Development of a Hop with European Aroma Characteristics[1]

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ABSTRACT

In recent years, the well-known German aroma hop Hallertauer mittelfrüher has become increasingly scarce in world hop markets. In 1985 it occupied only about 10% of West Germany's hop acreage, compared to nearly 80% 20 years ago. Despite strong demand from the brewing industry, low yields and susceptibility to Verticillium wilt have made this hop unattractive to German hop growers. Mass selection and elimination of major hop viruses have not significantly improved Hallertauer's yield potential. Therefore, the development of a higher yielding, disease-resistant Hallertauer-type aroma hop is a desirable alternative. In 1983, a tetraploid Hallertauer was crossed to nine aroma-type male hops. The males were selected over a 10-year period based on agronomic and chemical evaluation. All showed a high breeding potential for aroma characteristics, and most were derived from known European aroma hops. Five selections, USDA 21455, 21456, 21457, 21458, and 21459, representing four different crosses, were identified in 1985 as having aroma properties similar to their parent Hallertauer mittelfrüher. All five are genetically seedless triploids, early to medium early in maturity, with an indicated yield potential at least twice that of their female parent. This paper presents detailed agronomic and quality data on the five aroma selections in comparison with the Hallertauer and Tettnanger controls.

Key words: α-Acids, β-Acids, Cohumulone, Essential oil, Humulus, Yield

Most brewers agree that hops contribute unique taste and flavor properties to fermented malt beverages. Taste, perceived organoleptically as "bitterness," is mainly due to the hop bittering compounds, primarily α-acids. These substances are measured relatively easily either spectrophotometrically or conductometrically. The industry term "brewing value," derived mainly from analysis of bittering compounds, is often used in trade negotiations. Hop breeders in various countries have made significant progress in recent years in raising the brewing value of hops to levels that only a few years earlier were thought to be unattainable (4,9,11,15,20,21).

Aroma or hop flavor, on the other hand, is much more difficult to measure, and opinions differ greatly on the value of hop aroma constituents in beer. Whereas most of the nearly 300 components of hop essential oils disappear during boiling and fermentation, some have been detected in the finished product (14,16–18,22,23).

There is a great diversity among hop cultivars in the composition of hop essential oils. Native European hops and particularly cultivars that arose from these indigenous hops are known in the trade as "noble aroma" hops. The best known among these are the German cultivars Hallertauer mittelfrüher and Tettnanger, and the Czechoslovakian Saazer (Table I). All are relatively low in α-acids and particularly in cohumulone, an α-acid analog. In addition, the proportions of α- and β-acids are nearly equal, resulting in an α-to-β ratio near 1. Rigby (19) suggested that such a quality profile is characteristic of all noble aroma hops. The essential oil of such hops, compared to other cultivars, is low in myrcene but high in humulene. Humulene epoxides have recently been shown to be among the major aroma constituents of hops in finished beer (14). In addition to humulene, other components of hop essential oils may be significant for hop aroma (13). Foster and Nickerson (3) identified three groups of hoppy compounds in 19 different hop cultivars that they believe collectively determine the total hoppiness potential.

Most traditional European noble aroma hops are low in both yield and α-acids when grown in the United States (Table I). Efforts by hop research centers in several countries to develop a substitute for one or more of the traditional noble aroma hops have thus far been unsuccessful.

In Germany, Hallertauer mittelfrüher has largely been replaced by Hersbrucker, a previously little-known, late-maturing hop cultivar from the Hersbruck mountains. The production of Tettnanger, in a small area near Lake Constance, has remained relatively stable. Czechoslovakia has slightly increased the acreage of Saazer, the only hop grown commercially in that country, but production has been stable, and yield levels have averaged under 900 lb/acre in recent years (2). Such low yields are not economically feasible for American hop growers.

Hallertauer mittelfrüher was chosen for our research. Until about 12 years ago, Hallertauer was the leading German hop cultivar and accounted for most of the German hop acreage (Fig. 1). Attempts to grow this cultivar in the United States have been largely unsuccessful, mainly due to low yields (Table I). Yields in Germany are somewhat higher, ranging from 900 to 1,500 lb/acre, with substantial year-to-year variations. In recent years, German hop growers switched to higher yielding cultivars with more consistent yields. For example, average yields of Hallertauer mittelfrüher in Germany in 1984 were 1,160 lb/acre, compared to those of Brewer's Gold (1,878 lb/acre) and Northern Brewer (1,775 lb/acre) (5). The production and acreage of Hallertauer mittelfrüher in Germany has declined rapidly in recent years, with no signs of leveling off. The decrease averaged about 10% per year between 1982 and 1985. The main reason for acreage decrease is that Hallertauer mittelfrüher is very susceptible to Verticillium wilt, a soilborne fungus disease prevalent in German hop-growing

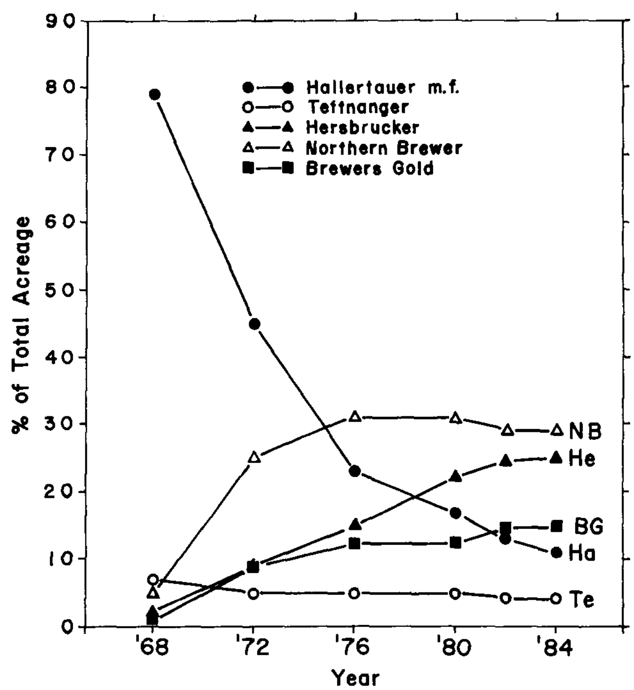

Fig. 1. Changes in the hop variety distribution in West Germany since 1968 (5).

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areas, and particularly in the Hallertau area of Bavaria where most German hops are grown. Although Verticillium has been found in U.S. hop-growing areas, the devastating strain of the fungus attacking Hallertauer mittelfrüher in Germany has never been found in the United States.

This paper describes an attempt to develop a European-type noble aroma hop by using techniques that have been successful in developing the cultivar Willamette as a replacement for Fuggle, another low-yielding aroma hop (8).

EXPERIMENTAL

Through the cooperation of R. A. Neve, director of the Department of Hop Research, Wye College, England, a tetraploid Hallertauer mittelfrüher was obtained in the spring of 1983. Softwood cuttings of tetraploid Hallertauer mittelfrüher were planted in a greenhouse soil bed in May 1983 and trained on a single string. They flowered in early August and were crossed with nine different males (Table II). The males were selected from the USDA hop germ plasm collection at Corvallis, OR, during the preceding 10 years. They were judged to be among the most promising males for aroma crosses based on analysis of isolated lupulin glands collected over several growing seasons. Among these were three genotypes, USDA 64033M, 64035M, and 64037M, which were selected at Corvallis from an open pollinated seedling progeny of a female German aroma hop grown at Wye College, England (10). Seeds were harvested in the fall of 1983 and pretreated and germinated in early spring 1984 as described previously (7). Seedlings were grown in greenhouse pots and propagated by softwood cuttings to establish four-hill yield plots in June 1984. Plants were trained on a three-foot bamboo stake. About 10% of the seedlings flowered late in the season and produced cone samples for laboratory analysis. The following year, plants were trained on two strings per hill like commercial hops. Agronomic data were obtained during the growing season and, based upon visual evaluation, 213 of the highest yielding selections were machine-harvested. Green plot weights were

TABLE I
Yield and Quality of European Noble Aroma Hops Grown at Corvallis, OR, over a 10-Year Period, 1975–1985

Variety	Maturity	Yield (lb/A)	Alpha Acids[a] (%)	Beta Acids[a] (%)	Alpha Ratio[b]	Cohumulone (%)	α-Acid (6-month storage) % Orig.	Oil (ml/100 g)	H/C[c]	H/F[c]	Myrcene (%)	Humulene (%)
Hallertauer mittelfrüher	early	536	4.6	4.5	51	21	51	0.77	3.5	0	35.1	37.6
Tettnanger	early	329	4.5	4.1	52	23	60	0.58	3.4	1.6	44.2	21.1
Hersbrucker	late	781	5.0	6.9	42	23	59	0.61	3.3	0	48.3	28.3
Saazer	med. early	205	4.3	3.6	54	24	51	0.62	3.5	1.7	33.6	27.7

[a] Spectrophotometric analysis.
[b] Alpha/(alpha + beta).
[c] H = humulene, C = caryophyllene, and F = farnesene.

TABLE II
Crosses for European Noble Aroma Hops, 1983

Cross No.	Pedigree	Total No. of Seeds	No. of Seedlings	Sex: F	Sex: (M + F)	Sex: M	Sex: ?
8301	Tetraploid Hallertauer mittelfrüher × 19058M (Early Green × Unknown)	347	75	67	8	0	0
8302	× 64033M (German Seedling)	95	18	11	6	0	1
8303	× 64035M (German Seedling)	347	143	115	15	9	4
8304	× 64037M (German Seedling)	466	134	106	21	3	4
8305	× 21237M (Saazer Seedling)	138	29	22	7	0	0
8306	× 21337M (Northern Brewer × Bullion-German Seedling)	92	6	3	2	0	1
8307	× 21361M [Cascade × [(Brewer's Gold × Early Green-Unknown) × German Seedling]]	126	4	1	3	0	0
8308	× 21362M [Cascade × [(Brewer's Gold × Early Green-Unknown × German Seedling]]	298	72	36	31	3	2
8309	× 21381M [Cascade × [(Brewer's Gold × Early Green-Unknown) × German Seedling]]	205	39	28	9	2	0
Totals		2,114	520	389	102	17	12

converted to yields per acre expressed in pounds. Hops were kiln-dried, rehumidified to about 9% moisture, compressed into 1-lb bale samples, and stored in a freezer until analysis. Loose cone samples were saved separately for determination of seed content and hand evaluation by interested brewers. Chemical analyses were done as follows: α- and β-acids by the official ASBC method (1), α-acid composition according to the method of Verzele et al (24), total oil content by steam distillation according to Wright and Connery (25), oil composition by gas-liquid chromatography as described in Figure 2, and storage stability of the soft resins according to Likens et al (12).

RESULTS

Seed production among crosses involving the nine different males varied considerably. This was largely caused by differences in receptiveness of female flowers under greenhouse conditions and fluctuating temperatures at the time of day when pollinations were made. Some male parents also seemed to produce a larger number of empty seeds, a situation that is occasionally observed in hop crosses.

On average, female seedlings (F) in each progeny outnumbered males (M) about fourfold (Table II). However, the sex ratio differed widely among crosses for reasons that are not understood at this time. Most male plants produced ample pollen but also developed normal appearing female flowers primarily at the tips of lateral branches (M + F). These flowers developed into normal hop cones. Strictly male plants without female flowers among the progeny were very rare, a situation previously found in progenies of tetraploid × diploid crosses (6). None of the plants identified as females produced any male flowers.

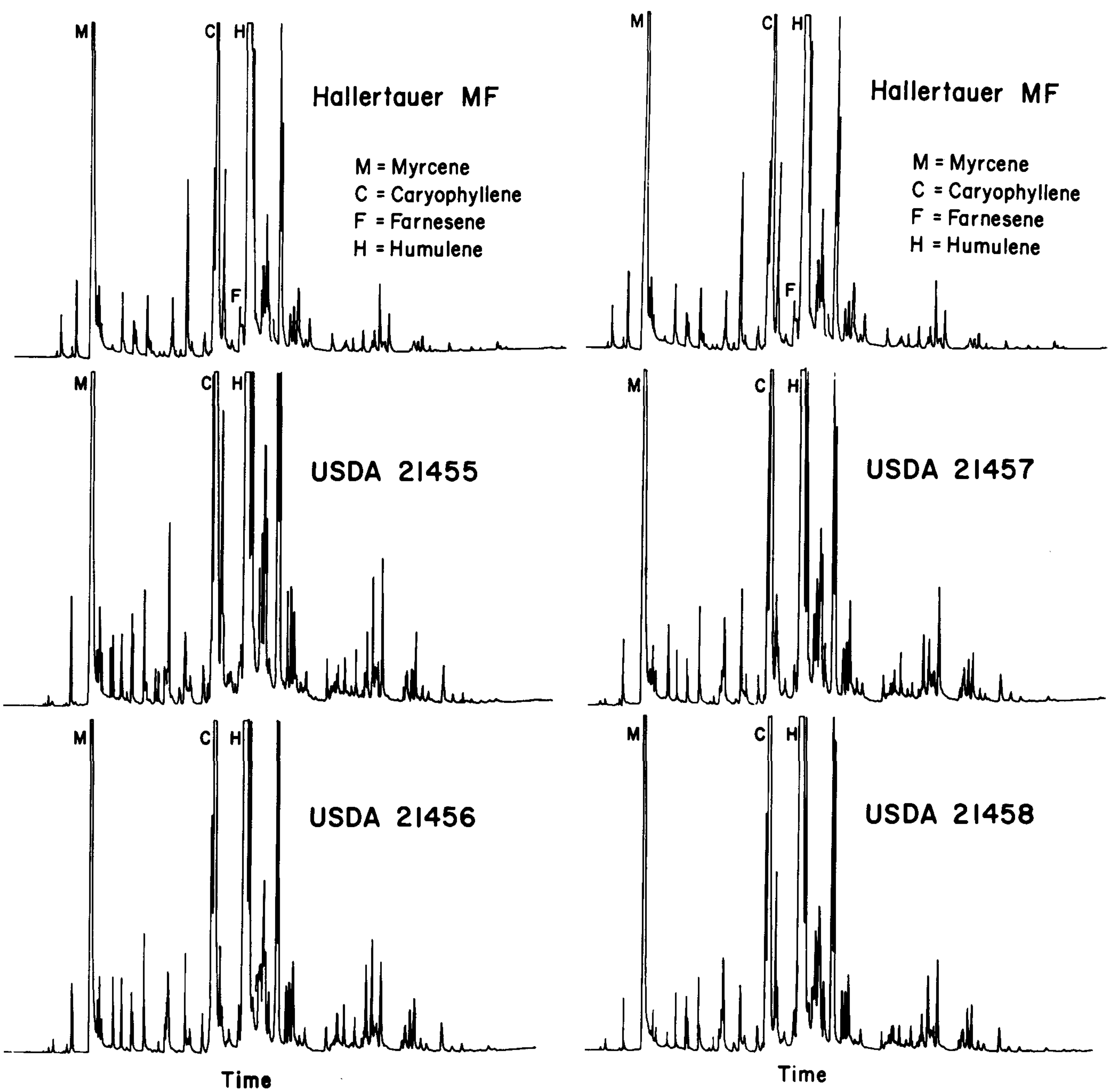

Fig. 2. Gas-liquid chromatograms of four triploid Hallertauer seedling selections in comparison with the Hallertauer mittelfrüher control. Note almost complete lack of farnesene. Conditions: 30 m SP-1000 0.32 mm i.d., fused capillary column, isothermal at 80° C for 5 min, then to 180° C at 3° C/min, flame ionization detector.

A total of 520 seedlings was transplanted to the field. This number is smaller than the actual germination percentage, because only vigorously growing genotypes were propagated for field planting. Weak seedlings or those with growth abnormalities were discarded. Cytological analysis of root tip samples obtained in 1984 showed that over 90% of all seedlings were triploids with a chromosome number of 2n = 30. Although triploids are expected from tetraploid × diploid crosses, selection may have been in favor of triploid genotypes, which are known to be more vigorous than those with missing or extra chromosomes (6). If so, it may also explain the extraordinarily large number of triploid genotypes as compared to an earlier study (6) involving crosses of tetraploid × diploid hops where even weak seedlings were saved that were found to be aneuploids with little practical value. Among the crosses with the largest number of surviving seedlings were those involving the two German-derived male genotypes 64035M and 64037M, the male USDA 19058M, which has the English aroma hop Early Green in its pedigree and USDA 21362M, a relatively new USDA male aroma selection developed from Cascade, Early Green, and Brewer's Gold.

By visual selection and evaluation during the 1985 growing season and particularly shortly before harvest, nearly one half of the 389 female genotypes represented in Table II were eliminated before machine harvesting. The remaining 213 genotypes, which came mostly from the five crosses with the largest seedling progenies, were machine harvested at optimum maturity, and cone samples were stored at −15° C for laboratory analyses.

Table III presents detailed data for each individual progeny from these five crosses, the overall yield and quality data of the whole female Hallertauer triploid seedling population, the parental diploid Hallertauer control, and averages from a group of 140 unrelated genotypes grown under identical conditions that served as unselected yield and quality control.

Average yield levels among the five crosses were nearly triple that of the Hallertauer parent. Yields varied substantially within each cross. Under our experimental conditions, yields in excess of 900 lb/acre are considered commercially acceptable. Each cross listed in Table III produced many seedlings that exceeded this level, sometimes by a substantial margin. The α- and β-acids levels of the individual progenies were generally in a range expected for aroma hops. A number of seedings had a soft resin profile and a ratio of α- to β-acids very similar to that of Hallertauer mittelfrüher.

The average cohumulone content of each seedling population and of the overall triploid Hallertauer seedling population in comparison with the Hallertauer parent was very favorable. Few individuals showed substantially higher cohumulone content than the Hallertauer parent, and some were significantly lower as shown by the range in Table III. A comparison with the unselected control shows that the quality profile of the seedling progeny can be altered significantly by carefully choosing parents to reach a given objective. This is particularly well illustrated by the cohumulone content, which until recently received little attention in hop breeding. The unselected control averaged significantly higher in cohumulone than any of the Hallertauer seedling populations, and the range within the unselected group was also much greater.

The content of essential oils as measured in milliliters per 100 g of dried hop cones was similar for all crosses studied. The population mean was also similar to the Hallertauer parent but substantially lower than that of the unselected control.

Myrcene, one of the undesirable components of hop essential oils, was as low or lower than that of the Hallertauer parent in all crosses studied (Table III). It rarely exceeded 50% of the total oil content, with a mean of 32% for the triploid population. This compares favorably to a mean myrcene content of 55% for the essential oil of the unselected control group, which ranged from 18 to 82% myrcene. Humulene, a desirable aroma component, had very high levels in all crosses studied and frequently exceeded the mean of the Hallertauer parent. The average humulene content of the overall triploid Hallertauer seedling population was 40%, more

TABLE III
Yield and Quality of Triploid Seedling Populations of Hallertauer mittelfrüher Crosses Made in 1983, Grown at Corvallis, OR, in 1985

Cross No.	Yield (lb/A)	α-Acids (%)	β-Acids (%)	Alpha Ratio[a]	Cohumulone (%)	Oil (ml/100 g)	Myrcene (%)	Humulene (%)	H/C[b]	No. of Seedlings
8301										
$\bar{x}$	1,251	6.2	4.6	57	26.5	0.82	33	34	2.88	43
range	246-2,346	3.0–12.0	3.3–7.4	38–71	18–34	0.13–1.47	10–57	13–47	2.48–3.54	
8303										
$\bar{x}$	1,204	5.8	3.8	60	23.3	0.83	31	41	3.52	74
range	626-2,293	2.4–8.3	2.3–5.8	31–71	19–37	0.19–1.52	15–44	32–53	3.36–3.72	
8304										
$\bar{x}$	1,039	5.2	4.9	51	24.1	0.82	30	43	3.50	46
range	384–1,685	2.1–7.1	3.2–6.0	30–60	20–39	0.12–1.40	13–47	33–54	3.41–3.64	
8308										
$\bar{x}$	994	6.3	3.5	65	25.7	0.92	33	37	3.46	22
range	544–2,048	4.7–7.7	2.7–4.2	58–71	22–31	0.40–1.93	22–43	32–43	3.32–3.56	
8309										
$\bar{x}$	1,436	7.3	4.5	62	23.6	1.24	46	27	3.26	16
range	544–2,368	5.7–8.6	3.3–6.1	53–69	18–29	0.69–1.79	30–65	9–43	2.01–3.61	
Hallertauer										
$\bar{x}$	395	4.2	4.1	50	22.5	0.70	44	31	3.42	2
All triploids										
$\bar{x}$	1,156	5.9	4.2	58	24.5	0.87	32	40	3.42	213
range	246–2,368	2.1–12.0	2.3–7.4	30–71	18–39	0.12–1.93	10–65	9–54	2.01–3.72	
Unselected control										
$\bar{x}$	1,238	8.1	4.8	62	33.5	1.40	55	19	2.47	140
range	74–3,652	1.7–18.3	1.2–9.7	22–77	19–55	0.17–3.38	18–82	0.5–43	0.03–4.08	

[a] Alpha/(alpha + beta).
[b] H = humulene, C = caryophyllene.

than twice that of the unselected and unrelated control.

The ratio of the two essential oil components humulene (H) and caryophyllene (C) is sometimes used to characterize noble aroma hops. The average H/C ratio of all crosses except cross 8301 was above 3.2, a level comparable to that of noble aroma European hops listed in Table I. The progeny of three crosses, 8303, 8304, and 8308, averaged higher than the Hallertauer mittelfrüher parent. In 1985, the overall triploid Hallertauer seedling progeny of 213 machine-harvested genotypes had an H/C ratio identical to that of the Hallertauer parent. The unselected control, however, had a substantially lower H/C ratio and a much greater range for individual genotypes from near zero to over four (Table III).

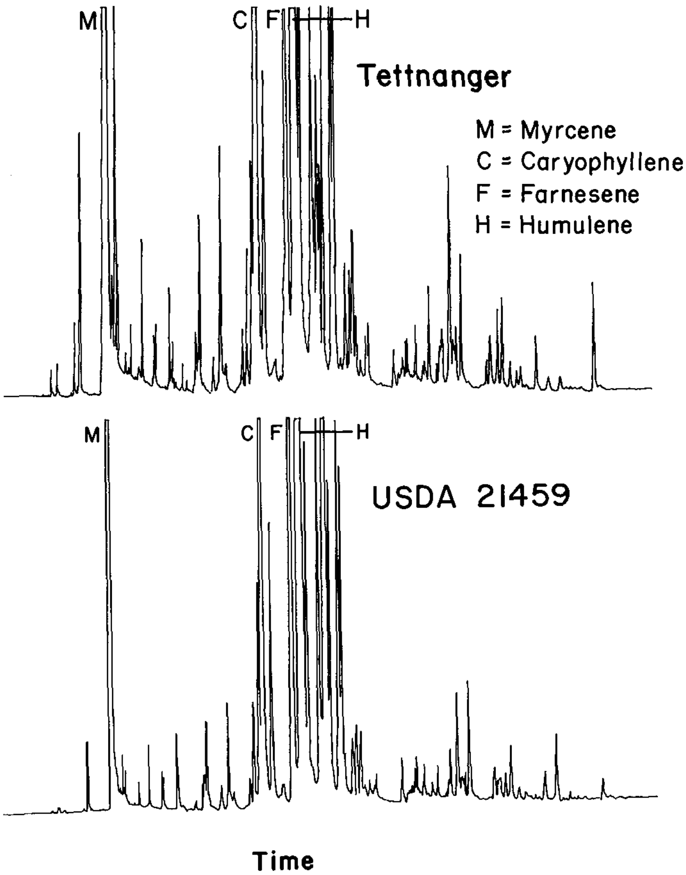

Fig. 3. Gas-liquid chromatogram of USDA 21459 in comparison with the Tettnanger control. Note pronounced farnesene peak. Conditions identical to those of Fig. 2.

Based on laboratory analyses in conjunction with yield data, the number of selections was reduced by applying the following criteria: yield level above 900 lb/acre, α-acids content under 7%, H/C ratio above 3, cohumulone content below 25%, α ratio ($\alpha/(\alpha + \beta)$) between 40 and 60 (equivalent to ratio of α to β between 0.8 and 1.2), and absence of farnesene, a compound not found in the oil of Hallertauer mittelfrüher. Because Tettnanger, another highly desirable German aroma hop, contains farnesene, some genotypes with farnesene were also saved.

This evaluation process reduced the number of selections to 47. The majority came from crosses 8303 and 8304 (21 and 14 selections, respectively), and none from crosses 8306 and 8307.

Loose cone samples from each of these 47 selections were submitted to experienced hop flavor taste panels of two large U.S. brewers who use imported noble aroma hops. In addition, oil chromatograms of each selection were compared with those of Hallertauer mittelfrüher and Tettnanger hops grown under identical conditions. After organoleptic and gas-liquid chromatographic analysis, the list was further reduced to 21 selections, from which the final five genotypes were chosen for commercial off-station trials in 1986 to be followed by commercial-scale brewing trials.

The five genotypes listed in Table IV represent four crosses, 8301, 8303, 8304, and 8308. All five have now received permanent USDA accession numbers for future testing. Three of the five selections mature early (about August 18–22 in the Willamette Valley, OR), a distinct advantage for growers; one appears to be medium early, and one has medium maturity, probably about 8–10 days later than the early selections. Yield levels ranged from 1,500 to over 2,000 lb/acre, about four times the Hallertauer control. The 1985 α-acids level of all five selections was slightly higher than that of Hallertauer mittlefrüher. Experience from other commercial trials shows that the α-acids content may likely be somewhat lower in commercial tests than in nursery plots and, therefore, comparable to Hallertauer mittelfrüher. The α ratio was also somewhat higher than the Hallertauer control except for accession 21458, but may be lower under commercial conditions. The cohumulone content differed little from that of Hallertauer mittelfrüher, whereas the oil content, with the exception of accession 21459, was slightly higher. The H/C ratio was almost identical to that of Hallertauer mittelfrüher, with the exception of accession 21455.

Like Hallertauer, four genotypes did not contain any farnesene. Although the fifth contained farnesene, it was chosen because of its

TABLE IV
Yield and Quality of Five Triploid Hallertauer mittelfrüher Seedling Selections, Corvallis, OR, 1985

Accession (Selection)	Maturity	Yield (lb/A)	α-Acids (%)	β-Acids (%)	Alpha Ratio[a]	Cohumulone (%)	Oil (ml/100 g)	H/C[b]	H/F[b]	Myrcene (%)	Humulene (%)	Brewer Taste Panel[c] 1	2
21455 (8301-10)	early	2058	7.7	5.8	57	23	1.04	2.5	0	32.0	38.0	VG	G
21456 (8303-46)	early	1376	5.1	4.4	54	24	1.00	3.5	0	26.1	46.3	VG	G
21457 (8303-117)	medium early	1866	6.6	3.7	64	21	0.80	3.5	0	37.4	37.4	Ex	G
21458 (8304-119)	early	1322	5.5	5.8	49	22	0.94	3.4	0	29.3	43.8	VG	F
21459 (8308-66)	medium	2048	4.7	3.4	58	24	0.57	3.5	1.3	29.0	32.6	P	G
Hallert. control	early	395	4.2	4.1	50	22	0.70	3.4	0	44.3	31.4		

[a] Alpha/(alpha + beta).
[b] H = humulene, C = caryophyllene, F = farnesene.
[c] Representing two major U.S. breweries. Ex, VG, G, F, P = excellent, very good, good, fair, poor.

similarity in the oil chromatogram to Tettnanger. The myrcene content of all five accessions was lower than that of Hallertauer mittelfrüher, a welcome development, whereas humulene was higher.

The brewery taste panels agreed on three selections with a good to excellent rating, but opinions differed for accessions 21458 and 21459. Oil chromatograms in comparison with the Hallertauer control showed that four genotypes gave a good match with Hallertauer (Fig. 2) and one with Tettnanger (Fig. 3).

These five accessions are now being increased for commercial testing in Washington, Oregon, and Idaho. Planting should be completed by mid 1986, and the first mature crop of each selection will be available for brewing trials in 1987.

ACKNOWLEDGMENTS

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