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A comparison of the composition of epicuticular wax from red raspberry (*Rubus idaeus* L.) and hawthorn (*Crataegus monogyna* Jacq.) flowers

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Abstract

Epicuticular waxes have been characterised from the flowers of raspberry and hawthorn, on both of which adult raspberry beetles (*Byturus tomentosus*) can feed. The flower wax from both species had similar alkane profiles and also contained long-chain alcohols, aldehydes and fatty acids. The range of the carbon numbers detected for these classes of compounds was broadly similar in both but the relative amounts of each differed between species. Raspberry flower wax also contained fatty acid methyl esters, a group of compounds that has rarely been detected in plant epicuticular waxes, however, these were not observed in hawthorn flower wax. Long-chain alcohol-fatty acid esters with carbon numbers ranging from C36 to C48 were also detected in both plant species. However, an examination of their constituent acids indicated that in hawthorn the esters based on the C16 fatty acid predominated, whilst in raspberry flower wax, esters based on the C20 fatty acid were most abundant. Both species also contained pentacyclic triterpenoids, which accounted for, on average, over 16 and 48% of the total wax extracted from raspberry and hawthorn flowers respectively. In the former, ursolic and oleanolic acids accounted for over 90% of the pentacyclic triterpenes, whilst hawthorn flower wax, in addition to containing these acids, also contained high relative concentrations of both free and esterified α- and β-amyrins.

Keywords: Raspberry; *Rubus idaeus*; Hawthorn; *Crataegus monogyna*; Flowers; Epicuticular wax composition

1. Introduction

The raspberry beetle (*Byturus tomentosus*) is a major pest in commercial raspberry production, primarily due to the damage caused to the fruit by the developing larvae. Adult beetles over-winter in soil at the base of raspberry plants and on emergence tend to remain in the young foliage at the base of the plant until sufficiently developed to fly. In temperate locations, conditions may be such that the adult beetles become airborne before the buds of the raspberry flowers are open, in which case they may migrate to other, earlier flowering, rosaceous plants such as hawthorn (*Crataegus monogyna*). After feeding on the hawthorn flowers they then return to the raspberry flowers to breed (Gordon et al., 1997).

A study of the flower volatiles from both plant species (Robertson et al., 1993) revealed that the range of compounds detected was broadly similar, although the volatiles released from hawthorn flowers were notably richer in aromatic compounds. A recent study examining both the volatiles and epicuticular wax from faba bean flowers indicated that the high proportion of phenylpropenyl compounds detected in the volatiles was also reflected in the composition of the epicuticular wax, which was found to contain a high proportion of cinnamyl alcohol esters. (Griffiths et al., 1999). Comparatively little information appears available regarding either the ecological significance or chemical composition of flower epicuticular waxes. It has been suggested that they may aid in pollen transfer and/or improve drainage within the flower (Juniper, 1995).

The objectives of this study were to chemically characterise and compare the epicuticular waxes from raspberry and hawthorn flowers and to determine if the

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differences previously reported in volatile composition was reflected in their respective wax chemistry.

2. Results and discussion

2.1. Alkanes

Long-chain hydrocarbons accounted for between 27 and 31% of the total wax (Table 1) extracted from the flowers of the three raspberry cultivars. The individual hydrocarbons ranged from C21 to C33, with odd-numbered hydrocarbons predominating (Table 2). In all three cultivars, C29 was the predominant homologue, which was the same as that reported in a recent study of raspberry leaf epicuticular waxes (Shepherd et al., 1999a). The distribution of the individual alkanes was almost identical in Glen Prosen and Glen Ample but the hydrocarbon fraction isolated from Malling Leo flowers contained higher proportions of the lower odd-carbon-numbered compounds (C23 and C25). The relative amount of total hydrocarbons in wax derived from hawthorn flowers was of the same order of magnitude as that from the raspberry flowers and as in the latter, the hydrocarbon profile was dominated by the C29 alkane. The distribution of the individual alkanes, which ranged from C19 to C31, was broadly similar to that found in the raspberry flower waxes. However, the hawthorn flower wax contained much less of the C27 alkane, which accounted for less than 10% of the total hydrocarbons as compared with over 20% in the three raspberry cultivars.

Table 1
The distribution (expressed as a relative apparent percentages)[a] of different classes of compounds in epicuticular wax extracted from raspberry and hawthorn flowers

	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
Alkanes	31	31	27	37
Alcohols	15	9	10	8
Aldehydes	4	1	2	1
Fatty acids	9	10	11	1
Fatty acid methyl esters	4	1	1	nd
Long chain alcohol esters	14	32	36	3
Tocopherols	1	1	1	2
Pentacyclic triterpenes	22	15	13	48

[a] (Combined areas of peaks for compounds of given class/combined areas of all identified peaks from all classes)×100.

Table 2
The relative concentrations of hydrocarbon (as % total hydrocarbons) in epicuticular wax extracted from raspberry and hawthorn flowers

Hydrocarbon carbon no.	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
C19	nd[a]	nd	nd	3.4
C20	nd	nd	nd	0.9
C21	4.9	1.1	0.8	8.9
C22	0.9	0.3	tr[b]	1.8
C23	14.5	7.6	4.8	12.5
C24	1.1	0.4	0.4	1.8
C25	11.9	6.8	6.5	7.7
C26	1.2	0.5	0.5	0.5
C27	20.9	21.1	20.9	8.4
C28	3.8	1.8	1.5	2.6
C29	24.6	41.6	42.9	44.6
C30	1.9	1.6	2.1	1.3
C31	12.7	15.3	18.0	5.7
C32	nd	nd	nd	nd
C33	1.9	1.8	1.5	nd

[a] nd, not detected.
[b] tr, trace.

2.2. Alcohols and aldehydes

The relative concentrations of long-chain alcohols (Table 3), determined as their trimethylsilyl (TMSi) derivatives, was similar in both the hawthorn and raspberry flower wax. In the case of the former, long-chain alcohols accounted for 8.4% of the total wax as compared with an average value of 11.2% for the three raspberry cultivars. However, the predominant alcohols present in raspberry flower wax were the C26 and C28 alcohols whilst in hawthorn flower wax shorter-chain alcohols (C20–C24) predominated. Wax from both species also contained detectable levels of long-chain aldehydes (Table 2) with carbon numbers ranging from C18 to C26. In contrast to the alcohols, the longer-chain aldehydes (C24 and C26) were more prevelant in hawthorn, whilst raspberry flower wax contained higher proportions of the shorter-chain-length aldehydes.

Table 3
The relative concentrations of individual alcohols and aldehydes (as % individual class) in epicuticular wax extracted from raspberry and hawthorn flowers

	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
Alcohol carbon no.				
C20	0.4	0.4	0.8	28.4
C22	13.7	8.5	13.5	27.3
C24	19.8	14.3	21.8	22.9
C26	28.3	28.8	29.1	15.4
C28	24.8	32.6	20.1	6.0
C30	13.0	15.5	14.6	nd[a]
Aldehyde carbon no.				
C18	2.2	1.6	1.5	nd
C20	19.9	13.3	18.4	4.7
C22	36.2	36.0	49.4	10.0
C24	31.8	27.5	20.4	58.0
C26	9.9	21.6	10.3	27.3

[a] nd, not detected.

2.3. Fatty acids and their methyl esters

Free fatty acids, which were quantified and identified as TMSi derivatives,were present in the floral wax of both species with, as expected, even-carbon-numbered acids predominating. As in raspberry leaf wax (Shepherd et al., 1999a), the most common free fatty acid in raspberry flower wax was the C24 acid (Table 4) but in the hawthorn flower wax the highest individual relative concentration was found for the C16 acid. However, in hawthorn flower wax, the free fatty acids accounted for less than 1% of the total wax as compared with an average value of over 10% for the three raspberry cultivars.

In addition to free fatty acids, flower wax from all three raspberry cultivars also contained methyl esters of the even-carbon-numbered acids in the range C18–C30. The distribution of the methyl esters broadly reflected that of the free fatty acids, with the methyl esters of the C24 and C26 acids predominant. Methyl esters accounted for over 4% of the total wax extracted from Malling Leo flowers as compared with less than 1% of the flower waxes from Glen Ample and Glen Prosen. Fatty acid methyl esters have rarely been reported as constituents of natural plant waxes (Hamilton, 1995). Trace amounts (0.25% of total wax) have, however, been detected in epicuticular waxes from eragrostoid grasses (Tulloch, 1984) and at levels of around 7% in waxes from the needles of white spruce (*Picea gauca*) and balsam fir (*Abies balsamea*) trees (Tulloch, 1987).

Table 4
The relative concentrations of individual fatty acids and fatty acid methyl esters (as % individual class) in epicuticular wax extracted from raspberry and hawthorn flowers

	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
Fatty acid carbon no.				
C13	0.9	1.2	1.0	[a]nd
C14	1.6	0.9	1.4	7.5
C15	0.8	0.8	1.7	nd
C16	7.2	5.2	7.3	30.8
C17	0.6	0.8	0.7	nd
C18	6.1	5.1	3.8	7.8
C19	0.2	0.3	0.2	5.5
C20	10.1	12.6	19.2	4.3
C22	25.1	24.8	29.4	3.6
C24	27.7	27.4	20.2	15.8
C26	15.2	14.7	10.8	13.3
C28	4.8	6.1	4.1	11.3
Fatty acid methyl ester carbon no.				
C18	0.8	0.6	0.8	nd
C20	4.7	3.1	5.8	nd
C22	10.9	11.5	17.7	nd
C24	29.8	25.8	24.9	nd
C26	33.5	41.9	30.4	nd
C28	15.1	11.5	10.7	nd
C30	5.2	5.6	9.7	nd

[a] nd, not detected.

2.4. Long-chain alcohol-fatty acid esters

In total, long-chain alkyl esters accounted for 14, 32 and 36% of the wax extracted, respectively, from the flowers of the raspberry cultivars Malling Leo, Glen Ample and Glen Prosen (Table 1). Although Malling Leo flowers contained relatively less esters than the other cultivars examined, the chain length distribution of the detected esters was identical in all three cultivars ranging from C36 to C48 with C42 esters being consistently the most abundant as shown for the cultivar Glen Ample in Table 5. Esters with a similar range of carbon numbers were also detected in hawthorn flower wax but these accounted for only 3% of the total wax with C40 esters predominating (Table 6). Long-chain alkyl esters with carbon numbers ranging from C36 to C54 have been reported to account for between 50 and 80% of the total wax extracted from raspberry leaves (Shepherd et al., 1999a,b). In faba bean flower wax, esters in the range C32–C46 have been detected at levels corresponding to 7% of the total wax (Griffiths et al., 1999).

Esters with identical carbon numbers may be produced from different combinations of long-chain alcohols and acids. The identity and relative amounts of each individual ester found within the ester peak of given carbon number was determined by mass spectral analysis based on the characteristic $[RCO_2H_2]^+$ ions derived from the acid portions of the constituent esters by McLafferty rearrangement (Shepherd et al., 1995). The results for the three raspberry cultivars gave similar results (results for Glen Ample shown in Table 5) with,

Table 5
The relative concentration of individual fatty acid–fatty alcohol esters (as % total esters) in the epicuticular wax extracted from raspberry flowers (cv Glen Ample)

	Fatty acid							
Fatty alcohol	C14	C16	C18	C20	C22	C24	C26	Total
C14	nd[a]	nd	nd	nd	nd	tr[b]	tr	0.1
C16	nd	nd	nd	nd	nd	0.1	tr	0.1
C18	nd	nd	nd	0.3	0.5	0.6	0.2	1.5
C20	nd	0.3	0.3	8.7	4.7	2.1	0.5	16.5
C22	0.1	2.0	0.9	23.8	19.1	9.6	1.8	57.3
C24	0.2	1.7	0.5	7.1	2.7	0.7	nd	12.8
C26	0.4	1.5	0.4	5.0	1.1	nd	nd	8.3
C28	0.3	0.6	0.2	1.6	nd	nd	nd	2.7
C30	0.2	0.3	tr	nd	nd	nd	nd	0.5
C32	0.1	0.1	nd	nd	nd	nd	nd	0.2
Total	1.2	6.4	2.3	46.4	28.1	13.1	2.6	

[a] nd, not detected.
[b] tr, trace (< 0.05%).

in each case, the predominant constituents of the esters consisting of even-numbered acids in the range C14–C26 combined with even-numbered alcohols (C14–C32). In the esters of all three cultivars C20 was the predominant fatty acid which was present in over 48% of all esters, when averaged over the three cultivars, whilst the C22 alcohol was detected, on average, in over 55% of all esters. This alcohol has also been reported as the most common constituent of raspberry leaf wax esters, being found in over 60% of all esters. Similarly, in the raspberry leaf waxes the major acids found in the constituent esters were C20, C22 and C24 fatty acids (Shepherd et al., 1999b).

The carbon numbers of the acids and alcohols present in the esters of hawthorn flowers (Table 6) were broadly similar to those found for the raspberry flower esters, and differed only in that C32 alcohols were not detected in hawthorn flower wax. However, palmitic acid (C16) was the predominant acid constituent of the hawthorn flower esters, being found in almost 80% of the esters detected, most commonly in combination with the C24 alcohol (Table 6).

In a recent study of the epicuticular wax of faba bean flowers (Griffiths et al., 1999), fatty acid esters derived from both phytol and cinnamyl alcohol were identified, the latter class of compounds apparently reflecting the relatively high concentration of phenylpropenoids detected in the floral bouquet of faba bean flowers. In this study, neither class of compounds was detected in flower wax from either raspberry or hawthorn, and no evidence for other ester classes based on either aromatic acids or aromatic alcohols was observed. Consequently it would appear that the high levels of aromatic compounds detected in hawthorn flower volatiles (Robertson et al., 1993) was not reflected in the chemistry of the wax esters.

Table 6
The relative concentration of individual fatty acid-fatty alcohol esters (as % total esters) in the epicuticular wax extracted from hawthorn flowers

	Fatty acid							
Fatty alcohol	C14	C16	C18	C20	C22	C24	C26	Total
C14	nd[a]	nd	nd	nd	0.1	0.1	0.4	0.5
C16	nd	nd	nd	0.1	nd	0.1	tr[b]	0.2
C18	nd	nd	0.2	0.4	nd	0.1	0.1	0.7
C20	nd	18.9	3.1	2.2	0.6	0.2	nd	25.0
C22	0.8	11.8	1.8	1.7	0.5	0.2	nd	16.8
C24	0.7	38.7	2.3	2.9	0.5	nd	nd	45.1
C26	0.4	8.3	0.4	1.2	nd	nd	nd	10.3
C28	0.1	1.0	nd	nd	nd	nd	nd	1.2
C30	nd	0.2	nd	nd	nd	nd	nd	0.2
Total	2.0	79.0	7.7	8.5	1.7	0.6	0.5	

[a] nd, not detected.
[b] tr, trace (< 0.05%).

2.5. Tocopherols

Tocopherols appear to have been rarely reported as constituents of epicuticular plant wax. However, in a recent study of raspberry leaf wax (Shepherd et al., 1999a) α-, δ- and γ-tocopherol were detected and together accounted for between 1 and 2% of the total wax. These three tocopherols were also detected in raspberry flower wax (Table 7) but in contrast to the raspberry leaf wax, where γ-tocopherol predominated (Shepherd et al., 1999a), the highest relative concentration in the flower wax was found for δ-tocopherol. Hawthorn flower wax contained the three tocopherols detected in raspberry flowers with α-tocopherol being the most abundant; additionally, small quantities of β-tocopherol were also detected.

2.6. Pentacyclic triterpenes

The identity of the constituent non-esterified pentacyclic triterpenes were confirmed by comparison of the mass spectra and retention times of their trimethylsilyl derivatives with those similarly prepared from authenticated standards. In the raspberry flower wax, pentacyclic triterpenoids and their esters accounted for between 13 and 22% of the total wax (Table 1) with ursolic and oleanolic acids being the most abundant compounds in all three cultivars. Hawthorn flower wax also contained pentacyclic triterpenes, which together accounted for almost 50% of the total wax (Table 1). Although the same classes of compounds were detected in the wax of both species (Table 8), hawthorn wax contained a much higher proportion of amyrins and amyrin esters. Within each class of terpenoids, and in both plant species, compounds based on the ursane skeleton predominated, with α-amyrin and α-amyrin esters being more abundant than β-amyrin and β-amyrin esters. Similarly uvaol and ursolic acid were found at relatively higher concentrations than their respective oleane-based equivalents, erythrodiol and oleanolic acid.

High levels of ursolic acid have been previously reported in epicuticular waxes of other Rosaceous species

Table 7
The relative concentrations of tocopherols (as % total tocopherols) in epicuticular wax extracted from raspberry and hawthorn flowers

Compound	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
α-Tocopherol	11.1	7.3	8.6	53.8
β-Tocopherol	nd[a]	nd	nd	4.4
δ-Tocopherol	54.9	67.9	57.0	20.0
γ-Tocopherol	34.0	24.9	34.4	22.1

[a] nd, not detected.

Table 8
The relative concentrations of pentacyclic triterpenes (as % total pentacyclic triterpenes) in epicuticular wax extracted from raspberry and hawthorn flowers

Compound	Malling Leo	Raspberry Glen Ample	Glen Prosen	Hawthorn
α-Amyrin	53.0	61.5	63.7	81.4
β-Amyrin	47.0	38.5	36.3	18.6
Total Amyrins[a]	0.6	0.1	0.2	15.5
α-Amyrin ester				
C14 acid	17.8	17.7	20.3	10.2
C15 acid	nd[b]	nd	nd	1.5
C16 acid	59.8	58.9	58.4	57.2
C18acid	nd	nd	nd	18.5
β-Amyrin esters				
C14 acid	5.7	4.8	7.5	1.5
C16 acid	16.7	18.6	13.8	8.7
C18acid	nd	nd	nd	2.4
Total esters[a]	0.1	0.1	0.1	13.7
Ursolic acid	70.6	76.8	80.5	76.8
Oleanolic acid	25.5	20.5	18.1	15.9
Uvaol	3.4	2.4	1.2	6.9
Erythrodiol	0.5	0.3	0.3	0.5
Total[a]	20.8	15.1	13.1	19.0

[a] Expressed as % of the total ion current.
[b] nd, not detected.

including leaf (Silva Fernandes et al., 1964) and fruit (Belding et al., 1998) waxes from apples (*Malus* spp) and in leaf waxes from peach (*Prunus persica*) leaves (Bukovac et al., 1979). Likewise, analysis of fruit wax from plums (*Prunus domestica* L.) revealed the presence of both oleanolic and ursolic acids (Ismail et al., 1977). Although the objective of this study was only to chemically characterise epicuticular flower waxes from raspberry and hawthorn, it is of interest to note that adult raspberry beetles can also be found in the flowers of many of these species (Gordon et al., 1997). Consequently, it is tempting to conclude that the pentacyclic triterpenes, in particular ursolic acid located in the flower wax of both raspberries and hawthorn, may play a part in host-plant recognition and may be worthy of further investigation. However, it should be stressed that the fact that these terpenoid acids have been detected in fruit and leaf samples of other members of the Rosaceae does not necessarily mean that they must also be present in the flowers. It has been frequently demonstrated that wax from different organs from the same plant can differ substantially in composition (Bianchi, 1995) and indeed no terpenoid acids were detected in raspberry leaf wax (Shepherd et al., 1999a). Additionally, ursolic and oleanolic acids have been detected in the epicuticular waxes of other plant species including the olive (*Olea europea*) fruit (Bianchi et al., 1992) and on the leaves of *Ilex aquifolium* (Van Genderen and Jaarsma, 1990).

3. Experimental

3.1. Epicuticular wax extracts

Samples of hawthorn flowers, each consisting of 40 panicles of approximately eight flowers per panicle, were randomly picked from locally growing bushes in May 1999. All associated leaf material was manually removed and each panicle was individually dipped for a maximum of 5 s into 40 ml dichloromethane which after filtration, was concentrated to a final volume of 10 ml. A 2 ml aliquot was then taken and reduced to 0.1 ml using a gentle stream of gaseous nitrogen and subsequently analysed by gas chromatography–mass spectrometry (GC–MS).

Raspberry flowers (approx. 200 per sample) from each of three cultivars (Malling Leo, Glen Ample and Glen Prosen) were hand-picked from a replicated field trial in the summer of 1999. Care was taken to select flowers prior to petal drop and to ensure the resulting samples were devoid of any leaf material. Each flower was individually dipped into dichloromethane and concentrated as for the hawthorn flower samples.

3.2. Derivatisation

Trimethylsilyl (TMSi) derivatives of both the epicuticular wax extracts and standard compounds were prepared using either *N,O*-bis (trimethylsilyl)-trifluoroacetamide (Shepherd et al., 1995) or *N*-trimethylsilylimidazole.

3.3. Gas chromatography–mass spectrometry

Extracts were analysed using a Hewlett Packard 5989 GC–MS fitted with a DB-5MS column (30 m×0.25 mm id, 0.25 μm film, J & W Scientific, Fulsom, CA, USA). Samples (1–1.5 μl) were loaded by cold on-column injection and electronic pressure programming was used to maintain a constant flow (1 ml min^{-1}) of the helium carrier gas. The oven temperature was held at 55°C for 5 min and then increased at a rate of 4°C min^{-1} to a final temperature of 350°C. The mass spectrometer was used in EI mode (ionization energy of 70 eV; trap current of 300 μA) and set to scan the mass range 35–900 amu at the rate of 1 scan per s, with source and quadrupole temperatures held at 250 and 100°C, respectively. The resulting data was processed using the Hewlett Packard G1034 MS Chemstation Software package. The relative apparent percentage of each compound within its given chemical class was determined by dividing the integrated area of the peak for the compound in

question (× 100), by the summed value for the areas of all peaks of the same chemical class. Component identification was carried out using the Wiley 138 K mass spectral data base and where available confirmed by comparison of both retention times and mass spectra of authenticated standards.

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References

Belding, R.D., Blankenship, S.M., Young, E., Leidy, R.B., 1998. Composition and variability of epicuticular waxes in apple cultivars. Journal of the American Society of Horticultural Science 123, 348–356.

Bianchi, G. 1995. Plant waxes. In: Hamilton, R.J. (Ed.), Waxes: Chemistry, Molecular Biology and Functions, Dundee: The Oily Press, pp. 175–222.

Bianchi, G., Murelli, C., Vlahov, G., 1992. Surface waxes from olive fruits. Phytochemistry 31, 3503–3506.

Bukovac, M.J., Flore, J.A., Baker, E.A., 1979. Peach leaf surfaces: changes in wettability, retention, cuticular permeability and epicuticular wax chemistry during expansion with special reference to spray application. Journal of the American Society of Horticultural Science 104, 611–617.

Gordon, S.C., Woodford, J.A.T., Birch, A.N.E., 1997. Arthropod pests of *Rubus* in Europe: pest status, current and future control strategies. Journal of Horticultural Science 72, 831–862.

Griffiths, D.W., Robertson, G.W., Shepherd, T., Ramsay, G., 1999. Epicuticular waxes and volatiles from faba bean (*Vicia faba*) flowers. Phytochemistry 52, 607–612.

Hamilton, R.J., 1995. Analysis of waxes. In: Hamilton, R.J. (Ed.), Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, pp. 311–342.

Ismail, H.M., Brown, G.A., Tucknott, O.G., Holloway, P.J., Williams, A.A., 1977. Nonanal in epicuticular wax of Golden Egg plums (*Prunus domestica*). Phytochemistry 16, 769–770.

Juniper, B.E., 1995. Waxes on plant surfaces and their interaction with insects. In: Hamilton, R.J. (Ed.), Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, pp. 157–174.

Robertson, G.W., Griffiths, D.W., Woodford, J.A.T., Birch, A.N.E., Pickett, J.A., Wadhams, L.J., 1993. A comparison of the flower volatiles from hawthorn and four raspberry cultivars. Phytochemistry 33, 1047–1053.

Shepherd, T., Robertson, G.W., Griffiths, D.W., 1995. Compositional analysis of intact alkyl esters in leaf epicuticular wax of swede by capillary gas chromatography and electron impact-mass spectrometry. Phytochemical Analysis 6, 65–73.

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E., 1999. Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L). Phytochemistry 52, 1239–1254.

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E., 1999b. Epicuticular wax ester and triacylglycerol composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L). Phytochemistry 52, 1255–1267.

Silva Fernandes, A.M., Barker, E.A., Martin, J.T., 1964. Studies on plant cuticle VI. The isolation and fractionation of cuticular waxes. Annals of Aplied Biology 53, 43–58.

Tulloch, A.P., 1984. Epicuticular waxes from four eragrostoid grasses. Phytochemistry 23, 1619–1623.

Tulloch, A.P., 1987. Epicuticular waxes of *Abies balsamea* and *Picea glauca*: Occurrence of long-chain methyl esters. Phytochemistry 26, 1041–1043.

Van Genderen, H.H., Jaarsma, J., 1990. Triterpenes and alkanes in developing variegated and albino leaves of *Ilex aquifolium* L. (Aquifoliaceae). Plant Science 72, 165–172.