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ONTOGENETIC VARIATIONS IN THE COMPOSITION OF PEACH LEAF WAX*

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Abstract—The composition of the epicuticular waxes from the adaxial and abaxial surfaces of peach leaves varies considerably during one season's growth. Triterpenoid acids are major components (84–95%) of the waxes from the youngest leaves but the proportions of these constituents decrease as the leaves expand. The waxes from the abaxial surfaces of fully expanded leaves consist primarily of hydrocarbons (C_{22}–C_{34}) and triterpenoid acids, whereas the adaxial surface waxes also contain large proportions of primary alcohols (C_{26}–C_{34}) and esters (C_{42}–C_{52}). The latter include sitosteryl esters of hexacosanoic, octacosanoic and eicosanoic acids. Variations were also noted between fully expanded leaves of different ages, the abaxial surface waxes of the oldest leaves containing the highest proportions of hydrocarbons, whilst the wax from the adaxial surface of the corresponding leaves contained the largest amounts of esters, sitosterol and hydrocarbons.

INTRODUCTION

During recent years the constitution, site of formation, biosynthesis and physicochemical properties of plant epicuticular waxes have been the subject of intensive investigations [1–3]. These studies have demonstrated that waxes from different species and from different organs of the same species can be substantially different. Additional investigations with a limited range of plants have shown that factors such as growth conditions [4–7] and age of tissue [7–15] can also influence the form and distribution of wax constituents. Such observations have led to speculations that changes in cuticular components during growth may be responsible for the variations observed in the retention and redistribution of foliar applied chemicals [16–18]. In order to examine this possibility, we have studied seasonal changes in the physical and chemical properties of waxes on the adaxial and abaxial surfaces of peach leaves. The results of the chemical investigations are described in this paper.

RESULTS

The composition of the leaf waxes at the termination of shoot extension are summarized in Table 1.

Surface variations

Marked differences were noted between the compositions of the adaxial and abaxial surface waxes of individual leaves at all stages of growth. The waxes on the abaxial surface consisted primarily of triterpenoid acids and hydrocarbons together with smaller amounts of

Table 1. Distribution of wax classes (μg/cm²) on the adaxial and abaxial surfaces of peach leaves

Surface and leaf position	Average leaf area (cm²)	Constituent class: Hydrocarbon	Primary alcohol	Sterol	Ester	Triterpenoid acid
Adaxial						
Node 4	24	0.7	5.5	2.3	tr	45.0
5	34	1.0	7.0	3.2	2.2	29.0
6	38	1.0	7.7	4.3	2.8	17.6
7	41	1.2	6.7	4.4	4.1	12.8
8	43	1.7	7.8	5.3	6.6	11.3
9	43	2.0	7.5	4.0	5.7	9.2
10	46	2.0	6.4	4.1	7.4	5.9
11	44	2.3	6.5	4.2	7.9	5.9
12	42	3.9	6.0	4.2	8.8	6.3
13	42	3.4	5.9	4.7	8.2	6.2
14	38	3.3	5.5	4.0	7.9	5.2
15	34	3.9	6.9	4.8	10.0	6.2
Abaxial						
Node 4	24	1.5	0.4	nd	nd	39.8
5	34	3.7	0.4	nd	nd	33.0
6	38	9.3	0.6	nd	nd	40.3
7	41	10.1	0.6	nd	nd	29.6
8	43	12.4	0.6	nd	nd	27.6
9	43	15.7	0.9	nd	nd	27.1
10	46	15.8	0.8	nd	nd	19.2
11	44	18.8	0.8	nd	nd	18.1
12	42	16.9	0.9	nd	nd	18.4
13	42	17.9	1.2	nd	nd	20.7
14	38	20.8	1.2	nd	nd	18.3
15	34	20.5	1.4	nd	nd	19.0

tr = trace; nd = not detected.

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primary alcohols. In contrast, the waxes on the adaxial surfaces contained, in addition to these three classes, variable quantities of steryl esters, alkyl esters and free sterols. Of the constituents which were common to both surfaces, primary alcohols were found in greater quantities on the adiaxial (5.5–7.8 μg/cm²) than the abaxial surface (0.4–1.4 μg/cm²) whilst hydrocarbons were formed mainly on the abaxial surface.

Variations with leaf age

Wax compositions altered steadily as the age of the leaf increased, the most notable changes affecting the distribution of triterpenoid acids. These constituents were dominant components of the surface deposits of immature leaves (84–95% of wax) but were greatly reduced on fully expanded leaves (20 48% of wax). Extensive variations were also observed in the distribution of esters on the adaxial surface (>0.1–10 μg/cm²) and in hydrocarbons on the abaxial surface (1.5–20.8 μg/cm²). As a consequence of these changes, the quantities of the various constituents present on individual leaves were also markedly different. For example, the abaxial surface of leaves from node 5 carried 126 μg hydrocarbons and 1123 μg triterpenoid acids whereas the amounts on the corresponding surface of leaves from node 15 were 690 and 640 μg, respectively. Similarly the amounts of the various constituents on the adaxial surfaces of the leaves from nodes 5 and 15 were respectively triterpenoid acids (991; 211 μg), esters (75; 336 μg), hydrocarbons (34; 132 μg), sterols (102; 162 μg) and primary alcohols (240; 206 μg). It is apparent that the variations in wax composition with age resulted from differences in the rate at which the component classes were transported to the leaf surface rather than through the redistribution of constituents formed at successive stages during leaf expansion.

Composition of hydrocarbon fractions

The hydrocarbon fractions consisted of *n*-alkanes ranging between C_{22} C_{34}, in which the odd carbon-numbered homologues were dominant (Table 2). Hentriacontane was the major constituent of all fractions except those from the abaxial surface of leaves from node 4; the C_{25}, C_{27} and C_{29} homologues were also prominent. Variations in the composition of the hydrocarbon fractions with age followed a similar pattern on both surfaces, showing a gradual shift towards the longer chain length homologues during the final stages of leaf expansion.

Table 2. Relative percentages of major *n*-alkanes in epicuticular waxes from the adaxial and abaxial surfaces of peach leaves

Surface and leaf position		% Hydrocarbon										
		C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}
Adaxial												
Node	4	2	2	15	3	18	5	20	2	27	2	4
	5	2	1	14	1	15	4	20	1	37	1	5
	6	2	1	14	1	14	3	21	1	36	1	5
	7–15	2	1	14	1	14	1	19	1	40	1	6
Abaxial												
Node	4	1	1	26	2	19	3	29	1	15	1	1
	5	1	1	18	1	14	1	19	1	35	1	2
	6	1	1	16	1	12	1	21	1	40	1	5
	7–15	tr	tr	14	tr	11	tr	18	1	42	1	8

tr = Trace (<0.5%).

Table 3. Relative percentages of major alcohols in the epicuticular waxes from the adaxial surfaces of peach leaves

Leaf position		% Alcohol			
		C_{26}	C_{28}	C_{30}	C_{32}
Node	4	29	19	26	26
	5	37	14	26	23
	6	41	16	19	23
	7	42	19	14	25
	8	38	22	17	23
	9–15	35	20	20	25

The hydrocarbon fractions from immature leaves were richer both in homologues of shorter chain length (C_{22}–C_{29}) and in even carbon-number constituents, the latter forming 14% of the fraction from the youngest leaves (node 4) compared to 5% for mature leaves. The GLC profiles of the fractions from older leaves (nodes 7–15) were almost identical.

Composition of primary alcohol fractions

The primary alcohol fractions on both the adaxial and abaxial surfaces consisted almost entirely of even chain length homologues (C_{26}–C_{32}). Since the abaxial surface waxes contained only small amounts of primary alcohols, detailed studies of the effect of age on homologue composition were confined to the fractions from the adaxial surfaces. The alcohol fractions from older leaves (nodes 9–15) were remarkably similar and age effects were therefore evident only in the fractions from the younger leaves (Table 3). Although relatively small differences in homologue composition were noted between the fractions of leaves from adjacent nodes, the steady rise or fall in the proportions of individual homologues produced clearly defined differences between the GLC profiles of the fractions from more widely separated leaves.

For example, the decline in triacontanol (from 26 to 14%) between nodes 4 and 7 was balanced by an increase in the proportions of hexacosanol (from 29 to 42%). Similarly, comparing nodes 5 and 8, the increase in the octacosanol content (from 14 to 22%) compensated for the decrease in the proportions of triacontanol (from 26 to 17%). On the other hand, the dotriacontanol content of the fractions showed little variation (23–26%).

Composition of ester fractions

The ester fractions from leaves at all stages of development contained steryl and alkyl esters with a chain length distribution corresponding to a series (C_{42}–C_{52}) of esterified primary alcohols (Table 4).

Sitosteryl esters of C_{16}, C_{18} and C_{20} saturated fatty acids were the major constituents of the steryl ester fractions. On the Dexsil column these esters co-eluted with alkyl esters of chain length C_{46}, C_{48} and C_{50}, respectively. The proportion of the C_{50} homologues (mainly sitosteryl eicosnoate and hexacosyl tetracosanoate) declined gradually from 61% in the fractions from the youngest leaves (node 4) to 45% in the older leaves whilst the C_{44} and C_{46} homologues increased. The ester fractions from the older leaves (nodes 9–15)

Table 4. Relative percentages of the major esters in the epicuticular waxes from the adaxial surface of peach leaves

Leaf position	% Ester C_{44}	C_{46}	C_{48}	C_{50}	C_{52}
Node 4	3	4	17	61	15
5	4	3	17	59	17
6	5	7	18	50	20
7	6	8	18	48	20
8	7	9	20	46	18
9–15	8	12	20	45	15

were very similar in composition consisting primarily of sitosteryl (58%) and hexacosyl esters (24%). A detailed examination of the hydrolysis products of the ester fractions from the older leaves revealed clear differences between the profiles of the fatty acids from the steryl esters C_{16} (20%), C_{18}(32%) and C_{20}(46%) and the hexacosyl esters C_{18} (24%), C_{24} (40%) and C_{26} (22%).

Compositions of triterpenoid acid and sterol fractions

Although the quantities of triterpenoid acids on the different aged leaves varied widely, the compositions of the fractions showed little variation. The proportions of the dominant component ursolic acid (72%) and of oleanolic acid (20%) were consistent in all the fractions. Dihydroxy acids of the ursane and oleanane series, identified as minor constituents (8%), were also present in similar proportions (3.5:1) as the parent acids. The triterpenoids were identified by comparison with GLC R_t data of authentic reference compounds and by GC–MS (see Experimental). Typical fragments for the monohydroxy acids were those for the molecular ion *m/e* 542 and for the elimination of Me (*m/e* 527) and TMSiOH (*m/e* 452), whilst the corresponding fragments from the dihydroxy acids were found at *m/e* 630, 615 and 540, respectively. Diagnostic ions for members of the ursane and oleanane series [19] included the fragments at *m/e* 262 arising from the retro-Diels Alder involving the Δ^{12} double bond [20] and at *m/e* 203 (*m/e* 262 − 59). The relative abundance of the fragment at *m/e* 262 (base peak for ursolic acid) compared to *m/e* 203 was greater for members of the ursane series. Sitosterol was the sole constituent of the sterol fraction in all leaf samples.

DISCUSSION

The progressive change in the composition of the wax from peach leaves located at successive nodes along fully extended shoots emphasizes the complexity of the process controlling the formation of these epicuticular deposits. Ontogenetic variations in wax composition have been reported previously for ivy [7], cypress [8], wheat [9], apple [10], coffee [11] and pine [13–15] leaves, and for apple [10] and tomato [12] fruits. With these plants, the variable distribution results from the sequential formation of specific wax classes during leaf expansion, whereas for peach leaves an increase in one wax class is balanced by compensatory decreases in others. The differences in the composition of the surface deposits of fully expanded peach leaves could result from weathering or from seasonal fluctuations in growth conditions although evidence from previous studies would not support these proposals [4, 21, 22]. Alternatively, constituents formed during the initial stages of growth might be re-metabolized and the products transported to the wax layer [23]. However, the most likely explanation would appear to be that the steady increase in the production of triterpenoid acids, particularly during the latter part of the season, reflects the gradual decrease in the metabolic activity of the plant.

The contrast between the distribution of wax constituents on the adaxial and abaxial surfaces of the same leaf is a further example of the major differences which can exist between the waxes from adjacent areas of tissue [24, 25] and emphasizes the importance of using isolation procedures which provide separate extracts from the individual surfaces. Such differences were not identified in a previous analysis of peach leaf wax [16]. A possible explanation for this discrepancy may be that in the previous study the waxes were analysed by TLC and IR; chemical differences between waxes are less apparent using these techniques, By comparison, in the present survey, the GLC profiles of the waxes from the adaxial and abaxial surfaces were quite distinct.

The occurrence of steryl esters in the surface extracts from peach leaves is worthy of further comment since these constituents have not been commonly reported as components of epicuticular waxes. Exceptions include the steryl acetates identified in the leaf waxes of *Rhododendron* spp. [26]. The contrast between the esterification patterns of the primary alcohols and sitosterol suggests that these ester classes utilize separate pools of fatty acids, the latter showing a preference for acids of shorter chain length. The differences in intensity of the peaks at *m/e* 262 and 203 in the MS of the triterpenoid acids provided a satisfactory means by which to distinguish between members of the ursane and oleanane series; identification had previously been achieved from the MS of the acetoxy-toluidides [27].

In conclusion it should be stressed that whereas the qualitative composition of the epicuticular waxes may be consistent within a species, the extent of the quantitative variations identified in the present investigation seems likely to preclude the use of these cuticular components for chemotaxonomic studies. Future investigations should be concerned more with the distribution of wax constituents over small areas of tissue. Analyses of the bulk waxes obtained by batch extraction of large numbers of leaves or whole plants would appear to be of limited value.

EXPERIMENTAL

Leaves of peach (*Prunus persica* L. Batsch cv Redhaven), selected by node number counting the youngest leaf primordia adjacent to the apical meristem as node 1, were obtained at terminal bud set from mature trees grown in the orchards of Michigan State University. Samples taken from nodes 4 to 15 included leaves of the following ages *ca* 12, 18, 24, 28, 32, 36, 42, 49, 56, 64, 72 and 80 days.

Isolation and analysis. Waxes were removed separately from the adaxial and abaxial surfaces by running $CHCl_3$–Et_2O (1:1), delivered from a burette, over the leaves held vertically by the petiole. The chromatographic techniques used for the qualitative and quantitative analyses of the wax constituents (TLC, PLC, GLC and GC–MS) have been described previously [28]. Primary alcohols and sterols were analysed as the TMSi ethers and triterpenoid acids as the TMSi ether Me ester [29, 30].

Esters were hydrolysed using NaOMe [31] and the products recovered in Et_2O after acidification [32].

Mass spectral data. Ursolic acid TMSi Me ester *m/e* (rel. int.): 542 (M^+)(3), 527(2), 483(2), 452(2), 437(2), 279(14), 262(100), 249(6), 233(5), 203(85), 190(27), 189(33), 175(6), 147(9), 133(45), 75(18), 73(28). Oleanolic acid TMSi Me ester 542 (M^+)(2), 527(1), 483(1), 452(1), 437(1), 279(8), 262(72), 247(6), 203(100), 190(30), 189(30), 175(11), 147(11), 133(15), 75(29), 73(34). Hydroxyursolic acid diTMSi Me ester, 630 (M^+)(1), 615(1), 540(5), 525(3), 512(1), 497(3), 483(2), 393(3), 367(6), 277(6), 278(14), 262(86), 203(100), 190(10), 189(29), 147(24), 133(26), 75(19), 73(52). Hydroxyoleanolic acid diTMSi Me ester, 630 (M^+)(1), 615(1), 540(2), 525(2), 512(2), 497(2), 483(5), 392(2), 279(10), 262(20), 203(100), 190(39), 189(41), 147(15), 133(38), 75(38), 73(56).

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