Alkaloid and Nitrate Nitrogen Concentration of Two Isogenic Strains of Burley Tobacco

John L. Sims, Lowell P. Bush, and William O. Atkinson

Nitrate nitrogen, total nitrogen, nicotine, nornicotine, total alkaloids, and dry matter production were determined in cured leaves of two strains of Burley 21 tobacco. The tobacco had been produced under different nitrogen nutrition and suckering practices. Concentration of nicotine, nornicotine, total alkaloids, and total nitrogen was lowest in leaves from the lowest stalk position. The reverse was true for nitrate concentration. Nitrogen fractions, other than the isogenic factors nicotine and nornicotine, and dry matter were similar in the two strains. Nitrogen components except nicotine were significantly higher in leaves from plants treated at the rate of 448 kg of N per ha than from plants treated with 112 kg of N per ha. Nitrate concentration was two to three times greater at the high nitrogen fertilizer level. Generally plants treated with maleic hydrazide (MH-30) for axillary bud control had lower concentrations of nitrate, nornicotine, nicotine, and total alkaloids than plants that had been hand suckered. The percentage of total alkaloids converted to nornicotine was greater at the high than low nitrogen fertilizer level.

Nitrate and secondary amine concentrations of cigarette tobacco are of interest from a human health standpoint in at least three ways. First, both nitrate and secondary amines are precursors of carcinogenic nitrosamines (Clapp and Craig, 1967; Druckrey and Preussmann, 1962; Magee and Barnes, 1956; Neurath *et al.*, 1964; Serfontein and Hurter, 1966a), possible constituents of tobacco smoke (Serfontein and Hurter, 1966b; Wynder and Hoffmann, 1967). Secondly, oxides of N may have pharmacological activity. The concentration of oxides of N in the main stream smoke of cigarettes is closely related to the nitrate content of cigarettes (Broaddus *et al.*, 1965). And third, nitrate salts added to cigarettes prior to smoking reduced the levels of certain toxic and tumor-causing compounds in the particulate phase of smoke (Benner *et al.*, 1968; Hoffmann and Wynder, 1967; Muller *et al.*, 1968).

Broaddus *et al.* (1965) reported that nitrate contents of tobacco could be influenced by N fertilizer rate, soil moisture, soil type, and, to a lesser extent, by genetic differences. Recent work by Sheen *et al.* (1968) revealed wide differences in nitrate contents between tobacco types but lesser differences within types. Studies by Peterson (1968) revealed nitrate concentration of tobacco to be influenced by N fertilizer but not by P or K fertilizer added to the growing crop.

Concentrations of nicotine and total alkaloids in tobacco leaf are well documented (Stedman, 1968). The four main alkaloids in tobacco are nicotine, nornicotine, anabasine, and anatabine (Pailer, 1965). Of interest in the current study is the fact that nornicotine, a secondary amine, may be nitrosated to nitrosonornicotine under certain conditions and as such may be health related (Boyland and Roe, 1966). Since nicotine is a tertiary amine, it is not nitrosated but may be of interest to health in ways other than carcinogenicity This paper reports the concentration of total N, nitrate, and alkaloids in cured leaf tissue of two strains of Burley 21 (isogenic for nornicotine concentration) tobacco grown under different conditions of N nutrition and sucker control practices.

Kentucky Agricultural Experiment Station, University of Kentucky, Lexington, Ky. 40506

EXPERIMENTAL

Cultural Procedures. A high nornicotine line (strain) and the commercial variety of Burley 21 (normal or low nornicotine) tobacco (*Nicotiana tabacum* L.) were grown in field plots at Lexington, Ky., during 1966. Plants used in the experiment were started in exploded vermiculite moistened with Hoagland's nutrient solution (Hoagland and Arnon, 1950) and grown in growth cabinets and in the greenhouse until about 20 cm high. Plants were started in this manner to insure uniformity of size before being transferred to the field. The soil used was Maury silt loam with little or no slope. The experimental area had been in mixed-grass sod the two previous years.

A split-split plot experimental design was utilized and treatments were replicated three times. Treatments consisted of two rates of N fertilizer (112 and 448 kg of N per ha), two strains of the tobacco variety Burley 21 (normal nornicotine and high nornicotine), and two sucker (axillary bud) control practices (hand suckered and chemically with maleic hydrazide). N rate was the whole plot, burley strain the subplot, and sucker control practices the sub-subplots. Individual plots consisted of three rows that were 1.1 m apart and 11 m long, and plants were set 41 cm apart in rows.

All fertilizer was broadcast on the soil surface and incorporated 1 week prior to transplanting. Fertilizer rates and kinds were 100 kg of P per ha as 46% superphosphate, 280 kg of K per ha as potassium sulfate, and 112 or 448 of N per ha as ammonium nitrate.

All plots received irrigation water (sprinkler system) to

Table I. Nitrate N and Total N (Dry Weight Basis) in Cured Leaves of Two Strains of Burley 21 Tobacco Grown Using Two N Fertilizer Levels and Suckering Practices

N Fertilizer Level	Leaf Position	Low Nornicotine Burley 21		High Nornicotine Burley 21		
		Hand Suckered	MH-30	Hand Suckered	MH-30	Avg[a]
kg/ha				% Nitrate N		
112	Lower	0.55 g–j[b]	0.47 h–k	0.54 g–j	0.56 g–j	0.55 d
	Middle	0.32 h–k	0.26 j–k	0.28 i–k	0.41 h–k	0.35 e
	Upper	0.19 k	0.21 k	0.28 i–k	0.26 j–k	0.27 e
	Avg	0.35	0.31	0.37	0.41	
448	Lower	1.47 a	1.21 a–c	1.46 a	1.13 b–d	1.30 a
	Middle	1.08 b–d	0.88 d–f	1.31 a–b	0.79 e–g	1.05 b
	Upper	0.94 c–e	0.49 g–k	0.96 c–e	0.61 f–h	0.79 c
	Avg	1.16	0.86	1.24	0.84	
				% Total N		
112	Lower	2.67	3.15	3.25	3.73	3.20
	Middle	3.21	3.59	3.39	3.91	3.53
	Upper	3.47	3.93	4.07	4.33	3.95
	Avg	3.12	3.56	3.57	3.99	3.56
448	Lower	4.76	4.72	4.83	4.95	4.82
	Middle	4.84	4.89	5.31	5.08	5.03
	Upper	5.19	5.15	5.56	5.39	5.32
	Avg	4.93	4.92	5.23	5.14	5.06

[a] Averages in this column for nitrate at the 5% level.
[b] Means at the 5% level.

supplement rainfall when soil moisture dropped below about 60% of available moisture holding capacity. Soil moisture was determined using a Nuclear-Chicago D/M gage.

Conventional cultural practices were carried out to reduce nutritive competition and to have whole, healthy plants for sampling. For hand suckered plots, suckers were removed by hand when about 10 cm long, and for chemically suckered plots, maleic hydrazide (MH-30) was applied soon after topping at rates of 170 mg of active ingredient per plant. After harvesting and air-curing, the leaves were separated into upper, middle, and lower groups, each group containing approximately equal numbers of leaves. Subsamples of the cured leaf lamina were oven dried at 70° C for 48 hr, weighed, and ground in a Wiley mill fitted with a 40-mesh screen. Nitrate and total N were determined in duplicate on these samples. Additional subsamples were taken and the three replicates of each treatment combined into one sample per treatment. After drying and grinding, nicotine, nornicotine, and total alkaloids were determined on each sample.

Analytical Methods. Nicotine, nornicotine, and total alkaloids in tobacco were determined in duplicate following methods of Cundiff and Markunas (1955). Nitrate N was determined by the method of Lowe and Hamilton (1967). Total N was calculated by adding nitrate N and Kjeldahl N as determined with Technicon autoanalyzer following a modified procedure of Ferrari (1960).

Letters in tables for nitrate are used in accordance with Duncan's Multiple Range test. Any two individual means in the body of the table, or any two averages within a column that are not followed by the same letter or letter ranges, are significantly different at the probability level indicated.

RESULTS AND DISCUSSION

The concentration of nitrate N was greatest in cured tobacco leaves from the lower stalk position and decreased with ascending leaf position on the stalk (Table I). Concentrations in tissue from the upper stalk positions ranged from 32 to 63% of the quantities found in the lower positions. The influence of leaf position was less in leaves from plants treated with N fertilizer at the 448 kg per ha rate than in leaves from the 112 kg per ha treatment.

Average values for concentration of nitrate N were two to three times greater in leaves from plants treated with the 448 than 112 kg per ha N fertilizer rate. Nitrate N concentration averaged less in leaves from plants receiving MH-30 for sucker control than from plants hand suckered. However, a significant nitrogen X suckering practice interaction existed, and whereas suckering practice had little effect on nitrate N at the low N fertilizer rate, at the high N fertilizer rate values for MH-30 treated tobacco were much lower than for hand suckered tobacco. Nitrate N concentration in leaves was not influenced significantly by varietal strain.

The concentration of total N was lowest in leaves from the lower stalk position and increased with ascending leaf position on the stalk (Table I). Concentration of total N with respect to leaf position was just the reverse of nitrate N: leaves containing highest concentration of total N had lowest concentration of nitrate N and leaves containing highest nitrate levels had lowest total N.

Statistical analysis of the total N data revealed that, in addition to leaf position, all other main effects were significant. Highest average concentrations of total N were found in leaves treated with the high rate of N fertilizer, in the high nornicotine strain, and in leaves from plants treated with MH-30. The significant nitrogen X suckering practice interaction revealed that suckering practice had no effect on total N concentration at the high N fertilizer level, but at the low N fertilizer level, use of MH-30 resulted in higher total N concentrations.

The ratio of nitrate N to total N was calculated from the

Table II. Concentration of Nornicotine, Nicotine, and Total Alkaloids in Two Strains of Cured Burley 21 Tobacco Grown Using Two Nitrogen Fertilizer Levels and Suckering Practices

Nitrogen Rate	Leaf Position	Low Nornicotine Burley 21		High Nornicotine Burley 21	
		Hand Suckered	MH-30	Hand Suckered	MH-30
kg/ha		% Nornicotine			
112	Lower	0.48	0.42	2.24	1.56
	Middle	0.52	0.68	2.06	3.22
	Upper	0.42	0.54	3.10	2.40
	Avg	0.47	0.55	2.47	2.39
448	Lower	1.10	0.58	2.56	2.38
	Middle	0.68	0.68	3.34	3.46
	Upper	1.24	0.66	3.44	2.78
	Avg	1.01	0.64	3.11	2.87
		% Nicotine			
112	Lower	3.34	2.93	1.96	2.16
	Middle	4.98	4.60	3.30	0.87
	Upper	4.88	3.70	1.54	0.69
	Avg	4.40	3.74	2.27	1.24
448	Lower	3.07	3.70	1.36	0.71
	Middle	5.30	4.38	1.68	0.82
	Upper	4.69	3.42	1.44	0.64
	Avg	4.35	3.83	1.49	0.72
		% Total Alkaloids			
112	Lower	3.82	3.40	4.41	3.86
	Middle	5.56	5.35	5.55	4.40
	Upper	5.33	4.28	5.04	3.32
	Avg	4.90	4.34	5.00	3.86
448	Lower	4.27	4.04	4.16	3.31
	Middle	6.06	5.13	5.35	4.45
	Upper	6.04	4.37	5.75	3.74
	Avg	5.46	4.51	5.09	3.83

data of Table I to obtain evidence for changes in the relative concentrations of the two N fractions among treatments. Treatments influenced the ratio in a manner similar to that for nitrate N concentration(%), *e.g.*, highest ratios resulted from the high N fertilizer level, hand suckering practice, and lower stalk position, but were unaffected by varietal strain. This indicates nitrate N concentration was influenced by a given treatment to a much greater degree than total N.

Nornicotine concentration was three to five times greater in cured leaves from the high nornicotine strain than from the normal commercial strain of Burley 21 tobacco (Table II). Concentrations were lowest in leaves from the lower stalk positions and in leaves from plants fertilized at the 112 kg per ha N rate. Values for the middle and upper leaf positions did not differ significantly. Generally, concentration was lower in leaves from plants treated with MH-30 than from plants hand suckered. The effect of suckering practice was not consistent between leaf positions nor N fertilizer levels.

The concentration of both nicotine and total alkaloids was greater in leaves of the commercial strain than the high nornicotine strain (Table II). N fertilizer level influenced concentrations little, but concentrations of both nicotine and total alkaloids were lower in leaves of plants chemically suckered with MH-30.

Current work with plants of *Nicotiana tabacum* suggests that nicotine is synthesized in the roots and translocated to the leaves, where it is partially converted to nornicotine. Conversion may take place during the growing season and also during the curing process in some species (Jeffrey, 1959). A dominant gene controlling the conversion of nicotine to nornicotine in tobacco has been reported by a number of workers. Terrill (1965) utilized a value calculated as the percent nicotine divided by percent total alkaloids multiplied by 100 to represent the degree of conversion of nicotine to nornicotine in tobacco. Using this value to evaluate the influence of environmental factors on conversion in flue-cured tobacco types, Terrill showed that low-topping of tobacco influenced conversion, but N fertilizer had no effect.

In the present study the degree of conversion to nornicotine in each of the two strains was calculated as the percent nornicotine divided by percent total alkaloids multiplied by 100 (Table III). This method of calculation leads to expression of conversion with positive values. As expected, conversion was much higher in the high nornicotine strain. Conversion for MH-30 treated plants was somewhat higher than hand suckered plants and conversion in plants treated with N fertilizer at rates of 448 kg per ha was higher than in plants treated at the 112 kg rate.

Quality of tobacco often is associated with level of nornicotine, since tobaccos containing high levels of nornicotine may result in cigarettes with unsatisfactory smoke taste (Mann and Weybrew, 1958). Generally tobaccos with nornicotine contents less than 5–8% of the total alkaloid fraction are commercially acceptable. Nornicotine level of leaf from the low nornicotine strain in this study exceeded 8% of total alkaloids at both N fertilizer levels.

Table III. The Percentage of Total Alkaloids Converted to Nornicotine in Two Strains of Cured Burley 21 Tobacco Grown With Varying Nitrogen Nutrition and Suckering Practice

Nitrogen Rate kg/ha	Leaf Position	Low Nornicotine Burley 21		High Nornicotine Burley 21		Avg
		Hand Suckered	MH-30	Hand Suckered	MH-30	
112	Lower	13	12	51	40	29
	Middle	9	13	37	73	33
	Upper	8	13	62	72	38
	Avg	10	13	50	62	33
448	Lower	26	14	62	72	44
	Middle	11	13	62	78	41
	Upper	21	15	60	74	43
	Avg	19	14	61	75	43

Table IV. Dry Weight of Cured Leaves From Two Strains of Burley 21 Tobacco Grown Using Two N Fertilizer Levels and Suckering Practices

N Fertilizer Level	Low Nornicotine Burley 21		High Nornicotine Burley 21		
	Hand Suckered	MH-30	Hand Suckered	MH-30	Avg
kg/ha	Grams Per Plant				
112	97	110	97	105	102
448	124	133	122	135	129
Avg	111	122	110	120	

Dry weight of leaves per plant was greater for plants receiving the high N fertilizer level and for plants treated with MH-30 (Table IV). These data partially explain the lower alkaloid and nitrate N concentrations of MH-30 treated tobaccos (Tables I, II) through a dilution effect. Furthermore, MH-30 treated plants had greater amounts of photosynthesis and may have assimulated greater amounts of N in the leaves during the growing season (Table I), thus lowering nitrate levels.

ACKNOWLEDGMENT

We acknowledge the services of the personnel of the Analytical Services Laboratory (Supported by USDA Contract 12-14-100-9530-73) of the University of Kentucky Tobacco and Health Institute for making the determinations of alkaloids.

LITERATURE CITED

Benner, J. F., Burton, H. R., Burdick, D., *Tobacco Sci.* **12,** 37–40 (1968).
Boyland, E., Roe, F. J. C., "Lung Tumors in Animals." L. Severi, Ed., pp. 667–76, Div. of Cancer Res., Perugia, Italy, 1966.
Broaddus, G. M., York, J. E., Moseley, J. M., *Tobacco Sci.* **9,** 149–57 (1965).
Clapp, N. K., Craig, H. W., *J. Nat. Cancer Inst.* **39,** 903 (1967).
Cundiff, R. H., Markunas, P. C., *J. Ass. Anal. Chem.* **27,** 1650–53 (1955).
Druckrey, H., Preussmann, R., *Naturwissenschaften* **21,** 498 (1962).
Ferrari, A., *New York Acad. Sci.* **87,** 792 (1960).
Hoagland, D. R., Arnon, D. I., Calif. Agr. Exp. Sta. Cir. 347 (1950).
Hoffmann, D., Wynder, E. L., *Cancer Res.* **27,** 172 (1967).
Jeffrey, R. N., *Tobacco Sci.* **3,** 89–93 (1959).
Lowe, R., Hamilton, J., J. AGR. FOOD CHEM. **15,** 359 (1967).
Magee, P. N., Barnes, J. M., *Brit. J. Cancer* **10,** 114–22 (1956).
Mann, T. J., Weybrew, J. A., *Tobacco Sci.* **2,** 29–34 (1958).
Muller, R., Moldenhauer, W., Schleminer, P., *Inst. Tabakforsch. Ber.* **15,** 126–90 (1968).
Neurath, G., Pirmann, B., Wichern, H., *Beitr. Tabakforsch.* **2,** 311–19 (1964).
Pailer, M., "Tobacco Alkaloids and Related Compounds," U.S. von Euler, Ed, p. 346, Macmillan, New York, 1965.
Peterson, L. A., *Agron. J.* **60,** 26–9 (1968).
Serfontein, P. S., Hurter, P. S., *Afr. Cancer Bull.* **10,** 62–70 (1966a).
Serfontein, W. J., Hurter, P., *Cancer Res.* **26,** 575–9 (1966b).
Sheen, S. J., Calvert, J., Stokes, G. W., *Tobacco Sci.* **12,** 81–85 (1968).
Stedman, R. L., *Chem. Rev.* **68,** 153–207 (1968).
Terrill, T. R., *Diss. Abstr.* **26,** 605 (1965).
Wynder, E. L., Hoffmann, D., "Tobacco and Tobacco Smoke," p. 432, Academic Press, New York, 1967.

Received for review November 5, 1969. Accepted February 6, 1970 The investigation reported in this paper (No. 69-3-140) was in connection with a project of the Kentucky Agricultural Experiment Station and Agricultural Research Service, U.S. Department of Agriculture. This paper is published with the approval of the Director and was supported by Crops Research Division, ARS-USDA contract Nos. 12-14-100-8878(34) and 12-14-100-9523(34).