

The identification of potential aeroallergen/irritant(s) from oilseed rape (*Brassica napus* spp. *oleifera*): volatile organic compounds emitted during flowering progression

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Summary

Volatile organic compounds emitted by growing intact oilseed rape plants have been detected using an entrainment apparatus enabling volatile headspace analysis by thermal desorption coupled to capillary gas chromatography-mass spectrometry. In total, 22 volatile compounds were identified as being emitted during the flowering period. The main constituents were α -farnesene (a sesquiterpene); β -myrcene (a monoterpene); linalool (a monoterpene alcohol) and the 'green leaf' volatile (E)-3-hexen-1-ol acetate. These compounds constituted between 50 and 87% (mean 68%) of the total volatiles emitted in all of the entrainments carried out with flowering oilseed rape plants. The remaining constituents consisted of a range of compounds including other terpenoids, the characteristic 'green leaf' volatile (E)-3-hexen-1-ol, short chain alcohols and ketones, organic sulphides and nitrogen-containing compounds. These were generally present as minor constituents but some plant entrainments revealed that higher relative amounts could be emitted. This was particularly apparent for dimethyl disulphide, 3-methyl-2-pentanone, 3-hydroxy-2-butanone, sabinene, isomyrcenol and (E)-3-hexen-1-ol. The possible role of the 22 compounds in respiratory mucosa and conjunctiva irritation associated with airborne releases from oilseed rape is discussed.

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Introduction

Within the last 15 years autumn sown OSR (oilseed rape —*Brassica napus* ssp. *oleifera*) has become a very common arable crop in the UK, its distinctive yellow flowers in spring/early summer providing clear evidence of the fact that it is now grown on around 10% of arable land. The crop has increasingly been accused by rural populations of being responsible for respiratory and hay fever-like symptoms during its peak flowering period. However, proof of this involvement and the identification of the airborne released agent(s) from OSR which could potentially cause the symptoms have not been determined [1,2]. Complaints received from members of

the public living in close proximity to the crop suggest that upper respiratory mucous membrane and conjunctiva irritation may be involved. At present it remains unclear whether this may be caused by an IgE-mediated allergic reaction or by a non-immunological irritant mechanism. As indicated [1], studies into the likely involvement of OSR pollen have proved inconclusive, although it is apparent that potential allergens are carried by the pollen [3]. This has led to speculation that some other airborne antigen released from the OSR crop may be responsible for the majority of reported symptoms [1]. Indeed, the flowering period of OSR is not only the time of pollen release but also of maximum odour release by the plants and coincides with the period when both fungal and pest infestations can be at their highest and consequently fungicide and insecticide application at their greatest.

In this paper we report on the identification of the

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VOCs (volatile organic compounds) emitted by OSR during the flowering period, some of which will make up the characteristic odour of the crop. We have monitored the release of VOCs from intact OSR plants before flowering and as they progress through flowering, which is the first occasion this has been reported. The OSR cultivars entrained included those among the most commonly grown in the UK in the last several years. The double-low cultivars, so named because they have low levels of erucic acid in the seed and low levels of glucosinolates in the seed pod, have become by far the most dominant, cultivars. Tapidor, cultivar Falcon and cultivar Envol being among the most commonly grown cultivars in 1989, 1991 and 1992 respectively. Single-low cultivars (low only in seed erucic acid) are represented by cultivars Pasha which was one of the most commonly grown OSR cultivars in 1987. These four cultivars are all sown in the autumn but since 1990 the amount of spring-sown cultivars grown has markedly increased and accounted for nearly 50% of total OSR grown in Scotland in 1993. Two spring-sown OSR cultivars (cultivar Forte and cultivar Comet) were therefore also examined to allow comparison. Together with

earlier investigations of OSR volatiles [4–6], these results now allow an assessment of any likely health effects caused by the above emissions and suggest candidate VOCs for clinical investigation.

Method

Plant cultivation

OSR plants were raised from seed in Jiffy pots containing compost within a glasshouse at 15°C under a 12 h light regime and were repotted into individual 4 inch pots after 10 days. After 3 further days, plants were vernalized at 5°C in a cold room with a 7 h light regime for a 6 week period prior to being returned to the glasshouse and repotted into 6 inch pots. Plants were taken into the entrainment room when required. In the entrainment room the plants were placed under a mercury lamp and subjected to a 12 h light regime similar to that in the glasshouse, a treatment necessary to achieve flowering progression. The temperature within the room varied over a daily range of 11°C–18°C. OSR cultivars examined were Tapidor, Falcon, Envol, Pasha, Forte and

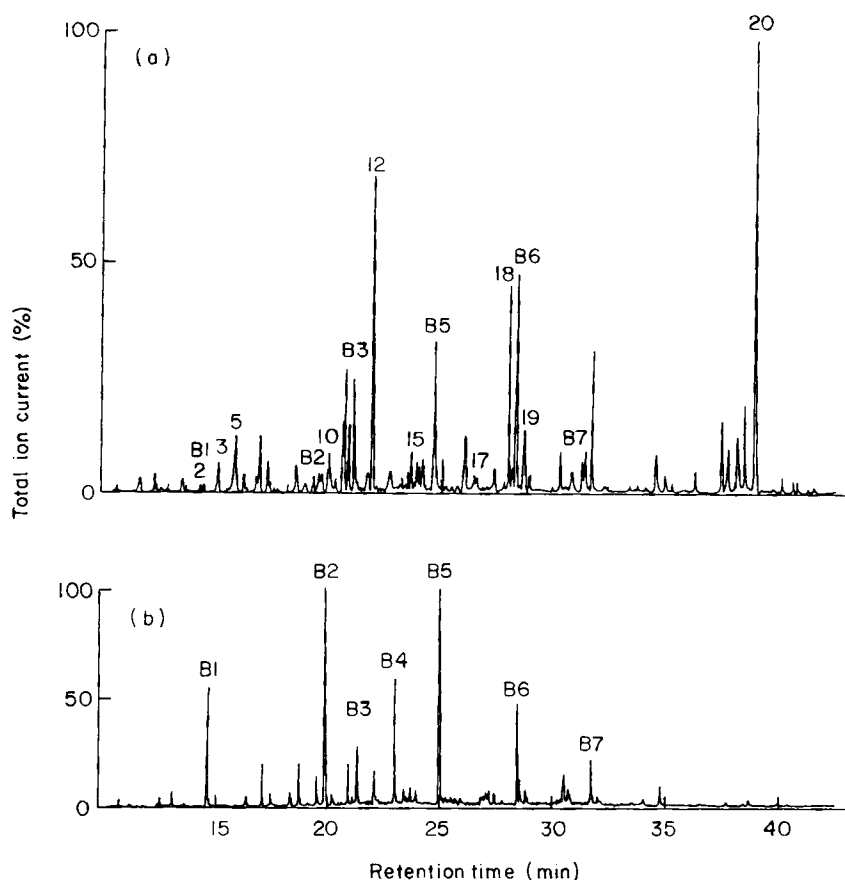


Fig. 1. Chromatograms of the volatile organic compounds trapped on Tenax-TA after 24 hours of sampling of the air from (a) a flowering raceme of an intact oilseed rape plant (cultivar Tapidor growth stage 4:5, 5:05), (b) the room in which the entrainment was performed. Peak numbers refer to those given in Table 1. Peaks labelled B1–B7 refer to compounds that were major background contaminants (B1 = toluene, B2 = α -pinene, B3 = *n*-decane, B4 = Δ -3-carene, B5 = octanal + siloxane (GC column bleed), B6 = *n*-dodecane, B7 = tridecane).

Table 1. Percentage composition of volatile organic compounds identified as emanating from an intact living OSR (cultivar Tapidor) flowering raceme followed through its flowering period

Peak number	Compound	Retention time (min)	Growth stage							
			4:10	4:20	4:30	4:50,5:05	4:70,5:10	4:70,5:15	4:65,5:20	4:60,5:25
1	2-Methyl-propanenitrile	11.67	ND	ND	ND	ND	ND	ND	ND	ND
2	Dimethyl disulphide	14.48	ND	ND	0.7	0.7	0.4	0.9	0.3	0.5
3	3-Methyl-2-pentanone	15.15	6.3	3.7	5.9	8.9	5.5	5.3	5.0	6.0
4	N-Methylene ethanamine	15.52	0.6	0.8	1.2	0.3	1.6	1.1	0.8	1.3
5	3-Hydroxy-2-butanone	15.75	ND	ND	9.5	9.0	3.6	9.8	11.7	0.9
6	Thiocyanic acid, methyl ester	16.17	ND	ND	ND	ND	ND	ND	ND	ND
7	3-Methyl-2-pentanol	16.82	2.1	1.9	1.9	0.9	0.7	0.5	0.5	0.5
8	Unknown	18.05	ND	ND	ND	ND	ND	ND	ND	ND
9	α -Thugene	19.32	1.3	1.5	0.4	0.4	0.8	0.9	0.5	0.7
10	(E)-3-Hexen-1-ol	20.10	11.9	6.3	6.1	5.1	5.8	6.1	4.7	5.9
11	Sabinene	21.80	1.7	2.6	2.3	1.5	1.0	1.7	1.8	1.7
12	β -Myrcene	22.01	38.1	34.4	25.2	20.9	27.7	28.8	26.7	29.4
13	α -Terpinene	23.34	ND	ND	ND	ND	ND	ND	ND	ND
14	Dimethyl trisulphide	24.10	ND	ND	ND	ND	ND	ND	ND	ND
15	(E)-3-Hexen-1-ol acetate	24.29	6.5	3.3	4.9	2.4	16.5	4.9	1.7	9.2
16	1-8,Cineole	24.79	ND	ND	ND	ND	ND	ND	ND	ND
17	(E)-4,8-Dimethyl-1,3,7-nonatriene	26.77	ND	ND	ND	ND	ND	ND	ND	ND
18	Linalool	28.19	15.2	18.4	18.4	16.3	12.7	12.8	16.0	15.4
19	Isomyrcenol	28.85	ND	6.0	7.1	4.0	5.0	4.7	5.6	4.1
20	α -Farnesene	39.17	16.2	21.3	16.4	29.8	18.7	22.6	24.6	24.4
21	1H-Indole	39.89	ND	ND	ND	ND	ND	ND	ND	ND
22	Unidentified sesquiterpene	41.64	ND	ND	ND	Trace	ND	ND	ND	ND
Total peak area*			10.3	10.5	9.7	12.0	7.0	8.0	9.4	8.9

* Relative amount of total plant volatile organic compounds emitted in 24 h as determined by summing the peak areas for each of the compounds in the table. ND. Not detected.

Comet. The growth stage of individual plants was assessed visually and expressed using a code based on that developed for OSR by Sylvester-Bradley [7]. Briefly, flower bud development is represented by growth stage 3.0–3.9, when the first flower is open the growth stage is 4, the scale then progresses through flowering referring to the percentage of buds which are in flower (e.g. 50% of flower buds open = 4:50) as well as to the % of seed pods which are more than 2 cm long (growth stage 5), e.g. 50% of flower buds open and 10% of all possible seed pods over 2 cm = 4:50, 5:10).

Sampling of volatiles

VOCs emitted from plants were collected and characterized by dynamic headspace analysis. The laboratory entrainment apparatus used for the collection of plant volatiles was based on that described by Heath *et al.* [8]

which enabled the volatiles emitted from intact plants to be collected. This apparatus was improved by using a Rylsan II forensic nylon sheet or a 36 inch $\times$ 24 inch bag of the same material (Keygrove Marketing) sealed across the open end of the glass entrainment apparatus. Elastic bands were secured on the outside of the nylon sheet or bag to seal around the sample tube(s) projecting into the chamber and loosely around the plant stem. This reduced the amount of unpurified air entering the collection chamber and allowed lower flow rates of 180 ml/min to be used for the collection of plant volatiles and thus minimized adsorbent breakthrough [9]. Air was pumped through 2 filters (one containing molecular sieve 5A, the other activated charcoal) into the entrainment chambers at 50 ml/min in excess of that being drawn out and passed into two sample tubes, the first containing Tenax-TA (60–80 mesh), the second Haysep-Q (60–80 mesh) adsorbent. Sampling times were for 24 h

Table 2. Percentage composition of volatile organic compounds identified as emanating from the complete aerial section of intact living autumn-sown double-low OSR plants (cultivar type and growth stage of plant as indicated)

Peak number	Compound	Retention time (min)	Cultivar Growth stage	Tapidor			Falcon			Envol	
				3:4	4:15	4:60,5:10	3:9	4:1	4:7,5:2	4:2	4:6,5:1
1	2-Methyl-propanenitrile	11.67		ND	0.9	0.4	3.8	0.8	0.4	0.4	0.5
2	Dimethyl disulphide	14.48		0.7	0.1	4.0	0.8	2.2	6.2	9.1	1.2
3	3-Methyl-2-pentanone	15.15		9.0	17.4	4.6	13.1	3.0	5.0	6.2	5.6
4	N-Methylene ethanamine	15.52		ND	0.9	1.0	ND	0.2	0.3	0.7	0.1
5	3-Hydroxy-2-butanone	15.75		ND	ND	ND	ND	0.9	6.6	3.9	2.2
6	Thiocyanic acid, methyl ester	16.17		ND	ND	ND	ND	ND	0.2	0.2	0.2
7	3-Methyl-2-pentanol	16.82		1.9	2.8	1.0	ND	0.2	0.2	ND	0.6
8	Unknown	18.05		ND	0.3	1.5	ND	ND	ND	ND	ND
9	α -Thugene	19.32		ND	ND	ND	2.2	0.8	5.0	0.9	2.7
10	(E)-3-Hexen-1-ol	20.10		ND	ND	ND	ND	ND	ND	ND	0.4
11	Sabinene	21.80		ND	6.5	ND	2.6	2.6	3.9	2.4	7.4
12	β -Myrcene	22.01		60.3	57.5	52.4	48.0	27.6	42.7	27.1	28.8
13	α -Terpinene	23.34		ND	ND	ND	ND	ND	4.8	ND	ND
14	Dimethyl trisulphide	24.10		ND	ND	ND	ND	Trace	0.6	Trace	Trace
15	(E)-3-Hexen-1-ol acetate	24.29		23.4	6.0	8.1	27.0	8.0	4.5	11.4	16.3
16	1-8,Cineole	24.79		ND	0.8	ND	1.3	ND	4.5	1.3	2.5
17	(E)-4,8-Dimethyl-1,3,7-nonatriene	26.77		ND	ND	ND	ND	2.5	0.7	0.9	0.5
18	Linalool	28.19		ND	2.5	3.7	ND	27.7	6.9	17.9	11.1
19	Isomyrcenol	28.85		ND	Trace	Trace	ND	6.7	0.6	2.9	3.6
20	α -Farnesene	39.17		4.7	4.3	23.3	1.2	4.6	1.1	12.7	13.6
21	1H-Indole	39.89		ND	ND	ND	ND	12.3	5.1	2.0	2.6
22	Unidentified Sesquiterpene	41.64		ND	ND	ND	ND	ND	0.5	ND	ND
Total peak area*				9.0	19.6	34.0	7.0	32.6	37.6	22.9	22.2

* Relative amount of total plant volatile organic compounds emitted in 24 h as determined by summing the peak areas for each of the compounds in the table. ND. Not detected.

periods after which the sample tubes were either replaced or the entrainment was ended. The details of materials, fitting and associated methods were described in a previous report [4] with the addition that Tenax-TA filled tubes were preconditioned at 240°C instead of 180°C used for Haysep-Q. In addition, prior to thermal desorption tubes were flushed with N₂ at 25 ml/min for 10 min to remove any trapped water vapour.

The background volatiles associated with the entrainment apparatus itself were determined prior to the plant entrainment by running the system without a plant present. On each sampling day a control adsorbent tube line was set up (flow rate 180 ml/min) in order to monitor the volatiles present in the same room as the entrainment experiment. Furthermore, the purity of the air being pumped into the entrainment apparatus was periodically monitored for the presence of any volatiles using an adsorbent tube. In this way it was possible to account for the source of the volatiles detected.

Gas chromatography (GC) — mass spectrometry (MS)

The volatiles trapped on the adsorbent tubes were analysed with a Perkin Elmer ATD50 automated thermal desorption unit linked to a Hewlett Packard 5890 capillary gas chromatograph and VG TRIO-1000 quadrupole mass spectrometer by the method described in our earlier report [4]. Sample tubes were desorbed over a period of 15 min at a temperature of 200°C for Tenax-TA and 130°C for Haysep-Q.

Results

A typical chromatogram from an entrainment of an intact growing OSR plant's flowering raceme is shown in Fig. 1a and revealed the presence of over 150 different compounds. However, since it was possible that a number of these compounds originated from sources other than the plant, an assessment was made of the

Table 3. Percentage composition of volatile organic compounds identified as emanating from the complete aerial section of intact living (a) autumn-sown single-low OSR plant (cultivar Pasha, growth stage of plant as indicated) (b) spring-sown double-low OSR plants (cultivar type and growth stage of plant as indicated)

Peak number	Compound	Retention time (min)	Cultivar Growth stage	a)	Pasha	b)	Forte	Comet
					4:1	4:6, 5:1	4:50, 5:05	4:35, 5:05
1	2-Methyl-propanenitrile	11.67			0.9	0.2	ND	1.4
2	Dimethyl disulphide	14.48			0.6	2.9	4.4	3.0
3	3-Methyl-2-pentanone	15.15			4.0	1.7	2.0	7.9
4	N-Methylene ethanamine	15.52			0.1	ND	ND	ND
5	3-Hydroxy-2-butanone	15.75			Trace	20.0	1.6	17.2
6	Thiocyanic acid, methyl ester	16.17			Trace	0.2	3.8	0.5
7	3-Methyl-2-pentanol	16.82			ND	ND	ND	Trace
8	Unknown	18.05			ND	ND	ND	ND
9	α -Thugene	19.32			1.0	0.5	1.2	3.3
10	(E)-3-Hexen-1-ol	20.10			ND	ND	3.3	ND
11	Sabinene	21.80			Trace	ND	5.0	6.0
12	β -Myrcene	22.01			38.8	14.6	17.3	21.6
13	α -Terpinene	23.34			2.2	ND	ND	1.6
14	Dimethyl trisulphide	24.10			0.2	2.5	2.1	0.7
15	(E)-3-Hexen-1-ol acetate	24.29			2.0	1.6	38.6	18.5
16	1-8,Cineole	24.79			0.8	1.1	1.1	4.2
17	(E)-4,8-Dimethyl-1,3,7-nonatriene	26.77			0.3	0.1	1.9	2.2
18	Linalool	28.19			17.2	12.5	1.8	2.5
19	Isomyrcenol	28.85			0.6	2.3	ND	ND
20	α -Farnesene	39.17			15.9	31.7	14.4	7.5
21	1H-Indole	39.89			15.6	7.1	1.3	1.7
22	Unidentified Sesquiterpene	41.64			ND	1.0	ND	ND
Total peak area*					18.7	56.2	11.1	14.3

* Relative amount of total plant volatile organic compounds emitted in 24 h as determined by summing the peak areas for each of the compounds in the table. ND. Not detected.

volatile constituents present in the room used for the entrainment (Fig. 1b). These potential contaminants were seen at lower levels in the entrainment apparatus when no plant was present and included aliphatic and aromatic hydrocarbons, aldehydes and monoterpenes. Other contaminants from the analytical process included a number of siloxanes originating from the chromatographic column as well as adsorbent polymer breakdown products. When the presence of these contaminants was taken into consideration a total of 22 compounds could be identified as being uniquely attributable to the OSR plant material under investigation (Tables 1–3). An assessment of the reproducibility of the method with respect to the relative proportions of these unique compounds was undertaken by running two adsorbent lines in parallel. A comparison of the results indicated that there was good reproducibility, a representative example of the variation is shown in Table 4.

In the initial experiment the relative proportions of the VOCs emitted from the flowering raceme of a single plant were determined over the flowering period. Entrainment began when the flowering period commenced, at growth stage 4:10 (i.e. when 10% of flower buds on the raceme were open) and continued on until growth stage 4:6,5:25 (i.e. when 60% of flower buds on the raceme were open and 25% of the potential seed pods exceeded 2 cm in length). During this period 14 compounds were identified as being emitted by the flowering raceme (Table 1). The predominant products found over this period were the sesquiterpene α -farnesene, the monoterpene β -myrcene and the monoterpene alcohol linalool, which when combined accounted for between 59 and 74% (mean 66%) of the total unique volatiles emitted. As the raceme progressed through flowering only comparatively minor changes were observed in the relative proportions of the emitted compounds. For

Table 4. A representative example of the reproducibility of the method based on duplicate analyses of trapped volatile organic compounds. The example shown is of an intact living OSR (cultivar Tapidor) flowering raceme (growth stage 4:20, Table 1)

Peak number	Compound	% composition			Standard error	Coefficient of variance
		Data 1	Data 2	Mean		
3	3-Methyl-2-pentanone	3.5	3.8	3.65	0.15	4.1
4	<i>N</i> -Methylene ethanamine	0.9	0.7	0.8	0.1	12.5
7	3-Methyl-2-pentanol	2.1	1.6	1.85	0.25	13.5
9	α -Thugene	1.4	1.6	1.5	0.1	6.7
10	(E)-3-Hexen-1-ol	5.9	6.6	6.25	0.35	5.6
11	Sabinene	2.8	2.3	2.55	0.25	9.8
12	β -Myrcene	33.9	34.8	34.35	0.45	1.3
15	(E)-3-Hexen-1-ol acetate	2.8	3.8	3.3	0.5	15.2
18	Linalool	18.4	18.4	18.4	0	0
19	Isomyrcenol	5.8	6.1	5.95	0.15	2.5
20	α -Farnesene	22.3	20.3	21.3	1	4.7
Total peak area*		11.2	9.7	10.45	0.75	7.2

* Relative amount of total plant volatile organic compounds emitted in 24 h as determined by summing the peak areas for each of the compounds in the Table.

instance, between growth stages 4:10 and 4:20, decreases were seen in the amounts of the 'green leaf' volatiles (E)-3-hexen-1-ol acetate and (E)-3-hexen-1-ol. Additionally at growth stage 4:20, isomyrcenol was first detected at levels of around 6% and thereafter remained fairly constant. Dimethyl disulphide did not appear until growth stage 4:30 and in subsequent entrainments was found at levels of about 1% of the total volatiles emitted from the raceme. The fluctuations observed in the relative proportions of detected volatile compounds may not necessarily be solely linked to the stage of raceme maturity but may also reflect minor changes in environmental and ambient conditions although all available means were employed to minimize these effects.

Since human exposure to OSR derived volatiles would include those from all parts of the plant, the volatile compounds emitted from the entire aerial section of intact plants were also examined. A second series of entrainment experiments were undertaken with an individual plant from each of the autumn-sown cultivars (Tapidor, Falcon, Envol, Pasha) and the spring-sown cultivars (Forte and Comet).

The results for the autumn-sown double-low cultivar Tapidor (Table 2) indicated that the major volatile constituent was again the monoterpene β -myrcene, and with the exception of 3-hydroxy-2-butanone, (E)-3-hexen-1-ol, α -thugene and the unidentified sesquiterpene (peak number 22) all compounds previously

detected in the raceme entrainment (Table 1) were found. Three additional compounds were found namely 2-methyl propanenitrile, 1-8 cineole and an unidentified peak with a retention time of 18.05 min (peak number 8) but in total these accounted for less than 2% of the VOCs emanating from the plant. As with the flowering raceme, increasing plant maturity during flowering had no major effect on the type of compounds detected and also showed fluctuations in their relative amounts. However, at the pre-flowering growth stage (3:4), considerably less total volatiles were detected as indicated by comparing the total peak areas obtained at the different growth stages as shown in Table 2 (9.0 compared with 19.6 and 34.0 as the plant matured). In addition, only six volatiles were found to be emanating from the pre-flowering growth stage, with β -myrcene and (E)-3-hexen-1-ol acetate accounting for 83.7% of the total volatiles emitted.

A similar set of entrainments with a plant from another autumn-sown cultivar (Falcon) at one slightly later pre-flowering and two flowering growth stages equivalent to those examined with the Tapidor cultivar, revealed that the same general pattern of volatiles were emitted (Table 2). Three compounds were detected which although not detected in the whole cultivars Tapidor entrainment were found in the flowering raceme work (Table 1). These were 3-hydroxy-2-butanone, α -thugene and the unidentified sesquiterpene (peak number 22). Five additional compounds identified as thiocyanic acid

methyl ester, α -terpinene, dimethyl trisulphide, 1H-indole and the homomonoterpene (E)-4,8-dimethyl-1,3,7-nonatriene which had not been found in any of the cultivar Tapidor entrainments were also present at relatively low amounts. An increase in the total amount of volatile compounds detected during plant maturation was apparent when comparing the total peak areas obtained for each entrainment which was consistent with that seen in the cultivar Tapidor whole plant entrainment (Table 2). A third autumn-sown double-low cultivar (Envol) was also examined at two flowering stages and revealed the same series of volatile compounds previously identified with the relative amounts of each falling within the ranges previously found for the other two cultivars (Table 2).

Entrainment of the autumn-sown single-low cultivar Pasha revealed that 19 of the previously identified volatile compounds and no new compounds could be detected at two stages of flowering equivalent to those examined in the other cultivars (Table 3). The three compounds not detected were 3-methyl-2-butanone, (E)-3-hexen-1-ol and the unidentified compound (peak number 8). This was consistent with the results previously obtained with the double-low cultivars (Table 2), with the most abundant compounds detected being broadly the same, although slightly more 3-hydroxy-2-butanone and α -farnesene and less β -myrcene and (E)-3-hexen-1-ol acetate were detected in the more mature plant entrainment.

Entrainment of the spring-sown cultivars Forte and Comet at a growth stage equivalent to that of the most advanced flowering stages examined for the other cultivars once again showed that the same set of compounds were detected (Table 3). Only *N*-methylene ethenamine, isomyrcenol, the unidentified sesquiterpene (peak number 22) and the unidentified compound peak number 8 were not detected. The relative amounts of each compound were within the variation previously shown for the autumn-sown double-low cultivars (Table 2), except that in the cultivar Forte entrainment slightly more thiocyanic acid methyl ester and (E)-3-hexen-1-ol acetate were emitted.

The volatiles emitted from leaves of non-flowering stages of plants were also examined to determine whether these were different to the whole plant entrainments undertaken on pre-flowering plants. In these whole plant entrainments the plants were at stages just before flowering and thus included immature flower buds in various stages of development (growth stage 3.0–3.9). Leaf entrainments from several of the above cultivars of OSR consistently resulted in (E)-3-hexen-1-ol acetate and (E)-3-hexen-1-ol being the major constituents, at around 93% and 3–4% respectively.

Discussion

Cruciferous plants including OSR are characterized by high contents of sulphur-containing compounds and glucosinolates which can be metabolized to various biologically active volatile compounds especially when plants are damaged [4 and references therein]. In particular, isothiocyanates have been suggested to have the potential to cause toxic, non-specific reactions, as well as the induction of IgE antibody [1,10,11]. However, OSR also produces many other volatiles, some of which may be common to other plants [12]. Since OSR is extensively grown on arable land in dense monoculture it may be found that the levels of these common volatiles are much greater in close proximity to the crop than would normally be experienced, perhaps sufficient to act as irritants or allergens.

In this paper the dynamic headspace analysis method was used to detect the VOCs emitted from OSR plants during the flowering period. In addition, a comparison was made to the VOCs emitted at earlier growth stages in order to define specific VOCs that may play a role in the reported respiratory symptoms experienced by members of the public in close proximity to the crop at the time of flowering. A total of 22 VOCs were found to be emitted by OSR plants and overall the major constituents, which together accounted for 50–87% (mean 67.6%) of the total volatiles emitted in all the whole plant entrainments carried out during flowering were α -farnesene, β -myrcene, linalool and the 'green leaf' volatile (E)-3-hexen-1-ol acetate. Of these four compounds only linalool was not detected in plants at growth stages just before flowering commenced, when β -myrcene and (E)-3-hexen-1-ol acetate accounted for 75–84% of the total volatiles. There was a fourfold increase in the total quantity of volatiles emitted as plants progressed from this pre-flowering stage to the peak flowering stage. This was paralleled by an increase in the number of compounds detected from 6 to up to 20. With the exception of the characteristic 'green leaf' volatiles, (E)-3-hexen-1-ol acetate and (E)-3-hexen-1-ol, the compounds found in the flowering period or from whole plants with immature flower buds were either not detected or were present at very low levels when only leaves were entrained. This suggests that immature flower buds may be responsible for the relatively high levels of β -myrcene found in entrainments which included immature flower buds but no open flowers. However, unlike (E)-3-hexen-1-ol acetate, the actual level of β -myrcene emitted was found to substantially increase as the plant progressed into flowering (Table 2). Thus, of the major constituents emitted from OSR, the amounts of β -myrcene and α -farnesene were shown to increase in the flowering

period and linalool was only emitted from flowering plants. Of the other 17 compounds detected all but 2-methyl-propanenitrile were either emitted only during flowering or showed increases in the flowering period. Variable amounts of 2-methyl-propanenitrile were detected prior to and during flowering but it was detected at much lower levels in leaf entrainments. Overall, these findings confirm the changes in odour sensations commonly experienced from the crop at these different growth stages.

In some flowering plant entrainments, OSR plant volatiles that were generally present in low amounts showed marked increases, e.g. dimethyl disulphide, 3-methyl-2-pentanone, 3-hydroxy-2-butanone, sabinene and isomyrcenol. The reason for this variation may not be solely related to the growth stage of the plant since other as yet undefined influences may be acting. For instance, it has been reported that dimethyl disulphide may also be released from cruciferous plant material by fungal enzymes [13,14]. Such mechanisms may be important when decaying vegetative matter such as that resulting from dropped flower petals is present along with fungal pathogens. The determination of whether the predominant influence resulting in this variation was micro-environmental and stress related, or due to plant or cultivar differences, although of importance was not the main purpose of this set of experiments. All such causes of variation could be expected in fields to which members of the public are exposed. Since our experience to date suggests that it is technically very difficult to detect the volatile compounds present in ambient OSR field air, at present it is only possible to identify the range of compounds likely to be encountered from such work as reported here. These difficulties appear to result from an inability to adequately trap the VOCs, possibly because water vapour or VOCs from non-OSR sources interfere with the trapping efficiency of the polymeric adsorbent and can result in polymer breakdown. These problems are substantially reduced by our current method employing air purifying filters to purge pure air through plant entrainments.

The volatiles identified from the flowering raceme of cultivar Tapidor included many of those reported from our earlier work using cut racemes [4]. However, the relative proportions were considerably different particularly with respect to dimethyl disulphide, β -myrcene and α -farnesene. These differences may be due in part to the fact that in the work with cut racemes only Haysep-Q was used. This polymeric adsorbent fails to efficiently trap volatiles with relatively higher boiling points. This would be likely to result in an underestimation of compounds such as α -farnesene, consequently resulting in a relatively higher proportion of

dimethyl disulphide. By using both Tenax-TA followed by Haysep-Q adsorbent tubes in series it was possible to efficiently trap both high and low boiling point volatiles. In addition the damage caused to flowering racemes by being cut would be expected to alter the metabolic processes occurring in the raceme resulting in changes in the composition of VOCs being emitted [15,16]. Other workers have concentrated on identifying the VOCs emitted from OSR plants at the time of flowering. When our current results are compared with those [5,6], there is general agreement in the types of compound emitted but variation in their precise identity and their relative amounts, which may be due to cut plants being used. Unlike Evans and Allen-Williams [6] we did not detect any isothiocyanates (3-butenyl-; sec-butyl-; 4-pentenyl-) which presumably arose from the enzymic breakdown of glucosinolate. However, in our work with cut flower racemes [4] we too identified trace levels of 3-butenyl- and 4-pentenyl isothiocyanates. Furthermore, in entrainments of macerated OSR stem tissue we identified these and other isothiocyanates as well as nitriles as was also reported by Tollsten and Bergström [5]. This would appear to confirm previous reports that the products of glucosinolate breakdown are variable and probably dependent on both environmental factors and plant damage [17,18]. Although at least some of these compounds appear capable of inducing respiratory symptoms, their occurrence at all, or the levels released by intact OSR plants in fields according to our present work would be relatively low compared with some of the other compounds. We did confirm that dimethyl disulphide, 2-methyl-propanenitrile and thiocyanic methyl ester are commonly released by intact apparently undamaged plants at the time of flowering, these being classified as respiratory irritants [19].

The involvement of the other volatiles that we have detected in inducing irritation of the respiratory mucosa and conjunctiva remains uncertain due to the lack of comprehensive toxicological and exposure data for the individual compounds. Many of the compounds identified belong to chemical groups which are components of essential oils (monoterpenes and sesquiterpenes, aromatics, simple aliphatic alcohols, ketones and esters) used in perfumes [12]. Public exposure to other compounds (organic sulphides and glucosinolate breakdown products) could also be expected to be widespread due to their release during food preparation of cabbage and related species of cruciferae [10,11,20]. This does not, however, exclude the distinct possibility that individual compounds within these chemical groups might be more potent respiratory irritants, especially since many plant volatiles have evolved as feedant deterrent against insect

and herbivore attack and as defences against fungal or bacterial infection [17,21,22].

It is also worth noting that allergy to perfume ingredients does occur [23], that terpenes can induce macrophage and mast cell migration into broncho-alveolar lavage fluid [24] and that sesquiterpene-lactones are considered to be the agents involved in compositae dermatitis and in some photodermatitis reactions [25]. The pharmacological properties of sesquiterpene lactones as antimicrobial and antitumoural agents as well as their effects on vascular smooth muscle have also received attention [26–28]. Recent interest in alternative medicines has aroused greater research and use of plant essential oils and fragrances in aromatherapy which has suggested distinct biological activity for some component compounds [29]. As well as the compounds emitted directly from the plant it is also important to consider their stability and products of biodegradation in air with or without contact with particulate or gaseous air pollutants. For example, the maximum life of terpenes in air is suggested as 10 h and may be influenced by ozone levels [30]. Myrcene has been reported to breakdown to acetaldehyde, formaldehyde and acetone under certain conditions [31], possible mechanisms of formaldehyde-induced discomfort in the upper respiratory tract having been investigated [32]. One further point of relevance is that it has been shown that relatively non-reactive VOCs have pungency thresholds in humans and that it is possible that the presence in air of subthreshold levels of a wide array of VOCs may evoke pungent sensations by a cumulative process [33]. These sensations appear to result from stimulation of the trigeminal, glossopharyngeal, and vagus nerves (cranial nerves V, IX and X respectively), and could be consistent with at least some of the symptoms.

From this work we have identified a number of volatile compounds emitted from intact growing OSR plants and have indicated those that appear to be specific to, or increased over the flowering period of this crop. We found only minor differences between the six cultivars of OSR which we investigated. This suggests that if the volatiles are involved in an allergic or irritant response in humans then exposure to the volatiles could be expected to be the same now as when the crop first began to become more common. Secondly, since the spring sown cultivars also showed that similar volatiles were emitted, then the latest agricultural trend towards increased acreage of this crop, which flowers at a later time than the autumn sown crop, will result in an increased period over which public exposure could occur (April–July). However, the possible role of the identified volatile compounds in the link between OSR and respiratory mucosa and conjunctiva irritation is at present unclear

but their identification represents an important step in future work to define their allergenic or irritant potential.

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