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Epicuticular wax ester and triacylglycerol composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.)

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Abstract

Epicuticular waxes from two cultivars of red raspberry (*Rubus idaeus*) were collected from the newly emerging crown leaves, and also from the group of four more mature leaves immediately below the crown. One cultivar, Autumn Bliss, was identified as aphid-resistant, and the other, Malling Jewel, as aphid-susceptible following bioassay with the large raspberry aphid, *Amphorophora idaei*, just prior to collection of the wax. Biological activity was primarily associated with the more mature leaves. Epicuticular wax esters consisted predominantly of long-chain aliphatic compounds in which even-carbon-number acids were esterified to even-carbon-number alcohols. Lesser amounts of odd-carbon-number esters were also present. The acid : alcohol combinations of the major esters were C_{38}: 14 : 24, 16 : 22, 20 : 18; C_{40}: 14 : 26, 16 : 24, 18 : 22, 20 : 20; C_{42}: 16 : 26, 20 : 22; 22 : 20, C_{44}: 20 : 24, 22 : 22, 24 : 20; C_{46}: 20 : 26, 22 : 24, 24 : 22; C_{48}: 20 : 28, 22 : 26, 24 : 24, 26 : 22; C_{50}: 20 : 30, 22 : 28, 24 : 26, 26 : 24, 28 : 22 and C_{52}: 22 : 30, 24 : 28, 26 : 26. Terpenyl esters were also present and these consisted of α- and β-amyrin and cycloartenol esterified to C_{16}, C_{18} and C_{20} acids. Compositional differences between the more mature leaves which may relate to resistance to *A. idaei* were higher levels of cycloartenyl esters and α-amyryl esters in wax from the resistant cultivar Bliss. There were also differences between the cultivars in the distribution of individual alkyl esters and their component acids and alcohols. Esters with longer acid : shorter alcohol combinations were more abundant in Jewel than Bliss. There were compositional differences between leaves at the different developmental stages. Alkyl esters were more abundant and cycloartenyl esters were not detected in wax from the immature leaves. Small amounts of an unusual class of triacylglycerol were found only on leaves of the aphid-susceptible cultivar, Jewel, which had been subject to bioassay with raspberry aphid. These compounds, which have a C_6 acid at C-2 of the glycerol backbone, were derived from the aphid, and are the major component in the insect's cornicle secretions. The triacylglycerols probably arise from the presence on the leaf surface of shed aphid skins, or by incorporation of cornicle fluid into the leaf wax. The abundance of aphid triacylglycerols on the leaf surface may provide a measure of aphid-susceptibility.

Keywords: *Rubus idaeus*; Red raspberry; Rosaceae; Leaf epicuticular wax; Wax composition; Wax esters; Triacylglycerols; *Amphorophora idaei*; Raspberry aphid; Aphidae; Aphid resistance; Aphid susceptibility

1. Introduction

The microcrystalline layer of wax present on the leaf surface is believed to play an important role in the process of host-plant recognition by herbiverous insects, including aphids (Eigenbrode & Espelie, 1995; Juniper, 1995). The large raspberry aphid, *Amphorophora idaei* (Börner) is a major pest of red raspberry, *Rubus idaeus*, L. (family: Rosacaea), an important soft fruit crop in temperate climates (Jones, 1979, 1986; Birch & Jones, 1988). As part of our studies of the ecological role of plant cuticular waxes, we demonstrated a possible relationship between cuticular

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Table 1
General distribution of the different classes of ester in epicuticular wax from raspberry cultivars, Autumn Bliss and Malling Jewel

	Autumn Bliss (resistant)[a]			Malling Jewel (susceptible)[a]		
	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
Rest of Wax	40.66	44.09	20.95	34.76	34.26	25.35
	0.35	*4.94*		*3.44*	*2.90*	
Alkyl esters	58.05	54.18	78.22	64.20	64.52	73.40
	0.28	*5.13*		*3.14*	*3.11*	
Amyryl esters	0.46	0.66	0.82	0.82	1.03	0.95
	0.04	*0.08*		*0.30*	*0.18*	
Cycloartenyl esters	0.68	0.91	n.d.[g]	0.07	0.06	n.d.[g]
	0.35	*0.34*		< [h]	*0.01*	
Triacylglycerols[f]	n.d.[g]	n.d.[g]	n.d.[g]	n.d.[g]	0.85	0.52
					0.44	

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are expressed as a percentage of total wax content, excluding triacylglycerols.
[b] Values are means and standard deviations for three replicates each of four or five plants.
[c] Control plants, not subject to bioassay with *A. idaei*.
[d] Plants subject to bioassay with *A. idaei*.
[e] Combined sample from all plants.
[f] Values are expressed relative to the total for all other wax components.
[g] Not detected.
[h] Standard deviation < 0.005.

wax composition and resistance to *A. idaei*, based on a partial analysis of the wax from several cultivars of red raspberry (Robertson, Griffiths, Birch, Jones, McNicol & Hall, 1991). We have subsequently carried out a more detailed comparison between the aphid-resistant raspberry cultivar, Autumn Bliss, and the aphid-susceptible cultivar, Malling Jewel, in which the composition of epicuticular wax from both was analysed in full. Samples of wax were obtained from the crown of emerging leaves, and also from the four fully unfolded leaves just below the crown. The plants were subject to bioassay with *A. idaei* to demonstrate resistance and susceptibility to the insect prior to collection of the wax. The results of these experiments are described here and also in the preceding paper, in which full details of the collection of wax and the bioassay with *A. idaei* are given. Samples were also taken from a similar control group of plants which were not subjected to bioassay, in order to assess whether the presence of aphids had any effect on the composition of the leaf wax. In the preceding paper we considered the possible role of the various chemical components found within the cuticular waxes in relation to resistance or susceptibility to *A. idaei*. All the major classes of chemical compound were included with the exception of the long-chain esters.

In many plant species, including raspberry, long-chain esters are major components (Tulloch, 1976; Bianchi, 1995; Reiderer & Schreiber, 1995; Shepherd et al., 1999, preceding paper), and in some plants, their presence may be a significant factor in conferring resistance to insect pests (Atkin, Hamilton & Bernays, 1982; Woodhead, 1983; Dillwith & Berberet, 1990; Bergman, Dillwith, Zarrabi, Caddel & Berberet, 1991; Shepherd, Robertson, Griffiths, Birch & Duncan, 1995b; Shepherd, Robertson, Griffiths & Birch 1997). Here we consider the possible significance of long-chain esters in relation to the interaction between red raspberry and *A. idaei*.

2. Results and discussion

2.1. Wax composition

Three classes of long-chain ester were found in the wax of both raspberry cultivars (Table 1). Of these, saturated straight-chain *n*-alkyl esters were the most abundant, and they constituted the bulk (54–78%) of the wax. Triterpenoid esters were found in all samples, at up to 1.5% abundance, and these consisted of amyryl and cycloartenyl alkanoates. The third type of esters were triacylglycerols, and these were found exclusively in wax from *A. idaei*-susceptible Jewel that had been infested with the aphid prior to wax collection. Alkyl esters consisted of a homologous series in the range C_{36}–C_{54} (Table 2). Each homologue consisted of several positional isomers which varied in the length of the acid and alcohol moieties. The distribution of individual esters, and their component acids and alcohols are shown in Tables 3–5 respectively, and the distribution of individual terpenyl esters and triacylglycerols are given in Table 6.

The maximum degree of biological activity,

Table 2
Distribution of long-chain alkyl esters in epicuticular wax from raspberry cultivars, Autumn Bliss and Malling Jewel

Esters	Autumn Bliss[a]			Malling Jewel[a]		
	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
C_{36}	0.06	0.08	0.60	0.12	0.10	1.18
	0.02	*0.01*		*0.02*	*0.03*	
C_{37}	0.07	0.06	0.42	0.14	0.12	0.74
	0.02	*0.01*		*0.03*	*0.03*	
C_{38}	0.91	1.00	3.13	1.66	1.58	4.73
	0.07	*0.08*		*0.10*	*0.33*	
C_{39}	0.12	0.12	0.48	0.16	0.16	0.72
	0.02	*0.01*		*0.01*	*0.04*	
C_{40}	2.55	2.88	5.32	2.74	2.75	5.67
	0.19	*0.29*		*0.24*	*0.41*	
C_{41}	0.24	0.27	0.57	0.24	0.26	0.64
	0.02	*0.03*		*0.02*	*0.03*	
C_{42}	10.87	12.09	15.82	11.57	12.00	16.16
	0.47	*0.91*		*0.14*	*1.08*	
C_{43}	0.51	0.55	0.74	0.51	0.51	0.71
	0.02	*0.03*		*0.06*	*0.04*	
C_{44}	23.55	24.04	25.31	23.15	23.83	22.27
	0.34	*0.28*		*0.69*	*0.80*	
C_{45}	1.04	1.02	0.81	0.93	1.00	0.84
	0.08	*0.01*		*0.04*	*0.03*	
C_{46}	32.39	31.20	27.40	31.66	31.31	26.12
	0.61	*1.26*		*0.92*	*1.36*	
C_{47}	0.96	0.87	0.66	0.83	0.86	0.69
	0.14	*0.04*		*0.08*	*0.04*	
C_{48}	17.51	15.86	13.61	17.94	17.25	14.11
	0.55	*0.96*		*0.48*	*1.22*	
C_{49}	0.41	0.35	0.27	0.38	0.36	0.19
	0.04	*0.04*		< [f]	*0.03*	
C_{50}	6.21	6.53	3.30	5.71	5.74	3.49
	0.12	*0.51*		*0.56*	*0.13*	
C_{51}	0.33	0.64	0.15	0.33	0.34	0.07
	0.06	*0.24*		*0.18*	*0.06*	
C_{52}	1.54	1.63	0.92	1.33	1.27	1.05
	0.08	*0.10*		*0.02*	*0.05*	
C_{53}	0.13	0.22	0.11	0.08	0.06	0.13
	0.03	*0.02*		*0.02*	*0.02*	
C_{54}	0.59	0.57	0.39	0.51	0.48	0.47
	0.05	*0.04*		*0.05*	*0.04*	

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are expressed as a percentage of total alkyl esters.
[b] Values are means and standard deviations for three replicates each of four or five plants.
[c] Control plants, not subject to bioassay with *A. idaei*.
[d] Plants subject to bioassay with *A. idaei*.
[e] Combined sample from all plants.
[f] Standard deviation < 0.005.

expressed during bioassay as an increase in the aphid population on the susceptible cultivar, Jewel, was observed for the group of four more mature leaves in the mid-foliar region below the crown of the plant. Consequently, consideration of the role of leaf surface chemicals in resistance and susceptibility to *A. idaei* was centred on the mid-foliar region. As a comparison between the cultivars, selected data representing the major components in the waxes from the mid-foliar regions of Bliss and Jewel are also shown in diagrammatic form for the homologous series of esters (Fig. 1(a)), their component acid (Fig. 1(b)) and alcohol (Fig. 1(c)) moieties, for the major individual esters (Fig. 2) and for the terpenyl esters and triacylglycerols (Fig. 3).

2.1.1. Saturated alkyl esters

Alkyl esters were of slightly greater abundance in wax from mature leaves of Jewel than Bliss (Table 1). The overall distribution of ester chain lengths were

Table 3
Distribution of individual esters in epicuticular wax from raspberry cultivars, Autumn Bliss and Malling Jewel

Esters (C_n)	Acid : Alcohol	Autumn Bliss[a]			Malling Jewel[a]		
		Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
C_{36}	14 : 22	100.00	n.d.[f]	10.67	36.39	18.23	20.57
	16 : 20	n.d.[f]	n.d.[f]	89.33	63.61	81.77	79.43
C_{38}	14 : 24	3.27	5.47	5.81	6.99	5.96	5.85
	16 : 22	89.06	87.71	87.87	89.08	91.09	88.06
	18 : 20	n.d.[f]	n.d.[f]	3.09	1.43	n.d.[f]	2.51
	20 : 18	7.67	6.82	1.98	1.85	2.00	1.93
	22 : 16	n.d.[f]	n.d.[f]	0.61	n.d.[f]	n.d.[f]	0.16
	24 : 14	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	0.95	1.19
C_{40}	14 : 26	6.97	7.41	4.27	9.24	9.05	4.03
	16 : 24	41.12	40.62	30.76	42.70	45.81	34.93
	18 : 22	5.78	8.18	6.57	9.02	8.08	6.00
	20 : 20	41.82	43.78	55.19	35.28	35.42	53.64
	22 : 18	4.30	n.d.[f]	3.20	n.d.[f]	n.d.[f]	n.d.[f]
	24 : 16	n.d.[f]	n.d.[f]	n.d.[f]	3.76	1.64	0.38
C_{41}	16 : 25	100.00	n.d.[f]	n.d.[f]	100.00	100.00	36.82
	20 : 21	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	41.52
	21 : 20	n.d.[f]	53.85	81.55	n.d.[f]	n.d.[f]	21.66
	22 : 19	n.d.[f]	n.d.[f]	18.45	n.d.[f]	n.d.[f]	n.d.[f]
	23 : 18	n.d.[f]	46.15	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
C_{42}	12 : 30	0.18	n.d.[f]	n.d.[f]	0.29	0.49	0.40
	13 : 29	0.22	0.19	0.16	0.50	0.24	0.57
	14 : 28	0.39	1.24	0.91	1.53	1.92	1.04
	15 : 27	n.d.[f]	n.d.[f]	n.d.[f]	0.24	0.44	0.47
	16 : 26	11.97	12.12	5.77	7.52	8.17	5.60
	17 : 25	n.d.[f]	n.d.[f]	n.d.[f]	0.08	0.20	0.20
	18 : 24	2.57	2.01	0.92	2.82	2.68	0.98
	20 : 22	64.12	64.18	68.46	72.39	70.02	69.24
	21 : 21	0.15	n.d.[f]	0.13	n.d.[f]	0.16	0.12
	22 : 20	18.56	18.31	21.56	12.32	13.16	19.17
	23 : 19	0.06	n.d.[f]	n.d.[f]	n.d.[f]	0.15	0.14
	24 : 18	1.79	1.95	2.10	2.31	2.37	2.06
C_{43}	20 : 23	n.d.[f]	n.d.[f]	n.d.[f]	18.92	24.58	33.40
	21 : 22	76.20	31.75	55.59	72.10	75.42	54.64
	22 : 21	2.42	57.14	44.41	8.97	n.d.[f]	7.45
	23 : 20	21.38	11.11	n.d.[f]	n.d.[f]	n.d.[f]	4.51
C_{44}	12 : 32	0.09	0.10	0.33	0.15	0.30	0.44
	13 : 31	0.10	0.18	0.38	0.37	0.24	0.36
	14 : 30	0.11	0.59	0.46	0.75	0.97	0.67
	15 : 29	0.25	0.36	0.28	0.61	0.44	0.51
	16 : 28	1.75	1.54	1.22	1.55	1.85	1.40
	17 : 27	0.21	0.10	0.10	0.35	0.25	0.22
	18 : 26	2.77	2.88	0.56	1.87	1.85	0.62
	19 : 25	n.d.[f]	0.10	0.10	0.18	0.06	0.18
	20 : 24	18.21	18.82	17.72	18.85	22.12	19.51
	22 : 22	68.05	67.13	68.84	65.91	62.79	63.06
	23 : 21	0.07	0.05	0.04	0.03	0.01	0.09
	24 : 20	8.06	7.91	9.98	8.83	8.53	12.16
	26 : 18	0.24	0.26	n.d.[f]	0.55	0.58	0.72
C_{45}	20 : 25	34.53	39.34	23.78	51.23	42.63	23.01
	21 : 24	n.d.[f]	15.53	n.d.[f]	17.57	20.19	18.02
	22 : 23	34.41	24.82	22.95	3.10	14.21	9.13
	23 : 22	31.06	20.31	53.27	13.20	20.08	32.54
	24 : 21	n.d.[f]	n.d.[f]	n.d.[f]	14.90	2.88	17.30
C_{46}	12 : 34	0.07	0.10	0.09	0.23	0.09	0.24
	13 : 33	0.19	0.07	0.00	0.16	0.13	0.28
	14 : 32	0.37	n.d.[f]	0.15	0.50	0.40	n.d.[f]
	15 : 31	0.07	0.06	n.d.[f]	0.27	0.38	0.36
	16 : 30	0.86	0.86	0.56	0.97	0.92	0.97

Table 3 (*continued*)

Esters (C_n)	Acid : Alcohol	Autumn Bliss[a]			Malling Jewel[a]		
		Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
	17 : 29	0.18	n.d.[f]	n.d.[f]	0.31	0.35	0.30
	18 : 28	0.67	0.53	0.24	0.48	0.52	0.34
	19 : 27	n.d.[f]	n.d.[f]	n.d.[f]	0.18	0.33	0.21
	20 : 26	14.65	16.21	10.44	9.17	11.35	7.42
	21 : 25	n.d.[f]	0.21	n.d.[f]	0.38	0.23	0.10
	22 : 24	14.03	14.23	15.87	11.87	13.98	13.19
	23 : 23	0.11	0.03	0.09	0.04	0.01	0.18
	24 : 22	66.55	65.24	69.07	72.48	68.12	71.77
	26 : 20	2.25	2.46	3.48	2.92	3.11	4.60
	28 : 18	n.d.[f]	n.d.[f]	n.d.[f]	0.03	0.08	0.04
C_{47}	21 : 26	n.d.[f]	20.16	53.97	n.d.[f]	10.24	n.d.[f]
	22 : 25	86.54	57.22	21.25	10.40	28.41	32.55
	23 : 24	13.46	6.17	24.79	8.50	15.11	n.d.[f]
	24 : 23	n.d.[f]	n.d.[f]	n.d.[f]	26.25	13.99	n.d.[f]
	25 : 22	n.d.[f]	16.45	n.d.[f]	54.85	25.93	51.71
	26 : 21	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	6.31	15.74
C_{48}	12 : 36	n.d.[f]	n.d.[f]	n.d.[f]	0.08	0.11	n.d.[f]
	14 : 34	n.d.[f]	0.17	n.d.[f]	0.09	0.10	0.44
	16 : 32	n.d.[f]	n.d.[f]	0.24	0.44	0.33	0.83
	17 : 31	n.d.[f]	n.d.[f]	n.d.[f]	0.40	n.d.[f]	0.17
	18 : 30	0.19	n.d.[f]	n.d.[f]	0.36	0.10	0.23
	19 : 29	n.d.[f]	n.d.[f]	n.d.[f]	0.08	0.09	0.15
	20 : 28	5.81	7.27	3.98	4.43	5.50	3.64
	22 : 26	17.30	16.84	15.72	8.81	11.95	8.72
	23 : 25	0.14	n.d.[f]	0.13	0.08	0.05	0.09
	24 : 24	21.33	22.77	22.99	21.82	21.95	22.13
	26 : 22	55.05	52.78	56.94	63.16	59.48	62.91
	28 : 20	0.19	0.17	n.d.[f]	0.08	0.20	0.28
C_{49}	22 : 27	23.15	100.00	62.50	40.67	11.71	37.07
	23 : 26	76.85	n.d.[f]	37.50	32.33	28.38	n.d.[f]
	24 : 25	n.d.[f]	n.d.[f]	n.d.[f]	17.64	19.90	32.82
	26 : 23	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	40.01	30.11
C_{50}	20 : 30	3.82	6.78	1.48	6.12	9.96	4.13
	22 : 28	17.68	11.10	10.42	7.40	13.42	7.74
	23 : 27	n.d.[f]	n.d.[f]	n.d.[f]	0.19	1.21	n.d.[f]
	24 : 26	44.15	44.29	35.52	31.57	1.28	29.50
	26 : 24	30.12	26.45	35.30	36.37	49.51	38.89
	28 : 22	4.22	11.38	17.27	18.35	24.62	19.74
C_{52}	22 : 30	27.27	28.30	23.08	19.23	15.61	18.95
	24 : 28	39.39	37.74	42.31	48.08	46.41	47.37
	26 : 26	33.33	33.96	34.62	32.69	37.97	33.68

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are for individual esters of overall carbon number C_n consisting of acid : alcohol combinations of carbon numbers C_{nac} : C_{nalc}, and are expressed as percentages of the total for each overall ester C_n given in Table 2.

[b] Values are for replicate sample 2.

[c] Control plants, not subject to bioassay with *A. idaei*.

[d] Plants subject to bioassay with *A. idaei*.

[e] Combined sample from all plants.

[f] Not detected.

generally very similar (Table 2, Fig. 1(a)), except that Jewel had more esters of the shortest chain lengths (C_{36}–C_{38}) and Bliss more esters of the longest chain lengths (C_{52}–C_{54}). Esters were more abundant in wax from the younger emerging crown leaves, which also had proportionally more of the shorter (C_{36}–C_{42}) and less of the longer (C_{46}–C_{50}) homologues than wax from the older more mature leaves (Table 2).

Individual esters within each homologue were identified from the molecular ions ($[M]^+ = [RCO_2R']^+$) and the fragment ions $[RCO_2H_2]^+$ and $[R'\text{-}1]^+$ derived respectively from the acid and alcohol moieties of the ester by McLafferty rearrangement (Shepherd et al., 1995a) following analysis by GC–MS. Quantification was based on a combination of GC data (Table 2) for each homologue, and data from selected ion chromato-

Table 4
Distribution of acids in in epicuticular wax esters from raspberry cultivars, Autumn Bliss and Malling Jewel

Acids C_n	Autumn Bliss[a]			Malling Jewel[a]		
	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
C_{12}	0.08	0.06	0.15	0.19	0.20	0.26
C_{13}	0.13	0.11	0.14	0.24	0.15	0.32
C_{14}	0.51	0.64	0.91	1.04	1.09	1.47
C_{15}	0.10	0.12	0.08	0.31	0.32	0.37
C_{16}	4.87	4.51	7.61	4.91	5.07	11.00
C_{17}	0.12	0.03	0.03	0.28	0.22	0.19
C_{18}	1.63	1.55	0.94	1.45	1.34	1.03
C_{19}	n.d.[f]	0.03	0.03	0.12	0.14	0.12
C_{20}	22.24	23.22	25.04	21.18	22.53	25.42
C_{21}	0.11	0.21	0.16	0.36	0.45	0.32
C_{22}	30.63	29.78	30.39	25.87	26.08	24.10
C_{23}	0.23	0.13	0.13	0.13	0.21	0.22
C_{24}	30.05	30.43	26.17	32.09	30.33	25.29
C_{25}	n.d.[f]	0.02	n.d.[f]	0.09	0.06	0.06
C_{26}	9.17	8.85	7.92	11.28	11.30	9.43
C_{28}	0.12	0.29	0.31	0.46	0.53	0.41

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are expressed as a percentage of total fatty acids within the esters.
[b] Values are derived from those determined experimentally (Table 3) for individual esters containing these acids of carbon number C_n.
[c] Control plants, not subject to bioassay with *A. idaei*.
[d] Plants subject to bioassay with *A. idaei*.
[e] Combined sample from all plants.
[f] Not detected.

Table 5
Distribution of alcohols in in epicuticular wax esters from raspberry cultivars, Autumn Bliss and Malling Jewel

Alcohols C_n	Autumn Bliss[a]			Malling Jewel[a]		
	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]
C_{14}	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	0.02	0.10
C_{15}	0.03	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	0.01
C_{16}	0.02	n.d.[f]	0.02	0.11	0.05	0.04
C_{18}	0.55	0.44	0.68	0.54	0.56	0.74
C_{19}	0.01	n.d.[f]	0.01	n.d.[f]	0.02	0.03
C_{20}	7.31	7.19	12.06	6.41	6.49	12.89
C_{21}	0.04	0.06	0.09	0.09	0.05	0.25
C_{22}	61.28	60.68	62.74	65.93	62.82	62.57
C_{23}	0.15	0.10	0.05	0.11	0.20	0.25
C_{24}	15.61	15.88	15.00	15.32	17.22	14.43
C_{25}	0.20	0.31	0.08	0.46	0.44	0.35
C_{26}	11.47	12.11	7.04	7.19	7.92	5.10
C_{27}	0.07	0.09	0.04	0.22	0.30	0.24
C_{28}	2.19	2.12	1.37	1.71	2.14	1.27
C_{29}	0.17	0.16	0.11	0.36	0.29	0.34
C_{30}	0.61	0.68	0.41	0.82	0.89	0.62
C_{31}	0.06	0.07	0.11	0.25	0.20	0.20
C_{32}	0.16	0.03	0.16	0.28	0.27	0.20
C_{33}	0.07	0.03	n.d.[f]	0.08	0.05	0.22
C_{34}	0.03	0.06	0.03	0.09	0.04	0.11
C_{35}	n.d.[f]	n.d.[f]	n.d.[f]	0.01	n.d.[f]	0.02
C_{36}	n.d.[f]	n.d.[f]	n.d.[f]	0.01	0.02	n.d.[f]

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are expressed as a percentage of total alcohols within the esters.
[b] Values are derived from those determined experimentally (Table 3) for individual esters containing these alcohols of carbon number C_n.
[c] Control plants, not subject to bioassay with *A. idaei*.
[d] Plants subject to bioassay with *A. idaei*.
[e] Combined sample from all plants.
[f] Not detected.

Table 6
Distribution of terpene esters and triacylglycerols found in epicuticular wax from raspberry cultivars, Autumn Bliss and Malling Jewel

	Autumn Bliss[a]			Malling Jewel[a]		
	Aphid-free[b,c]	Plus aphids[b,d]	Tops[e]	Aphid-free[b,c]	Pluss aphids[b,d]	Tops[e]
Terpene esters						
Terpenol : acid						
β-Amyrin : C_{14}	4.80	5.04	n.d.[f]	12.62	9.56	n.d.[f]
	2.86	*0.66*		*10.05*	*1.64*	
α-Amyrin : C_{14}	2.15	0.85	n.d.[f]	0.69	0.53	n.d.[f]
	1.28	*0.10*		*0.55*	*0.15*	
β-Amyrin : C_{16}	7.37	8.84	13.73	23.75	21.72	54.93
	0.29	*2.40*		*0.64*	*1.13*	
Cycloartenol : C_{16}	9.38	10.43	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
	1.19	*3.20*				
α-Amyrin : C_{16}	16.95	11.06	78.20	9.98	7.80	39.06
	8.82	*2.19*		*5.65*	*0.88*	
β-Amyrin : C_{18}	4.23	9.28	4.36	28.37	31.35	2.25
	0.60	*4.35*		*16.21*	*0.44*	
Cycloartenol : C_{18}	34.40	27.49	n.d.[f]	8.67	5.88	n.d.[f]
	18.48	*17.57*		*3.12*	*1.40*	
α-Amyrin : C_{18}	0.21	1.25	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
	0.10	*0.58*				
β-Amyrin : C_{20}	8.24	7.00	3.71	15.93	23.16	3.75
	4.33	*2.75*		*2.81*	*2.21*	
Cycloartenol : C_{20}	12.28	18.74	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
	2.37	*5.21*				
Triacylglycerols (acids at C-1, C-2 and C-3 of the glycerol backbone)						
$C_{12}C_6C_{14}$	–	–		–	11.66	18.04
					2.56	
$C_{14}C_6C_{14}$	–			–	72.32	52.26
					2.78	
$C_{14}C_{6:2}C_{14}$	–			–	2.96	7.51
					0.33	
$C_{14}C_6C_{16}$	–			–	13.06	22.19
					0.63	

[a] Samples were taken from the crown of emerging leaves (tops) and from groups of four leaves immediately below the crown. Values are expressed as a percentage of total terpene esters, and as a percentage of total triacylglycerols.
[b] Values are means and standard deviations for three replicates each of four or five plants.
[c] Control plants, not subject to bioassay with *A. idaei*.
[d] Plants subject to bioassay with *A. idaei*.
[e] Combined sample from all plants.
[f] Not detected.

grams (SIC) for $[RCO_2H_2]^+$ as described previously for wax esters from swede (Shepherd et al., 1995a).

The distributions of individual esters are shown (Table 3, Fig. 2) as a proportion of each homologue in the range C_{36}–C_{52}. Compositional data for the individual C_{53} and C_{54} esters were not available as these lay beyond the range of chromatographic separation achievable during analysis by GC–MS. From these data, the distributions of component acids (C_{12}–C_{28}) (Table 4, Fig. 1(b)) and alcohols (C_{14}–C_{36}) (Table 5, Fig. 1(c)) within the esters were determined.

Even-carbon number esters were predominant, with lesser amounts of odd-carbon number compounds. The major esters (acid : alcohol combinations) were C_{38}: 14 : 24, 16 : 22, 20 : 18; C_{40}: 14 : 26, 16 : 24, 18 : 22, 20 : 20; C_{42}: 16 : 26, 20 : 22; 22 : 20, C_{44}: 20 : 24, 22 : 22, 24 : 20; C_{46}: 20 : 26, 22 : 24, 24 : 22; C_{48}: 20 : 28, 22 : 26, 24 : 24, 26 : 22; C_{50}: 20 : 30, 22 : 28, 24 : 26, 26 : 24, 28 : 22 and C_{52}: 22 : 30, 24 : 28, 26 : 26 (Table 3, Fig. 2).

From these data, the distributions of component acids (C_{12}–C_{28}, with C_{16} and C_{20}–C_{26} predominant; Table 4, Fig. 1(b)) and alcohols (C_{14}–C_{36}, with C_{20}–C_{26} predominant; Table 5, Fig. 1(c)) within the esters were determined. Their distribution in the esters differed from those of the free fatty acids and primary alcohols also present in the raspberry wax (see the preceding paper). Acids (Table 4, Fig. 1(b)) and alcohols

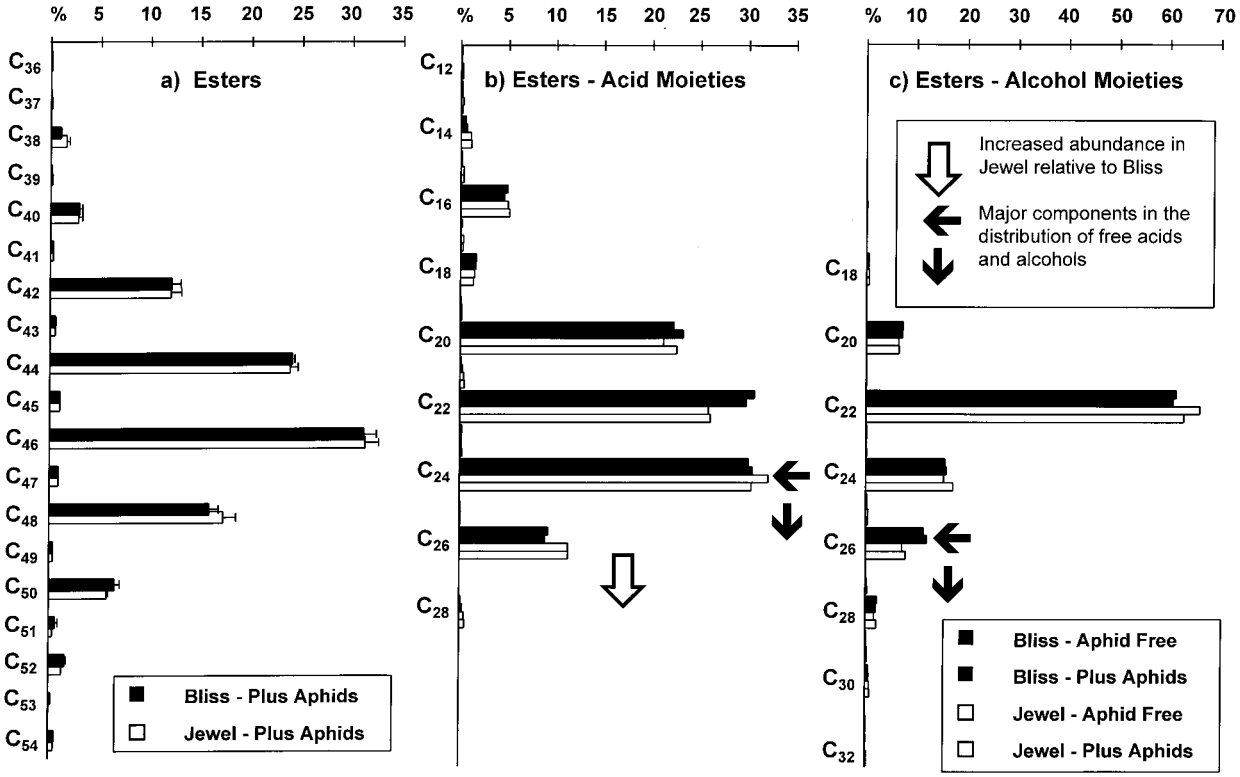

Fig. 1. Distribution of epicuticular wax components from mature biologically active leaves of the raspberry cultivars, Autumn Bliss and Malling Jewel. (a) Long-chain alkyl esters by overall chain length, (b) acid moieties present in the esters and (c) alcohol moieties present in the esters.

(Table 5, Fig. 1(c)) of medium chain length (C_{20}–C_{24}) were more abundant within the esters, whereas the shorter (C_{14}–C_{18}) and longer (C_{24}–C_{30}) acids and longer alcohols (C_{26}–C_{32}) were more abundant in the free form. This is consistent with the depletion of mid-range acids and alcohols from a common pool of precursors for incorporation into esters, and is a feature of wax composition that is often observed for the alcohol fraction, but less frequently for the acid fraction. The acid chain length distribution shows the pattern of double maxima (at C_{16} and C_{22}–C_{24}) also observed for the free acids, which is suggestive of the utilisation of acyl chains from the two distinct elongation systems, the synthesis de novo of chains up to C_{16}–C_{18}, and the subsequent elongation to longer chain lengths.

The overall distribution of individual esters in wax from the more mature biologically active leaves was similar for both cultivars. However, compounds with longer acid–shorter alcohol combinations were generally more abundant in wax from Jewel than Bliss, and this was most noticeable as the overall ester chain length increased in the range C_{45}–C_{52} (Table 3, Fig. 2). A slight shift in the distribution of ester fatty acids towards components of longer chain length was also observed for Jewel, relative to Bliss (Table 4, Fig. 1(b)), and this resembled the similar variation between the cultivars in the chain length distribution found for the free acids (preceding paper). There were also minor differences between the the cultivars in the distribution of the ester alcohols. The longest alcohols (C_{29}–C_{36}) were more abundant and the C_{20} and C_{26} alcohols less abundant in Jewel than Bliss (Table 5, Fig. 1(c)). In addition, odd-carbon acids and alcohols were more abundant in esters from Jewel than Bliss (Tables 4 and 5).

Differences between the young immature crown leaves and the more mature leaves were manifest as a greater abundance in the former of esters with acid–alcohol combinations of mid-chain length, and also of esters with long acid–short alcohol combinations. Esters from the immature leaves had relatively more of the shorter acids (C_{12}–C_{16}) and alcohols (C_{18}–C_{21}) and less of the longer acids (C_{22}–C_{26}) and alcohols (C_{26}–C_{30}) than esters from the more mature leaves.

Formation of long-chain alkyl wax esters is apparently sensitive to acid and alcohol chain length, and the distribution can differ from that for a purely random combination of acids with alcohols (Kolattukudy, 1969; Kolatukudy, Croteau & Buckner, 1976; Avato, Bianchi & Pogna, 1990; Shepherd et al., 1997). A theoretical distribution of esters based on the random combination of acids and alcohols was calculated using the data for individual acids and alcohols present within the esters. Combinations outside the experimentally observed range (C_{36}–C_{52}) were excluded from the

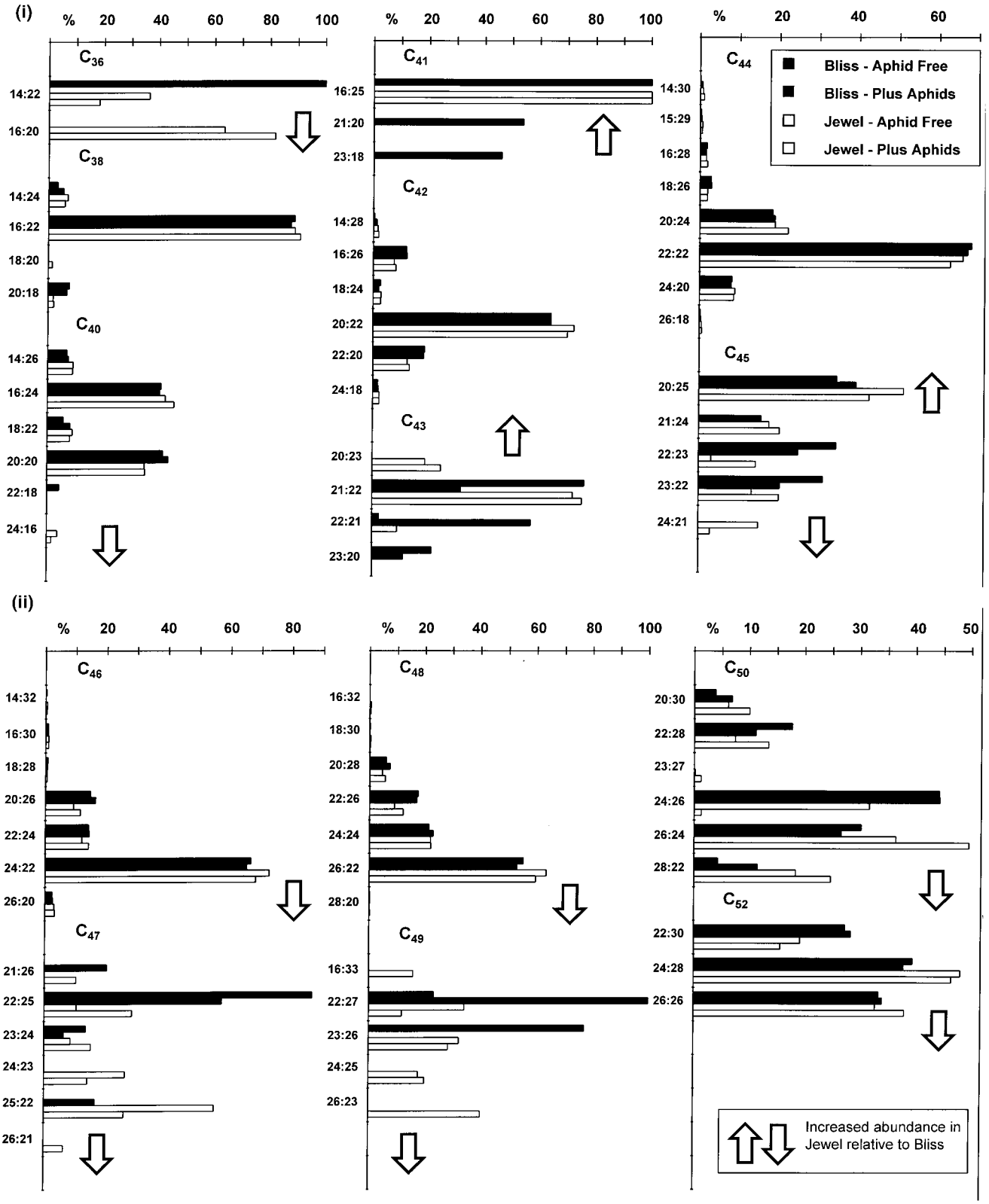

Fig. 2. Distribution of the individual long-chain alkyl esters in epicuticular wax from mature biologically active leaves of the raspberry cultivars, Autumn Bliss and Malling Jewel.

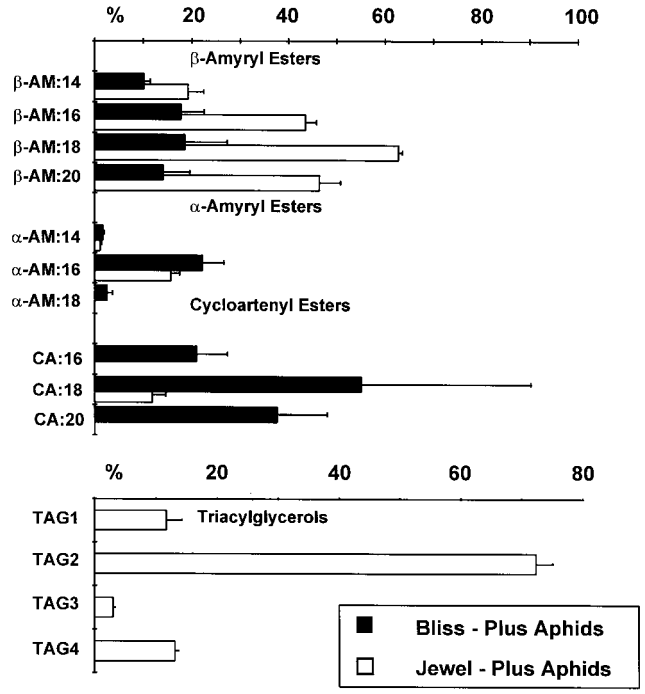

Fig. 3. Distribution of β-amyryl (β-AM), α-amyryl (α-AM) and cycloartenyl (CA) esters in epicuticular wax from mature biologically active leaves of the raspberry cultivars, Autumn Bliss and Malling Jewel which had been exposed to raspberry aphid. Esters have the terpenol : acid (C_n) combinations shown. Distribution of triacylglycerols (TAG) found in wax of the aphid-infested raspberry cultivar, Jewel. TAG 1: 1-dodecanoyl-2-hexanoyl-3-tetradecanoylglycerol, TAG 2: 1,3-ditetradecanoyl-2-hexanoylglycerol, TAG 3: 1,3-ditetradecanoyl-2-(2*E*,4*E*-hexadienoyl)glycerol, TAG 4: 1-hexadecanoyl-2-hexanoyl-3-tetradecanoylglycerol.

calculation of the distribution. The results (not shown) were similar for the immature and the more mature leaves of both cultivars. Esters of mid-chain length (C_{44}–C_{48}) were more abundant than predicted for random combination, whereas the shorter (C_{36}–C_{42}) and longer (C_{50}–C_{52}) esters were less abundant than predicted. Shorter acid : longer alcohol combinations were more favoured than predicted, although combinations of mid-range acids and alcohols were most abundant. Similar preferences were observed in wax esters from pea (Kolattukudy, 1969; Kolatukudy et al., 1976), from young and mature maize plants (Avato et al., 1990) and from the brassicas, kale and swede (Shepherd et al., 1997). For odd-carbon esters, shorter acid : longer alcohol combinations were particularly disfavoured. Optimum chain lengths and limiting chain lengths for participation of acids and alcohols during the esterification process were estimated from the data. The lower and upper limits for acids were approximately C_{12} and C_{30} respectively, with an optimum range of C_{16}–C_{28}, and for alcohols the limits were C_{14} and C_{36}–C_{38} with an optimum range of C_{20}–C_{28}/C_{30}. The esterification system is apparently optimised for

Table 7
Triacyglycerols from the cuticular lipid of raspberry aphid

C_n	Acids[a]		C_n	Acids[a]		C_n	Acids[a]	
C_{33}	$C_{12}C_6$ C_{12}	0.11	C_{37}	$C_{14}C_6C_{14}$	66.93[b]	C_{41}	$C_{14}C_8C_{16}$	0.02
	$C_{12}C_4C_{14}$	0.05		$C_{16}C_6C_{12}$	2.80		$C_{14}C_6C_{18}$	0.37
	$C_{14}C_2C_{14}$	0.05		$C_{14}C_{6:1}C_{14}$	0.31		$C_{16}C_6C_{16}$	0.27
	$C_{12}C_{6:1}C_{12}$	tr		$C_{14}C_{6:2}C_{14}$	1.92		$C_{18:1}C_6C_{14}$	0.03
	$C_{12}C_{6:2}C_{12}$	0.01		$C_{14}C_{6:2}C_{14}$	7.77[b]		$C_{14}C_{6:1}C_{18}$	0.04[c]
							$C_{16}C_{6:1}C_{16}$	
C_{35}	$C_{12}C_6C_{14}$	5.50[b]	C_{39}	$C_{14}C_8C_{14}$	0.18			
							$C_{14}C_{6:2}C_{18}$	
	$C_{14}C_4C_{14}$	0.02		$C_{14}C_6C_{16}$	10.08[b]			0.05[c]
							$C_{16}C_{6:2}C_{16}$	
							$C_{14}C_{6:2}C_{18}$	
	$C_{14}C_{6:1}C_{14}$	0.32		$C_{12}C_6C_{18}$	0.35		$C_{16}C_{6:2}C_{16}$	0.08[c]
	$C_{12}C_{6:1}C_{14}$	0.13		$C_{14}C_{6:1}C_{16}$	0.06			
	$C_{12}C_{6:2}C_{14}$	0.51		$C_{14}C_{6:2}C_{16}$	0.35			
				$C_{14}C_{6:2}C_{16}$	1.50	C_{43}	$C_{16}C_6C_{18}$	0.02[c]
				$C_{14}C_{8:3}C_{14}$	0.04		$C_{14}C_6C_{20}$	
				$C_{14}C_{8:3}C_{14}$	0.18			

[a] Acids esterified at C-1, C-2 and C-3 of the glycerol backbone of triacylglycerols of overall carbon number C_n. The major $C_{6:2}$ acid is considered to be (2*E*, 4*E*)-hexa-2,4-dienoic acid (sorbic acid), and the major $C_{8:3}$ acid (2*E*, 4*E*, 6*E*)-octa-2,4,6-trienoic acid by analogy with triacyl glycerols found in other aphid species (Callow et al., 1973; Dillwith et al., 1993).

[b] Compounds also identified in epicuticular wax of the aphid-infested raspberry genotype, Malling Jewel.

[c] Isomeric composition could not be determined.

combination of mid range acids and alcohols, which accounts for the lower abundance of longer and shorter esters than predicted for random combination.

2.1.2. Terpene esters

The individual α- and β-amyrin alkanoates and cycloartenol alkanoates in the wax (Table 6, Fig. 3) were identified from their mass spectra, which show the characteristic fragmentation patterns of the free terpenes, α- and β-amyrin (*m*/*z* 218, 203, 189) and cycloartenol (*m*/*z* 411, 409, 408, 393, 365, 286). The acid moieties (C_{14}–C_{20}) were identified from the molecular ions $[M]^+$ and the fragment $[M\text{-}15]^+$, if present (Elias, Simoneit, Pereira & Cardoso, 1997), or by inference. The chromatographic elution order of the terpene esters was assumed to be the same as that of the free terpenes, as previously observed for amyrin alkanoates (Elias et al., 1997), and identities were assigned on that basis.

2.1.2.1. Amyrin alkanoates. Amyrin esters were of similar abundance in wax of both cultivars and their levels were similar for both the emerging leaves and the more mature leaves (Table 1). In the most abundant of

the individual homologues, β-amyrin was esterified to the C_{16}–C_{20} acids and α-amyrin was esterified to the C_{16} acid (Table 6, Fig. 3). The two cultivars differed in the proportions of total α- and β-amyrin esters, there were more α-amyryl compounds in mature leaves of Bliss (α : β ratio 4 : 5 to 7 : 16) than Jewel (α : β ratio 2 : 15 to 1 : 10), and also in wax from immature leaves (Bliss, α : β ratio 7 : 2; Jewel, α : β ratio 4 : 6). These isomeric distributions of the most abundant amyrin esters were similar to those of the free terpenes in the wax (see preceding paper).

2.1.2.2. Cycloartenol alkanoates. Cycloartenol alkanoates were ten times more abundant in wax from the more mature leaves of Bliss than Jewel, and were not detected in wax from immature leaves of either cultivar. Three homologues were detected in Bliss, in which the terpene was esterified to acids in the range C_{16}–C_{20}. Of these, cycloartenol octanoate was the major component detected in Bliss, and was the only compound detected in Jewel (Table 6, Fig. 3). The higher abundance of cycolartenol esters in Bliss relative to Jewel reflects the similar distribution of free cycloartenol in the wax of immature leaves.

Amyrin and sterol alkanoates have been found in cuticular wax of other plant species including leaves of silver lime, *Tilia tomentosa* (Moench) (Gülz, Muller & Prasad, 1991) and flower petals of decorative rose, *Rosa* spp. (Mladenova, Stoianova-Ivanova & Camaggi, 1977).

2.1.3. Triacylglycerols

Four different triacylglycerols were identified in wax from the aphid-susceptible cultivar, Jewel (Table 6, Fig. 3). These belong to an unusual class of triacylglcyerols which have a short C_6 acid moiety at C-2 of the glycerol backbone, and have been previously reported in wax from various grasses and Canada thistle, *Cirsium arvense* L. (Tulloch, 1981a,b; Tulloch & Hoffman, 1981). Identification of the acids was made on the basis of ions corresponding to the acyl group $[CH_3(CH_2)_nCO]^+$, and to loss of the acyloxy group from the molecular ion $[M^+\text{-}CH_3(CH_2)_nCO_2]$. The position of the acids on the glycerol backbone, particularly that of the C_6 acid at C-2, was deduced from the ions corresponding to loss of the acyloxymethylene group from the molecular ion $[M^+\text{-}CH_2OCO(CH_2)_nCH_3]$ by cleavage of the triacylglycerol backbone between C-1 and C-2 and between C-2 and C-3 (Tulloch & Hoffman, 1981). In studies with synthetic triacylglycerols, Tulloch & Hoffman (1981) showed that under electron-impact conditions, acyl groups were more readily lost from a secondary position than a primary position. For example, the ion at 99 m/z corresponding to a 2-hexanoyl group was the base peak, whereas m/z 99 corresponding to a 3-hexanoyl group was of 60% abundance, and m/z 43 was the base peak. In the mass spectra of all triacylglycerols found in the raspberry wax, m/z 99 corresponding to C_6, hexanoate, and m/z 95 corresponding to $C_{6:2}$, sorbate, were the base peaks, giving further confirmation of their location at C-2 of the glycerol backbone.

Other than in the above mentioned instances, triacylglycerols are not usually reported as constituents of plant epicuticular waxes. Furthermore, if triacylglycerols were plant-derived, they could reasonably be expected to resemble those found in internal plant lipids and have three long-chain fatty acid moieties. The presence of triacylglycerols only in wax from the aphid-susceptible cultivar, Jewel, which had been subject to bioassay with aphids, and not in aphid-free Jewel, was strongly indicative that the triacylglycerols were related to the presence of aphids. To test this possibility, we extracted the surface lipids of the total population of *A. idaei* that had developed on Jewel during bioassay. On analysis by GC–MS, the surface lipids were found to consist of two distinct groups of compounds. The first was a complex mixture of long-chain fatty acids, alcohols, alkanes and aldehydes similar to those found in plant epicuticular waxes. The second group consisted of a range of triacylglycerols, all of which had a short acid moiety at C-2, predominantly C_6, although C_2, C_4, C_8, $C_{6:1}$, $C_{6:2}$ and $C_{8:3}$ acids were also found at C-2 in some instances (Table 7). Of these, the four major components were found to have a similar distribution (underlined in Table 7) and the same chromatographic and mass spectral characteristics as the triacylglycerols found in the raspberry wax and were therefore assumed to be identical with those present in the leaf wax. It is most likely that in our study, and in those of Tulloch (1981a,b) and Tulloch & Hoffman (1981), the presence on the leaf surface of triacylglycerols with C_6 acids was due solely to the presence of aphids. In the earlier studies, the source plants had been grown outdoors and were sampled when flowering, a time when the probability of aphid infestation was high.

2.2. Role of epicuticular lipid as a determinant of insect behaviour

2.2.1. Saturated alkyl esters

The abundance of wax esters has been linked with antixenotic resistance of alfalfa, *Medicago sativa*, to spotted alfalfa aphids, *Therioaphis maculata* (Buckton) (Dillwith & Berberet, 1990; Bergman et al., 1991) and wax esters deterred feeding of *Locusta migratoria* (L.) on sorghum (Atkin et al., 1982; Woodhead, 1983). Antixenotic resistance of various *Brassica* species to turnip rootfly, *Delia floralis* (Fall.), may also be linked to the presence of wax esters

(Shepherd et al., 1995b; Shepherd et al., 1997). The similar abundance of alkyl esters in wax from both raspberry genotypes suggests that the biological activity towards *A. idaei* was not related to the overall levels of the esters (Table 1). However, as the single most abundant component in the wax, the esters will have a major influence on wax structure and morphology. Subtle variations in ester structure, for example the greater abundance of compounds with longer acid : shorter alcohol combinations found for the susceptible cultivar, Jewel (Table 3, Fig. 2), may have significant effects on wax morphology and the spatial distribution of other wax components. This may in turn affect the way in which the aphid perceives specific stimulants or deterrents within the wax. Such synergism between leaf surface chemicals has been suggested for a number of plant-insect interactions (Städler & Schöni, 1990; Eigenbrode & Espelie, 1995; Shepherd et al., 1997).

2.2.2. Terpene esters

The relative distributions of the different terpene esters (Table 6, Fig. 3) within the wax of both cultivars were similar to those of the free terpenes (see preceding paper). However, any similarity, or otherwise, in the effects of free and esterified terpenes on the behaviour of *A. idaei*, remains to be determined. Increased levels of amyrins have been associated with resistance of cabbage, azalea (*Rhododendron* spp.) and sorghum to the diamondback moth, *Plutella xylostella* L., azalea lace bug, *Stephanitis pyriodes* (Scott) and various aphids, and they also inhibit feeding by *L. Migratoria* (Heupel, 1985; Eigenbrode, Espelie & Shelton, 1991; Eigenbrode & Espelie, 1995). The higher abundance of α-amyrin alkanoates in wax from the resistant cultivar, Bliss, may be significant, since the most abundant of these, α-amyryl palmitate, when isolated from suberin wax of the sandal tree, *Santalum album* L., adversely effected the development of several lepidoptera species (Shankaranaryana, Ayyar & Krishna Rao, 1980). The much higher abundance of cycloartenol alkanoates in Bliss (Table 6, Fig. 3) may also be a significant factor in resistance to *A. idaei*.

Investigations continue in our laboratories of the involvement of alkyl esters and terpenyl esters in resistance to *A. idaei*, particularly the possibility of synergism between these and the other wax components. Other factors may be of importance, including the spatial heterogeneity of these wax components between upper and lower leaf surfaces. For example, esters have been found only on the upper leaf surface of peach, another member of the Rosacea, but not on the lower surface (Baker et al., 1979). This is of significance since aphids such as *A. idaei* feed on the lower leaf surface, although initial contact and some elements of host selection occur on the upper surface.

2.3. Significance of the presence of triacylglycerols

It is clear that the triacylglycerols found within the wax of the aphid-susceptible cultivar, Jewel, originate from the raspberry aphid rather than the raspberry plant. These aphid triacylglycerols are found as part of the internal lipid and also in secretions produced by the cornicle glands located on the insect's upper abdomen. Production of cornicle secretions can be a response to stress, and aphids cover their cuticular surface with a supercooled fluid which rapidly solidifies on contact, producing a physiochemical barrier which embeds and immobilises parasites and other pathogens (Edwards, 1966; Callow et al., 1973; Dillwith et al., 1993). In a detailed investigation of the cuticular lipids of raspberry aphid, which will be described elsewhere, we found that the characteristic chemical signature of the cuticle, including the triacylglycerols, was retained by the exuviae shed by the insects during their growth and development. We believe that aphid exuviae present on the leaf surface of host plants are the primary source of the triacylglycerols, although the direct incorporation of droplets of cornicle fluid into the plant cuticular wax is also a possibility. Triacylglycerols were also found on the young immature leaves of the same plants (Table 6), although these leaves were not a significant site of aphid feeding and reproduction. It is likely that there would be significant short-term exploration of the young leaves by insects from the adjacent population. Exuviae produced by these insects prior to their return to the older leaves could then account for the presence of the aphids' triacylglycerol signature on the immature leaves. We have also detected the same triacylglycerols on leaves of field-grown plants of the cultivar Jewel, but not Bliss, at a time when aphids were known to have been present in the locality. Similarly, the triacylglycerols were found in wax from field-grown plants of other species, including brassica and potato (Shepherd, Robertson, Griffiths & Birch, unpublished results). Our findings suggest that measurement of the relative levels of triacylglycerols in waxes from field-grown or glass house-grown plants might provide a chemical index of the levels of aphid-infestation, and hence susceptibility, and might be a useful tool for screening plants for aphid-resistance.

The triacylglyerols constitute the major part (90%) of the aphid cuticular lipids. The remaining part which is chemically similar to the raspberry epicuticular wax, is present in insufficient quantity to affect the measured composition of the plant wax.

3. Experimental

3.1. Collection of epicuticular wax from raspberry plants, sample preparation and analysis by GC and GC–MS and conduct of biosassay with aphids

Details of plant growth, sample collection, analytical instrumentation, chromatographic conditions and analytical methodology for chemical analysis of the wax by GC and GC–MS, are given in the preceding paper and by Shepherd et al. (1995a). Details of the bioassay with aphids, conducted prior to sample collection, are given in the preceding paper.

3.2. Collection and analysis of cuticular lipids from raspberry aphid, A. idaei

3.2.1. Collection of cuticular lipids

Aphids (1.045 g) collected from leaves of the aphid-susceptible cultivar, Malling Jewel, at the end of the period of bioassay with *A. idaei*, were immersed in dichloromethane (100 ml) for one minute. The aphids were removed by filtration and the filtrate was warmed gently on a hotplate and reduced slowly to 10 ml and then evaporated to dryness under a flow of nitrogen. The cuticular lipid was obtained as a pale brown waxy solid (16.5 mg, 15.8 mg g^{-1} aphid).

3.2.2. Sample preparation and analysis by GC and GC–MS

Samples (1.4 mg) were prepared by derivatization with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and analysed by capillary GC and GC–MS as described previously for brassica and raspberry waxes (Shepherd et al., 1995a, previous paper).

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