Beiträge zur Tabakforschung International · Volume 13 · No. 2 · December 1985 DOI: 10.2478/cttr-2013-0560

Influence of Genetic and Cultural Factors on Chemical and Physical Properties of Tobacco

II. Cell Wall Biopolymers *

by

William S. Ryan, Jr., G. H. Bokelman and H. H. Sun
Philip Morris Research Center, Richmond, Virginia, U.S.A.

and

T. R. Terrill
Virginia Polytechnic Institute and State University, Blackstone, Virginia, U.S.A.

SUMMARY

The influence of genetic factors and cultural management conditions on the cell wall biopolymer composition of tobacco was investigated. Five tobacco cultivars — Pennbel 69 (cigar filler), Catterton (Maryland), Coker 319 (bright), Burley 21 (Burley), and Little Sweet Orinoco (sun-cured) — were grown and cured under both flue-cured and dark fire-cured cultural management systems. The cell wall biopolymer composition of both freeze-dried mature (ripe) leaf and cured tobacco samples was determined by our standard fractionation procedure.

For all five tobacco cultivars the levels of most cell wall biopolymers in the freeze-dried mature leaf did not vary significantly as a function of cultural management conditions. However, for Pennbel 69, Catterton and Coker 319 changing from flue-cured to dark fire-cured growing conditions relatively lowered starch contents by values between 32% and 74% while increasing the quantities of ethanol solubles and protein. The following general trends were noted for changes in chemical composition as a function of curing: protein decreased, lignin increased, soluble ash decreased and insoluble ash increased. Coker 319 and Little Sweet Orinoco were found to be generally lower in pectin, lignin, and cellulose than the other cultivars regardless of cultural regime.

ZUSAMMENFASSUNG

Es wurde bestimmt, inwieweit biopolymere Bestandteile der Zellwand von Tabakpflanzen durch genetische Faktoren und Anbaubedingungen beeinflußt werden. Fünf Tabaksorten — Pennbel 69 (Fülltabak für Zigarren), Catterton (Maryland), Coker 319 (heller Tabak), Burley 21 (Burley) und Little Sweet Orinoco („sun-cured") — wurden gemäß dem „flue-curing"-Verfahren einerseits und dem „dark fire-curing"-Verfahren andererseits angebaut und getrocknet. Ausgewachsene reife Blätter wurden gefriergetrocknet oder einem der beiden genannten Trocknungsverfahren unterzogen und unter Einsatz der kürzlich von den Autoren entwickelten, standardisierten Fraktionierungsmethode auf den Gehalt an Biopolymeren in der Zellwand untersucht.

Die meisten Biopolymere waren in der Zellwand des gefriergetrockneten reifen Blattes aller fünf Tabakkultivare unabhängig von der Kulturtechnik in nahezu gleicher Menge enthalten. Beim Übergang von den „flue-curing"-Anbaubedingungen zu „dark fire-curing"-Anbaubedingungen verminderte sich jedoch bei den Sorten Pennbel 69, Catterton und Coker 319 der Stärkegehalt relativ um Werte zwischen 32% und 74%, während sich die Mengen an Eiweiß und an in Ethanol löslichen Verbindungen erhöhten. Bei Anwendung der beiden Trocknungsverfahren zeigte sich gegenüber der Gefriertrocknung in der chemischen Zusammensetzung der Zellwand der Kultivare der Tendenz nach folgendes: der Eiweißgehalt nahm ab, der Ligningehalt stieg an, die Menge an löslicher Asche ging zurück und die unlöslicher Asche nahm zu. Die Proben der Kultivare Coker 319 und Little Sweet Orinoco wiesen un-

* Received: 17th May 1985 — accepted: 7th October 1985.
Presented at the 38th Tobacco Chemists' Research Conference, Atlanta, Georgia, U.S.A., in 1984.
For Part I, see Ref. 14.

abhängig von den Anbau- und Trocknungsbedingungen im allgemeinen einen geringeren Gehalt an Pektin, Lignin und Cellulose auf als die anderen Sorten.

RESUME

L'objet de cette étude consistait à déterminer quelle était l'influence exercée sur les composants biopolymères de la paroi cellulaire des plantes de tabac par des facteurs d'ordre génétique ainsi que par les conditions de culture. Cinq variétés de tabac: Pennbel 69 (tabac de remplissage pour cigares), Catterton (Maryland), Coker 319 (tabac clair), Burley 21 (Burley) et Little Sweet Orinoco («sun-cured») ont été cultivées et séchées selon les procédés de «flue-curing» d'une part et de «dark fire-curing» de l'autre. Arrivées à maturité, les feuilles ont été lyophilisées ou soumises à l'un des deux procédés de séchage mentionnés ci-dessus, et la teneur en biopolymères de leur paroi cellulaire a été déterminée au moyen du procédé de fractionnement standardisé récemment mis au point par les auteurs de l'étude.
La plupart des biopolymères sont présents en quantités pratiquement égales dans la paroi cellulaire des feuilles lyophilisées des cinq variétés de tabac étudiées, et ce, indépendamment du mode de culture. Dans le cas du Pennbel 69, du Catterton et du Coker 319, la teneur en amidon accuse toutefois une diminution relative variant de 32% à 74% lorsqu'on passe du «flue-curing» au «dark fire-curing», alors que les quantités de protéines et de composés solubles dans l'éthanol augmentent. En ce qui concerne la composition chimique de la paroi cellulaire selon le procédé de séchage, on observe les modifications suivantes par rapport à la lyophilisation: la teneur en protéines diminue, la teneur en lignine augmente, la quantité de cendres solubles décroît et celle de cendres insolubles croît. D'une façon générale et indépendamment des conditions de culture et de séchage, les échantillons de Coker 319 et Little Sweet Orinoco ont une teneur respective en pectine, lignine et cellulose inférieure à celle des autres variétés.

INTRODUCTION

Although tobacco has been one of the most thoroughly analyzed of all plant materials, tobacco cell wall biopolymers have received relatively little attention (1—11). One recent advance has been the development of a general fractionation procedure for the separation and analysis of tobacco biopolymers (12, 13). The present study was designed to utilize this fractionation procedure in order to determine and compare the cell wall biopolymer composition of five tobacco cultivars grown under two different cultural management systems. Both freeze-dried mature (i.e. ripe) and cured leaf samples were analyzed. One goal of this study was to gain insights into changes in cell wall biopolymer composition occurring during curing. In addition it was hoped that examination of analytical data for the different cultivars would reveal ranges of genetic variability for the cell wall biopolymers.

EXPERIMENTAL

The following five cultivars of *Nicotiana tabacum* L. were grown and lyophilized or cured at the Virginia Polytechnic Institute and State University, Southern Piedmont Center, Blackstone, Virginia, during the 1982 crop year: Pennbel 69 (cigar filler), Catterton (Maryland), Coker 319 (bright), Burley 21 (Burley), and Little Sweet Orinoco (sun-cured). Each cultivar was grown under both flue-cured and dark fire-cured management conditions (14). For the flue-cured regime, samples for lyophilization and flue-curing were collected when the leaves in the mid-stalk position were judged to be mature (third harvest). For the dark fire-cured regime, samples for lyophilization were also collected when the middle leaves were judged to be mature, which was one day before the plants were cut to start the dark fire-curing process.
Tobacco samples, whether lyophilized or cured, consisted of the lamina only from the middle leaf of each respective cultivar. Although the middle leaf might not represent the true mean for a particular tobacco cul-

Figure 1.
Fractionation of tobacco for determination of cell wall components.

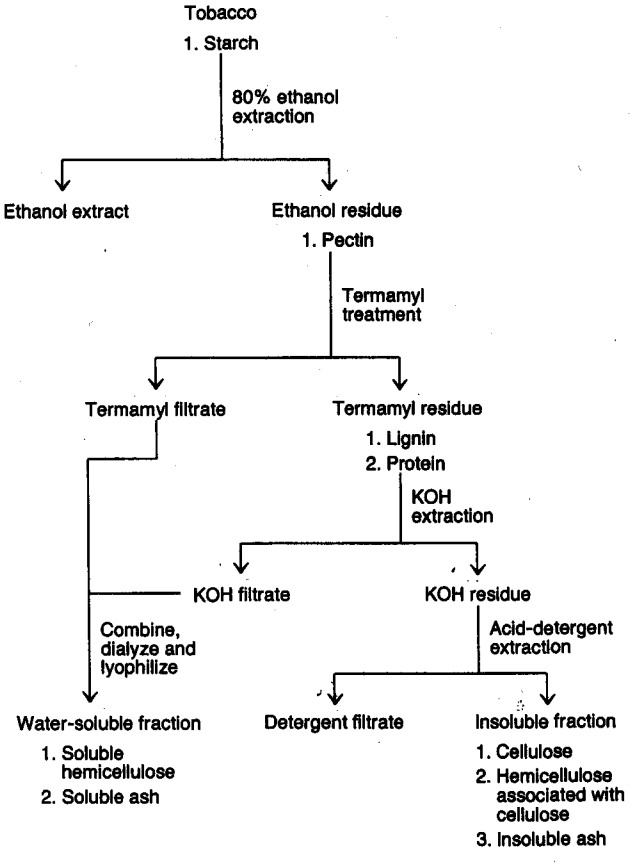

Table 1.
Cell wall biopolymer composition of tobaccos from flue-cured management, not corrected for weight loss during curing (all values expressed on a percentage dry weight basis).

Tobacco components	Pennbel 69		Catterton		Coker 319		Burley 21		Little Sweet Orinoco	
	ripe	cured	ripe	cured	ripe	cured	ripe	cured	ripe	cured
Ethanol solubles	38.2	52.1	28.4	56.4	29.9	65.1	45.5	41.9	40.7	58.8
Aqueous solubles lost during dialysis	11.1	6.8	~ 0	3.3	~ 0	2.6	7.1	12.2	6.6	6.4
Acid-detergent solubles	3.5	4.3	3.6	3.5	2.6	2.7	4.8	7.2	3.5	3.4
Pectin	7.1	9.2	8.3	8.1	5.2	7.2	8.6	11.9	5.2	4.8
Starch	17.6	0.9	34.3	6.8	42.2	6.4	9.0	~ 0	20.9	1.4
Protein	10.9	6.3	9.6	5.1	7.7	5.0	13.8	7.4	11.4	5.9
Hemicellulose										
soluble	3.0	3.4	2.8	2.3	2.5	1.9	2.4	2.4	2.2	2.1
associated with cellulose	0.7	0.7	0.6	0.7	0.4	0.4	0.9	1.0	0.3	0.4
Lignin	1.1	1.7	1.1	2.0	0.7	1.0	1.0	1.9	0.9	0.9
Cellulose	5.2	5.4	4.8	5.2	3.4	4.1	5.7	7.3	3.5	4.2
Ash										
soluble	4.2	3.9	3.8	3.9	3.8	2.0	3.3	3.1	0.7	1.9
insoluble	0.5	2.9	1.4	1.6	0.6	0.9	0.7	1.9	1.4	2.7
Total	103.1	97.6	98.7	98.9	99.0	99.3	102.8	98.2	97.3	92.9

Table 2.
Cell wall biopolymer composition of tobaccos from dark fire-cured management, not corrected for weight loss during curing (all values expressed on a percentage dry weight basis).

Tobacco components	Pennbel 69		Catterton		Coker 319		Burley 21		Little Sweet Orinoco	
	ripe	cured	ripe	cured	ripe	cured	ripe	cured	ripe	cured
Ethanol solubles	42.7	40.8	38.5	36.9	34.6	44.1	43.3	42.5	42.4	45.4
Aqueous solubles lost during dialysis	9.7	9.8	6.3	16.4	7.1	11.1	9.0	14.6	7.0	14.1
Acid-detergent solubles	6.0	7.4	3.9	7.5	2.9	5.9	4.1	5.9	3.4	6.2
Pectin	8.7	9.7	6.7	11.7	4.5	10.5	8.2	11.1	5.7	8.9
Starch	4.6	~ 0	17.0	~ 0	28.8	~ 0	8.8	~ 0	16.1	~ 0
Protein	14.1	9.3	11.9	6.6	9.4	7.1	12.3	6.4	12.6	6.7
Hemicellulose										
soluble	2.6	3.5	2.8	3.0	2.3	3.5	2.1	2.5	1.8	2.6
associated with cellulose	0.8	0.8	0.7	0.9	0.5	0.6	0.6	0.5	0.4	0.7
Lignin	1.1	2.2	1.5	2.8	0.7	2.0	0.7	1.6	0.8	1.6
Cellulose	5.2	6.6	4.9	6.6	4.1	6.3	5.0	5.3	3.7	6.0
Ash										
soluble	3.7	2.9	3.9	2.6	3.4	2.8	3.9	2.2	2.8	0.8
insoluble	2.3	2.7	0.6	2.1	0.6	1.4	1.4	5.7	2.0	3.7
Total	101.5	95.7	98.7	97.1	98.9	95.3	99.4	98.3	98.7	96.7

Table 3.
Comparison of cell wall biopolymer composition of freeze-dried mature tobaccos
(all values expressed on a percentage dry weight basis).

Tobacco components	Pennbel 69		Catterton		Coker 319		Burley 21		Little Sweet Orinoco	
	flue-cured	dark fire-cured	flue-cured	dark fire-cured	flue-cured	dark fire-cured	flue-cured	dark fire-cured	flue-cured	dark fire-cured
Ethanol solubles	38.2	42.7	28.4	38.5	29.9	34.6	45.5	43.3	40.7	42.4
Aqueous solubles lost during dialysis	11.1	9.7	~ 0	6.3	~ 0	7.1	7.1	9.0	6.6	7.0
Acid-detergent solubles	3.5	6.0	3.6	3.9	2.6	2.9	4.8	4.1	3.5	3.4
Pectin	7.1	8.7	8.3	6.7	5.2	4.5	8.6	8.2	5.2	5.7
Starch	17.6	4.6	34.3	17.0	42.2	28.8	9.0	8.8	20.9	16.1
Protein	10.9	14.1	9.6	11.9	7.7	9.4	13.8	12.3	11.4	12.6
Hemicellulose										
soluble	3.0	2.6	2.8	2.8	2.5	2.3	2.4	2.1	2.2	1.8
associated with cellulose	0.7	0.8	0.6	0.7	0.4	0.5	0.9	0.6	0.3	0.4
Lignin	1.1	1.1	1.1	1.5	0.7	0.7	1.0	0.7	0.9	0.8
Cellulose	5.2	5.2	4.8	4.9	3.4	4.1	5.7	5.0	3.5	3.7
Ash										
soluble	4.2	3.7	3.8	3.9	3.8	3.4	3.3	3.9	0.7	2.8
insoluble	0.5	2.3	1.4	0.6	0.6	0.6	0.7	1.4	1.4	2.0
Total	103.1	101.5	98.7	98.7	99.0	98.9	102.8	99.4	97.3	98.7

tivar, it was thought to be the least biased position for single-sample comparisons. When counted from the lowest leaf on the stalk, the middle leaf was the 8th leaf for Little Sweet Orinoco, the 10th or 11th leaf for Coker 319, Catterton and Pennbel 69, and the 12th leaf for Burley 21. The lamina to be lyophilized from mature tobacco leaves was frozen with dry ice immediately after harvesting.

All the tobacco samples were ground to pass a 20 mesh screen. Cell wall biopolymer composition of the ground tobacco samples was determined by a slightly modified version of our standard fractionation procedure (13) shown in Figure 1. The tobacco samples were initially Soxhlet extracted with 80% aqueous ethanol for 18 h to remove low molecular weight materials. The ethanol extracted tobaccos were then treated with the thermophilic amylase Termamyl® 60-L (Novo Laboratories) to remove starch. The Termamyl treated residues were then subjected to an alkaline extraction with 0.1 N KOH. The alkaline extracted residues were then detergent extracted with cetyltrimethylammonium bromide in 0.1 N H_2SO_4 and the residue from this final extraction was termed the insoluble fraction. The aqueous filtrates from the Termamyl® treatments and the alkaline extractions were combined, dialyzed (12,000-dalton molecular weight cut-off tubing) and lyophilized to produce the water-soluble fractions.

The following analyses were performed on the indicated fractions to determine the various biopolymers. The starch content was determined by analysis of the initial tobacco materials. The amount of pectin in the tobacco was calculated from the uronic acid content of the ethanol extracted residues. The protein content was obtained by multiplying the total nitrogen content of the Termamyl® treated residues by the conventional factor of 6.25. The lignin content of the samples was calculated from the Klason residue values for the Termamyl® residues. The water-soluble fractions were analyzed for soluble hemicellulose and soluble ash. The insoluble fractions were analyzed for cellulose, hemicellulose associated with cellulose and insoluble ash. A more detailed discussion of the method of calculation of these values has been reported previously (12).

RESULTS AND DISCUSSION

The major objective of the present study was to examine the range of composition of very diverse commercially grown tobaccos. The cell wall biopolymer compositions are summarized in Tables 1 and 2 on an "as is" basis, i.e. no corrections were made for the weight loss that occurred during curing. In Table 3 a side-by-side comparison is shown for the freeze-dried mature tobaccos under both cultural management conditions. It may be seen that for all five tobacco cultivars the levels of most cell wall biopolymers in the freeze-dried mature leaf do not vary significantly as a function of cultural management conditions. However, for Pennbel 69, Catterton, and Coker 319 changing from flue-cured

Table 4.
Comparison of cell wall biopolymer composition of tobaccos from flue-cured management
(all values expressed on a percentage dry weight basis).

Tobacco components	Pennbel 69		Catterton		Coker 319		Burley 21		Little Sweet Orinoco	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Ethanol solubles	56.0	52.1	46.0	56.4	41.3	65.1	73.9	41.9	63.6	58.8
Aqueous solubles lost during dialysis	16.3	6.8	~ 0	3.3	~ 0	2.6	11.5	12.2	10.3	6.4
Acid-detergent solubles	5.1	4.3	5.8	3.5	3.6	2.7	7.8	7.2	5.5	3.4
Pectin	10.4	9.2	13.5	8.1	7.2	7.2	14.0	11.9	8.1	4.8
Starch	25.8	0.9	55.6	6.8	58.3	6.4	14.6	~ 0	32.6	1.4
Protein	16.0	6.3	15.6	5.1	10.6	5.0	22.4	7.4	17.8	5.9
Hemicellulose										
soluble	4.4	3.4	4.5	2.3	3.5	1.9	3.9	2.4	3.4	2.1
associated with cellulose	1.0	0.7	1.0	0.7	0.6	0.4	1.5	1.0	0.5	0.4
Lignin	1.6	1.7	1.8	2.0	0.9	1.0	1.6	1.9	1.4	0.9
Cellulose	7.6	5.4	7.8	5.2	4.7	4.1	9.3	7.3	5.5	4.2
Ash										
soluble	6.2	3.9	6.2	3.9	5.2	2.0	5.4	3.1	1.1	1.9
insoluble	0.7	2.9	2.3	1.6	0.8	0.9	1.1	1.9	2.2	2.7

a: freeze-dried mature tobacco, corrected for weight loss that would have occurred during curing.

b: cured tobacco ("as is").

Table 5.
Comparison of cell wall biopolymer composition of tobaccos from dark fire-cured management
(all values expressed on a percentage dry weight basis).

Tobacco components	Pennbel 69		Catterton		Coker 319		Burley 21		Little Sweet Orinoco	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Ethanol solubles	52.1	40.8	75.0	36.9	54.3	44.1	56.4	42.5	72.9	45.4
Aqueous solubles lost during dialysis	11.8	9.8	12.3	16.4	11.1	11.1	11.7	14.6	12.0	14.1
Acid-detergent solubles	7.3	7.4	7.6	7.5	4.6	5.9	5.3	5.9	5.8	6.2
Pectin	10.6	9.7	13.1	11.7	7.1	10.5	10.7	11.1	9.8	8.9
Starch	5.6	~ 0	33.1	~ 0	45.2	~ 0	11.5	~ 0	27.7	~ 0
Protein	17.2	9.3	23.2	6.6	14.8	7.1	16.0	6.4	21.7	6.7
Hemicellulose										
soluble	3.2	3.5	5.5	3.0	3.6	3.5	2.7	2.5	3.1	2.6
associated with cellulose	1.0	0.8	1.4	0.9	0.8	0.6	0.8	0.5	0.7	0.7
Lignin	1.3	2.2	2.9	2.8	1.1	2.0	0.9	1.6	1.4	1.6
Cellulose	6.3	6.6	9.5	6.6	6.4	6.3	6.5	5.3	6.4	6.0
Ash										
soluble	4.5	2.9	7.6	2.6	5.3	2.8	5.1	2.2	4.8	0.8
insoluble	2.8	2.7	1.2	2.1	0.9	1.4	1.8	5.7	3.4	3.7

a: freeze-dried mature tobacco, corrected for weight loss that would have occurred during curing.

b: cured tobacco ("as is").

to dark fire-cured growing conditions relatively lowers the starch content by values between 32% and 74% while increasing the quantity of ethanol solubles found in the mature leaf. It appears that increased nitrogen fertilization reduced the starch levels in these cultivars. Such an inverse relationship between starch accumulation and the use of nitrogen fertilizer has been reported previously for bright tobacco (15, 16). For Catterton and Coker 319, the change to the dark fire-cured regime produced a significant increase in the aqueous solubles lost during dialysis. For these three cultivars it may be that a decreased production of starch is accompanied by an increased production of low molecular weight oligo- or polysaccharides that would be found in the ethanol solubles or aqueous solubles lost during dialysis. In addition, for these three cultivars the protein levels were increased under the dark fire-cured regime.

It is interesting to note for Burley 21 that the level of starch is low ($\leq$ 9%) and the level of protein is high ($>$ 12%) in the freeze-dried mature leaf regardless of how the tobacco is grown. Thus for this cultivar it appears that genetic factors are predominant in controlling the cell wall biopolymer composition.

Little Sweet Orinoco has starch concentrations in the freeze-dried mature leaf which are intermediate between those found for Burley 21 and Coker 319. It may be that Little Sweet Orinoco represents an "old line" tobacco from which other cultivars were developed.

Certain general trends for changes occurring in the flue-curing process may be noted by examining Table 4, which is corrected for weight loss resulting from curing. The corrections for the loss in solids caused by curing were calculated on the basis of total ash in the initial starting tobacco. For all the tobacco cultivars large decreases in starch values occur as a function of flue curing. This effect is a reflection of the well known conversion of starch to glucose. It is also clear that all the cultivars undergo degradation of protein during flue curing. In addition, all the cultivars except for Little Sweet Orinoco show increased values for Klason lignin after flue curing. Lignin is presently understood to be a three-dimensional polymeric natural product produced by an enzyme-initiated dehydrogenative polymerization of *trans*-coniferyl, *trans*-sinapyl and *trans-p*-coumaryl alcohols (17). However, in the present fractionation procedure, the lignin value is defined to be the Klason residue corrected for both protein and ash contents. The Klason residue represents an H_2SO_4 acid-insoluble subfraction of the Termamyl® residue. It is doubtful that the flue-curing process produced any lignin. Rather, it is thought that during the curing process, condensation reactions involving protein or glycoprotein generated acid-insoluble products and increased the Klason lignin values.

Two of these same trends are also found in the dark fire-curing process, as shown in Table 5. Every cultivar except Catterton demonstrates both a decrease in protein concentration and an increase in lignin concentration as a result of dark fire curing.

It is also apparent from an examination of Tables 4 and 5 that soluble ash tends to decrease and insoluble ash tends to increase as a function of curing, by either curing process. These changes in ash values may be indicative of structural changes occurring in the cell wall biopolymers. The soluble and insoluble ash values may reflect the levels of inorganics which are associated in some manner with the cell wall biopolymers. The increased levels of soluble ash for the cured samples suggest that pectin may have been converted from the methyl ester form to the ionic salt form which would bind calcium.

Other, subtle differences may be discerned from the data in Table 5. For example, Catterton and Burley 21 undergo a more pronounced degradation of their cellulose during the curing process under dark fire-cured management than do the other cultivars. The result is that dark fire-cured Burley 21 has the lowest level of cellulose. On the other hand, the protein in Coker 319 and Pennbel 69 is hydrolyzed less efficiently during dark fire curing than is the case for the other cultivars. In the freeze-dried mature leaf under dark fire-cured management Coker 319 has the lowest level of protein. However, after dark fire curing Coker 319 has the second highest residual level of protein.

These last two examples illustrate that differences exist, perhaps in the activity or concentration of endogenous enzymes, among the cultivars which can markedly affect chemical changes occuring in an air-curing process.

Several trends are evident from a side-by-side comparison of the cell wall biopolymer compositions in Tables 1 and 2 which appear to be independent of growing conditions and curing treatments. For example, Coker 319 and Little Sweet Orinoco are lowest in pectin, lignin and cellulose. Little Sweet Orinoco is also lowest in soluble ash, which suggests that the pectin in this cultivar may have a low content of calcium and other cations. Coker 319 is lowest in protein and acid detergent solubles. Coker 319 and Catterton are highest in starch, while Burley 21 is lowest in starch. These trends indicate that genetic differences may exist among the five tobacco cultivars which influence or control the concentrations of cell wall biopolymers.

An attempt was made to utilize such trends in order to evaluate the potential of particular cultivars to produce

Table 6.
General trends for relative concentrations of cell wall biopolymers.

Tobacco components	Pennbel 69 (cigar filler)	Catterton (Maryland)	Coker 319 (bright)	Burley 21 (Burley)	Little Sweet Orinoco (sun-cured)
Pectin	high	high	low	high	low
Starch	low	high	high	low	high
Protein	high	low	low	high	high
Cellulose	high	high	low	high	low

cured tobaccos. The cured tobaccos were assigned official market grades by a U.S.D.A. Marketing Service Tobacco Inspector and market values established from the average seasonal price obtained for comparable grades in the appropriate market (14). The highest prices assigned were for the flue-cured cultivar (Coker 319) produced as flue-cured tobacco, but acceptable flue-cured grades were assigned to many lots of sun-cured and Maryland tobacco (14). From a comparison of the concentrations of starch, pectin, protein, and cellulose in the five cultivars, as shown in Table 6, it appears that a relatively high concentration of starch and relatively low concentrations of pectin, protein, and cellulose may be desirable for the production of flue-cured tobacco. The relationship between cell wall biopolymer composition and the production of dark fire-cured tobacco was less well defined. However, it was clear that the Burley cultivar grown in Blackstone, Virginia, failed to produce quality tobacco under either the flue-cured or dark fire-cured system.

REFERENCES

1. Bourne, E. J., J. B. Pridham and H. G. J. Worth: Pectic substances in cured and uncured tobacco; Phytochemistry (Oxf.) 6 (1967) 423—431.
2. Christy, M. G., and M. Samfield: The average degree of polymerization of cellulose in various tobacco types, Part I. Experimental; Tob. Sci. 4 (1960) 33—37.
3. Eda, S., Y. Akiyama, K. Katō, A. Ishizu and J. Nakano: Methylation analysis of cell wall polysaccharides from suspension-cultured cells of *Nicotiana tabacum;* Agric. Biol. Chem. 47 (1983) 1783—1789.
4. Eda, S., and K. Katō: An arabinoxyloglucan isolated from the midrib of the leaves of *Nicotiana tabacum;* Agric. Biol. Chem. 42 (1978) 351—357.
5. Eda, S., and K. Katō: Pectin isolated from the midrib of leaves of *Nicotiana tabacum;* Agric. Biol. Chem. 44 (1980) 2793—2801.
6. Eda, S., H. Kodama, Y. Akiyama, M. Mori, K. Katō, A. Ishizu and J. Nakano: An arabinoxyloglucan from the cell walls of suspension-cultured tobacco cells; Agric. Biol. Chem. 47 (1983) 1791—1797.
7. Eda, S., A. Ohnishi and K. Katō: Xylan isolated from the stalk of *Nicotiana tabacum;* Agric. Biol. Chem. 40 (1976) 359—364.
8. Eda, S., F. Watanabe and K. Katō: 4-*O*-Methylglucuronoxylan isolated from the midrib of *Nicotiana tabacum;* Agric. Biol. Chem. 41 (1977) 429—434.
9. Mori, M., S. Eda and K. Katō: Two xyloglucan oligosaccharides obtained by cellulase-degradation of tobacco arabinoxyloglucan; Agric. Biol. Chem. 43 (1979) 145—149.
10. Mori, M., S. Eda and K. Katō: Structural investigation of the arabinoxyloglucan from *Nicotiana tabacum;* Carbohydr. Res. 84 (1980) 125—135.
11. Mori, M., and K. Katō: An arabinoglucuronomannan from suspension-cultured cells of *Nicotiana tabacum;* Carbohydr. Res. 91 (1981) 49—58.
12. Bokelman, G. H., W. S. Ryan, Jr., and E. T. Oakley: Fractionation of bright tobacco; J. Agric. Food Chem. 31 (1983) 897—901.
13. Bokelman, G. H., and W. S. Ryan, Jr.: Analyses of Bright and Burley tobacco laminae and stems; Beitr. Tabakforsch. Int. 13 (1985) 29—36.
14. Terrill, T. R., G. H. Bokelman, W. S. Ryan, Jr., and H. H. Sun: Influence of genetic and cultural factors on chemical and physical properties of tobacco, I. Agronomic measurements; Tob. Sci. 29 (1985) 40—43.
15. Long, R. C., and W. G. Woltz: Environmental factors affecting the chemical composition of tobacco; in: Proc. Am. Chem. Soc. Symp., 173rd American Chemical Society Meeting (Agricultural and Food Chemistry Division), Symposium on Recent advances in the chemical composition of tobacco and tobacco smoke, New Orleans, Louisiana, 1977, pp. 116—163.
16. Wan Ismail, W. A.: Effect of *N*-rates, soil moisture regimes, and tillage on flue-cured tobacco production; M.S. thesis, North Carolina State University, Raleigh, N.C., 1981.
17. Sarkanen, K. V., and C. H. Ludwig: Lignins — Occurrence, formation, structure and reactions; Wiley-Interscience, New York, N.Y., 1971.

Acknowledgements

The technical assistance of Mr. Wilton Suiter and Mr. Robert Esperdy is gratefully acknowledged.

Authors' address:

Philip Morris Research Center,
P. O. Box 26583,
Richmond, Virginia, 23261, U.S.A.