

# ***Cannabis sativa*: volatile compounds from pollen and entire male and female plants of two variants, Northern Lights and Hawaiian Indica**

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Sixty-eight compounds were identified by coupled gas chromatography and mass spectrometry (GC-MS) in the chemosphere of *Cannabis sativa* L. pollen and entire male and female plants of two cultivated varieties, Northern Lights and Hawaiian Indica. Twenty-one and 28 substances, respectively, were present in pollen of the two forms. To conserve the natural composition of volatiles a delicate headspace method was employed. The two varieties represent different chemotypes which distinguish themselves, in the main quantitatively, in the setup of volatiles from pollen and entire male and female plants. Twenty compounds were monoterpenes, including the five major components:  $\beta$ -myrcene (E)- $\beta$ -ocimene, terpinolene,  $\beta$ -pinene and limonene; 25 were sesquiterpenes, and the other 23 were of mixed biogenetic origin, including 3-methyl-1-butanol and benzylalcohol which occurred only in pollen; two pyrazines occurred only in Northern Lights females. Besides being of interest in natural products chemistry, the results should have relevance for plant systematics and for the pharmaceutical and technical applications of *Cannabis*. We demonstrate that the pollen has a distinct chemical character in possessing two exclusive volatiles, while lacking seven compounds occurring in males and females of both variants. © 2005 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2005, 147, 387–397.

**ADDITIONAL KEYWORDS:** Cannabaceae – chemosphere – chemotypes – GC-MS – headspace – monoterpenes – pyrazines – scent – sesquiterpenes – sorption.

## **INTRODUCTION**

*Cannabis sativa* L. is a dioecious plant, which, together with Hop (*Humulus*) is now classified as a separate family, Cannabaceae (Hutchinson, 1959; Bremer, Bremer & Thulin, 2000). *Cannabis* has probably been cultivated for over 5000 years and by artificial selection an optimal production of fibre, oil, and intoxicating resin has been obtained. Interest in its narcotic qualities has engendered some 7000 publications on various aspects of this unusual plant.

Today there is a renewed interest in *Cannabis* owing to the novel application of hemp as household insulation material and more importantly for its

approved use in pharmaceutical preparations for the relief of severe pain (Williamson & Evans, 2000; Williamson, 2001). We were primarily interested in the composition of volatiles given off by males and females of the *Cannabis* plant, especially in regard to the possibility of the two horticultural varieties Northern Lights and Hawaiian Indica representing different chemotypes. Special attention was paid to their pollen volatiles. These are of interest due to their possible role as insect or vertebrate repellents or attractants (Dobson & Bergström, 2000). Although insects from 14 orders have been reported feeding on pollen only the volatiles of pollen from c. 12 species have been identified, of which only six species have been studied in detail (Dobson & Bergström, 2000).

A few publications describe *Cannabis* volatiles but not one has concentrated on examining the chemosphere around the plant. The various techniques used

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and different plant preparations have shown that they can produce variable results. A good example is that demonstrated by Hood, Dames & Barry (1973), which compared an analysis of the headspace of whole plants with that obtained by steam distillation. Eighteen volatiles were found in the former case and 26 in the second. The most complete compilation of *Cannabis* essential oil components was presented by Turner, ElSohly & Boeren (1980) in which they record 421 volatiles and nonvolatiles obtained by steam distillation.

In order to obtain as true a picture as possible of those compounds released to the chemosphere surrounding *Cannabis* plants we used a delicate adsorption/desorption method for the isolation of volatiles (see Material and methods).

## MATERIAL AND METHODS

### EXPERIMENTAL

#### *Plant material*

Seeds of two horticultural hybrids of *Cannabis sativa* L., Northern Lights (NL) and Hawaiian Indica (Hi), were obtained from Sensi Seed B.V., P.O. Box 1771, 3000 BT Rotterdam, the Netherlands. The plants were grown at Ashton Wold, UK, in an unheated greenhouse with open air ventilation. In March the seeds were planted in clay pots (15 cm height, 20 cm diameter) in John Innes potting compost no. 1. The soil and the pots were completely covered with aluminium foil (from rolls of Skultuna foil, 30 cm × 20 m). The plastic bags (see below) were, in their lowest point fastened to the stem of the plant by a short external metal wire and in their upper part closed, apart from a hole for the inert surgical tubing connecting with the adsorbent cartridges. These measures ensured that there was no contamination from pot or soil. The volatiles were collected in late August/September, when the plants were 180–210 cm in height and were flowering. The volatiles were collected from the whole upper part of the plant, enclosed in the plastic bag, i.e. stem, green parts, flower, and microamounts of resin exudate.

#### *Isolation of volatiles*

A list of the samples prepared, with the respective collection methods for volatiles, is given in Table 1. For collection of the volatiles of samples 1 and 3–11, the upper part of the plant (approximately 40 cm) or china bowls (12 cm height, 20 cm diameter) (samples 3, 4, and 11) were enclosed in plastic bags (trademark Penquin, frying bag), which emit very small amounts of background volatiles, less than any compound in Table 2, i.e. less than 0.01% of total. Air was then drawn through the bags by micro pumps at flow rates of 70–110 mL min<sup>-1</sup> for 4–8 h (Bergström, Rothschild

**Table 1.** Samples used in the chemical analysis

N	Description	Collection method
1	1 plant (H.i. f) in pot	encl. in plastic bag, sorption
2	40 plants (N.l./H.i., f/m, 10 each)	sorption from ambient air
3	pollen (N.l.) in bowl	air over bowl, sorption
4	pollen (H.i.) in bowl	"
5	upper part (N.l. m) in pot	encl. in plastic bag, sorption
6	upper part (N.l. f) in pot	"
7	upper part (H.i. m) in pot	"
8	upper part (H.i. f) in pot	"
9	upper part (H.i. m) cut	"
10	upper part (H.i. f) cut	"
11	20 florettes/buds (H.i. f)	in bowl air over bowl, sorption

*et al.*, 1995b). Volatiles were adsorbed from the stream of air on to Porapak Q, 80–100 mesh adsorbent, Supelco 60 mg, packed into small, precleaned Teflon cartridges, 3 mm i.d., 40 mm in length, sealed with polypropylene wool, and afterwards rinsed with 0.3 mL pure distilled diethyl ether. The respective ends of the cartridges were connected to the surgical tubing from the plastic bag, and to the minipump. The samples were concentrated to 30 µL. For two of the collections, samples 9 and 10, carbon microfilters were employed instead. A total of 11 collections were made.

After solvent extraction of the adsorption plugs they formed samples 1–11. Samples 1, 2 and 11 were used for orientative analyses, giving a preliminary idea of the types of compounds encountered and their amounts. Samples 9 and 10 were used for complementary analyses employing the carbon microfilters. For best results (collection of compounds of different volatilities and polarities, consistency for comparisons between samples) we chose Porapak Q plugs, and they were used in the analyses for samples 3–8, which form the backbone of the investigation.

#### *GC-MS analyses*

After concentrating the samples, a reference (dodecyl acetate) was added to aid quantification and chemical analyses were carried out by gas chromatography – mass spectrometry (GC-MS) using a Hewlett-Packard 6890–5973 combination instrument. The analytical column was fused Silica HP-Innowax coated with polyethyleneglycol. Conditions were 50 °C isothermal for 3 min, programming 10 °C per min to 220 °C, and then isothermal. Identification of all the volatiles was carried out by comparing mass spectra with computer library references. For 46 of the substances, marked R

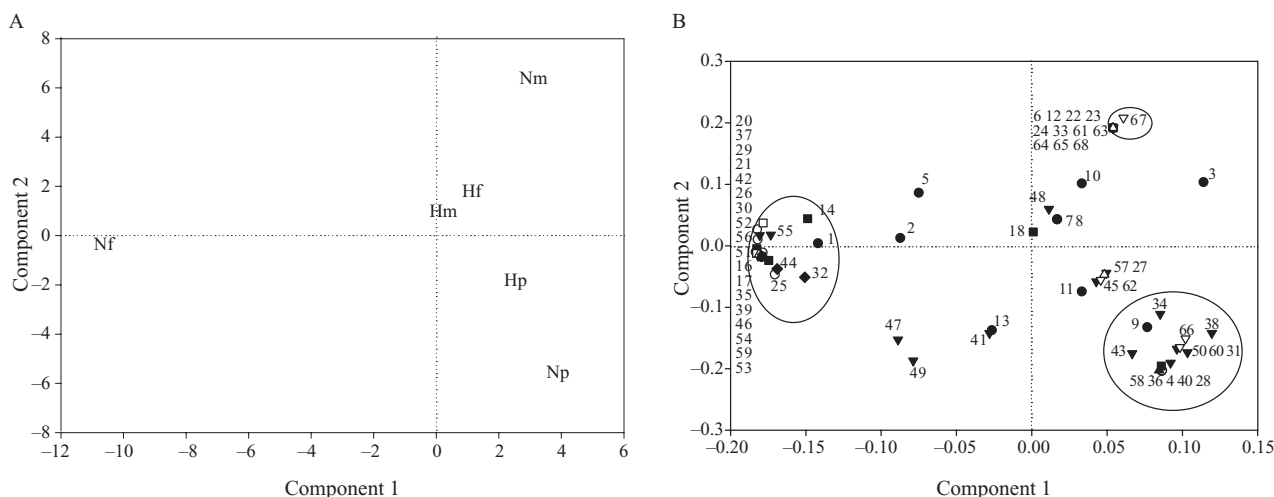
**Table 2.** Relative amounts (% of total) of 68 volatile compounds identified in the head space of six samples of *Cannabis sativa*. The substance's name, its number (N) in this paper (order of gas chromatographic elution), the method of identification (R, reference; M, mass spectrum), the gas chromatographic retention time (Rt), the relative content of each substance in the six samples, chemical class and quantitative rank (starting with the largest component as no. 1)

Substance	N	I	Rt	Nf	Nm	Np	Hf	Hm	Hp	Class	Quantitative rank order
$\alpha$ -pinene	1	R	4.53	1.54	0	0	0	1.52	0	A1-1	16
$\beta$ -pinene	2	R	5.24	10.7	1.12	1.57	7.45	18.8	4.31	A1-1	4
$\beta$ -myrcene	3	R	6.43	14.6	64.8	27.7	25.8	23.5	56.2	A1-1	1
3-methyl-1-butanol	4	R	7.00	0	0	5.29	0	0	4.74	B	8
limonene	5	R	7.07	9.61	4.33	0	9.71	18.6	0	A1-1	5
eucalyptol	6	R	7.14	0	5.75	0	0	0	0	A1-2	10
sabinene	7	R	7.15	0	0	0	6.13	0	0	A1-1	9
$\beta$ -phellandrene	8	R	7.31	0	0	0	0.99	0	0	A1-1	43
(Z)- $\beta$ -ocimene	9	R	7.61	1.09	1.56	3.50	0.68	3.09	1.78	A1-1	7
3-carene	10	R	7.78	0	0.20	0	0.68	0	0	A1-1	45
(E)- $\beta$ -ocimene	11	R	7.93	12.6	11.8	20.7	18.4	19.8	7.57	A1-1	2
$\alpha$ -terpinene	12	R	8.39	0	3.13	0	0	0	0	A1-1	14
terpinolene	13	R	8.46	16.3	0	18.2	27.6	8.26	14.1	A1-1	3
3-hexenyl acetate	14	R	8.89	1.38	0.28	0	0.22	1.24	0	B	15
(Z)-3-hexen-1-ol	15	R	9.83	1.11	0.04	0	0.10	0.41	0.40	B	20
1,3,8-p-menthatriene	16	R	10.10	1.39	0	0	0	0	0	A1-1	32
2-methylhexyl butanoate	17	R	10.48	0.49	0	0	0	0	0	B	52
3-methyl-3-cyclohexen-1-one	18	M	10.63	0	0	0	0	0.18	0	B	56
3-methyl-4-methylene-bicyclo(3,2,1)oct-2-ene	19	M	10.81	0.87	0.13	0	0.35	0.21	0	C1-1	27
epoxyterpinolene	20	M	11.06	1.38	0.22	0	0.21	0.29	0	A1-2	19
(Z)-3-hexenyl- $\alpha$ -methyl butyrate	21	M	11.11	0.90	0.04	0	0.03	0.12	0	B	40
ylangene	22	R	11.29	0	0.05	0	0	0	0	A2-1	59
$\alpha$ -cubebene	23	R	11.41	0	0.04	0	0	0	0	A2-1	62
$\alpha$ -gurjunene	24	M	11.97	0	0.01	0	0	0	0	A2-1	67
linalool	25	R	12.02	1.42	0.07	0.21	0.01	0.02	0.21	A1-2	21
(Z)-2-pinanol	26	R	12.11	0.61	0.02	0	0	0	0	A1-2	50
(E)- $\alpha$ -bergamotene	27	R	12.38	0	0.02	0	0	0.02	0.19	A2-1	53
longifolene	28	R	12.45	0	0.05	0.59	0	0	0.42	A2-1	41
exo-fenchol	29	R	12.53	0.93	0.10	0	0.06	0.14	0	A1-2	38
2,6-dimethyl-6bicyclo(3,1,1)hept-2-ene	30	M	12.59	0.83	0	0	0	0.19	0	C1-1	42
$\alpha$ -zingiberene	31	R	12.60	0	0.20	0.95	0.06	0.05	0.47	A2-1	23
1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-azulene	32	M	12.69	1.05	0	0	0	0.02	0.58	C1-2	24
germacrene D	33	R	12.72	0	0.02	0	0	0	0	A2-1	65
$\beta$ -caryophyllene	34	R	12.81	1.35	3.11	5.80	0.72	1.54	1.70	A2-1	6
5,5-dimethyl-2(5H)-furanone	35	M	12.92	0.88	0	0	0	0	0	C1-3	46
menthol	36	R	13.21	0	0	0.80	0	0.04	0.45	A1-2	33

**Table 2.** *Continued*

Substance	N	I	Rt	Nf	Nm	Np	Hf	Hm	Hp	Class	Quantitative rank order
allo-aromadendrene	37	R	13.39	1.19	0.17	0	0.05	0.21	0	A2-1	26
(E)- $\beta$ -farnesene	38	R	13.52	0	0.18	0.53	0.06	0.35	0.29	A2-1	31
alkyl pyrazine*	39	M	13.64	0.84	0	0	0	0	0	C2	47
ipsdienol	40	R	13.64	0	0	0.39	0.01	0	0.24	A1-2	49
$\alpha$ -humulene ( $\alpha$ -caryophyllene)	41	R	13.68	1.32	0.78	1.79	0.17	0.35	0.58	A2-1	11
1,8-menthadien-4-ol	42	R	13.79	0.99	0	0	0.03	0.26	0	A1-2	34
vidiflorene	43	M	14.21	0	0	0.18	0	0.02	0	A2-1	54
1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-azulene	44	M	14.20	0.96	0	0	0.02	0	0.30	C1-2	35
$\beta$ -bisabolene isomer	45	M	14.28	0	0	0	0	0.32	0.94	A2-1	36
2,4-dimethylanisol	46	R	14.40	1.50	0	0	0	0	0	C1-4	29
(E,E)- $\alpha$ -farnesene	47	R	14.50	1.03	0.25	0.92	0.17	0.10	0.38	A2-1	17
$\gamma$ -murolene	48	R	14.71	0	0.03	0	0	0.16	0	A2-1	55
(Z)- $\alpha$ -bisabolene	49	R	14.81	1.72	0	1.76	0.11	0.16	0.59	A2-1	13
$\beta$ -cedrene	50	R	14.95	0	0.64	3.04	0	0	1.22	A2-1	12
2,6-dimethyl-3,5,7-octatrien-(E)-2-ol	51	M	15.08	0.95	0	0	0	0.01	0	C1-5	44
2,6-dimethyl-3,5,7-octatrien-(Z)-2-ol	52	M	15.27	1.49	0	0	0.10	0.04	0	C1-5	25
2,5-dimethoxytoluene	53	R	15.43	2.53	0	0	0.05	0	0.14	C1-4	18
methoxypyrazine*	54	M	15.44	1.25	0	0	0	0	0	C2	37
$\gamma$ -elemene	55	R	15.52	1.28	0.23	0	0.01	0	0	A2-1	28
o-methoxyacetophenone	56	M	15.61	1.81	0	0	0.04	0	0	C1-4	22
geranylacetone	57	M	15.68	0	0.01	0	0	0	0.10	C1-5	57
benzylalcohol	58	R	15.93	0	0	0.75	0	0	0.40	C1-4	39
5tert.butyl-2,2-dimethyl-3(2H)-furanone	59	M	17.12	0.07	0	0	0	0	0	C1-3	58
caryophyllene oxide	60	R	17.15	0	0.10	0.49	0.05	0.03	0.14	A2-2	48
nerolidol	61	M	17.53	0	0.04	0	0	0	0	C1-5	63
guaiaol	62	R	18.08	0	0.06	0	0.05	0	1.32	A2-2	30
$\gamma$ -eudesmol	63	M	18.30	0	0.05	0	0	0	0	A2-2	60
6,10,14-trimethyl-2-pentadecanone	64	M	18.39	0	0.02	0	0	0	0	B	66
agarospirol	65	M	18.97	0	0.01	0	0	0	0	A2-2	68
$\alpha$ -bisabolol	66	R	19.27	0	0.09	0.33	0.04	0	0.10	A2-2	51
$\alpha$ -eudesmol	67	R	19.37	0	0.02	0	0.01	0	0	A2-2	64
$\beta$ -eudesmol	68	R	19.45	0	0.05	0	0	0	0	A2-2	61





**Figure 1.** A, plot of scores for the first two Principal Components. B, plot of loadings from the two first Principal Components. Classification of substances into classes are indicated by different symbols (see Table 3). The boundaries of the three identified clusters are indicated. Numbers refer to the substance numbers given in Table 2.

**Table 5.** Probability that the similarity in ranking between pairs of samples was by chance, estimated as Kendall  $\tau$ .

	NF	NM	NP	HF	HM	HP
NF	–	52.06%	17.83%	0.00%	0.00%	11.71%
NM		–	0.11%	0.00%	0.07%	3.24%
NP			–	0.01%	0.00%	0.00%
IF				–	0.00%	0.02%
IM					–	0.01%
IP						–

– MS), see further the Material and Methods section above.

The three major compounds collected from the two varieties

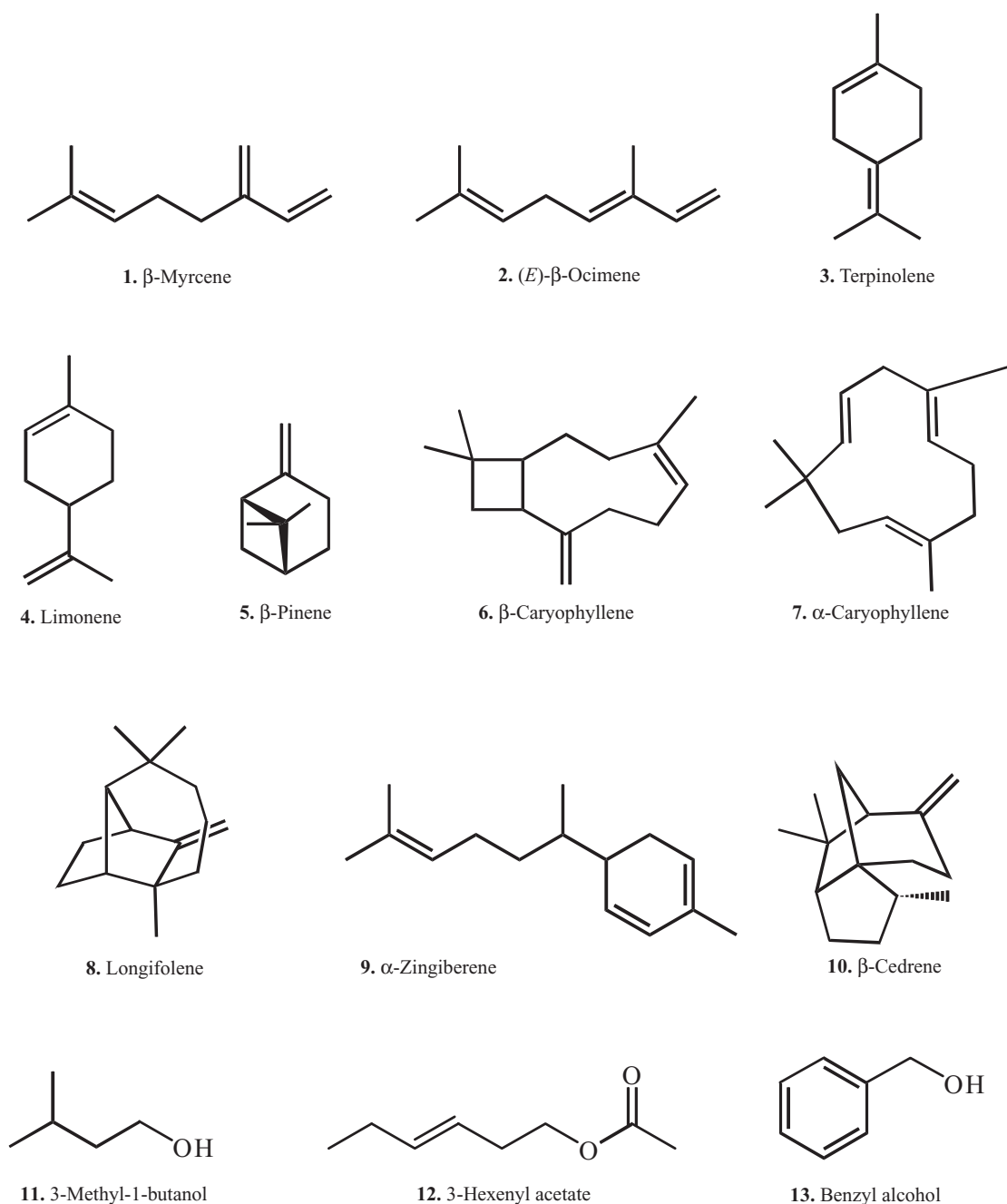
Northern Lights (Nl) and Hawaian Indica (Hi) are the simple and common monoterpene hydrocarbons  $\beta$ -myrcene (E)- $\beta$ -ocimene and terpinolene (compound numbers 3, 11 and 13). The first two are acyclic, the third one is monocyclic. Altogether 68 volatiles were identified. 20 were monoterpenes of which 12 are hydrocarbons and eight oxygenated. 25 were sesquiterpenes, of which 18 are hydrocarbons and eight are oxygenated. The 68 compounds identified have been grouped in three main categories and subcategories according to structural and biogenetic relationships (see Table 2, column 10, Tables 3 and 4). Category A is composed of terpenes (isoprenoids), 46 compounds in all. They comprise monoterpenes (A1) of which 12 are hydrocarbons (A1-1) and eight are oxygenated sub-

stances (A1-2), seven of them alcohols. The sesquiterpenes (A2) also consist of hydrocarbons (A2-1), 18 of them, and eight oxygenated compounds (A2-2), seven of them alcohols. The next category are represented by eight 'simple' fatty acid derivatives (B). All the other 14 compounds make up category C, in which the two pyrazines are designated group C2. The C1 category includes the following subcategories: C1-1 unsaturated bicyclo alkenes; C1-2 azulenes; C1-3 furanones; C1-4 benzenoids and C1-5 isomeric dimethyloctatrienols.

In Scheme 1 a few examples of the identified compounds are given. Numbers 1–5 represent the most common monoterpene hydrocarbons. Numbers 6–10 are five prominent sesquiterpene hydrocarbons. The two substances found exclusively in pollen, of both cultivars, have numbers 11 and 13. Compound 12 represents one of the substances characteristically absent from pollen. Many of the compounds found are common in floral scents (Knudsen, Tollsten & Bergström, 1993) and in essential oils of green plants generally, including trees and shrubs. It should be noted that eucalyptol (compound number 6) is identical with 1,8-cineole, an oxygenated bicyclic monoterpene possessing a pyrane ring. This explains its relatively short gas chromatographic retention time; it appears among the monoterpene hydrocarbons.

Twenty-one compounds were found in the pollen volatiles of Nl and 28 in Hi. Twenty compounds were identical in both varieties. Two of the compounds, 3-methyl-butanol (4) and benzylalcohol (58) are confined to the pollen of both varieties and are found nowhere else in the plant. The first one occurred in the two variants in 5.3% and 4.7%, respectively; the





**Scheme 1.** Scheme of prominent compounds of the volatiles found in *Cannabis*. Compounds 1–5: major monoterpenes; compounds 6–10: major sesquiterpenes; substances 11 and 13: exclusive in pollen; 12: not present in pollen.

second in 0.75% and 0.40%, respectively. Four other compounds, longifolene (28), menthol (36), ipsdienol (40) and  $\beta$ -cedrene are virtually peculiar to *Cannabis* pollen since each one of them is only present in one other collection (presented in the columns of Table 2), and then in much smaller quantities (< 1/10 of the occurrence in pollen). It is worth noting that seven compounds (numbers 5, 14, 19, 20, 21, 29 and 37) are

absent from pollen of both varieties although present in both sexes of both cultivars. They belong to different chemical classes. The largest component of them is limonene, which occurs in 4–19% in the different samples.

The two pyrazines (39 and 54) occurring in considerable quantities, 0.84% and 1.25%, respectively, were found exclusively in the female plant of NL.

They are very likely an alkyl pyrazine and a methoxy pyrazine, tentatively identified as 3,5-dimethyl-2-propylpyrazine and 2-methoxy-3-(1-methylethyl)pyrazine, respectively. The definitive determination of their chemical identity requires further study. The alkylmethoxypyrazines have a phenomenally low olfactory threshold (0.002 p.p.b.) and produce one of the most powerful odours known to man. The possible function of pyrazine in *Cannabis* and its possible influence on man is, at present, unknown. It would be of interest to investigate the possible effect of their presence on the action of T.H.C. (tetrahydrocannabinol), C.B.D. (cannabidiol) and CB (cannabinol).

In the nonterpenoid category of volatiles there are certain differences between the sexes of the two varieties other than the pyrazines in female Nl. For example, the two furanones (35 and 59), 1, 3, 8-p-menthetriene (16), 2-methylhexyl butanoate (17) and 2,4-dimethylanisol (46) were found only in female Nl; eucalyptol/1,8-cineol (6) occurs exclusively in male Nl (5.8%) together with several small compounds close to our set detection limit of 0.01% (numbers 22, 23, 24, 33, 61, 63, 64, 65, 68); sabinene (7) and  $\beta$ -phellandrene (8) is found only in female Hi (6.1% and 1%, respectively); 3-methyl-3-cyclohexenone (18) is only present in Hi males.

#### STATISTICAL ANALYSIS

The qualitative analysis showed that only eight substances were found in all samples (Table 4). They are all isoprenoids. While for females and males, of both forms, only a single substance was unique, nine substances were unique for pollen samples. In the samples Nf and Nm, a long range of substances were positively or negatively unique for the respective form. For the Hf and Hm samples only three substances were unique, both by their presence. No substance was found in all samples from one form and in no sample of the other form. Looking at all substances combined, the relative concentration of the different substances was very similar in all samples except for Nf, when examined using Kendall *tau* (Table 5). The Nf sample showed very low similarity with the other N-samples and the Hp sample, but ranked the substances in the same order as both the female and male H-samples.

The plot of the scores of the two first PC-components confirms that the relative concentrations in Nf separates from the other samples (Fig. 1A). The first component explained 42% and the second 24% of variation. While the H-samples were close to one another, the three N-samples were spread, especially in the second component. The plot of loadings (Fig. 1B) revealed a cluster of the 24 substances with low values in the first component corresponding to the Nf sample. This cluster

includes all substances in the chemical groups C1-1, C1-2 and C1-3 but no A2-2. A second cluster was identified in the lower right corner, corresponding to the Np sample. It included 13 substances mostly from the chemical A-groups but only one B substance and one C substance (benzylalcohol, a C1-4 substance). A third cluster are those substances that are positively unique for the Nm sample and have a position corresponding to the position of Nm in Figure 1B.

Together this analysis shows:

- The chemical content of the H cultivar showed a lower degree of variation than did the N cultivar. It is seen both in that a low number of substances was unique (positively or negatively) for all samples from it, and that the samples showed a high degree of similarity both in the ranking test and in the PCA analysis.
- Of the samples from the N cultivar both the female and the male samples included a large number of substances which were unique. Both the ranking test and the PCA analysis showed this, especially the female that differed from the others, and the PCA analysis indicated that it was especially due to substances within the groups C1-1, C1-2 and C1-3.
- The ranking test showed that the female N sample differed more from the male and pollen samples from the same cultivar than from any of the samples from the H cultivar.
- While only a few substances were unique for either the two female samples or the two male samples, eight substances were unique (positively or negatively) for the two pollen samples. A similarity between the pollen samples is also indicated by their closeness in the PCA plot.

#### STUDIES OF *CANNABIS* VOLATILES

Several reports have been published which include descriptions of *Cannabis* volatiles but none has been concentrated on the examination of the chemosphere. The various techniques used and different plant preparations have shown that these can produce variable results. A good example is that demonstrated by Hood *et al.* (1973) who compared an analysis of the headspace of whole plants with the volatiles extracted by steam distillation from *Cannabis* oil. Eighteen volatiles were found in the former case and 26 by the second. All compounds identified were mono- or sesquiterpenes. The main components in the former was  $\alpha$ -pinene, followed by  $\beta$ -pinene and thirdly by myrcene, whereas the essential oil volatiles gave  $\beta$ -caryophyllene as the major compound followed by  $\beta$ -humulene and caryophyllene oxide.

These results agree with those of Nigam *et al.*, (1965) who found the first two plus  $\alpha$ -selinene as



major compounds in the essential oil distillates of an Indian variety of *C. sativa*. The most complete compilation of *Cannabis* essential oil components was presented by Turner *et al.* (1980) in which they record 414 volatiles and nonvolatiles, which are arranged according to their chemical classes. They include 58 mono- and 38 sesquiterpenes. Ross & ElSohly (1996) found 68 components in the volatile oil of *Cannabis*, of which 57 were identified, prepared from fresh and dried buds.

#### SEXUAL DIFFERENCES

Comparison between the sexes of the same variety or between different varieties (Fig. 1, Scheme 1) indicate that the variance is mainly of a quantitative nature apart from the specific pollen volatiles. Nigam *et al.* (1965) found that the essential oil compositions from male and female were 'almost identical'. It should be noted that the difference between individual plants grown in a greenhouse can be considerable.

#### DISCUSSION

##### ANALYSES OF POLLEN VOLATILES

Few analyses of the pollen volatiles from specific plants have been made. They indicate that the number of these volatiles can vary from species to species but usually variability is less than that between the flowers themselves. In an earlier compilation of data from head-space analysis of flower odours (Knudsen *et al.*, 1993), over 700 volatile compounds from 441 taxa were reported, showing that most of the volatiles from *Cannabis* are known, especially the mono- and sesquiterpenes. Some of the pollen compounds are common, but a few are quite specific. One such is the small volatile lactone protoanemonin (5-methylene-2(5H)-furanone), found in the pollen of *Ranunculus acris* (Bergström, Dobson & Groth, 1995a). In preparations from some years it was dominant (75%) while in others it fell below  $\alpha$ -farnesene (6% and 69%, respectively) at only 18%. This compound is a skin irritant to humans and is considered a deterrent for herbivores, e.g. livestock.

3-methyl-butanol is the largest of the volatile components characteristic for *Cannabis* pollen, with 5.3% in NI and 4.7% in HI (Table 1). It seems, at present, to be known only in the pollen of *Cannabis* but it has been found in other parts of plants. In our review of head-space studies of floral scents (Knudsen *et al.*, 1993) we referred to it as occurring in 14 plant genera. We have ourselves identified it in *Actaea erythrocarpa*, 1.2% (Pellmyr, Groth & Bergström, 1984) and in *A. rubra*, 2.0% (Pellmyr, Bergström & Groth, 1987); in *Rosa rugosa*, in medium amounts, 4–20%, from sam-

ples including green portions, such as sepals and flower buds (Dobson *et al.*, 1987); and in other species.

Benzylalcohol, occurring in 0.8% in NI and 0.4% in HI (Table 1), is a common floral scent. Of the three other characteristic *Cannabis* pollen compounds, longifolene, menthol and ipsdienol, the first two are well known components of essential oils, and have also been found in floral scents of *Rosa* (Chen, Li & Li, 1987) and *Osmanthus* (Ding *et al.*, 1989); ipsdienol is known as a pheromone component in insects, e.g. bark beetles.

##### SYSTEMATICS: RELATIONSHIPS TO *HUMULUS*

Owing to the close relationships of *Cannabis* and *Humulus* (for up-to-date information on the systematics of Cannabaceae, etc. see Wilmot-Dear, 1999, and Bremer *et al.*, 2000) it is of interest to compare their volatiles. Eri *et al.* (2000) have reported 280 volatile compounds from four Hop varieties of which two thirds (more than 100 for each variety) have been identified. The volatiles were isolated from the essential oil by way of direct thermal desorption using Tenax TA adsorbent and, simultaneously, by conventional steam distillation-extraction. The major compounds were found to be  $\beta$ -myrcene, caryophyllene and humulene. Fatty acid derivatives and mono and sesquiterpenes are the dominant chemical classes. Hops in this analysis have more fatty acid derivatives, for example several esters, in small amounts and a few sulphur-containing compounds. There is an extensive literature on the composition of the essential oil of hops. One recent work is that by Steinhaus & Schieberle (2000) who analysed odour-active compounds from hop cones and identified 20 odourants of which *trans*-4,5-epoxy-(E)-2-decenal, linalool and myrcene were the most potent ones. A similar study by Mediavilla & Steinemann (1997), identifying 16 major mono- and sesquiterpenes, finds myrcene and *trans*-caryophyllene as dominant components. Although our preparation was different from the one employed in these studies, both specific compounds and overall similarity in composition can be noted. Comparisons among the set-up of volatiles in *Cannabis* and *Humulus* and other plants certainly point to a close systematic relationship between the two.

##### PYRAZINES IN NI FEMALES

Pyrazines have varied functions. Their smell can potentiate learning in mammals (Kaye *et al.*, 1989), improve recall of past events for chickens (Barnea & Rothschild, 2002) and produce alerting signals to draw attention to food and prey available in plants and insects (Woolfson & Rothschild, 1990). The study by Barnea & Rothschild demonstrated that the

mean egg mass of pyrazine exposed (2-methoxy-3-isobutylpyrazine) of leghorn chickens was significantly increased as compared to a control group.

Eri *et al.* (2000) suggest, tentatively, the presence of a dimethyl pyrazine isomer in one of the four varieties of hop tested, which is noteworthy in view of our finding of two pyrazines in the *Cannabis* variety NI. The function of this widely distributed highly potent odourant is not always understood. In many cases it acts as a warning signal for herbivores of the presence of dangerous chemicals, and in other situations as an attractant for edible fruit which simultaneously assists seed dispersal for the plant.

In the related species, *Urtica dioica*, the nettle, pyrazines are only present when the plant is in flower (Moore *et al.*, 1990). In an unrelated but toxic plant, *Asclepias curassavica*, it is only present in the plant's foliage in summer months (M. Rothschild, pers. observ.). It is quite possible that the pyrazines will be found in *Cannabis* at other times of the year.

#### CHEMICAL RELATIONSHIPS TO THE CANNABINOID MOLECULES

There are clear resemblances, indicating biogenetic closeness, between parts of the nonvolatile cannabinoid molecules, such as tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CB), and some of the volatiles we have identified. Such are, for instance, the acyclic monoterpenes  $\beta$ -myrcene and  $\beta$ -ocimene, the monocyclic terpinolene and limonene, and the pollen compounds 3-methylbutanol and benzylalcohol, perhaps also linalool,  $\beta$ -pinene and the bicyclic monoterpene 1,8-cineole (= ucalyptol). They include the major volatile compounds. The relational structures and their activity have been summarized by McPartland & Priutt (1999). Several of them have antibacterial and antifungal effects and some are sedative, e.g. linalool. It could well be that the pyrazines enhance and activate the cannabinoids present in *Cannabis*.

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