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CHANGES IN THE CHEMICAL COMPOSITION OF VOLATILES RELEASED BY THE FLOWERS AND FRUITS OF THE RED RASPBERRY (*RUBUS IDAEUS*) CULTIVAR GLEN PROSEN

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Key Word Index—*Rubus idaeus*; Rosaceae; raspberry; flower volatiles; fruit volatiles; thermal desorption; GC-MS; raspberry beetle; *Byturus tomentosus*.

Abstract—Volatiles at various stages of inflorescence development, bud formation, flowering, fruit formation and ripening of a red raspberry, were entrained on the porous polymer Tenax TA and analysed by thermal desorption-gas chromatography-mass spectrometry. Major classes of compound identified included aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols and esters, monoterpenes and sesquiterpenes. As the inflorescences matured, levels of green leaf volatiles such as *trans*-β-ocimene and *cis*-3-hexenyl acetate declined and the monoterpenes, α-pinene, camphene, β-myrcene and limonene increased. During fruit ripening several additional compounds appeared including α- and β-ionone, α- and β-phellandrene and hexanoic acid ethyl ester. Ethyl acetate at 12–18% was the major detectable volatile product of the ripe fruit.

INTRODUCTION

The aroma of raspberry fruit is well documented in the scientific literature and has been reviewed several times [1–3]. Early work showed that the characteristic odour associated with the fruit was primarily due to 4-(4-hydroxyphenyl)-butan-2-one [4], and described both the volatile carbonyl [5] and acid fractions [6]. The neutral fraction from raspberry fruit oil has also revealed the presence of new chemical constituents [7], and the odour thresholds of a number of major constituents have recently been determined [8].

Previous studies [9] of the volatiles released by the flowers of four raspberry cultivars at similar stages of growth had, however, revealed that the compounds detected differed markedly from those in ripe fruit. The complex mixture of terpenoid, aliphatic and aromatic volatiles emitted by the plant during the flowering period may attract pollinating insects [10, 11] or in the case of raspberry, specific insect pests such as the raspberry beetle *Byturus tomentosus*. Volatiles emitted by the plant at other stages of development can also be of biological significance [12].

The objective of this study was to identify the changes in the volatile profile likely to be perceived by insects in close proximity to the raspberry inflorescence as it developed from the green bud stage to full fruit maturity.

RESULTS

Volatile compounds were sampled at six sequential stages of inflorescence development in the red raspberry (*Rubus idaeus*) cultivar Glen Prosen—as green buds, flowers, old flowers/early green fruit, green fruit, pink fruit and mature red fruit. The compounds were entrained on a porous polymer (Tenax-TA) and then analysed by thermal desorption-gas chromatography-mass spectrometry (ATD-GC-MS). The compounds entrained consisted mainly of saturated and unsaturated aliphatic aldehydes, ketones, alcohols and esters, monoterpenes, sesquiterpenes and α- and β-ionones. The changes in the relative levels of these volatile compounds over the period of inflorescence development are detailed in Table 1 together with their chromatographic retention times.

Several changes are apparent in the abundance of certain classes of compound detected as the inflorescence matures. Volatiles normally associated with green leaves such as *cis*-β-ocimene (R_t 23.75) and *trans*-β-ocimene (R_t 24.40) decline with fruit ripening and this is paralleled by a decrease in several sesquiterpenes (R_ts 34.55, 37.40, 38.70, 38.90, 39.40, 39.70). Other green leaf volatiles such as *cis*-3-hexen-1-ol (R_t 20.10) and 2-hexenal (R_t 17.20) which are derived from α-linolenic and linoleic acids by enzymatic pathways [13], do not appear to follow this trend. Indeed, *cis*-3-hexen-1-ol levels appear to rise with the increasing alcohol content of the fruit volatiles. In contrast, certain other monoterpenes, α-pinene (R_t 19.80), camphene (R_t 20.80), β-myrcene (R_t 22.05) and limonene (R_t 23.82) appear to rise steadily throughout the period of maturation and this is further emphasized by the appearance of high levels of α-phellandrene (R_t 23.01) in the ripening fruit. Traces of β-phellandrene were also detected. This increase in the concentration of certain terpene

Table 1. Compounds entrained on Tenax TA from cv Glen Prosen at six stages of inflorescence development

Compound	R_t (min)	Total volatile peak area (%) Green buds	Flowers	Flowers/ green fruit	Green fruit	Pink fruit	Ripe red fruit
Ethanol	6.47	tr	tr	tr	tr	tr	tr
Methyl acetate	7.17	nd	tr	tr	nd	0.5	2.5
Ethyl acetate	9.32	0.4	0.9	0.2	0.7	18.5	11.8
3-Methyl-2-butanone	9.58	nd	0.4	1.2	tr	1.1	tr
2-Butanone	9.70	tr	0.3	0.3	0.2	tr	tr
2-Butanol	9.97	tr	tr	tr	nd	nd	nd
3-Methylbutanal	11.40	nd	0.1	0.2	0.9	0.4	tr
2-Ethylfuran	11.48	nd	tr	tr	0.1	tr	tr
2-Methylbutanal	11.60	tr	0.1	0.3	0.6	tr	tr
Propyl acetate	12.68	nd	nd	nd	nd	0.4	tr
2-Pentanone	12.78	nd	nd	tr	nd	0.6	tr
Unknown	12.93	nd	tr	0.2	tr	tr	2.0
3-Pentanone	13.01	1.2	5.2	5.5	6.3	2.3	3.8
3-Pentanol	13.27	0.5	2.1	3.0	1.6	2.1	1.9
Octane	13.52	nd	nd	0.1	0.4	0.5	0.6
Unknown A*	14.01	tr	0.1	0.2	nd	nd	0.5
Unknown B*	14.28	6.5	5.9	7.0	5.9	tr	1.7
3-Methyl-1-butanol	15.07	tr	0.2	0.7	0.2	tr	0.9
2-Methyl-1-butanol	15.18	0.1	0.4	1.3	0.8	0.9	4.2
N-Methylene-ethanamine	15.58	4.6	1.1	1.6	2.2	tr	1.8
3-Hydroxy-2-butanone	15.98	tr	0.5	14.7	nd	5.1	tr
Unknown	16.12	0.3	tr	0.1	nd	tr	nd
1-Pentanol	16.32	nd	tr	0.2	nd	0.4	tr
Unknown	16.80	nd	tr	0.2	nd	tr	0.4
Hexanal	17.01	0.5	0.6	1.8	2.4	2.9	1.6
2-Hexenal	17.20	nd	0.2	0.2	nd	tr	0.6
Nonane	17.40	tr	tr	tr	tr	0.4	0.6
Unknown	19.57	5.2	0.9	1.5	0.9	tr	0.8
α-Pinene	19.80	0.1	0.1	0.1	0.6	1.8	4.6
Unknown	19.98	1.3	0.2	0.4	0.2	tr	tr
cis-3-Hexen-1-ol	20.10	0.8	0.7	1.1	1.4	2.0	2.4
Unknown	20.20	tr	tr	1.0	tr	2.1	2.5
Camphene	20.80	tr	0.2	0.3	0.4	2.1	3.5
Heptanal	21.00	0.3	0.2	1.5	0.9	2.1	0.5
Decane	21.20	0.3	0.1	tr	0.2	0.4	tr
Methoxybenzene	21.70	tr	0.2	tr	0.5	tr	nd
β-Myrcene	22.05	0.4	0.2	0.3	tr	0.8	1.4
2-Pentylfuran	22.67	nd	tr	0.2	tr	tr	0.5
Unknown	22.80	2.9	0.5	0.6	tr	nd	tr
α-Phellandrene	23.01	tr	tr	tr	nd	1.5	3.7
Hexanoic acid ethyl ester	23.41	nd	nd	nd	nd	6.0	2.5
cis-β-Ocimene	23.75	0.4	0.9	0.7	0.4	tr	tr
Limonene	23.82	0.4	0.3	0.1	0.4	0.7	0.8
cis-3-Hexenyl acetate	24.19	14.5	21.1	11.9	1.1	4.7	1.1
trans-β-Ocimene	24.40	38.4	44.5	25.7	34.0	3.6	5.6
6-Methyl-5-hepten-2-one	24.60	0.6	0.1	0.4	0.5	1.1	0.4
Octanal	24.95	1.4	0.5	0.4	2.4	3.3	1.6
Benzaldehyde	25.32	0.2	tr	0.3	0.1	0.6	0.4
Unknown	26.19	tr	tr	tr	0.4	1.5	1.0
(*E*)-4,8 Dimethyl-1,3,7-nonatriene	26.70	2.8	3.1	5.7	1.4	1.2	0.6
Linalool	28.20	1.5	0.1	0.4	0.2	1.1	0.5
Duodecane	28.43	tr	0.1	tr	0.2	0.5	tr
Nonanal	28.55	1.2	0.7	1.0	3.8	5.6	2.5
Benzyl alcohol	28.98	nd	tr	0.1	nd	nd	tr
Tridecane	31.70	tr	tr	tr	tr	1.2	1.0
Decanal	31.98	0.4	0.4	0.4	1.8	3.8	2.5
Sesquiterpene	34.55	0.7	0.2	0.2	tr	tr	0.6*
Tetradecane	34.75	tr	tr	tr	0.9	1.0	0.39

Table 1. *Continued*

Compound	R_t (min)	Total volatile peak area (%) Green buds	Flowers	Flowers/ green fruit	Green fruit	Pink fruit	Ripe red fruit
Sesquiterpene	35.40	tr	tr	tr	tr	tr	1.2
α-Copaene	35.75	0.3	2.0	1.5	10.4	2.2	2.8
β-Bourbonnene	36.34	0.7	0.8	0.8	1.0	0.5	0.8
β-Ionone†	36.97	nd	nd	nd	nd	0.8	3.3
Sesquiterpene	37.40	0.5	tr	tr	nd	tr	tr
β-Caryophyllene	37.90	5.5	2.6	2.7	12.7	6.8	4.0
Sesquiterpene	38.70	0.3	tr	tr	nd	nd	nd
Sesquiterpene	38.90	0.5	0.1	tr	nd	tr	tr
α-Farnesene	39.05	2.8	0.9	1.3	1.0	0.7	1.9
α-Muurolene	39.40	0.2	tr	tr	nd	nd	nd
Sesquiterpene	39.70	0.6	tr	tr	nd	nd	2.8[x]
Cadinene	39.90	0.7	0.2	0.2	0.3	0.5	nd
α-Ionone	40.04	nd	nd	nd	nd	3.2	5.2
β-Ionone†	41.70	nd	nd	nd	nd	0.6	1.8

*Previously identified as C_6 ketoximes.

†Mass spectra of isomers.

tr, Present at trace level below peak area threshold and verified from mass spectral data.

nd, Not detected.

[x], Multi component peaks containing two sesquiterpenes.

hydrocarbons in ripening fruit was previously reported by Guichard [14]. The changes in monoterpene composition found in this study are illustrated in Fig. 1a. The level of the terpene alcohol linalool (R_t 28.20) showed no significant change with fruit development.

During the flower and flower/green fruit stage several compounds described previously in raspberry [9] appear to reach peak levels. Loosely termed the 'flower' volatiles (Fig. 1b), they include Unknown A (R_t 14.01), Unknown B (R_t 14.28) the homomonoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (R_t 26.70) first described by Maurer *et al.* [15] and several ketones, 3-methyl-2-butanone (R_t 9.58), 2-butanone (R_t 9.70), 2-pentanone (R_t 12.78) and 3-pentanone (R_t 13.01).

The level of the green leaf volatile *cis*-3-hexenyl acetate (R_t 24.19) also declines with inflorescence maturity while methyl acetate (R_t 7.17), ethyl acetate (R_t 9.32) and propyl acetate (R_t 12.68) all increase after fruit ripening (Fig. 1c). Ethyl acetate, first documented by Palluy *et al.* [6] appears to be the major volatile constituent of the ripe fruit [~ 12–18%]. Fruit ripening is marked also by the appearance of the ionones, β-ionone isomers (R_ts 36.97, 41.70) and α-ionone (R_t 40.04) together with hexanoic acid ethyl ester (R_t 23.41). There appears also to be a general increase in the aliphatic aldehydes [3.5–18.3%]: 3-methyl butanal (R_t 11.40), 2-methyl butanal (R_t 11.60), hexanal (R_t 17.01), 2-hexenal (R_t 17.20), heptanal (R_t 21.00), octanal (R_t 24.95), nonanal (R_t 28.55), decanal (R_t 31.98); aliphatic alcohols [1.6–9.6%]: ethanol (R_t 6.47), 2-butanol (R_t 9.97), 3-pentanol (R_t 13.27), 3-methyl-1-butanol (R_t 15.07), 2-methyl-1-butanol (R_t 15.18), 1-pentanol (R_t 16.32) and aliphatic hydrocarbons [0.3–2.6%]: octane (R_t 13.52), nonane (R_t 17.40), decane (R_t 21.20), duodecane (R_t 28.43), tridecane (R_t 31.70) and tetradecane (R_t 34.75) with inflorescence maturity (Fig. 1d). Under the analytical conditions used in this study the main character compound of ripe raspberry fruit, 4-(4-hydroxyphenyl)-butan-2-one, could not be detected. Tests utilizing the authentic compound demonstrated either that it was not trapped by the porous polymer used or that it did not elute from the column due to chromatographic conditions (stationary phase polarity or temperature programme limitations). A previous study by another research group [16] using similar dynamic head space analysis conditions also failed to detect the presence of this compound for probably the same reasons.

DISCUSSION

Although previously published work has followed changes in the volatile release by raspberry fruit during ripening [14] this study is the first to follow changes in the volatile profile from the green bud stage to full fruit maturity utilizing the combination of adsorption on porous polymer and thermal desorption. Because this procedure gives a good estimate of the compounds released naturally into the environment it may have particular value in insect behavioural studies by determining the volatile profile actually perceived by insects in close proximity to the plant. Many of the compounds entrained during the early stages of inflorescence development have already been identified in the volatile profiles of four flowers from raspberry cultivars [9]. Several of these 'flower volatiles' appear to peak during the flower

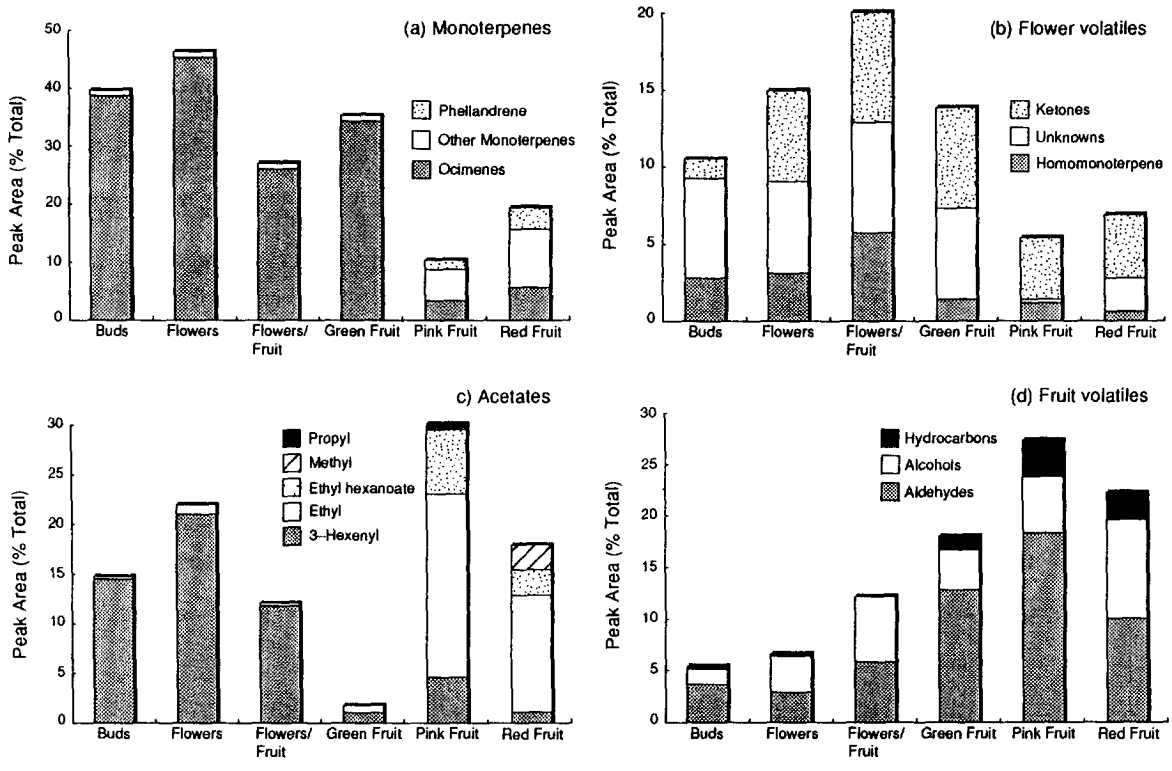

Fig. 1. Changes in volatile composition with inflorescence development.

and the flower/green fruit stage. Unknown A and Unknown B remain to be identified and their mass spectra are detailed in the Experimental. These compounds were tentatively identified as C_6 ketoximes in a previous paper [9]. The case for the possible presence of this type of compound is strengthened by documented evidence in the scientific literature of the closely related, amino acid-derived oximes found in the floral scent of 14 orchid species [17]. As the inflorescence matured the 'green leaf' volatile content decreased and this appeared to be paralleled by a gradual increase in certain monoterpenes. The picture changes rapidly however at the fruit stage with the appearance of several compounds unique to ripe raspberry fruit such as α- and β-ionone, α- and β-phellandrene hexanoic acid ethyl ester together with higher levels of methyl ethyl and propyl acetates. It remains to be shown if these changes in the volatile profile explain observed differences in behavioural responses demonstrated by the raspberry beetle during flower and fruit development (J. A. T. Woodford *et al.* unpublished observations).

EXPERIMENTAL

Collection of volatiles. The methods used were published previously [9, 18]. Plant material was obtained from the raspberry cultivar Glen Prosen grown at the Scottish Crop Research Institute during May–July 1993. For the entrainment of bud, flower and green fruit samples, the pedicels were inserted through an aluminium foil seal into water-filled Petri dishes. Ripe fruit samples were placed directly into the glass entrainment vessel with their receptacles and stalks intact. The Tenax TA tubes were pre-conditioned as before except that high purity helium was employed to back flush them at 240°.

GC-MS analysis. All samples were analysed as previously described [9]. For the purposes of quantification a threshold (1% of the largest peak area in each chromatogram) was again imposed. Each remaining peak was then normalized with respect to the sum of the total peak areas above the threshold. Identification of the volatile components was achieved by comparison with the retention time and mass spectral data of standard materials and where these were unobtainable from mass spectral data bases or published reports.

The two volatile components Unknown A (R_t 14.01) and Unknown B (R_t 14.28) yielded mass spectra as follows (A): EIMS 70 eV, *m/z* (rel. int.): 115 (6), 100 (32), 87 (71), 73 (100), 57 (28), 56 (89), 55 (51), 54 (53), 41 (86), 39 (36), 29 (93), 27 (57); and (B): EIMS 70 eV, *m/z* (rel. int.): 115 (2), 100 (33), 87 (65), 73 (100), 56 (59), 55 (33), 54 (41), 42 (29), 41 (89), 39 (37), 29 (70), 27 (44). Minor changes in the retention times of all compounds to those in previous work [9] are due to a change of chromatographic column.

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