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VARIATION IN LEAF WAX ALKANES IN CYPRESS TREES GROWN IN KENYA*

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Abstract—Gas–liquid chromatographic analysis of alkane fractions isolated from the leaf waxes of Cypress trees has shown the proportions in which they occur to be variable. Variation with species, seed provenance and between individual trees within a plantation is evident. To use the leaf wax alkane composition as a taxonomic discriminant requires careful replicated sampling with concurrent reference sampling of authenticated stands.

INTRODUCTION

IN A PREVIOUS paper[1] the authors showed that, although alkanes were not the major constituents of the leaf waxes of *Cupressus* species, the proportions in which they were present could be used to identify species and interspecific hybrids. Variation in the proportions of alkanes was noticed between species and between individual trees within a species, and replicated samples from at least seven individuals of each species were necessary to obtain mean values of alkane proportions which could be validly compared for specific discrimination.

In continuation of this work, further analyses of leaf waxes of cypresses of species not previously examined were carried out, using the sampling procedure and laboratory methods already described.[1,2] Repeat sampling of certain stands and individual trees, for checking purposes, which had been examined in 1966–67 yielded estimates of alkane proportions inconsistent with the earlier work. Statistical analysis of our earlier data had shown that variation arising from chance differences in extraction and chromatographic techniques was small compared with intrinsic variation of the alkane proportions in Cypress leaf waxes, and further study of the variation within trees and stands seemed desirable.

RESULTS

Variation Between Seed Sources Within a Species

Paired foliage samples were collected from the current season's growth on seven individuals of each of two seed provenances of three Californian species of *Cupressus*. The trees were established in 1942 in adjacent trial plots on a uniform site at Elburgon, Kenya,

* "Studies on Plant Cuticular Waxes" (VII). For the previous paper (VI) see G. A. HERBIN and P. A. ROBINS, *Phytochem.* **8**, 1985 (1969).

[1] W. G. DYSON and G. A. HERBIN, *Phytochem.* **7**, 1339 (1968).

[2] G. A. HERBIN and P. A. ROBINS, *Phytochem.* **7**, 239 (1968).

from seed collected from known localities which has been fully documented by Wolf.[3] The mean alkane ratios found are given in Table 1.

Table 1. Proportions of leaf wax alkanes in Californian cypresses

	Provenance					
	C. forbesii		*C. arizonica*		*C. sargentii*	
	Wolf nos.:		Wolf nos.:		Wolf nos.:	
Alkane ratio	2320	2331	2357	3370	3375	2157
C_{29}/C_{27}	2·0	1·7	1·7	1·1	1·6	2·2
C_{31}/C_{29}	5·5	9·3	4·5	5·4	8·9	3·0
C_{35}/C_{31}	0·5	0·9	0·7	0·5	0·4	1·8
$(C_{25} + C_{26})/C_{27}$	0·5	0·5	0·4	0·5	0·7	0·6

Inspection of the data in Table 1 immediately shows that, while individual provenances may be distinguishable by reference to their alkane ratios, the two provenances of a single species differ more from one another than they do from provenances of a different species. In passing, it may be noted that all values of the $(C_{25} + C_{26})/C_{27}$ ratio are substantially less than unity as is characteristic of New World Species.[1] Statistical analysis of the data from the forty-two pairs of samples analysed chemically yielded the variance estimates and variance ratios shown in Table 2.

Table 2. Variance estimates and variance ratios of proportions of leaf wax alkanes in six Californian cypresses

		Alkane ratios							
		C_{29}/C_{27}		C_{31}/C_{29}		C_{35}/C_{31}		$(C_{25} + C_{26})/C_{27}$	
Source of variation	d.f.	Variance	*F*	Variance	*F*	Variance	*F*	Variance	*F*
Between species	2	2·205		43·224		1·679		0·344	
			1·10		0·37		0·36		2·24
Between provenances within species	3	2·009		115·581		4·668		0·154	
			3·80*		31·04‡		35·82‡		1·23
Between trees within provenances	36	0·329		3·723		0·130		0·125	
			7·98‡		3·33‡		2·04†		1·61
Within trees	42	0·066		1·118		0·061		0·077	
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*, †, ‡; Statistically significant at the 5%, 1% and 0·1% level respectively.

The low variance estimates obtained between pairs of samples within trees again give confidence in the sampling and laboratory techniques used, and it is clear that individual plots of trees of uniform seed provenance can be successfully distinguished by sampling seven individuals in the stand and comparing their alkane ratios. In all four ratios examined, however, there was large variation amongst individual trees within a provenance and, without replicated sampling, individual variation could easily have been mistaken for specific or provenance differences.

[3] C. B. Wolf and W. W. Wagener, *El Aliso* 1, 429 (1968).

Similar results were obtained when a further twenty-eight pairs of wax samples were examined from a second (incomplete) set of Californian Cypress plots at Uplands Forest Station. As at Elburgon there was large variation amongst trees within a provenance, but provenance plots could be distinguished on the basis of seven-tree mean ratios. Species could not be distinguished. Examination of four provenances of *Cupressus arizonica* growing on separate sites again yielded mean alkane ratios unique to the stand sampled.

Variation with Time of Sampling

In order to check whether the ratios found were unique to stands or to sample batches, two stands at Muguga which had been sampled in 1966, *C. arizonica*, and 1967, *C. funebris*, were resampled in 1968. Not all the fourteen individual trees sampled in 1966 and 1967 could be found again in 1968, but four *C. arizonica* and five *C. funebris* which had been previously examined were found and resampled. The mean ratios obtained are shown in Table 3.

TABLE 3. MEAN LEAF WAX ALKANE RATIOS FOR TWO SPECIES OF *Cupressus* SAMPLED AT DIFFERENT TIMES

		Alkane ratios			
		C_{29}/C_{27}	C_{31}/C_{29}	C_{35}/C_{31}	$(C_{25}+C_{26})/C_{27}$
Cupressus arizonica (plot 251)					
Nov. 1966:	7 trees	0·9	4·3	0·3	0·3
Sept. 1968:	4 trees	1·2	5·3	0·3	0·2
(L.S.D. 5%)		(0·12)	(0·08)	(0·21)	(0·13)
Cupressus funebris (plot 64)					
Feb. 1967:	7 trees	3·7	0·32	5·2	1·4
Sept. 1968:	5 trees	2·4	0·22	5·9	0·2
(L.S.D. 5%)		(0·79)	(0·04)	(0·76)	(0·18)

From Table 3 it is immediately clear that, while the two plots differ widely in their alkane proportions, successive samplings of the same plot are significantly different for seven of the eight ratios calculated. Because sampling had been confined to the current season's new growth and since the alkane constitution of leaf waxes may vary with age, one of the *C. funebris* trees was resampled for four ages of leaf. The mean values for the four alkane ratios examined are given in Table 4.

TABLE 4. MEAN VALUES OF LEAF WAX ALKANE RATIOS FOR FOUR AGES OF LEAF IN A SINGLE *Cupressus funebris* TREE

	Ratio			
Ages of leaf	$(C_{25}+C_{26})/C_{27}$	C_{29}/C_{27}	C_{31}/C_{29}	C_{35}/C_{31}
Young	0·2	4·9	0·22	2·0
Mature	0·8	4·1	0·36	6·3
Old	0·6	3·6	0·60	5·3
Dead	0·8	2·9	0·22	5·6
(L.S.D. 5%)	(0·17)	(1·28)	(0·08)	(0·93)

As will be seen from Table 4, the values for young foliage differ significantly from the old or dead leaves for all four ratios. There is some indication that the ratios rise with increasing age of leaf, with the exception of the C_{29}/C_{27} ratio. Clearly, however, the implication that alkanes of longer chain length are laid down with increasing age of leaf does not account for all the variation observed. It was decided, therefore, to examine a single tree more thoroughly.

Variation Within a Single Tree

For this, a single open grown *C. torulosa* tree was selected and foliage samples taken from the upper, lower and mid-crown in the four cardinal directions. Young, mature and senescent leaves were sampled from each position giving a total of thirty-six samples. The five alkanes C_{35}, C_{33}, C_{31}, C_{29}, C_{27} were measured on all chromatograms and the five ratios, 29/27, 33/27, 31/29, 35/31, 33/35, calculated. The first analyses of the data showed slight and insignificant variation with direction of sampling. Directional samples were therefore used as replicates for a more precise analysis of the variation with height on the crown and age of leaf. Highly significant variation with age was found for all ratios calculated, but differences with height of crown sampled were significant at the 5 per cent level only in the 33/35 and 35/31 ratios. The pattern of results was similar and for brevity only the mean values observed for the C_{31}/C_{29} ratio are shown in Table 5.

TABLE 5. MEAN VALUES OF THE ALKANE RATIO C_{31}/C_{29} IN A SINGLE *Cupressus torulosa* TREE

Age of leaf	Old	Mature	Young	Mean
Sampling height				
Top	0·79	0·51	0·21	0·50
Middle	0·46	0·50	0·32	0·43
Bottom	0·63	0·64	0·38	0·55
Mean	0·63	0·55	0·30	—

Least significant difference (5%) comparing two ages or two heights 0·057.

Least significant difference (5%) comparing two ages for the same height or two heights for the same age 0·098.

Whenever different sampling heights gave significantly different ratios, the height × age interaction term was also significant, and it is probable that the observed differences are due to inadvertent sampling of material of different ages rather than to genuine changes with position on the crown of the tree.

As far as the change of ratio with age is concerned, it was found that the longer chain-length alkanes, C_{35}, C_{33} and C_{31}, were present in greater proportions in the older leaves. Ratios involving the C_{27} alkane, however, do not follow this general pattern and cannot be precisely explained on the data so far obtained. The variation found in young leaves was much greater than that of old or mature foliage, in some cases up to ten times as great. The variation due to age of leaf is sufficient to account for the inconsistent results obtained when stands were resampled.

CONCLUSIONS

The results of this study show that the proportions of alkanes of given chain length in Cypress leaf waxes are extremely variable. They vary with species and seed provenance, between individual trees within a provenance, and within individual trees. In a single tree the greatest variation is attributable to age of leaf sampled, but they may perhaps also vary with the part of the crown sampled. With this extensive variation the leaf wax alkanes are not a suitable criterion for taxonomic discrimination in the genus *Cupressus*. With careful replicated sampling, preferably of the oldest foliage collectable, individual stands of trees may be discriminated, and it may be possible to allocate such stands to species as was done in the earlier work,[1] but the method cannot identify single trees, nor stands of trees unless known comparable reference material can be concurrently sampled.

EXPERIMENTAL PROCEDURES

The foliage sampling techniques are described in Dyson and Herbin[1] and laboratory procedures in Herbin and Robins.[2]

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