Composition of Male Hop Oil[1]

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ABSTRACT

Although the male hop flower is of no economic importance, information about its quality characteristics is useful in breeding new hop varieties. The lupulin glands of male genotypes like those of female hops contain volatile oils. The terpenes myrcene and farnesene are not present in the oil of males. The sesquiterpenes caryophyllene and humulene are present, and the proportion of humulene/caryophyllene is a varietal characteristic in male hops. Cadinene, selinene, calacorene, and calamenene were also detected in male hop oil by gas chromatography/mass spectroscopy. Composition of the oil in lupulin glands was determined by direct injection of a pentane extract and compared with the steam-distilled oil of female hops cones. The oil composition of male genotypes from the U.S. Department of Agriculture hop germ plasm collection at Oregon State University showed a wide range in the proportion of humulene and caryophyllene. The male parent's humulene/caryophyllene ratio was shown to have an effect on the humulene/caryophyllene ratio of the female progeny of a cross.

Key words: *Humulus lupulus* L., Male hops, Essential oil composition, Lupulin, Humulene, Caryophyllene

The hop, *Humulus lupulus* L., is a dioecious perennial with male and female flowers on separate plants. Hop cones used in brewing are the female flowers. The male flower is about 0.5 cm in diameter, much smaller than the female cone (Fig. 1). One hop cone contains as much resin as 150 male flowers. Male flowers may have from 5 to 66 lupulin glands per flower (3). The male flowers contain from 0.1 to 1.0% α- plus β-acids, with the α- plus β-acids constituting 73% of the lupulin (10,12). Although the male hop has no brewing value, male hops increase yield in commercial hop yards by stimulating larger cone size in females by pollination. Seed content of female cones can be as high as 18% by weight when the cones are completely pollinated (4). Unlike female varieties, which flower over a period of weeks, male hops develop flowers, dehisce (shed pollen), and become senescent (flowers dry up and fall off) in a matter of 7–10 days. Like female hops, some male hop genotypes mature earlier than others.

Male hops are used as pollinators to develop new varieties by breeding. There is no apparent relationship between the number of glands per flower or the amount of resins per flower and the resin content of a female progeny (8). However, the proportion of α- to β-acids and the percent cohumulone in α-acids in male parents does affect the female progeny of a cross (12). Because of the small amounts of resin in male flowers, the usual methods for hop analysis are not applicable, and analytical procedures have been developed by analyzing lupulin glands of males (11,15). This paper describes a method for determining the composition of male hop oil. Since 1979 we have used this technique to analyze male genotypes from the U.S. Department of Agriculture germ plasm collection and have obtained information about male hop oil composition and content.

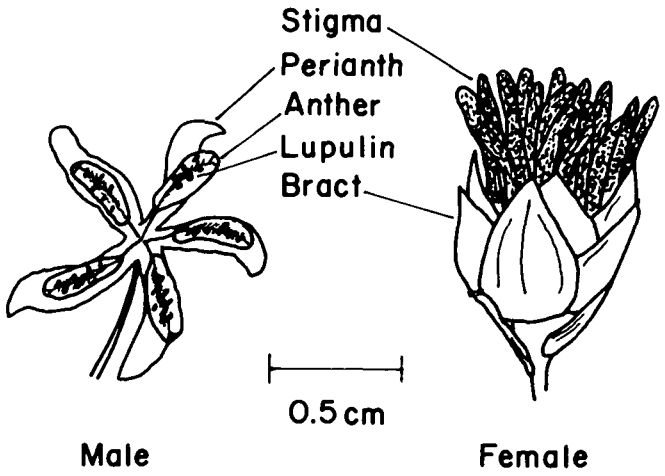

Fig. 1. Comparison of the morphology of male and female hop flowers at time of pollination.

EXPERIMENTAL

The male flowers were picked shortly after dehiscence, and the flowers were stripped from the sidearms and dried overnight in paper bags at 30–40° C. About 500 mg of lupulin was obtained from 50 g of green male flowers. The lupulin glands were separated from the flowers by gentle stirring in a blender with cold water. The slurry was poured through several sieves to separate the lupulin from the flowers and pollen. Lupulin passes through an 80-mesh (0.147-mm) screen and remains on a 200-mesh (0.074-mm) screen. The glands were washed onto a filter paper and most of the water removed by gentle suction. Lupulin was dried in a vacuum oven at 35° C overnight, placed in 4-ml vials, stoppered, and put into −4° C storage until analysis. After several years of cold storage, rehumidification was necessary for good extraction.

We tried several techniques for measuring oil composition. A solid sampler was used to introduce the lupulin glands into the gas-liquid chromatograph injection port. With this method, the injection port temperature was critical, and at temperatures less than 180° C the higher boiling oil constituents were not volatilized, whereas resins were pyrolyzed if the sample was overheated (11). Although methylene chloride and acetone extract more material from lupulin than pentane, they also extract more resins and waxes than pentane does. Gas-liquid chromatography analysis time is considerably longer if waxes must be eluted form the column.

For oil content and composition analyses, 24–50 mg of lupulin was accurately weighed (±0.1 mg) into a 0.1-ml vial. About 0.1 ml of pentane and exactly 0.5 μl of octanol-2 (internal standard) were added. The vial was sealed and placed in a sonic bath for 20 min. After equilibrating to room temperature, 5.0 μl was injected for gas-liquid chromatographic analysis at maximum detector sensitivity.

The pentane extract of lupulin was chromatographed on a 0.25 mm × 60 m fused silica column coated with Supelcowax 10 (Supelco no. 2-4081). A Hewlett-Packard model 5830A gas chromatograph with model 18835B capillary inlet system (1:100 split ratio) was used with a flame ionization detector in conjunction

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with a Hewlett-Packard 3393A integrator for data reduction and peak quantitation.

Two different temperature programs were used. For rapid determination of humulene/caryophyllene ratios, an initial temperature of 150° C was used, and after 5 min the temperature was increased to 250° C at 5° C/min. For detailed examination of the constituents, the initial temperature was set at 80° C and after 5 min programmed to 250° C at 2° C/min. The internal standard was used to quantify the data. The relative response factors were determined by using purified standards.

A large sample of male lupulin, 26 g, was extracted with 200 ml of methanol. Water (100 ml) and base (50 ml of 1.0*N* NaOH) were added, and the aqueous solution was partitioned into 50 ml of pentane. A sample of female lupulin was extracted in the same way. The hydrocarbons were separated from oxygenated compounds using silicic acid chromatography. The eluate was concentrated under vacuum at 30° C, and the fraction analyzed by gas chromatography/mass spectroscopy (GC/MS). Mass spectral data were acquired on a Finnigan model 4023 quadruple mass spectrometer using a 0.32-mm i.d. × 50 m Durawax-4 fused silica column (J&W Scientific).

RESULTS AND DISCUSSION

The major hydrocarbons in female hop oil are myrcene, caryophyllene, farnesene, and humulene. Farnesene may be present in all female hop varieties (16), but in most varieties it is less than 0.1% of the oil and considered absent. However, in some varieties usch as Tettnanger, Cascade, or Fuggle, farnesene is a major component of the essential oil. Other hydrocarbons detected in female hop oils include α- and β-pinene, limonene, copaene, cadinene, muurolene, and selinene (1,6). The structures of these compounds are shown in Figure 2. From mass spectral data alone, it is difficult to determine the difference between cadinenes and muurolenes. The only difference between these sesquiterpenes is the configuration of the rings, with cadinene having the "chair-chair" arrangement while muurolene has the "chair-boat" configuration. Although the hydrocarbons are usually lost during brewing, they are very useful in varietal identification. The proportion of humulene to caryophyllene (H/C) is a varietal characteristic used to distinguish among "kettle" and "aroma" hops. Aroma hops have high levels of humulene and high H/C ratios (13).

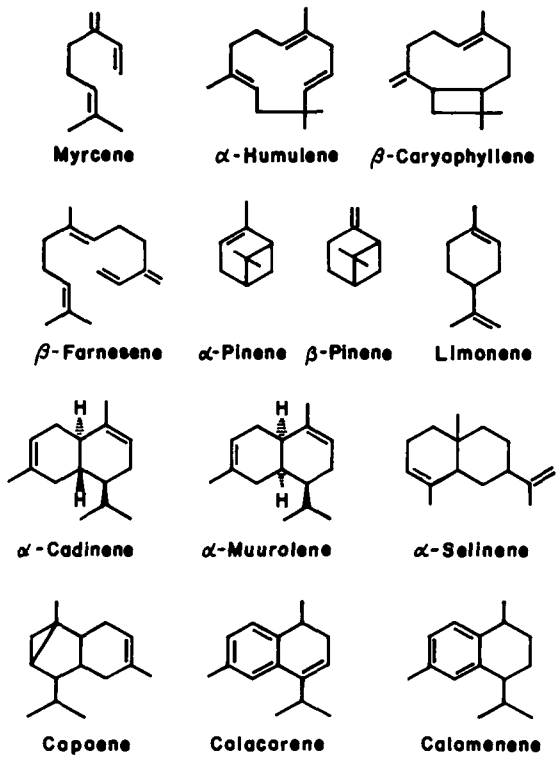

Fig. 2. Structure of terpenes found in hop oil.

A comparison of steam-distilled hop oil with volatiles extracted from lupulin is only possible with female varieties. The volatiles in the pentane extract of female lupulin are very similar to those in the steam-distilled hop oil. Figure 3 shows the chromatograms of steam-distilled oil and lupulin volatiles from the female variety Hallertauer mittelfrüh. Note the presence of α- and β-pinene and myrcene and absence of farnesene in both chromatograms. Table I shows the concentration of the major constituents of the volatiles. The lupulin sample has undergone some deterioration, as evidenced by the increase in the caryophyllene and humulene oxidation products and the decrease in myrcene concentration. The H/C ratio for the steam-distilled oil, 3.8, is slightly higher than the H/C found in the lupulin extract, 3.39. Farnesene is present in Cascade steam-distilled oil and lupulin extracts. The steam-distilled oil and lupulin extract of Cascade had H/C ratios of 2.86 and 2.98, respectively. Figure 4 shows the chromatograms of hydrocarbons from female lupulin and those extracted from male lupulin. The absence of myrcene and farnesene in male hop oil was previously noted (7,11), and this absence was verified by GC/MS. Cadinene/muurolene, selinene, calamene, α-calacorene, and copaene were identified in the hydrocarbon fraction by GC/MS.

Monoterpene synthesis is usually compartmentalized (2), and there is evidence that myrcene is concentrated in the secretory cells

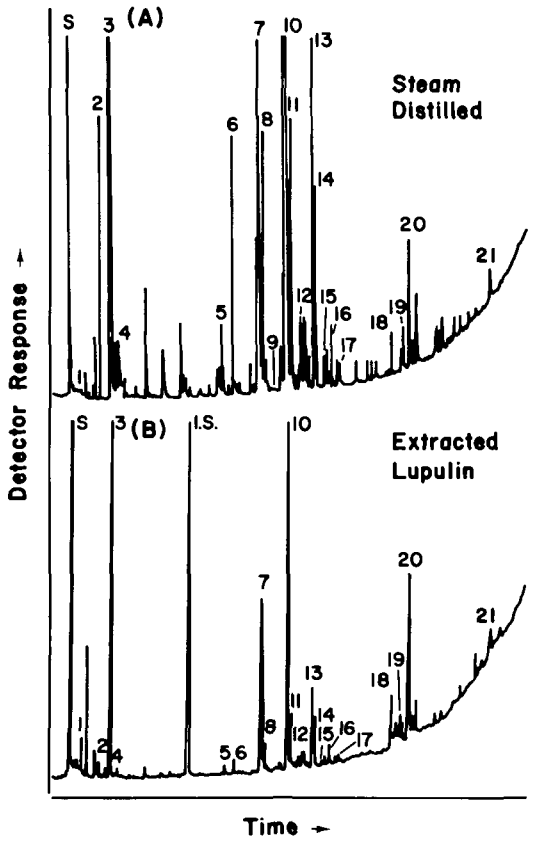

Fig. 3. Chromatograms of (A) steam-distilled hop oil and (B) lupulin volatiles extracted with pentane from the female variety Hallertauer mittelfrüh grown in Oregon. Gas-liquid chromatography conditions as described in text. Peak identifications as in Table I. S = solvent, I.S. = internal standard.

of the female lupulin glands and not in the secretory space (14). In mint, monoterpene concentration fluctuates diurnally, and radioactive-labeled carbon studies show that the monoterpenes are catabolized into primary metabolites (13). Myrcene is always present in female hop oil and increases during maturation, reaching a maximum after the α- and β-acid synthesis has ceased (5). It is possible that monoterpenes are an energy source utilized during the short maturation period of male hops. It is also possible that monoterpene biosynthesis does not take place in male flowers. It is interesting that the two terpenes absent in male oil, myrcene and farnesene, are acyclic and not cyclic.

The amount of lupulin and the amount of oil in lupulin of female hops may be estimated from whole hop analysis. Generally, the amount of oil in female hops increases with higher resin contents. We found a positive correlation, $r = 0.72$, between these two traits from 115 female genotypes. The oil content of female varieties ranged from 0.3 to 4.2 ml/100 g, or 4 to 15% volatiles in lupulin. The percent volatiles in male lupulin from 26 different genotypes ranged from 1.1 to 4.3%, but the relationship between resin content and volatiles was not statistically significant. Replicated extractions gave a 10% coefficient of variation for the percent volatiles in the lupulin. Caryophyllene and humulene were the main constituents and accounted for more than 75% of the volatiles. Howard and Slater examined Cluster lupulin and found 22% (w/w) oxygenated constituents in the volatiles (9). Because the amount of oxygenated constituents was small, no attempt was made to quantify individual oxygenated compounds.

Male genotypes show consistent H/C ratios. The H/C ratio is not affected by the picking date, as shown in Table II. The coefficient of variation for replicated H/C ratio determinations was 2.4%. The H/C ratios of six male genotypes for six years are shown in Table III. The difference between varieties is statistically significant at the 99% confidence level, whereas the year-to-year variation is not significant. Analyses of the progeny from different males crossed on the same female show that the male H/C ratio does affect the H/C ratio for female progeny. Table IV gives the mean H/C ratios for female progeny from three Hallertauer tetraploid crosses. Although the ranges overlap, choice of a specific male parent with a high H/C ratio increases the likelihood of obtaining progeny with high H/C ratios.

When female varieties without farnesene are crossed with

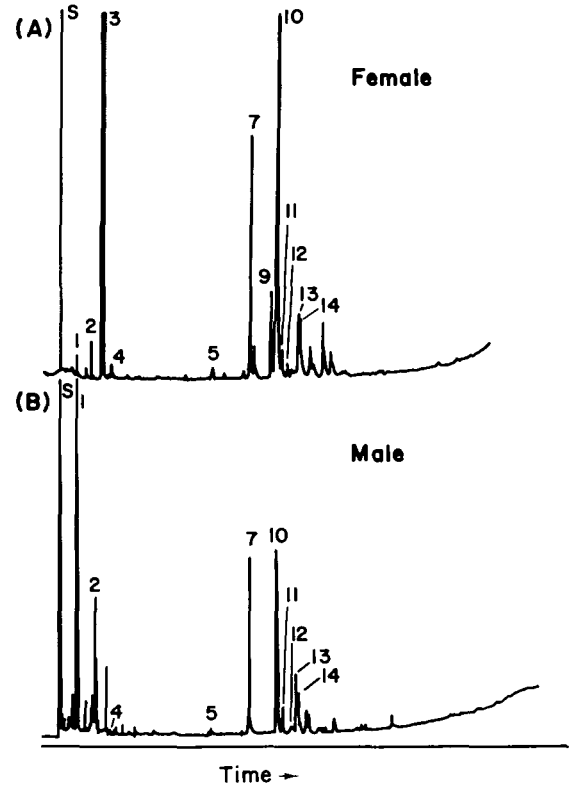

Fig. 4. Chromatograms of hydrocarbon fraction of (A) female lupulin extract and (B) male lupulin extract. Gas-liquid chromatography conditions as described in text. Peak identifications as in Table I. S = solvent.

TABLE I
Comparison of the Composition of Steam-Distilled Hop Oil and Pentane Lupulin Extract of 1985 Hallertauer mittelfrüh Grown at Corvallis, OR[a]

Peak	Compound	Area %[b] Steam-Distilled	Lupulin Extract
1	α-Pinene	0.06	3.05
2	β-Pinene	0.60	0.30
3	Myrcene	45.53	21.34
4	Limonene	0.28	0.30
5	Copaene	0.24	0.20
6	Linalool	0.76	0.40
7	Caryophyllene	9.23	13.54
8	Methyl dec-4-enoate	0.79	0.84
9	Farnesene	0.00	0.00
10	Humulene	32.00	41.73
11	Muurolene/cadinene	0.82	1.19
12	Selinene	0.34	0.40
13	γ-Cadinene	1.52	2.53
14	δ-Cadinene	0.88	1.62
15	Geranyl acetate	0.13	0.10
16	Geranyl isobutyrate	0.28	0.34
17	Geraniol	0.08	tr
18	Caryophyllene oxide	0.17	1.66
19	Humulene monoepoxide I	0.16	0.82
20	Humulene monoepoxide II	0.67	6.81
21	Humulene diepoxide A	0.12	0.65
Humulene/caryophyllene ratio		3.47	3.09
(corrected for relative response)		3.80	3.39
Oil content (%, v/w)		0.9	1.2 est.

[a]Oil steam distilled December 19, 1985; lupulin extracted May 18, 1987.
[b]Not corrected for response factors.

TABLE II
Effect of Picking Date on Amount and Proportion of Caryophyllene and Humulene in Male Hops

Genotype	Date	Caryophyllene	Humulene	Humulene/ Caryophyllene
19046M	July 11	1,410	2,214	1.57
	July 21	476	8,364	1.76
21087M	July 08	882	2,072	2.35
	July 21	1,951	4,637	2,36
63015M	July 16	750	1,608	2.15
	July 21	594	1,175	1.97

TABLE III
Humulene/Caryophyllene Ratios of Selected Male Genotypes[a]

Genotype	1980	1981	1983	1984	1986	Mean
64035M	3.86	4.22	3.81	3.92	3.89	3.95 a[b]
21361M	3.86	3.69	4.03	4.19	3.78	3.89 a
21337M	2.74	3.09	3.22	3.15	2.90	3.02 b
21087M	3.36	2.88	2.72	2.95	2.83	2.95 b
19058M	2.69	1.69	2.50	2.55	2.08	2.30 c
21089M	0.19	0.02	0.21	0.08	0.08	0.11 d

[a]Values corrected for relative detector response.
[b]Values followed by different letters are statistically different at the 95% confidence level.

TABLE IV
Humulene/Caryophyllene (H/C) Ratios for Female Progeny from Hallertauer mittelfrüh Tetraploid Crosses

Hallertauer H/C Ratio	Cross	H/C Ratio	H/C Ratio in Female Progeny		
			Mean	SD	*n*
4.25	× 19058M	2.30	2.96	0.61	1
4.25	× 21381M	3.95	3.57	0.50	9
4.25	× 64035M	3.07	3.61	0.10	13

selected male parents, the female progeny may or may not have farnesene. A Hallertauer tetraploid was crossed with two different males. The female and male progeny of cross 8301 did not have farnesene, whereas some female offspring of cross 8309 had farnesene in the essential oil. A previous Cascade cross produced the male parent used in cross 8309. Figure 5 shows the chromatograms of male and female progeny from these crosses. All the the progeny of cross 8309, tetraploid Hallertauer mittelfrüh × diploid male, were triploids.

CONCLUSIONS

Myrcene, an acyclic monoterpene, and farnesene, an acyclic sesquiterpene, do not occur in male hop oil. The major hydrocarbons of male hop oil are caryophyllene and humulene. Other sesquiterpenes identified in male hop oil are cadinene/muurolene, selineses, copaene, calamenene, and calacorene. The oil content ranges from 1.0 to 4.3% of the male lupulin glands. The ratio of humulene to caryophyllene is a varietal characteristic of male genotypes, and varies from 0.1 to 4.5 depending upon the genotype. The H/C ratio of female progeny is directly influenced by the H/C ratio of the male parent.

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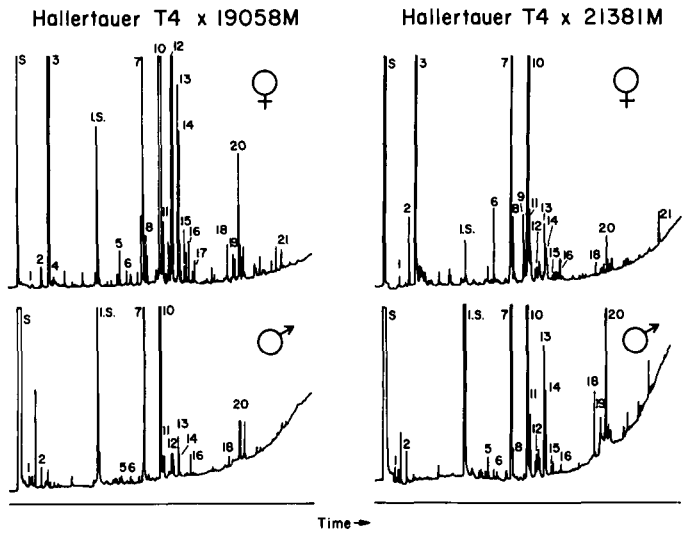

Fig. 5. Chromatograms of steam-distilled oil and lupulin extract for female (top) and male (bottom) progeny of Hallertauer tetraploid × two male parents. Gas-liquid chromatography conditions as described in text. Peak identifications as in Table I. S = solvent, I.S. = internal standard.