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EFFECT OF LIGHT AND TEMPERATURE ON EPICUTICULAR FATTY ACID AND FATTY ALCOHOL OF TOBACCO

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Abstract—Genetically uniform burley tobacco (*Nicotiana tabacum*) was grown under field and various controlled-environment conditions to determine whether environment influenced epicuticular alkane, fatty acid, and fatty-alcohol composition of the leaves. Quantity and quality of alkanes, fatty acids, and fatty alcohols were greatly influenced by environmental conditions. Highest light intensity did not result in the largest total long aliphatic carbon-chain production. Generally, long photoperiod and cool temperature were associated with highest long aliphatic carbon-chain production on a leaf area basis. Quantity of the individual alkane, fatty acid, or fatty alcohol classes present under the different growth conditions varied in relation to the leaf metabolic status and not leaf size.

INTRODUCTION

Leaf epicuticular-wax quantity and quality were shown to be controlled by environmental parameters and chemicals in *Cassia obtusifolia* L. [1]. Tobacco (*Nicotiana tabacum* L.) epicuticular alkane quality is influenced by photoperiod, temperature and leaf age [2]. Fatty acids are precursors to alkanes in peas (*Pisum sativum* L.) and spinach (*Spinacia oleracea* L.) [3] and to primary alcohols in broccoli (*Brassica oleracea* L.) [4]. Also, alkanes can be converted to secondary alcohols and ketones in broccoli [5]. Hence, the quantity of each of these constituents in leaf epicuticular wax appears to depend upon the plant species and the environment during leaf development.

Tobacco leaf quality has been evaluated on the basis of lipid extracts [6]. Leaf waxes have been studied relative to their role in smoking and health [7]. Stage of maturity and curing of flue-cured tobacco leaves influence the fatty acid content [8, 9], and yearly differences have been reported [10]. However, data on environmental influence on burley tobacco epicuticular wax quantity and quality were not available. Therefore, experiments were undertaken to measure leaf epicuticular wax contents on burley tobacco leaves developed under different temperature and light regimes.

RESULTS AND DISCUSSION

In order to measure effects of growth environment on wax composition of burley tobacco, leaves were first characterized for size and mass. These leaf characters were then related to the content of and relationship among alkanes, fatty acids and fatty alcohols accumulated on leaves that developed under the various environments.

Leaf size

Leaf area was influenced by the temperature and light environments under which the leaves developed (Table 1). Under the 18° controlled environments, leaves from plants grown under short photoperiods were smaller in area than leaves from plants grown under long photoperiods. However, under long photoperiods, increased temperature resulted in decreased leaf size. Thus, a temperature–photoperiod interaction existed in tobacco leaf area development. Field-grown tobacco leaves were much larger than growth chamber-grown leaves, and this is attributed to the increased quantity of light available for photosynthesis in the field.

Lamina density (mg dry wt/cm^2) was increased by long photoperiods at both 18 and 28°, being highest under long photoperiods at 28°. Density of field-grown leaves was intermediate to densities of leaves from plants grown at 18 and 28° under long photoperiods.

The plants were genetically uniform, and light intensities available for photosynthesis were essentially the same in the short and long photoperiod environments because the low-intensity light used as a night interruption to obtain long photoperiods was insignificant for photosynthesis. Thus, the increased size and density of leaves from plants grown at 18° were photoperiodic responses. At 28°, the leaves were smaller and heavier from plants grown under long photoperiods than were leaves from plants grown under short photoperiods. If we assume that each metabolic unit of leaf material contains the same inherent capacity for dry wt production at uniform temperature and light-intensity conditions, then photoperiodic modification of the capacity of that unit of leaf material to expand would decrease the dry wt per unit area in photoperiods which induce the greatest leaf growth. Similarly, under uniform photoperiods, increases in temperature would result in an increased size up to an optimum temperature; thereafter, increased temperature would result in reduced dry wt per unit area. The

Table 1. Physical characteristics of burley tobacco leaves obtained from different temperature and light regimes

Growth environment Temperature(°)	Photoperiod	Whole leaf area*(cm²)	Lamina mass/area*† Fresh (mg/cm²)	Dry	Total wax per leaf (mg)
18	Short	501.4‡	27.8	2.7	2.8
18	Long	576.7	24.0	3.1	4.2
28	Short	561.5	22.5	2.6	1.8
28	Long	436.0	24.0	4.4	1.9
35	Long	318.2	22.5	1.8	1.1
Field	Field	818.3	24.0	3.3	1.7
$S_{\bar{x}}$		18.7	0.4	0.2	

* Area based on one surface of leaf.

† Based on two 50 cm² leaf-discs taken from each leaf.

‡ Entries are means for 10 leaves (the 2 most recently fully expanded leaves from each of 5 plants).

data in Table 1 support such a hypothesis in relation to the temperature responses. Leaf area and leaf density responses varied in relation to photoperiod at 18 and 28°. For example, lamina density was greatest in leaves from plants grown under long photoperiods at 28°, yet the leaf area was significantly less than that of plants grown under shorter photoperiods or lower temperatures. It is apparent that growth and metabolism respond differently to variations in environmental parameters. Thus, the question arises as to whether epicuticular wax quantity and quality depend on the rates of growth or metabolism as influenced by environment.

Total epicuticular wax composition

Total wax (mg/leaf) on leaves varied with growth environments (Table 1). At 18°, leaves from plants grown under long photoperiods had more wax than leaves from plants grown under short photoperiods; however, when plants were grown at 28°, there was no difference in the total wax production on leaves grown in long or short photoperiods. Increased temperature resulted in decreased total wax content at both photoperiods.

On an area basis, increased photoperiod induced a higher content of total wax (ng/cm²) on leaves from plants grown at both 18° and 28° (Table 2). Increased temperature decreased the total wax under both long and short photoperiods. However, the quality of the wax was altered by the environment under which the plants were grown. Alkane and fatty-acid contents were decreased on leaves from plants grown under long photoperiods at 18° and increased at 28°, but fatty-alcohol content showed a response to photoperiod that was the reverse of that shown for the alkanes and fatty acids. Higher temperature resulted in a reduction of alkane and fatty-acid contents under short photoperiods; but, under long photoperiods, alkane and fatty-acid contents were highest at 28°. Again, fatty-alcohol content responses were inversely related to leaf alkane and fatty-acid contents. Thus, on an area basis, the quality of the epicuticular wax composition was a function of the metabolic status of each leaf rather than a uniform production per unit of leaf tissue whose size was varied as a response of the leaf growth to environmental parameters.

On a dry wt basis, the total epicuticular wax content was highest at 18° (Table 2). Alkane and fatty-alcohol contents showed the same relationships on a dry wt basis as were observed on a leaf-area basis; whereas, fatty-acid content was greatest under short photoperiods at 18° but was not significantly altered by photoperiod at 28°. Fatty-acid content was decreased by elevated temperature under short photoperiods and increased by elevated temperature when the plants were grown under long photoperiods.

Thus, wax quantity and quality were responsive to the metabolic status of the leaves and were not greatly

Table 2. Quantity of epicuticular alkanes, fatty acids, and fatty alcohols of tobacco leaves grown under different temperature and light regimes

Growth environment		Leaf area basis*				Dry wt basis				Ratio of components		
Temp. (°)	Photoperiod	Alkanes (ng/cm²)	Fatty acids (ng/cm²)	Fatty alcohols (ng/cm²)	Total (ng/cm²)	Alkanes (μg/g)	Fatty acids (μg/g)	Fatty alcohols (μg/g)	Total (μg/g)	Alkanes (%)	Fatty acids (%)	Fatty alcohols (%)
18	Short	1336	2334	1935	5605	495	864	717	2076	23.8	41.7	34.5
18	Long	411	1114	5779	7304	133	359	1864	2356	5.6	15.3	71.9
28	Short	325	1306	1524	3155	125	502	586	1213	10.3	41.4	48.3
28	Long	1030	2549	840	4419	234	579	191	1004	23.3	57.7	19.0
35	Long	233	1522	1582	3337	129	846	879	1854	7.0	45.6	47.4
Field	Field	500	967	557	2024	152	293	169	613	24.7	47.8	27.5
$S_{\bar{x}}$		115	291	99		42	107	37				

Each value is the mean of 10 analyses.

* Leaf area based on one surface of leaf.

influenced by the growth (i.e. increase in size) patterns. Consequently, leaf growth and epicuticular wax accumulation responded differentially to environment. Light intensity did not appear to be significant in the development of any factor except leaf size (Tables 1 and 2). These data suggest that the various leaf metabolic systems are highly responsive to environment during leaf growth and development.

Fatty acid and fatty alcohol composition

The various classes were influenced by environment (Table 3. Even-carbon-numbered fatty acids were slightly higher under long photoperiods and decreased in amount with increased temperature. Odd-carbon-numbered fatty acids were decreased under long photoperiods and increased by increasing temperature from 18 to 28°. Unsaturated fatty acids followed the same pattern as the even-carbon-numbered fatty acids. However, the amounts of *anteiso*- and *iso*-fatty acids were lower under long photoperiods at 18°, essentially unaltered by photoperiod at 28°, and increased from 18 to 35° under long photoperiods.

Fatty-alcohol contents exhibited differences in response among leaves grown under differing photoperiods and temperatures. Since epicuticular fatty acid and fatty alcohol class totals were altered by environment on genetically uniform leaves, the possibility arose of a random synthesis within a single subclass as opposed to the synthesis of a single product as a major unit within each subclass.

Individual epicuticular fatty acid and fatty alcohol contents

Tables 4 and 5 indicate that within each class the individual fatty-acid and fatty-alcohol contents varied between the various environmental conditions. These variations in contents were found in individual components and also developed different patterns of component accumulation within each class.

Current biosynthetic hypotheses suggest that short chain fatty-acid synthesis occurs with elongation of the aliphatic-chain to long carbon chains: (a) unsaturates (i.e. $-C_{14:1} \rightarrow C_{24:1}$) [11]; (i.e. $-C_{18:0} \rightarrow C_{18:1} \rightarrow C_{18:2}$) [12] (b) even-carbon-chain saturates (i.e. $-C_{12} \rightarrow C_{28}$) [11]; (c) odd-carbon-chain saturates (i.e. $-C_{13} \rightarrow C_{27}$) [11]; and (d) branched-chain aliphatic units are derived from deaminated valine and isoleucine [11]. Then, fatty acids are converted to alcohols and alkanes [11]. Thus, the total variability of epicuticular wax quality and quantity on these genetically uniform leaves appears to be a reflection of the activity of the individual enzymatic processes under each environmental condition. These processes include, but are not limited to, aliphatic carbon-chain synthesis, desaturation, reduction and other modification; amino-acid metabolism; and the multiplicity of factors influencing leaf growth. Consequently, the epicuticular wax present on naturally grown leaves will be a summation of these constantly changing synthetic systems which respond to environmental parameters.

EXPERIMENTAL

Nicotiana tabacum L. cv Burley 21 plants were started and grown for *ca* 6 weeks at 28° under 14-hr photoperiods at 16 000 lx from cool-white fluorescent lamps. Uniformly sized seedlings were transplanted to a field plot or transferred to pots of soil and placed in controlled-environment chambers. Plants transferred to the controlled-environment chambers were transplanted to the same type of soil as were those transplanted to the field.

Growth environments. Plants grown under the field environment were exposed to natural illumination and temperature at Lexington, KY from 23 June to 24 July. Others were exposed to the controlled environments for the same period. Temps in the controlled environments were 18, 28, and 35°. All 5 chambers were illuminated for 8 hr each day from VHO cool-white fluorescent lamps at an intensity of 22 000 lx. Plants that received short photoperiods remained in darkness for the other 16 hr in each diurnal cycle. The long-photoperiod treatment consisted of the basic 8-hr day plus a 4-hr interruption in the middle of the night at 250 lx.

Sampling procedure. The 5th and 6th leaves from the apex (excluding all leaves shorter than 8 cm) were collected from

Table 3. Percentages of fatty acid and fatty alcohol classes of burley tobacco leaves grown under different temperature and light regimes

Growth environment		Class				
Temp(°)	Photoperiod	Even	Odd	Unsaturated	*Anteiso*	*Iso*
				Fatty acid		
18	Short	43.2	18.1	25.0	9.8	3.9
18	Long	47.9	8.2	37.3	5.7	0.9
28	Short	40.5	24.3	27.0	7.7	0.5
28	Long	46.9	10.3	34.1	8.4	0.3
35	Long	44.2	17.8	15.2	7.2	15.6
Field	Field	44.3	23.8	21.4	6.1	4.4
				Fatty alcohol		
18	Short	30.8	26.8	3.5	9.3	29.6
18	Long	21.4	1.2	74.5	0.0	2.9
28	Short	36.3	15.9	7.7	11.6	28.5
28	Long	36.3	23.5	9.3	10.3	20.6
35	Long	32.1	3.7	59.1	0.0	5.1
Field	Field	31.8	4.2	51.7	0.2	12.1

Each value is the mean of 10 analyses.

Table 4. Percentages of total epicuticular fatty acids within classes on burley tobacco leaves grown under different temperature and light regimes

C_n	18° Short	18° Long	28° Short	28° Long	35° Long	Field	$S_{\bar{x}}$
11	0.3	0.3	0.3	0.4	0.4	0.3	0.08
13	0.1	0.2	0.4	0.4	0.4	1.5	0.42
15	0.6	0.4	1.9	1.1	0.8	5.0	0.78
19	1.7	1.8	7.5	1.6	2.0	7.6	0.82
21	3.2	2.1	6.2	0.9	1.7	6.3	1.27
23	2.3	2.0	4.4	1.0	2.7	3.1	1.27
25	7.1	0.6	3.0	2.2	5.4	0.0	0.97
27	2.9	0.7	0.7	2.8	4.3	0.0	0.92
12	0.1	0.1	0.3	0.3	0.2	0.7	0.10
14	0.1	0.1	0.2	0.4	0.7	2.9	0.55
16	2.4	4.2	3.0	3.1	3.0	5.2	0.31
18	15.6	19.5	10.9	18.9	9.6	9.0	1.84
20	4.4	5.4	6.9	5.8	2.6	9.1	0.69
22	3.8	3.3	9.7	3.0	3.7	3.8	1.22
24	7.4	6.2	7.2	8.2	12.9	10.5	1.64
26	7.2	6.8	2.3	3.8	6.6	3.0	1.56
28	2.3	2.4	0.0	3.5	4.5	0.0	1.22
i-14	0.0	0.0	0.2	+	0.2	0.1	0.09
i-16	0.1	0.0	+	+	0.2	1.0	0.26
i-18	0.0	0.2	0.0	0.0	7.5	1.0	0.94
i-20	0.0	0.0	0.3	0.0	0.5	0.0	0.14
i-22	1.5	0.8	0.0	0.2	0.7	1.0	0.43
i-24	1.1	0.0	0.0	0.0	3.5	1.3	0.74
i-26	1.2	0.0	0.0	0.0	3.0	0.0	0.91
a-13	0.0	0.0	0.0	0.0	0.2	0.0	0.04
a-15	0.1	0.0	+	0.2	+	1.3	0.21
a-17	0.0	0.0	0.0	0.0	0.1	0.5	0.13
a-19	0.2	0.0	0.3	0.0	0.5	0.0	0.16
a-21	0.9	1.0	0.9	1.1	1.8	2.1	0.26
a-23	3.6	4.7	6.5	5.4	4.2	2.3	0.67
a-25	5.0	0.0	0.0	0.8	0.5	0.0	1.05
a-27	0.0	0.0	0.0	0.8	0.0	0.0	0.31
16:1	0.6	1.3	0.3	0.5	1.1	2.9	0.38
18:1	11.1	15.6	10.1	12.8	5.8	7.6	1.22
18:2	9.9	16.2	12.4	17.7	3.9	7.5	1.44
18:3	3.5	4.3	4.2	3.1	4.4	3.4	0.45

+ = less than 0.05% of total; *i* = *iso*; *a* = *anteiso*.

each of 5 plants from each of the 6 environments. The leaves included in these samples were the most recent to attain max expansion, and their entire growth period occurred under the respective controlled and field environments. The leaves were weighed and areas determined by planimetry. A 50 cm^2 leaf sample was removed from each half of each leaf to obtain fr and dry wts. The leaves were transferred to Experiment, Georgia, for analyses.

Extraction. Epicuticular waxes from each of 10 leaves were extracted with boiling $CHCl_3$ in two 20-sec washes. After the addition of: (a) 1 mg *n*-docosane (*n*-C_{22}), (b) 1 mg *n*-heptadecanoic acid (*n*-C_{17}) and (c) 1 mg *n*-heptadecanol (*n*-C_{17}) as int. standards for alkanes, fatty acids, and fatty alcohols, respectively, each total sample was split into 3 aliquots for analyses.

Analyses. Alkanes were separated from the remainder of the lipids by TLC [13] and separated and quantitated by GLC using dual FID apparatus equipped with 183 cm × 3.2 mm od stainless steel columns containing 10% OV-1 on Chromosorb W(AW) (DMCS). Oven temps were prog from 70 to 300° at 6°/min with an upper limit hold of *ca* 5 min. Identification of the compounds was made by the relative elution technique [14], using previously-purified tobacco alkanes for comparison.

Fatty acid Me esters were produced by introducing the $CHCl_3$ soln into a 100 ml vol flask, evaporating the $CHCl_3$ at 40° to a vol of *ca* 3 ml, adding 20 ml MeOH + 1 ml conc.H_2SO_4 + 5 ml C_6H_6 and heating for 16 hr at 60° [1]. Me esters were separated by TLC [13] and quantitated by GLC using 183 cm × 3.2 mm od stainless steel columns packed with 10% stablized DEGS in Chromosorb G(AW) (DMCS). Oven temps were prog from 70 to 220° at 4°/min with an upper limit hold of 12 min. Additionally, Me esters were analysed by OV-1 columns used for alkane analysis. Identification of the compounds was made by the relative elution technique [14] using authentic compounds for comparison.

Fatty-alcohol formyl esters were produced by introducing the $CHCl_3$ soln into a 100 ml vol. flask, evaporating the

Table 5. Percentages of total epicuticular fatty alcohols within classes on burley tobacco leaves grown under different temperature and light regimes

C_n	18° Short	18° Long	28° Short	28° Long	35° Long	Field	$S_{\bar{x}}$
13	0.0	0.0	0.1	0.0	0.3	0.0	0.07
15	0.6	0.4	1.1	0.8	3.4	2.1	0.42
19	0.9	0.0	1.4	5.2	0.0	0.0	0.40
21	0.7	0.3	1.5	3.8	0.0	0.6	0.15
23	3.2	0.5	3.4	2.2	0.0	0.7	0.54
25	3.6	0.0	6.5	7.6	0.0	0.7	0.96
27	17.8	0.0	1.9	3.8	0.0	0.1	2.22
14	2.0	0.0	2.6	3.4	1.2	0.2	0.10
16	0.5	13.6	6.1	1.0	13.9	11.5	0.80
18	9.2	4.5	11.5	16.6	5.5	5.9	0.58
20	3.3	1.6	2.2	1.2	1.2	4.9	0.91
22	2.4	0.4	2.8	3.8	0.2	6.5	1.50
24	0.0	0.6	1.4	3.7	7.9	1.6	0.53
26	9.3	0.7	8.5	4.5	2.3	0.9	0.32
28	4.1	0.0	1.1	2.0	0.0	0.2	0.42
i-14	+	0.0	0.1	+	0.0	0.1	0.02
i-16	0.2	0.0	0.5	0.1	0.0	0.1	0.14
i-18	3.0	1.3	4.6	4.2	0.0	4.3	0.50
i-20	3.5	0.0	4.8	1.9	1.8	0.9	0.22
i-22	3.0	0.0	2.4	3.3	+	4.1	0.97
i-24	3.4	1.3	3.9	1.2	1.7	2.6	0.29
i-26	5.9	0.3	11.0	7.2	1.5	0.1	0.45
i-28	10.6	+	1.2	2.7	0.0	0.0	1.48
a-15	0.1	0.0	1.1	0.3	0.0	+	0.11
a-17	1.0	0.0	3.8	3.1	0.0	0.0	0.51
a-19	0.2	0.0	1.8	2.3	0.0	0.0	0.24
a-21	0.4	0.0	0.9	1.5	0.0	0.0	0.11
a-23	2.0	0.0	1.9	0.0	0.0	0.2	0.26
a-25	1.3	0.0	0.0	2.7	0.0	0.0	0.20
a-27	4.4	0.0	2.1	0.5	0.0	0.0	0.68
16:1	0.0	+	0.0	0.0	0.1	+	0.02
18:1	2.5	35.5	7.7	6.3	28.4	23.7	2.24
18:2	1.0	38.8	0.0	2.2	30.7	27.5	2.25
20:1	0.0	0.2	0.0	0.1	0.0	0.6	0.09
24:1	0.0	0.0	0.0	0.8	0.0	0.0	0.28

+ = present but less than 0.05% of total; *i* = *iso*; *a* = *anteiso*.

$CHCl_3$ at 40° to a vol. of *ca* 3 ml, adding 20 ml HCOOH + 1 ml conc. H_2SO_4 + 5 ml *p*-dioxane and heating for 16 hr at 60°. Fatty acid formyl esters were separated by TLC [13] and quantitated by the same procedures used for Me esters. Identification of the compounds utilized the relative elution technique [14] and authentic primary alcohols for comparison.

Quantitation was carried out using a digital integrator and the int standards. Statistical analyses were conducted on each component from each leaf on a randomized block design. S.e.m.'s were used to separate means of the various component concns.

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