They were calculated by using weighted averages of the lamina and midvein values obtained from the gas chromatographic analyses. They were included in the figure since leaf is generally separated into only lamina and midvein for most analyses. The data from this surface plot clearly show that nicotine concentration is greatest on the periphery of the leaf. The concentration of nicotine does decrease from a maximum in segment 7 to a minimum in segment 1 of the lamina. Analyses of the lateral vein for nicotine show that this alkaloid increased toward the outer edges of the leaf (Table I). Along the midrib the concentration of nicotine increased from the base (segment 10) to the tip of the leaf (segment 1). Nornicotine distribution in the leaf is similar

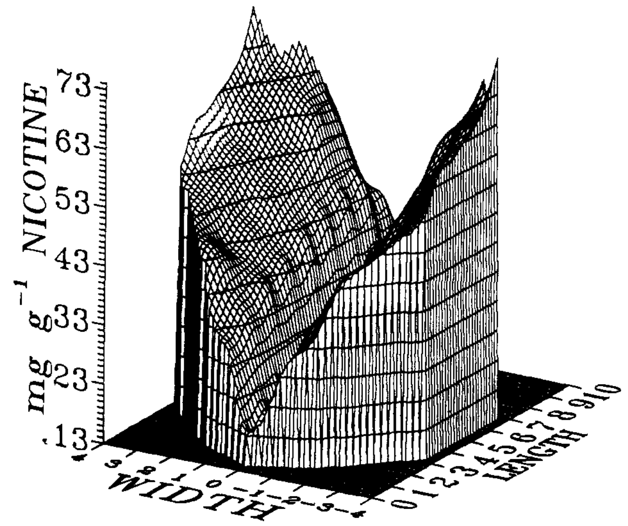

Figure 4. Distribution of nicotine in leaf.

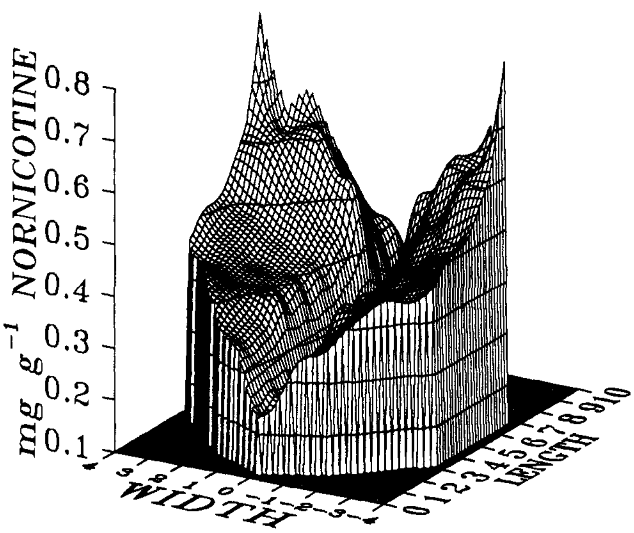

Figure 5. Distribution of nornicotine in leaf.

to that of nicotine (Figure 5) since the midvein contains the lowest concentration of this alkaloid and nornicotine is highest at the outer edge of the lamina. However, the difference in nornicotine concentration between segment 1 and segment 4 (Figure 5) is not as great as that for nicotine (Figure 4). The other minor alkaloids (myosmine, anabasine, and anatabine) have the same distribution pattern as nicotine (figures not presented). This would be predicted since one would expect their physical and chemical characteristics to be similar to those of nicotine and, therefore, their distribution within the leaf to be the same as that of nicotine. It should be noted that the concentration for the individual alkaloids is greatest in segments 7 and 8. This is the section where the leaf has its greatest width. The reasons for the higher accumulation within these segments are not known.

Since the distributions of individual alkaloids, nitrate, and nitrite, within the lamina were quite different, it should be possible to discern if the concentrations of these constituents within the leaf are correlated with the concentration of the TSNAs in tobacco. One would predict that alkaloids and nitrite would influence nitrosamine accumulation since they are both necessary intermediates for nitrosamine formation.

Distribution of Tobacco-Specific Nitrosamines. Data for distribution of nitrosamines in leaf are presented in Table II. In lamina the concentration of nitrosamines was lowest at the tip of the leaf and then increased to maximum levels at the base of the leaf. Also, nitrosamine concentration decreased from the center of the leaf to the outer periphery. The midrib follows the same trend. Segments 1–6 of midvein contained the lowest concentrations of nitrosamines in the leaf. There was a marked increase of individual nitrosamines from segments 7–10, and these segments contained nitrosamine levels as high as those of the lamina on a microgram per gram basis. Lateral vein data showed approximately the same trend of having the lowest concentrations of TSNAs at the tip of the leaf.

To better present the distribution of TSNAs in the leaf,

Table II. Distribution of Nitrosamines in Leaf

leaf segment	tissue[a]	NNN, $\mu g\ g^{-1}$	NAT, $\mu g\ g^{-1}$	NNK, $\mu g\ g^{-1}$	TSNA, $\mu g\ g^{-1}$
1A	L	0.313	0.509	0.028	0.850
1B	L	0.302	0.484	0.010	0.796
2A	L	0.279	0.479	0.007	0.765
2B	L	0.249	0.388	0.012	0.649
2C	L	0.340	0.547	0.022	0.909
3A	L	0.390	0.692	0.011	1.093
3B	L	0.241	0.484	0.010	0.735
3C	L	0.206	0.374	0.013	0.593
3D	L	0.246	0.434	0.003	0.683
4A	L	0.532	1.102	0.043	1.677
4B	L	0.363	0.696	0.018	1.077
4C	L	0.380	0.757	0.044	1.159
4D	L	0.358	0.612	0.026	0.996
5A	L	0.592	1.271	0.018	1.881
5B	L	0.476	0.967	0.018	1.461
5C	L	0.387	0.695	0.026	1.108
5D	L	0.342	0.617	0.024	0.982
6A	L	0.519	1.066	0.013	1.598
6B	L	0.545	1.171	0.016	1.733
6C	L	0.490	0.890	0.019	1.400
6D	L	0.507	1.033	0.024	1.563
7A	L	0.567	1.272	0.020	1.859
7B	L	0.526	1.044	0.020	1.590
7C	L	0.413	0.843	0.033	1.289
7D	L	0.530	0.979	0.014	1.523
8A	L	0.506	1.178	0.024	1.708
8B	L	0.509	1.071	0.019	1.598
8C	L	0.488	0.917	0.011	1.416
9A	L	0.675	1.616	0.025	2.316
9B	L	0.468	0.925	0.016	1.410
10A	L	0.681	1.331	0.041	2.053
1	M	0.288	0.301	0.005	0.591
2	M	0.288	0.301	0.005	0.591
3	M	0.182	0.204	0.008	0.394
4	M	0.107	0.146	0.004	0.255
5	M	0.142	0.117	ND[b]	0.259
6	M	0.164	0.299	0.008	0.466
7	M	0.375	0.831	0.006	1.212
8	M	0.531	1.087	0.014	1.632
9	M	0.654	1.130	0.036	1.821
10	M	0.770	0.592	0.069	1.431
2A	V	0.457	0.558	0.180	1.196
3A	V	0.353	0.294	0.018	0.664
3B	V	0.281	0.342	0.028	0.651
4A	V	0.304	0.431	0.020	0.754
4B	V	0.299	0.299	0.103	0.702
4C	V	0.383	0.478	0.028	0.890
5A	V	0.297	0.460	0.015	0.771
5B	V	0.371	0.474	0.015	0.861
5C	V	0.621	0.846	0.115	1.582
6A	V	0.465	0.541	0.022	1.029
6B	V	0.542	0.637	0.043	1.222
6C,D	V	0.409	0.439	0.022	0.870
7A	V	0.407	0.464	0.042	0.913
7B	V	0.422	0.533	0.034	0.989
7C,D	V	0.830	0.908	0.129	1.867
8A	V	0.501	0.726	0.014	1.241
8B,C	V	0.654	0.623	0.027	1.304
9A,B	V	0.531	0.791	0.034	1.356

[a] L, lamina; M, midvein; V, lateral vein. [b] ND, not detected.

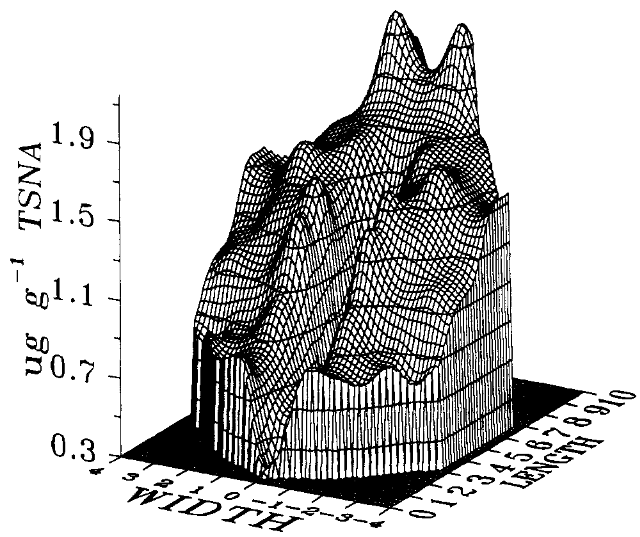

Figure 6. Distribution of tobacco-specific nitrosamines in leaf.

data for the lateral vein and lamina were combined using the weighted average of each segment. A surface graph for the total TSNA is presented in Figure 6 and gives a more complete view of the distribution of TSNAs in the tobacco leaf. The tip of the leaf contained the lowest concentration and the base of the leaf the highest level of TSNAs. Also, the TSNA concentration was lowest for the midrib in segments 1–6. In segment 8, the concentrations of TSNAs in lamina and midvein were equivalent. Along the length of the leaf, the TSNA concentration was highest in the leaf segments adjacent to the midvein. Higher concentration adjacent to the midvein may be the result of the slower dry down of the inner portion of the lamina during curing. Elevated moisture in cured lamina has been shown to result in higher concentrations of TSNAs (Burton et al., 1989a,b, 1990).

The data from surface plot of Figure 6 also showed the apparent complexity of nitrosamine accumulation in tobacco and conflicting data from previous studies. Burton et al. (1989b, 1990) reported that the midvein of tobacco contained higher levels of TSNAs than lamina. These observations were in agreement with data from a study by Chamberlain et al. (1986). The above data were for samples taken from whole leaf. Conversely, data presented by Djordjevic et al. (1985, 1989) indicated that the midvein contains the lowest levels of nitrosamines. From the experimental data, their samples were a subsample taken from the center of the leaf (segments 3–7). Midvein in those samples would contain the lowest levels of TSNAs. If the TSNAs were calculated on a percent distribution, it would more clearly show that the sampling for TSNAs was not representative for the whole leaf. TSNA data based on percent of distribution in the leaf (Figure 7) show that the highest distribution is in the last three segments of the midvein. Sampling from middle segments of the leaf would result in low values for the TSNAs in the midvein which would not be indicative of the TSNA concentration in the whole leaf.

Relationships between Nitrite, Alkaloids, and TSNA Accumulation. Previous studies have indicated there is a relationship between alkaloid content and TSNA accumulation (Djordjevic et al., 1989). Also, there is a positive relationship between nitrite concentration and TSNA accumulation in the cured leaf (Burton et al., 1989a,b, 1990). Data from this indicate that nitrite content may be a better indicator of potential TSNA accumulation in the leaf than alkaloid concentration. From Figure 5, nornicotine concentration is greatest at the periphery of the leaf whereas nitrosonornicotine (NNN) concentration is highest along the segments adjacent to the midrib and at the base of the leaf. Except for the midrib, nitrite levels are generally uniform across the leaf, but nitrite increases from the tip to the base of the leaf. This increase of nitrite is also observed for the midrib. When nitrite data are presented as percent distribution in the leaf (Figure 8), its profile closely resembles the percent distribution of TSNAs (Figure 6). The TSNA distribution in leaf more closely parallels the nitrite levels than it does the alkaloid content of the leaf. Therefore, from this study, nitrite, and not alkaloids or nitrate, appears to be the limiting factor for TSNA accumulation.

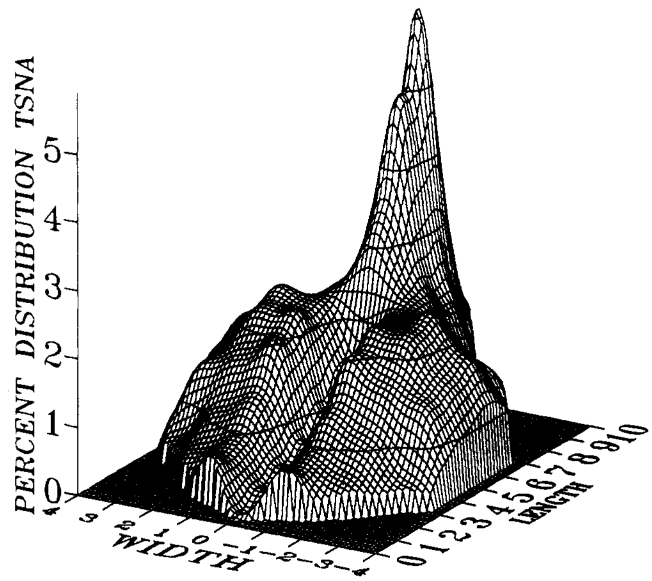

Figure 7. Percent distribution of tobacco-specific nitrosamines in tobacco leaf segments.

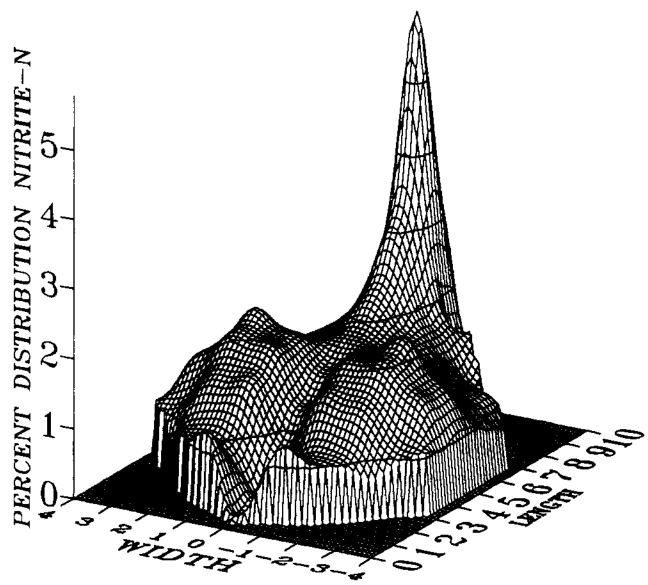

Figure 8. Percent distribution of nitrite nitrogen in tobacco leaf segments.

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Registry No. NO_3^-, 14797-55-8; NO_2^-, 14797-65-0; nicotine, 54-11-5; myosmine, 532-12-7; nornicotine, 494-97-3; anabasine, 494-52-0; anatabine, 581-49-7; *N*-nitrosonornicotine, 16543-55-8; *N'*-nitrosoanatabine, 71267-22-6; 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, 64091-91-4.