Shade During Growth—Effects on Chemical Composition and Leaf Color of Air-Cured Burley Tobacco[1]

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ABSTRACT

Development of off-color in air-cured burley tobacco (*Nicotiana tabacum* L.) leaf may adversely affect the quality and value of the crop. Shading from other vegetation during growth may be a contributing factor. The purpose of this investigation was to determine the effects of shading during field growth on leaf color and chemical composition of air-cured burley tobacco. Whole leaves and lamina from air-cured burley tobacco (cv. Kentucky 14) grown under 0, 45, or 65% shade (reduced light) conditions in field (Maury silt loam soil; clayey, mixed, mesic, Typic Paleudalf) experiments were quantitatively analyzed for parameters of color (determined by reflectance), chlorophyll isomers, carotenoids, brown pigment, total phenols, total alkaloids, and nitrate-N. Whole leaves and lamina from tobacco grown under increased shade generally had more red hue, less yellow hue, less brightness, and more total color change. Lamina from tobacco grown under increased shade had higher levels of chlorophyll *a*, chlorophyll *b*, lutein, β-carotene, neoxanthin, violaxanthin, and nitrate-N, but lower contents of brown pigment and total alkaloids. Whole leaf grown under increased shade had generally higher contents of nitrate-N and lower brown pigment, total phenols, and total alkaloids. The color changes in air-cured whole leaf and lamina associated with increased shade during growth support the hypothesis that shade is one causative factor for the undesirable "pink leaf" characteristic that sometimes develops in air-cured burley tobacco, although the data do not show that it is the only factor involved.

Additional index words: ***Nicotiana tabacum*** **L., Light reflectance, Tobacco quality, "Pink leaf" characteristic, Chlorophyll, Carotenoids, Brown pigment, Total alkaloids, Total phenols, Nitrate-N.**

THE QUALITY and value of tobacco (*Nicotiana tabacum* L.) in domestic and export markets is partly determined by color of the cured leaf. Color development in the leaf depends on tobacco cultivar and type, field growth conditions, and postharvest processing.

One color problem in air-cured burley tobacco that adversely affects its quality and value is the "pink leaf" characteristic. Affected leaves have a pale pink hue. The cause of the disorder is unknown, but it appears to result from the growth of tobacco under partial-shade conditions. This may occur in low lying fields near large trees, on shaded hillsides, or in other locations that provide shading sometime during the day, other than that attributable to the tobacco plant canopy itself. Shading caused by the tobacco leaf canopy itself decreases light intensity and alters the ratios of incident light wavelengths that reach leaf photoreceptors (Kasperbauer, 1971) and additional shading from other plants that act as light filters may also alter spectral distributions of light. Chen and Huang (1970) found that the rate of change in tobacco leaf weight per unit increase in leaf area was positively related to light intensity. The purpose of this investigation was to determine the quantitative effects of controlled levels of shading during field growth on leaf color and chemical composition of air-cured burley tobacco.

MATERIALS AND METHODS

Plants

Experiments were conducted in 1980 and 1982. In the 1980 experiment, burley tobacco (*Nicotiana tabacum* L. cv Kentucky 14) was grown in field plots on the Kentucky Agricultural Experiment Station (KAES) Spindletop Farm in Lexington. The soil was a Maury silt loam (clayey, mixed, mesic, Typic Paleudalf). Recommended cultural and fertilization practices were followed during the growing season (Atkinson et al., 1976). A randomized complete block experimental design was used. Each treatment plot was a row of 12 plants spaced 45 cm apart with parallel border rows 1 m apart. When plants reached a height of 50 cm, each plot was either left unshaded (control treatment) or a light barrier was erected that transmitted either 55 or 35% of the incident light (i.e., 45 or 65% shade, respectively) and increased the transmittance of far red (735 nm) relative to red (650 nm) radiation by about 5% (Table 1). The rectangular fabric light barrier was green polyvinylidene chloride mesh mounted as a canopy on a wooden frame. It extended from ground level to 2 m in height and was 1 m wide and 5.5 m long at the top.

Three treatments were used in the experiment, i.e. no shade, 45% shade, and 65% shade, and each treatment was replicated three times. Inflorescences and small upper leaves were removed when 25% of the plants had one or more open flowers. Axillary buds were removed from all plants by hand. After harvest the tobacco was conventionally air-cured. Air-cured whole leaf samples were prepared from 12 plants of each treatment at three stalk positions. The upper leaf position on the stalk corresponded to the topmost section on the growing plant that had one-third of the total leaves; the middle and lower positions corresponded to the two successively lower sections that had one-third of the total leaves each. Separate leaf samples were prepared for each replicate of shade treatment × stalk position. Samples were ground to 100- to 200-mesh size and then equilibrated overnight to ambient moisture content. The samples were then stored in sealed containers in a refrigerator until analyzed.

The 1982 experiment was carried out with the same cultivar, cultural conditions, treatments, and curing conditions used in the 1980 experiment, except that field plots were located on the KAES South Farm in Lexington. The soil was a Maury silt loam. There were three replicates. Five plants from each treatment were conventionally air-cured and then sampled. Leaves were grouped into the top, middle, and bottom stalk positions. Midveins were separated from lamina immediately after curing. Cured samples of lamina were ground and stored in the same manner described for whole leaves in the first (1980) experiment.

Leaf Color Measurements

A Model D25M/L-2 Hunter Color Difference Meter[3] was used to measure differences in reflected color from samples of cured leaf samples ground to 100- to 200-mesh size. The instrument was calibrated with a pink tile (CMR0060) that had color coordinate values of $L = 54.0$, $a = +31.8$, and

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Table 1. Relative transmission of sunlight and shift in far-red/red (FR/R) light ratios below shade materials† relative to unshaded control.

Shade material	Wavelength, nm								FR/R ratio‡
	500	550	600	650	700	735	750	800	
	Transmission, % of unshaded control								
1	54.2	54.1	53.0	53.8	55.5	55.4	56.5	58.5	1.03
2	35.3	35.7	32.9	33.3	34.6	35.3	35.4	38.3	1.06

† as measured with a Model LI-1800 LiCor spectroradiometer placed 30 cm below shading material.
‡ Transmission ratio (far-red/red) 735 nm/650 nm relative to value in direct sunlight that was arbitrarily set at 1.00.

$b = +10.0$. The a and b chromaticity parameters and the L coordinate parameter are defined in the laboratory manual for this instrument (Hunter Assoc. Laboratory, Inc., Fairfax, VA). Total color difference ($\Delta\epsilon$) was estimated according to the relationship $\Delta\epsilon = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2$.

Chemical Analysis

Beta-carotene, lutein, neoxanthin, violaxanthin, chlorophyll *a*, and chlorophyll *b* were determined by modifications of the high-performance liquid chromatography (HPLC) plant pigment analytical method (Eskins et al., 1977) and sample preparation procedure (Eskins and Dutton, 1979). A 500-mg sample of cured tobacco was extracted with 20 mL of acetone for 1 h on a micro-Soxhlet extractor. The acetone extract and acetone washings were passed through a Waters Sep-Pak μBondapak C_{18} reverse-phase cartridge, and the eluate was diluted to 25 mL with acetone. A 100-μL aliquot was injected into a Varian Model 5000 HPLC system equipped with a 3 by 300 mm μBondapak C_{18} column and a Vari-Chrom variable wavelength detector set for 436 nm. The following solvent system was used at a 5 mL/min flow rate: 90:10 methanol/H_2O from 0 to 5 min, then solvents were programmed linearly to reach 45:5:50 methanol/H_2O/ethyl acetate at 15 min and this solvent composition was maintained until 35 min. Chromatographic peaks obtained with a sample were compared to standard curves based on peak height responses of reference compounds.

Quantitative estimations of brown pigment (main pigment band) in cured lamina were made according to the previously described method (Andersen et al., 1970), except that a 500 mg sample was used and spectrophotometric readings were made at 205 nm. Total soluble phenols were estimated by spectrophotometric measurement of the reduction of Folins phenol reagent and determination of the difference in absorbance before and after the addition of insoluble polyvinylpyrollidone (Andersen and Todd, 1968). Total alkaloids were determined spectrophotometrically at 460 nm after reaction with cyanogen bromide and aniline (Harvey et al., 1969). Results were expressed as nicotine equivalent. Nitrate analyses were carried out by a spectrophotometric method as previously described (Anderson et al., 1982) after reduction of nitrate to nitrite with a nitrate reductase preparation isolated from *E. coli*. Corrections for endogenous nitrite were performed. Concentrations of all determined components were on an ambient moisture–equilibrated basis.

RESULTS AND DISCUSSION

Leaf Color

Color measurement values of air-cured tobacco grown under nonshaded and shaded conditions are given in Table 2. The a and b chromaticity parameters and the L coordinate parameter are mathematically related to the tristimulus values adopted by the International Commission on Illumination (Hardy, 1936). The parameters designate color as follows: a measures redness when positive (+), grey when zero, and greenness when negative (−); b measures yellowness when positive (+), grey when zero, and blueness when negative (−); L measures lightness or brightness and can vary from 100 for perfect white to zero for black, approximately as the human eye would evaluate it. The results indicate the presence of more red and yellow hues and more brightness in leaf lamina from lower leaf positions. Unshaded and 45% shaded whole leaves from the upper and middle positions showed no significant differences in redness (a values), but lower a values were obtained in these positions for the 65% shaded whole leaves. Lower a values occurred in whole leaves from the lower leaf position on the stalk for all shade treatments. Thus, the direction of the stalk position effect on a values in whole leaf differed from that for lamina. There was a statistically significant ($p < 0.05$) shade treatment × stalk position interaction for the a chromaticity values in whole leaf samples, but not in lamina samples. Successively lower positions on the stalk for a given shade treatment yielded whole leaf samples with either no significant change in b values (yellowness) or increasingly larger positive (+) values; lower positions on the stalk yielded whole leaf with increasingly larger L values. Thus, the direction of the stalk position effect on b and L values was the same for samples of whole leaf and lamina.

Table 2. Effects of shade during growth on values of color parameters for air-cured tobacco leaves.

% Shade†	Leaf position on stalk	Chromaticity a‡ Whole leaf	Chromaticity a‡ Lamina	Chromaticity b§ Whole leaf	Chromaticity b§ Lamina	Lightness, L¶ Whole leaf	Lightness, L¶ Lamina
		1980	1982	1980	1982	1980	1982
		meter readings					
None	Upper	+11.2	+5.45	+19.0	+12.1	42.4	42.0
45	Upper	+12.2	+5.60	+19.2	+12.0	42.1	42.0
65	Upper	+12.3	+4.53	+17.8	+10.6	39.6	40.7
None	Middle	+11.1	+6.68	+20.0	+12.7	45.2	42.5
45	Middle	+12.2	+7.57	+19.0	+12.6	43.1	43.1
65	Middle	+11.9	+6.53	+18.7	+11.5	43.1	42.1
None	Lower	+9.42	+7.28	+20.2	+13.1	49.2	44.0
45	Lower	+9.67	+7.62	+19.3	+13.1	47.7	45.1
65	Lower	+9.43	+6.83	+19.0	+12.4	46.9	44.6
LSD 0.05		0.31	0.61	0.6	0.6	1.7	0.9

† Approximate value (see Table 1).
‡ Increased positive value = increased red hue.
§ Increased positive value = increased yellow hue.
¶ Increased positive value = increased brightness.

Because of the stalk position effect on color values, the interpretation of the effects of shading during growth on the a, b, and L values of air-cured leaf (Table 2) were based on comparisons made at the same stalk position. Air-cured lamina and whole leaf grown in 45% shade had increased redness, i.e., larger positive (+) a values when compared with corresponding a values for lamina and whole leaf from unshaded plants. These differences were not always significant at the 5%

[3] The mention of proprietory products or materials does not imply endorsement of these products or materials by any parties, including the USDA or the Univ. of Kentucky.

Table 3. Effects of shade during growth on total color of air-cured tobacco leaves.

% Shade†	Leaf position on stalk	Total color difference ($\Delta\epsilon$)‡ between air-cured shade-grown and nonshade-grown leaves from same stalk position	
		Whole leaf (1980)	Lamina (1982)
45	Upper	1.06	0.17
45	Middle	2.66	1.12
45	Lower	1.68	1.12
65	Upper	3.24	2.11
65	Middle	2.60	1.08
65	Lower	2.48	1.04

† Approximate value (see Table 1). ‡ $\Delta\epsilon = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2$.

Table 4. Effects of shade during growth on chlorophyll and carotenoid pigments in air-cured tobacco.

% Shade†	Leaf position on stalk	Chlorophyll		Carotenoid			
		a	*b*	Lutein	β-Carotene	Neoxanthin	Violaxanthin
		μg pigment/g air-cured leaf lamina					
None	Upper	60	29	164	137	10	2
45	Upper	91	20	314	177	23	16
65	Upper	310	123	440	197	40	17
None	Middle	51	20	157	115	6	2
45	Middle	41	20	323	169	17	13
65	Middle	110	43	419	194	25	17
None	Lower	9	ND‡	76	58	ND	ND
45	Lower	23	20	138	89	7	ND
65	Lower	27	22	187	92	13	7
LSD 0.05		42	34	40	9	4	4

† Approximate value (see Table 1). ‡ ND = not detected.

level. Lamina and whole leaf grown in 65% shade, on the other hand, generally had lower positive (+) *a* values than 45% shade-grown or unshaded samples. In general, air-cured leaf grown under increased shade conditions had lower positive (+) *b* values, i.e., less yellowness, although the differences were not always significant at the 5% level. Also, air-cured whole leaf samples grown under increased shade conditions had lower *L* values, i.e., less brightness. Air-cured lamina, however, did not exhibit the same trend in *L* values (cf. lamina from 45% shade-grown and unshaded tobacco). In the latter case, *L* values were significantly higher than those for lamina from non shade-grown tobacco.

Total color difference ($\Delta\epsilon$) values between shade-grown and nonshade-grown lamina and whole leaf samples from different stalk positions are given in Table 3. The results show more color difference in the 65% shade-grown than in the 45% shade-grown lamina of the upper leaf position, but little difference due to shade occurred at the other stalk positions. Air-cured whole leaf samples generally showed more total color difference than corresponding lamina samples and the magnitudes of the differences were greater in the 65% shade-grown samples than in the 45% shade-grown samples. Total color difference values are probably dependent upon net changes in leaf pigment levels.

Chemical Composition

The effects of shade during growth on concentrations of chlorophyll and carotenoid pigments in air-cured tobacco leaf lamina are given in Table 4. The mean concentration ratios of chlorophyll *a*/chlorophyll *b*/lutein/β-carotene/neoxanthin/violaxanthin were 9:4:30:16:2:1. Lamina from successively lower positions on the stalk for a given shade treatment had decreased levels of chlorophyll *a*, chlorophyll *b*, lutein, β-carotene, neoxanthin, and violaxanthin. There were few exceptions to this trend, although in some instances the pigment concentration differences were not significant at the 95% level of confidence. A similar direction of stalk position × pigment concentration effects occurred in leaves sampled at harvest (Andersen et al., 1980, unpublished results).

There were significant ($p < 0.05$) shade treatment × stalk position interactions for chlorophyll *a*, lutein, β-carotene, neoxanthin, and violaxanthin in lamina. The stalk position effect on chlorophyll and carotenoid pigment concentrations made it necessary to interpret the effects of different degrees of shading at the same stalk position (Table 4). In general, air-cured leaf lamina from tobacco grown in 45% shade had higher levels of individual chlorophyll and carotenoid pigments than lamina from unshaded tobacco. Similarly, lamina from 65% shaded plants had higher individual chlorophyll and carotenoid levels than from 45% shaded plants. The higher orange-yellow–colored carotenoid concentrations in lamina from plants grown in more shade (Table 4) seemed consistent with the positive increases in the *a* color (red) coordinate values found in lamina from the 45% shade-grown compared to nonshaded plants. However, the *b* coordinate values (yellow) were less positive in lamina from the more shaded plants. This was contrary to the expectation that increased carotenoid levels would increase the net amount of yellow hue. It, therefore, seemed probable that components other than chlorophylls and carotenoids contributed to air-cured leaf color.

The effects of stalk position and shade during growth on concentrations of brown pigment, total phenols, total alkaloids, and nitrate-N in air-cured tobacco are given in Table 5. Successively lower positions on the stalk for a given shade treatment generally yielded samples with decreased levels of brown pigment and total alkaloids. In the case of total phenols, however, there was no consistent stalk position effect in lamina. Whole leaf from lower positions on the stalk corresponding to the same shade treatment generally had lower levels of total phenols. Specific phenols such as anthocyanins would be expected to contribute significantly to the color of tobacco. The presence of anthocyanins in cured tobacco leaf, however, has not been reported. In contrast with the direction of the stalk position effect on brown pigment, total phenols, and total alkaloids in leaf, nitrate-N contents were increased in lamina and whole leaves from successively lower positions on the stalk for a given shade treatment.

In general, air-cured leaf lamina taken from the same stalk position of tobacco grown under increased shade had lower levels of brown pigment and total alkaloids, but higher levels of nitrate-N. Whole leaf samples of tobacco grown under increased shade had generally lower levels of brown pigment, total phenols, and total alkaloids, but higher contents of nitrate-N. There were significant ($p < 0.05$) shade treatment × stalk position interactions for nitrate-N in lamina and whole leaf samples.

Table 5. Effects of shade during growth on phenols, brown pigment, alkaloids, and nitrate in air-cured tobacco leaves.

% Shade†	Leaf position on stalk	Brown pigment, absorbance/60 mL/0.5 g		Total phenols (chlorogenic acid equivalent)		Total alkaloids (nicotine equivalent)		Nitrate-N	
		Whole leaf	Lamina	Whole leaf	Lamina	Whole leaf	Lamina	Whole leaf	Lamina
		1980	1982	1980	1982	1980	1982	1980	1982
						mg/g			
None	Upper	2.41	1.81	3.6	1.6	38.5	54.2	0.7	2.2
45	Upper	1.70	1.96	2.3	4.0	42.0	56.3	1.1	3.0
65	Upper	1.41	1.46	2.1	3.3	40.5	50.5	2.9	6.1
None	Middle	1.50	2.21	2.7	6.2	37.5	56.7	1.5	2.8
45	Middle	1.26	1.52	1.9	4.1	36.0	51.0	3.9	5.6
65	Middle	1.28	0.91	1.8	2.6	34.5	44.0	6.0	10.0
None	Lower	1.37	1.09	1.7	4.2	24.5	39.5	3.1	5.2
45	Lower	1.15	0.94	2.0	2.4	25.0	33.0	7.6	12.1
65	Lower	1.29	0.67	1.9	3.1	43.5	29.8	9.7	17.9
LSD 0.05				0.6	0.6	4.1	7.1	0.9	1.6

† Approximate value (see Table 1).

Brown pigments and total phenols are known to play a role in the color of cured tobacco leaves (Andersen et al., 1970). It is also possible that total alkaloids influence the color of tobacco leaves based on the report of Chaplin and Weeks (1976) and the visual observations of Gupton et al. (1972) that indicated an increased wood color of burley tobacco stalk cross sections was associated with higher leaf alkaloid levels for samples from tobaccos of divergent alkaloid genotype. Nitrate-N presumably does not affect color directly, but it is a biochemical precursor of alkaloids.

The general trend of color change in air-cured leaves associated with different shade treatments of the tobacco during growth was that increased shade produced more red hue, less yellow hue, less brightness, and more total color change. It is suggested that some of these color changes associated with increased shading may be analogous to the undesirable "pink leaf" characteristic in burley tobacco. The *a* and *b* chromaticity values for shaded leaves more nearly approximated the pink tile standard values than those for unshaded leaves. Color changes were associated with quantitative differences in chemical composition for leaves grown under different amounts and quality of incident daylight. The shade materials caused some alteration of far-red relative to red radiation reaching the plants; both 45 and 65% shading caused a small increase in far-red relative to red radiation. Increased far red/red ratios were recently shown to alter tobacco chloroplast development and leaf morphology (Kasperbauer and Hamilton, 1984). In addition to chemical composition changes in cured leaves caused by shade during growth, there were some physical differences in leaves. Leaves from shaded plants were thinner and had longer internodes than from nonshaded plants (Andersen and Kasperbauer, 1982, unpublished data). It is probable that differences in leaf thickness contributed to differences in the concentrations of some chemical components.

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