usual sodium chlorite procedure (*5*). Practically all of the hemicelluloses may be removed from holocellulose by extraction with a 10% solution of NaOH. Analyses indicate that the residue is almost pure α-cellulose.

Neutralization of the alkaline hemicellulose solution causes precipitation of the higher molecular weight polysaccharides and leaves in solution most of the glycuronans and polysaccharides of comparatively low molecular weight. The precipitated material is called hemicellulose A. Most of the soluble material remaining in the neutral solution is precipitated by the addition of two volumes of alcohol and is called hemicellulose B (*2*).

Results in this laboratory show that practically all of hemicellulose A consists of xylan. The mixture contains only 3% of combined hexuronic acid anhydride. Hemicellulose A, 25% of the total holocellulose, can be esterified with acetic anhydride in the presence of 0.25% of nitric acid (*4*) to produce a white fibrous acetate containing 38.6% of acetyl groups (calculated for diacetyl xylan, 39.8%). The acetate is soluble in dioxane, pyridine, or in a chloroform-methanol 9:1 mixture. When the acetate is cast from any of these solvents, clear films are produced which show an average tensile strength of 7.2 kg/mm² when measured on a Scott IP-4 inclined-plane serigraph. Films from commercial cellulose triacetate, when prepared in a similar manner, have a tensile strength of 8.6 kg/mm². Films cast from solutions of mixtures of hemicellulose A acetate and cellulose triacetate are clear and strong provided that incorporation of hemicellulose A acetate does not exceed 50%. Larger amounts of hemicellulose A acetate produce cloudy films.

Hemicellulose B, 6% of which is glycuronan material, may be esterified easily by a mixture of acetic anhydride and pyridine, provided that it is first swelled in formamide (*1*). The resulting acetate is a light brown, powdery material having an acetyl content of 35.0%. When cast from a chloroform solution, it produces clear films having a tensile strength of 7.5 kg/mm². While films can be produced by mixing hemicellulose B acetate with either cellulose acetate or hemicellulose A acetate, they are all cloudy, indicating the immiscibility of hemicellulose B acetate with either of the other two acetates.

These results show that the presence of hemicellulose A acetates does not have an adverse effect on the production of clear films of high quality. It is only the hemicellulose B acetates and possibly other low molecular weight polysaccharides that are responsible for cloudy films. The hemicellulose B fraction is present in most plant tissues in small quantities only. It represents approximately 10% of corncob holocellulose. It may be easily and completely removed through short extraction with alkaline solutions of 1–2% concentration. The residue obtained from corncob holocellulose after such an extraction may be acetylated and cast into good films.

These observations suggest the desirability of revising pulping techniques in order to retain more of the higher molecular weight hemicellulose fraction and thereby permit the use of these hemicelluloses, which are now discarded in commercial practice.

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A Possible Explanation of Symptom Formation in Tobacco with Frenching and Mineral Deficiencies

Robert A. Steinberg, John D. Bowling, and James E. McMurtrey, Jr.

Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland

Studies with Maryland Medium Broadleaf tobacco seedlings in aseptic culture (*3*, *4*) have disclosed that slight excesses of amino acids in the medium led to the formation of characteristic growth abnormalities and chloroses. The symptoms of toxicity covered a wide range but were specific for each amino acid. Admixtures in some cases led to appearance of new abnormalities. Most effective was L(−)-hydroxyproline (3 ppm), the seedlings being killed at 5 ppm. A close approximation of the extreme symptoms of frenching was produced with the natural amino acid, L(+)-isoleucine (100 ppm); the unnatural isomer being relatively ineffective. These growth abnormalities included inhibition of stem and branch elongation, accelerated development of the leaves in the axillary buds, and reticular chlorosis of newly expanded leaves, together with greatly reduced leaf laminae ("strap leaves"). Increased leaf number was a prominent feature.

Similar responses to amino acids have now been obtained with tobacco plants growing in water-culture and soil. Frenching of oriental Xanthi tobacco was obtained with DL-isoleucine at 20 ppm in water-culture. Partially sterilized soil required very large quantities, however, in order to produce the symptoms of frenching in Connecticut Broadleaf and Xanthi tobacco.

Large scale analytical studies with field plants of Maryland Medium Broadleaf tobacco have afforded some confirmation of the interpretation that excessive accumulation of free amino acids in the plant may be a primary cause of symptom formation in certain abnormalities. Free amino acids in leaf laminae of mildly frenched plants increased to a maximum of 121% above normal. J. L. Stokes of Merck and Company estimated the corresponding increase for L(+)-isoleucine to be 50%. Free amino acids in leaf laminae of plants showing symptoms of mineral deficiencies increased with nitrogen deficiency by 32%; phosphorus, 48%; potassium, 587%; calcium, 120%; magnesium, 283%; and boron, 27%. These values are maxima. They confirm and extend the

data of mineral deficiency studies with green plants (*1*, *2*).

The increases in free amino acids are considered to be evidence that these chemical elements participate in protein metabolism, and that formation of symptoms is primarily due to the localized action of excessively accumulated normal metabolites. Growth responses and chloroses with amino acids are of sufficient variety to include many of the individual symptoms comprising the syndromes displayed with mineral deficiencies. Except possibly for magnesium, breakdown of chlorophyll in mineral deficiency is, therefore, not necessarily indicative of mineral participation in chlorophyll formation, as has often been assumed. The probable participation of the chemical elements in the enzymes regulating protein metabolism warrants the exercise of caution in associating specific mineral deficiencies with physiological processes in the plant. Drastic interference in the basic function of the plant should cause a breakdown in all physiological processes at varying rates.

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Annual Variation in Nicotine Content of Tobacco

Earl W. Flosdorf and Arthur W. Palmer

Bayuk Cigars Inc., Philadelphia

In connection with the change in nicotine content during fermentation of cigar leaf tobacco of the Pennsylvania Seedleaf variety, Frankenburg (*1*) has given data for crops of four different years. This percentage is given before and after "sweat" by the manufacturer, which is usually referred to as "final forced fermentation." In addition to reduction in nicotine content during this stage, there is a reduction in the curing by the farmer before the manufacturer purchases the tobacco. Then there is reduction during storage fermentation by the manufacturer before the tobacco is put into final forced fermentation. In our laboratory, tobacco, as received by the manfacturer from the farmer after shed-curing, has been analyzed for alkaloids routinely for a period of more than 20 years. Table 1 shows the variation from year to year over that period.

The data in the table for the years 1936, 1938, 1939, and 1941 are somewhat higher than those reported by Frankenburg for the same years. This is to be expected, inasmuch as the data were obtained with tobaccos as received from the farmers, so there was no loss from any processing by the manufacturer.

The results include nor-nicotine and other alkaloids which are present in trace amounts and for that reason the percentage is reported as total alkaloids. Each year's crop represents an average of tobaccos grown by about 100 different farmers. Five leaves were taken from each of three hands distributed throughout one bundle from each farmer's tobacco. The leaves from all these locations were ground and mixed to produce a uniform sample. By quartering, a final representative sample was obtained for analysis.

TABLE 1

ALKALOIDS IN TOBACCO AFTER CURING

Crop	Total alkaloids (oven-dried basis)	pH
	%	
1927	3.1	..
1928	2.8	..
1929	4.6	..
1930	6.0	5.8
1931	3.3	6.9
1932	5.0	5.2
1933	2.5	5.8
1934	3.0	7.3
1935	4.5	6.5
1936	4.3	6.4
1938	3.1	..
1939	3.0	..
1940	3.2	6.2
1941	4.1	5.4
1942	3.1	6.7
1943	5.0	6.2
1944	3.6	6.5
1945	2.8	6.8
1946	2.9	6.5
1947	4.2	6.3
1948	3.15	6.5

In addition to the representative sample for each year's crop, individual farmers' tobaccos have been analyzed. It has been found that from farmer to farmer there is variation in any given year, with some tobaccos having as little as half of the average for the year and others as much as 50% more. However, normally over 90% of the individual farmers' tobaccos do not vary more than about ±10% from the average for the year. This uniformity is to be expected, inasmuch as the tobacco represents only that grown in the general vicinity of Lancaster, Pennsylvania. Where variations do occur, they represent differences from field to field, depending upon the soil, fertilizer, seed, and variations in rainfall occurring between the time of early and late harvesting of crops by different farmers. There is no relation between pH and alkaloid content.

Plotting the average alkaloid content for the various years against the amount of rainfall during the growing season shows a definite trend of higher alkaloid content in tobaccos grown in dry seasons. By breaking this down into months, it is found that the influence of rainfall on the alkaloid content is somewhat greater towards the end of the growing season, as the time of harvest approaches.

As a further point of interest, individual crops have been followed through complete manufacturing operations, including all fermentation. The average amount of alkaloid reduction is about 40%, all based on oven-dried samples. In certain extreme conditions, this may be as little as 20%, or as much as 80% reduction. This