# Factors Affecting the Levels of Nitrate[1] Nitrogen in Cured Tobacco Leaves

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The wide variations in nitrate content of cured tobacco leaves from various sources has long been recognized. Among the four principal types used in the manufacture of American cigarettes the levels of nitrate are highest in Burley, intermediate in Maryland, least in Oriental and flue-cured (6). In early experiments, Shedd (15) found that the nitrate content varied widely among grades of Burley tobacco and tended to be slightly higher in the better grades. Garner et al. (4) found that the nitrate content of Maryland tobacco increased markedly with high rates of fertilizer nitrogen. It has been shown (10) that the nitrate content of Burley tobacco was highest in leaves from the bottom of the plant and decreased with ascending stalk position. Phillips and Bacot (14), in studies made with Burley tobacco grown in the 1951 and 1952 seasons and representing a wide range of qualities, found little correlation between nitrate content and qualities within official U. S. Government grade groups and little correlation with other chemical leaf components.

These early studies were made by tedious and time consuming analytical methods. The rapid colorimetric methods customarily used for the determination of nitrates were not applicable to the analysis of cured tobacco leaves because of the interference posed by the leaf pigments. Rapid and precise methods are now available for the determination of nitrates in cured tobacco leaves. Neurath and Ehmke (11) recently described a photometric method in which 3, 4-dimethylphenol is used followed by distillation. These authors reported on the results of an extensive survey of the nitrate content of various types including Oriental, flue-cured and Burley, and various countries of origin. Confirming earlier work, Burley had the highest levels of nitrate among these types. The nitrates were higher in the midribs of the leaves. Concurrently, Lipp and Dolberg (7) using the same method, for which they reported a very high precision, found values for nitrates in Virginia, Oriental and Burley generally consistent with those reported by Neurath and Ehmke (11). Other types were analyzed with results intermediate between flue-cured and Burley. Only traces of nitrites were found. The German workers found essentially no effect on nitrate levels as a result of the method of curing and some increase upon fermentation in comparison with redrying.

The factors affecting the levels of nitrates in cured tobacco are of especial interest at this time because of

**Table 1. Recovery of nitrate nitrogen to tobacco.[a]**

| Addod mg. | Recovered[b] mg. | Recovery % |
|---|---|---|
| 1.00 | 0.9813 | 98.1 |
| 1.50 | 1.4917 | 99.4 |
| | | Mean 98.8 |

[a]Flue-cured tobacco containing 0.0436 mg nitrate N.
[b]Mean of 5 determinations.

**Table 2. Precision of the colorimetric method for nitrate nitrogen.**

| Tobacco Sample | Range[a] | Mean[a] | Std. Dev. | Rel. Std. Dev. |
|---|---|---|---|---|
| Flue-cured | 0.018-0.019 | $0.018_4$ | 0.00052 | 2.81 |
| Burley (low level) | $0.26_9$-$0.27_2$ | $0.26_5$ | 0.0043 | 1.63 |
| Burley (high level) | $1.12_5$-$1.17_0$ | $1.15_2$ | 0.014 | 1.22 |
| | | | Mean R.S.D. | 1.89 |

[a]Range and mean of ten determinations (% nitrate nitrogen).

[1] Presented at the Twentieth Tobacco Workers' Conference, Lexington, Ky., July 12-15, 1965.

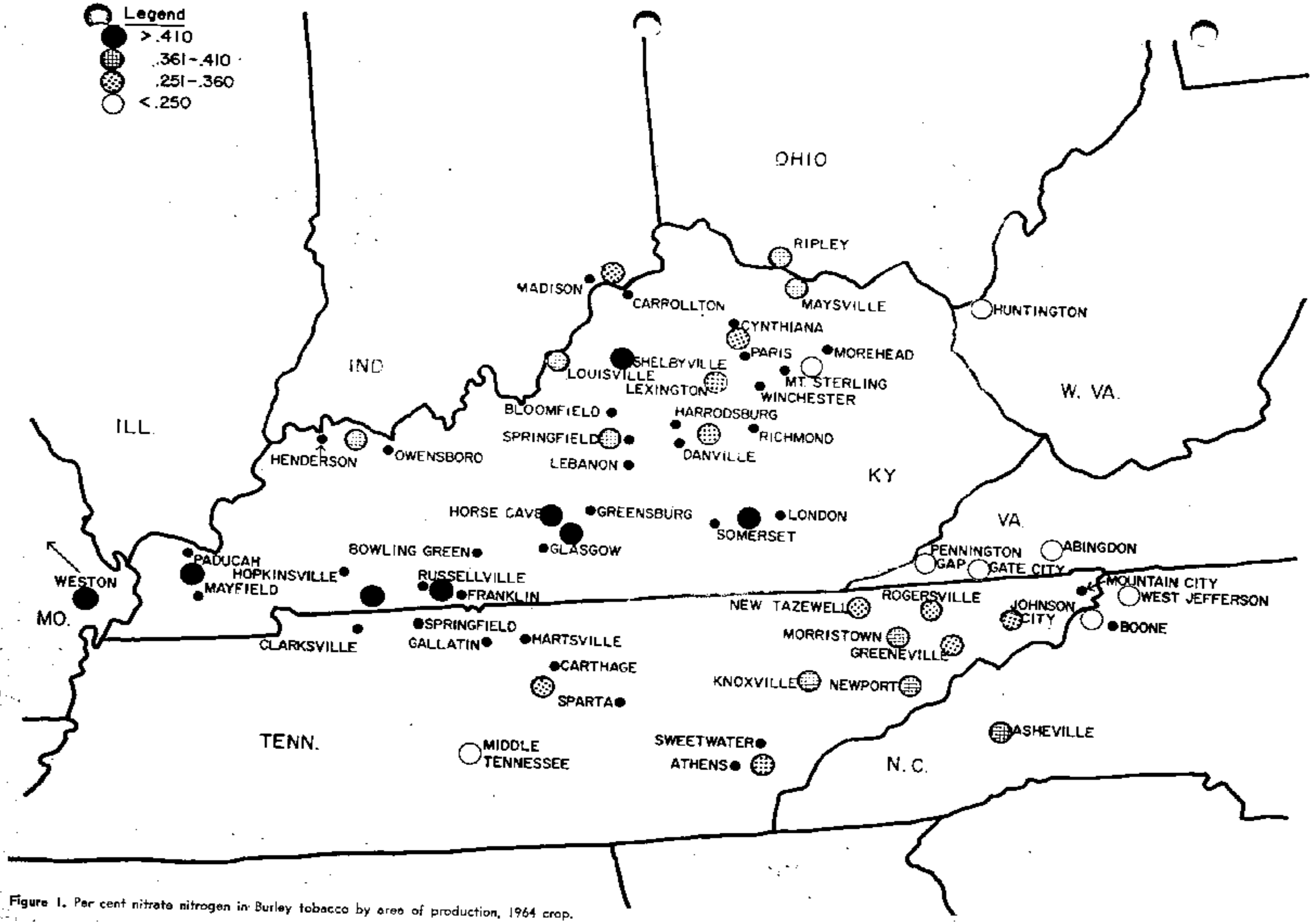


Figure 1. Per cent nitrate nitrogen in Burley tobacco by area of production, 1964 crop.

**Table 3. Nitrate nitrogen content of commercial tobaccos.**

| Type - Source | Crop Year | No. Samples | % Nitrate Nitrogen Range | % Nitrate Nitrogen Mean |
|---|---|---|---|---|
| *Flue-cured*[a] | | | | |
| USA, Georgia | 1960-64 | 5 | 0.016-0.031 | 0.025 |
| USA, SC | 1960-64 | 5 | .014- .033 | .025 |
| USA, ENC | 1960-64 | 5 | .016- .036 | .026 |
| USA, MB | 1960-64 | 5 | .012- .032 | .022 |
| USA, OB | 1960-64 | 5 | .010- .029 | .019 |
| | | | Mean | .023 |
| *Burley* | | | | |
| USA[a] | 1960 | 14 | .25 - .50 | .37 |
| USA[a] | 1961 | 14 | .090- .28 | .18 |
| USA[a] | 1962 | 14 | .30 - .57 | .40 |
| USA[a] | 1963 | 14 | .24 - .58 | .34 |
| USA[a] | 1964 | 14 | .16 - .58 | .33 |
| | | | Mean | .32 |
| Canada | 1963-64 | 8 | .072- .34 | .20 |
| Colombia | Unknown | 3 | .067- .17 | .11 |
| Greece | 1964 | 3 | .16 - .48 | .33 |
| Mexico | 1963 | 5 | .077- .20 | .13 |
| Rhodesia | 1964 | 5 | .018- .17 | .054 |
| Venezuela | 1963-64 | 8 | .073- .39 | .24 |
| *Oriental* | | | | |
| Smyrna[a] | Various | 4 | .019- .027 | .022 |
| Samsun[a] | Various | 4 | .026- .031 | .029 |
| Macedonia[a] | Various | 4 | .035- .051 | .044 |
| Rhodesia (Var. Samsun) | 1962 | 1 | | .024 |
| *Maryland*[a] | | | | |
| USA | 1959-63 | 5 | .048- .086 | .063 |
| *Cigar Types, Fermented* | | | | |
| C.S.G. Wrapper | 1960 | 3 | .66 -1.05 | .80 |
| Penn. Filler | Various | 3 | .37 - .48 | .41 |
| Havana | Various | 2 | .28 - .38 | .33 |
| Puerto Rican | Various | 3 | .41 - .45 | .43 |
| Colombian | Various | 2 | .49 - .58 | .54 |
| *Other Types*[b] | | | | |
| Green River | 1962-64 | 3 | .14 - .15 | .15 |
| One Sucker | 1962-64 | 3 | .14 - .17 | .16 |

[a]Blend of domestic cigarette grades, one company.
[b]Blend of farm grades.

**Table 4. Nitrate nitrogen content of *Nicotiana* species.**

| Species | Source: N. C. 1959 | N. C. 1964 | S. C. 1964 | Tenn. 1964 | Tenn.-Md. 1964 |
|---|---|---|---|---|---|
| | % Nitrate Nitrogen | | | | |
| *glauca* | 0.025 | 0.042 | 0.052 | 0.27 | |
| *glutinosa* | | .18 | .17 | | 0.31 |
| *tomentosiformis* | .037 | .031 | | | |
| *otophora* | .023 | .045 | | | |
| *sylvestris* | .040 | .059 | | | |
| *rustica* | .015 | | .020 | | .23 |
| *tabacum*, | | | | | |
| var. PD 97 | .020 | | | | |
| var. C 139 | | .022 | .023 | | |
| var. SC 58 | | .022 | .026 | | |
| var. NC 95 | | | .027 | | |
| var. OX 402 | | | .023 | | |
| var. Hicks | | | .021 | | |
| var. PD 33 | | | .023 | | |

the reported relationship between nitrate content of the tobacco and the levels of oxides of nitrogen (NO and $NO_2$) in cigarette smoke, and various hypotheses concerning the pharmacological activity of these substances and compounds which may be derived therefrom. Philippe and Hackney (13) showed qualitative evidence for the presence of nitrous oxide in the smoke derived from Burley and a blend of flue-cured and Burley. The range of nitrogen oxides in the smoke of commercial cigarettes was found by Haagen-Smit et al. (5) to be 145-655 p.p.m. and by Bokhoven and Niessen (1) to be 170-210 p.p.m. Westcott (17) reported much lower levels of nitrogen oxides in the smoke of United Kingdom cigarettes in comparison with those manufactured in the United States. Tada (16) stated that the major nitrogen oxide in cigarette smoke is NO. Norman and Keith (12) reported that essentially all of the nitrogen oxides in fresh, normally-obtained cigarette smoke is the less pharmacologically active nitrogen oxide, NO.

## Materials and Methods

Some of the samples used in this study were grown under controlled conditions by personnel of the USDA and State Experiment Stations. The results are being reported with the permission of the personnel and agencies concerned. We also used samples of commercial grades and blends, some of these supplied by dealers in leaf tobacco. All data are reported on a moisture-free basis and, unless otherwise noted, on the leaf lamina.

The analytical method is that described by Middleton (8, 9) as modified for tobacco by Connors (3). It is based upon the colorimetric estimation of an azo dye, Orange I, which is formed by the reaction of the modified Griess reagent with nitrites.

The method gave good accuracy and precision in our hands. Good agreement was obtained with the present method and the Devarda's alloy method (10) upon analyzing tobacco samples having a range of 0.02-1.97 per cent nitrate nitrogen. The per cent recovery, 98.8 and the mean relative standard deviation, 1.89 per cent, **Tables 1 and 2**, compare favorably with the results reported on the 3, 4-dimethylphenol method by Lipp and Dolberg (7). The greater speed of the present method, which does not require distillation, appears to be a distinct advantage.

**Table 5. Nitrate nitrogen content of commercial Burley varieties.[a]**

| Variety | 1963 Tobacco Exp. Sta. Greeneville, Tenn. | 1963 Godwin Grainger Co., Tenn. | 1964 Tobacco Exp. Sta. Greeneville, Tenn. | 1964 Godwin Grainger Co. Tenn. | Mean |
|---|---|---|---|---|---|
| | *% Nitrate Nitrogen* | | | | |
| Bur 1 | 0.23 | 0.46 | 0.57 | 0.54 | 0.45 |
| Bur 21 | .19 | .47 | .55 | .37 | .39 |
| Bur 37 | .16 | .48 | .62 | .20 | .37 |
| HLN 21 | .18 | .42 | .57 | .25 | .36 |
| Ky 9 | .26 | .40 | .58 | .32 | .39 |
| Ky 10 | .27 | .52 | .53 | .26 | .39 |
| Ky 12 | .10 | .37 | .65 | .44 | .39 |
| Ky 16 | .18 | .38 | .57 | .44 | .39 |
| MS21 X Ky 9 | .20 | .41 | .64 | .33 | .40 |
| MS21 X Ky 10 | .17 | .52 | .46 | .34 | .37 |
| MS21 X Ky 12 | .17 | .40 | .49 | .43 | .37 |
| MSL8 X Bur 21 | .14 | .42 | .54 | .36 | .36 |
| Gr 49 | | | .34 | .35 | |
| Mean (12 entries) | .19 | .44 | .57 | .36 | .39 N.S.[b] |
| Rainfall Pattern | Seasonable through topping, then dry | Seasonable | Very dry until Aug. 3, then seasonable | Seasonable except two weeks in July | |

[a]Weighted composites, entire production.
[b]NS—Not statistically significant.

### Procedure for Determination of Nitrate Nitrogen in Tobacco

*Apparatus*

Wrist action shaker, Burrell Spectrophotometer, Beckman Model B

*Reagents*

1. (a) Extraction Mixture: Dilute 34 ml $NH_4OH$ with distilled $H_2O$ to one liter (0.5 *N*)
   (b) Reaction Mixture: Dilute 50 ml $NH_4OH$ with distilled $H_2O$ to one liter (0.745 *N*)
2. $MnSO_4$ Solution: Dissolve exactly 10 grams $MnSO_4 \cdot 4H_2O$ (7.5772 grams $MnSO_4 \cdot H_2O$) in 51 ml $CH_3COOH$ and dilute to one liter with distilled $H_2O$
3. Zinc Metal: dust
4. Griess Reagent (Modified): Dissolve exactly one gram of sulfanilic acid in 200 ml distilled $H_2O$. Add 0.8000 gram alpha naphthol and 752 ml $CH_3COOH$. Dilute to one liter with distilled $H_2O$. Refrigerate.
5. Stock Diluent: To 103 ml $NH_4OH$ add 400 ml distilled water and then cautiously, with stirring, add 321 ml $CH_3COOH$. Dilute to two liters with distilled $H_2O$
6. Nitrate Standard Solution: Dissolve exactly 360.9 mg. $KNO_3$ in 34 ml $NH_4OH$. Dilute to one liter with distilled $H_2O$. One ml of solution is equivalent to 0.05 mg nitrate nitrogen as nitrogen.

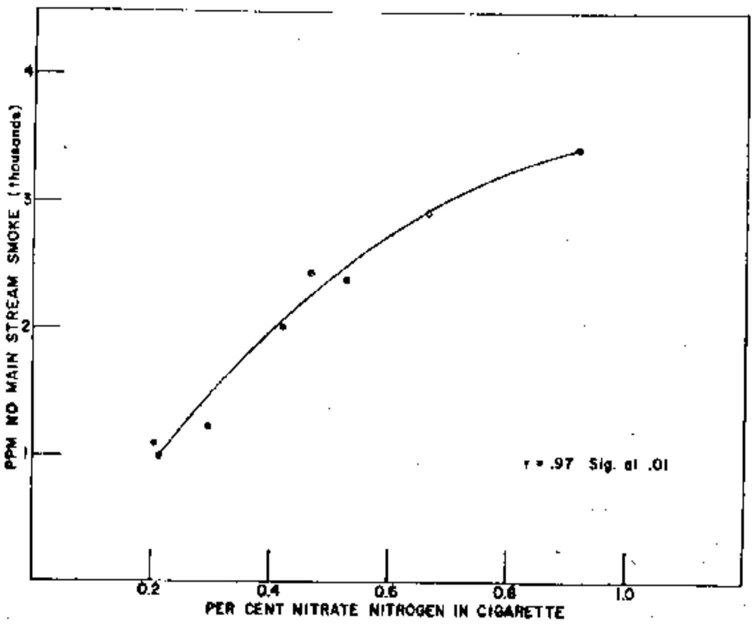


Figure 2. Oxides of nitrogen in smoke of cigarettes with differing levels of nitrate nitrogen.

**Table 6. Effects of fertilizer and soil fumigation on per cent nitrate nitrogen in flue-cured tobacco.[a]**

| Year | 1962 | 1963 | 1964 |
|---|---|---|---|
| *Source of N* | | *Not Fumigated* | |
| All Ammonium | 0.004 | 0.007 | 0.006 |
| 50% Am.–50% Nitrate | .005 | .005 | .006 |
| All Nitrate | .004 | .007 | .006 |
| | | *Fumigated, EDB 40[b]* | |
| All Ammonium | .004 | | |
| 50% Am.–50% Nitrate | .004 | | |
| All Nitrate | .004 | | |

[a]*Experiments conducted at Georgia Coastal Plain Experiment Station, Tifton, Ga.*
[b]*Ethylene dibromide.*

*Preparation of Standard Curve*

(a) Place 40 ml of reaction mixture (1b) in each five 250 ml glass stoppered Erlenmeyer flasks.

(b) Add one, two, three and four ml of KNO, standard solution to first four flasks respectively.

(c) Add eight, seven, six and five ml distilled water to the respective flasks, making a total of 49 ml.

(d) Add nine ml of distilled $H_2O$ to fifth flask to be used as a blank.

(e) Add one ml of reagent 2 to each flask followed by a small amount (ca 200 mg) of zinc dust. Stopper flask and shake for one hour.

(f) Filter through Whatman #1 paper

(g) Place 5 ml of reagent 4 in each of five 25 ml glass stoppered volumetric flasks.

(h) Dilute to volume with filtrate from step f. Stopper, mix and allow 30 minutes for color to develop.

(i) Dilute one ml of solution from step h to volume in a 10 ml volumetric flask with reagent 5. Allow 30 minutes for color to develop.

(j) Read absorbance of sample vs reagent blank in spectrophotometer at 475 millimicrons.

(k) Plot the absorbance vs mg nitrate nitrogen in each sample. A straight line should be obtained.

Method

Place 300 mg of tobacco in a 250 ml glass stoppered Erlenmeyer flask. Add 100 ml of reagent 1a, stopper and shake for one hour. Filter Place nine ml of filtrate in a 250 ml glass stoppered Erlenmeyer flask followed by 40 ml of reagent 1b, one ml of reagent 2 and ca 200 mg of zinc dust. Stopper and shake for one hour. Follow procedure for standard curve starting with step g.

It has been found that absorbances of 0.2-0.4 are in the desired range. This range may be obtained by varying sample size or the final dilution.

Experimental Results

The nitrate nitrogen content of commercial tobaccos from various sources is shown in Table 3. Among types examined, flue-cured and Oriental had the lowest levels with only minor differences among the flue-cured belts. Burley contained, on the average, about fourteen times as much nitrate nitrogen as flue-cured. The lowest level was recorded in 1961, a season of plentiful rainfall. However, the levels were not significantly different for 1963, a seasonable year and 1964, a dry year. The highest levels were found in 1962 which was dry early, followed by late rains and second growth. Among samples from other countries

$$\text{Wt. Sample} \times \left(\frac{9}{100} \times \frac{20}{50} \times \frac{1}{10}\right) =$$

$$\text{Wt. Sample} \times .0036 = \text{Wt. sample in aliquot}$$

$$\frac{\text{Mg nitrate nitrogen}}{\text{Wt. Sample in Aliquot}} \times 100 = \%\ \text{nitrate nitrogen as N}$$

**Table 7. Effects of fertilizer and leaching on per cent nitrate nitrogen in flue-cured tobacco.[a]**

| Water Treatment | Post Water Fertilizer Treatment[b] | | | Interval Between Transplating and Post Water Fertilizer Treatment | | |
|---|---|---|---|---|---|---|
| | N | $K_2O$ | MgO | 5 Weeks | 7 Weeks | Mean |
| | Pounds per Acre | | | % Nitrate Nitrogen | | |
| | 0 | 0 | 0 | 0.003 | 0.008 | 0.006 |
| | 20 | 60 | 10 | .006 | .005 | .006 |
| Seasonal | 40 | 120 | 20 | .007 | .007 | .007 |
| | 60 | 180 | 30 | .013 | .010 | .012 |
| | 0 | 0 | 0 | .007 | .007 | .007 |
| 2 in. excess one week | 20 | 60 | 10 | .008 | .009 | .009 |
| before Fert. Treatment | 40 | 120 | 20 | .007 | .007 | .007 |
| | 60 | 180 | 30 | .019 | .010 | .015 |
| Mean | | | | .009 | .008 | .009 |

[a]*Experiments conducted at Oxford, North Carolina Tobacco Research Station, 1964.*
[b]*Basic Fertiliser Treatment: 70 lbs. N, 96 lbs. $P_2O_5$, 163 lbs. $K_2O$, 24 lbs MgO.*

of origin, Rhodesian Burley had the lowest levels of nitrate nitrogen followed by Colombian and Mexican. The data shown for foreign-grown Burley are based on a limited number of samples supplied by leaf dealers and may not be representative of the production in these countries. Among other air-cured types, the levels of nitrates were lowest in Maryland and highest in Connecticut Shade-grown Wrappers.

Samples of Burley tobacco were drawn from representative markets throughout the Burley producing area in the 1964 season and analyzed for nitrate nitrogen with results as shown in **Figure 1.** Each dot represents fifty or more samples. The markets were separated into four groups according to levels of nitrate nitrogen. The highest levels of nitrates were found in southern and western Kentucky; while the mountainous areas of production tended to have lower levels of nitrates.

Among seven *Nicotiana* species surveyed, the levels of nitrates for a given location were highest in *N. glutinosa* and least in *N. rustica*. There were only minor differences among representative flue-cured varieties of *N. tabacum* **(Table 4).**

A survey of twelve of the more widely grown Burley varieties produced at two locations in East Tennessee in the 1963 and 1964 seasons, revealed no significant differences in levels of nitrate nitrogen among the varieties **(Table 5).** Among the four crops, the lowest levels occurred at the Greeneville Station, 1963. The rainfall pattern was seasonable through time of topping, then dry. The highest levels occurred at the

**Table 8. Nitrate nitrogen content in Burley tobacco, individual farms.[a]**

| State | Variety | Year | No. Farms | % Nitrate Nitrogen Range | Mean |
|---|---|---|---|---|---|
| Kentucky | Bur 21 | 1962 | 11 | 0.35 –0.96 | 0.66 |
| | | 1963 | 10 | .17 –1.05 | .43 |
| | | 1964 | 12 | .33 – .96 | .64 |
| | | | | Mean | .58 |
| Tennessee | Bur 37 | 1962 | 8 | .18 – .73 | .34 |
| | | 1963 | 9 | .081– .70 | .33 |
| | | 1964 | 10 | .22 – .78 | .42 |
| | | | | Mean | .36 |
| Virginia | Bur 21 | 1962 | 4 | .12 – .50 | .30 |
| | | 1963 | 4 | .13 – .52 | .29 |
| | | 1964 | 4 | .11 – .43 | .26 |
| | | | | Mean | .29 |
| Mean | | 1962 | 23 | (Dry, Late Rains) | .49 |
| Mean | | 1963 | 23 | (Seasonal) | .37 |
| Mean | | 1964 | 26 | (Dry) | .50 |

Correlation Coefficient Nitrate Nitrogen vs:

| | |
|---|---|
| Per cent total production graded (mean 3-6 companies) | 0.19 NS[b] |
| Grade index of sample (one company) | .09 NS |
| Per cent total nitrogen | .58 ** |
| Per cent nicotine | –.13 NS |
| Per cent total nitrogen less nicotine N and nitrate N | .40 ** |

[a]Weighted composite of cigarette grades, one company.
[b]NS—Not statistically significant.
**Significant at .01.

**Table 9. Effect of fertilization on nitrate nitrogen content of Burley tobacco.[a]**

| Fertilizer Added lbs/acre *N* | $P_2O_5$ | $K_2O$ | FA | FB | FH | FM | FW | FY | *Mean* |
|---|---|---|---|---|---|---|---|---|---|
| | | | Location — *% Nitrate Nitrogen* | | | | | | |
| | | | 0.27 | 0.16 | 0.19 | 0.14 | 0.15 | 0.15 | 0.17 |
| 120 | 80 | 170 | .46 | .22 | .23 | .40 | .24 | .27 | .30 |
| 220 | 80 | 170 | .52 | .32 | .48 | .47 | .37 | .38 | .42 |
| 320 | 80 | 170 | .28 | .16 | .19 | .15 | .14 | .24 | .19 |
| 120 | 80 | 270 | .33 | .17 | .33 | .26 | .14 | .15 | .23 |
| 120 | 80 | 370 | .30 | .14 | .24 | .18 | .15 | .28 | .22 |
| 120 | 180 | 170 | .56 | .54 | .36 | .42 | .36 | .25 | .42 |
| 320 | 80 | 270 | .64 | .48 | .30 | .48 | .38 | .33 | .44 |
| 420 | 280 | 370 | .42 | .27 | .29 | .31 | .24 | .26 | .30 |
| | | *Mean* | | | | | | | |

Correlations Per Cent Nitrate Nitrogen vs:

| | |
|---|---|
| Fertilizer nitrogen added | r = 0.76 ** |
| Per cent total nitrogen | r = .77 ** |
| Per cent nicotine | r = .06 NS[b] |
| Per cent total nitrogen less nicotine N and nitrate N | r = .67 ** |

[a]Experiments conducted on farm locations in North Carolina by Mountain Research Station, Waynesville, N. C. 1963, weighted composites, entire production.
[b]NS—Not statistically significant.
**Significant at .01.

**Table 10. Effects of fertilization and cover crop on per cent nitrate nitrogen in Burley tobacco.[a]**

| Fertilizer Nitrogen[b] lbs/acre | 1 & 2 | 3 | Stalk Position 4 | 5 | 6 | Weighted Mean | Ratio $NO_3$ N Midribs / $NO_3$ N Lamina |
|---|---|---|---|---|---|---|---|
| | | | Vetch Cover Crop | | | | |
| | 12.5 | 16.5 | 29.5 | 24.7 | 16.8[c] | | |
| 0 | 0.51 | 0.30 | 0.26 | 0.16 | 0.13 | 0.26 | 6.8 |
| 75 | .60 | .46 | .36 | .25 | .22 | .36 | 7.7 |
| 150 | 1.26 | .94 | .50 | .46 | .40 | .61 | 5.6 |
| 225 | .81 | .92 | .66 | .59 | .60 | .67 | 5.5 |
| 300 | .85 | .75 | .69 | .54 | .52 | .62 | 5.3 |
| 125[b] | 1.12 | .66 | .51 | .35 | .28 | .52 | 4.7 |
| Mean | .86 | .67 | .48 | .39 | .34 | .51 | 5.9 |
| $NO_3$ N Midribs / $NO_3$ N Lamina | 4.4 | 5.2 | 6.3 | 6.6 | 6.5 | 5.7 | |
| | | | Rye Cover Crop | | | | |
| | 16.1 | 19.2 | 27.4 | 19.4 | 17.9[c] | | |
| 0 | 0.038 | 0.023 | 0.026 | 0.036 | 0.024 | 0.029 | |
| 75 | .25 | .16 | .10 | .090 | .072 | .14 | |
| 150 | .58 | .28 | .19 | .19 | .14 | .25 | |
| 225 | .79 | .65 | .44 | .35 | .30 | .47 | |
| 300 | 1.41 | .82 | .58 | .45 | .25 | .62 | |
| 125[b] | .50 | .25 | .14 | .12 | .11 | .20 | |
| Mean | .59 | .36 | .25 | .21 | .15 | .28 | |
| $NO_3$ N Midribs / $NO_3$ N Lamina | 3.9 | | | | 9.1 | | 7.5 |

Correlation Nitrate Nitrogen vs Fertilizer Nitrogen $r = 0.58$ **

[a]*Experiments conducted at Tobacco Experiment Station, Greeneville, Tennessee, 1964.*
[b]*75 lbs. from mineral sources and 50 lbs. from manure.*
[c]*Per cent total production represented by respective stalk positions.*
***Significant at .01.*

Greeneville Station, 1964. The rainfall pattern was the reverse of that described above, that is, very dry until August 3, then seasonable.

In a three year study of the effects of sources of nitrogen and soil fumigation with ethylene dibromide at the Georgia Coastal Plain Experiment Station, 1962-1964, no differences in nitrate levels were found as a result of sources of nitrogen, whether ammonium or nitrate, and no differences were found as a result of soil fumigation (**Table 6**).

Experiments were conducted at the Oxford, North Carolina Experiment Station, 1964 season, in which varying rates of supplementary fertilizer were added five and seven weeks after transplanting, with and without excess water one week prior to fertilization. No difference in nitrate content was found between the two fertilizing schedules of five and seven weeks and no difference as a result of the excess water. Only minor differences were found among fertilizer rates except at the highest rate, which was greatly in excess of that normally employed in the production of flue-cured tobacco (**Table 7**).

In cooperative variety evaluation tests, Burley tobacco was grown on a total of 72 locations in the 1962, 1963, and 1964 seasons. Weighted composites of cigarette grades in the check variety, Burley 21 or Burley 37, as judged by one domestic company, were analyzed for nitrate nitrogen (**Table 8**). There was a thirteenfold difference between the extremes among individual crops. Among the three states, Kentucky had the highest nitrates, Virginia the lowest. There was no significant correlation between nitrate content of the tobacco and the average amount graded by the participating companies or the grade index as computed by one domestic company. There was a highly significant correlation between per cent nitrate nitrogen and per cent total nitrogen and between per cent nitrate nitrogen and per cent total nitrogen less nicotine nitrogen and nitrate nitrogen. There was no significant correlation between per cent nitrate nitrogen and per cent nicotine. The crops which were grown in a rotation tended to have higher nitrate content than those which followed tobacco.

Fertilization tests with Burley tobacco were conducted at six farm locations in North Carolina in 1963, and nitrate nitrogen determined on weighted composites of entire production from each plot (**Table 9**). There was a highly significant correlation between nitrate nitrogen in the cured leaves and the fertilizer nitrogen added. There was a highly significant correlation between nitrates and total nitrogen and between nitrates and total nitrogen less nicotine and nitrate nitrogen. There was virtually no correlation between per cent nitrates and per cent nicotine.

Fertilization and cover crop experiments with Burley tobacco were conducted at the Tobacco Experiment Station, Greeneville, Tennessee in the 1964 season (**Table 10**). The cured tobacco from each plot was divided into six farm grades, according to stalk position, grade 1 representing the lowermost leaves, or flyings, and grade 6 the uppermost leaves, or tips. Grades 1 and 2 were combined prior to stemming and analysis. Nitrates were also determined on the midribs of the samples grown after a vetch cover crop and the lowermost and uppermost stalk positions following the rye cover crop. The nitrates were much higher in those plots which followed a vetch crop, the differences being very large at the low rates of fertilizer nitrogen and disappearing at the highest, 300 pound, rate of fertilizer nitrogen. Consistent with results reported by Moseley et al. (10), the per cent nitrates were highest in the lowermost leaves and decreased with successive stalk positions. There was a highly significan correlation between nitrate content of the cured leaves and the fertilizer nitrogen added. The midribs contained, on the average, about six times as much nitrate nitrogen as the lamina. Relative to the lamina, the midribs did not show as much variation with stalk position or with rates of fertilizer nitrogen.

**Table 11. Effects of fertilizer, spacing and irrigation on per cent nitrate nitrogen in Burley tobacco.**[a]

| Spacing (in) / Fertilization N lbs/acre | 12 | 16 | 20 | 24 | Mean |
|---|---|---|---|---|---|
| **Replication 1** | | | | | |
| *Not Irrigated* | | | | | |
| 100 | 0.94 | 0.88 | 1.02 | 0.89 | 0.93 |
| 200 | .99 | 1.34 | 1.36 | 1.32 | 1.25 |
| 300 | 1.42 | 1.42 | 1.54 | 1.55 | 1.48 |
| *Irrigated* | | | | | |
| 100 | .68 | .50 | .87 | .43 | .62 |
| 200 | .93 | 1.03 | 1.03 | 1.31 | 1.08 |
| 300 | 1.49 | 1.40 | 1.43 | 1.50 | 1.46 |
| **Replication 2** | | | | | |
| *Not Irrigated* | | | | | |
| 100 | .73 | .94 | .98 | .91 | .89 |
| 200 | 1.56 | 1.34 | 1.20 | 1.11 | 1.30 |
| 300 | 1.72 | 1.49 | 1.22 | 1.20 | 1.41 |
| *Irrigated* | | | | | |
| 100 | .55 | .86 | .66 | .57 | .66 |
| 200 | 1.28 | 1.17 | 1.15 | .99 | 1.22 |
| 300 | 1.48 | 1.58 | 1.52 | 1.28 | 1.46 |

Correlation Coefficient Nitrate Nitrogen vs:
Fertilizer nitrogen added   r = 0.86 **
Spacing   r = -.06 NS[b]

[a] *Experiments conducted at Kentucky Agricultural Experiment Station, Lexington, Kentucky, 1964, Weighted composite of entire production, whole leaves.*
[b] *NS—Not statistically significant.*
** *Significant at .01.*

A fertilizer — spacing—irrigation experiment was conducted with Burley tobacco at the University of Kentucky Experiment Station, Lexington, Kentucky in the 1964 season. Nitrate nitrogen was determined on whole leaves representing weighted composites of the entire production on each plot (**Table 11**). There was a highly significant correlation between per cent nitrates and rates of fertilizer nitrogen, no significant correlation between per cent nitrates and spacing. Irrigation resulted in a large reduction in nitrates at the low rate of fertilizer nitrogen, less at the intermediate rate, and no reduction at the high rate.

Certain of the four principal types were grown in other belts in the 1964 season and cured by two methods, with results shown in **Table 12**. The environment during growth had a far greater effect upon nitrate nitrogen content than did genotype or curing method. Thus, all types grown in the Burley area, Lexington, Kentucky and Waynesville, North Carolina, had levels of nitrates approaching those of Burley and all types grown in the flue-cured area, Oxford, North Carolina and Whiteville, North Carolina, had levels of nitrates approaching those of flue-cured. The large difference between the nitrate content of flue-cured tobacco grown in the Burley area as compared with that grown in the flue-cured area, even with massive application of fertilizer nitrogen, suggests that factors other than nitrogen supply, such as soil type, may have important influences on the nitrate levels.

Eight lots of Burley tobacco having a wide range of nitrate nitrogen were selected from the farm grown variety tests. These were aged, cased, resampled, and manufactured into 70 mm cigarettes. Cigarettes of uniform weight were smoked mechanically according to the technique described by Bradford et al. (2). The concentration of oxides of nitrogen as NO was determined in the mainstream smoke obtained from each of four cigarettes in each lot using an unpublished method involving absorption of smoke in alkaline $KMnO_4$, which oxidizes the oxides of nitrogen to nitrate and colorimetric determination of nitrate by the metaxylenol procedure. The method is not specific for nitrogen oxides and may include other substances present in the gas phase smoke if they can be oxidized to nitrate by the absorbing medium. Since the nitrogen oxides of smoke occur in the gaseous phase, there is no problem of interference due to pigments. As shown in **Figure 2**, there was a highly significant correlation between the per cent nitrates in the tobacco and the concentration of oxides of nitrogen in the mainstream smoke.

The eight lots of cigarettes were divided into four pairs. The members of each pair had approximately the same nicotine content but widely different nitrate content. The four pairs were submitted to a 20 member, expert smoking panel and evaluated on the basis of mildness, flavor, and overall acceptability. There appeared to be no consistent relationship between these attributes and the levels of nitrates in the tobacco.

## Summary

Genetic differences appear to have only minor influences on the nitrate content of cured tobacco leaves. The wide variations among and within tobacco types appear to result pri-

**Table 12. Effects of location and curing method on per cent nitrate nitrogen, 1964.**

| Location - Type | Curing Method Air-cured | Flue-cured |
|---|---|---|
| Lexington, Ky. | | |
| Burley | 0.49 | |
| Flue-cured | .33 | |
| Waynesville, N. C. | | |
| Burley | .53 | 0.53 |
| Flue-cured | .34 | .46 |
| Oriental | .21 | .23 |
| Maryland | .25 | .26 |
| Oxford, N. C. | | |
| Burley | .031 | .033 |
| Flue-cured | .029 | |
| Oriental[a] | .026 | .026 |
| Maryland | .027 | .024 |
| Whiteville, N. C. | | |
| Burley | .035 | |

[a]Whole leaves.

marily from environmental influences, especially the availability of fertilizer nitrogen. There is evidence of a relationship of nitrates with water supply, especially at low rates of fertilizer nitrogen and when applied early in the growing season. There is evidence also of a relationship with soil type and previous cropping history. Confirming the experiments of Neurath (11), nitrates appear to be the principal source of oxides of nitrogen in the smoke. The grading record of the Burley tobaccos grown in farm grown variety trials (Table 8) and the results of smoke taste evaluation tests on selected samples from this group, suggest that the levels of nitrates, per se, do not exert a dominant influence upon smoking quality, as judged by traditional subjective methods.

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# Factors Affecting the Levels of Nitrate[1] Nitrogen in Cured Tobacco Leaves

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# Factors Affecting the Levels of Nitrate Nitrogen in Cured Tobacco Leaves[1]

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The wide variations in nitrate content of cured tobacco leaves from various sources has long been recognized. Among the four principal types used in the manufacture of American cigarettes the levels of nitrate are highest in Burley, intermediate in Maryland, least in Oriental and flue-cured (6). In early experiments, Shedd (15) found that the nitrate content varied widely among grades of Burley tobacco and tended to be slightly higher in the better grades. Garner et al. (4) found that the nitrate content of Maryland tobacco increased markedly with high rates of fertilizer nitrogen. It has been shown (10) that the nitrate content of Burley tobacco was highest in leaves from the bottom of the plant and decreased with ascending stalk position. Phillips and Bacot (14), in studies made with Burley tobacco grown in the 1951 and 1952 seasons and representing a wide range of qualities, found little correlation between nitrate content and qualities within official U. S. Government grade groups and little correlation with other chemical leaf components.

These early studies were made by tedious and time consuming analytical methods. The rapid colorimetric methods customarily used for the determination of nitrates were not applicable to the analysis of cured tobacco leaves because of the interference posed by the leaf pigments. Rapid and precise methods are now available for the determination of nitrates in cured tobacco leaves. Neurath and Ehmke (11) recently described a photometric method in which 3, 4-dimethylphenol is used followed by distillation. These authors reported on the results of an extensive survey of the nitrate content of various types including Oriental, flue-cured and Burley, and various countries of origin. Confirming earlier work, Burley had the highest levels of nitrate among these types. The nitrates were higher in the midribs of the leaves. Concurrently, Lipp and Dolberg (7) using the same method, for which they reported a very high precision, found values for nitrates in Virginia, Oriental and Burley generally consistent with those reported by Neurath and Ehmke (11). Other types were analyzed with results intermediate between flue-cured and Burley. Only traces of nitrites were found. The German workers found essentially no effect on nitrate levels as a result of the method of curing and some increase upon fermentation in comparison with redrying.

The factors affecting the levels of nitrates in cured tobacco are of especial interest at this time because of

**Table 1. Recovery of nitrate nitrogen to tobacco.[a]**

| Added | Recovered[b] | Recovery |
|---|---|---|
| mg. | mg. | % |
| 1.00 | 0.9813 | 98.1 |
| 1.50 | 1.4917 | 99.4 |
| | | Mean 98.8 |

[a]Flue-cured tobacco containing 0.0436 mg nitrate N.
[b]Mean of 5 determinations.

**Table 2. Precision of the colorimetric method for nitrate nitrogen.**

| Tobacco Sample | Range[a] | Mean[a] | Std. Dev. | Rel. Std. Dev. |
|---|---|---|---|---|
| Flue-cured | 0.018-0.019 | $0.018_4$ | 0.00052 | 2.81 |
| Burley (low level) | $0.26_0$-$0.27_2$ | $0.26_5$ | 0.0043 | 1.63 |
| Burley (high level) | $1.12_5$-$1.17_0$ | $1.15_2$ | 0.014 | 1.22 |
| | | | Mean R.S.D. | 1.89 |

[a]Range and mean of ten determinations (% nitrate nitrogen).

[1]Presented at the Twentieth Tobacco Workers' Conference, Lexington, Ky., July 12-15, 1965.

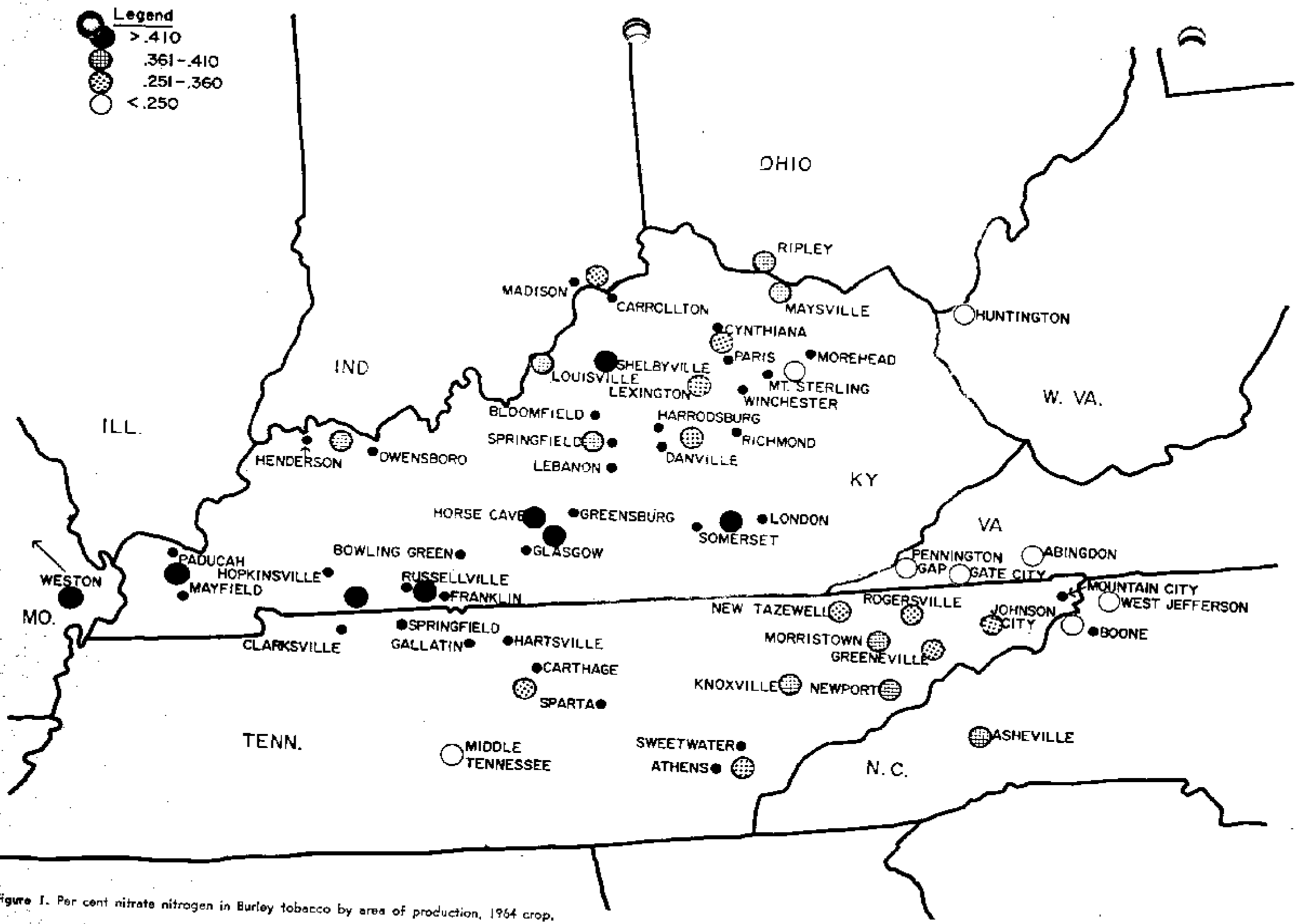


Figure 1. Per cent nitrate nitrogen in Burley tobacco by area of production, 1964 crop.

**Table 3. Nitrate nitrogen content of commercial tobaccos.**

| Type - Source | Crop Year | No. Samples | % Nitrate Nitrogen Range | % Nitrate Nitrogen Mean |
|---|---|---|---|---|
| *Flue-cured*[a] | | | | |
| USA, Georgia | 1960-64 | 5 | 0.016-0.031 | 0.025 |
| USA, SC | 1960-64 | 5 | .014- .033 | .025 |
| USA, ENC | 1960-64 | 5 | .016- .036 | .026 |
| USA, MB | 1960-64 | 5 | .012- .032 | .022 |
| USA, OB | 1960-64 | 5 | .010- .029 | .019 |
| | | | Mean | .023 |
| *Burley* | | | | |
| USA[a] | 1960 | 14 | .25 - .50 | .37 |
| USA[a] | 1961 | 14 | .090- .28 | .18 |
| USA[a] | 1962 | 14 | .30 - .57 | .40 |
| USA[a] | 1963 | 14 | .24 - .58 | .34 |
| USA[a] | 1964 | 14 | .16 - .58 | .33 |
| | | | Mean | .32 |
| Canada | 1963-64 | 8 | .072- .34 | .20 |
| Colombia | Unknown | 3 | .067- .17 | .11 |
| Greece | 1964 | 3 | .16 - .48 | .33 |
| Mexico | 1963 | 5 | .077- .20 | .13 |
| Rhodesia | 1964 | 5 | .018- .17 | .054 |
| Venezuela | 1963-64 | 8 | .073- .39 | .24 |
| *Oriental* | | | | |
| Smyrna[a] | Various | 4 | .019- .027 | .022 |
| Samsun[a] | Various | 4 | .026- .031 | .029 |
| Macedonia[a] | Various | 4 | .035- .051 | .044 |
| Rhodesia (Var. Samsun) | 1962 | 1 | | .024 |
| *Maryland*[a] | | | | |
| USA | 1959-63 | 5 | .048- .086 | .063 |
| *Cigar Types, Fermented* | | | | |
| C.S.G. Wrapper | 1960 | 3 | .66 -1.05 | .80 |
| Penn. Filler | Various | 3 | .37 - .48 | .41 |
| Havana | Various | 2 | .28 - .38 | .33 |
| Puerto Rican | Various | 3 | .41 - .45 | .43 |
| Colombian | Various | 2 | .49 - .58 | .54 |
| *Other Types*[b] | | | | |
| Green River | 1962-64 | 3 | .14 - .15 | .15 |
| One Sucker | 1962-64 | 3 | .14 - .17 | .16 |

[a]Blend of domestic cigarette grades, one company.
[b]Blend of farm grades.

**Table 4. Nitrate nitrogen content of *Nicotiana* species.**

| Species | Source N. C. 1959 | N. C. 1964 | S. C. 1964 | Tenn. 1964 | Tenn.-Md. 1964 |
|---|---|---|---|---|---|
| | % Nitrate Nitrogen | | | | |
| *glauca* | 0.025 | 0.042 | 0.052 | 0.27 | |
| *glutinosa* | | .18 | .17 | | 0.31 |
| *tomentosiformis* | .037 | .031 | | | |
| *otophora* | .023 | .045 | | | |
| *sylvestris* | .040 | .059 | | | .23 |
| *rustica* | .015 | | .020 | | |
| *tabacum*, | | | | | |
| var. PD 97 | .020 | | | | |
| var. C 139 | | .022 | .023 | | |
| var. SC 58 | | .022 | .026 | | |
| var. NC 95 | | | .027 | | |
| var. OX 402 | | | .023 | | |
| var. Hicks | | | .021 | | |
| var. PD 33 | | | .023 | | |

the reported relationship between nitrate content of the tobacco and the levels of oxides of nitrogen ($NO$ and $NO_2$) in cigarette smoke, and various hypotheses concerning the pharmacological activity of these substances and compounds which may be derived therefrom. Philippe and Hackney (13) showed qualitative evidence for the presence of nitrous oxide in the smoke derived from Burley and a blend of flue-cured and Burley. The range of nitrogen oxides in the smoke of commercial cigarettes was found by Haagen-Smit et al. (5) to be 145-655 p.p.m. and by Bokhoven and Niessen (1) to be 170-210 p.p.m. Westcott (17) reported much lower levels of nitrogen oxides in the smoke of United Kingdom cigarettes in comparison with those manufactured in the United States. Tada (16) stated that the major nitrogen oxide in cigarette smoke is NO. Norman and Keith (12) reported that essentially all of the nitrogen oxides in fresh, normally-obtained cigarette smoke is the less pharmacologically active nitrogen oxide, NO.

## Materials and Methods

Some of the samples used in this study were grown under controlled conditions by personnel of the USDA and State Experiment Stations. The results are being reported with the permission of the personnel and agencies concerned. We also used samples of commercial grades and blends, some of these supplied by dealers in leaf tobacco. All data are reported on a moisture-free basis and, unless otherwise noted, on the leaf lamina.

The analytical method is that described by Middleton (8, 9) as modified for tobacco by Connors (3). It is based upon the colorimetric estimation of an azo dye, Orange I, which is formed by the reaction of the modified Griess reagent with nitrites.

The method gave good accuracy and precision in our hands. Good agreement was obtained with the present method and the Devarda's alloy method (10) upon analyzing tobacco samples having a range of 0.02-1.97 per cent nitrate nitrogen. The per cent recovery, 98.8 and the mean relative standard deviation, 1.89 per cent, **Tables 1** and **2**, compare favorably with the results reported on the 3, 4-dimethylphenol method by Lipp and Dolberg (7). The greater speed of the present method, which does not require distillation, appears to be a distinct advantage.

**Table 5. Nitrate nitrogen content of commercial Burley varieties.**[a]

| *Variety* | 1963 Tobacco Exp. Sta. Greeneville, Tenn. | 1963 Godwin Grainger Co., Tenn. | 1964 Tobacco Exp. Sta. Greeneville, Tenn. | 1964 Godwin Grainger Co. Tenn. | *Mean* |
|---|---|---|---|---|---|
| | *% Nitrate Nitrogen* | | | | |
| Bur 1 | 0.23 | 0.46 | 0.57 | 0.54 | 0.45 |
| Bur 21 | .19 | .47 | .55 | .37 | .39 |
| Bur 37 | .16 | .48 | .62 | .20 | .37 |
| HLN 21 | .18 | .42 | .57 | .25 | .36 |
| Ky 9 | .26 | .40 | .58 | .32 | .39 |
| Ky 10 | .27 | .52 | .53 | .26 | .39 |
| Ky 12 | .10 | .37 | .65 | .44 | .39 |
| Ky 16 | .18 | .38 | .57 | .44 | .39 |
| MS21 X Ky 9 | .20 | .41 | .64 | .33 | .40 |
| MS21 X Ky 10 | .17 | .52 | .46 | .34 | .37 |
| MS21 X Ky 12 | .17 | .40 | .49 | .43 | .37 |
| MSL8 X Bur 21 | .14 | .42 | .54 | .36 | .36 |
| Gr 49 | | | .34 | .35 | |
| Mean (12 entries) | .19 | .44 | .57 | .36 | .39 N.S.[b] |
| Rainfall Pattern | Seasonable through topping, then dry | Seasonable | Very dry until Aug. 3, then seasonable | Seasonable except two weeks in July | |

[a]*Weighted composites, entire production.*
[b]*NS—Not statistically significant.*

Procedure for Determination of Nitrate Nitrogen in Tobacco

*Apparatus*

Wrist action shaker, Burrell Spectrophotometer, Beckman Model B

*Reagents*

1. (a) Extraction Mixture: Dilute 34 ml $NH_4OH$ with distilled $H_2O$ to one liter (0.5 *N*)
   (b) Reaction Mixture: Dilute 50 ml $NH_4OH$ with distilled $H_2O$ to one liter (0.745 *N*)
2. $MnSO_4$ Solution: Dissolve exactly 10 grams $MnSO_4 \cdot 4H_2O$ (7.5772 grams $MnSO_4 \cdot H_2O$) in 51 ml $CH_3COOH$ and dilute to one liter with distilled $H_2O$
3. Zinc Metal: dust
4. Griess Reagent (Modified): Dissolve exactly one gram of sulfanilic acid in 200 ml distilled $H_2O$. Add 0.8000 gram alpha naphthol and 752 ml $CH_3COOH$. Dilute to one liter with distilled $H_2O$. Refrigerate.
5. Stock Diluent: To 103 ml $NH_4OH$ add 400 ml distilled water and then cautiously, with stirring, add 321 ml $CH_3COOH$. Dilute to two liters with distilled $H_2O$
6. Nitrate Standard Solution: Dissolve exactly 360.9 mg. $KNO_3$ in 34 ml $NH_4OH$. Dilute to one liter with distilled $H_2O$. One ml of solution is equivalent to 0.05 mg nitrate nitrogen as nitrogen.

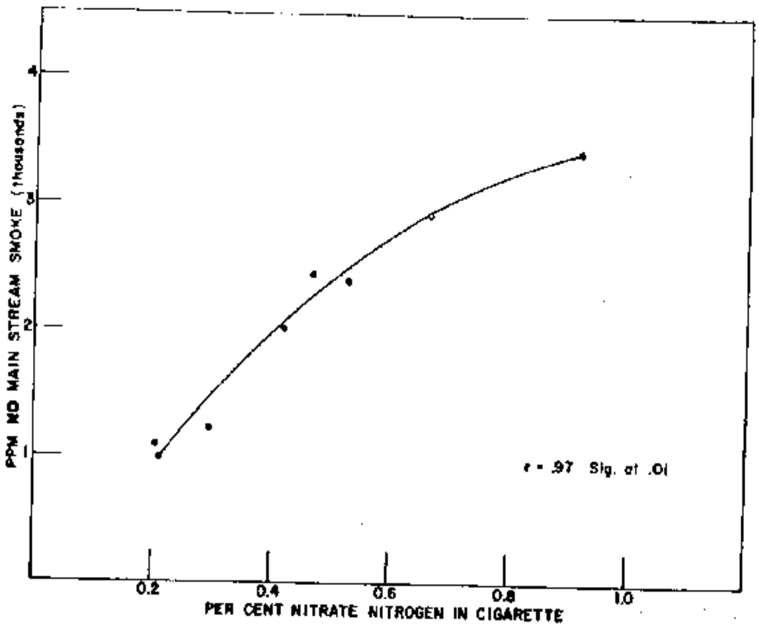


Figure 2. Oxides of nitrogen in smoke of cigarettes with differing levels of nitrate nitrogen.

each sample. A straight line should be obtained.

**Table 6. Effects of fertilizer and soil fumigation on per cent nitrate nitrogen in flue-cured tobacco.**[a]

| Year | 1962 | 1963 | 1964 |
|---|---|---|---|
| *Source of N* | | *Not Fumigated* | |
| All Ammonium | 0.004 | 0.007 | 0.006 |
| 50% Am.–50% Nitrate | .005 | .005 | .006 |
| All Nitrate | .004 | .007 | .006 |
| | | *Fumigated, EDB 40*[b] | |
| All Ammonium | .004 | | |
| 50% Am.–50% Nitrate | .004 | | |
| All Nitrate | .004 | | |

[a]*Experiments conducted at Georgia Coastal Plain Experiment Station, Tifton, Ga.*
[b]*Ethylene dibromide.*

*Preparation of Standard Curve*

(a) Place 40 ml of reaction mixture (1b) in each five 250 ml glass stoppered Erlenmeyer flasks.

(b) Add one, two, three and four ml of $KNO_3$ standard solution to first four flasks respectively.

(c) Add eight, seven, six and five ml distilled water to the respective flasks, making a total of 49 ml.

(d) Add nine ml of distilled $H_2O$ to fifth flask to be used as a blank.

(e) Add one ml of reagent 2 to each flask followed by a small amount (ca 200 mg) of zinc dust. Stopper flask and shake for one hour.

(f) Filter through Whatman #1 paper

(g) Place 5 ml of reagent 4 in each of five 25 ml glass stoppered volumetric flasks.

(h) Dilute to volume with filtrate from step f. Stopper, mix and allow 30 minutes for color to develop.

(i) Dilute one ml of solution from step h to volume in a 10 ml volumetric flask with reagent 5. Allow 30 minutes for color to develop.

(j) Read absorbance of sample vs reagent blank in spectrophotometer at 475 millimicrons.

(k) Plot the absorbance vs mg nitrate nitrogen in each sample. A straight line should be obtained.

### Method

Place 300 mg of tobacco in a 250 ml glass stoppered Erlenmeyer flask. Add 100 ml of reagent 1a, stopper and shake for one hour. Filter Place nine ml of filtrate in a 250 ml glass stoppered Erlenmeyer flask followed by 40 ml of reagent 1b, one ml of reagent 2 and ca 200 mg of zinc dust. Stopper and shake for one hour. Follow procedure for standard curve starting with step g.

It has been found that absorbances of 0.2-0.4 are in the desired range. This range may be obtained by varying sample size or the final dilution.

### Experimental Results

The nitrate nitrogen content of commercial tobaccos from various sources is shown in **Table 3.** Among types examined, flue-cured and Oriental had the lowest levels with only minor differences among the flue-cured belts. Burley contained, on the average, about fourteen times as much nitrate nitrogen as flue-cured. The lowest level was recorded in 1961, a season of plentiful rainfall. However, the levels were not significantly different for 1963, a seasonable year and 1964, a dry year. The highest levels were found in 1962 which was dry early, followed by late rains and second growth. Among samples from other countries

$$\text{Wt. Sample} \times \left(\frac{9}{100} \times \frac{20}{50} \times \frac{1}{10}\right) =$$

$$\text{Wt. Sample} \times .0036 = \text{Wt. sample in aliquot}$$

$$\frac{\text{Mg nitrate nitrogen}}{\text{Wt. Sample in Aliquot}} \times 100 = \%\ \text{nitrate nitrogen as N}$$

**Table 7. Effects of fertilizer and leaching on per cent nitrate nitrogen in flue-cured tobacco.**[a]

| Water Treatment | Post Water Fertilizer Treatment[b] N | K$_2$O | MgO | Interval Between Transplating and Post Water Fertilizer Treatment 5 Weeks | 7 Weeks | Mean |
|---|---|---|---|---|---|---|
| | Pounds per Acre | | | % Nitrate Nitrogen | | |
| | 0 | 0 | 0 | 0.003 | 0.008 | 0.006 |
| | 20 | 60 | 10 | .006 | .005 | .006 |
| Seasonal | 40 | 120 | 20 | .007 | .007 | .007 |
| | 60 | 180 | 30 | .013 | .010 | .012 |
| | 0 | 0 | 0 | .007 | .007 | .007 |
| 2 in. excess one week before Fert. Treatment | 20 | 60 | 10 | .008 | .009 | .009 |
| | 40 | 120 | 20 | .007 | .007 | .007 |
| | 60 | 180 | 30 | .019 | .010 | .015 |
| | | | Mean | .009 | .008 | .009 |

[a]*Experiments conducted at Oxford, North Carolina Tobacco Research Station, 1964.*
[b]*Basic Fertilizer Treatment: 70 lbs. N, 96 lbs. $P_2O_5$, 163 lbs. $K_2O$, 24 lbs. MgO.*

of origin, Rhodesian Burley had the lowest levels of nitrate nitrogen followed by Colombian and Mexican. The data shown for foreign-grown Burley are based on a limited number of samples supplied by leaf dealers and may not be representative of the production in these countries. Among other air-cured types, the levels of nitrates were lowest in Maryland and highest in Connecticut Shade-grown Wrappers.

Samples of Burley tobacco were drawn from representative markets throughout the Burley producing area in the 1964 season and analyzed for nitrate nitrogen with results as shown in **Figure 1**. Each dot represents fifty or more samples. The markets were separated into four groups according to levels of nitrate nitrogen. The highest levels of nitrates were found in southern and western Kentucky; while the mountainous areas of production tended to have lower levels of nitrates.

Among seven *Nicotiana* species surveyed, the levels of nitrates for a given location were highest in *N. glutinosa* and least in *N. rustica*. There were only minor differences among representative flue-cured varieties of *N. tabacum* (**Table 4**).

A survey of twelve of the more widely grown Burley varieties produced at two locations in East Tennessee in the 1963 and 1964 seasons, revealed no significant differences in levels of nitrate nitrogen among the varieties (**Table 5**). Among the four crops, the lowest levels occurred at the Greeneville Station, 1963. The rainfall pattern was seasonable through time of topping, then dry. The highest levels occurred at the

**Table 8. Nitrate nitrogen content in Burley tobacco, individual farms.**[a]

| State | Variety | Year | No. Farms | % Nitrate Nitrogen Range | % Nitrate Nitrogen Mean |
|---|---|---|---|---|---|
| Kentucky | Bur 21 | 1962 | 11 | 0.35 –0.96 | 0.66 |
| | | 1963 | 10 | .17 - 1.05 | .43 |
| | | 1964 | 12 | .33 – .96 | .64 |
| | | | | Mean | .58 |
| Tennessee | Bur 37 | 1962 | 8 | .18 – .73 | .34 |
| | | 1963 | 9 | .081– .70 | .33 |
| | | 1964 | 10 | .22 – .78 | .42 |
| | | | | Mean | .36 |
| Virginia | Bur 21 | 1962 | 4 | .12 – .50 | .30 |
| | | 1963 | 4 | .13 – .52 | .29 |
| | | 1964 | 4 | .11 – .43 | .26 |
| | | | | Mean | .29 |
| Mean | | 1962 | 23 | (Dry, Late Rains) | .49 |
| Mean | | 1963 | 23 | (Seasonal) | .37 |
| Mean | | 1964 | 26 | (Dry) | .50 |

Correlation Coefficient Nitrate Nitrogen vs:

| | |
|---|---|
| Per cent total production graded (mean 3-6 companies) | 0.19 NS[b] |
| Grade index of sample (one company) | .09 NS |
| Per cent total nitrogen | .58 ** |
| Per cent nicotine | –.13 NS |
| Per cent total nitrogen less nicotine N and nitrate N | .40 ** |

*[a]Weighted composite of cigarette grades, one company.*
*[b]NS- -Not statistically significant.*
***Significant at .01.*

**Table 9. Effect of fertilization on nitrate nitrogen content of Burley tobacco.**[a]

| Fertilizer Added lbs/acre N | $P_2O_5$ | $K_2O$ | FA | FB | FH | FM | FW | FY | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | | % Nitrate Nitrogen | | | | | | |
| 120 | 80 | 170 | 0.27 | 0.16 | 0.19 | 0.14 | 0.15 | 0.15 | 0.17 |
| 220 | 80 | 170 | .46 | .22 | .23 | .40 | .24 | .27 | .30 |
| 320 | 80 | 170 | .52 | .32 | .48 | .47 | .37 | .38 | .42 |
| 120 | 80 | 270 | .28 | .16 | .19 | .15 | .14 | .24 | .19 |
| 120 | 80 | 370 | .33 | .17 | .33 | .26 | .14 | .15 | .23 |
| 120 | 180 | 170 | .30 | .14 | .24 | .18 | .15 | .28 | .22 |
| 320 | 80 | 270 | .56 | .54 | .36 | .42 | .36 | .25 | .42 |
| 420 | 280 | 370 | .64 | .48 | .30 | .48 | .38 | .33 | .44 |
| | | *Mean* | .42 | .27 | .29 | .31 | .24 | .26 | .30 |

(Location: FA, FB, FH, FM, FW, FY)

Correlations Per Cent Nitrate Nitrogen vs:

| | |
|---|---|
| Fertilizer nitrogen added | r = 0.76 ** |
| Per cent total nitrogen | r = .77 ** |
| Per cent nicotine | r = .06 NS[b] |
| Per cent total nitrogen less nicotine N and nitrate N | r = .67 ** |

*[a]Experiments conducted on farm locations in North Carolina by Mountain Research Station, Waynesville, N. C. 1963, weighted composites, entire production.*
*[b]NS –Not statistically significant.*
***Significant at .01.*

**Table 10. Effects of fertilization and cover crop on per cent nitrate nitrogen in Burley tobacco.[a]**

| Fertilizer Nitrogen[b] lbs/acre | Stalk Position 1 & 2 | 3 | 4 | 5 | 6 | Weighted Mean | Ratio $NO_3$ N Midribs / $NO_3$ N Lamina |
|---|---|---|---|---|---|---|---|
| | | | Vetch Cover Crop | | | | |
| | 12.5 | 16.5 | 29.5 | 24.7 | 16.8[c] | | |
| 0 | 0.51 | 0.30 | 0.26 | 0.16 | 0.13 | 0.26 | 6.8 |
| 75 | .60 | .46 | .36 | .25 | .22 | .36 | 7.7 |
| 150 | 1.26 | .94 | .50 | .46 | .40 | .61 | 5.6 |
| 225 | .81 | .92 | .66 | .59 | .50 | .67 | 5.5 |
| 300 | .85 | .75 | .69 | .54 | .52 | .62 | 5.3 |
| 125[b] | 1.12 | .66 | .51 | .35 | .28 | .52 | 4.7 |
| Mean | .86 | .67 | .48 | .39 | .34 | .51 | 5.9 |
| $NO_3$ N Midribs / $NO_3$ N Lamina | 4.4 | 5.2 | 6.3 | 6.6 | 6.5 | 5.7 | |
| | | | Rye Cover Crop | | | | |
| | 16.1 | 19.2 | 27.4 | 19.4 | 17.9[c] | | |
| 0 | 0.038 | 0.023 | 0.026 | 0.036 | 0.024 | 0.029 | |
| 75 | .25 | .16 | .10 | .090 | .072 | .14 | |
| 150 | .58 | .28 | .19 | .19 | .14 | .25 | |
| 225 | .79 | .65 | .44 | .35 | .30 | .47 | |
| 300 | 1.41 | .82 | .58 | .45 | .25 | .62 | |
| 125[b] | .50 | .25 | .14 | .12 | .11 | .20 | |
| Mean | .59 | .36 | .25 | .21 | .15 | .28 | |
| $NO_3$ N Midribs / $NO_3$ N Lamina | 3.9 | | | | 9.1 | | 7.5 |

Correlation Nitrate Nitrogen vs Fertilizer Nitrogen $r = 0.58$ **

[a] *Experiments conducted at Tobacco Experiment Station, Greeneville, Tennessee, 1964.*
[b] *75 lbs. from mineral sources and 50 lbs. from manure.*
[c] *Per cent total production represented by respective stalk positions.*
** *Significant at .01.*

Greeneville Station, 1964. The rainfall pattern was the reverse of that described above, that is, very dry until August 3, then seasonable.

In a three year study of the effects of sources of nitrogen and soil fumigation with ethylene dibromide at the Georgia Coastal Plain Experiment Station, 1962-1964, no differences in nitrate levels were found as a result of sources of nitrogen, whether ammonium or nitrate, and no differences were found as a result of soil fumigation (Table 6).

Experiments were conducted at the Oxford, North Carolina Experiment Station, 1964 season, in which varying rates of supplementary fertilizer were added five and seven weeks after transplanting, with and without excess water one week prior to fertilization. No difference in nitrate content was found between the two fertilizing schedules of five and seven weeks and no difference as a result of the excess water. Only minor differences were found among fertilizer rates except at the highest rate, which was greatly in excess of that normally employed in the production of flue-cured tobacco (Table 7).

In cooperative variety evaluation tests, Burley tobacco was grown on a total of 72 locations in the 1962, 1963, and 1964 seasons. Weighted composites of cigarette grades in the check variety, Burley 21 or Burley 37, as judged by one domestic company, were analyzed for nitrate nitrogen (Table 8). There was a thirteen-fold difference between the extremes among individual crops. Among the three states, Kentucky had the highest nitrates, Virginia the lowest. There was no significant correlation between nitrate content of the tobacco and the average amount graded by the participating companies or the grade index as computed by one domestic company. There was a highly significant correlation between per cent nitrate nitrogen and per cent total nitrogen and between per cent nitrate nitrogen and per cent total nitrogen less nicotine nitrogen and nitrate nitrogen. There was no significant correlation between per cent nitrate nitrogen and per cent nicotine. The crops which were grown in a rotation tended to have higher nitrate content than those which followed tobacco.

Fertilization tests with Burley tobacco were conducted at six farm locations in North Carolina in 1963, and nitrate nitrogen determined on weighted composites of entire production from each plot (Table 9). There was a highly significant correlation between nitrate nitrogen in the cured leaves and the fertilizer nitrogen added. There was a highly significant correlation between nitrates and total nitrogen and between nitrates and total nitrogen less nicotine and nitrate nitrogen. There was virtually no correlation between per cent nitrates and per cent nicotine.

Fertilization and cover crop experiments with Burley tobacco were conducted at the Tobacco Experiment Station, Greeneville, Tennessee in the 1964 season **(Table 10).** The cured tobacco from each plot was divided into six farm grades, according to stalk position, grade 1 representing the lowermost leaves, or flyings, and grade 6 the uppermost leaves, or tips. Grades 1 and 2 were combined prior to stemming and analysis. Nitrates were also determined on the midribs of the samples grown after a vetch cover crop and the lowermost and uppermost stalk positions following the rye cover crop. The nitrates were much higher in those plots which followed a vetch crop, the differences being very large at the low rates of fertilizer nitrogen and disappearing at the highest, 300 pound, rate of fertilizer nitrogen. Consistent with results reported by Moseley et al. (10), the per cent nitrates were highest in the lowermost leaves and decreased with successive stalk positions. There was a highly significan correlation between nitrate content of the cured leaves and the fertilizer nitrogen added. The midribs contained, on the average, about six times as much nitrate nitrogen as the lamina. Relative to the lamina, the midribs did not show as much variation with stalk position or with rates of fertilizer nitrogen.

**Table 11. Effects of fertilizer, spacing and irrigation on per cent nitrate nitrogen in Burley tobacco.**[a]

| Spacing (in) Fertilization N lbs/acre | 12 | 16 | 20 | 24 | Mean |
|---|---|---|---|---|---|
| | | Replication 1 *Not Irrigated* | | | |
| 100 | 0.94 | 0.88 | 1.02 | 0.89 | 0.93 |
| 200 | .99 | 1.34 | 1.36 | 1.32 | 1.25 |
| 300 | 1.42 | 1.42 | 1.54 | 1.55 | 1.48 |
| | | *Irrigated* | | | |
| 100 | .68 | .50 | .87 | .43 | .62 |
| 200 | .93 | 1.03 | 1.03 | 1.31 | 1.08 |
| 300 | 1.49 | 1.40 | 1.43 | 1.50 | 1.46 |
| | | Replication 2 *Not Irrigated* | | | |
| 100 | .73 | .94 | .98 | .91 | .89 |
| 200 | 1.56 | 1.34 | 1.20 | 1.11 | 1.30 |
| 300 | 1.72 | 1.49 | 1.22 | 1.20 | 1.41 |
| | | *Irrigated* | | | |
| 100 | .55 | .86 | .66 | .57 | .66 |
| 200 | 1.28 | 1.17 | 1.15 | .99 | 1.22 |
| 300 | 1.48 | 1.58 | 1.52 | 1.28 | 1.46 |

Correlation Coefficient Nitrate Nitrogen vs:
Fertilizer nitrogen added $r = 0.86$ **
Spacing $r = -.06$ NS[b]

[a]*Experiments conducted at Kentucky Agricultural Experiment Station, Lexington, Kentucky, 1964. Weighted composite of entire production, whole leaves.*
[b]*NS—Not statistically significant.*
***Significant at .01.*

A fertilizer — spacing—irrigation experiment was conducted with Burley tobacco at the University of Kentucky Experiment Station, Lexington, Kentucky in the 1964 season. Nitrate nitrogen was determined on whole leaves representing weighted composites of the entire production on each plot **(Table 11).** There was a highly significant correlation between per cent nitrates and rates of fertilizer nitrogen, no significant correlation between per cent nitrates and spacing. Irrigation resulted in a large reduction in nitrates at the low rate of fertilizer nitrogen, less at the intermediate rate, and no reduction at the high rate.

Certain of the four principal types were grown in other belts in the 1964 season and cured by two methods, with results shown in **Table 12.** The environment during growth had a far greater effect upon nitrate nitrogen content than did genotype or curing method. Thus, all types grown in the Burley area, Lexington, Kentucky and Waynesville, North Carolina, had levels of nitrates approaching those of Burley and all types grown in the flue-cured area, Oxford, North Carolina and Whiteville, North Carolina, had levels of nitrates approaching those of flue-cured. The large difference between the nitrate content of flue-cured tobacco grown in the Burley area as compared with that grown in the flue-cured area, even with massive application of fertilizer nitrogen, suggests that factors other than nitrogen supply, such as soil type, may have important influences on the nitrate levels.

Eight lots of Burley tobacco having a wide range of nitrate nitrogen were selected from the farm grown variety tests. These were aged, cased, resampled, and manufactured into 70 mm cigarettes. Cigarettes of uniform weight were smoked mechanically according to the technique described by Bradford et al. (2). The concentration of oxides of nitrogen as NO was determined in the mainstream smoke obtained from each of four cigarettes in each lot using an unpublished method involving absorption of smoke in alkaline $KMnO_4$, which oxidizes the oxides of nitrogen to nitrate and colorimetric determination of nitrate by the metaxylenol procedure. The method is not specific for nitrogen oxides and may include other substances present in the gas phase smoke if they can be oxidized to nitrate by the absorbing medium. Since the nitrogen oxides of smoke occur in the gaseous phase, there is no problem of interference due to pigments. As shown **in Figure 2,** there was a highly significant correlation between the per cent nitrates in the tobacco and the concentration of oxides of nitrogen in the mainstream smoke.

The eight lots of cigarettes were divided into four pairs. The members of each pair had approximately the same nicotine content but widely different nitrate content. The four pairs were submitted to a 20 member, expert smoking panel and evaluated on the basis of mildness, flavor, and overall acceptability. There appeared to be no consistent relationship between these attributes and the levels of nitrates in the tobacco.

## Summary

Genetic differences appear to have only minor influences on the nitrate content of cured tobacco leaves. The wide variations among and within tobacco types appear to result pri-

**Table 12. Effects of location and curing method on per cent nitrate nitrogen, 1964.**

| Location - Type | Curing Method Air-cured | Flue-cured |
|---|---|---|
| Lexington, Ky. | | |
| Burley | 0.49 | |
| Flue-cured | .33 | |
| Waynesville, N. C. | | |
| Burley | .53 | 0.53 |
| Flue-cured | .34 | .46 |
| Oriental | .21 | .23 |
| Maryland | .25 | .26 |
| Oxford, N. C. | | |
| Burley | .031 | .033 |
| Flue-cured | .029 | |
| Oriental[a] | .026 | .026 |
| Maryland | .027 | .024 |
| Whiteville, N. C. | | |
| Burley | .035 | |

[a]*Whole leaves.*

marily from environmental influences, especially the availability of fertilizer nitrogen. There is evidence of a relationship of nitrates with water supply, especially at low rates of fertilizer nitrogen and when applied early in the growing season. There is evidence also of a relationship with soil type and previous cropping history. Confirming the experiments of Neurath (11), nitrates appear to be the principal source of oxides of nitrogen in the smoke. The grading record of the Burley tobaccos grown in farm grown variety trials (Table 8) and the results of smoke taste evaluation tests on selected samples from this group, suggest that the levels of nitrates, per se, do not exert a dominant influence upon smoking quality, as judged by traditional subjective methods.

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