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VOLATILE CONSTITUENTS FROM CAULIFLOWER AND OTHER CRUCIFERS

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Key Word Index—*Brassica oleracea* var. *botrytis*; cauliflower; *B. rapa* ssp. *rapa*; turnip; *B. juncea*; mustard; *Raphanus sativus*; radish; *Cheiranthus cheiri*; wallflower; Cruciferae; volatiles; allyl isothiocyanate; *cis*-hex-3-enyl acetate.

Abstract—Vapour emanating from intact turnip and radish plants contained small amounts of only one component, probably hexenyl acetate, distinguishable from background vapours. From disrupted leaf tissues of cauliflower, turnip, radish and wallflower at 30°, the predominant vapour component was *cis*-hex-3-enyl acetate, whereas allyl isothiocyanate was the chief component from *Brassica juncea*. Other minor components were also identified. Apart from isothiocyanates, the only sulphur-containing component identified from these crucifers was dimethyl disulphide which was detected only from cauliflower, particularly in vapour from disrupted curd tissue.

INTRODUCTION

Chemicals associated with cruciferous host-plants of the cabbage root fly (*Erioischia brassicae* (Bouché)) determine, at least in part, the egg-laying behaviour of this insect [1–3]. The gravid female fly has been observed to congregate on the upwind side when confined in a cage outdoors downwind of, but near to, a plot of brassicas [3]. The proportion of flies responding in this way declined with increasing distance from the crop up to 30 m but at greater distances, or upwind of the crop, they did not respond. These observations are consistent with the mature female flies moving upwind in response to wind-borne odours emanating from the host-plants and studies were undertaken to detect and identify in the vapour from certain cruciferous host-plants of the cabbage root fly any components which might attract the pest or otherwise influence its behaviour. Vapours from intact plants and from disrupted tissues were analysed as a complementary study to that of Cole [4].

RESULTS

Intact plants

Vapour samples from groups of intact turnip and radish plants contained only one main component clearly detectable by GLC at a concentration of about 40 ng/l. in the atmosphere of the collecting box. The MS obtained with the amounts of this component available did not include the M^+ but was otherwise similar to the published spectra [5] of hexenyl acetate isomers (prominent ions at *m/e* 82, 67, 43) and to that of synthetic hexenyl acetate measured under similar conditions. GLC analysis was also consistent with this identification, synthetic hexenyl acetate in dilute aqueous solution having a R_t of 16.5 ± 0.5 min compared with 16.4 min for the main component in a very dilute aqueous sample of trapped vapour from the intact plants (Table 1). The very small amounts of the component collected precluded further confirmation of its identity or identification of the isomer present. Assuming that the plant vapour was evenly distributed in the collecting box, the approximate mean rate of release of hexenyl acetate from turnip or radish plants necessary to establish a concentration of 40 ng/l. was about 6 ng/plant/min, corresponding to *ca* 0.3 ng/g plant tissue or 20 pg/cm² leaf area/min.

GLC analysis of vapour samples from intact cauliflower and wallflower also indicated the presence of hexenyl acetate but the amount was only about 15% of that obtained from turnip. Vapour samples from *B. juncea* could not be distinguished from samples of background air which always contained several unidentified components associated with the plant container, the compost mixture and the collection apparatus. These background components were usually present at concentrations of about 0.005–0.010 μg/l. and could have obscured any minor components emanating from the plants.

Disrupted tissue

Much larger amounts of vapour were obtained when leaf tissues from the five crucifers were cut into small pieces and the vapour accumulating in 30 min at 30° was collected (Table 1). The major component from cauliflower, turnip, radish and wallflower was identified by GLC and MS as *cis*-hex-3-enyl acetate. However this compound was only a minor component in the vapour from *B. juncea*, the major component from the leaves of this species being allyl isothiocyanate. Minor components from *B. juncea* vapour included allyl cyanide and also a component tentatively identified by MS as prop-1-enyl isothiocyanate which is isomeric with allyl isothiocyanate. However, since both isomers appeared to be present in synthetic allyl isothiocyanate obtained

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Table 1. Concentrations of components detected in vapours from five crucifers

Component	GLC R_t (min)	Concentration (μg/l.) Cauliflower	Turnip	Mustard	Radish	Wallflower
Intact plants						
hexenyl acetate isomer (aqueous)	16.5	0.006	0.040*	—	0.040*	0.006
Disrupted leaf tissues						
acetone, methanol, ethanol, pentan-3-one	6.1–8.4	33*	19	37	30	40
dimethyl disulphide	10.7	< 1*	—	—	—	—
ethyl cyanide	11.6	—	5*	—	—	—
hexane-3,4-dione (?) ¶	14.4	3†	3	2	2	4
allyl cyanide	14.9	—	—	20*	—	—
a pentenol (?)¶	17.0	8†	4†	<1	1	2
sec-butyl isothiocyanate	18.6	—	<1*	14*	—	—
hexyl acetate	18.9	4*	2*	4*	2*	3
cis-hex-3-enyl acetate	20.4	330*‡	320*	58*	320*	81*
allyl isothiocyanate	21.5	—	—	1400*	1‖	1
cis-hex-3-en-1-ol	22.7	7*	15†	27	6†	6
prop-1-enyl isothiocyanate (?)¶	25.3	—	1‖	65†	—	—

R_t for the intact plant component was modified by water of transpiration (refer to experimental procedure). R_t for other synthetic and natural components differed by less than 0.2 min when analysed in similar conditions.

Results were based mainly on GLC analysis and identifications were confirmed as follows: *unequivocal MS identification by comparison with published spectra [5] and/or synthetic chemicals; †tentative MS identification; ‡spectra were consistent only with the component being both a straight chain 3-isomer (PMR) and a *cis*-isomer (IR); ‖GLC identification inconclusive; ¶GLC retention times not available for authentic compounds; —none detectable; the limit of detection was 3 ng/l. for intact plants and 0.5 μg/l. for disrupted tissue.

commerically, the prop-1-enyl isomer may have been either a true component or an artifact of the GLC analysis. Other components identified by MS included ethyl cyanide in vapour from disrupted turnip leaves and a trace of dimethyl disulphide from cauliflower. No other sulphides were detected in vapour from disrupted leaves of any other species examined.

To investigate further the unexpectedly low concentrations of sulphides and isothiocyanates present in the leaf vapours, the components in vapours from disrupted root, stem or leaf tissues, or from the uppermost 30 mm of cauliflower plants (including any curd present) were analysed (Table 2). Dimethyl disulphide was detected at greatest concentrations in the vapour from disrupted curd tissue and also at lower concentrations from the uppermost portions of young plants and from the stems of older plants. Very little could be detected from any leaf or root tissue. A moderate amount of allyl isothiocyanate was detected in vapour from roots of 14-week-old glasshouse-grown cauliflower plants, but only traces were found in vapour from younger plants. By comparison, the concentrations of allyl isothiocyanate in vapours from roots of mature outdoor-grown plants were highly variable, ranging from 〈1–〉 1000 μg/l. The reason for such large variations in otherwise apparently similar plants is not known. Almost all of the *cis*-hex-3-enyl acetate in the glasshouse grown plants was associated with leaf-tissue, particularly the small leaves surrounding the immature curds of young plants.

DISCUSSION

Bailey *et al.* [6] found that the major volatile constituents from finely disrupted cabbage tissue at 60° con-

Table 2. Concentrations of components detected in vapour from disrupted tissues from different parts of 9 and 14 week old cauliflowers

	Concentration (μg/l.) Plants 9 weeks old				Plants 14 weeks old			
	Top*	Leaf	Stem	Root	Top*†	Leaf	Stem†	Root
Acetone, methanol, ethanol, pentan-3-one	24	31	21	44	28	20	18	25
Dimethyl disulphide	3.7	<0.1	0.9	0.9	25	0.2	4.6	0.3
Hexane-3,4-dione (?)	0.2	0.2	0.2	0.5	0.4	0.2	0.5	—
Allyl cyanide	—	—	—	—	—	—	—	0.5
A pentenol (?)	0.8	0.9	—	—	—	1.0	—	—
Hexyl acetate	2.2	0.4	0.3	—	0.2	0.2	0.1	—
cis-hex-3-enyl acetate	84	66	1.6	<0.1	0.5	59	0.7	0.1
Allyl isothiocyanate	0.1	<0.1	0.2	<0.1	0.3	<0.1	<0.1	3.8
cis-hex-3-en-1-ol	0.3	0.5	0.7	—	2.4	0.4	0.6	—

Identifications were based on GLC R_ts only

* The "Top" samples were of the uppermost 30 mm of the plant including any curd present. † Means of 2 samples only; all other results represent means of 3 samples.

tained sulphur and they identified fifteen mono-, di- and tri-sulphides as well as five isothiocyanates. MacCleod and MacCleod [7, 8] studied the volatiles from boiling cabbage and identified seven sulphur-containing components accounting for 37–43% of the total vapour. From boiling cauliflower tissues they also reported [8] nine sulphur-containing compounds representing 34% of the total vapour but these were dominated by dimethyl sulphide and allyl isothiocyanate which accounted for 26.5 and 4.5% respectively. In these studies [6–8] the disrupted tissues were held at relatively high temperatures while the volatiles were formed and evolved. At the more natural temperature of 30° for 30 min employed for the present work only low concentrations of volatile sulphides and isothiocyanates were usually encountered. *B. juncea* tissue was exceptional in yielding a large amount of allyl isothiocyanate as well as other isothiocyanates, suggesting that the appropriate thioglucosidases or glucosinolate precursors [9], or both, were more readily available in this species than in the other four examined. *sec*-Butyl isothiocyanate, which also occurred in leaf vapour from *B. juncea*, and to a very minor extent in vapour from turnip, has been detected in seeds of several *Brassica* species [10], but the occurrence of the tentatively identified prop-1-enyl isothiocyanate has apparently not been reported previously. Although but-3-enyl isothiocyanate has been detected in *B. juncea* seeds originating from certain geographical locations [11], it was not detected in the present study. The presence of *cis*-hex-3-enyl acetate in vapour from disrupted leaf tissue of all the crucifers tested, and as the major component in four species, was unexpected as it has apparently not been identified in other studies of volatiles from crucifers [6–8, 12]. The related component, *cis*-hex-3-en-1-ol, was also present at low concentrations in all the crucifers examined, but *trans*-hex-2-enal was not detected in any.

Although isothiocyanates have been generally believed to be the principal characteristic vapours emanating from intact cruciferous plants [13], any isothiocyanate vapour produced from intact plants in the present study was at concentrations below the limit of detection. Field experiments have shown that coloured traps with a source of synthetic allyl isothiocyanate vapour can be more attractive to cabbage root flies than coloured traps alone [14, 15]. Synthetic *cis*-hex-3-enyl acetate, which was the only component identified in vapour from intact turnip and radish and, tentatively, cauliflower and wallflower, appeared not to be attractive to flies in laboratory tests. Any vapour components from intact *B. juncea* could not be distinguished from background, although disrupted leaf-tissue of this species released substantial amounts of allyl isothiocyanate. Thus, if these compounds play a part in the attraction of cabbage root fly to its host-plant for egg-laying, they do so at very low concentrations or only close to the plant where higher concentrations may occur. In vapours from disrupted tissues of *B. juncea* leaf and of some samples of cauliflower roots, appreciable amounts of allyl isothiocyanate occurred together with relatively minor amounts of allyl cyanide, a pattern of occurrence consistent with both components being produced as a result of the hydrolysis of allyl glucosinolate (sinigrin) [9]. However, in vapour from disrupted turnip leaf, ethyl cyanide was detected in moderate amounts without any accompanying ethyl isothiocyanate.

More allyl isothiocyanate was produced by disrupting older (14 week-old) than younger (9 week-old) tissues of cauliflower plants. Whereas in the younger plants the stems were the richest source of this chemical, in the older plants it was more prevalent from the root and the young growing tissues (uppermost 30 mm) of the plants. When MacCleod and MacCleod [16] cooked tissues of mature cauliflower either conventionally or by microwave radiation, they also recovered larger quantities of allyl isothiocyanate from younger (inner) that from older (outer) leaves.

Differences in the relative amounts of individual vapour constituents are to be expected from different tissues but the physiological age of the plant and the factors influencing its rate of growth may also induce differences in the biochemical composition of the tissues which will be reflected in the composition of the resulting vapour. Although some qualitative differences can often be found, it is not usually certain whether these are real or arise from limitations in the characteristics and sensitivities of the analytical techniques employed. Before meaningful generalisations can be deduced, more attention will need to be paid to factors influencing the physiological condition of the plant tissues and also to limitations of specific techniques used to characterize plant constituents in relation to the evolution of volatiles. This is particularly so since the oviposition behaviour of the cabbage root fly is probably dependent on the interplay of many chemical stimuli provided by its host-plants [2] and there is no reason to believe that its host-finding behaviour is any less complicated.

EXPERIMENTAL

Plant material. Plants of cauliflower (*Brassica oleracea* L. var. *botrytis* L. cv. Finney's 110), turnip (*Brassica rapa* L. ssp *rapa* L. cv. Snowball), *B. juncea* Czern and Coss. (a type of mustard), radish (*Raphanus sativus* L. cv. Cherry Belle) and wallflower (*Cheiranthus cheiri* L. cv. Persian Carpet) were grown throughout the year in a sand/peat mixture in 8 cm pots in a glasshouse. Plants with at least 6 true leaves were used.

Cauliflower plants grown in a heated glasshouse for 9 or 14 weeks during late autumn and winter were used to determine the distribution of the volatile components. Vapour samples were taken from disrupted plant material from the root, the stem including the petiole and the hypocotyl above the compost surface, the leaves up to the 6th leaf, and the curd including the uppermost 30 mm of stem with associated small leaves.

Vapour collection. Volatiles from intact plants were collected by enclosing 20 potted plants, or 16 of larger-sized turnip plants, in an 80 l. box maintained at 20°. 15 l. of the box atatmosphere was then displaced at 0.6 l./min through glass wool contained in a tube cooled with solid CO_2, thereby minimizing contamination by background components in the air. Transpired H_2O vapour was not removed from these samples before analysis.

To collect vapour from disrupted tissue, 15 g of leaf was cut into small pieces and placed in a 250 ml flask. For root samples, the tissues were cut and also coarsely minced. Volatiles were allowed to accumulate for 30 min at 30° and were then displaced by 500 ml air onto a 6 mm o.d. tube containing Porapak Q. H_2O vapour was removed by purging with 500 ml N_2 at room temp, the volatile organic compounds remaining adsorbed on the Porapak Q [14].

GLC analysis. A FID instrument was used and the tube containing the sample was coupled in-line at the beginning of the GLC column with the N_2 carrier flow stopped. The samples on Porapak Q were heated concentrically for 4 min

to 150° and were then purged onto the GLC column by recommencing the carrier flow at 40 ml/min. For aq samples on glass wool, the carrier was recommenced at the beginning of the preheating period to prevent back-distillation of up to 250 mg transpired H_2O present in the samples. The analysis was performed using 8% Carbowax 1540 on Chromosorb W in a 150 cm × 4 mm i.d. glass column with the temp held at 50° for 1 min after the 4 min sample-heating period, and then programmed at 4°/min to 114°. R_t's were calculated from the beginning of the sample-heating period. To obtain reference R_t's, synthetic vapours were collected in the same way as the plant volatiles and subjected to the same conditions of purging and analysis according to whether they were collected on Porapak Q or glass wool. The relatively large amounts of H_2O (up to 5×10^5 excess) present in vapour samples collected from intact plants considerably shortened the R_t of hexenyl acetate compared with its R_t when the drier samples from the disrupted tissues were chromatographed (16.5 compared with 20.4 min; Table 1), a similar effect occurring when synthetic hexenyl acetate was chromatographed in the presence or absence of comparable amounts of H_2O (16.5 ± 0.5 compared with 20.4 min).

Identification. Vapour components were analysed on a high resolution mass spectrometer after "on-line" separation using a coupled GLC fitted with a 50 m SCOT column of Carbowax 20M. Samples containing H_2O were first purged through an empty U-tube and a tube containing a protein mixture [17]. Low resolution MS were obtained at 70 eV and a source temp of 200°. The identifications deduced from the spectra obtained were confirmed by comparison with those of synthetic compounds analysed under identical conditions or with published MS [5]. The isomeric form of hexenyl acetate was determined by PMR and IR analysis. *Ca* 3 mg of the natural compound was obtained from several collections of vapour from finely-cut cauliflower leaf onto Porapak Q and re-collecting the component at about 98% purity after separation by GLC. The PMR spectrum (60 MHz) of the sample in CCl_4–1% TMS was obtained after computer enhancement of the signal. The IR spectrum was obtained on the same sample after recovery and concentration by partial evaporation of the solvent. The isomeric form was determined by analysis of the spectra and by comparison with spectra of a synthetic *cis trans*-hex-3-enyl acetate mixture.

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