

Burley Tobacco

Burley Tobacco: Chemistry & Toxicity of Unburnt Material

Lewis, Ramsey S.; Parker, Robert G.; Danehower, David A.; Andres, Karen; Jack, Anne M.; Whitley, D. Scott; Bush, Lowell P. (2012), "Impact of alleles at the Yellow Burley (Yb) loci and nitrogen fertilization rate on nitrogen utilization efficiency and tobacco-specific nitrosamine (TSNA) formation in air-cured tobacco" Journal of Agricultural and Food Chemistry, 60(25), 6454-6461

Tobacco-specific nitrosamine (TSNA) formation in tobacco is influenced by alkaloid levels and the availability of nitrosating agents. Tobacco types differ in their potential for TSNA accumulation due to genetic, agronomic, and curing factors. Highest TSNA concns. are typically measured in burley tobaccos. One of the main genetic differences between burley and all other tobacco types is that this tobacco type is homozygous for recessive mutant alleles at the Yellow Burley 1 (Yb1) and Yellow Burley 2 (Yb2) loci. In addn., burley tobacco is typically fertilized at higher nitrogen (N) rates than most other tobacco types. This study utilized nearly isogenic lines (NILs) differing for the presence of dominant or recessive alleles at the Yb1 and Yb2 loci to investigate the potential influence of genes at these loci on TSNA accumulation. Three pairs of NILs were evaluated at three different nitrogen fertilization rates for alkaloid levels, nitrogen physiol. measures, and TSNA accumulation after air-curing. As previously obsd. by others, pos. correlations were obsd. between N application rates and TSNA accumulation. Recessive alleles at Yb1 and Yb2 were assocd. with increased alkaloid levels, reduced nitrogen use efficiency, reduced nitrogen utilization efficiency, and increased leaf nitrate nitrogen (NO₃-N). Acting together, these factors contributed to significantly greater TSNA levels in genotypes possessing the recessive alleles at these two loci relative to those carrying the dominant alleles. The chlorophyll-deficient phenotype conferred by the recessive yb1 and yb2 alleles probably contributes in a substantial way to increase available NO₃-N during curing and, consequently, increased potential for TSNA formation.

Moldoveanu, Serban C.; Byrd, Crystal H.; Gerardi, Anthony R, (2011), " Analysis of certain nitrogenous compounds in tobacco. Part 1: adenosine, 2,5- and 2,6-deoxyfructosazines, mannosamine and glucosamine" - Beitraege zur Tabakforschung International, 24(5), 233-242

Nitrogenous compds. such as amino acids and proteins are frequently analyzed in tobacco since they are considered precursors of toxicants in cigarette smoke. However, much less attention is given to other nitrogenous compds. such as amino sugars and deoxyfructosazines, although their concn. in tobacco can be equal to or even higher than that of most free amino acids. These nitrogenous compds. may contribute to the formation of toxicants in smoke, or may contribute to the sensory properties of cigarette smoke, reasons for which their anal. is important. This study describes a procedure for the anal. of adenosine, 2,5- and 2,6-deoxyfructosazines (DFs), mannosamine and glucosamine in tobacco. The anal. uses a liq. chromatog.-tandem mass spectrometry (LC/MS/MS) technique. Sample prepn. for anal. consists of the extn. of the tobacco with a soln. of 90% water and 10% methanol, followed by filtration. The sepn. of the analytes was done on a hydrophilic interaction liq. chromatog. HILIC column using an isocratic procedure with a solvent consisting of 78% CH₃CN, 22% H₂O, that also contained 0.1% HCOOH and 0.143 g/L CH₃COONH₄. The measurements were done using electrospray pos. ionization mass spectrometric detection. The anal. procedure was validated and was proven very reliable. A no. of tobaccos were analyzed, including several flue-cured and Burley

USA tobaccos, off-shore tobaccos, two Oriental tobaccos, two green tobaccos, as well as tobaccos from com. and Kentucky ref. cigarettes. The ranges for the analytes per g tobacco were found between 0.4 and 20.3 µg/g for adenosine, between 0.0 and 608.5 µg/g for 2,5-DF, between 0.0 and 424.5 µg/g for 2,6-DF, between 12.5 and 415.5 µg/g for mannosamine and between 25.9 and 1885.7 µg/g for glucosamine. The study also indicated that the levels of DFs and that of the amino sugars in tobacco show a very good correlation. This correlation can be explained by the same source of the two classes of compds., namely the reaction of (reducing) sugars and ammonia

Golia, E. E.; Dimirkou, A.; Mitsios, I. K. (2009) "Heavy-metal concentration in tobacco leaves in relation to their available soil fractions". Communications in Soil Science and Plant Analysis, 40(1-6), 106-120

Tobacco (*Nicotiana tabacum*) is one of the most important agricultural products in Greece. The accumulation of heavy metals in tobacco leaves in accordance with a possible risk of transferring them to people by smoke is well known. To monitoring heavy-metal contents in soils, as well as in tobacco leaves, a 6-yr survey was conducted in the Thessaly region (central Greece). The concn. of zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), iron (Fe), and manganese (Mn) was measured both in soils and in air-cured (Burley), flue-cured (Virginia), and sun-cured (Oriental-filling) tobacco leaves in lower and upper leaves (primings). Regression anal. was conducted between soil and tobacco heavy-metal content to examine the possibility of predicting tobacco heavy-metal levels based on the results of soil anal. DTPA-extractable levels. The results showed a high correlation between Oriental tobacco heavy-metal content and DTPA-extd. heavy-metal level in soils. Although regressions based on the pooled data from the three tobacco types adequately predicted tobacco heavy-metal levels, better fits were obtained when regression models were used for Oriental tobacco sep. Statistically significant neg. correlations were recorded between heavy-metal concns. in Oriental tobacco leaves and soil pH, in all primings. Therefore, low soil pH value leads to an increased availability of heavy metals in tobacco leaves.

Lugon-Moulin, Nicolas; Martin, Florian; Krauss, Marc R.; Ramey, Patrice B.; Rossi, Luca. (2008) " Arsenic Concentration in Tobacco Leaves: A Study on Three Commercially Important Tobacco (*Nicotiana tabacum* L.)" Water, Air, & Soil Pollution, 192(1-4), 315-319

In recent years, arsenic (As) has received increased attention as humans may be exposed to it through occupational and environmental exposure. Tobacco (*Nicotiana tabacum* L.) like other crops can uptake this element from the soil, which may lead to human exposure. Here, we report on a survey on arsenic in cured or processed tobacco leaves obtained from Africa, Asia, Europe, South and North America. A total of 1,431 leaf samples of flue-cured, burley, and Oriental tobaccos were obtained from various sampling locations during 2002 to 2004. Arsenic concn. in the samples averaged $0.4 \pm 0.6 \mu\text{g g}^{-1}$ as detd. by inductively coupled plasma-mass spectrometry. Recorded values from most samples showed that concns. of arsenic were usually found at the lower end of the distribution. Significant differences were found among tobacco types, sampling locations, and crop years. Arsenic concns. were rather low in the majority of regions investigated, which is compatible with data from the literature. However, sample size was small and sampling geog. restricted. Our results would need to be validated with a larger dataset.

Liu, Baizhan; Chen, Chaoying; Wu, Da; Su, Qingde. (2008). " Enantiomeric analysis of

anatabine, nornicotine and anabasine in commercial tobacco by multi-dimensional gas chromatography and mass spectrometry". Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences, 865(1-2), 13-17

A fully automated multi-dimensional gas chromatog. (MDGC) system with a megabore precolumn and cyclodextrin-based anal. column was developed to analyze the enantiomeric compns. of anatabine, nornicotine and anabasine in com. tobacco. The enantiomer abundances of anatabine and nornicotine varied among different tobacco. S-(-)-anatabine, as a proportion of total anatabine, was 86.6% for flue-cured, 86.0% for burley and 77.5% for oriental tobacco. S-(-)-nornicotine, as a proportion of total nornicotine, was 90.8% in oriental tobacco and higher than in burley (69.4%) and flue-cured (58.7%) tobacco. S-(-)-anabasine, as a proportion of total anabasine, was relatively const. for flue-cured (60.1%), burley (65.1%) and oriental (61.7%) tobacco. A simple solvent extn. with dichloromethane followed by derivatization with trifluoroacetic anhydride gave relative std. deviations of less than 1.5% for the detn. of the S-(-)-isomers of all three alkaloids. The study also indicated that, a higher proportion of S-(-)-nornicotine is related to the more active nicotine demethylation in the leaf.

Lugon-Moulin, Nicolas; Martin, Florian; Krauss, Marc R.; Ramey, Patrice B.; Rossi, Luca. (2006). "Cadmium concentration in tobacco (*Nicotiana tabacum* L.) from different countries and its relationship with other elements" Chemosphere, 63(7), 1074-1086

Cadmium accumulation in crop plants, such as tobacco (*N. tabacum* L.), can lead to human exposure to this carcinogenic metal. To better define actual Cd distribution in cured or processed tobacco leaves from chief tobacco-producing regions, the authors analyzed 755 leaf samples of 3 major types (Flue-cured, Burley, and Oriental) obtained from 13 countries during 2001-2003. This survey may help identify regions with low- or high-Cd concns. in tobacco to obtain insight into the cause of the concn. extremes and to assist in defining strategies to reduce Cd in tobacco. Cadmium concns. in the samples ranged from 0 to 6.78 $\mu\text{g g}^{-1}$, as detd. by ICP-MS. Significant differences were found among types and among countries, but significant interactions between type and country were found. Variations in Cd concns. were also found in all countries. The results suggest an important contribution of the field (e.g., bioavailable Cd in soil, other soil characteristics) to the Cd concn. in tobacco. Finally, the correlation between the concn. of Cd and that of other elements differed among the types, which could be effectively discriminated based on the concns. of 20 elements.

Wisniewska-Kielian, Barbara; Kielian, Artur. (2003). "Comparison of trace element contents in leaves of different tobacco types". Chemia i Inżynieria Ekologiczna, 10(9), 1031-1038

Microelement and heavy metal contents were compared in leaves of Polish flue-cured tobacco (Virginia and Burley cvs.) and air-cured tobacco (Mocny Skroniowski and Kentucky cvs.). These two types of tobacco differ in their soil requirements and nutritional needs. Both types require intensive phosphorus and potassium treatment, so far applied as single component fertilizers, but recently recommended as multi-component ones. These fertilizers bring to the soil as ballast easily available forms of metallic trace elements, which may be absorbed in excessive quantity and accumulate in tobacco leaves. A considerable diversification in tobacco contents of trace elements (Zn, Cu, Cd, Pb, Ni and Cr) was obsd. depending on the usable type, cultivars variety, place of cultivation, date of harvest and quality of tobacco raw material.

Armstrong, Daniel W.; Wang, Xiande; Lee, Jauh-Tzuoh; Liu, Yan-Song. (1999),. "Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco". Chirality (1999), 11(1), 82-84

Literature reports on the optical purity of the minor alkaloids in tobacco leaf and its products often contradict one another. The enantiomeric compns. of nornicotine, anatabine, and anabasine were measured using gas chromatog./mass spectrometry (GC/MS) (with a chiral stationary phase) in three types of tobacco leaf (Burley, Turkish, and Virginia); three types of smokeless tobacco (loose-leaf, dry snuff, and moist snuff); and four types of cigarettes. Regardless of the tobacco type or product, anabasine always had the highest relative percentage of the minor (R)-(+)-enantiomeric component (between 40 and 46% vs. 54-60% of the (S)-(-)-enantiomer). Of the four common tobacco alkaloids, nicotine seems to have the highest enantiomeric excess (e.e.) while anabasine has the lowest (in the tobacco leaf and tobacco products analyzed). Nornicotine and anatabine have intermediate e.e. values

Cui, Mingwu; Burton, Harold R.; Bush, Lowell P.; Sutton, Tommy G.; Crafts-Brandner, Steve J. (1994) " Effect of Maleic Hydrazide Application on Accumulation of Tobacco-Specific Nitrosamines in Air-Cured Burley Tobacco" Journal of Agricultural and Food Chemistry, 42(12), 2912-16.

A two year study was initiated to det. the effect of maleic hydrazide application rates (0.5×, 1.0×, 1.5×, and 2.0× the recommended rate) and methods (single, split, and reduced vol. application) on accumulation of tobacco-specific nitrosamines (TSNA) and the correlation between TSNA and their precursors in burley tobacco. With increased MH application rates, alkaloids and nitrate levels decreased, whereas nitrite level was not affected. TSNAs were significantly lower (30-50%) in the lamina from the top stalk position than the hand-suckered control. N'-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT) were lowered by 15-51%, 36-65%, 38-61%, and 30-58%, resp., at the top stalk position. TSNA content was significantly correlated with the content of alkaloid in the lamina. These results suggest that MH application may alter precursors-TSNA relationship which results in decreases of TSNA in air-cured burley tobacco.

Chamberlain, W. J.; Chortyk, O. T. (1992)." Effects of curing and fertilization on nitrosamine formation in Bright and Burley tobacco". Beitraege zur Tabakforschung International. 15(2), 87-92.

A Bright and a Burley tobacco were grown at four fertilization rates and each tobacco was then both flue-cured and air-cured. Levels of alkaloids and nitrosamines increased with increasing fertilization levels. Levels of alkaloids, N-nitrosonornicotine (NNN), and other tobacco-specific nitrosamines (TSNA) were consistently higher in the Burley tobacco than in the Bright tobacco, regardless of curing method. In comparing the effects of curing, NNN and total TSNA levels were higher in the midrib than in the lamina of the air-cured samples, while just the opposite was found for the flue-cured samples. Flue-curing Bright tobacco produced three times the level of TSNA vs air-curing the same tobacco. On the other hand, flue-curing Burley tobacco reduced the alkaloids, but greatly increased the TSNA in the lamina. As midribs from the air-cured Burley leaves had three times the TSNA concn. of the lamina, the use of air-cured midribs in tobacco products should be avoided. Thus, lower fertilization levels and

careful manipulations of curing parameters could lower nitrosamine levels in cured tobacco.

Burton, Harold R.; Bush, Lowell P.; Djordjevic, Mirjana V. (1989) " Influence of temperature and humidity on the accumulation of tobacco-specific nitrosamines in stored burley tobacco". Journal of Agricultural and Food Chemistry, 37(5), 1372-7

Burley tobacco was stored in a const.-temp. humidity chamber at either 32°/83% relative humidity (RH) or 32°/90% RH. Sampling of the tobacco over a 3-wk period and analyses of the lamina and midvein showed that at 90% RH there was a significant increase of nitrite, N'-nitrosonornicotine, N'-nitrosoanatabine, and 4-[N'-(nitrosomethyl)amino]-1-(3-pyridyl)-1-butanone within 7 days, which was followed by decreased amts. of these constituents. Alkaloid and nitrate concns. were markedly decreased during the 21-day storage at 32°/90% RH. Treatment of the tobaccos with streptomycin and rifampicin did not inhibit nitrite or nitrosamine formation. During this 21-day period, approx. half of the original wt. was lost due to microbial activity and leaching. Storage at 32°/83% RH resulted in a redn. of alkaloids, but no increase of nitrite or tobacco-specific nitrosamines and no change in nitrate concn. over a 28-day period.

Fischer, Sophia; Spiegelhalder, Bertold; Preussmann, Rudolf. (1989) " Preformed tobacco-specific nitrosamines in tobacco-role of nitrate and influence of tobacco type". Carcinogenesis, 10(8), 1511-17

Fifty-five types of com. cigarettes on the open market in the Federal Republic of Germany and several samples of pure tobacco types were analyzed for preformed tobacco-specific nitrosamines (TSNA) and nitrate in the tobacco. For the cigarette tobaccos the obsd. range for N'-nitrosonornicotine (NNN) was 50-5316 ng/cigarette and for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) from not detected (<50 ng/cigarette) to 1120 (ng/cigarette). Nitrate levels ranged from 0.6 to 14.4 mg/cigarette. The highest TSNA values were obtained for cigarettes made of dark tobaccos and the lowest for Oriental type cigarettes. There is a correlation between TSNA and nitrate levels. The tobacco type is another important influencing factor, esp. for NNK. In Virginia tobaccos unexpectedly high NNK values were obsd. For the samples of the pure tobacco types, NNN levels ranged from 20 to 8850 ppb and NNK levels from not detected (<50 ppb) to 1400 ppb. The obsd. range for nitrate was from traces (<0.005%) to 4.1%. Pure Oriental tobaccos which are low in nitrate showed the lowest TSNA concns. In the high nitrate Burley tobaccos the highest TSNA concns. could be detd. Virginia tobaccos which show a low nitrate content and low in NNN but rather high NNK concns. were found. The results from these pure tobacco types demonstrate that the nitrate content of the tobacco has a great influence on the TSNA level. However, Virginia tobaccos show higher NNK concns. than expected according to their low nitrate content.

Burton, Harold R.; Childs, George H., Jr.; Andersen, Roger A.; Fleming, Pierce D. (1989). " Changes in chemical composition of burley tobacco during senescence and curing. 3. Tobacco-specific nitrosamines". Journal of Agricultural and Food Chemistry, 37(2), 426-30

Burley tobacco was harvested at 3 stages of maturity and cured in 2 curing environments [24°/70% relative humidity (RH) and 32°/83% RH]. Tobacco samples taken throughout the curing process were analyzed for N'-nitrosonornicotine, N'-

nitrosoanatabine, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, nitrate N, and nitrite N. There was a pos. correlation between nitrite N and nitrosamines when cured in a normal environment (24°/70% RH). Curing tobacco at a higher temp. and humidity (32°/83% RH) dramatically increased the accumulation of individual nitrosamines and nitrite. Again, a significant, pos. correlation was obsd. between tobacco-specific nitrosamines and nitrite.

Andersen, Roger A.; Fleming, Pierce D.; Burton, Harold R.; Hamilton-Kemp, Thomas R.; Sutton, Tommy G. (1989). " N'-Acyl and N'-nitroso pyridine alkaloids in alkaloid lines of burley tobacco during growth and air-curing". Journal of Agricultural and Food Chemistry, 37(1), 44-50

N'-Acyl and N'-nitroso pyridine alkaloids were quantified by gas chromatog. (GC) in burley tobacco genotypes varying in accumulation of pyridine alkaloids. Leaves were sampled during field growth and air-curing. N'-substituted alkaloid identities were confirmed by GC and GC-mass spectroscopy; N'-acetylanatabine was newly identified. Alkaloid derivs. in lamina were in the following order of decreasing content averaged over the sampling dates: acylated compds. formylhornicotine, n-octanoylhornicotine, n-hexanoylhornicotine, formylanatabine, acetylhornicotine, n-butanoylhornicotine, and acetylanatabine; nitrosamines nitrosoanatabine, nitrosonornicotine, and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. Generally higher levels of N'-substituted pyridine alkaloids were found in lamina of the KY 78379 line (high nornicotine and total alkaloids) and B21 cultivar (high nicotine and total alkaloids) compared to LAB21 (low total alkaloids). N'-Acyl alkaloids occurred in green as well as cured leaves; N'-nitroso alkaloids were mainly present during curing. Thus, acylated alkaloids, unlike nitroso alkaloids, are apparent products of plant metab. during late growth stages.

Nielsen, M. T.; Collins, G. B. (1985). " Genotypic and environmental influences on smoke components and leaf chemical constituents in burley tobacco" Tobacco International, 187(24), 37-41.

Genotypic and environmental influences on smoke components and leaf chem. constituents were evaluated as part of an effort to alter genetically the smoke condensate concn. in burley tobacco (*Nicotiana tabacum*). To identify a rapid, accurate screening method several leaf sampling techniques were evaluated and the particulate matter index (PMI) procedure was compared to cigarette smoke analyses. Four burley tobacco cultivars grown for 2 yr at 2 Kentucky locations provided the leaf material. Cigarettes were manufd. from cured leaves and subjected to smoke analyses. The PMI procedure was used to obtain ests. of smoke condensate levels in cured leaf samples and 2 different samples (noncured) taken at harvest. Smoke condensate nicotine concn. and the total alkaloid, total N, and nitrate levels in the cured and noncured samples were measured. Tobacco grown during the dry, hot 1983 season had greatly elevated smoke condensate, nicotine, and total alkaloid levels. Other cigarette parameters such as burntime and filling value were also greatest for tobacco grown in 1983. Genotypic differences were mostly nonsignificant for all measured smoke and leaf components. The lack of significant genotype-environment interactions may reflect negligible genetic variability. Comparisons of the PMI detns. with the cigarette smoke analyses suggested that cured-leaf samples must be used for PMI estn. Total alkaloid concns. in cured leaves were highly correlated with PMI ests. Smoke evaluation of advanced breeding lines of burley tobacco should be based on results of cigarette analyses.

Andersen, Roger A.; Kemp, Thomas R. (1985). " Accumulation of 4-(N-methyl-n-

nitrosamino)-1-(3-pyridyl)-1-butanone in alkaloid genotypes of burley tobacco during postharvest processing: comparisons with N'-nitrosonornicotine and probable nitrosamine precursors" Cancer Research, 45(11, Pt. 1), 5287-93.

The concn. relationships of 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)(I) [64091-91-4], N'-nitrosonornicotine (NNN) [16543-55-8], and their probable precursors, i.e., nitrite, nitrate, and alkaloids, were detd.: (a) after the growth of Ky 14 burley tobacco under different shade conditions followed by air curing; and (b) during prepn. of air-cured and homogenized-leaf-cured (HLC) burley tobaccos from conventionally grown tobaccos of different alkaloid genotypes. NNK contents ranged from 0.2 to 0.5 µg/g in air-cured Ky 14 tobacco lamina from leaves grown under 0-65% shade (100, 65, and 35% of natural daylight). The highest NNK concns. were from 45% shade-grown lamina from lower leaf positions on stalks. Concns. of NNK did not correlate significantly with those of either nitrate or total alkaloids calcd. over all shade treatments and stalk positions. During HLC tobacco processing, the following significant correlations of NNK with precursor content changes were found for each of 4 burley alkaloid genotypes calcd. over the 4 successive stages of processing: NNN ($r = -0.9$). NNK also correlated neg. with nicotine [54-11-5] concn. changes ($r = -0.9$) in the low-alkaloid and high-alkaloid isoline [30000-36-3] during processing. After a 20-h incubation period under aerobic conditions followed by a 1-h standing period without aeration, substantial increases of NNK were obsd. in each burley line. The increased NNK contents ranged from 9-fold for the low-alkaloid isoline to 99-fold for the nornicotine [494-97-3]-converter line. Increases in NNK content (27-69%) also occurred during the air drying stage; further increases occurred during a 15-mo storage period at ambient conditions. After the HLC process and prolonged storage, maximal NNK contents were obsd. in each tobacco genotype in the following order of increasing NNK content: Ky 14 cultivar, 79 µg/g; low-alkaloid line, 80 µg/g; nornicotine converter line, 102 µg/g; and high-alkaloid line, 177 µg/g. At the beginning of a controlled environmental storage period used for high-alkaloid and low-alkaloid isoline air-cured and HLC tobaccos, NNK contents correlated with nitrite ($r = 1.0$) and nitrate ($r = -0.9$) calcd. over the 2 curing regimens. Increased NNK contents were obsd. in HLC high-alkaloid line tobacco but not in the air-cured high-alkaloid line after 52 wk of storage at 20° and 12% moisture. NNK accumulation varied among tobacco alkaloid genotypes and occurred mainly during the following HLC processing step: standing period following incubation; forced-air drying and prolonged storage (aging) for HLC tobaccos. It seems probable that low levels of NNK and NNN in burley tobacco could be achieved by growth of a low-alkaloid genotype and use of postharvest treatment procedures that minimize nitrite formation and nitrate and alkaloid conversion (disappearance).

Nagaraj, G.; Chakraborty, M. K.; Someswararao, M.; Venkatachari, M. V. V. S. S. (1982). "The effect of methods of harvest and curing on yield and physicochemical characteristics of burley tobacco" Tobacco Research, 8(2), 160-73

The effect of partial priming and complete priming methods of harvest followed by sun- and air-curing on the burley tobacco yield and quality was examd. Complete priming with air-curing gave significantly higher yield than complete priming with sun-curing and produced cured leaves having improved physicochem. characteristics; in lower primings it produced leaves with high burn rate and in upper primings leaves had higher pore vol. and less nitrate-N. Methods of harvest and curing did not affect nicotine content of burley tobacco, but lower primings had lower nicotine content than upper primings. Lower priming tobaccos, in general, had higher filling value and leaf burn; however, upper primings were more flavorful. Sun-curing of complete primings decreased yield and affected several quality characters. Partially priming with stalk-cut

decreased yield drastically without affecting quality of burley tobacco, when cured. Curing method affected chlorogenic acid and total volatile bases, and the interaction of curing and harvesting affected K percentage.

Matsushima, Sanji; Ohsumi, Tomoko; Sugawara, Shiro (1983). "Composition of trace alkaloids in tobacco leaf lamina" Agricultural and Biological Chemistry, 47(3), 507-10.

Changes were investigated in trace alkaloid composition. of MC-1 (bright) and Burley-21 leaf lamina during curing. Seventeen alkaloids were identified. The total level of trace alkaloids decreased by 60% in both MC-1 (flue-cured) and Burley-21 (air-cured). Although the level of most alkaloids also decreased, 2,3'-bipyridyl and 4 nor nicotine derivs., i.e. N'-hexanoyl-, N'-octanoyl-, 1'-(6-hydroxyoctanoyl)-, and 1'-(7-hydroxyoctanoyl)nornicotine, increased during curing in both varieties. Differences in the trace alkaloid compn. of cured leaf lamina among MC-1, Burley-21 and Basma (Orient) were also studied. In all varieties, N'-formyl nornicotine, nicotyrine, anabasine, and cotinine were present at high concns. relative to the other trace alkaloids. The alkaloids which were present in a relatively high concn. in particular varieties, were N'-formylanabasine in MC-1, N'-hexanoyl-N'-octanoylnornicotine in Burley-21, and cotinine and N'-formylanatabine in Basma.

Campbell, J. S.; Chaplin, J. F.; Boyette, D. M.; Campbell, C. R.; Crawford, C. B. (1982) "Effect of plant spacings, topping heights, nitrogen rates, and varieties of tobacco on nicotine yield and concentration". Tobacco International. 184(11), 72-5

Expts. were conducted in 1974-1976, with tests modified each year after appraisal of results from the previous year, with Ky 16 (burley), Ky 160 and Ky 170 (dark fired), and SC 58 and Speight G-23 (flue-cured) tobacco. The N rates ranged 112-224 kg/ha, topping heights 12-18 leaves and within-row spacings 30.5-61.0 cm. Ky 160, Ky 170, and SC 58 produced higher nicotine [54-11-5] levels in leaves and yield of nicotine per ha than other varieties tested. It would appear these varieties have similar genetic potentials for nicotine prodn. N rates >112 kg/ha did not increase nicotine concn. of leaves or nicotine yield per ha. Plant spacings of 45.7 and 61.0 cm in the row resulted in higher nicotine levels than spacing plants 30.5 cm apart. Lower plant toppings, 12 and 15 leaves, resulted in a higher nicotine percentage, but topping the plants to 15 and 18 leaves resulted in higher nicotine prodn. per ha. The increase in nicotine per ha was due to higher yields of dry matter in the higher topped plants. Generally, the varietal differences more affected leaf concn. and prodn. of nicotine than any of the other variables studied. However, N rates, topping heights, and within-row spacings were important.

Hamilton, J. L.; Lowe, R. H. (1978) "Changes in the concentration of proteins, amino acids and ammonia in Burley tobacco during air curing". Tobacco Science, 22(85-93), 89-93.

About one-half of the initial leaf protein was degraded during curing. This occurred during the early stages of curing and was completed by the time when the largest part of the leaf was yellow. Apparently most of the protein degraded was Fraction 1 protein. Total free amino acids of leaves increased during the early stages of curing when protein was rapidly decompd., and decreased when protein hydrolysis stopped. The increase in total free amino acids did not account for all the amino acids hydrolyzed from protein. Thus, during the time of rapid increases in free amino acids there were also losses. About 70% of the total free amino acids in cured burley leaves

were the amino acid amides. The equiv. of all the amino acids hydrolyzed from protein were degraded during curing, if the amino acid amides are not included. More proline accumulated during the early stages of curing than was released by protein hydrolysis. Thus, some of the chlorophyll was degraded to proline. Ammonia concn. began to increase rapidly after the 3rd day and continued to increase until most of the leaf became brown. It did not accumulate the 1st 2 days of curing, although amino acids were being deaminated. The ammonia released was probably used to synthesize amides.

Andersen, Roger A.; Litton, Columbus C. (1975) "Variation of carbohydrate, phenolic, and alkaloid contents in different parts of air-cured tobacco" Tobacco Science, 19, 69-70.

stalk vs. representative whole leaf, (2) lamina vs. midveins vs. whole leaf, and (3) lamina, midvein, or whole leaf as a function of grade. Fiber was the most abundant of the leaf substances assayed, followed in decreasing order by total alkaloids, lignin, total sol. phenols, sucrose, and glucose. Abundance of compds. in stalk was in decreasing order fiber, lignin, glucose, fructose, total alkaloids, sucrose, and rhamnose. The results are discussed in terms of the effects of the use of different proportions of tobacco plant parts in the prodn. of Chem. compn. of conventionally cultured and air-cured Burley 21 cultivar tobacco differed in decreasing order in: reconstituted tobacco products.

Wassef, Momtaz K.; Hendrix, James W. (1974) "Lipid content of cured tobacco" Journal of Botany, 52(5), 1123-6.

Samples from cured burley and flue-cured tobacco were examd. for total lipid content and for lipid compn. of fractionated lipid classes. Cured burley tobacco contained 6-8% lipids compared with 14-15% in cured flue-cured tobacco. Neutral lipids constituted 70-80% of burley lipids but only half the flue-cured lipids; the latter had 75% more neutral lipids than the former. Flue-cured tobacco contained considerably more (5-10 times) medium- to high-polarity lipids than burley. Stalk position had only a minor effect on the lipid content of both tobacco types.

Sheen, S. J.; Grunwald, C.; Sims, J. L. (1973) "Effects of nitrogen fertilization and stalk position on polyphenols and carbohydrates in the air-cured leaf of three tobacco genotypes". Journal of Plant Science, 53(4), 897-905

Air-cured leaves from 3 stalk positions of the tobacco (*Nicotiana tabacum*) cv Burley 21 and 2 breeding lines LA Burley 21 and LP Burley 21 cultured with 112, 224, and 336 kg N/ha were analyzed for the concn. of polyphenols, carbohydrates, and related compds. LA Burley 21 was a low-alkaloid and LP Burley 21 a low-polyphenol line. Concns. of polyphenols and brown pigments were distributed in a decreasing gradient from upper to lower stalk positions. Distribution of cellulose content showed a reverse pattern. Lignin was highest in the upper leaves. In comparison with Burley 21, LA Burley 21 contained more polyphenols and carbohydrates, whereas LP Burley 21 was high in total sol. sugars. Polyphenol accumulation was greater with low than high N fertilizer treatments, and, generally, an inverse relation existed between N rate and concns. of the carbohydrates measured. Starch was not detected in the leaf samples. Results point to the importance of N fertilization, genotype, and selection of leaf grades for altering the polyphenol and carbohydrate fractions of tobacco leaves, many of which are suspected precursors of tumorigenic agents in cigarette smoke.

Henson, W. H., Jr.; Walton, L. R.; Bunn, J. M. (1972) "Relation of leaf moisture content and certain chemical changes during curing of burley tobacco". Transactions of the ASAE, 15(2), 342-5

Drying of freshly harvested leaf at low temp. and low humidity caused the greatest damage to leaf quality. Low temps. and low humidities during the cure caused overdrying of the leaf as manifested by high levels of protein N, total sugars, and Ca. The max. recommended drying rates for unwilted leaves at various temps. for the 1st 4 days are given, corresponding to the following conditions: (1) at 50°F, dry leaves at 82% relative humidity, removing 0.27 of the original moisture; (2) at 65°F, dry leaves at 55% relative humidity, removing 0.48 of the original moisture; and (3) at 80°F, dry leaves at 38% relative humidity, removing 0.74 of the original moisture.

Nadkarni, R. A.; Ehmann, William D. (1969) "Determination of trace elements in tobacco products by neutron activation analysis". Proc. Tob. Health Workshop, 47-57

Neutron activation analysis was used to det. trace elements in burley tobacco leaves and stalks, 19 brands of com. cigarette tobacco, 2 brands of pipe and cigar tobacco, and various cigarette papers. La, Sb, and Zn were lower in stalks than in leaves. The reverse was found for Br, Co, and Se. Other elements detd. were As, Cr, Ag, Zr, Sc, Fe, Au, and Hg. Tobacco leaves and cigarette fillers contained about the same concns. of most elements except for higher amts. of Sb and Fe in the fillers. The higher value of Fe is attributed to the contact with iron and steel machinery in cigarette manuf. A few of the tobaccos were ashed at 90° for 1 h- and the ashes analyzed for some of the trace elements. As expected, most of the Br was lost on ignition. Also lost were 75% of the La, 50% of the Se, 90% of the Zn, 96% of the Co, and 75% of the Sb.

Schmeltz, Irwin; Stedman, R. L.; Chamberlain, W. J.; Burdick, D. (1964). "Composition studies on tobacco. XX. Bases of cigarette smoke" Journal of the Science of Food and Agriculture, 15(11), 774-81

cf. CA 61, 12340h. The bases of cigaret smoke from tobaccos of different types and of different organoleptic properties were investigated. The volatile bases found were pyridine, α -picoline, 2,6-lutidine, β - and (or) γ -picoline, 2,4-and (or) 2,5-lutidine, 2,3-lutidine and (or) 2,4,6-collidine, 3-ethylpyridine, and 3-vinylpyridine. Alkaloids found were nicotine, nor nicotine, myosmine, anabasine, and 2,3'-bipyridyl. Flue-cured, Burley, Turkish, and Maryland tobaccos were examd., as well as blends. The presence of Turkish tobaccos in a cigarette blend appeared to enhance the evolution of some of the more volatile bases compared to the individual tobaccos. Nicotine was the major component of the alkaloidal fraction. There appeared to be some relation between smoke flavor and the amts. of volatile bases and nicotine. However, in the case of the volatile bases, the differences among samples were relatively small. The values were compared with those for cigar smoke, and both cigarette and cigar smokes were found to be similar on the basis of y/g. tobacco smoked.

Schmeltz, I., S. Abidi, et al. (1977). "Tumorigenic agents in unburned processed tobacco: N-nitrosodiethanolamine and 1,1-dimethylhydrazine." Cancer Lett. (Amsterdam) 2(3): 125-131.

The tumorigenic agents N-nitrosodiethanolamine [1116-54-7], a weak hepatic carcinogen in rats, and MeNNH₂ [57-14-7] were isolated from unburned processed tobacco. N-nitrosodiethanolamine varied in amts. from 0.1 ppb in flue-cured tobacco not treated with the herbicide MH-30 (maleic hydrazide) [123-33-1] to 173 ppb in Burley tobacco to which MH-30 had been applied prior to harvesting. MH-30 is commonly formulated as the diethanolamine salt. Me₂NNH₂ ranged 60-147 ppb, except in the case of Burley tobacco, where none was detected. The source of the nitrosamine in the tobacco appears to be the MH-30. [on SciFinder(R)]

Variation dynamics of total nitrogen and nicotine of burley tobacco at various growing stages under different nitrogen nutrition levels. By Chai, Jiarong. From Agricultural Science & Technology (2012), 13(11), 2327-2331. Language: English, Database: CAPLUS

[Objective] This study aimed to investigate the variation dynamics of total nitrogen and nicotine of burley tobacco at various growing stages under different nitrogen nutrition levels to provide ref. for optimizing fertilization technologies for burley tobacco. [Method] Fresh burley tobacco leaves were collected at various growing stages and dried to measure the content of total nitrogen, protein and nicotine by using INTEGRAL automated chem. analyzer and analyze the correlation with nitrogen application level. [Result] Regardless of the nitrogen application level, the content of total nitrogen and protein showed a downward trend since root extending stage and reached the min. at mature stage; the content of nicotine showed an upward trend since early vigorous growing stage and increased to the max. at mature stage; the content of total nitrogen, protein and nicotine all increased after air curing; the content of total nitrogen, protein and nicotine showed pos. correlation with nitrogen application level, while total nitrogen/nicotine was neg. correlated. The results indicate that nitrogen level is closely related to the total nitrogen and nicotine of burley tobacco at growing period and post-air curing, rational application of nitrogen is an important measure to regulate the content of total nitrogen and nicotine of tobacco. [Conclusion] This study provides scientific basis for rational fertilization of burley tobacco.

Assessment of tobacco specific nitrosamines in tobacco genotypes. By Guo, Peiguo; Xia, Yanshi; Li, Ronghua; Lu, Yonghua; Qiu, Miaowen; Zhao, Weichai; Yu, Yiwen. From Advanced Materials Research (Durnten-Zurich, Switzerland) (2013), 641-642, 871-874, 5 pp.. Language: English, Database: CAPLUS, DOI:10.4028/www.scientific.net/AMR.641-642.871

The tobacco-specific nitrosamines (TSNAs) are one of the most important groups of carcinogens in tobacco leaf, knowing the difference of TSNAs contents in tobacco genotypes should be beneficial for breeding low TSNAs cultivars which could decrease the harmfulness to human health in tobacco products. For this purpose, 8 genotypes

(include burley, sun-cured and flue-cured tobacco) were selected as materials, the TSNAs contents of uncured and cured leaves were assessed with LC-MS/MS. The results showed that TSNAs contents were significantly increased after curing in all genotypes. The mean TSNAs contents of leaves before and after curing in burley were the highest among three types of tobacco and the contents in sun-cured tobacco were the lowest. Coeff. of variation (CV) of TSNAs among genotypes in burley was higher than that in flue-cured and sun-cured tobacco and the CV in sun-cured tobacco was the lowest. The pos. correlation was obsd. in NNN, NAT and total TSNAs contents between uncured and cured leaves in three types of tobacco. Therefore, the TSNAs contents in uncured leaves could be considered as evaluation index to screen tobacco cultivars with the low TSNAs contents.

Burley Tobacco: Pyrolysis

Torikai, K., K. Torikai, et al. (2005). "Study on tobacco components involved in the pyrolytic generation of selected smoke constituents." Food Chem Toxicol **43**(4): 559-568.

The aim of this study was to investigate the contribution of various tobacco components to the generation of smoke constituents using a tobacco pyrolysis model. We analyzed the amounts of primary tobacco components (sugars, protein, polyphenols, alkaloids, organic acids, inorganics etc.) in flue-cured and burley tobacco leaves. Each of the components was added to the tobacco leaves at the 0.5-fold and 1.0-fold amount naturally present in the leaves. The treated tobacco samples were pyrolyzed at 800 degrees C in a nitrogen atmosphere with an infrared image furnace, and the selected smoke constituents (benzo[a]pyrene, hydrogen cyanide, carbonyl compounds, aromatic amines, volatile organic compounds and phenolics) were quantitatively analyzed by several methods, including high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC-MS). The contribution of each tobacco component to the generation of selected smoke constituents was estimated from a regression line determined by the three yields (no addition, 0.5-fold addition, and 1.0-fold addition). The results of this study can provide useful and comprehensive information on the relationship between tobacco components and selected smoke constituents during pyrolysis.

Bell, J. H., A. O. Saunders, et al. (1966). "The contribution of tobacco constituents to phenol yield of cigarettes." Tob. Sci. **10**: 138-142.

A study of pyrolysis conditions made possible a selection of exptl. variations which, when tested with flue-cured and burley tobacco, produced a PhOH yield similar to that obtained when these tobaccos are smoked. The results indicate that the carbohydrates of tobacco are important precursors of PhOH in cigarette smoke, but they alone do not account for the total PhOH yield, probably only ~25%. Pyrolysis of tobacco exts. indicates that the more polar components of the leaf contribute greatly to the total PhOH yield. Cigarettes impregnated with glucose-14C were smoked, and the PhOH which was obtained established that the carbohydrate conversion is greater than that estd. from the pyrolysis of the pure compds, being perhaps as high as 41%. [on SciFinder(R)]

Oja, Vahur; Hajaligol, Mohammad R.; Waymack, Bruce E. (2006) "The vaporization of semi-volatile compounds during tobacco pyrolysis" Journal of Analytical and Applied Pyrolysis, 76(1-2), 117-123

Pyrolysis of tobacco, a complex biomass matrix, was investigated to further understand thermal decompn. processes that are accompanied by evapn. of relatively stable non-polymeric endogenous compds. Pyrolysis of two types of tobacco, bright and burley were studied using thermo-gravimetry mass spectrometry (TG-MS) and field ionization mass spectrometry (FIMS) analyses. Tobacco contains biopolymers and many non-polymeric compds. Unlike many biomass pyrolysis tars derived from wood or cellulose, tobacco pyrolysis tars can contain significant amts. of high mol. wt. endogenous constituents such as waxes and terpenes that are transferred intact. The phenomenon of evapn. of high mol. wt. non-polymeric compds. is illustrated by tobacco micro-sample pyrolysis in FIMS under vacuum (at a pressure of 10⁻⁴ Torr). These expts. indicate that the evapn. of relatively stable high mol. wt. species occurs below about 220°C generating 300 Da and higher mol. wt. products; and, decompn. of tobacco biopolymers such as starch, cellulose, hemicellulose, lignin, and pectin occurs mostly at temps. higher than 220°C producing species mostly with mol. wt. below 300 Da. Some of the high mol. wt. compds., such as stigmaterol (412 Da), α -tocopherol (430 Da), and solanesol (630 Da), were tentatively identified using the FIMS spectra.

Shin, Eun-Jae; Hajaligol, Mohammad R.; Rasouli, Firooz. (2003). "Characterizing biomatrix materials using pyrolysis molecular beam mass spectrometer and pattern recognition". Journal of Analytical and Applied Pyrolysis, 68-69, 213-229.

Fingerprinting techniques have been applied to pyrolysis mol. beam mass spectra of a complex biomatrix material such as tobacco. Pyrolysis products of three major tobaccos (burley, bright and oriental) and their blends were scanned from m/z 25 to 500 by a mol. beam mass spectrometer (MBMS) in high vacuum and soft ionization (25 eV). The resultant complex mass spectra were further analyzed using multivariate data anal. (pattern recognition) to discover the underlying chem. differences that may not be obvious by comparison of such complex mass spectra. Using the MBMS and pattern recognition techniques, we were able to statistically distinguish among different major tobaccos and their blends and show their characteristic components by only looking at their primary pyrolysis products. Burley was signified by its nitrogen contg. products, oriental by its abundant carbohydrate-derived products and bright by its intermediate behavior. Blended tobacco samples of burley and oriental with a ratio of 66:34 resulted in similar product compn. to that of bright. Classes of pyrolysis products of a tobacco sample at different reaction conditions were also extd. by using the same anal. These results indicate that this approach could be used to rapidly predict biomass pyrolysis product distribution.

Schlotzhauer, W. S.; Chortyk, O. T.; Severson, R. F. (1979). "Rapid pyrolytic method for evaluating effect of tobacco variety, growing, harvesting, and post-harvest treatment on smoke composition". Tobacco International, 181(19), 63-6.

A pyrolytic method (Higman, E. B et al., 1977; Smith, W. T. et al., 1975) was improved to more closely stimulate the prodn. of combined smoke condensate of cigarettes. The silylated pyrolyzate was analyzed by gas chromatog. using a flame-ionization detector and a column contg. 6% OV-17 on Chromosorb W, temp. programmed for 50-280° (2°/min). Tobacco blends obtained from representative com. cigarettes differed

widely in levels of selected pyrolyzate constituents, including dihydroxybenzenes and lipids. Pyrolytic profiling showed higher levels of the latter components in Bright tobaccos than in Maryland or Burley tobaccos. Close-grown, modular-cured, Speight G-28 tobacco gave a pyrolyzate contg. relatively higher levels of phenolics and lower amts. of nicotine, sterols, and paraffins than pyrolyzates of conventionally grown and cured G-28. Pale-yellow tobacco yielded higher levels of phenolics, nicotine, and fatty acids when low-profile grown and bulk-barn cured than when conventionally grown and cured. Homogenized leaf curing processes resulted in redn. of paraffins in pyrolyzates of close-grown tobacco and redns. in a majority of components detd. in pyrolyzates of low-profile grown tobaccos.

Adam, T.; Ferge, T.; Mitschke, S.; Streibel, T.; Baker, R. R.; Zimmermann, R. (2005) "Discrimination of three tobacco types (Burley, Virginia and Oriental) by pyrolysis single-photon ionization-time-of-flight mass spectrometry and advanced statistical methods". *Analytical and Bioanalytical Chemistry*, 381(2), 487-499

Pyrolysis single-photon ionization (SPI)-time-of-flight mass spectrometry (TOFMS) and statistical anal. techniques have been applied to differentiate three major tobacco types, Burley, Virginia and Oriental, by means of the gas phase. SPI is known as a soft ionization technique that allows fast and comprehensive online monitoring of a large variety of aliph. and arom. substances without fragmentation of the mol. ions. The tobacco samples were pyrolyzed at 800°C in a nitrogen atm. The resulting pyrolysis gas contained signals from more than 70 masses between m/z 5 and 170. Mass spectra obtained were analyzed by principal component anal. (PCA) and linear discriminant anal. (LDA) to distinguish between different tobacco types. Prior variable redn. of the data set was carried out by calcn. of the Fisher ratios. Results achieved give information about chem. compn. and characteristics of the smoke derived from each tobacco type and enable conclusions on plant cultivation to be drawn. Based on LDA, a model for tobacco type recognition of unknown samples was established, which was cross-checked by addnl. measurements of each tobacco type. Furthermore, first results on the recognition of tobacco mixts. based on principal component regression (PCR) are presented.

Time-resolved analysis of the emission of sidestream smoke (SSS) from cigarettes during smoking by photo ionization/time-of-flight mass spectrometry (PI-TOFMS): towards a better description of environmental tobacco smoke. By Streibel, T.; Mitschke, S.; Adam, T.; Zimmermann, R. From *Analytical and Bioanalytical Chemistry* (2013), 405(22), 7071-7082. Language: English, Database: CAPLUS

This work detd. the chem. compn. of side-stream smoke (SSS) emissions from cigarettes by laser-based, single-photon ionization time-of-flight mass spectrometry. SSS is generated from various cigarette types (2R4F research cigarette, Burley, Oriental and Virginia single tobacco-type cigarettes) smoked on a single-port smoking machine and collected using a fish-tail chimney device. With this set-up, puff-resolved quantification of several SSS components was performed. Investigations of SSS emission dynamics showed the concn. profiles of various substances can be categorized into several groups, depending on the occurrence of a puff or unaffected by changes in the burning zone during puffing. SSS emissions occurring directly after a puff strongly

resembled the compn. of mainstream smoke (MSS). In the smoldering phase, clear differences between MSS and SSS were obsd. Changed chem. profiles of SSS and MSS might also be important for environmental tobacco smoke which is largely detd. by SSS. Also, the SSS chem. compn. SSS is strongly affected by the tobacco type. Thus, the higher N content of Burley tobacco led to detection of increased N-contg. compd. concns. in SSS.

Burley Tobacco: Smoke Chemistry

Adam, Thomas; Mitschke, Stefan; Streibel, Thorsten; Baker, Richard R.; Zimmermann, Ralf. (2006) "Puff-by-puff resolved characterisation of cigarette mainstream smoke by single photon ionisation (SPI)-time-of-flight mass spectrometry (TOFMS): Comparison of the 2R4F research cigarette and pure Burley, Virginia, Oriental and Maryland tobacco cigarettes". Analytica Chimica Acta (2006), 572(2), 219-229.

Soft single photon ionization (SPI)-time-of-flight mass spectrometry (TOFMS) is applied for the characterization and comparison of puff-by-puff resolved and total yields of cigarette mainstream smoke from single tobacco type cigarettes (Virginia, Oriental, Burley, and Maryland) and the 2R4F University of Kentucky research cigarette. Puff-by-puff characteristics of various smoke components within one cigarette type as well as between different cigarette types can differ tremendously. This is demonstrated by means of a few selected compds. Puff yields vary between 15 and 106 μm for acetaldehyde, 6 and 57 μm for NO, and between 1 and 8 μm for butadiene. Thereby, cigarettes contg. 100% Oriental and Burley tobacco exhibit a very unique behavior for the first and last puff. Different cultivation and processing methods as well as burning characteristics are most likely responsible for this. Since the 2R4F cigarette contains all four tobacco types it combines features of all of them. However, for some smoke constituents, smoking of the 2R4F ref. cigarette results in exceptionally high yields which might not be attributable to the four pure tobacco types, but to other factors. In addn., comparison of the different cigarettes was also carried out by normalizing the yields to puff resolved particulate matter. This procedure minimises effects caused by unequal smoke formation and represents another approach in evaluating the data.

Griest, W. H.; Guerin, M. R. (1977) "Influence of tobacco type on smoke composition". Recent Advances in Tobacco Science, 3, 121-44.

A review and discussion with 42 refs. on the comparison of Bright and Burley tobacco cigaret smoke and smoke condensate and on the influence of origin, variety, curing practice, and stalk position of tobacco on smoke compn

Tso, T. C.; Sims, J. L.; Johnson, D. E. (1975) "Agronomic factors affecting N-dimethylnitrosamine content in cigaret smoke" Beitraege zur Tabakforschung, 8(1), 34-8.

Exptl. cigarettes from tobaccos varying in genotype, N nutrition, stalk position, suckering practice, and curing methods were used to examine the levels of N-dimethylnitrosamine (DMN) in smoke. Measurable amts. of DMN were found in all

exptl. samples, ranging from 1.7 to 115 ng/g of tobacco burned. DMN content in smoke generally increased as rate of N fertilization increased. However, there were wide seasonal, cultural, and varietal effects. Burley-type tobacco produced a much higher level of DMN than the bright-type tobacco. DMN content in smoke was significantly and pos. related to leaf total N, total alkaloids, nicotine, nornicotine, total volatile bases and nitrate N, but neg. related to reducing sugars. Reconstituted sheet tobaccos made with homogenized-leaf-curing samples produced much lower amts. of DMN than conventionally cured leaf. Addnl. information is needed to elucidate the primary leaf constituents that serve as precursors of DMN.

Thornton, R. E.; Massey, S. R. (1975) " Effects of adding sugar to tobacco" Beitrag zur Tabakforschung, 8(1), 11-15

A series of cigarettes made from Burley tobacco contg. different levels of added reducing sugar (10.5 to 17.8%) were examd. Compared to the control cigarette there was virtually no change in the deliveries of aldehydes and carbonyl constituents. However, an increase in the delivery of 2-furfural was obsd. esp. when fructose was the added sugar: even so, the conversion efficiency was only 1-2%. A similar increase in the delivery of 2-furfural was also obsd. when glucose was added to flue-cured tobacco. An addnl. finding was that the addn. of glucose and fructose reduced the delivery of nicotine. Radioactivity balance expts. on flue-cured cigarettes with added glucose indicated that this was probably due to an increase in the nicotine filtration efficiency of the cigarette rod. Filtration studies using air-cured cigarettes demonstrated that, on addn. of glucose, there was a significant increase in the nicotine filtration efficiency of the tobacco rod and that less of the available nicotine was directed into the main-stream.

Benner, John F.; Burton, Harold R.; Burdick, Donald (1968) " Composition of cigaret smoke from high- and low-nitrate Burley tobacco" Tobacco Science, 16(10), 21-5.

Two lots of Burley No. 37 tobacco were grown on adjacent plots, one having low, the other high N fertilization. The nitrate levels of the 2 lots were 0.098 and 1.66%, resp. Cigarets (85 mm.) were made with each lot and 20 from each were smoked to a butt length of 23 mm., by using a 35-ml. puff of 2 sec. duration, once a min. The smoke was collected at room temp. in 50 ml. of vigorously stirred cyclohexane, in a 250-ml. flask. A Cambridge filter located behind the collection flask did not collect any significant amt. of particulate matter during the smoking of 20 cigarettes. On the basis of an equal vol. of smoke, the high nitrate cigarettes yielded considerably more nicotine than the low nitrate cigarettes. The levels of most other smoke constituents detd. (total particulate matter, nitromethane-sol. solids, and total phenols) did not differ greatly. Slightly lower levels of benzo[a]pyrene and m- and p-cresols were found in the smoke from the high-nitrate tobacco. Phenol was somewhat lower in the low-nitrate cigarettes. Com. cigarettes were also smoked for comparison but the phys. characteristics between them and Burley cigarettes were sufficiently different to make comparisons difficult. The nitrate content of the high-nitrate tobacco is equiv. to the addn. of 2.28% NaNO₃ to the low-nitrate tobacco.

Schmeltz, Irwin; Stedman, R. L.; Chamberlain, W. J.; Burdick, D. (1964) " Composition studies on tobacco. XX. Bases of cigarette smoke". Journal of the Science of Food and Agriculture 15(11), 774-81

cf. CA 61, 12340h. The bases of cigaret smoke from tobaccos of different types and of

different organoleptic properties were investigated. The volatile bases found were pyridine, α -picoline, 2,6-lutidine, β - and (or) γ -picoline, 2,4-and (or) 2,5-lutidine, 2,3-lutidine and (or) 2,4,6-collidine, 3-ethylpyridine, and 3-vinylpyridine. Alkaloids found were nicotine, nornicotine, myosmine, anabasine, and 2,3'-bipyridyl. Flue-cured, Burley, Turkish, and Maryland tobaccos were examd., as well as blends. The presence of Turkish tobaccos in a cigarette blend appeared to enhance the evolution of some of the more volatile bases compared to the individual tobaccos. Nicotine was the major component of the alkaloidal fraction. There appeared to be some relation between smoke flavor and the amts. of volatile bases and nicotine. However, in the case of the volatile bases, the differences among samples were relatively small. The values were compared with those for cigar smoke, and both cigarette and cigar smokes were found to be similar on the basis of y/g. tobacco smoked.

Tso, T. C.; Chaplin, J. F.; Adams, J. D.; Hoffmann, D. (1982) " Simple correlation and multiple regression among leaf and smoke characteristics of Burley tobaccos" Beitraege zur Tabakforschung International, 11(3), 141-50

Leaves from 8 stalk positions from 4 Burley tobaccos [cultivars Burley 21, Ky 14, and Ky 12, and 1 breeding line of low-alkaloid Burley 21 (LA Burley 21)] were tested. A total of 116 variables, including leaf and smoke characteristics, were detd. by collaborating scientists using established methods. Simple correlations and multiple regressions among selected variables were detd. to examine the relation among the variables. Generally, Burley tobaccos did not differ from bright tobaccos, in that the N fractions and combustibility were the major factors affecting nicotine (I) [54-11-5] and dry total particulate matter (TPM) delivery as well as other major smoke constituents. Stalk position and varieties also demonstrated significant differences in I and dry TPM delivery. Benzo[a]pyrene content, however, did not show significant differences among these exptl. Burley tobaccos or stalk positions.

Matsushima, Sanji; Ishiguro, Shigeo; Sugawara, Shiro (1979) 'Composition studies on some varieties of tobacco and their smoke. I. Major components in smoke condensate' Beitraege zur Tabakforschung International, 10(1), 31-8

The relative proportions of the major smoke components and N-contg. components in the semi-volatile phase and the non-volatile phase from some varieties of tobacco, i.e. flue-cured, Burley, and Turkish tobacco, were detd. In flue-cured tobacco smoke, 2-furfural, 5-methyl-furfural, 5-hydroxymethylfurfural, and other carbohydrate pyrolyzates, catechols, and org. acids were present in higher concns. than in the smoke from the other tobaccos. In Burley tobacco smoke, myosmine was present in a much higher concn. than in the smoke from the other tobaccos. In Turkish tobacco smoke, glycerol and β -methylvaleric acid were both present in much higher concns.

Yoshida, D. (1978) " Effect of cultural and curing practices of tobacco on the contents of tar in the smoke" Tobacco Research, 4(2), 1-6

Smoke of cigarettes made from flue-cured tobacco cultivars Bright Yellow and Hicks contained more tar than that from the air-cured Japanese cultivars Matsukawa and Daruma and from Burley 21. The tar contents of smoke from cigarettes made with air-cured Bright Yellow, Hicks and Shiroensyu tobacco leaves, however, did not differ from those of flue-cured leaves of the resp. cultivars. Tar content of smoke increased with advancing leaf maturity. Smoke of cigarettes made from untopped plants contained less tar than that from topped plants. The tar content of smoke of cigarettes

made from middle leaves of the plant decreased with increasing rate of N fertilization, but variable results were obtained with the upper leaves of the plant. Tar content of cigarette smoke was significantly pos. correlated with cigarette wt. and the nicotine content of the tobacco as well as with the content of alc.- benzene-, hexane-, and Me₂CO-extractable components in the leaves; tar content was neg. correlated with hot water-extractable residues in the leaves.

Burley Tobacco: In vitro studies

Bombick, D. W., K. Putnam, et al. (1998). "Comparative cytotoxicity studies of smoke condensates from different types of cigarettes and tobaccos." Toxicol In Vitro **12**(3): 241-249.

The neutral red assay, a rapid and accurate method for estimating the cytotoxicity of chemicals, has been used to assess the cytotoxicity of cigarette smoke condensate (CSC), a complex chemical mixture containing over 3000 identified compounds. The first objective was to optimize the neutral red assay for evaluation of CSCs. This study also assessed and compared the cytotoxicity of smoke condensates from three reference cigarettes which differ in 'tar' content; cigarettes of different tobacco type composition; an ultra-low tar cigarette (R1); and an RJR test cigarette which heats but does not burn tobacco. Finally, this study investigated the cytotoxicity of a specific CSC component, nicotine, and its metabolite, cotinine. Exposure times of 24 hours or longer using CHO cells provided optimal conditions for evaluation of CSC cytotoxicity. The cytotoxicity of CSCs from reference cigarettes was similar. CSC from cigarettes comprised of flue-cured tobacco exhibited greater cytotoxicity than CSC from cigarettes comprised of burley tobacco. CSC from the R1 cigarette exhibited similar cytotoxicity compared with 1R4F CSC. The CSC from a cigarette that heated but did not burn tobacco (RJR test cigarette) demonstrated no cytotoxicity in CHO cells. Finally, nicotine and cotinine were not cytotoxic to CHO cells. The neutral red assay has been proved useful for quantifying differences in cytotoxicity of smoke condensates from cigarettes which vary in 'tar' yield and for assessing specific smoke constituents.

Gairola, C. (1982). "Genetic effects of fresh cigarette smoke in *Saccharomyces cerevisiae*." Mutat Res **102**(2): 123-136.

Ability of fresh cigarette smoke from University of Kentucky reference cigarette 2R1 to induce gene conversion, reverse mutation and mitotic crossing-over in strain D7 of *Saccharomyces cerevisiae* was examined. A closed cell suspension-recycle system using 2 peristaltic pumps interconnected to a single-port reverse-phase smoking machine was developed to provide complete exposure of cells to smoke within 0.2--10 sec of its generation. The exposed cells showed a dose-dependent increase in the frequency of all the 3 genetic endpoints examined. Cell age was an important factor with younger cells being more sensitive than older. Filtration studies showed that the gas phase possessed as much as 25% of the total whole-smoke activity. Activated charcoal reduced the activity of smoke in direct proportion to its amount in the filter. Acetate filter did not appreciably alter the activity. A comparison of whole smoke from various cigarettes showed that: (1) the nicotine content of a cigarette does not affect the genetic activity of smoke; (2) burley and flue-cured tobaccos have differential activity in gene conversion and reverse mutation systems; and (3) the genetic effects of whole smoke are not peculiar to tobacco pyrolysis because similar effects are produced by smokes from lettuce and other non-tobacco cigarettes. It is concluded that the yeast D7 system can be used effectively for the quantitative evaluation of

genetic effects of smoke from different cigarettes, and both whole cigarette smoke and its gas phase possess mutagenic as well as recombinogenic activity that can be modified by the use of filters.

Bridges, R. B. and L. Hsieh (1986). "Effects of cigarette smoke fractions on the chemotaxis of polymorphonuclear leukocytes." J Leukoc Biol **40**(1): 73-85.

The effects of cigarette smoking fractions on polymorphonuclear leukocyte (PMN) chemotaxis were determined using the 51Cr-assay. Water-soluble fractions (WSF) of cigarette smoke produced from several tobacco types differed in inhibitory potencies (i.e., flue cured greater than or equal to Maryland greater than or equal to blended greater than Burley greater than or equal to Turkish) corresponding to the respective unsaturated aldehyde content of the smoke from these tobaccos. Fractionation of cigarette smoke condensate (CSC) demonstrated that the more polar fractions were potent inhibitors of chemotaxis whereas those containing nicotine and the polycyclic hydrocarbons were weak inhibitors of chemotaxis. Unlike the inhibitory effects of WSFs, CSC fractions did not inhibit random migration and their inhibition of chemotaxis could not be completely prevented by reduced glutathione. These data suggest that while the alpha, beta-unsaturated aldehydes present in the vapor phase of smoke are among the most potent inhibitors of in vitro PMN chemotaxis, other polar, nonvolatile constituents of cigarette smoke also inhibit chemotaxis and by a mechanism which differs from that of the unsaturated aldehydes.

Fukano, Y., M. Oishi, et al. (2006). "Analysis of the expression of heme oxygenase-1 gene in human alveolar epithelial cells exposed to cigarette smoke condensate." J. Toxicol. Sci. **31**(2): 99-109.

Airway epithelium is exposed to inhaled exogenous sources. Injury of the alveolar epithelium by cigarette smoking is presumed to be an important process in the pathogenesis of smoking-related pulmonary diseases. Current mechanistic assays that measure the toxicity of cigarette smoke focus on carcinogenesis. However, there is a need to design assays relevant to other disease processes. Oxidative stress is implicated in the pathogenesis of many respiratory diseases including chronic obstructive pulmonary disease. Therefore, we evaluated whether in vitro studies of cigarette smoking are appropriate to examine HO-1 mRNA expression. The human lung epithelial cell line A549 was exposed to the particulate fraction of cigarette smoke (Cigarette Smoke Condensate; CSC) and examd. for the induction of HO-1 mRNA. HO-1 gene expression by CSC is increased dose-dependently. In comparison of the induction of HO-1 mRNA by CSC prepd. from flue-cured or Burley tobacco, CSC from flue-cured tobacco seems to tend to induce an mRNA of HO-1 higher than CSC from Burley tobacco. The adaptation of HO-1 mRNA expression assay as a biol. relevant indicator of cigarette smoke-induced stress may be exemplified in this study whereby CSC derived from cigarette smoke pos. correlated with an increase in HO-1 expression and the difference of the type of tobacco can be detected. [on SciFinder(R)]