

Capillary Chromatography: Evaluation of Volatiles from Flue-Cured Tobacco Varieties

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Flue-cured varieties that were popular from the 1940s through the 1970s and some currently grown varieties were grown for chemical analysis and manufacture of cigarettes for subjective data. The objective of this study was to address the charge that the quality of U.S.-grown flue-cured tobaccos has deteriorated as a result of the introduction of disease-resistant varieties for growers. NC 2326, a flue-cured variety approved by the Minimum Standards Program of the Tobacco Workers' Conference, was used as the standard against which each variety in the study was compared. Significant statistical differences were found among varieties, both chemically and subjectively. No evidence was obtained to support the claim that currently grown resistant varieties are inferior quality-wise to the old susceptible varieties grown earlier.

The chemical composition of the tobacco leaf is influenced by genetics, environment, cultural practices, and curing. Genetics determines the potential of a cultivar to produce or not produce certain compounds and the amount it produces in certain cases (Chaplin, 1975; Heath and Reineccius, 1986; Matzinger et al., 1984). Prior to 1950, flue-cured tobaccos had similar characteristics and were susceptible to common diseases that plagued tobacco. Breeding programs began in the 1930s to develop disease-resistant varieties that were released in the 1940s, but they were not widely grown following their introduction.

Dixie Bright 101 (DB 101) was released in 1950, and it was the first variety resistant to bacterial wilt and black shank. Coker 139, resistant to these diseases, was released in 1955 and was widely planted in 1956 and 1957. In 1957, the variety discount program was initiated by the USDA, and the support price for Coker 139 was discounted to 50% because of low nicotine and poor quality. The Minimum Standards Program of the Tobacco Workers' Conference was initiated in 1963 to ensure development of good-quality varieties. This program established limits for certain chemistries and smoke evaluations and considered all aspects of tobacco production to enhance tobacco quality.

NC 95, resistant to black shank, bacterial wilt, *Fusarium* wilt, and root knot, was released in 1960. By 1970, most of the tobacco planted in the United States had some level of resistance to common tobacco diseases. There were still tobacco growers who considered the quality of resistant varieties inferior to that of old susceptible varieties grown before the release of resistant varieties (Campbell, 1978).

Of the flue-cured tobacco grown in the United States, 90% is consumed in the form of smoke; therefore, smoke flavor is an important consideration in tobacco quality. Technology has changed, and chemists have a better understanding of tobacco leaf and smoke. To date, 1300 compounds have been identified from the cured leaf, 3875 from smoke, and 1200 common to both (Dube and Green, 1982). Pyrolysis of high molecular weight compounds from the leaf and direct transfer of volatiles account for the compounds in smoke.

Major efforts in recent years have focused on volatiles in the leaf that transfer directly to smoke. Demole and

Berthet (1972) characterized volatiles and semivolatiles from burley tobacco that influence smoke flavor. Kimland et al. (1972) identified specific volatiles from Greek tobacco that have been identified in smoke. Lloyd et al. (1976) identified 323 volatile compounds from the flue-cured leaf that are also found in tobacco smoke. Wahlburg et al. (1977) identified several hundred compounds from aging tobacco important to smoke flavor. Sakai et al. (1984) investigated volatiles from the headspace of flue-cured tobacco that correlated with subjective smoke analyses. Conclusions from these studies were that volatiles from the tobacco leaf influence the taste and aroma of tobacco smoke and are important in tobacco quality.

The first objective of this study was to compare volatiles in the tobacco leaf and subjective smoke data from disease-resistant and all susceptible flue-cured varieties to determine whether the introduction of disease resistance reduces tobacco quality. The second objective was to develop a method by which agronomists could chemically measure tobacco volatiles from the leaf that transfer to smoke and use the method as a tool to select genetic material for a breeding program to improve tobacco quality.

MATERIALS AND METHODS

Fifteen flue-cured tobacco varieties were chosen to represent those planted during different times in U.S. production history. Gold Dollar, Hicks, and 402 were common varieties grown before 1950 and were susceptible to the common root diseases such as black shank, bacterial wilt, and root knot. Dixie Bright 101, Golden Cure, and Golden Harvest were chosen to represent varieties grown in the 1950s. Dixie Bright 101 was the first variety released to have resistance to both black shank and bacterial wilt. Golden Cure and Golden Harvest were susceptible to the major diseases.

Coker 187-Hicks, NC 95, and NC 2326 were chosen to represent varieties planted in the 1960s. Coker 187-Hicks and NC 95 were both released before the Minimum Standards Program was initiated and have resistance to black shank and bacterial wilt. NC 95 also has resistance to the common strain of the southern root knot nematode. NC 2326 was one of the first varieties released from the Minimum Standards Program. The varieties chosen to represent the 1970s were Speight G-28, Coker 347, and McNair 944, and those chosen to represent the 1980s were Speight G-70, K 399, and NC 82. All of these varieties have been approved by the Minimum Standards Program and have varying levels of resistance to diseases such as bacterial wilt, black shank, and root knot.

The experiment was conducted at the Upper Piedmont Research Station, Reidsville, NC, during the summer of 1986. Three replications of each variety were grown in a completely random design. Cultural practices, including fertilizer rates, harvesting,

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Table I. Compounds Identified by Mass Spectrometry from the Different Groups

| no. | compound | structure | no. | compound | structure | | | |
|-----------|--|-----------|----------|---|-----------|--|--|--|
| Group I | | | | | | | | |
| 1 | 3-methyl-4,5-dihydrofuran | | 11 | linalool | | | | |
| 2 | 2-methyl-3-keto-4,5-dihydrofuran | | 12 | 5-methylfurfural | | | | |
| 3 | acetoin | | 13 | 6-methyl-3,5-heptadien-2-one | | | | |
| 4 | 6-methyl-5-hepten-2-one | | 14 | β -methyl- γ -butyrolactone | | | | |
| 5 | γ -angelica lactone | | 15 | furfuryl alcohol | | | | |
| 6 | linalool oxide | | 16 | β -angelica lactone | | | | |
| 7 | furfural | | 17 | 4-ketoisophorone | | | | |
| 8 | 2,4-hexadienal | | 18 | 2-(hydroxymethyl)-5-methylfuran | | | | |
| 9 | furfuryl methyl ketone | | 19 | 2-acetonyl-3-isopropyl-6-methyltetrahydro-2-pyran | | | | |
| 10 | benzaldehyde | | Group II | | | | | |
| 20 | solanone | | 23 | 7-methylenetriecane | | | | |
| 21 | 2-butenolide | | 24 | damascone | | | | |
| 22 | 4-hydroxy-2-hexenoic acid ethyl ester | | 25 | damascenone | | | | |
| Group III | | | | | | | | |
| 26 | cyclotene | | 29 | solanol | | | | |
| 27 | geranylacetone | | 30 | phenethyl alcohol | | | | |
| 28 | benzyl alcohol | | 31 | phenol | | | | |
| Group IV | | | | | | | | |
| 32 | neophytadiene and isomers | | Group V | | | | | |
| 33 | 2-(2-keto-6-methylhexanyl)-5-methylfuran | | 46 | methylethylmaleimide | | | | |
| 34 | 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene | | 47 | nicotyrine | | | | |
| 35 | 2-formylpyrrole | | 48 | dihydroactinidiolide | | | | |
| 36 | 4-hydroxy-2-nonenic acid | | 49 | farnesylacetone | | | | |
| 37 | <i>p</i> -cresol | | 50 | indole | | | | |
| 38 | <i>p</i> -vinylphenol | | | | | | | |

Table I (Continued)

| no. | compound | structure | no. | compound | structure |
|-----|--|-----------|-----|--|-----------|
| 39 | 2-methyl-3-pyrrolicarboxaldehyde | | 51 | 3-hydroxy- β -damascone | |
| 40 | phytone | | 52 | 3,4-dihydro-1,5,6-trimethyl-2-(hydroxymethyl)naphthalene | |
| 41 | oxysolanone | | 53 | 4-keto- α -ionol | |
| 42 | 4,6,8-megastigmatrien-3-one and isomers | | 54 | 3-hydroxy- β -ionol | |
| 43 | farnesyl acetate | | 55 | 4-hydroxy- β -ionol | |
| 44 | <i>o</i> -methoxy- <i>p</i> -vinylphenol | | 56 | 5,8-oxido-9-methylene-3,13-duvidien-1-ol | |
| 45 | solanascone | | 57 | 6,7-dimethyl-5-hydroxybenzofuran | |
| | | | 58 | dehydro- β -ionone | |

and curing, were those practiced for flue-cured tobacco at the experiment station. It is noted that some of these tobaccos may not have been managed as they would have been if grown in a commercial setting; however, the data should be comparable. The plots were harvested individually, when the leaves were judged to be mature and ripe. The cured primings and lugs were discarded because of sand and other debris associated with tobacco from the lower stalk positions. The rest of the cured leaves from each variety were hand-picked to eliminate leaves that were scorched, were mildewed, were excessively injured, were off-color, or were of poor quality. Following cleanup, tobacco from each replication was composited over stalk position, the midrib was removed by machine, and the tobacco was cut into rag, blended, and made into 85-mm nonfiltered cigarettes. Two pounds of cut leaf from each replication was stored at 35 °F inside a walk-in cooler in plastic zip-lock bags and saved for chemical analyses.

The cigarettes were smoked by three members of a trained smoking panel. Cigarettes made from NC 2326, the check variety, were smoked several times by the panelists to identify the flavor. For comparison of varieties, a base of 10 was assigned to NC 2326. After each panelist was conditioned to the check, cigarettes from each variety were smoked one replication at a time against a check. Smoking was done at the discretion of the panelist to avoid fatigue and obtain accurate comparisons. A rank for each replication was entered on a score card. The scores of the panelist were totaled, and the mean of each replication was accepted as the score for the replication. Scores greater than 10 assigned to a replication indicated that the panel considered smoke from the cigarette better than the check, and scores lower than 10 indicated the smoke was inferior to the check. The data obtained were analyzed statistically by analysis of variance for completely random design.

Neutral volatiles were obtained from 10-g samples of each replication by steam distillation (Weeks and Seltmann, 1986). Gas chromatography was performed with a Varian 3700 capillary GC equipped with a flame ionization detector, a CDS 111 integrator

for temperature control, and a Hewlett-Packard 3393-A integrator interfaced with a Zenith XT computer for data collection. A Supelcowax 10 bonded-phase megabore column was used for routine analyses. Helium was used as carrier and makeup gases at 8 and 30 mL/min, respectively. Injector and detector temperatures were run at 250 and 270 °C. The injector was glass-lined and dead volume eliminated for direct injections. The volume of each sample was adjusted to 1 mL, and 1 μ L was injected, approximating the quantity of tobacco from a single puff of an 85-mm nonfiltered cigarette smoked on a standard smoking machine.

The oven temperature of the gas chromatograph was programmed from 60 to 210 °C with multilinear temperature programming, allowing a 5-min delay at 140 and 180 °C to enhance separation, for an overall program of 1.5 °C/min from 60 to 210 °C.

A new capillary column previously described, following the manufacturer's recommendation for conditioning, was calibrated and checked for reproducible retention times and response over the defined temperature range by running duplicate samples prepared from NC 2326, the check variety. The two samples were spiked with known quantities of compounds, previously identified from the distillate of flue-cured tobacco, with retention times over the entire temperature range. The relative response factors of the added compounds and the internal standard, tetradecane, were determined.

The spiked samples were also run on a Hewlett-Packard 5890A capillary gas chromatograph equipped with a Supelcowax 10 60-m, 0.53-mm-i.d., wall-coated column interfaced with a Model 800 Finnigan ion trap mass spectrometer. The elution patterns from the two columns were comparable. Unknown peaks were identified by library search and by comparing previous spectra identified by R. J. Reynolds Tobacco Co. from similar preparations.

Table II. Concentration of Group V Compounds in 15 Flue-Cured Tobacco Varieties and ANOVA of Volatiles

| variety | concn ($\mu\text{g/g}$) for replications | | | ξ | \bar{x} |
|-----------------|--|-----|-----|-------|------------------|
| | 1 | 2 | 3 | | |
| 1940s | | | | | |
| Gold Dollar | 230 | 226 | 236 | 692 | 231 ^b |
| Hicks | 329 | 370 | 361 | 1060 | 353 |
| 402 | 336 | 343 | 406 | 1085 | 362 |
| 1950s | | | | | |
| DB 101 | 221 | 239 | 231 | 682 | 228 ^b |
| Golden Cure | 198 | 204 | 166 | 568 | 190 ^b |
| Golden Harvest | 355 | 365 | 369 | 1089 | 363 |
| 1960s | | | | | |
| Coker 187-Hicks | 336 | 299 | 347 | 982 | 328 |
| NC 95 | 414 | 416 | 486 | 1316 | 439 ^a |
| NC 2326 (check) | 335 | 342 | 359 | 1036 | 345 |
| 1970s | | | | | |
| Speight G-28 | 424 | 440 | 402 | 1266 | 422 ^a |
| Coker 347 | 472 | 422 | 474 | 1368 | 456 ^a |
| McNair 944 | 433 | 436 | 446 | 1315 | 438 ^a |
| 1980s | | | | | |
| Speight G-70 | 500 | 489 | 515 | 1504 | 501 ^a |
| K 399 | 459 | 407 | 461 | 1327 | 442 ^a |
| NC 82 | 395 | 353 | 329 | 1077 | 359 |

ANOVA

| source of variation | DF | SS | MS | F | tab F |
|---------------------|----|---------|-------|---------|--------------------|
| replications | 2 | 2052 | 1026 | 1.94 NS | 3.34 \times 5.45 |
| varieties | 14 | 350 177 | 25012 | 47.21 | 2.06 \times 2.80 |
| error | 28 | 14 835 | 530 | | |
| total | 44 | 367 065 | | | |

mean = 364; CV = 6; LSD (0.05) = \pm 38; LSD (0.01) = \pm 52

^aSignificantly greater. ^bSignificantly less.

Table IV. Concentration of Neophytadiene in 15 Flue-Cured Varieties and ANOVA Data

| variety | concn ($\mu\text{g/g}$) for replications | | | ξ | \bar{x} |
|-----------------|--|-----|-----|-------|------------------|
| | 1 | 2 | 3 | | |
| 1940s | | | | | |
| Gold Dollar | 336 | 287 | 334 | 957 | 319 ^b |
| Hicks | 407 | 307 | 353 | 1067 | 355 |
| 402 | 335 | 392 | 252 | 979 | 326 |
| 1950s | | | | | |
| DB 101 | 379 | 297 | 366 | 1042 | 347 |
| Golden Cure | 267 | 239 | 260 | 766 | 256 ^b |
| Golden Harvest | 360 | 354 | 386 | 1100 | 367 |
| 1960s | | | | | |
| Coker 187-Hicks | 410 | 443 | 473 | 1326 | 442 ^a |
| NC 95 | 556 | 543 | 558 | 1656 | 552 ^a |
| NC 2326 (check) | 359 | 377 | 337 | 1073 | 358 |
| 1970s | | | | | |
| Speight G-28 | 297 | 329 | 349 | 975 | 325 |
| Coker 347 | 367 | 384 | 447 | 1198 | 399 |
| McNair 944 | 448 | 372 | 454 | 1274 | 425 |
| 1980s | | | | | |
| Speight G-70 | 320 | 318 | 408 | 1046 | 350 |
| K 399 | 372 | 457 | 448 | 1277 | 426 |
| NC 82 | 540 | 456 | 402 | 1398 | 466 ^a |

ANOVA

| source of variation | DF | SS | MS | F | tab F |
|---------------------|----|---------|-------|---------|--------------------|
| replications | 2 | 2637 | 1318 | 0.72 NS | 3.34 \times 5.45 |
| varieties | 14 | 220 604 | 15750 | 8.61 | 2.06 \times 2.80 |
| error | 28 | 51 236 | 1830 | | |
| total | 44 | 274 477 | | | |

mean = 381; CV = 11; LSD (0.05) = \pm 72; LSD (0.01) = \pm 96

^aSignificantly greater. ^bSignificantly less.

Table III. Concentration of Total Neutral Volatiles in 15 Flue-Cured Varieties and ANOVA of Volatiles

| variety | concn ($\mu\text{g/g}$) for replications | | | ξ | \bar{x} |
|-----------------|--|------|------|-------|-------------------|
| | 1 | 2 | 3 | | |
| 1940s | | | | | |
| Gold Dollar | 708 | 667 | 717 | 2092 | 697 ^b |
| Hicks | 926 | 775 | 798 | 2499 | 833 |
| 402 | 911 | 1038 | 811 | 2760 | 920 |
| 1950s | | | | | |
| DB 101 | 760 | 786 | 790 | 2336 | 779 |
| Golden Cure | 617 | 606 | 669 | 1892 | 631 ^b |
| Golden Harvest | 944 | 852 | 975 | 2771 | 924 |
| 1960s | | | | | |
| Coker 187-Hicks | 917 | 817 | 1057 | 2791 | 930 |
| NC 95 | 1001 | 1201 | 1057 | 3259 | 1086 ^a |
| NC 2326 (check) | 905 | 901 | 840 | 2646 | 882 |
| 1970s | | | | | |
| Speight G-28 | 951 | 1006 | 1090 | 3051 | 1017 |
| Coker 347 | 951 | 1009 | 1186 | 3146 | 1048 ^a |
| McNair 944 | 1117 | 1056 | 1143 | 3316 | 1105 ^a |
| 1980s | | | | | |
| Speight G-70 | 1020 | 950 | 1170 | 3140 | 1047 ^a |
| K 399 | 948 | 1037 | 1138 | 3123 | 1041 ^a |
| NC 82 | 1140 | 933 | 915 | 2988 | 996 |

ANOVA

| source of variation | DF | SS | MS | F | tab F |
|---------------------|----|-----------|-------|------|--------------------|
| replications | 2 | 18 737 | 9 | | 3.34 \times 5.45 |
| varieties | 14 | 856 794 | 61200 | 8.41 | 2.06 \times 2.80 |
| error | 28 | 203 660 | 7274 | | |
| total | 44 | 1 079 191 | | | |

mean = 929; CV = 9; LSD (0.05) = \pm 142.61; LSD (0.01) = \pm 192.38

^aSignificantly greater. ^bSignificantly less.

Table V. Panel Evaluation of Flue-Cured Character from Cigarettes Made from 15 Varieties and ANOVA Data

| variety | concn ($\mu\text{g/g}$) for replications | | | ξ | \bar{x} |
|-----------------|--|------|------|-------|-------------------|
| | 1 | 2 | 3 | | |
| 1940s | | | | | |
| Gold Dollar | 9.0 | 9.0 | 9.0 | 27.0 | 9.0 ^b |
| Hicks | 9.5 | 9.5 | 9.5 | 28.5 | 9.5 |
| 402 | 7.5 | 8.0 | 8.0 | 23.5 | 7.83 ^b |
| 1950s | | | | | |
| DB 101 | 7.0 | 8.5 | 7.5 | 23.0 | 7.67 |
| Golden Cure | 6.6 | 6.0 | 6.0 | 18.6 | 6.2 ^b |
| Golden Harvest | 9.5 | 9.0 | 9.0 | 27.5 | 9.17 ^b |
| 1960s | | | | | |
| Coker 187-Hicks | 9.0 | 9.0 | 9.0 | 27.0 | 9.0 ^b |
| NC 95 | 10.0 | 10.0 | 9.0 | 30.0 | 10.0 |
| NC 2326 (check) | 10.0 | 10.0 | 10.0 | 30.0 | 10.0 |
| 1970s | | | | | |
| Speight G-28 | 9.5 | 9.0 | 9.0 | 27.5 | 9.17 ^b |
| Coker 347 | 8.5 | 8.5 | 9.0 | 26.0 | 8.67 ^b |
| McNair 944 | 9.5 | 9.5 | 9.5 | 28.5 | 9.5 |
| 1980s | | | | | |
| Speight G-70 | 10.0 | 10.0 | 10.0 | 30.0 | 10.0 |
| K 399 | 10.5 | 10.0 | 10.0 | 30.5 | 10.17 |
| NC 82 | 9.5 | 9.0 | 9.0 | 27.5 | 9.17 ^b |

ANOVA

| source of variation | DF | SS | MS | F | tab F |
|---------------------|----|-------|-------|-----------|--------------------|
| replications | 2 | 0.03 | 0.015 | 0.1339 NS | 3.34 \times 5.45 |
| varieties | 14 | 23.13 | 1.65 | 14.73 | 2.06 \times 2.80 |
| error | 28 | 3.14 | 0.112 | | |
| total | 44 | 26.30 | | | |

mean = 9.23; CV = 4; LSD (0.05) = \pm 0.553; LSD (0.01) = \pm 0.746

^aSignificantly greater. ^bSignificantly less.

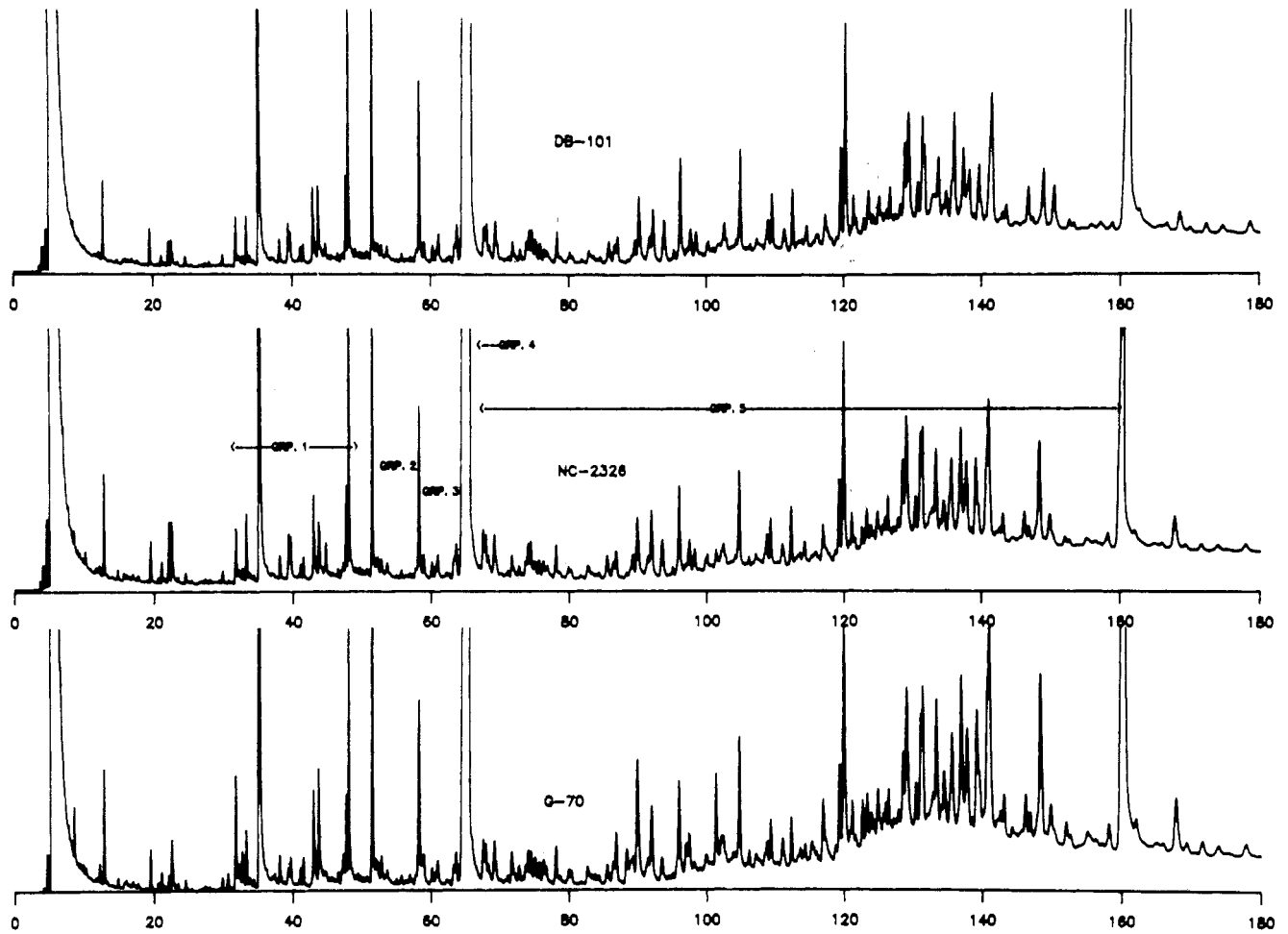


Figure 1. Chromatographic profile of DB 101, NC 2326, and Speight G-70.

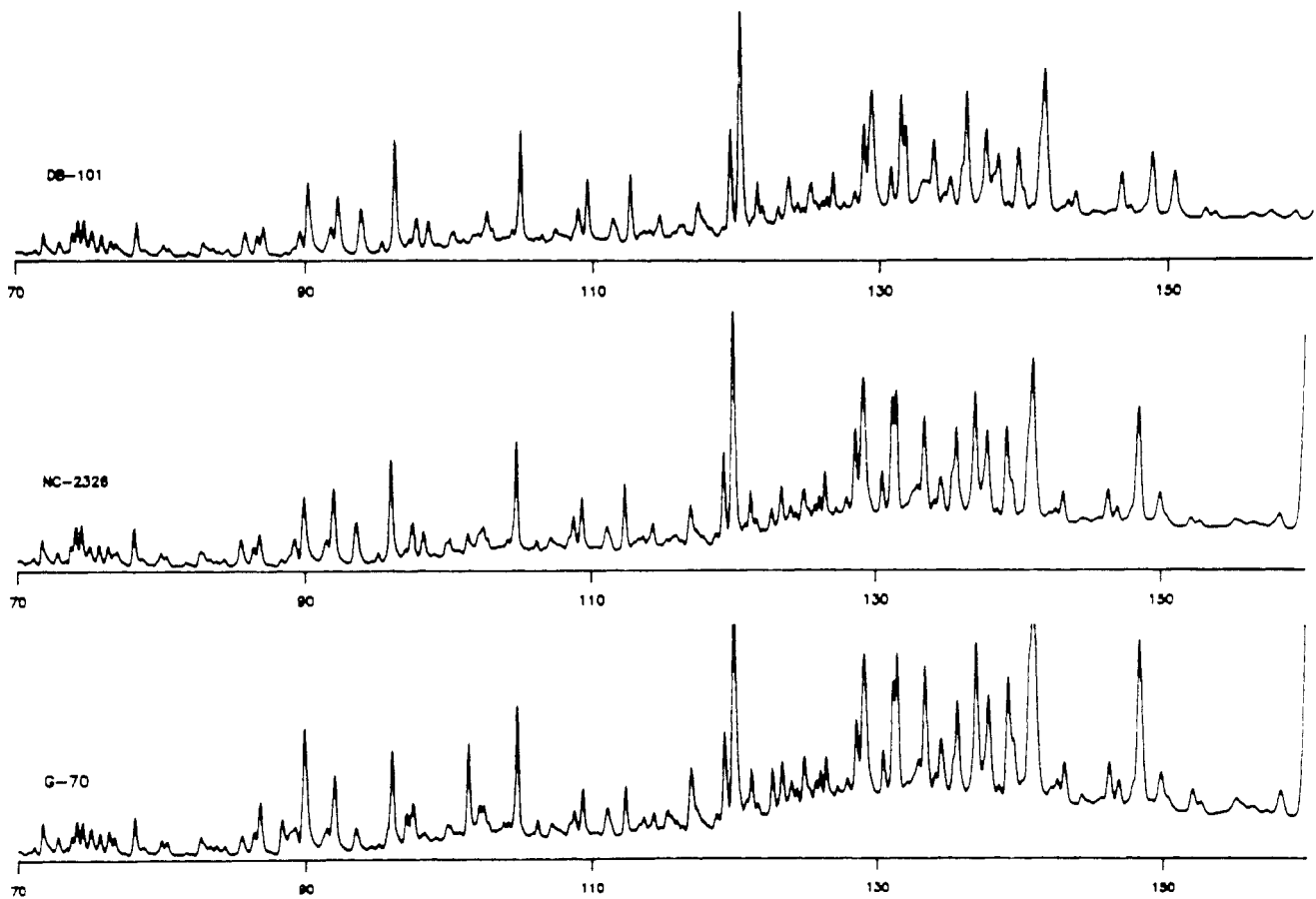


Figure 2. Comparison of varieties from Figure 1 (group V).

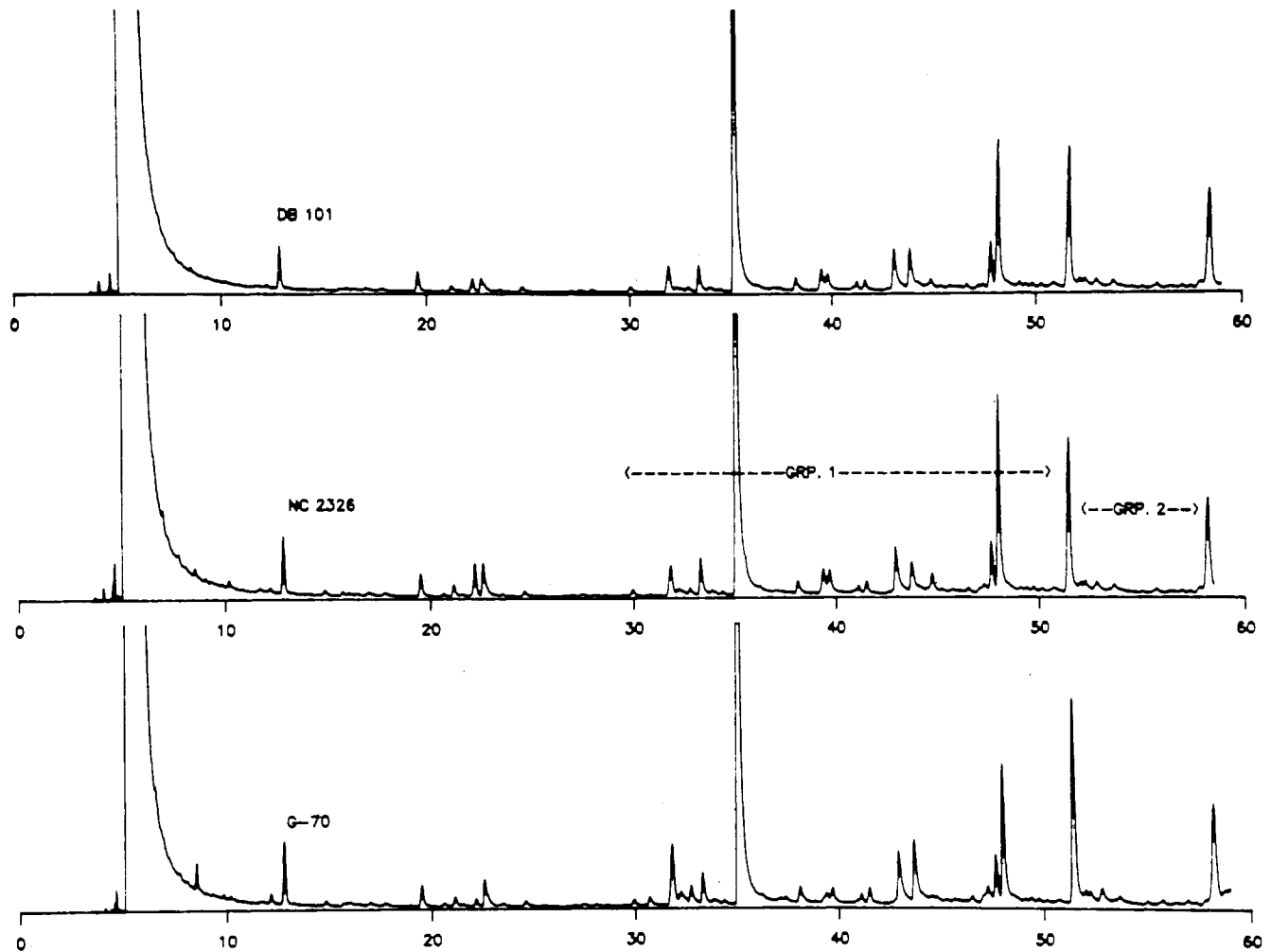


Figure 3. Comparison of varieties from Figure 1 (groups I and II).

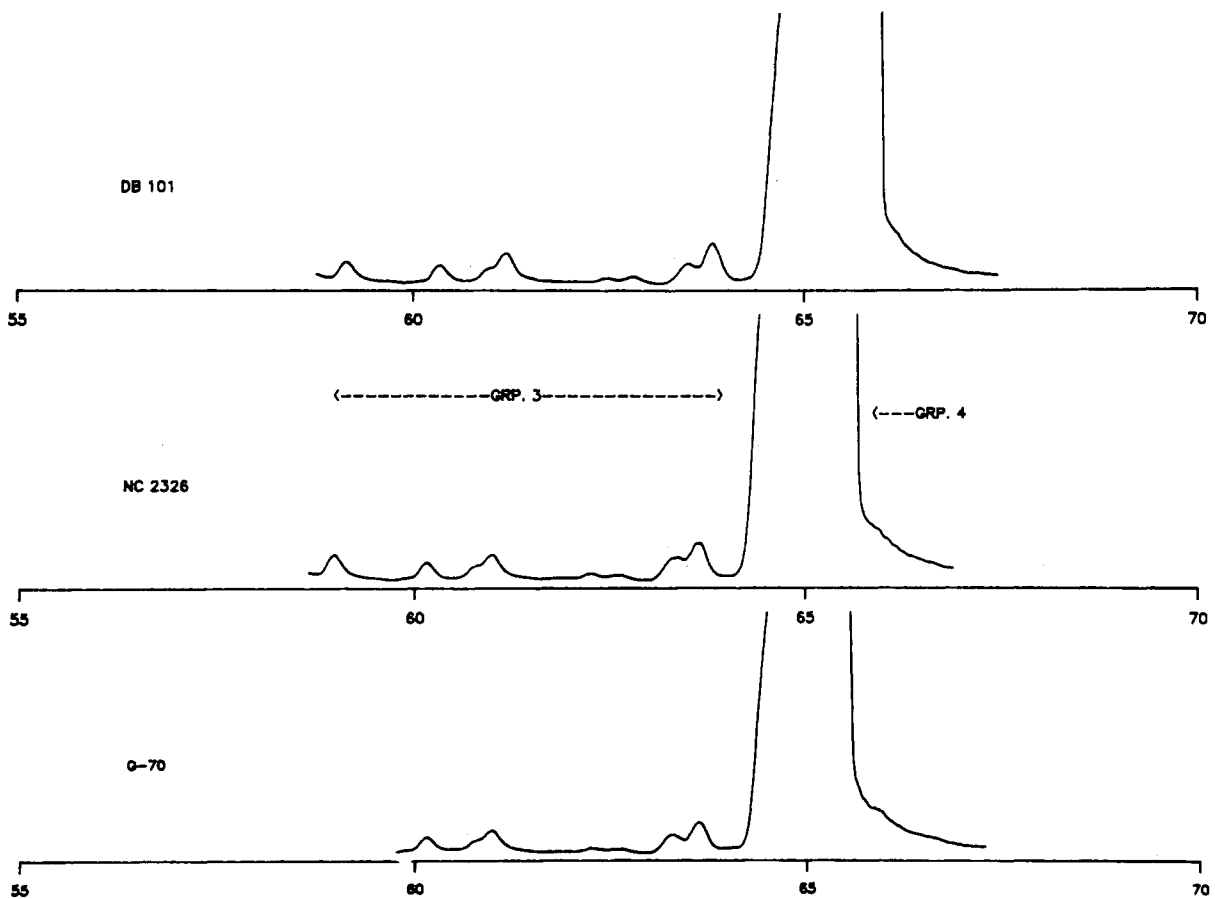


Figure 4. Comparison of varieties from Figure 1 (groups III and IV).

Table VI. Comparison of 17 Volatile Compounds in 15 Flue-Cured Varieties^a

| variety | volatile peaks | | | | | | | | | | | | | | | | |
|-----------------|----------------|-------|-------|------|-------|------|-------|-------|------|-------|-------|------|-------|-------|-------|-------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| Gold Dollar | + | -- | | | - | | | - | | -- | | | | | -- | | - |
| Hicks | - | -- | - | ++ | | | | | | -- | | | | | -- | - | - |
| 402 | ++ | -- | - | | -- | ++ | | -- | | | -- | | | | -- | | - |
| DB 101 | ++ | | -- | ++ | | | | | ++ | -- | | | | | -- | | - |
| Golden Cure | - | | | ++ | | | | -- | | -- | | | | | -- | | - |
| Golden Harvest | ++ | -- | | | -- | | | | | | | | | | -- | | - |
| Coker 187-Hicks | ++ | | | | | ++ | | -- | | | | | | | -- | | - |
| NC 95 | | -- | | ++ | | | | ++ | | ++ | | | | | | | - |
| NC 2326 | 6.50 | 12.39 | 15.92 | 6.27 | 16.83 | 6.01 | 16.46 | 13.92 | 6.64 | 21.13 | 17.35 | 9.20 | 12.29 | 32.72 | 28.92 | 24.36 | 6.49 |
| Speight G-28 | | -- | | | | | | -- | ++ | -- | | | | | -- | | - |
| Coker 347 | -- | -- | + | | -- | ++ | | | | | | | | | | | - |
| McNair 944 | -- | | | | -- | | | | ++ | ++ | ++ | | | | -- | | - |
| Speight G-70 | - | -- | ++ | ++ | -- | | | - | ++ | | ++ | ++ | + | | -- | | - |
| K 399 | -- | -- | ++ | | | ++ | | | | | | | | | -- | | - |
| NC 82 | ++ | -- | ++ | ++ | | ++ | | | | | | | | | -- | | - |

^aKey: + = significantly greater at 5%; ++ = highly significantly greater; - = significantly less at 5%; -- = highly significantly less.

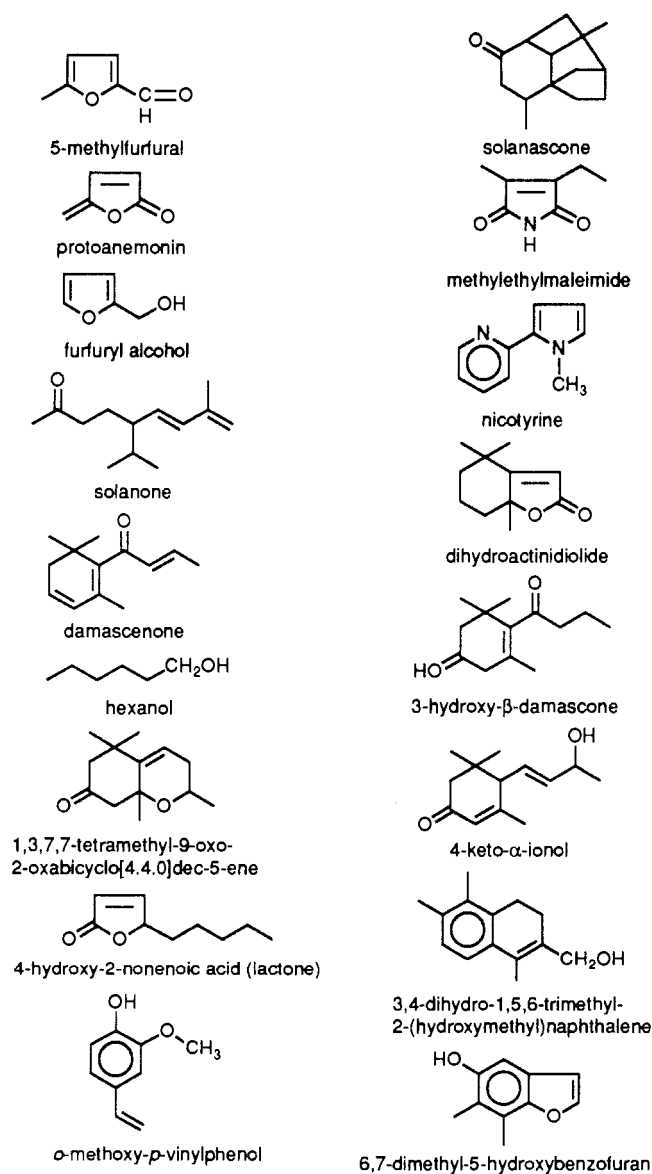


Figure 5. Molecular structures of 17 compounds greater than 5 ppm. Concentration ranges of the 17 most concentrated peaks (ppm): 1, 5.83–12.10; 2, 5.61–12.86; 3, 6.04–23.94; 4, 5.10–14.07; 5, 6.39–25.49; 6, 5.00–12.13; 7, 8.00–28.87; 8, 6.15–21.00; 9, 5.34–18.67; 10, 7.00–32.00; 11, 8.49–35.67; 12, 6.37–13.78; 13, 8.57–17.27; 14, 25.02–36.45; 15, 12.13–29.14; 16, 20.24–28.13; 17, 5.00–7.03.

RESULTS AND DISCUSSION

Selection of NC 2326 as the variety to use as a check by the panelists was in keeping with the policy of the Minimum Standards Program, which uses this variety as one of the standards for release of flue-cured varieties. Varieties common in the 1940s and 1950s evaluated in this test are no longer grown, primarily because of diseases that have discouraged their growth, and for this reason, there are few available data to repudiate the claim that old flue-cured varieties are superior quality-wise to current varieties.

Documents are available that relate a number of chemical ratios to taste and flavor of smoke (Shmuk, 1953), but there is no general agreement as to the exact contribution of any specific source or chemical factor to smoking flavor. There is, however, agreement that "volatile oils" have a positive influence on quality and contribute to flavor and aroma (Mendell et al., 1984). When the tobacco was enclosed for a short period of time, more aroma was associated with samples having better physical qualities (Speight G-70, Coker 347, McNair 944, Speight G-28, K 399). These were varieties with the highest measurable volatiles from group V, Table I.

Steam distillation and denicotinization of the extracted distillate gave very reproducible data for neutral volatiles when the samples were chromatographed. Differences among varieties were quantitative rather than qualitative. Separation of components by gas chromatography and mass spectrometry for a sample prepared from NC 2326 exhibited structures of compounds derived from identical precursors (Table I). A total of 130 compounds were separated. The concentrations of many of these compounds were too small for quantitation from the small samples available. During routine chromatographic analysis, the integrators were calibrated to quantify only compounds that were greater than 1 ppm in the NC 2326 sample, and to simplify comparison of the chromatograms from different varieties, each chromatogram was divided into groups. Group V (Table I) contained the largest number of carotenoid derivatives, aromatic compounds, and terpenones that have been identified as tobacco flavorants (Kimland et al., 1972).

The peaks in group V were compiled into totals, and an analysis of variance (in methods) was applied to the data (Table II). To keep the analytical data comparable to the smoke data, NC 2326 was compared to the volatile data for each variety with the LSD test. Gold Dollar, DB 101,

and Golden Cure were statistically lower in volatiles associated with flue-cured character than was NC 2326. These are older varieties, released prior to 1963. They are not currently grown, primarily because of low yield and poor quality. NC 95, Speight G-28, Coker 347, McNair 944, Speight G-70, and K 399 were significantly greater in group V volatiles than was NC 2326. These varieties were developed and released after 1963. Most of these are still grown and are popular for their high yield, ease of handling, and disease resistance. NC 82 did not differ from NC 2326 statistically, but it was higher in volatiles than was Gold Dollar, DB 101, and Golden Cure.

The analysis of variance of the total neutral volatiles (Table III) indicates that four out of six varieties produced prior to 1960 were lower in total volatiles than was NC 2326, while six out of nine varieties produced after 1960 were higher than was NC 2326.

Table IV includes the data for neophytadiene, the compound having the highest profile concentration. Comparison of the means indicates that NC 95, Coker 347, McNair 944, K 399, and NC 82 had higher concentrations of neophytadiene than did NC 2326. The chemical origin of neophytadiene is not known, but one postulate is that it occurs as a result of chemical degradation of chlorophyll (Davis, 1976). No information is available on the genetics of neophytadiene.

The smoke panel evaluation data showed a small range separating the rankings among varieties (Table V). Analysis of variance of panelists' rankings showed no difference among cigarettes made of tobacco from different replications. Comparison of the varietal means of cigarettes over replications, using LSD to compare NC 2326 with each variety, showed significant differences in panel rankings between NC 2326 and seven of the varieties. These rankings were significantly less than was the case for NC 2326, indicating poorer flue-cured smoking quality. Six varieties did not differ from the check statistically, although they were ranked as good or slightly better than the check by the panelists. Two of these were current varieties (Speight G-70, K 399).

The selection used in preparing the tobacco prior to blending and cigarette making resulted in the use of only the best tobacco from each plot. This may have been a contributing factor to the small differences the panelists found among the varieties. Moreover, the cigarettes were made from tobaccos that were not aged. This may have influenced the chemistry and taste.

The chromatograms in Figure 1 show the total profile for Speight G-70, DB 101, and NC 2326. Speight G-70 gave the highest quantitative yield of volatiles of all the varieties in group V (Table II). A comparison of group V (Figure 2) profiles illustrates chromatographic differences in varieties from the 1950s (DB 101), 1960s (NC 2326), and one that is currently grown (Speight G-70). The chromatographic differences in the other four groups from the same varieties are shown in Figures 3 and 4. No major differences are illustrated in these groups among the three varieties. Seventeen compounds from NC 2326, each greater than 5 ppm, were used for comparison among the varieties (Table VI; Figure 5). The range of concentration for each compound is also shown. Each of these components was analyzed statistically, and the mean of the three replications of each variety was compared as previously described (Table VI).

CONCLUSIONS

Numerous quantitative differences in composition exist in tobaccos with varying genetic backgrounds. This is exhibited in the gas chromatography profiles of steam-distillable compounds from flue-cured tobacco. Differences in aroma that are easily detected by the nose can be separated into meaningful qualitative and quantitative results. Capillary gas chromatography can be used to select for chemistries that correlate with sensory response, and geneticists can identify breeding material with chemical character that improves smoke flavor and aroma. On the basis of this work, presently grown flue-cured tobacco varieties appear to have superior flavor and aroma to the older ones, and the resistant varieties being grown today are not responsible for deterioration of the U.S.-grown flue-cured tobaccos.

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Received for review June 6, 1988. Accepted February 23, 1989. Paper No. 11620, Journal Series, North Carolina Agricultural Research Service, Raleigh, NC. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or USDA of the products named nor criticism of similar ones not mentioned.