

## EFFECT OF LEAF AGE ON EPICUTICULAR WAX ALKANES IN *RHODODENDRON*

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**Key Word Index**—*Rhododendron* cv; Ericaceae; epicuticular wax; *n*-alkanes; effect of leaf age.

**Abstract**—Alkane distribution pattern in epicuticular wax of *Rhododendron fortunei* cv Admiral Piet Hein leaves remains unchanged from the age of 6 weeks to abscission.

### INTRODUCTION

The potential of alkane composition in epicuticular wax as a taxonomic indicator has been pointed out by several workers over the past two decades, e.g. refs. [1,2]. However, it has been noted that the wax composition, including the alkane pattern, may vary within species according to leaf age. Evidence of the increase of hydrocarbon chain lengths with increasing leaf age has been presented for the epicuticular waxes of *Kalanchoe dyeri* N.E. Br. (Crassulaceae), *Solandra grandiflora* Sw. (Solanaceae), *Monstera deliciosa* Liebm. (Araceae), *Pelargonium* cv (Geraniaceae) and *Jatropha curcas* L. (Euphorbiaceae) [3], *Coffea arabica* L. (Rubiaceae) [4], and for *Beta vulgaris* cv (Chenopodiaceae), *Phaseolus vulgaris* cv (Leguminosae), *Malus hupehensis* (Rosaceae) and *Centranthus ruber* (Valerianaceae) [5], whereas no apparent effect of age has been reported for *Rosa* cv (Rosaceae), *Canna indica* cv (Cannaceae) and *Strelitzia regina* Banks (Strelitziaceae) [3], and for *Eucalyptus globulus* (Myrtaceae) [5]. Alkanes in epicuticular wax of *Prunus persica* cv (Rosaceae) leaves showed an increase in chain lengths up to the age of 28 days, whereas no further change was observed in leaves aged 28–80 days [6]. In the genus *Khaya* (Meliaceae), where alkane patterns were compared in the wax of 'young' and 'old' leaves [7], and of leaves aged 6 and 19 weeks [8], there was a general trend of increased  $C_{31}$  and decreased  $C_{29}$  alkane content in the wax from older leaves; however, some species showed a decline in the percentage of  $C_{31}$  and/or  $C_{33}$  alkanes over that period. Similarly a decrease in the hentriacontane content was observed in *Trifolium repens* L. (Leguminosae) between the ages of 10 and 42 days [5].

No information regarding the effect of age has been reported from the order Ericales. In view of our work on the epicuticular wax of Ericaceae [9] and Epacridaceae [10], the study of the effect of age on alkane pattern in the wax of a species from this order, a *Rhododendron* cv (Ericaceae), was undertaken.

### RESULTS AND DISCUSSION

The following common pattern emerges for *Rhododendron* leaves aged 6 weeks and over (samples 2–14): the major alkane is hentriacontane (43–55%), followed by tritriacontane (11–26%) and nonacosane

(13–20%), with a few cases where the latter two are present in equal amounts or nonacosane exceeds tritriacontane (Table 1). Variations within these ranges show no definite trends with age. Neither are any trends obvious in minor constituents. However, the wax from half-open leaf buds (sample 1) is completely different: it was obtained in the highest yield and it contains the lowest percentage of hydrocarbons, with the  $C_{23}$ ,  $C_{25}$  and  $C_{27}$  alkane content higher, and the  $C_{31}$  and  $C_{33}$  alkane content lower, than in older leaves.

The above results indicate that the alkane distribution pattern in *Rhododendron* leaf wax remains constant, once the leaves have attained their normal shape. Further growth in leaf size does not affect the pattern.

This pattern agrees with that of *R. macrophyllum* G. Don [9], the only other *Rhododendron* species for which the detailed alkane distribution pattern in epicuticular wax has been reported. It is also in agreement with the data of Evans *et al.* [11] who, having analysed the epicuticular wax of leaves of ca 300 *Rhododendron* species, subspecies and varieties, reported the three major alkanes for *R. fortunei* Tindl. to be  $C_{31}H_{64}$ ,  $C_{33}H_{68}$  and  $C_{29}H_{60}$ , and the majority (ca 70%) of the waxes to have hentriacontane as the major hydrocarbon.

### EXPERIMENTAL

All leaf material was collected from one shrub of *Rhododendron fortunei* cv Admiral Piet Hein (voucher specimen No. UNSW 12781 kept in the Herbarium of the School of Botany, University of New South Wales) growing in Roseville, a northern suburb of Sydney, New South Wales. After an initial collection of all green leaves from a number of branches, with young but almost full-size leaves accounting for ca 50% of the total (sample 14), the later collections either comprised leaves of only one age group (e.g. yellow), or the leaves were separated according to age before the wax was extracted.

The wax was removed by immersing the fr. leaves in redistilled petrol (bp 60–80°) at room temp. for 2–5 min. The filtered solns were concd by distillation and evaporated to dryness to determine the yield. A 200–300 mg portion of the wax (or all the wax where the total yield was less than 300 mg) was dissolved in hot petrol (50 ml), the soln allowed to stand at least 18 hr and chromatographed (after filtration if necessary) on  $Al_2O_3$  (Spence 'H', 15 g). The first fraction (petrol, 240 ml) containing hydro-

Table 1. Alkane distribution in epicuticular wax\*

Sample	Date	Age (weeks)	Wax yield†	Hydrocarbonst	Carbon chain length of alkanes																	
					18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1	15.10.80	0§	0.53	1.70	—	—	—	tr	tr	2	1	8	2	24	3	18	4	25	2	10		
2	22.11.81	6	0.17	8.30	—	—	—	—	—	tr	1	1	1	5	1	20	1	55	2	13		
3	01.01.81	11	0.15	17.45	—	—	—	—	—	tr	1	1	2	4	1	14	4	46	6	21		
4	04.05.81	20	0.11	10.32	—	—	—	—	—	tr	tr	1	1	4	1	13	2	51	3	24		
5	22.11.81	58	0.13	6.10	—	tr	tr	tr	1	1	2	2	2	7	2	14	4	46	3	14		
6	01.01.81	63	0.16	4.83	—	tr	tr	tr	1	1	1	2	2	7	2	15	3	47	3	15		
7	04.05.81	80	0.18	3.00	—	tr	tr	tr	tr	1	tr	1	1	6	3	13	5	43	8	18		
8	22.11.81	110	0.10	4.70	tr	tr	tr	tr	1	1	1	2	2	6	2	15	4	52	3	11		
9	05.01.80		0.12	4.96	—	—	—	—	1	tr	tr	1	1	5	2	14	4	51	3	16		
10	16.03.80		0.06	16.50	—	—	—	—	—	tr	tr	1	1	4	2	14	3	50	3	21		
11	01.01.81		0.12	6.03	—	—	tr	tr	tr	1	1	2	2	7	2	15	4	47	3	14		
12	19.04.80	, ¶	0.06	18.44	—	tr	—	tr	tr	tr	tr	1	1	4	1	13	3	45	4	26		
13	04.05.81	¶	0.11	5.83	—	tr	tr	tr	tr	1	1	2	1	5	2	14	4	50	3	16		
14	21.11.79	Mixed	0.13	4.07	—	—	—	—	tr	tr	tr	1	tr	4	1	17	1	55	3	16		

\* Percentages (by wt) are rounded off to the nearest 1%. Trace (tr) = 0.1–0.5%.

† Expressed as percentage of wt of fresh leaves.

‡ Percentage of hydrocarbons in wax.

§ Half-open buds.

|| Partly yellow leaves.

¶ Completely yellow leaves.

carbons was analysed by GC at 160–280° [9], with N<sub>2</sub> at 40 ml/min. Chain lengths were determined by comparison with known mixtures, and by GC/MS.

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