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EPICUTICULAR ALKANE CONTENT OF TOBACCO AS INFLUENCED BY PHOTOPERIOD, TEMPERATURE AND LEAF AGE

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Abstract—Genetically-uniform tobacco (*Nicotiana tabacum* L.) was grown in the field and under various controlled-environments to determine whether growth environment could influence epicuticular composition. In leaves of the same physiological age, leaf epicuticular alkane content was influenced by the photoperiod and temperature conditions under which the leaves developed. Also, leaves of different ages collected at the same time from field-grown plants differed in epicuticular alkane contents.

INTRODUCTION

PREVIOUS work on the utilization of leaf alkane constituents in chemotaxonomy[1] has indicated that the chemical composition must be specific to the plant species, and that the hydrocarbon pattern must be independent of season, age, or place of growth of the individual plant.[2] However, physiological changes occur in plants due to changes in environment. For example, cuticles developed during leaf expansion remain relatively uniform morphologically, but the total cuticle developed is dependent upon season and place of growth.[3] Amino acid and α-keto acid contents of mint (*Mentha piperita* L.) are responsive to photoperiod and temperature.[4] Branched paraffin biosynthesis in broccoli (*Brassica oleracea* L.) is linked to deaminated amino acid carbon chains as the skeletons for extension into fatty acids.[5] Alkane chain length increases with plant age in *Solandra grandiflora* Sw.[6]

Since fatty acids are precursors of hydrocarbons,[7] modification of plant metabolism by environmental parameters may possibly result in an altered plant epicuticular alkane content. Tobacco (*Nicotiana tobacum* L.) was one of the species whose flowering characteristics led to the discovery of photoperiodism,[8] and its developmental responses to tempera-

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[3] J. D. SKOSS, *Bot. Gaz.* **117,** 55 (1955).
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[5] P. E. KOLATTUKUDY, *Plant Physiol.* **43,** 1423 (1968).
[6] G. A. HERBIN and P. A. ROBINS, *Phytochem.* **8,** 1985 (1969).
[7] P. E. KOLATTUKUDY, *Ann. Rev. Plant Physiol.* **21,** 163 (1970).
[8] W. W. GARNER and H. A. ALLARD, *J. Agric. Res.* **18,** 553 (1920).

ture and photoperiod have been studied.[9] Additionally, the alkane content of this species has been characterized by GLC and MS.[10] Thus, if plant epicuticular alkane content is subject to modification by altered environment, such modification should be observable in a plant, such as tobacco, in which developmental characteristics are markedly influenced by photoperiod and temperature. Therefore, epicuticular alkane contents of leaves from tobacco plants grown in the field and under controlled environments were analyzed to determine whether these compounds were constant for tobacco, or if they varied with environment.

RESULTS AND DISCUSSION

In order to determine whether tobacco leaf epicuticular alkane contents could be modified by growth environment, studies were conducted with leaves of different physiological ages from the same field-grown plants, and from genetically uniform plants grown under various controlled conditions.

Different Physiological Ages from Same Plants

Leaves were collected from conventionally-grown field tobacco in 1969. Under normal tobacco production procedures the plants are 'topped' (the inflorescence and small uppermost leaves are removed when the plants begin to flower) and allowed to continue growth for several weeks before the leaves are harvested. After the apical portion of the plant is removed, 'suckers' (lateral branches originating from axillary buds) develop. Leaves used in this study were collected in mid-July, about four weeks after topping.

Epicuticular alkane contents of leaves from stalks and suckers were compared. The contents of leaves from the stalks were essentially the same as described by Eglinton *et al.*[10] However, analyses of leaves from suckers growing from these plants showed that the concentration of iso-alkanes progressively decreased until they were not detectable as separate components by programmed temperature GLC (Table 1). All the determined components except *n*-heptacosane (*n*-C_{27}) and 3-methylnonacosane (*a*-C_{30}) had significantly different concentrations between the stalk and sucker leaves (Table 1). These differences may have

TABLE 1. RELATIVE CONCENTRATIONS OF EPICUTICULAR ALKANE COMPONENTS IN OLD (STALK) AND YOUNG (SUCKER) LEAVES COLLECTED AT THE SAME TIME FROM FIELD-GROWN TOBACCO

Source of leaves	Epicuticular alkane content (% of total)										
	Normal				iso			anteiso			
	C_{27}	C_{29}	C_{31}	C_{33}	C_{29}	C_{31}	C_{33}	C_{28}	C_{30}	C_{32}	C_{34}
Stalk	7·31	6·45	26·23	8·55	2·13	16·85	5·73	1·18	9·55	16·02	tr
Sucker	8·08	5·43	19·31	22·88	1·38	10·90	tr	2·53	9·67	19·83	tr
Signif*	NS	+	+	+	+	+	+	+	NS	+	NS

* + Differences are statistically significant at the 1% level: NS = not significant at the 1% level.

been associated with physiological age and/or with growth environment that occurred during leaf development. The environmental differences during stalk and sucker leaf development included photoperiod, temperature and, perhaps, light intensity and quality since the sucker leaves were partly shaded by other plants during their development.

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[10] G. EGLINTON *et al.*, *Advances in Organic Geochemistry*, Vol. 2, Pergamon Press, Oxford (1965).

Previous work with tobacco has shown that effects of photoperiod, temperature and light quality interact to influence floral induction, leaf geometry and content of alkaloids, soluble phenols, sugars, organic acids and amino acids.[9,11–13] Thus, it is highly probable that epicuticular leaf alkanes may also be modified by the environment in which the leaves develop.

Same Physiological Age from Different Environments

Leaves grown to the same physiological age (the most recent to attain full expansion) under the four controlled environments did not differ significantly in area or fresh weight. However, those collected from field-grown plants were larger and heavier. The weights and percentages of alkanes extracted per unit area of leaf illustrate very clearly that epicuticular alkane contents were significantly influenced by the light and temperature conditions under which the plants were grown (Table 2). The controlled environments used in our experiment are known to differ in their stimulation of processes leading to floral induction.[9] Thus, the selected environments (Table 2) exerted different degrees of influence on metabolic patterns that lead to a transition from the vegetative to the reproductive phase of plant development.

TABLE 2. EPICUTICULAR ALKANE CONTENT OF TOBACCO LEAVES GROWN TO THE SAME PHYSIOLOGICAL AGE UNDER DIFFERENT PHOTOPERIODS AND TEMPERATURES

Conditions during leaf development		Epicuticular alkane content										
		Normal				iso			anteiso			
Photoperiod (hr)	Temp. (°)	C_{27}	C_{29}	C_{31}	C_{33}	C_{29}	C_{31}	C_{33}	C_{28}	C_{30}	C_{32}	C_{34}
		μg alkane/cm² leaf area										
16	28	45·4ab*	50·0b	586·4a	710·4a	0·5d	194·5bc	245·5a	9·3b	53·7d	493·6a	131·2a
8	28	12·5d	39·5d	266·0c	221·3b	4·9cd	169·1c	256·7a	9·1b	55·4d	255·7c	41·1b
8	18	32·9bc	107·3a	390·8b	207·0b	74·4a	337·9a	178·9a	33·1a	269·ab	339·6bc	60·2b
8 + 4	18	16·7cd	39·1b	351·2bc	250·3b	16·7a	238·7bc	228·2a	11·6b	115·3c	343·8bc	31·4b
Field	Field	59·8a	106·6a	346·0bc	176·4b	47·6b	252·1b	121·3a	13·7b	333·6a	400·1a	37·2b
		% of total alkanes										
16	28	1·8b	2·0c	23·9a	28·0a	tr d	7·4c	9·9b	0·4b	2·1e	19·6a	5·0a
8	28	1·0b	3·4bc	20·5bc	16·3b	0·4cd	13·5b	21·3a	0·6b	4·5d	20·1a	3·1a
8	18	1·5b	5·1ab	19·2bc	10·0cd	3·7a	16·7a	9·4b	1·5a	13·2b	16·9b	2·9a
8 + 4	18	1·0b	2·4c	21·7ab	14·4bc	1·1c	15·0ab	14·0b	0·7b	7·3c	21·1a	1·50
Field	Field	3·2a	5·7a	18·6c	9·4d	2·6b	13·5b	6·5b	0·7b	17·8a	21·4a	1·9a

* Values in the same column (within each sub-table) followed by the same letter or letters are not significantly different at the 1% level.

The data were obtained with short-term leaf washes which do not extract subepidermal lipid components from plant leaves.[14,15] Thus, our data are explicable only on the basis of altered plant metabolism caused by light and temperature combinations. These data substantiate the hypothesis[16] that plant leaf epicuticular alkane content reflects a steady state condition wherein the building blocks present under a given set of environmental conditions are used for the formation of the respective types of alkanes. As the environmental conditions change, a new array of carbon chain skeletons are available, a new steady state condition is established, and the quality of the leaf epicuticular alkanes reflects the new steady state condition.

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Use of leaf epicuticular alkane content as a taxonomic character requires that the composition of those alkanes must be independent of age, season, or location.[1,2] The data presented herein (Tables 1 and 2) and previous,[16] amply demonstrate that leaf epicuticular alkane content is strongly influenced by light and temperature. How, then, may components whose concentrations are changed by environmental parameters be utilized as taxonomic characters which must be specific to the species and uniform under all field conditions? During each season, plants grow in a continuum of changing photoperiod and temperature conditions. Therefore, leaves from plants exposed to naturally occurring combinations of photoperiod and temperature should exhibit a seasonal composite of epicuticular alkanes. However, if leaf epicuticular alkane content varies in a fashion similar to that of bud dormancy, floral induction, and stem elongation between different ecotypes,[17] taxonomic separation of closely allied species or hybrids on the basis of leaf epicuticular alkane content may be unreliable.

EXPERIMENTAL

First experiment. Conventionally started tobacco (*Nicotiana tabacum* L. cv. Hicks) seedlings were transplanted to a field plot near Tifton, Georgia, in spring of 1969. The plants were 'topped' (inflorescence and small upper leaves removed) in mid-June. Leaves were collected from the main stalks and from 'suckers' (regrowth from axillary buds) in mid-July. Thus, two physiological ages of leaves were obtained from the same plants.

Second experiment. Plant material. Plants of a uniform line of burley tobacco (cv. Burley–21) were started and grown for about 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 (Maury silt loam) as were those transplanted to the field plot.

Growth environments. Plants grown under the 'Field Environment' were exposed to natural illumination and temperature combinations at Lexington, Kentucky, from 17 June to 14 July, 1971. Others were exposed to the controlled-environments for the same period. The controlled-environments involved the following photoperiod and temperature combinations: (I) 16-hr/28°; (II) 8-hr/28°; (III) 8-hr/18°; and (IV) 8-hr/18° with an additional 4-hr exposure to low-intensity illumination in the middle of the 16-hr night. All illuminations, except the 4-hr night interruption in (IV), were from VHO cool-white fluorescent lamps at an intensity of 20 000 lx. The 4-hr night interruption used in (IV) was at 250 lx from white incandescent-filament lamps.

Sampling procedure. The fifth and sixth leaves from the apex (excluding all leaves shorter than 8 cm) were collected from each of five plants from each of the five environments (i.e. ten leaves of each of five environments) on 14 July 1971. The leaves included in these samples were the most recent to attain maximum expansion, and their entire growth period occurred under the respective controlled and field environments. The leaves were placed in plastic bags and transferred to Experiment, Georgia for analyses.

Extraction and analyses. Epicuticular alkanes were extracted with $CHCl_3$.[14] After the addition of 1 mg *n*-docosane (*n*-C_{22}) as an internal standard, the alkanes were separated from the remainder of the lipids by TLC,[16] and the alkanes were separated and quantitated via GLC using a 5751 A Hewlett Packard Dual Hydrogen Flame apparatus equipped with 183 cm × 3·2 mm i.d. stainless steel columns containing 10% OV–I on 100/120 mesh chromosorb W(AW) (DMCS). Oven temperatures were programmed from 70° to 300° at 6°/min with an upper limit hold of about 5 min at a range of 10 and an attenuation of 16. Identification of the compounds was made by the relative elution technique,[18] using previously purified tobacco alkane for comparison.[19] Quantitation was the area under the curves as measured by an Infotronics CRS-100 Digital Integrator in proportion to the internal standard (*n*-C_{22}). Statistical analyses were conducted on each component on a randomized block design, and the Duncan's multiple range test was used to separate means of the various component concentrations.

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[19] Courtesy of P. E. KOLATTUKUDY.