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Relationship between Tobacco-Specific Nitrosamines and Nitrite from Different Air-Cured Tobacco Varieties

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Three tobacco varieties of differing agronomic characteristics were grown and air-cured during the 1988–1990 growing seasons. The leaves were separated into lamina and midvein from three stalk positions. The replicated samples were analyzed for individual alkaloids, nitrate, nitrite, N′-nitrosonornicotine, N′-nitrosoanatabine, N′-nitrosoanabasine, and 4-[(methylnitroso)amino]-1-(3′-pyridyl)-1-butanone. The air-cured flue-type tobacco contained consistently lower quantities of the individual tobacco-specific nitrosamines (TSNA). There were no statistical differences in TSNA and stalk position or TSNA and curing season. Correlation coefficients were determined between nitrite and individual TSNA. Their coefficients showed there was a positive relationship between nitrite levels and individual TSNA concentrations in the tobacco tissue. From this 3-year study there was no relationship between alkaloids or nitrate and individual TSNAs. This study indicates that accumulation of TSNA in tobacco is limited by the amount of nitrite accumulated during air-curing.

Keywords: *Nicotiana tabacum L.; nicotine; tobacco-specific nitrosamines (TSNA); nitrite; nitrate*

INTRODUCTION

Accumulations of tobacco-specific nitrosamines (TSNAs) in tobacco have been of considerable interest during the past several years. This interest has been a result of the report that the TSNAs induced malignant tumors in mice, rats, and hamsters (Hoffmann and Hecht, 1985). The concentrations of TSNAs in tobaccos (Burton et al., 1989a; Chamberlain and Chortyk, 1992; Djordjevic et al., 1989) and processed tobaccos have been well documented (Fischer et al., 1989; MacKown et al., 1988; Brunnemann and Hoffmann, 1991). Even though TSNA concentrations in tobacco have been reported, there is little information available on the conditions that control their accumulation. Since TSNAs by definition are formed from the pyridine alkaloids, it has been postulated that higher levels of alkaloids in tobacco will result in enhanced accumulation of TSNA. Djordjevic et al. (1989) reported there was a positive correlation between alkaloids and TSNAs. However, in their studies there was a 3000-fold excess of the alkaloid in the tobaccos in comparison to the level of TSNAs that were analyzed. This large excess of alkaloid concentration implied alkaloids were limiting TSNA accumulation. Chamberlain and Chortyk (1992) studied the influence of curing on the accumulation of TSNAs from a flue-type and a burley-type tobacco. They reported that air-curing effected an increase of TSNA in lamina, while nitrogen fertilization effected an increase of TSNA in cured tobacco. Their results were from only a 1-year study.

Another possible plant constituent that could limit nitrosamine accumulation is nitrate, the precursor for nitrite. Brunnemann et al. (1983) reported there was a positive correlation between nitrate and TSNA in tobacco and cigarette smoke. In a survey of domestic and European cigarette brands Fischer et al. (1989) also reported there was a positive correlation between nitrate and TSNAs in the tobaccos. It should be noted there was over a 1000-fold excess of nitrate in comparison to the TSNAs found in tobacco. Because of the large excess of nitrate in tobacco, it is proposed that nitrate does not necessarily limit accumulation of nitrosamines.

It seems the component in tobacco that limits nitrosamine accumulation is nitrite. Burton et al. (1989b) reported that cured tobacco stored at 32 °C and 90% relative humidity had not only a large increase of TSNA accumulation but a significant increase of nitrite. This supported the data obtained when tobacco was air-cured at an elevated temperature and relative humidity (Burton et al., 1989a). The above studies showed there was a positive correlation between nitrosamine accumulation and nitrite in plant material. However, the environment required for increased accumulation of nitrosamines was substantially hotter and contained more moisture than the environment tobacco is generally exposed to during curing. The purpose of this study was to determine if there was a relationship between nitrite levels and the accumulation of TSNAs during air-curing of tobaccos. Three different tobacco varieties, a flue type, a burley type and a dark type, were used to determine if there was a varietal influence on nitrite formation and nitrosamine accumulation during curing of tobacco from three growing seasons.

EXPERIMENTAL PROCEDURES

Tobacco Production. Speight 28 (G28), a flue tobacco variety, a burley tobacco variety (KY 14), and dark tobacco variety (KY 171) were grown at the University of Kentucky Agricultural Experimental Station Farm in 1988, 1989, and 1990. The tobaccos were grown using cultural practices for the production of burley tobacco. Tobacco was harvested 4 weeks after topping. The tobacco plants were cut and placed on sticks (five plants per stick). Fifteen sticks (five sticks per replicate) from each of the three tobacco varieties were cured in a conventional curing barn. After curing, the leaves were removed from the plants and separated equally into top, middle, and bottom grades from the stalk. Leaves from 10 plants per replicate were taken for each variety. The leaves were separated into lamina and midvein tissue, dried, ground to pass a 40-mesh wire screen, and stored at −40 °C until analyses.

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Table 1. Nitrate-N, Nitrite-N, and Individual Nitrosamines in Lamina and Midvein from Three Tobacco Varieties from Three Growing Seasons[a]

year	variety		NNN	NAT	NNK	TSNA	NO_3-N	NO_2-N
				Lamina				
1988	KY 171	top	0.59	1.06	0.12	1.79	1280	2.74
		middle	0.78	1.25	0.19	2.24	1770	3.48
		bottom	1.18	1.40	0.33	2.94	2250	4.40
	G28	top	0.17	0.40	0.06	0.64	1110	2.63
		middle	0.18	0.46	0.07	0.72	1910	2.74
		bottom	0.33	0.87	0.09	1.31	3430	5.48
	KY 14	top	0.46	1.51	0.08	2.08	3280	3.72
		middle	0.41	1.20	0.09	1.72	3950	2.90
		bottom	0.60	1.97	0.17	2.76	5900	5.65
1989	KY 171	top	0.51	1.26	0.03	1.82	860	1.35
		middle	0.62	1.48	0.07	2.21	1850	1.58
		bottom	0.67	1.23	0.07	1.98	2960	2.79
	G28	top	0.18	0.49	0.01	0.69	1050	1.53
		middle	0.15	0.40	0.02	0.57	2040	1.58
		bottom	0.17	0.34	0.01	0.52	3410	3.81
	KY 14	top	0.63	2.34	0.06	3.02	2890	2.23
		middle	0.53	1.59	0.04	2.16	6210	3.58
		bottom	0.39	0.95	0.01	1.36	9130	3.88
1990	KY 171	top	0.39	0.55	0.01	0.09	760	2.27
		middle	0.47	0.77	0.04	1.29	1370	2.35
		bottom	0.62	0.77	0.06	1.45	1710	4.45
	G28	top	0.17	0.21	0.00	0.38	1100	4.73
		middle	0.19	0.32	0.01	0.53	1610	3.23
		bottom	0.34	0.50	0.03	0.87	1690	4.03
	KY 14	top	0.40	0.91	0.01	1.33	2200	3.50
		middle	0.50	1.18	0.01	1.69	4140	2.91
		bottom	0.64	1.39	0.03	2.07	6550	8.14
lsd (05)			0.13	0.35	0.05	0.48	643	1.17
				Midvein				
1988	KY 171	top	1.80	2.25	0.76	4.85	9050	6.67
		middle	1.77	2.04	0.68	4.53	13390	10.67
		bottom	1.55	1.63	0.77	3.98	14400	17.97
	G28	top	0.83	0.87	0.21	1.91	9390	6.15
		middle	0.45	0.56	0.13	1.14	15660	4.93
		bottom	0.49	0.49	0.07	1.06	28900	8.58
	KY 14	top	0.37	0.89	0.04	1.31	18860	5.58
		middle	0.43	0.80	0.15	1.40	23140	8.38
		bottom	0.46	0.85	0.12	1.43	26970	11.51
1989	KY 171	top	1.70	2.39	0.64	4.79	7930	25.27
		middle	1.94	2.36	0.82	5.18	11580	44.20
		bottom	1.12	1.49	0.18	2.79	18590	40.13
	G28	top	0.32	0.27	0.01	0.60	9780	10.87
		middle	0.26	0.22	0.00	0.49	17160	5.07
		bottom	0.31	0.55	0.02	0.88	20310	19.00
	KY 14	top	2.50	4.62	0.59	7.80	17770	104.33
		middle	1.49	2.39	0.45	4.33	22150	41.07
		bottom	0.82	1.26	0.06	2.14	25930	37.67
1990	KY 171	top	1.03	1.15	0.08	2.27	6670	13.60
		middle	1.78	1.58	0.46	3.82	9290	20.17
		bottom	1.37	1.13	0.13	2.64	9900	17.83
	G28	top	1.35	0.94	0.17	2.45	9490	23.50
		middle	0.43	0.37	0.03	0.83	11050	11.83
		bottom	0.53	0.49	0.04	1.06	9040	23.83
	KY 14	top	1.21	2.39	0.54	4.14	11020	49.33
		middle	0.77	1.18	0.19	2.14	17190	34.67
		bottom	0.79	1.16	0.06	2.01	21440	23.00
lsd (05)			0.76	0.80	0.41	1.87	22770	26.30

[a] All values are micrograms per gram of dry tobacco.

Table 2. TSNA, Nitrite-N, and Nitrate-N in Lamina and Midvein across Stalk Positions[a]

	NNN	NAT	NNK	TSNA	NO_2-N	NO_3-N
			Lamina			
top	0.39	0.97	0.04	1.41	1610	2.75
middle	0.43	0.96	0.06	1.46	2760	2.71
bottom	0.55	1.05	0.09	1.70	4120	5.85
lsd (0.05)	NS	NS	NS	NS	2450	2.94
			Midvein			
top	1.24	1.75	0.34	3.35	11110	27.3
middle	1.04	1.28	0.32	2.65	15620	20.1
bottom	0.83	1.01	0.16	2.00	19490	22.2
lsd (0.05)	NS	NS	NS	NS	7560	NS

[a] Rep × var × year. All values are micrograms per gram of dry tobacco.

Table 3. Effect of Variety on Accumulation of TSNAs, Nitrate-N, and Nitrite-N over Three Growing Seasons[a]

variety	NNN	NAT	NNK	TSNA	NO_3-N	NO_2-N
			Lamina			
KY 171	0.65	1.09	0.10	1.85	1650	2.83
G 28	0.21	0.44	0.03	0.69	1930	4.42
KY 14	0.51	1.45	0.06	2.02	4920	4.06
lsd (0.05)	0.22	0.54	NS	0.77	1950	NS
			Midvein			
KY 171	1.56	1.78	0.50	3.87	11200	21.83
G 28	0.55	0.53	0.08	1.16	14530	12.64
KY 14	0.98	1.73	0.25	2.97	20500	35.06
lsd (0.05)	0.89	1.22	NS	2.42	7160	NS

[a] Rep × stalk position × year. All values are micrograms per gram of dry tobacco.

Analyses. Nitrate-N concentration was determined using a Technicon autoanalyzer and Technicon procedure (Technicon Industrial Method 100−70W/B). Nitrite-N concentrations were determined using a colormetric procedure described by Crutchfield and Burton (1989). Individual alkaloids were analyzed by capillary chromatography using the procedure according to Madsen et al. (1984). Individual TSNAs were determined using procedures described by Burton et al. (1992). Because of the potential carcinogenic activity of the nitrosamines, all extractions and concentrations were carried out in laboratory hoods. The four TSNAs analyzed were *N*′-nitrosonornicotine (NNN), *N*′-nitrosoanatabine (NAT), *N*′-nitrosoanabasine (NAB), and 4-[(methylnitroso)amino]-1-(3′-pyridyl)-1-butanone (NNK). NAB eluted from the column as a shoulder on the larger NAT peak and was not completely resolved. Therefore, the concentration of NAT is reported as the summation of NAT and NAB. It should be noted in commercial tobaccos the concentration of NAB is approximately 10% of the concentration of NAT (Burton et al., 1989a).

RESULTS AND DISCUSSION

Influence of Variety, Stalk Position, and Year. Because of the large number of samples taken for this study, complete data are not presented. Average replicate values for TSNA, nitrate-N, and nitrite-N concentrations in cured lamina from the three varieties, three stalk positions, and three years are presented in Table 1. There are significant differences in the lamina between individual nitrosamines of the varieties. The level of NNN in cured lamina is usually less than 1 μg g^{-1}, yet there is a 4-fold difference in the concentration of NNN. The same trends are exhibited by NAT, NNK, and total TSNAs. For all 3 years the air-cured flue-type (G28) tobacco contained the lowest levels of TSNAs in the lamina. The nitrite concentrations from the tobacco lamina generally were less than 5 μg g^{-1} and did not show any trend between variety and year; however, the cured lamina from the bottom of the plant generally contained higher quantities of nitrite. Statistically, the lamina from the bottom of the plant contained significantly higher levels of nitrite and nitrate (Table 2). There was no statistical difference between stalk position and individual or total TSNAs. The higher levels of nitrite may be due, in part, to the higher concentration of nitrate in the bottom of the plant. The greater availability of nitrate may have resulted in the increased accumulation of nitrite. There was a significant effect of variety on the accumulation of nitrosamines in cured lamina (Table 3). Except for NNK, the flue-type tobacco contained lower levels of individual TSNAs in lamina and midvein in comparison to the other tobacco types. This agrees with the data reported by Chamberlain and Chortyk (1992) and Fis-

Table 4. Alkaloid Concentrations from Three Tobacco Varieties over Three Growing Seasons[a]

variety	nicotine	nornicotine	anabasine	anatabine	total
		Lamina			
KY 171	40.00	0.24	0.11	1.91	42.26
G 28	13.11	0.10	0.06	1.09	14.36
KY 14	27.97	0.17	0.10	2.13	30.37
lsd (0.05)	10.90	NS	0.03	0.83	11.78
		Midvein			
KY 171	10.93	0.16	0.04	0.63	11.76
G 28	4.29	0.10	0.02	0.41	4.82
KY 14	7.67	0.12	0.03	0.72	8.54
lsd (0.05)	3.51	NS	NS	NS	3.97

[a] Rep × stalk position × year. All values are micrograms per gram of dry tobacco.

Table 5. TSNA, Nitrate-N, and Nitrite-N Levels in Cured Lamina and Midvein from Three Growing Seasons[a]

	NNN	NAT	NNK	TSNA	NO_3-N	NO_2-N
lamina	0.46	0.99	0.06	1.52	3070	3.23
midvein	1.03	1.35	0.27	2.67	17270	16.98
lsd (0.05)	NS	NS	NS	NS	6540	1.44

[a] Rep × var × year × stalk position. All values are micrograms per gram of dry tobacco.

cher et al. (1989). The flue tobacco (G28) contains the lowest concentrations of nornicotine, anatabine, and anabasine (Table 4). The levels of these secondary alkaloids represent over a 200-fold excess of secondary amine to the nitrosamine. Even though there appears to be a relationship between the secondary amine alkaloids and individual TSNAs, the excess concentration of alkaloid suggests that alkaloids are not limiting the TSNA accumulation. There is no statistical significance between variety and nitrite accumulation (Table 3). This would suggest nitrite per se did not influence TSNA accumulation.

The data for nitrite-N and individual nitrosamines from midvein samples by variety, year, and stalk position are presented in Table 1. The midveins contain higher levels of TSNAs than the lamina. There was a 9-fold range of NNN levels and a 20-fold range of NAT concentration in the midvein, and these differences are much larger than those observed for lamina. Nitrite levels in cured midvein also exhibit a 20-fold range in their concentrations. The average midvein value for nitrite is 4.6 times higher than that in lamina, and the level of TSNAs is also higher in midvein than in lamina. However, there is no statistically significant difference between the levels of TSNAs in the lamina and midvein tissue (Table 5). When the nitrite values are transformed to $\log_{10}$ and statistical analyses of the transformed values are made, there is a significant difference between lamina and midrib values (Table 5). Midveins from the flue-type tobacco contain significantly lower levels of TSNAs (Table 3). From the lamina and midvein TSNA data, variety influences accumulation of nitrosamines (Table 3). From the present information it is difficult if not impossible to provide a rationale for the observed lower levels of TSNAs in the flue-type tobacco. It could be due to the lower levels of alkaloids in the flue-type tobacco; however, absence of statistical differences in midvein for the levels of the secondary pyridine alkaloids, nornicotine and anatabine, in the tobacco types (Table 4) makes it difficult to draw an unequivocal conclusion. There are no statistical differences between variety and nornicotine in the lamina, whereas G28 contains a statistically significant lower amount of anatabine than either the burley or dark tobaccos. In this study, nornicotine levels are approximately the same in midvein as they are in lamina (Table 4). In 1988 the concentration of nornicotine was higher in midvein of all varieties in comparison to lamina. In 1989 and 1990 the laminas contain higher levels of nornicotine, which is the usual observation for tobacco. Nicotine, anabasine, and anatabine concentrations are higher in the lamina than in the midvein tissue. This difference may be predicted since nicotine, anabasine, and anatabine are biosynthesized in the root during vegetative growth, whereas nornicotine is formed by demethylation of nicotine in the leaf during senescence and death of the leaf tissue (Bush et al., 1993). The higher levels of nornicotine in midvein for 1988 may be due to the observation that midvein is the last portion of the leaf to lose its cell integrity. The delays in drydown in the midvein may have allowed the nicotine to be demethylated, increasing the net accumulation of nornicotine in the midvein. There is an approximate 2-fold increase of NNN in the midvein in comparison to the lamina values, whereas there is only a 1.6-fold increase of NAT. This follows the trend observed for the relative levels of nornicotine and anatabine in the lamina and midvein.

Data for nitrite-N across this 3-year study show there was no statistical difference in the concentration of nitrite-N in the midvein and the three varieties of tobacco (Table 3). There also is no statistical significance between nitrite and year or stalk position (data not shown). Data from this study suggest nitrite accumulation did not limit TSNA accumulation. This is in contrast to our previous studies indicating there was a positive relationship between nitrite and nitrosamine accumulation (Burton et al., 1989). However, there is another possible relationship between TSNAs and nitrite levels in the cured lamina. Determining correlation coefficients between nitrite and individual nitrosamines should show if there are relationships between nitrosamines and nitrite concentrations in tobacco.

Correlation between Nitrite-N and Tobacco-Specific Nitrosamines. Nitrite accumulation in tobacco may not be uniform during the curing process and thereby would affect a scatter in the data among different tobacco types, stalk positions, and plant parts, resulting in insignificant relationships in nitrite accumulation in these varieties. To test this hypothesis, correlation coefficients were calculated for nitrite-N versus individual TSNAs. The data for nitrite-N versus NNN from the three varieties, three stalk positions, three years, and lamina and midvein are presented in Figure 1 as a log–log plot. Plotting NO_2-N vs NNN on a log–log scale gives the best visual representation of the data and correlation coefficients. Since midveins contained higher quantities of nitrosamines than the lamina, the lamina and midvein data are presented separately. There is a significant correlation between nitrite and NNN. From this figure one can visualize the clusters of NNN for lamina and midvein represented in Table 5. The correlation coefficient between nitrite-N and NNN for only lamina was not significant. There was a significant positive correlation between NO_2-N and NNN in the midvein tissue ($R = 0.5741$). Therefore, midvein data were contributing significantly to the positive relationship observed between nitrite-N and NNN from the combined lamina and midvein values. There was a significant positive correlation between nitrite-N and NNK (Figure 2), which parallels the

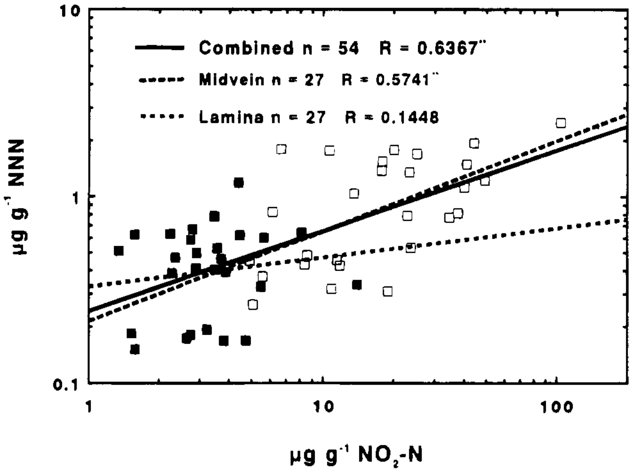

Figure 1. Nitrite-N versus NNN in lamina and midvein of air-cured tobacco varieties: (■) lamina; (□) midvein.

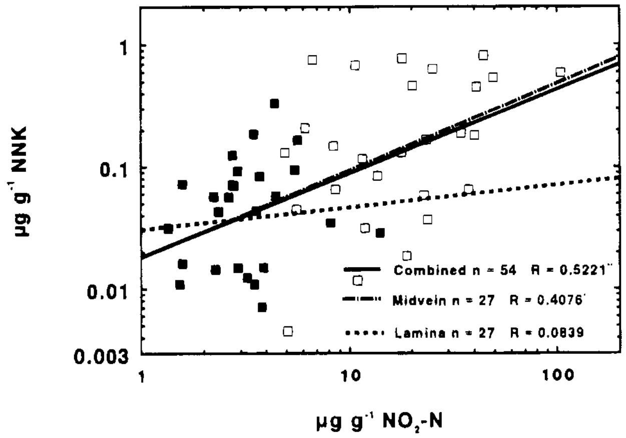

Figure 2. Nitrite-N versus NNK in lamina and midvein of air-cured tobacco varieties: (■) lamina; (□) midvein.

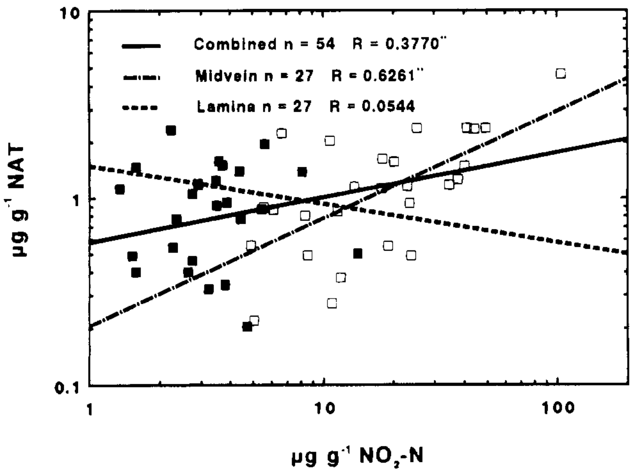

Figure 3. Nitrite-N versus NAT in lamina and midvein of air-cured tobacco varieties: (■) lamina; (□) midvein.

results obtained for NNN. Again there was no significant correlation between NO_2-N and NNK in the lamina. Nitrite versus NAT has a correlation coefficient of 0.3770 (Figure 3). This low but significant correlation coefficient was due to the poor correlation between NO_2-N and NAT in the lamina. The correlation coefficient (0.6261) was highest for the midvein. Summation of the individual TSNAs also gave a significant positive correlation ($R = 0.5053$) with nitrite-N. Again the midvein data have the highest correlation coefficient ($R = 0.5934$), and there was no significant correlation between nitrite content and TSNAs in the lamina ($R = 0.0493$). At this time there is no definitive explanation for this lack of correlation. Perhaps the conditions for these three curing seasons were not sufficient to allow a wide range of nitrite accumulation to occur.

Table 6. Correlation Coefficients between Nitrite-N and Individual Tobacco-Specific Nitrosamines[a]

		KY 171	G28	KY 14
		Lamina		
NO_2-N	NNN	0.595	0.777*	0.347
	NAT	0.108	0.123	0.042
	NNK	0.576	0.098	0.132
	TSNA	0.205	0.309	0.042
		Midvein		
NO_2-N	NNN	0.102	0.317	0.951**
	NAT	0.025	0.304	0.878**
	NNK	0.064	0.040	0.700*
	TSNA	0.048	0.282	0.906**
		Lamina and Midvein		
NO_2-N	NNN	0.827**	0.733**	0.847**
	NAT	0.569**	0.275	0.455*
	NNK	0.693**	0.250	0.728**
	TSNA	0.714**	0.512**	0.651**

[a] Significance: **, $P \leq 0.01$; *, $P \leq 0.05$.

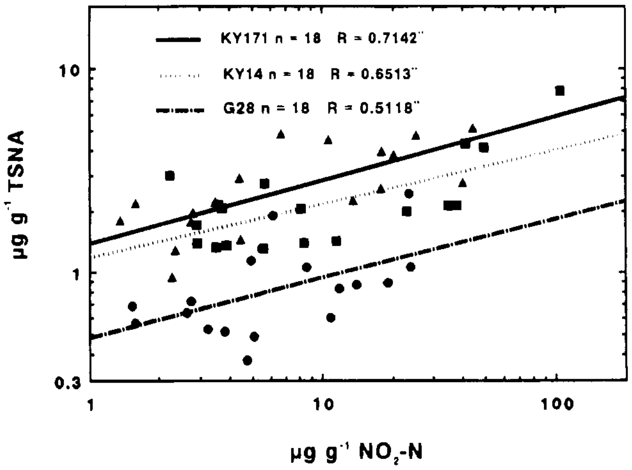

Figure 4. Nitrite-N versus TSNA in lamina and midvein from three tobacco varieties: (△) KY 171; (■) KY 14; (●) G28.

Even though there was a correlation between nitrite and nitrosamines in the three tobacco varieties, there was a wide scatter of data points. This is most likely due to the differences in the levels of nitrosamines from these three varieties. Correlation coefficients were determined between nitrite and nitrosamines separately for each variety (Table 6). This included both lamina and midvein data across three growing seasons. There is a significant correlation between nitrite and TSNAs from each variety. The dark type tobacco and burley tobacco have the highest concentration of nitrosamines, whereas the air-cured flue-type tobacco contains the lowest levels of TSNAs (Figure 4). This is expected since the alkaloid data had the same trend (Table 4). The slopes of the lines for the dark and flue tobacco varieties are nearly parallel in this concentration range; only the intercepts are different. The flue tobacco had a significant correlation between only nitrite-N and NNN (Table 6).

Except for NNN in G28 tobacco, there was no significant correlation between nitrosamines and nitrite-N in the lamina tissue. This most likely was due to the curing conditions during these 3 years not being conducive to the accumulation of a high nitrite concentration. Perhaps under different curing conditions, there would be greater nitrite accumulation in the lamina, resulting in a greater accumulation of nitrosamines. A recent study has shown that if there is a wide range of nitrite accumulation, 2−4400 μg g^{-1}, there is a significant correlation between individual TSNA and nitrite-N (Burton et al, 1993). Burley tobacco had a high and significant correlation between nitrite and individual nitrosamines (Table 6). The lack of correlation from the dark and flue-type tobaccos would indicate there are factors other than nitrite and alkaloid accumulation that influence the accumulation of tobacco-specific nitrosamines. It should be noted in this study there was no correlation between individual alkaloids and nitrosamines.

This study has shown that an air-cured flue-type tobacco (G28) contains lower levels of TSNAs than air-cured dark (KY 171) or burley tobacco (KY 14) varieties. There were no statistical differences among the three growing seasons and nitrosamine accumulations, indicating that under normal climatic conditions there are no differences in accumulation of nitrite and nitrosamines. In nonlinear regression analysis of the data, there is a positive relationship between nitrite accumulation and nitrosamine formation. This is the first report of a relationship between nitrite and TSNAs from tobacco cured using on-farm curing of stalk cut tobaccos. Determination of correlation coefficients for nitrite and TSNA data from combined midvein and lamina shows there is a poorer correlation between nitrite and TSNAs when compared to midvein data only. Future studies on the accumulation of nitrosamines from different tobacco types may provide additional information on other factors that affect nitrosamine accumulation in tobacco.

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LITERATURE CITED

Brunnemann, K. D.; Hoffmann, D. Analytical studies on N-nitrosamines in tobacco and tobacco smoke. *Recent Adv. Tob. Sci.* **1991**, *17*, 71−112.

Brunnemann, K. D.; Masaryk, J.; Hoffmann, D. Role of tobacco stems in the formation of nitrosamines in tobacco and mainstream and sidestream smoke. *J. Agric. Food Chem.* **1983**, *31*, 1221−1224.

Burton, H. R.; Dye, N. K. Differences in composition of air-cured burley tobacco using modified field curing structures. 1. Changes in nitrogeneous constituents. In *Abstracts of Papers*, 47th Tobacco Chemists' Research Conference, Gatlinburg, TN; Tobacco Literature Service, NCSU: Raleigh, NC, 1993; Abstr. 34.

Burton, H. R.; Bush, L. P.; Djordjevic, M. V. Influence of temperature and humidity on the accumulation of tobacco-specific nitrosamines in stored burley tobacco. *J. Agric. Food Chem.* **1989a**, *37*, 1372−1377.

Burton, H. R.; Childs, G. H., Jr.; Andersen, R. A.; Fleming, P. D. Changes in chemical composition of burley tobacco during senescence and curing. 3. Tobacco-specific nitrosamines. *J. Agric. Food Chem.* **1989b**, *37*, 426−430.

Burton, H. R.; Dye, N. K.; Bush, L. P. Distribution of tobacco constituents in tobacco leaf tissue. 1. Tobacco-specific nitrosamines, nitrate, nitrite, and alkaloids. *J. Agric. Food Chem.* **1992**, *40*, 1050−1055.

Bush, L. P.; Fannin, F. F.; Chelvarajan, R. L.; Burton, H. R. Biosynthesis and metabolism of nicotine and related alkaloids. In *Absorption, Distribution, Metabolism and Excretion of Nicotine and Related Alkaloids*; Gorrod, J. W., Wahren, J., Eds.; Chapman and Hall: London, 1993; pp 1−30.

Chamberlain, W. J.; Chortyk, O. T. Effects of curing and fertilization on nitrosamine formation in bright and burley tobacco. *Beitr. Tabakforsch. Int.* **1992**, *15*, 87−91.

Crutchfield, J. D.; Burton, H. R. Modified automated colormetric procedure for quantification of nitrite in agricultural products. *Anal. Lett.* **1989**, *22*, 555−571.

Djordjevic, M. V.; Gay, S. L.; Bush, L. P.; Chaplin, J. F. Tobacco-specific nitrosamine accumulation and distribution in flue-cured tobacco isolines. *J. Agric. Food Chem.* **1989**, *37*, 752−756.

Fischer, S.; Spiegelhalder, B.; Preussmann, R. Preformed tobacco-specific nitrosamines in tobacco—role of nitrate and influence of tobacco type. *Carcinogenesis* **1989**, *10*, 1511−1517.

Hoffmann, D.; Hecht, S. Nicotine-derived N-nitrosamines and tobacco-related cancer: Current status and future directions. *Cancer Res.* **1985**, *45*, 435−444.

MacKown, C. T.; Douglass, B.; Djordjevic, M. V.; Bush, L. P. Tobacco specific N-nitrosamines; Formation during processing of midrib and lamina fines. *J. Agric Food Chem.* **1988**, *36*, 1031−1035.

Madsen, J. P.; Bush, L. P.; Gay, S. L. Effect of curing on polyamine content of Nicotiana tabacum L. genotypes with different alkaloid levels. *J. Agric. Food Chem.* **1984**, *33*, 1182−1185.

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