Profiling of raspberry cane wound volatiles using a combination of SPME and GC-TOF-MS

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Background

The raspberry cane midge, *Resseliella theobaldi*, is an important pest of red raspberry, *Rubus idaeus*, in the UK and northern and central Europe. Splits in young canes caused by damage or natural splitting are the sites of egg laying by adult females. Emerging larvae feed on the pith causing lesions which provide a means of entry of diseases such as cane blight fungus and midge blight. Methods for monitoring and biological control of midges involve development of mass trapping systems using natural chemical attractants as lures. For example, considerable effort has gone into development of traps using female sex pheromone for local monitoring of males. However, effective control requires identification of an attractant to lure the females as they emerge from the soil in early spring (first generation) and during the second generation which coincides with fruit harvest and the main period of fungal colonisation.

Behavioural analysis suggests that female midges are attracted to egg laying sites by volatile chemicals released from the split canes. We have developed a sampling technique which uses solid phase microextraction (SPME) fibres to entrain volatiles in the immediate vicinity of a split.

Methods

Sampling enclosures consisting of wire frames (a) supporting an inert plastic (PET) film (b) were positioned around the cane at sites of manually created splits (c). Sheathed SPME fibres were inserted into the enclosure and the fibres were exposed adjacent to the split for collection of volatiles (d).

Entrainments were carried out in pairs, one being an unwounded control plant and the other having a 2-3 cm manually created wound. Fibres were either deployed singly (d) or in pairs (e).

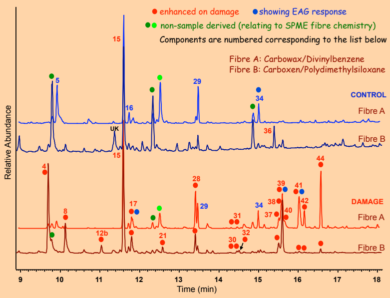
Following transfer of fibre holder assemblies to the GC-MS autosampler, the volatiles were desorbed via a PTV injector and analysed using a GC-TOF-MS.

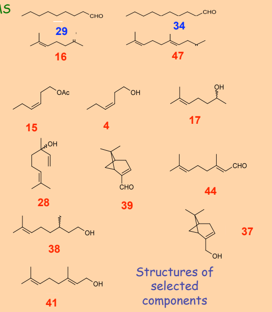
Separation of volatiles was effected on a DB 1701 GC column (30m x 0.32mm x 1.0 µm) using helium carrier gas at a flow rate of 1.2 ml/min. The GC -MS consisted of a ThermoFinnigan Tempus Time-of-flight (TOF) system operating at a data acquisition rate of 3 spectra/second. Data was acquired using the Xcalibur software package. Samples were desorbed for 2 minutes into a PTV injector assembly operating in splitless mode at temperatures varying from 200-280°C, depending on the type of SPME fibre used.

Results and Discussion

Volatile profiles obtained from both split and undamaged canes consist primarily of a mixture of monoterpenes, monoterpene alcohols, aliphatic alcohols, aldehydes and ketones.

Raspberry cane (Malling Promise) volatiles analysed using SPME and GC-MS





Structures of selected components

Profiles for the distribution of stem volatiles are shown for experiments conducted using the raspberry varieties Glen Ample, Glen Clova, Glen Prosen, Latham, Malling Delight and Malling Promise.

Components showing major enhancement in production following damage are numbered in red and are indicated: (orange shading)

Abundance values are reported as a percentage relative to the total combined measured amount of the C_7 - C_{11} *n* - aldehydes present in each sample.

Raspberry Varieties

- GA: Glen Ample
- GC: Glen Clova
- GP: Glen Prosen
- L: Latham
- MD: Malling Delight
- MP: Malling Promise

Plant Status

- CONT: Control (undamaged)
- DAMG: Damaged

SPME Fibre Adsorbent Phases

- PDMS: Polydimethylsiloxane
- PEG: Polyethyleneglycol
- CAR: Carboxen
- CW: Carbowax
- DVB: Divinylbenzene

Fibres used had the following adsorbents singly or in combination

(1) PDMS
(2) PEG
(3) CW/DVB
(4) CAR/PDMS
(5) CAR/PDMS/DVB

Date	Variety	Status	Rep	Fibre	Sampling time (min)	Sampling Type	1 Hexanal	2 tepene (ocimene? a-pinene?)	3 2- or 4-hexen1-ol	4 cis-3-hexen-1-ol	5 camphene	6 2-heptanone	7 n-heptanal	8 2-heptanol	9 2-hexen-1-ol or cyclohexanol	10 linalyl acetate	11 2-butanone or 2-ethyl-butanal	12 terpene (unknown)	13 limonene	14 5- or 4-hexene-1-ol acetate	15 cis-3-henen-ol-acetate	16 6-methyl-5-hepten-2-one	17 6-methyl-5-hepten-2-ol	18 n-octanal	19 benzaldehyde	20 2-ethyl-1-hexanol	21 5-ethyl-2(5H)-furanone or 5-methyl-4-hexen-3-one	22 4-hydroxybutanoate or dihdro-2(3H)-furanone	23 2-nonen-1-ol	24 linalool oxide	25 1-octanol	26 rose oxide	27 linalool oxide (2) or isomer?	28 linalool	29 n-nonanal	30 citronellal	31 t-pinocarveol or t-verbenol	32 like 5-ethyl-2(5H)-furanone etc.	33 11-nonanol	34 n-decanal	35 a-terpineol	36 methylsalicylate	37 myrtenol	38 b-citronellol	39 myrtenal	40 nerol	41 geraniol	42 neral (Z-citral)	43 n-undecanal	44 geranial (E-citral)	45 citronellyl acetate	46 b-bourbonene	47 geranyl acetate	48 a-muurolene	49 sesquiterpene (isoledene? Etc.)
04/12/07	MD	CONT	1	CW/DVB	180	A1	2.4	0.0	0.7	10.8	0.3	0.3	3.2	0.3	0.0	0.4	1.7	0.6	6.7	0.1	3.8	3.4	1.8	4.4	5.8	16.4	1.3	2.3	1.4	1.3	3.8	0.1	0.1	19.2	48.4	0.4	1.7	0.3	7.1	37.4	4.5	8.0	4.1	6.8	4.5	1.0	5.3	1.9	6.6	6.1	0.0	0.0	0.4	0.6	1.0
11/12/07	MD	CONT	2	CW/DVB	180	A1	3.0	0.0	0.7	26.5	0.6	0.4	2.2	0.2	0.0	0.4	1.4	0.6	2.8	0.3	69.3	9.6	4.3	4.0	8.1	40.0	7.0	1.8	1.0	6.1	7.7	0.3	0.0	20.1	57.8	0.7	11.0	1.0	6.2	29.5	18.8	5.3	8.0	4.9	9.2	2.0	3.9	4.9	6.5	16.6	0.0	0.0	0.0	0.0	0.0
04/12/07	MD	DAMG	1	CW/DVB	180	A1	4.0	0.0	0.3	10.0	0.5	0.3	3.3	0.2	0.1	0.4	1.3	0.4	5.2	0.1	12.9	6.6	5.6	4.4	6.8	37.1	2.8	30.4	1.1	1.7	6.7	0.2	0.5	69.0	51.0	1.0	1.7	0.4	12.0	32.3	6.9	48.9	8.5	45.6	7.5	3.1	32.7	10.0	9.1	58.4	4.3	0.0	1.4	0.7	3.7
11/12/07	MD	DAMG	2	CW/DVB	180	A1	3.6	0.0	0.6	18.4	0.3	0.1	2.1	0.1	0.1	0.2	1.4	0.5	2.0	0.2	68.1	10.2	6.6	5.0	6.5	13.7	6.0	6.0	1.0	2.8	5.9	0.4	1.3	87.9	48.2	5.4	6.5	1.0	10.7	38.1	14.9	21.2	18.7	66.1	35.5	8.9	58.5	41.7	6.6	209.7	8.9	0.0	4.8	0.6	1.5
11/12/07	MD	DAMG	2	CW/DVB	180	A1	25.4	0.0	5.5	13.2	2.3	1.2	2.4	1.0	0.4	0.0	7.5	0.6	0.3	0.0	3.4	1.5	5.5	2.7	27.2	11.7	8.5	152.9	18.3	15.5	2.2	0.0	0.0	0.0	41.8	5.7	17.6	3.5	4.1	17.0	17.7	47.1	71.7	109.9	84.5	12.9	87.5	77.1	36.0	390.2	7.5	0.0	11.7	52.6	12.0
07/12/07	Air			CW/DVB	180		2.5	0.0	0.5	0.0	5.5	0.5	1.9	0.1	0.0	0.1	1.7	0.6	1.9	0.0	0.1	0.8	0.0	3.4	10.4	47.7	0.0	19.7	0.9	0.0	3.6	0.0	0.0	0.0	60.4	0.0	2.7	0.0	3.2	24.2	6.8	0.0	0.0	0.0	0.0	0.6	3.3	0.0	10.1	0.0	0.0	0.0	1.2	0.0	0.0
27/02/08		BLANK-D		CAR/PDMS			23.3	0.0	0.0	0.0	0.0	0.0	16.9	12.0	7.1	0.0	25.9	0.0	0.0	0.0	0.0	4.8	0.0	3.9	164.9	43.7	0.0	471.1	0.0	0.0	0.0	0.0	0.0	0.0	49.8	0.0	0.0	0.0	11.4	19.8	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.6	0.0	0.0	0.0	0.0	3.6	0.0
27/02/08		BLANK-C		CAR/PDMS			16.4	0.0	0.0	0.0	0.0	0.0	7.8	6.6	0.8	0.0	7.7	0.0	0.0	0.0	0.0	9.2	0.0	3.7	50.1	57.7	0.0	136.8	0.0	0.0	0.0	0.0	0.0	0.0	39.8	0.0	0.0	0.0	6.2	36.8	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.9	0.0	0.0	0.0	0.0	4.6	5.1
27/02/08		BLANK-D		CAR/PDMS			17.2	0.1	0.1	0.1	0.1	0.1	26.5	9.3	4.5	0.1	57.0	0.1	0.1	0.1	0.1	10.8	0.1	5.4	196.7	48.1	0.1	588.4	0.1	0.1	0.1	0.1	0.1	41.8	30.5	0.1	0.1	40.9	0.1	20.9	4.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	16.7	0.1	0.1	0.1	0.1	0.1	0.1

Using six different raspberry varieties sampled using a range of different fibre chemistries, we have identified a suite of volatiles which show a consistent pattern of enhancement following damage to the cane. These compounds consist mainly of a family of structurally related terpenes, many of which are known to have behavioural effects on insects and plants (e.g. attract natural enemies) and/or are produced in response to insect herbivory.

Ongoing Work

The physiological activity of the entrained volatiles is being investigated by means of a GC-elecroantennography (EAG) system which identifies the metabolites which elicit a response from the antenna of female midges. Compounds showing enhanced production on damage to canes and those shown to elicit a response from the midge are being used to test and develop lures for biological control and monitoring. Some compounds identified from preliminary experiments are indicated in the chromatographic trace above.

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HDC Horticultural Development Company

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