CHANGES IN THE CHLOROPHYLL AND CAROTENE CONTENTS OF CURING BURLEY TOBACCO CUT AT DIFFERENT STAGES OF MATURITY[1]

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(WITH ONE FIGURE)

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Introduction

Changes in the chemical composition of burley tobacco which take place in curing are being investigated at the Kentucky Agricultural Experiment Station in an attempt to understand the physiology of curing and its effect on leaf quality. Color is recognized as one of the most important factors in the determination of leaf quality, any retention of green pigment in the cured leaf greatly reducing its value. The chlorophylls are the green pigments in normal tobacco, and the investigations reported in this paper were made primarily to determine the rate and amount of change in these pigments during air curing on the stalk of tobacco cut at three stages of maturity. The development of a method for determining carotene from the same solution used in the chlorophyll determinations made possible the study of this constituent.

This study of the catabolic changes of plant pigments is of general biochemical and physiological interest. Numerous articles have appeared reporting the rates of formation of these pigments under different conditions, but studies on pigment disappearance are rare. In 1918 WILLSTÄTTER and STOLL (5) reported that the chlorophyll content of yellowed leaves was less than of green leaves. They did not, however, follow closely the loss of either chlorophyll or carotenoids. GUTHRIE (2) investigated the pigment changes in potted plants when placed in the dark as compared with others remaining in the light. His results indicated that tomato plants lost about 25% of their chlorophyll in four days, soybean plants about 70% in eight days, and yellow coleus practically none in eight days.

Methods

HARVESTING AND CURING TECHNIQUE

Kentucky No. 16 burley tobacco was cut at three different stages of maturity as determined by experienced growers: (1) immature by about 10 days, (2) mature, and (3) overmature by 10 days. These lots of tobacco were topped to approximately 20 leaves, and to a height of about 136 cm. (4.5 ft.) two, 14, and 24 days respectively before cutting. The entire plant was cut just above the ground and the stalk "speared" onto a stick and hung to cure with the plant in an inverted position. Spearing

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

caused a split in the stalk about a foot long. Although the majority of the plant cells remained alive for some time, the more mature basal leaves, which had already started to turn yellow before cutting, died first, beginning at the tips. The yellowing and subsequent death of the cells then progressed gradually to the more immature leaves. The axillary buds near the tip of the stalk were still alive at the end of the curing period. Thus the curing process for this type of tobacco is one of gradual starvation and desiccation, accompanied by extensive chemical changes which may be catalyzed by the enzymes of the living plant.

The tobacco, after wilting in the field for 24 hours following cutting, was cured in the air-conditioned curing chambers described by O'BANNON (**4**) at 68% relative humidity and a temperature of 23.8° C. (74.9° F.). JEFFREY (**3**) found these conditions to be within the optimum range for the production of highest commercial quality cured leaf.

SAMPLING AND ANALYSIS

To sample two different stages of leaf maturity on each plant analytical samples were taken from two different levels. One, called the "basal leaf sample," was from each plant's basal three leaves which were sound and therefore likely to remain on the plant during curing; the other, designated as the "top leaf sample," was from the 5th, 6th, and 7th leaves from the point of topping. After curing, leaves from these positions on the stalk were graded chiefly as "trash" and "bright leaf," respectively. Leaves to be used as samples were measured and tagged in the field a day before cutting. Duplicate samples were taken in the field at cutting time, five days before the overmature plants were cut, and in the curing chambers. The frequency of sampling in the chambers was dependent on the rate of change observed. A sample consisted of four leaves, each taken from a different plant. The midribs were removed. The right halves of two of the leaves and the left halves of the other two were used for pigment extraction, and the remaining halves were placed in an oven at 65° C. for 48 hours to determine the percentage dry weight.

The leaf material was analyzed for chlorophylls *a* and *b* and for carotene using the method described by GRIFFITH and JEFFREY (**1**). By this method the pigments are extracted from the sample with acetone in a Waring blender and transferred to ether. Chlorophyll is determined in a dilution of the ether solution by means of spectrophotometric readings at the red absorption maxima of chlorophylls *a* and *b*, and carotene is determined spectrophotometrically after separation from the other pigments in the ether solution by the use of a chromatographic adsorption column. The estimation of xanthophyll had not been included in the method at the time this study was conducted.

EXPRESSION OF DATA

Since burley tobacco is cured on the stalk, the green or dry weight of the samples to be used for analysis could not be determined at cutting time.

YOUNG and JEFFREY (6) have shown that tobacco leaves cured under the same conditions used in this experiment lose 30% of their dry weight during the curing process; consequently, the oven dry weight does not constitute a satisfactory basis for reporting results. They found, however, that there was a high degree of correlation between the product of length and width of leaf and leaf area, and that the leaf area was a satisfactory basis for reporting changes that occur in curing tobacco. The leaf areas were calculated, using the equation:[2]

$$y = \frac{0.65\,x}{10{,}000} + 0.006$$

where y is the leaf area in square meters and x is the product of the length and width of the leaf expressed in centimeters.

Results

The chlorophyll and carotene contents of the top leaves at various times during the curing of plants cut when immature, mature and overmature are presented in table I. Similar results for basal leaves are presented in table II. The results expressed on an area basis are shown graphically

TABLE I

CHLOROPHYLL AND CAROTENE CONTENT OF TOP LEAVES AT VARIOUS STAGES OF CURING ON THE STALK FOR TOBACCO PLANTS CUT AT THREE STAGES OF MATURITY. RESULTS CALCULATED ON THE BASIS OF OVEN DRY WEIGHT AND OF FRESH LEAF AREA. LEAVES IN CURING CHAMBER EXCEPT AS NOTED

AGE	DAYS FROM FIRST CUTTING	OVEN DRY WEIGHT	WEIGHT BASIS		CHLOROPHYLL *a*	AREA BASIS	
			CHLOROPHYLL	CAROTENE		CHLOROPHYLL	CAROTENE
		%	*mg./gm.*	*mg./gm.*	%	*mg./m.*[2]	*mg./m.*[2]
	0.0*	16.2*	8.28 *	0.464*	72.0*	277.0*	15.4*
	2.0	16.1	7.18	0.444	70.1	227.0	14.0
	5.1	16.5	6.26	0.393	66.0	196.0	12.3
Immature	8.5	17.5	5.16	0.354	69.0	156.0	10.7
	15.1	24.6	1.46	0.263	68.5	39.0	7.0
	17.9	35.9	0.27	0.174	58.6	6.0	4.8
	21.0	51.4	0.52	0.183	67.3	13.0	4.5
	39.0	89.8	0.22	0.139	58.1	5.0	3.3
	10.0*	17.5*	5.10 *	0.299*	71.0*	188.0*	11.0*
	12.0	16.7	4.19	0.298	71.9	126.0	9.0
	15.1	17.8	2.45	0.190	64.3	78.0	6.1
Mature	18.5	34.4	0.24	0.119	67.1	6.4	3.2
	28.3	86.7	0.022	0.075	62.2	0.6	2.0
	34.0	86.1	0.020	0.070	58.3	0.5	2.0
	10.0†	17.5†	5.10 †	0.299†	71.0†	188.0†	11.0†
	14.8†	15.6†	4.46 †	0.265†	72.6†	160.0†	9.5†
	20.0*	16.6*	2.76 *	0.200*	72.6*	92.0*	6.7*
Overmature	21.9	18.5	1.88	0.156	71.6	62.0	5.2
	24.5	30.2	0.21	0.089	64.2	6.3	2.7
	27.9	79.1	0.027	0.079	61.4	0.8	2.3
	39.0	89.3	0.014	0.064	57.4	0.4	1.9

* Cut.
† In field.

[2] An error was present in the previously published form of this equation (6).

in figure 1. The left part of this figure shows the changes which take place in the chlorophyll content of the top leaves. In the first 17.9 days after cutting, the chlorophyll content of the immature tobacco decreased at an average rate of 15.1 mg./m.^{2} per day. Chlorophyll was lost by the mature tobacco at the rate of 21.4 mg./m.^{2} per day in the first 8.5 days of curing, and by the overmature tobacco at the rate of 16.9 mg./m.^{2} per day in the first 5.1 days of curing. The rate of chlorophyll loss in the tobacco growing in the field was 8.9 mg./m.^{2} per day in the first 10 days and 9.6 mg./m.^{2} per day in the second 10 days. The tobacco cut when immature and that

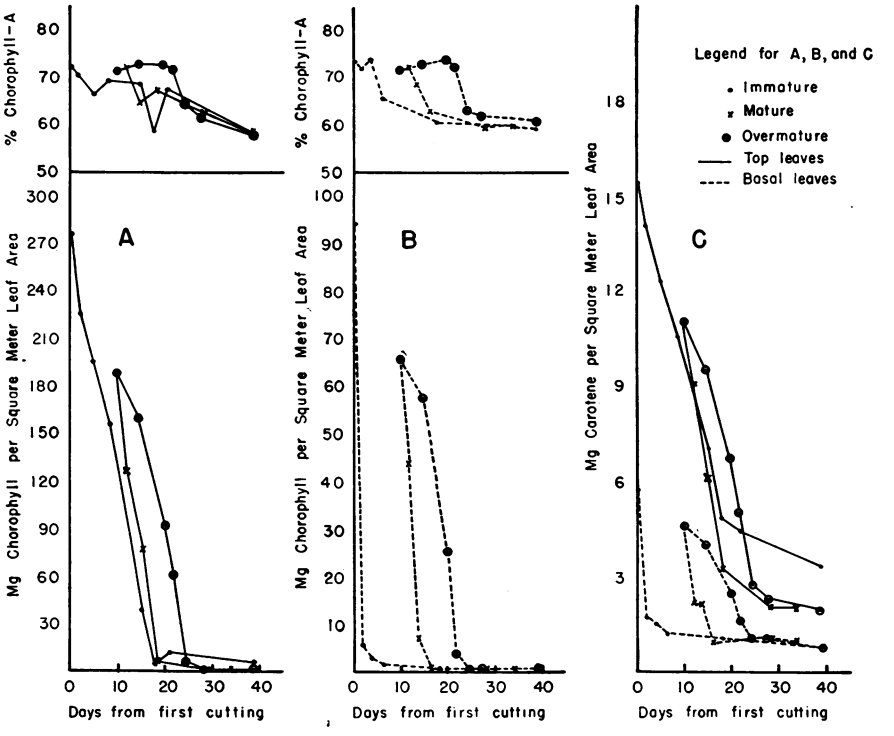

FIG. 1. Chlorophyll changes in top leaves (A), in basal leaves (B), and carotene changes in top and basal leaves (C) of curing burley tobacco cut when classified by experienced growers as immature, mature (10 days later), and overmature (20 days later).

cut when mature reached essentially the same chlorophyll content at the same time, even though the mature tobacco was cut 10 days later. Less time was required for chlorophyll destruction in the mature and overmature tobacco than in the immature tobacco, curing was more uniform, and the quality of leaf was better. The top leaves of the tobacco cut while immature still contained chlorophyll at the end of the curing period, incipient house-burn had occurred, and even the quality of the basal leaves was low.

The changes in the percentage of chlorophyll *a* based on total chlorophyll, are shown in the upper part of figure 1. The point representing the immature tobacco after 21 days is probably in error, since in the other five

instances in this and in the next graph representing basal leaves, the percentage chlorophyll *a* fell from about 70% to below 63% at about the same time that the total chlorophyll concentration ceased to fall. The oven dry weight in the leaf web was about 30% to 35% in all three cuttings of tobacco when the total chlorophyll content and the percentage chlorophyll *a* approached minimum values.

Changes in the chlorophyll content of the basal leaves are presented in the central part of figure 1. The initial chlorophyll content of these older leaves was much lower than that of the top leaves, and the time required

TABLE II

CHLOROPHYLL AND CAROTENE CONTENT OF BASAL LEAVES AT VARIOUS STAGES OF CURING ON THE STALK FOR TOBACCO PLANTS CUT AT THREE STAGES OF MATURITY. RESULTS CALCULATED ON THE BASIS OF OVEN DRY WEIGHT AND OF FRESH LEAF AREA. LEAVES IN CURING CHAMBER EXCEPT AS NOTED

AGE	DAYS FROM FIRST CUTTING	OVEN DRY WEIGHT	WEIGHT BASIS		CHLOROPHYLL *a*	AREA BASIS	
			CHLOROPHYLL	CAROTENE		CHLOROPHYLL	CAROTENE
		%	*mg./gm.*	*mg./gm.*	%	*mg./m.*^{2}	*mg./m.*^{2}
Immature	0.0*	11.3*	3.29*	0.211*	73.0*	94.2*	6.05*
	2.0	13.5	0.23	0.069	71.7	5.9	1.73
	3.9	31.0	0.11	0.051	73.1	3.3	1.57
	6.4	47.1	0.06	0.042	65.4	1.5	1.20
	18.1	86.9	0.03	0.043	60.1	0.8	1.02
	39.0	89.8	0.03	0.030	59.0	0.7	0.73
Mature	10.0*	12.3*	2.26*	0.158*	71.2*	65.5*	4.57*
	12.0	13.7	1.77	0.087	71.3	43.6	2.16
	13.9	21.2	0.30	0.085	67.9	7.4	2.07
	16.4	79.8	0.06	0.042	62.8	1.3	0.91
	28.3	87.4	0.03	0.043	59.0	0.8	1.06
	34.0	88.0	0.03	0.043	59.9	0.7	0.96
Overmature	10.0†	12.3†	2.26†	0.158†	71.2†	65.5†	4.57†
	14.8†	11.8†	1.71†	0.121†	72.5†	57.5†	4.02†
	20.0*	11.9*	0.92*	0.090*	73.6*	25.4*	2.47*
	21.9	20.7	0.16	0.063	72.0	4.0	1.58
	24.5	79.9	0.04	0.041	62.9	0.8	1.00
	27.9	86.0	0.04	0.043	61.5	1.0	1.06
	39.0	90.5	0.02	0.027	60.5	0.4	0.67

* Cut.
† In field.

for its loss was less than in the top leaves. In the first two days after cutting, the rate of chlorophyll loss in the basal leaves of the plants cut while immature was 44.3 mg./m.^{2} per day. In the first 3.9 days of curing the rate of loss in the basal leaves of the mature tobacco was 14.9 mg./m.^{2} per day. While the overmature tobacco was still in the field the rate of loss was 4.0 mg./m.^{2} per day and in the first 1.9 days after cutting the rate of loss was 11.3 mg./m.^{2} per day. Except for the immature tobacco the rate of loss per day in the basal leaves was less than the rate of loss in the top leaves, but because of lower initial chlorophyll values the length of time necessary for chlorophyll decomposition was less. The extremely rapid rate of 44.3 mg./m.^{2} per day of chlorophyll loss in the immature basal leaves was over

twice as great as any other rate of loss. Translocation of some substance or substances to the immature top leaves is the only explanation that can be offered for this rapid rate. The smaller rate of loss in the basal leaves of the plants cut when mature or overmature was probably due to the greater maturity and lower initial chlorophyll content of these plants. After five days of curing in plants of all stages of maturity there was very little change in chlorophyll content of the basal leaves.

The changes in percentage of total chlorophyll of the basal leaves which is chlorophyll *a* are shown in the upper part of figure 1. The sharp drop in percentage chlorophyll *a* in these basal leaves corresponded much more definitely than in the top leaves to the time at which the total chlorophyll reached a low value. It may be seen that the dry matter at this time is about 30% to 35% (table II), as it was also in the top leaf samples. However, decrease in chlorophyll values does not cease altogether at this point. In these experiments the chlorophyll concentrations reached a low value some time before the leaves became dry. Other experiments in the curing chambers have indicated that this would not have been true if the relative humidity of the air surrounding the curing plants had been low instead of optimum.

The results of the carotene determinations are presented in figure 1, C. The upper group of three curves represents the changes in the top leaves, while the lower group of three curves represents the changes in the basal leaves. Each set of curves is similar to the corresponding set of chlorophyll curves; however, the carotene does not appear to be so rapidly nor so completely destroyed as is the chlorophyll. Thus the yellowing of tobacco in the field and in curing is due in part to a more rapid and complete disappearance of chlorophyll than of carotene. At the time the break in the chlorophyll concentration curve occurs, the carotene content has been reduced to about one quarter its former value. A break occurs in the carotene concentration curves at this same time, corresponding to 30% to 35% dry matter, but these curves show a greater downward trend after this point has been reached than do the chlorophyll curves.

Discussion

The decrease in the calculated chlorophyll *a* percentage shown in the upper part of figure 1 may not appear to be very large, but there is reason to believe that the actual decrease is greater than is shown by these figures. All chlorophyll concentrations and percentage chlorophyll *a* values were calculated by means of equations based on the spectrophotometric absorption values at the red maxima of pure chlorophylls *a* and *b*. In green tobacco leaves no pheophytins were found, so satisfactory values were obtained by means of these equations. A chromatographic study of the pigments present in cured tobacco showed evidence of more pheophytin *a* than of chlorophyll *a*, though no pheophytin *b* was found. The absorption of pheophytin *a* at the red maximum of chlorophyll *a* is about half the latter, which would result in a calculated chlorophyll concentration by the method used, larger

than the true concentration of chlorophyll *a* plus *b*, but smaller than the concentration of the chlorophyll plus pheophytin *a*. This was confirmed by the concentration values calculated from the absorption values at 600 mμ, where the absorption of pheophytin *a* was more nearly equal to that of the chlorophylls. Close agreement was obtained between total chlorophyll concentration calculated from the readings at 600 mμ and at the *a* and *b* maxima on fresh leaf, but on the cured tobacco samples the concentration values determined at 600 mμ were larger than those calculated by the equations based on the chlorophyll maxima. Similarly, the percentage of chlorophyll *a* calculated by means of the equation is larger than the percentage of the chlorophyll which is really chlorophyll *a;* consequently the change in percentage chlorophyll *a* is really greater than that shown in the tables.

The break in the carotene curves is just as definite as that in the chlorophyll curves but the cause is more difficult to explain. At the time the break occurred the carotene values were about one-fourth as high as in the corresponding samples at cutting time. It does not seem probable that this concentration was low enough to affect the rate of the reaction sharply. Nor does it seem probable that the moisture content was low enough to cause such a marked reduction in reaction rate. The oven dry weight at this time was 30% to 35% and moisture was 65% to 70%. The moisture content threshold below which many other chemical reactions in cured tobacco leaf are inhibited, is approximately 80% dry matter and 20% moisture. It is possible that the carotene had undergone some change, but it was still sufficiently similar to true *beta* carotene to behave normally in the magnesium oxide-celite adsorption column. No light absorption studies were made of the carotene fraction from cured tobacco. Another possible explanation of the results is that the rate of carotene destruction may be connected in some way with the chlorophyll concentration, since the break in the two curves seems to occur at the same time. This could be a case of catalysis or it could be related to the existence of a definite chromoprotein molecule containing both chlorophyll and carotene, such as has been suggested by many workers.

Summary

1. A study was made of the changes in chlorophyll and carotene content of the leaves of stalk-cut burley tobacco during the air-curing process. Leaves from the upper and lower parts of plants, cut when immature, mature, and overmature, were analyzed. The initial chlorophyll content of the leaves decreased with increasing maturity, and was higher in the upper than in the corresponding basal leaves.

2. The rate of loss of chlorophyll in the top leaves was not greatly affected by maturity, and as a result, low chlorophyll concentrations were reached in a shorter time of curing in the overmature than in the immature plants.

3. The highest rates of chlorophyll loss were observed in the basal leaves of immature plants, indicating that translocation of some substances to the immature leaves may have occurred.

4. As the total chlorophyll content of the leaves reached low values, the percentage of chlorophyll *a* based on total chlorophyll decreased.

5. The shapes of the curves showing the carotene content of the leaves were very similar to the corresponding chlorophyll curves for leaves of each degree of maturity, though the carotene did not disappear so completely as the chlorophyll.

6. A change in rate of loss of carotene and of chlorophyll and in the proportion of chlorophyll *a* all occurred at a fairly definite moisture content, though the time required to reach this point depended upon the maturity of the leaves.

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LITERATURE CITED

1. GRIFFITH, R. B., AND JEFFREY, R. N. Determining chlorophyll, carotene, and xanthophyll in plants. Ind. & Eng. Chem., Anal. Ed. **16**: 438–440. 1944.
2. GUTHRIE, JOHN D. Effect of environmental conditions on the chloroplast pigments. Amer. Jour. Bot. **16**: 716–746. 1929.
3. JEFFREY, R. N. The effect of temperature and relative humidity during and after curing upon the quality of white burley tobacco. Kentucky Agr. Exp. Sta. Bull. **407**. 1940.
4. O'BANNON, L. S. A laboratory for use in determining the influence of various temperatures and relative humidities on the final quality of air-cured tobacco. Heating, piping and air conditioning **II**: 699–702. 1939.
5. WILLSTÄTTER, RICHARD, AND STOLL, ARTHUR. Untersuchungen über die Assimilation der Kohlensäure. Julius Springer, Berlin. 1918.
6. YOUNG, JAMES R., AND JEFFREY, R. N. Changes in certain water-soluble nitrogenous constituents of burley tobacco during curing. Plant Physiol. **18**: 433–448. 1943.