

Identification of volatile organic compounds emitted in the field by oilseed rape (*Brassica napus* ssp. *oleifera*) over the growing season

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Summary

Background Oilseed rape has been associated by rural dwellers with seasonal symptoms, such as sneezing, coughing, headache and eye irritation, during its flowering season, for a number of years. This study was performed to identify the volatile chemicals emitted from oilseed rape in the field.

Objective The objective of this study was to establish which volatile chemicals may be causative factors of oilseed rape allergy/toxicity.

Methods The volatile organic compounds were sampled over the flowering period using a modified entrainment technique for headspace analysis under field conditions. These volatiles were then identified using thermal desorption-gas chromatography-mass spectrometry.

Results The major constituents identified were the monoterpenes limonene, sabinene, β -myrcene, and *cis*-3-hexen-1-ol acetate, a 'green leaf' volatile. The minor constituents included monoterpenes, sesquiterpenes, short chain aldehydes and ketones, other 'green leaf' volatiles and organic sulphides including the respiratory irritant, dimethyl disulphide.

Conclusions This report highlights the diversity of volatile chemicals emitted by oilseed rape and confirms field emissions to be broadly similar to those found previously in laboratory studies. A review is carried out on the scientific literature already published on oilseed rape flower headspace analysis.

Keywords: allergy, gas chromatography-mass spectrometry, oilseed rape, thermal desorption, flower volatiles, volatile organic compounds

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Introduction

Oilseed rape [OSR, *Brassica napus*] is an increasingly important plant in the European economy due to the many uses for its main commercial product, vegetable oil. The crop has been identified as the most appropriate oilseed crop for the UK as it is well adapted to the northern European environment, production systems are well established and the plant species itself is easily manipulated genetically [1]. It is possible that further uses for the oil produced by this crop will be found in the future, giving it even greater

influence on the British and European economies. The crop has one very significant disadvantage however, in that it is believed to be responsible for allergic/irritant effects in a significant number of the population. Parratt *et al.* [2] have indicated from human studies that this crop was indeed detrimental to the health of some members of the public, especially at the mid-flowering stage of development.

It is in the interest of the public, the farming community and the environment in general that research be conducted to isolate the causative agents responsible for these allergic/irritant effects on humans. Possible agents include the pollen produced by the flowers, the fungi which infect the crop and the agrochemicals used to protect it, but at present interest is focused on the volatiles emitted by the crop,

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particularly at the flowering stage. Previous investigations have been conducted on the OSR volatile organic compounds [VOCs] produced under laboratory conditions [3–5], but this investigation is principally concerned with the characterization of VOCs in the environment. These studies were conducted in the field using crops grown by local farmers.

Methods and materials

Plants

The plants used for sampling in this experiment were selected from fields of OSR grown in the Tayside area during the 1994 and 1995 seasons. The cultivars sampled included Express, Rocket, Libravo, Cobra, Falcon [autumn sown or winter oilseed rape, WOSR] and Forte [spring sown or spring oilseed rape, SOSR]. The growth stages of these crops were assessed visually and expressed using a code based upon that developed by Sylvester-Bradley [6] and described in brief by Butcher *et al.* [4].

Sampling of volatiles

The VOCs produced by the plants were collected and characterized by dynamic headspace analysis. The field entrainment technique used was developed from the laboratory entrainment technique described in Butcher *et al.* [4]. This involved covering the top half of the selected plant with a 36 inch×24 inch Rylsan II forensic nylon bag [Keygrove Marketing] and tying it tightly around the middle of the plant with string, to minimize air movement. An air supply line was inserted into the bag, together with stainless steel sampling tubes containing Tenax-TA [60–80 mesh]. These were placed in close proximity to the flowers, and the bag entrance holes sealed with non-active tape. Purified air was supplied to the bag by a diaphragm pump [SKC Inc.] via two inline filters, [molecular sieve 5 A followed by activated charcoal]. Samples of the atmosphere within the bag were entrained by sucking air through the Tenax TA sampling tubes at 170 mL/min. The sampling time varied between 6 and 8 h for most of the cultivars sampled. However, the sampling of cv. Forte was more stringent in that samples were taken weekly using the same plant, while the other cultivars involved sampling randomly selected plants. The sampling time for this cultivar was also rigorously standardized at 4 h, to aid the interpretation of results obtained. Details of materials, fittings and associated methodology are described in previous publications [4,5].

The background volatiles emanating from the entrainment apparatus itself were determined by sampling an empty bag in the same manner.

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 Robertson *et al.* [5]. The Tenax TA sample tubes were desorbed in a stream of helium over a period of 15 min at a temperature of 200 °C. Analysis of the data provided by the GC-MS was conducted using VG Lab base computer software, the relative proportions of each VOC having been calculated from the peak area of the Total Ion Current [TIC] signal on the mass spectrometer.

Results

Tables 1 and 2 indicate the compounds identified from the field entrainments of the six OSR cultivars. Their relative concentrations were calculated from the peak area of the TIC signal recorded on the mass spectrometer.

Table 1 represents the data acquired from the cv. Forte SOSR field entrainments. This cultivar was sampled once a week for 6 weeks covering the period just before flowering [GS3.7/3.8, 21 June] to the seed development stage [GS 6.3, 26 July]. This shows that monoterpenes accounted for 60–90% of the total OSR VOC's identified. Of these, the monoterpenes, β -myrcene, sabinene and limonene, were most abundant throughout the series of entrainments, with limonene possessing the highest concentration and increasing its proportion significantly in the last entrainment [26 July]. The other monoterpenes present at proportionally lower levels did not appear to vary much over the sampling period.

Two sesquiterpenes, β -caryophyllene and α -farnesene, were also identified. The β -caryophyllene was only found in the first entrainment [21 June] while the α -farnesene was present at low levels throughout the growing season in all samples.

The non-terpenoid components, such as dimethyl disulphide, benzaldehyde, 3-methyl-2-butanone, N-methylene ethanamine, acetaldehyde, acetone and *cis*-3-hexen-1-ol, were also found at low levels and showed little variation in concentration. The 'green leaf' volatile, *cis*-3-hexen-1-ol acetate, was the most abundant of the non-terpene compounds and reached a proportion greater than 25% of the total OSR VOCs identified on two occasions. These growth stages were just before flowering [GS 3.7/3.8, 21 June] and at the end of flowering [GS4.9/5.0, 12 July].

Table 2 shows the VOCs originating from a wider variety of WOSR cultivars at their mid-flowering stage and it is clear that β -myrcene was the most abundant OSR VOC in all entrainments. The other major components do show variation in their abundance for different cultivars. The

Table 1. Spring oilseed rape cv. Forte field entrainments between 21 June and 26 July 1995

Peak Number	Compound	Retention Time [min]	Growth stage					
			3.7/3.8	4.1/4.2	4.4/4.5	4.9/5.0	6.1/6.2	6.3/6.3
1	Acetaldehyde	5.13	1.7	tr	tr	2.5	1.0	tr
2	Acetone	6.57	1.7	0.8	1.0	2.3	1.0	tr
3	3-Methyl-2-Butanone	11.25	tr	tr	tr	tr	tr	tr
4	Dimethyl disulphide	13.86	1.4	0.6	3.5	2.2	3.3	tr
5	N-Methylene ethanamine	15.07	ND	ND	ND	ND	ND	ND
6	α -Thujene	18.78	3.6	4.7	5.7	5.7	6.6	2.1
7	α -Pinene	19.21	3.4	ND	ND	8.0	3.4	tr
8	<i>cis</i> -3-Hexen-1-ol	19.46	2.7	tr	1.2	5.9	1.3	tr
9	Sabinene	21.25	15.5	26.3	18.0	12.7	25.2	9.1
10	β -Myrcene	21.43	5.9	14.4	10.8	7.2	8.8	4.7
11	Δ -3-Carene	22.30	1.1	tr	ND	1.5	tr	tr
12	Unidentified monoterpene	22.41	ND	tr	ND	tr	tr	tr
13	α -Terpinene	22.88	tr	1.4	2.6	1.2	1.1	tr
14	Limonene	23.23	24.3	41.8	29.0	15.6	27.5	75.1
15	<i>cis</i> -3-Hexen-1-ol-Acetate	23.52	27.7	3.9	5.1	27.0	7.3	2.4
16	<i>trans</i> - β -Ocimene	23.73	tr	1.0	2.2	tr	1.0	tr
17	1,8-Cineole	24.10	4.8	3.3	4.8	3.1	6.5	1.7
18	γ -Terpinene	24.37	ND	ND	9.5	ND	4.4	1.7
19	Benzaldehyde	24.62	1.7	tr	2.5	1.7	tr	tr
20	α -Terpinolene	27.42	tr	tr	tr	1.2	tr	tr
21	Linalool	27.58	1.2	tr	tr	ND	ND	tr
22	β -Caryophyllene	37.15	1.2	ND	ND	ND	ND	ND
23	α -Farnesene	38.30	tr	tr	3.0	1.6	1.0	tr

The relative VOC concentrations were calculated as a percentage of peak area of the TIC signal recorded on the mass spectrometer.

tr = Trace [values of less than 1%].

ND = Not Detected.

cultivars Cobra, Libravo and Express are similar to cv. Forte [SOSR] with β -myrcene, limonene and sabinene as major VOCs. In cv. Falcon, the monoterpene alcohol, linalool, is the second most abundant VOC followed by *cis*-hexen-1-ol acetate. The cultivar Rocket has limonene as the second most abundant VOC, followed by linalool and sabinene.

Other entrainments were carried out on these cultivars at the early and late flowering stages. These results showed that β -myrcene, sabinene and limonene were the most abundant VOCs, with the exception of a few samples which exhibited elevated levels of *cis*-3-hexen-1-ol acetate. In these late flowering entrainments, in common with the cv. Forte, limonene generally showed a large increase in concentration, making it the most abundant VOC in most cases at this growth stage. In addition, the levels of linalool in all of these entrainments consistently showed a significant increase at the mid-flowering stage.

Discussion

The possible allergic/irritant nature of OSR plants has been

attributed to several groups of particulates or chemicals, pollen, fungal infections and VOCs. Previous research has shown that relatively few people are affected by pollen and studies conducted on two of the common fungal infections of OSR, *Botrytis* and *Alternaria*, appear to create few problems [Galloway, pers. comm.]. While the number of people affected by plant derived VOCs has not been quantified, studies of similar chemicals have shown that they can have toxic or allergic effects in other circumstances [7–9].

All of the monoterpenes predominant in the VOC profile of OSR are also produced by other plants in higher quantities [10] and are widely found in perfumes and fragrances. These chemicals are responsible for allergic/irritant effect in some humans [11,12], but due to the ubiquitous nature of these compounds, it seems unlikely that they are the cause of this problem. However, OSR plants are grown in dense monoculture and this may lead to higher volatile concentrations of the minor non-terpenoid components in close proximity to the crop. These elevated levels may be

Table 2. Compounds identified from winter oilseed rape cultivar entrainments at mid flowering [GS 4.4/4.7]

Peak Number	Compound	Retention Time [min]	WOSR Variety				
			Falcon	Cobra	Libravo	Rocket	Express
1	Acetaldehyde	5.13	tr	tr	tr	tr	tr
2	Acetone	6.57	2.7	4.4	4.8	1.8	1.3
3	3-Methyl-2-Butanone	11.25	2.6	4.0	3.0	2.8	3.0
3	Dimethyl disulphide	13.86	tr	tr	1.7	tr	tr
5	N-Methylene ethanamine	15.07	tr	tr	tr	tr	tr
4	α -Thujene	18.78	2.4	4.5	3.4	2.3	4.9
5	α -Pinene	19.21	2.8	4.1	2.7	2.3	1.6
6	<i>cis</i> -3-Hexen-1-ol	19.46	4.9	1.4	tr	1.8	1.4
7	Sabinene	21.25	4.5	16.0	15.1	8.9	12.4
8	β -Myrcene	21.43	22.4	31.8	36.4	43.1	39.5
9	Δ -3-Carene	22.30	ND	tr	ND	tr	tr
10	Unidentified monoterpene	22.41	ND	tr	ND	tr	tr
11	α -Terpinene	22.88	tr	tr	tr	tr	1.0
12	Limonene	23.23	7.6	13.6	12.6	10.3	13.8
13	<i>cis</i> -3-Hexen-1-ol Acetate	23.52	13.3	4.0	1.1	5.3	4.1
14	<i>trans</i> - β -Ocimene	23.73	2.4	1.5	tr	2.8	2.0
15	1,8-Cineole	24.10	1.9	1.0	1.3	tr	tr
16	γ -Terpinene	24.37	ND	2.5	1.1	3.3	tr
17	Benzaldehyde	24.62	9.7	6.0	7.7	1.7	3.8
18	α -Terpinolene	25.42	tr	tr	tr	tr	tr
19	Linalool	27.58	19.9	3.3	5.7	10.0	8.1
20	β -Caryophyllene	37.15	tr	ND	ND	ND	ND
21	α -Farnesene	38.30	tr	tr	0.7	tr	tr

The relative VOC concentrations were calculated as a percentage of peak area of the TIC signal recorded on the mass spectrometer.

tr = Trace [values of less than 1%].

ND = Not Detected.

responsible for allergic/irritant reactions in humans. In common with other cruciferous plants, OSR produces a number of compounds from glucosinolate metabolism, especially when the plant is damaged [5]. The isothiocyanates are one such group of compounds which have been suggested as causing toxic, non-specific reactions, as well as the induction of IgE antibody [10–12]. These compounds were not detected in this study but may well be present at trace levels, particularly if the plants are damaged by wind, insects, fungal infection, animals and humans.

Unlike previous laboratory experiments, this was the first attempt to sample OSR volatiles from a variety of cultivars in a field situation over the flowering season. The results confirm that the volatiles emitted by the growing crop are similar in many respects to those found in previous laboratory studies and should influence future experimental approaches to OSR field VOC monitoring. Only chemicals previously documented as originating from plants were included in the study. This allowed the elimination of artefacts from the chemical analyses.

A consistent pattern emerges from these field entrainments, showing monoterpenes as the major VOCs emitted. The main monoterpenes common to most of the varieties examined were β -myrcene, sabinene and limonene. Variation in the relative levels of these compounds occurred throughout the growing season and with different cultivars, and may reflect the variation in the composition of essential oils in herb species and cultivars over the growing season [13,14]. Furthermore, it has been demonstrated that variation in the soil composition [15], the time of day at which the sample was taken [3] and weather conditions can alter the volatile terpene profile, so the variation shown here may well be due to some if not all of these factors. This may help explain why some sufferers of seasonal allergy/irritant problems have reactions that can vary from day to day and possibly from hour to hour.

To minimize the variation in VOC composition caused by slight deviations in the sampling protocol, a more rigorous system was used for one experiment involving cv. Forte [SOSR], which differed from the WOSR cultivars in that

limonene was the most abundant VOC throughout this series. In this extended time course, it is also noticeable that *cis*-3-hexen-1-ol acetate was found at a high relative proportion before [GS 3.7/3.8, 21 June] and at the end of the flowering period [4.9/5.7, 11 July]. These high concentrations of *cis*-3-hexen-1-ol acetate may punctuate the period where the highest overall atmospheric concentration of VOCs occurs, the mid-flowering stage, as the apparent decrease in its relative concentration may be due to an increase in chemicals such as the monoterpenes and so an increase in the overall atmospheric concentration of VOCs. The last two entrainments of this series show an increase in the relative levels of the major monoterpenes again, but this may not necessarily indicate an increase in the overall VOC concentration. Both these entrainments occurred at growth stages [GS 6.1/6.2, 18 July and GS6.3, 26 July], where leaf and then stem senescence is taking place, as the plant gives maximum input to the development of its seeds. As leaves are being shed, it is likely that the atmospheric

concentration of *cis*-3-hexen-1-ol acetate will decrease. These trends in the concentration of *cis*-3-hexen-1-ol acetate are mirrored by another 'green leaf' chemical reported here, *cis*-3-hexen-1-ol. Although this volatile occurs at a lower relative concentration, it also shows slightly elevated concentrations in the entrainments preceding and at the end of the flowering period. This may indicate that this crop has a peak in the emission of VOC's, especially monoterpenes, during the flowering period. As Parratt *et al.* [2] identified that maximum symptoms were experienced at peak flowering of OSR, it would therefore be reasonable to look at factors which also achieve their highest levels at or just prior to peak flowering. Accordingly, these chemicals should be given careful consideration as factors capable of causing ill health.

A comparison of the data obtained in previous papers investigating the dynamic headspace VOCs of OSR is shown in Table 3.

An examination of the results obtained by Robertson *et al.*

Table 3. Relative concentrations of VOC's emitted from OSR from previous literature

Compound	Cobra ¹	Libravo ¹	Tapidor ¹	Pasha ¹	Bienvenue ¹	Tapidor ²	Falcon ²	Envol ² SOSR	<i>B. napus</i> ³	<i>B. napus</i> ⁴
Acetaldehyde	1.37	2.99	1.18	0.39	tr	ND	ND	ND	ND	ND
Acetone	3.28	5.34	6.01	3.86	7.50	ND	ND	ND	ND	ND
3-Methyl-2-butanone	7.90	9.58	15.84	11.06	13.05	ND	ND	ND	ND	ND
Dimethyl disulphide	30.38	20.26	19.02	32.89	17.04	4.32	7.12	1.39	ND	ND
N-Methylene ethanamine	0.80	5.60	5.48	1.04	3.47	1.08	0.34	1.16	ND	ND
3-Hydroxy-2-butanone	44.17	17.47	25.96	27.20	17.04	ND	7.58	7.65	ND	ND
α -Thujene	tr	tr	tr	0.35	tr	ND	5.74	3.13	ND	5.00
α -Pinene	1.66	2.80	3.40	7.18	11.15	ND	ND	ND	2.23	11.05
<i>cis</i> -3-Hexen-1-ol	0.60	tr	tr	0.60	tr	ND	ND	0.46	ND	ND
Sabinene	ND	ND	ND	ND	ND	ND	4.48	8.57	11.37	13.68
β -Myrcene	2.73	16.45	9.23	5.32	15.81	56.64	49.02	33.37	13.60	31.05
Δ -3-Carene	tr	1.81	tr	1.85	1.90	ND	ND	ND	ND	ND
Unidentified monoterpene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
α -Terpinene	ND	ND	ND	ND	ND	ND	5.51	ND	ND	ND
Limonene	0.68	ND	1.91	3.54	3.54	ND	ND	17.35	17.35	14.47
Dimethyl Trisulphide	5.32	4.17	7.52	4.30	4.03	ND	0.69	ND	ND	ND
<i>cis</i> -3-Hexen-1-ol Acetate	ND	4.96	ND	ND	ND	8.75	5.17	ND	ND	ND
<i>trans</i> - β -Ocimene	tr	2.51	1.58	2.80	2.80	ND	ND	ND	2.70	ND
1,8-Cineole	ND	ND	ND	ND	ND	ND	5.17	2.90	3.75	4.47
γ -Terpinene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzaldehyde	1.11	3.91	1.94	2.18	2.65	ND	ND	ND	ND	ND
α -Terpinolene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Linalool	tr	2.13	0.90	0.79	tr	4.00	7.92	12.86	3.75	7.63
β -Caryophyllene	ND	ND	ND	ND	ND	ND	ND	ND	2.23	ND
α -Farnesene	ND	ND	ND	ND	ND	25.19	1.26	15.76	45.60	12.63

¹Robertson *et al.* [1993], ²Butcher *et al.* [1994], ³Tollston & Bergström [1988], ⁴Jackobsen *et al.* [1994]. Only compounds similar to those identified as originating from OSR in this study were used in this table. The relative concentrations being recalculated as a percentage of those VOC's selected.

[5] shows that the major VOCs from the five different varieties were non-terpenoid, these compounds being dimethyl disulphide and 3-hydroxy-2-butanone. This is in stark contrast to the results obtained in this field study, as dimethyl disulphide was found only at low concentrations and no 3-hydroxy-2-butanone was detected at all. Dimethyl trisulphide was also found at low concentrations by Robertson *et al.* [5]. This chemical was not detected in the present study and, of the literature examined, only Butcher *et al.* [4] detected this compound, and then at a low concentration and in only one of the cultivars examined. It was noticeable that of the monoterpenes detected by Robertson *et al.* [5], β -myrcene was the most abundant in most cases.

The studies of Butcher *et al.* [4] indicate that terpenes were the major VOCs, with β -myrcene, α -farnesene and linalool as the major components. Of the non-terpenoid compounds, dimethyl disulphide, 3-hydroxy-2-butanone and *cis*-3-hexen-1-ol acetate were found at reasonably high concentrations.

The studies conducted by Tollston and Bergström [16] on an unidentified SOSR cultivar are the most similar to those found in this work, as limonene, sabinene and β -myrcene were the most abundant monoterpenes. These findings parallel those in the cv. Forte [SOSR] in this study. The only major difference was in the higher levels of α -farnesene detected by Tollston and Bergström, making it the most abundant VOC. Jakobsen *et al.* [3] also obtained similar results, from the pooled data on three SOSR cultivars, although in this case the level of α -farnesene was lower, making β -myrcene the most abundant VOC.

Table 3 indicates the large variation in the perceived VOC composition from each of these studies. This may well be due to differences in sampling techniques, the porous polymer used, the technique used to extract the volatiles from the porous polymer, sampling time and the preparation of the OSR plant to facilitate the entrainment. The sampling time used varied from 3 to 96 h. In this study, the sampling period was between 4 and 8 h, to minimize problems caused by moisture being entrained into the Tenax porous polymer, leading to the production of artefacts in the GC-MS analysis. The result of studies conducted by Surburg *et al.* [17] suggest that it may be more representative to sample over a longer period of time, to permit the presence of larger, slightly less volatile chemicals, such as the sesquiterpene α -farnesene, to be determined. However, there is the problem of polymer breakthrough occurring during long sampling periods, resulting in chemicals of lower volatility being artificially concentrated while the more volatile compounds may be lost. Different porous polymers were also used in these reports. Robertson *et al.* [5] used Haysep Q, Tenax TA was used in the present study and by Butcher *et al.* [4] who also used Haysep Q, whereas both Tollston and Bergström [16]

and Jakobsen *et al.* [3] used Poropak Q. Each of these porous polymers has different properties and so will vary in its trapping efficiency for different chemical compounds. For the extraction of the volatile chemicals from these porous polymers, various techniques were again used. Some studies employed solvent elution to liberate the volatiles, this technique requiring the reconcentration of the sample by evaporation of the solvent where it was likely that some of the more volatile chemicals would be lost. Other studies, such as this one, employed thermal desorption which was likely to create artefacts due to chemical reactions taking place as a result of the heat applied to the porous polymer, especially if water was present. Finally the variation in the treatment of the plant will also lead to changes in the VOCs identified. Robertson *et al.* [5] used excised flower heads which were placed in the sampling chamber. This may explain the high concentration of dimethyl disulphide and dimethyl trisulphide, as these chemicals are naturally produced in a damaged *Brassica napus* plant. Mookherjee *et al.* [18] also demonstrated that prominent changes in VOCs occur rapidly when flowers are detached from the plant. Tollston and Bergström [16] used cut plants, in this case at soil level, and placed in water, while Jakobsen *et al.* [3] used intact plants. All these entrainments were carried out in a laboratory environment and were not subject to field conditions leading to differences in the entrainments conducted in this study.

The results of this work detail the main VOCs emitted by the growing crop in the field and their relative concentrations over the flowering season, using a modified sampling protocol. The VOCs identified in this study were found to be similar to those in other studies. This confirms the candidate VOCs, identified in previous laboratory studies, for clinical investigation. However, the possible effects of minor VOC components cannot be excluded and this may warrant further research to assess the strategy for sampling and quantification of these headspace VOCs accurately.

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