

Evaluation of Thermal Desorption and Solvent Elution Combined with Polymer Entrainment for the Analysis of Volatiles Released by Leaves from Midge (*Dasineura tetensi*) Resistant and Susceptible Blackcurrant (*Ribes Nigrum* L.) cultivars

D. W. Griffiths,* G. W. Robertson, A. N. E. Birch and R. M. Brennan

Scottish Crop Research Institute, Dundee DD2 5DA, Scotland, UK

Polymer entrainment combined with solvent elution or thermal desorption, steam distillation and solvent surface extractions has been applied to leaves from midge-resistant and -susceptible blackcurrant cultivars. Irrespective of the methods used, the predominant compounds detected were terpene hydrocarbons, but only minor quantitative differences were found in the relative concentrations of the individual mono- and sesqui-terpenes identified. Both steam distillation and thermal desorption resulted in the formation of a number of artefacts, whilst the use of polymer entrainment revealed the presence of a number of compounds, including a homoterpene not detected by either steam distillation or leaf surface solvent extraction. The relative merits of the various methods used are discussed in relation to their potential value for the identification and quantification of leaf surface volatiles. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: Leaf volatiles; thermal desorption; leaf curling midge; blackcurrant; *Ribes nigrum*.

INTRODUCTION

The blackcurrant (*Ribes nigrum* L.) is the most extensively grown bush fruit in Australasia and Europe. The berries are used in the large-scale production of fruit juices. In addition, absolutes of particular value as flavour enhancers in the food industry and in the manufacture of cosmetics are extracted commercially from blackcurrant buds (Le Quere and Latrasse, 1990).

The chemical compositions of absolutes extracted from macerated samples of blackcurrant buds and leaves, using both steam distillation and cold solvent extraction, have been reported for a number of commercial cultivars (Andersson *et al.*, 1963; Latrasse and Lantin, 1977; Kerslake and Menary, 1985a, b; Marriott, 1988; Nishimura and Mihara, 1988; Kerslake *et al.*, 1989; Le Quere and Latrasse, 1990; Piry *et al.*, 1995). Their results have shown that these products primarily contain compounds derived from the isoprenoid pathway. Indeed, differences in their mono- and sesqui-terpene contents have been used to define varietal chemotypes (Kerslake *et al.*, 1989; Latrasse *et al.*, 1990) and have also been used in the discrimination of blackcurrant genotypes resistant to gall mites (Brennan *et al.*, 1992). However, Marriot (1988) reported that during steam distillation the relative

concentrations of several mono- and sesqui-terpenes were significantly reduced, primarily due to acid-catalysed rearrangements or oxidation. Terpenes can also rearrange on exposure to high temperatures and such reactions may frequently be catalysed by their exposure to metal and other surfaces (Whittaker, 1972). Such conditions may well be experienced during the process of thermal desorption.

Moreover, owing to microclimate influences and plant physiological state, the chemical signature of the headspace surrounding plant organelles may differ significantly from the composition found on the surface of the plant. Dynamic headspace analysis employing porous polymer collection has been widely used for the study of plant odour plumes to derive a more accurate profile of the volatiles, but the results obtained are highly method-dependant (Raguso and Pellmyr, 1998).

Plant-derived terpenoids have long been known to modify the behaviour of both insect and mammalian herbivores. In many cases, these compounds may be acting as both feeding and oviposition deterrents, but for specialist adapted herbivores they may aid in host identification. The high volatility of the monoterpenes also suggests that they may act as long range cues in host location (Gershenson and Croteau, 1991; Pickett, 1991).

The leaf curling midge (*Dasineura tetensi*) is a serious pest of blackcurrant crops in the UK, particularly in warmer southern areas (Keep, 1985; Brennan, 1996). Its control can present some difficulties as it can produce four generations in a single summer. Genotypic differences in susceptibility have been reported and clearly a

* Correspondence to: D. W. Griffiths, Scottish Crop Research Institute, Dundee, Scotland, DD2 5DA, UK.

E-mail: wgriff@sari.ac.uk

Contract/grant sponsor: Scottish Office Agriculture, Environment and Fisheries Department.

Table 1. The monoterpene hydrocarbon composition of solvent extracts of leaf surfaces prepared from three blackcurrant cultivars

Compound	Retention time (min)	Monoterpene composition (% total monoterpenes)		
		Ben Alder	Ben Connan	Ben Tirran
α -Thujene	19.26	0.1	0.3	0.2
α -Pinene	19.62	1.1	1.0	1.1
Camphene	20.40	0.1	0.1	0.2
Sabinene	21.54	0.5	0.5	0.5
β -Myrcene	21.72	2.7	2.6	3.0
Δ^4 -Carene	22.13	0.4	0.5	0.5
Δ^3 -Carene	22.54	41.8	39.3	41.9
α -Terpinene	23.00	1.3 ^{a,b}	1.0 ^a	1.6 ^b
(Z) β -Ocimene	23.32	13.3 ^{a,b}	16.0 ^b	11.2 ^b
β -Phellandrene	23.76	0.9 ^a	1.5 ^{a,b}	1.7 ^b
(E)- β -Ocimene	23.87	8.8 ^b	10.3 ^c	7.3 ^a
Unidentified	24.20	0.6	0.6	0.6
γ -Terpinene	24.30	0.6	0.5	0.5
α -Terpinolene	25.39	27.5	25.8	29.7

^{a,b,c} Values which bear different superscript letters differ significantly ($p < 0.05$) from each other.

rapid screening method for plant resistance to this pest would be of value.

The objective of the present study was (a) to compare the results obtained by thermal desorption with solvent elution of polymer-entrained volatiles from leaves of both midge-resistant and -susceptible blackcurrant cultivars, and (b) to ascertain to what extent the compounds detected in the entrained odour plume differed from those present on the leaf surface.

EXPERIMENTAL

Plant material. Blackcurrant bushes from the cultivar Ben Connan, resistant to the leaf curling midge (*Dasineura tetensi*), and from two cultivars susceptible to midges, Ben Alder and Ben Tirran (Brennan, 1996), were grown under glasshouse conditions. Leaf samples for both extraction and entrainment were taken from the top of the plant.

Leaf surface extracts. Approximately 5 g of whole leaves were immersed for 5 s in 10 mL dichloromethane. The resulting solution was filtered and an aliquot immediately taken for analysis.

Leaf entrainments. Stainless steel adsorption tubes (ATD 50; Perkin-Elmer, Beaconsfield, UK) were packed with 250 mg of Tenax-TA (Enka Research, Arnhem, Netherlands) and thermally conditioned at 200°C with an oxygen-free helium flow of 15 mL/min for 18 h. The tubes were then exhaustively washed with high purity diethyl ether and again thermally conditioned. Prior to sampling, the adsorption tubes were analysed to check background levels.

The stems of the excised plant material (10–15 g) were sealed, using aluminium foil, into a 100 mL conical flask containing 80 mL water and placed inside a 2 L entrainment apparatus (Robertson *et al.*, 1993) fitted with one inlet and two exit ports. Two adsorption tubes, connected in series, were coupled to one exit port and a single adsorption tube fitted to the other. The two sample outlets were both pumped via independent flow con-

trollers at rates of 50 mL/min for a period of 8 h utilizing purified air introduced through the inlet port. The system was maintained at 20°C and illuminated by sodium lights. The adsorption tubes were then removed, back-flushed with purified nitrogen (10 mL/min) for 10 min to remove water vapour, and sealed using Swagelock[®] brass end caps. The two serially connected adsorption tubes were analysed by thermal desorption. The single adsorption tube was eluted with high-purity diethyl ether (2 × 2 mL). Both fractions were carefully concentrated to 0.1 mL under a nitrogen flow and analysed by solvent vapourization GC-MS.

Steam distillation. Approximately 4 g of coarsely shredded leaves were placed in a Likens and Nikerson micro-steam distillation/extraction apparatus together with 40 mL of glass-distilled water, and 0.5 mL of dichloromethane was used as the extraction solvent. The samples were distilled for 75 min and the dichloromethane refluxed for a further 15 min. After cooling, the dichloromethane fraction was removed and analysed by GC-MS.

Thermal desorption GC/MS. The thermal desorption system consisted of a Perkin-Elmer Model ATD 50 automated thermal desorption apparatus linked to a Finnigan (MassLab Group, Manchester, UK) Masslab Trio 1000 quadrupole GC-MS system. Primary sample desorption was set at 200°C for 15 min, with the Tenax-TA cold trap maintained at -25°C. Secondary desorption at 250°C transferred the concentrated volatiles onto a DB 1701 chromatographic column (60 m × 0.25 mm i.d.; 1.0 µm film; J&W Scientific, Folsom, California, USA). Pre-filtered helium (flow-rate 1 mL/min) was used as the carrier gas and the GC oven was programmed from 40 to 240°C at a rate of 5°C/min, and then held for 20 min at 240°C. The quadrupole MS, which scanned a mass range of 20–300 amu at 1 s intervals, was used in the electron impact mode (ionization energy, 70 eV; trap current, 200 µA; source temperature, 250°C). The GC-MS data was acquired using a VG Lab-Base software system and subsequently translated to the Hewlett Packard (Hewlett Packard Ltd, Stockport, UK) Chemstation format.

Table 2. The monoterpene hydrocarbon composition of polymer-entrained leaf volatiles prepared from three blackcurrant cultivars and determined by thermal desorption and solvent elution.

Monoterpene	Monoterpene composition (% total monoterpenes)					
	Ben Alder		Ben Connan		Ben Tirran	
	Thermal desorption	Solvent elution	Thermal desorption	Solvent elution	Thermal desorption	Solvent elution
α -Thujene	0.4	0.4	0.2	0.2	0.3	0.3
α -Pinene	1.4	1.2	1.0	1.0	0.9	1.2
Camphene	0.3	0.4	0.2	0.2	0.2	0.2
Sabinene	1.1	0.8	0.9	0.8	0.6	0.8
β -Myrcene	6.5	4.2	4.5	3.6	4.3	3.8
Δ^4 -Carene	0.7	0.5	0.6	0.3	0.4	0.4
Δ^3 -Carene	29.5	38.3	34.2	37.4	35.1	44.2
α -Terpinene	2.4	2.2	1.5	1.9	2.5	2.3
Sylvestrene	0.6	nd ^a	0.4	nd	0.4	nd
(Z) β -Ocimene	13.0	15.8	13.8	16.5	12.9	13.2
β -Phellandrene	1.6	0.9	1.9	1.1	1.8	1.3
(E) β -Ocimene	15.6	9.8	12.8	10.8	13.6	7.8
Unidentified	1.3	0.3	1.1	0.9	1.5	0.5
γ -Terpinene	1.5	0.3	1.2	0.9	1.6	0.5
Unidentified	1.0	nd	0.9	nd	1.1	nd
α -Terpinolene	19.3	25.1	20.7	24.3	19.4	23.6
Dimethyl octatriene	3.9	nd	4.2	nd	3.7	nd

^a nd, none detected.

Solvent vapourization GC-MS. Solvent eluted or extracted samples were analysed using an HP5989B mass spectrometer fitted with a DB 1701 capillary column identical to that described above. The samples (1 μ L) were introduced directly onto the column using a cold on-column injector. Electronic pressure programming was used to maintain a constant helium carrier gas flow of 1 mL/min. The oven temperature was held at 40°C for 3 min and then increased at 5°C/min for 48 min and maintained at 280°C for a further 5 min. The quadrupole MS scanned the mass range 20–300 amu at 1 s intervals and was used in the electron impact mode (ionization energy, 70 eV; trap current, 300 μ A; source temperature, 250°C; analyser temperature, 100°C). Data were analysed using the HP MS Chemstation software package. Compound identification was based on retention time (R_t) and comparison of MS of authenticated standards when available and/or by comparison of the MS with those in the Wiley 138K mass spectral database (McLafferty and Stauffer, 1989).

RESULTS AND DISCUSSION

Monoterpenes

Analysis of the dichloromethane extracts of the leaf surface revealed the presence of 13 known and one unidentified monoterpene hydrocarbon (Table 1). All of the identified monoterpenes, except Δ^4 -carene, have been reported previously in steam distillates of blackcurrant bud (Kerslake and Menary, 1985a,b; Piry *et al.*, 1995) and leaf oils (Anderson *et al.*, 1963; Marriot, 1988). In all three cultivars, the predominant monoterpenes were Δ^3 -carene, α -terpinolene and (Z) β -ocimene, which accounted for over 80% of the total monoterpenes present. The relative amounts of the individual monoterpenes were broadly similar in all three cultivars, although small

statistically significant differences were seen in the relative concentrations of α -terpinene, β -phellandrene, and (Z) and (E) β -ocimene.

The levels of sabinene in the three cultivars studied were considerably lower than those reported for leaf oils isolated from the cultivars Wellington XXX, Baldwin and Ben Lomond (Marriot, 1988). However, no sabinene was detected in leaf oil from the blackcurrant cultivar Brödtröp (Anderson *et al.*, 1963). Although degradation of sabinene by acid-catalysed hydration during the distillation process can occur, it is unlikely that the appropriate conditions for such reactions would have arisen during the cold solvent extraction process used in this study. The use of cold on-column injection also minimized the possibility of any thermal breakdown of sabinene or any other terpene.

The ether eluted volatiles entrained by the porous polymer Tenax-TA contained the same 14 monoterpene hydrocarbons (Table 2) as the extracts of the leaf surfaces. The relative concentrations were similar in all three cultivars, with Δ^3 -carene, α -terpinolene and (Z) β -ocimene again accounting for almost 80% of the total monoterpenes. The only statistically significant difference was in the relative concentrations of Δ^4 -carene.

The monoterpene hydrocarbon profile of the thermally desorbed polymer volatiles was broadly similar to that produced by solvent elution (Table 2) with all 14 monoterpenes being detected in similar orders of magnitude to those determined in the leaf surface dipped samples. No statistically significant differences were observed between the three cultivars. However, an additional three terpenoid compounds (Table 2), each with a molecular ion corresponding to m/z 136, were detected. The earliest eluting of these was tentatively identified as sylvestrene (R_t 13.14 min), possibly produced by the surface catalysed opening of the cyclopropane ring of Δ^3 -carene. The relative amounts of sylvestrene observed were low and insufficient to account for the consistently lower levels of Δ^3 -carene in

Table 3. The monoterpene hydrocarbon composition of steam distilled extracts prepared from leaves of two blackcurrant cultivars

Compound	Monoterpene composition (% total monoterpenes)	
	Ben Alder	Ben Connan
α -Thujene	0.5	0.5
α -Pinene	1.9	2.0
Camphene	0.5	0.5
Sabinene	0.8	1.0
β -Myrcene	5.0	5.3
Δ^4 -Carene	0.9	1.0
Δ^3 -Carene	26.6	26.3
α -Terpinene	3.6	3.1
(Z) β -Ocimene	14.9	15.2
β -Phellandrene	3.5	3.2
(E)- β -Ocimene	13.1	12.7
Unidentified	1.2	1.0
γ -Terpinene	1.5	1.3
α -Terpinolene	26.0	27.0

the thermally desorbed samples. No increase was found in the relative amounts of α -terpinene or α -terpinolene, which can both be similarly produced from Δ^3 -carene (Whittaker, 1972). The relative concentrations of sabinene and α -thujene detected by thermal desorption and solvent elution were not significantly different. However, the breakdown of these compounds would be consistent with the small but statistically significant increases in the relative concentrations of both β -phellandrene and γ -terpinene determined in the thermally desorbed samples.

The second identifiable artefact detected was an alloocimene (R_t 27.06 min) and this accounted for almost 4% of the total monoterpenes in each of the three cultivars. This compound may have arisen by heat-catalysed isomerization of (Z) β -ocimene, which, by a 1,5 hydrogen migration, would yield 4-(E)-6(Z) alloocimene (Whittaker, 1972). This would also explain the slightly lower relative concentration of (Z) β -ocimene consis-

tently detected in the thermally desorbed samples. The third compound (R_t 25.14 min) remains unidentified.

Leaf oils produced by steam distillation from both Ben Connan and Ben Alder were also analysed by cold on-column injection GC-MS. The results (Table 3) indicated no significant differences between the two cultivars with respect to any of the 14 monoterpene hydrocarbons detected and none of the artefacts found in the thermally desorbed samples were observed. The major difference in the monoterpene hydrocarbons in the steam distilled isolate was the somewhat low relative concentration of Δ^3 -carene. A number of oxygenated monoterpenes (m/z 150 or 152), presumably formed by acid catalysed hydration (Marriott, 1988), were also detected in the distillate in quantifiable amounts. These included linalool (R_t 27.41 min), 1-4 terpineol (R_t 30.29 min) and α -terpineol (R_t 31.08 min), none of which was detected by the other methods used. However, one oxygenated compound, *p*-cymene-8-ol (R_t 31.58 min), was detected in trace amounts in the leaf surface extracts of all three cultivars and was also found at similar levels (0.1% total ion current, TIC) in the steam distilled extracts.

Sesquiterpenes

Steam distillation of macerated blackcurrant leaves results in major rearrangements of the sesquiterpene hydrocarbons (Marriott, 1988). Consequently in this section the results presented and discussed will be restricted to those from the polymer entrainments and leaf surface solvent extracts. Irrespective of the method used, only four sesquiterpene hydrocarbons were identified in quantifiable amounts (Table 4), namely, caryophyllene (R_t 36.23 min), humulene (R_t 37.24 min), germacrene (R_t 37.95 min) and cadinene (R_t 38.19 min). This was in broad agreement with the study of Marriott (1988) who, utilizing cold solvent extraction, detected the same four sesquiterpenes in the cultivars Baldwin, Ben Lomond and Wellington XXX.

On the leaf surface, the ratio of sesqui to monoterpenes was significantly higher than that detected by polymer entrainment, presumably reflecting their relative

Table 4. The sesquiterpene hydrocarbon composition of leaf surface volatiles prepared from three blackcurrant cultivars and determined by polymer entrainment and solvent extraction

Method	Sesquiterpene	Sesquiterpene composition (% total sesquiterpenes)		
		Ben Alder	Ben Connan	Ben Tirran
Solvent extraction	β -Caryophyllene	59.2	54.4	56.6
	α -Humulene	26.1	34.3	25.7
	Germacrene-D	14.7	10.1	17.1
	Δ -Cadinene	nd ^a	1.2	0.6
	S/M ratio ^b	0.65	0.36	0.54
Polymer/ solvent	β -Caryophyllene	65.0	45.5	64.4
	α -Humulene	20.8	36.2	23.7
	Germacrene-D	13.3	15.1	11.9
	Δ -Cadinene	1.0	3.3	nd
	S/M ratio	0.13	0.40	0.06
Polymer/ heat	β -Caryophyllene	73.2	59.3	72.7
	α -Humulene	22.8	29.4	22.1
	Germacrene-D	0.3	7.2	3.3
	Δ -Cadinene	3.7	4.1	1.9
	S/M ratio	0.12	0.08	0.07

^a nd, none detected.

^b S/M ratio, ratio of sesquiterpenes to monoterpenes.

Table 5. The relative concentration of additional compounds detected by polymer entrainment from three blackcurrant cultivars.

Compound	Composition (% total ion in current)					
	Ben Alder		Ben Connan		Ben Tirran	
	Thermal desorption	Solvent elution	Thermal desorption	Solvent elution	Thermal desorption	Solvent elution
(Z) 3-Hexenol	1.1	0.1	0.2	nd ^a	0.4	0.2
(Z) 3-Hexenolacetate	24.4	29.6	18.0	17.6	24.2	28.8
Cyclohexenolacetate	0.8	nd	0.8	nd	2.8	nd
4,8 Dimethyl- 1,3,7-nonatriene	2.5	1.3	4.4	3.0	5.4	4.6

^a nd, none detected.

volatilities. The significantly reduced ratio of sesqui to mono-terpenes on the leaf surface of the midge-resistant cultivar Ben Connan was also not reflected in the values obtained by polymer entrainment. Comparatively good agreement was found between the relative concentrations of the four sesquiterpenes as determined in leaf surface solvent extracts or by solvent elution of the polymer-entrained volatiles. The major sesquiterpenes in all three cultivars were caryophyllene and humulene, which together accounted for over 80% of the total sesquiterpenes. In both methods Ben Connan contained less caryophyllene and relatively more humulene than either Ben Alder or Ben Tirran. This observation was also confirmed in the thermally desorbed samples, but the exposure of the sesquiterpene hydrocarbons to heat resulted in a significant loss of germacrene and an increase in the relative concentration of cadinene. The low thermal stability of germacrene has been previously reported (Njoroge *et al.*, 1996).

A later eluting compound (R_t 39.96 min) with MS characteristics of an oxygenated sesquiterpene [m/z (relative intensity): 81(100), 161(45), 123(25), 105(24), 204(19), 119(19), 80(17), 43(17), 93(16), 207(15), 121(14), 109(14); M^+ 222(3)] was found in quantifiable amounts in the leaf surface extracts. This compound was present at significantly higher levels in the midge-resistant cultivar Ben Connan, accounting for 9.8% of the TIC as compared with 4.9 and 3.0% in Ben Alder and Ben Tirran, respectively. The MS was not compatible with the published spectra (McLafferty and Stauffer 1989) of oxygenated sesquiterpenes previously reported in leaf oils of blackcurrant (Marriott, 1988) nor with those of spathulenol or spathulanol (LeQuere and Latrasse, 1986). Further studies are required to isolate and characterize this compound with respect to both its structure and possible biological activity.

Other compounds

The polymer-entrained volatiles contained substantial amounts (Table 5) of the so-called green leaf volatiles, namely (Z) 3-hexenol (R_t 19.86 min) and (Z) 3-hexenyl acetate (R_t 23.71 min), produced from the breakdown of unsaturated fatty acids (Hatanaka *et al.*, 1987). No hexenyl acetate and only trace amounts (<0.05% TIC) of the alcohol were detected in the leaf surface extracts. The thermally desorbed samples consistently contained higher amounts of the free alcohol and in all cultivars an additional compound, which was tentatively identified as cyclohexenyl acetate (R_t 24.98 min), was detected. Both these compounds may have been derived from (Z)

3-hexenyl acetate, although thermally desorbing a Tenax adsorption tube containing trace quantities of (Z) 3-hexenyl acetate failed to produce any detectable artefacts. The formation of these compounds may require the presence of small quantities of water vapour, which may not have been completely removed from the adsorption tubes used for the leaf volatile entrainments.

The other compound detected solely in the polymer-entrained samples was the homoterpene, 4,8-dimethyl-1,3,7-nonatriene (R_t 26.06 min). This compound is released in large quantities by a number of plant species when damaged by insect herbivores and can attract their natural enemies, including parasitoids (Dicke, 1994). A comparatively high concentration (3–5% TIC) of the nonatriene was noted (Table 5) in the polymer-entrained volatiles of all three cultivars, irrespective of the elution method used. This may be indicative of some undetected herbivorous damage or may have arisen from the mechanical damage produced during the excision of samples from the growing plant. However, in both corn (Turlings *et al.*, 1990) and Lima bean leaves (Takabayashi *et al.*, 1991), artificial damage induced only minor increases in homoterpene production. Alternatively, blackcurrant leaves may naturally release more of this compound.

Further evidence of possible thermal rearrangements was displayed by two isomers of methyl jasmonate, detected in trace amounts in the leaf oils produced by steam distillation and in the dichloromethane leaf surface extracts. Jasmonic acid and its derivatives are known to be involved in regulating wound-induced responses (Wasternack and Parthier, 1997). A MS library search suggested that the earlier eluting isomer (R_t 45.5 min) was (–)-methyl jasmonate, with the later eluting isomer (R_t 46.7 min) identified as (+)-7-iso-methyl jasmonate. In the extracts of the leaf surface, the later eluting isomer predominated, whilst in the steam distilled oils the reverse was found. Previously reported studies (Mueller and Brodschelm, 1994) have suggested that the epimerization of (+)-7-iso-jasmonic acid to (–)-jasmonic acid can occur in the presence of acids or high temperature. A similar mechanism may account for the increased levels of (–)-methyl jasmonate detected in the steam distilled samples.

CONCLUSIONS

From the results presented, it can be seen that the major compounds detected in the odour plume produced by

leaves of both midge-resistant and susceptible blackcurrant cultivars were identical, with only quantitative differences in their relative concentrations being observed. The relative concentrations of the individual mono- and sesqui-terpenes entrained by the porous polymer reflected those extracted from the leaf surface. However, the polymer-entrained volatiles also included a number of compounds which were not detected on the leaf surface, including a homoterpene and a number of C₆ aliphatic compounds derived from unsaturated fatty acids. Presumably these were continually synthesized and did not accumulate sufficiently to be detected on the leaf surface itself.

Comparisons of the results obtained by thermal desorption and solvent elution of the polymer-entrained volatiles indicated that the majority of compounds were detected by both techniques. Thermal desorption did produce a number of artefacts at least one of which, alloocimene, could readily be mistaken for a naturally occurring compound. It also resulted in a significant alteration in the relative concentrations of the hydrocarbon sesquiterpenes as typified by the increase in Δ -cadinene levels. However, compared with steam distillation, thermal desorption combined with polymer entrainment resulted in a more accurate reflection of the

true volatile profile. The conclusion to be drawn from this study would appear to be that the optimum method for fully characterizing the odour plumes released by blackcurrant leaves is solvent elution combined with polymer entrainment. However, for certain purposes such as in preliminary studies to select the optimal polymer or to establish the sampling conditions for minimal sample breakthrough, the relative simplicity and opportunity for automation would make thermal desorption the method of choice. Additionally, if polymer solvent elution indicated little or no degradation of a particular compound, such as the semiochemical 4,8-dimethyl 1,3,7-nonatriene, thermal desorption could be an effective technique to monitor changes in its levels of production in response to insect damage or to examine relatively rapid diurnal variations.

Acknowledgements

The authors thank Ms Elizabeth Brechany from the Hannah Research Institute (Ayr, Scotland) for undertaking the steam distillation, and the Scottish Office Agriculture, Environment and Fisheries Department for their financial support.

REFERENCES

- Andersson, J., Bosvik, R. and Von Sydow, E. (1963). The composition of the essential oil of blackcurrant leaves (*Ribes nigrum* L.). *J. Sci. Food Agric.* **14**, 834–840.
- Brennan, R. M. (1996). Currants and gooseberries. in *Fruit Breeding Vol. 11: Vine and Small Fruit Crops.* (Janick J. and Mare J. N. eds.), pp 191–295. Wiley, New York.
- Brennan R. M., Robertson G. W., McNicol, J. W., Fyffe, L. and Hall, J. E. (1992). The use of metabolic profiling in the identification of gall mite (*Cecidyphopsis ribis* Westw.)-resistant blackcurrant genotypes. *Ann. Appl. Biol.* **121**, 503–509.
- Dicke, M. (1994). Local and systemic production of volatile herbivore-induced terpenoids: their role in plant–carnivore mutualism. *J. Plant Physiol.* **143**, 465–472.
- Gershenzon, J. and Croteau, R. (1991). Terpenoids. in *Herbivores: their Interaction with Secondary Plant Metabolites Vol I: The Chemical Participants.* (Rosenthal G. R. and Berenbaum, M. R., eds.), pp 165–219. Academic Press, New York.
- Hatanaka, A., Kajiwara, T. and Sekiya, J. (1987). Biosynthetic pathway for C₆-aldehydes formation from linoleic acid in green leaves. *Chem. Phys Lipids* **44**, 341–361.
- Keep, E. (1985). The blackcurrant leaf curling midge, *Dasineura tetensi* Rubs.: its host range and inheritance of resistance. *Euphytica* **34**, 801–809.
- Kerslake, M. F. and Menary, R. C. (1985a). Varietal differences of extracts from blackcurrant buds (*Ribes nigrum* L.). *J. Sci. Food Agric.* **36**, 343–351.
- Kerslake, M. F. and Menary, R. C. (1985b). Aroma constituents of blackcurrant buds (*Ribes nigrum* L.). *Perfum. Flavor* **9**, 13–24.
- Kerslake, M. F., Latrasse, A. G. and Le Quere, J.-L. (1989). Hydrocarbon chemotypes of some blackcurrant cultivars (*Ribes* sp.). *J. Sci. Food Agric.* **47**, 43–51.
- Latrasse, A. and Lantin, B. (1977). Composition de l'huile essentielle de bourgeons de cassis (Variabilite et heritabilite). *Riv. Ita Essenze* **39**, 395–402.
- Latrasse, A., Schlich, P. and Le Quere, J. L. (1990). Chemotaxonomy of the essential oils of the blackcurrant buds (*Ribes* sp.) from major terpenes (hydrocarbons and oxygenated compounds). *Lebens. Wiss. Technol.* **23**, 377–385.
- Le Quere, J. L. and Latrasse, A. (1986). Identification of (+)-spathulenol in the essential oil of blackcurrant buds (*Ribes nigrum* L.). *Sci. Aliments* **6**, 47–59.
- Le Quere, J. L. and Latrasse, A. (1990). Composition of the essential oils of blackcurrant buds (*Ribes nigrum* L.). *J. Agric. Food Chem* **38**, 3–10.
- Marriott, R. J. (1988). Isolation and analysis of blackcurrant (*Ribes nigrum*) leaf oil. In *Flavors and Fragrances: a World Perspective.* (Lawrence, B. M., Mookherjee, B. D. and Willis, B. J. eds.), pp 387–403. Elsevier, Amsterdam.
- McLafferty, F. W. and Stauffer, D. B. (1989). The Wiley/NBS Registry of Mass Spectral Data. Vols. 1–7. Wiley-Interscience, Chichester.
- Mueller, M. J. and Brodschelm, W. (1994). Quantification of jasmonic acid by capillary gas chromatography-negative chemical ionisation-mass spectrometry. *Anal. Biochem.* **218**, 425–435.
- Nishimura, O. and Mihara, S. (1988). Aroma constituents of blackcurrant buds (*Ribes nigrum* L.). in *Flavors and Fragrances: a World Perspective.* (Lawrence, B. M., Mookherjee, B. D. and Willis, B. J. eds.), pp 375–386. Elsevier, Amsterdam.
- Njoroge, S. M., Ukeda, H. and Sawamura, M. (1996). Changes in the volatile composition of yuzu (*Citrus junos* Tanaka) cold pressed oil during storage. *J. Agric. Food Chem.* **44**, 550–556.
- Pickett, J. A. (1991). Lower terpenoids as natural insect control agents. in *Ecological Chemistry and Biochemistry of Plant Terpenoids.* (Harborne J. B. and Thomas-Barberan, F. A. eds.) pp 297–313. Clarendon Press, Oxford.
- Piry, J., Pribela, A., Durcanska, J. and Farkas, P. (1995). Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. *Food Chem.* **54**, 73–77.
- Raguso, R. A. and Pellmyr, O. (1998). Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* **81**, 238–254.
- Robertson, G. W., Griffiths, D. W., Macfarlane-Smith, W. H. and Butcher, R. D. (1993). The application of thermal desorption-gas chromatography-mass spectrometry to the analyses of flower volatiles from five varieties of oilseed rape. *Phytochem. Anal.* **4**, 152–157.
- Takabayashi, J., Dicke, M. and Posthumus, M. A. (1991).

- Induction of indirect defence against spider-mites in uninfested Lima bean leaves. *Phytochemistry* **30**, 1459–1462.
- Turlings, T. C., Tumlinson, J. H. and Lewis, W. J. (1990). Exploitation of herbivore-induced plant odours by host-seeking parasitic wasps. *Science* **250**, 1251–1253.
- Wasternack, C. and Parthier, B. (1997). Jasmonate-signalled plant gene expression. *Trends Plant Sci.* **2**, 302–307.
- Whittaker, D. (1972). The monoterpenes. in *Chemistry of Terpenes and Terpenoids*. (Newman, A. A. ed.), pp 11–87. Academic Press, New York.