Identification of Hop Varieties by Gas Chromatographic Analysis of Their Essential Oils

Constancy of Oil Composition under Various Environmental Influences

SAM T. LIKENS AND GAIL B. NICKERSON

The essential oil of several varieties of hops displayed good varietal uniformity of composition under the following environmental conditions: annual climatic conditions, climates and soils of widely different growing areas within the United States, abnormal ranges of nitrogen and phosphorus fertility over various moisture levels, development of the plant over a 4-year period (hops are perennial), maturity of the crop over a 21-day harvest period, while hosting any of several viruses, through processing, and during storage. These observations were offered as justification for accepting hop oil composition as a reliable criterion for a system of varietal identification.

Many systems of hop identification, based on botanical description, have been established. These range from methods which rely mainly on general descriptive terms—such as size, shape, maturity, and quality (*14*, *15*)—to highly technical systems utilizing measurement of strig angularity, types of glandular hairs, and the like (*4*). In all cases, within-plant and among-plant variation is substantial, a factor which frequently leads to overlapping of varietal traits.

During the recent years of gas chromatographic analysis, many implications or statements have been made regarding the varietal nature of the composition of hop oil (*3*, *5*, *6*, *9*, *11*, *12*). The authors' experience has indicated that certain components or ratios of components of hop oil are highly specific for individual varieties (*8*).

However, all of these sources of information have a common shortcoming. None has revealed the degree of variability to be expected in hops grown under different environmental conditions. The sole purpose of this paper is to establish that hop oil composition is sufficiently constant to serve as a reliable basis for a system of varietal identification. Two other papers will deal with specific identifying characteristics of chromatograms from capillary and packed columns, respectively.

Methods

Hop samples used for obtaining oil specimens were collected from many areas (Table I). Only samples taken by qualified scientific and technical personnel were considered sufficiently reliable for use in this study. All oil samples were steam distilled by the method of Wright and Connery (*16*). After the oil was collected, it was immediately sealed in glass ampoules and stored under refrigeration at $-5°$ F.

All gas chromatographic analyses were made within a 3-week period under identical conditions. Gas chromatographic parameters were selected according to a method of Buttery (*2*): columns, dual $^1/_8$-inch o.d. by 25-foot standard-wall aluminum, packed with 10% by weight Carbowax 20M on 60- to 80-mesh Chromosorb P; inlet temperature, 200° C.; detector temperature, 200° C.; column temperature, programmed at 2° per minute from 80° to 190° C.; detectors, hydrogen flame; carrier gas, helium; flow rate, 30 cc. per minute at start with constant inlet pressure throughout the run; sample size, 0.9 μl.; chart speed, 0.2 inch per minute. All data are presented in the form of gas chromatograms for the purpose of easy visual comparison of the effect of variables on oil composition.

Results

Annual Variation (Figure 1). While the percentage of oil in the cones varies appreciably from year to year, the composition remains relatively constant. Minor differences can be observed in the 1950 'Brewers Gold' chromatogram and in the 1965 late 'Cluster' chromatogram. The over-all characteristics of each variety are easily recognizable, however, over the 15-year period.

Unfortunately, it was impossible to avoid statistical confounding of variables in this study. For example, in this test, years are confounded with location. However, this does not detract from the validity of the conclusion. First, differences are not noted; and second, locations are cleared of involvement in a separate test (Figure 2).

Climates and Soils (Figure 2). Neither Brewers Gold nor late Cluster oils are affected by the contrasting climates or soils of the Yakima and Willamette Valleys (Table II). Similarly, oils from 'Fuggle,' raised in the Willamette Valley or the Puyallup area, adhere closely to a varietal pattern.

Fertility and Moisture (Figure 3). Excessive application of nitrogen (400 pounds of N per acre) lowered both the α-acid and oil content of Cluster hops (*10*), but did not alter the oil composition. Excessive application of phosphorus (172 pounds of P per acre) had no effect on oil composition. Maintaining high or low available moisture (54 to 18%) did not affect oil composition, and no interactions with fertility were apparent.

Crops Research Division, U. S. Department of Agriculture, Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore. 97331.

Table I. Source of Each Sample Reported

Figure	Variety[a]	Harvest Date	Source	Collected by
1	L.C.	9-18-1950	Seedless, Warren Dutton Ranch, Sacramento, Calif.	R. G. Wright
		9-13-1963	Seedless, USDA Plots, Corvallis, Ore.	
		9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
	B.G.	8-30-1950	Seeded, E. Clemens Horst Ranch, Sacramento, Calif.	R. G. Wright
		8-?-1963	Seeded, USDA Plots, Corvallis, Ore.	
		9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
2	B.G.	9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
		9-15-1965	Seedless, Patnode Ranch, Yakima, Wash.	E. Netter
	Fu.	9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
		8-30-1965	Seedless, Pincus Farm, Roy, Wash.	F. Pease
	L.C.	9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
		9-15-1965	Seedless, Yakima Golding Farms, Toppenish, Wash.	F. Pease
3	Wn. L-1	?	Seedless, Wash. State Univ. Plots Prosser, Wash.	C. E. Nelson
4	Ta.	9-?-1965	Seedless, first season, P. Batt Ranch, Caldwell, Idaho	R. R. Romanko
		9-?-1965	Seedless, second season, Enrose Farms, Caldwell, Idaho	R. R. Romanko
		9-?-1965	Seedless, third season, Top Farms, Caldwell, Idaho	R. R. Romanko
		9-?-1965	Seedless, fourth season, Wilder Farms, Caldwell, Idaho	R. R. Romanko
5	Fu.	8-17-1962	Seeded, USDA Plots, Corvallis, Ore.	
		8-29-1962	Seeded, USDA Plots, Corvallis, Ore.	
	L.C.	9-3-1962	Seedless, Batt Ranch, Caldwell, Idaho	R. R. Romanko
		9-12-1962	Seedless, Batt Ranch, Caldwell, Idaho	R. R. Romanko
	Bu.	8-22-1963	Seeded, Kerr Ranch, Salem, Ore.	S. T. Likens
		9-8-1963	Seeded, Kerr Ranch, Salem, Ore.	S. T. Likens
6	B.G.	9-2-1965	Seeded, USDA Plots, Corvallis, Ore.	
		9-6-1965	Seedless, USDA Plots, Corvallis, Ore.	
	Fu.	8-26-1965	Seeded, USDA Plots, Corvallis, Ore.	
		9-2-1965	Seedless, USDA Plots, Corvallis, Ore.	
	L.C.	8-27-1965	Seeded, USDA Plots, Corvallis, Ore.	
		9-3-1965	Seedless, USDA Plots, Corvallis, Ore.	
	Bu.	9-4-1965	Seeded, Kerr Ranch, Salem, Ore.	S. T. Likens
		9-6-1965	Seedless, USDA Plots, Corvallis, Ore.	
7	Wn. E-2	9-?-1965	Seedless, Wash. State Univ. Plots	C. B. Skotland

[a] B.G. = Brewers Gold, Fu. = Fuggle, L. C. = Late Cluster, Ta. = Talisman (certified virus-free root program), Bu. = Bullion, Wn. E-2 = selection from early Cluster not yet named (certified virus-free root program), Wn. L-1 = selection from late Cluster not yet named (certified virus-free root program).

Age of the Plants (Figure 4). 'Talisman,' a new variety just released in Idaho, was sampled for four successive seasons in the Parma area. The composition of the oil is unaffected by aging of the perennial crown. The oil composition of propagules of recently selected genotypes is uniform to minute details. This may emerge as being significant from the standpoint of studying certain somatic mutations.

Maturity of the Crop (Figure 5). The two dates shown for each of the varieties in Figure 5 represent the limits of the usual periods of commercial harvest. While small variations can be observed, varietal characteristics are retained throughout the harvest period.

Seeds (Figure 6). Again, minor variations in oil compositions can be noted, but the production of seeds by any of the varieties tested did not mask the varietal identity.

Diseases (Figure 7). Each of the viruses in Figure 7 (*13*) was transmitted by graft to clones of an early Cluster selection (Wn. E-2). The yellow-fleck virus and the line leaf pattern virus have little effect on yield or α-acid and no apparent influence on composition. The

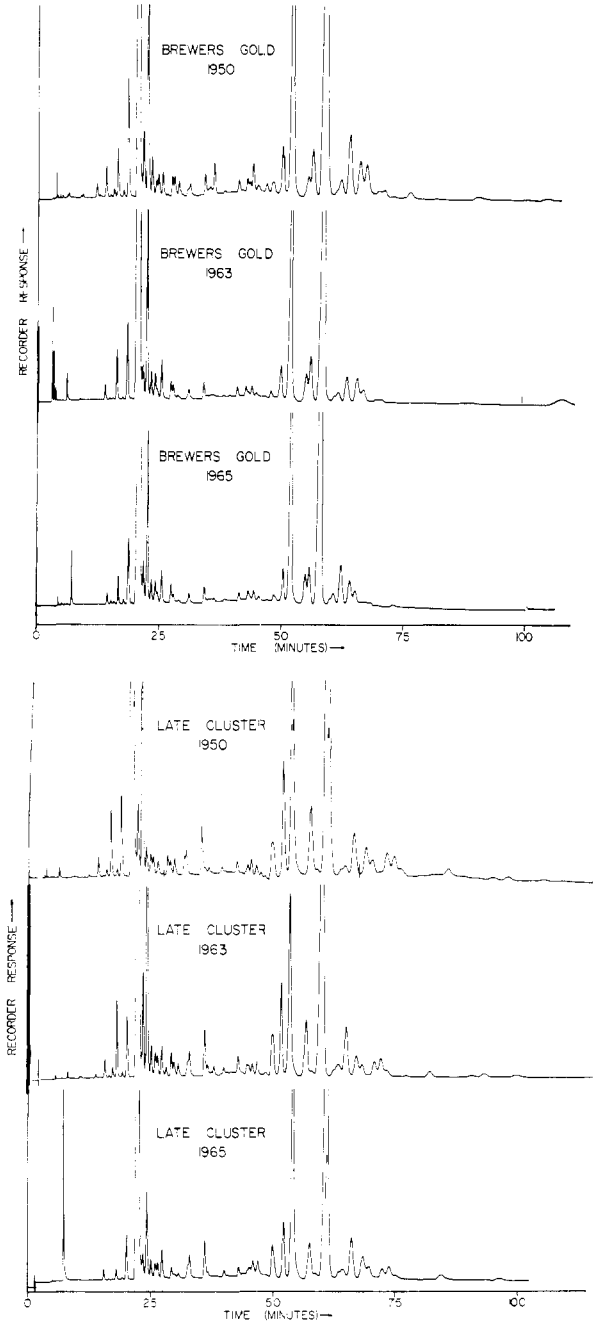

Figure 1. Annual variation in hop oil composition for two varieties over a period of 15 years

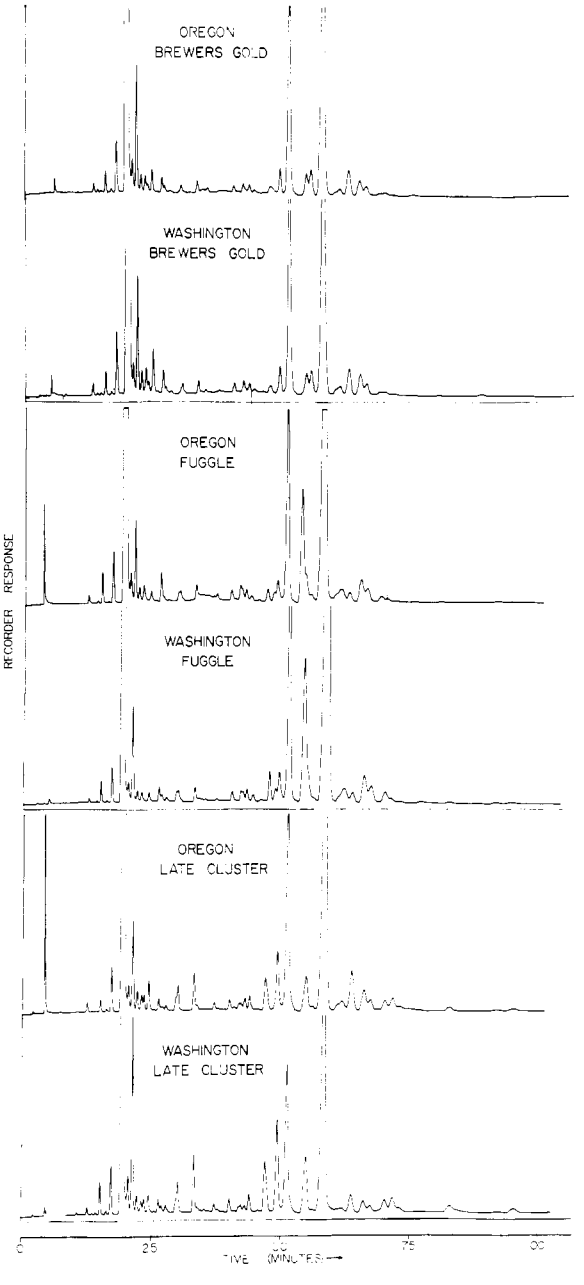

Figure 2. Climates and soils exert no detectable influence in hop oil composition

Table II. Description of Areas from Which Samples Were Taken

State	Area	Av. July Temp., °F.	Av. Rainfall, June-Sept., Inches	Soil Descriptions		
				Type	pH	O.M., %
Wash.	Yakima	74	1.1	Fine sandy loam	6-8	0.5
Wash.	Puyallup	64	5.3	Lake bed	5-6	20
Ore.	Salem	66	3.8	Clay loam	5-6	1.5
Idaho	Parma	75	0.3	Clay to silt loam	6-7	0.5

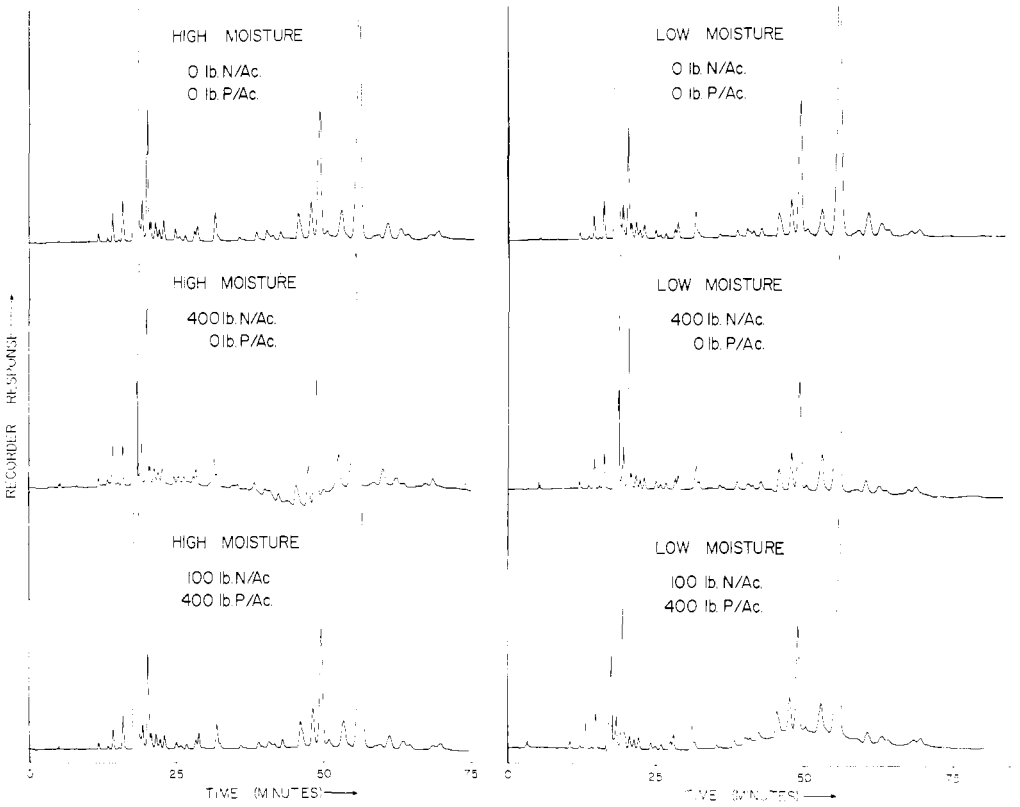

Figure 3. Neither fertility nor available moisture influences hop oil composition

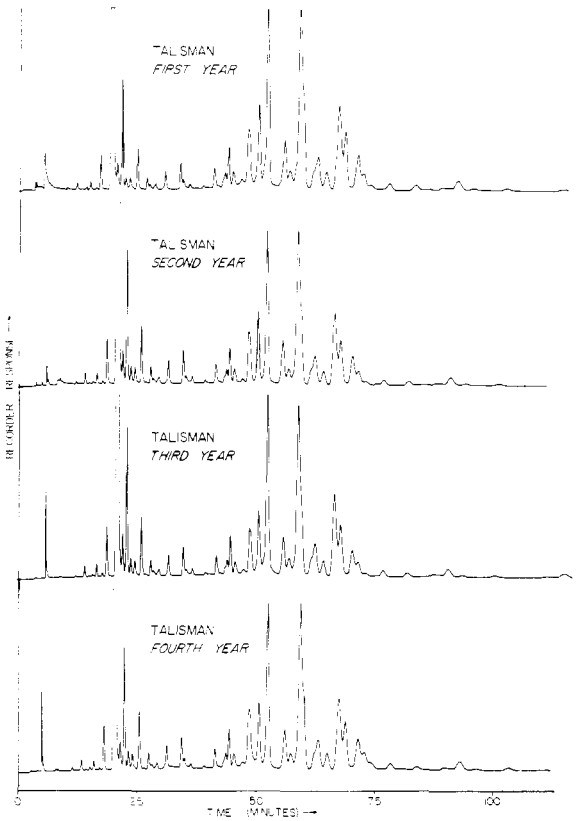

Figure 4. Age of plants has no effect on hop oil composition

two mosaic viruses produce distortion of the cones and reduce yield and α-acid. They do not, however, influence oil composition. Sterility (Black Hill) causes a severe loss in yield but, again, no change in oil composition.

Samples from a Fuggle plant with symptoms of a mosaic virus contained oil with normal composition. Another Fuggle plant with a suspected virus produced oil with very atypical composition. In a third case of suspected virus, late Cluster produced an atypical plant, but a recognizable oil.

Processing. Although no chromatograms of oil distilled from fresh, undried hops are available for this paper, the authors have observed repeatedly that normal drying and baling operations result in an oil loss of 10 to 30% without significant change in composition. This observation is in agreement with Howard and Slater, who found drying had little effect on oil composition (*7*).

Storage. Oils were distilled from late Cluster and Brewers Gold after extended storage at 3° C. The Cluster (18 months in storage) showed no change in oil composition. The Brewers Gold (uncertain storage period) was very low in all hydrocarbons but the oxygenated components retained their varietal proportions.

Discussion

Figures 1 through 7 indicate that hop oil composition is relatively stable under many environmental conditions. The samples were intentionally selected, how-

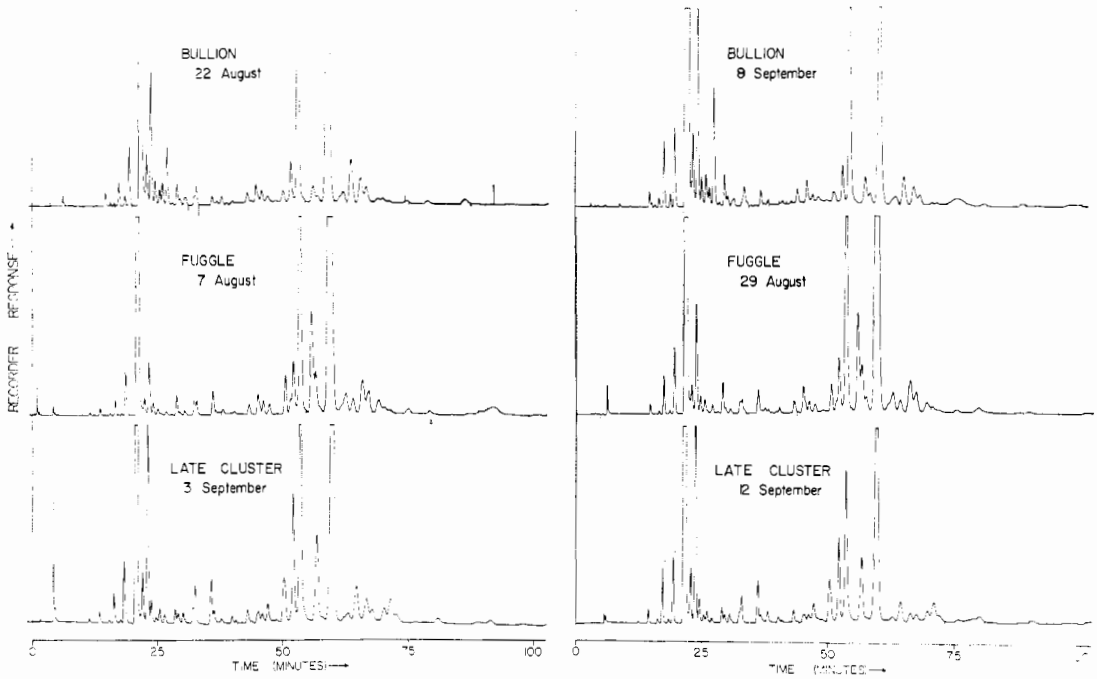

Figure 5. Maturity of the crop within the limits of usual harvest periods does not affect hop oil composition

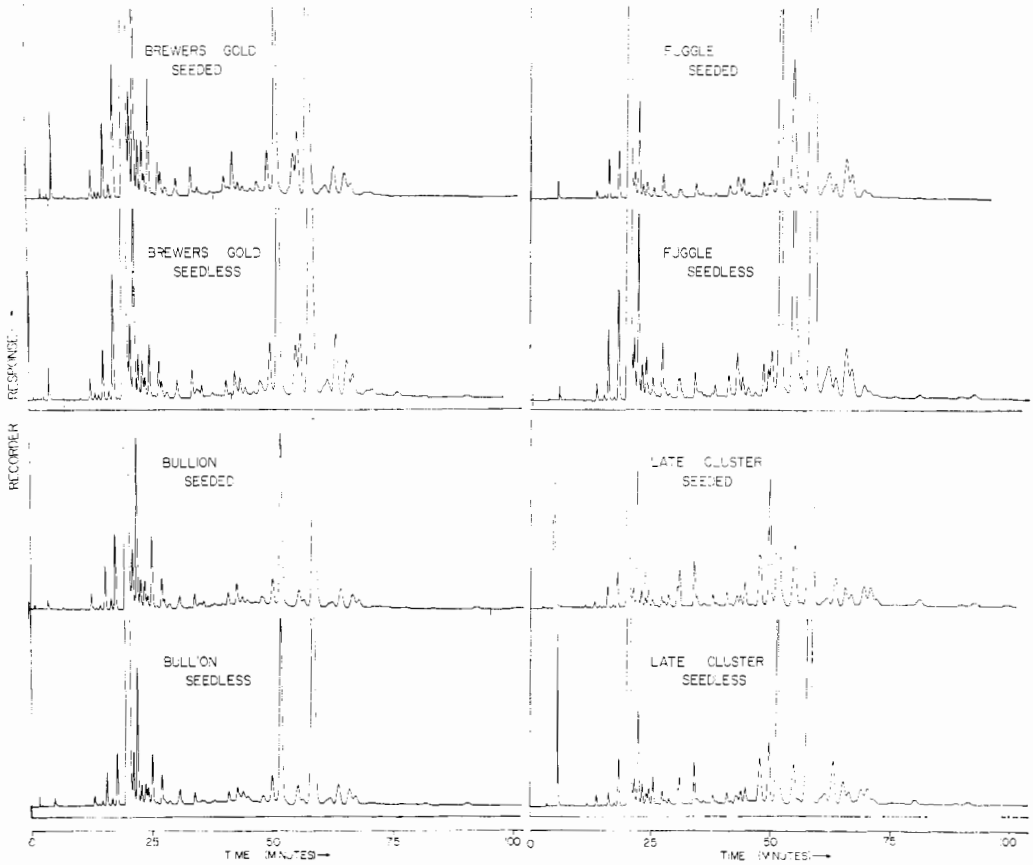

Figure 6. The presence or absence of seeds does not change the composition of hop oil

ever, for the best possible varietal uniformity. Fields were avoided if they appeared nonuniform owing to mixed varieties, off-types, disease, or any other reason. In spite of these precautions, noticeable variations occurred such as the extra peak at 35 minutes in the 1950 Brewers Gold or the out-of-proportion peak at 18 minutes in the 1965 late Cluster oil (Figure 1).

All commercial hop yards offer a possibility of some

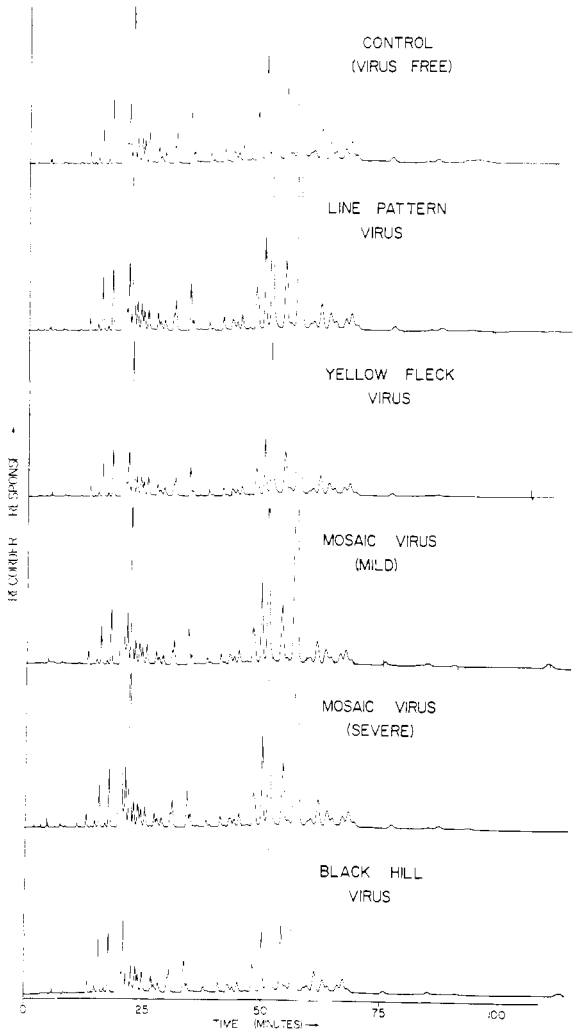

Figure 7. The presence of certain viruses does not alter the composition of late Cluster oil

degree of mixed plantings. Occasionally, seedlings may go unnoticed and after full development may even be used as a source for propagation. A different oil composition from the main variety could easily alter the average from which an oil sample was distilled. As a variety becomes older, more of this type variation is to be expected.

Zavarin (*17*) is using the composition of the essential oil of various firs as an index to races which botanists are unable to differentiate on morphological or botanical grounds. A similar situation may exist with hops. Somatic mutation has been suggested as being responsible for certain variations found in Fuggle (*1*). Variation in oil composition resulting from such mutations would also be more prevalent in old varieties.

It is significant that variation is found only in the older, established varieties. New hop plantings which have been propagated from a recently selected cone, such as Talisman, display remarkable uniformity of oil composition (Figure 4). The early Cluster selection in Figure 7 is also a recent selection and shows very uniform oil composition in spite of the presence of virus.

The irrigation-fertility trial from which samples were obtained for Figure 3 is planted with a recently selected medium-maturity Cluster (Wn. L-1), and, again, uniform oil composition is noted (Figure 3).

Conclusions

The oils of several varieties displayed good varietal uniformity under an array of environmental circumstances. The authors believe that this forms an adequate basis upon which to build a reliable system of varietal identification based on oil analysis. Minor within-variety differences in oil composition can be expected to occur among commercial samples, but these will not mask the genetic identity. Such minor variation may be a measure of genetic purity of particular fields, or may indicate the existence of races within varieties.

Acknowledgment

The authors thank the following for their contributions of sample material: R. G. Wright (P. Ballantine and Sons), Ernie Netter (Washington State Hop Producers Association), Fred Pease (John I. Haas, Inc.), C. E. Nelson (Washington State University), R. R. Romanko (University of Idaho), and C. B. Skotland (Washington State University).

Literature Cited

(1) Brooks, S. N., *Crop Sci.* **2,** 5 (1962).
(2) Buttery, R. G., Western Utilization Research and Development Division, U. S. Department of Agriculture, Albany, Calif., private communication, 1964.
(3) Buttery, R. G., Black, D. R., Guadagni, D. G., Kealy, M. P., *Am. Soc. Brewing Chemists Proc.* **1965,** p. 103.
(4) Davis, E. L., *Ann. Missouri Botan. Garden* **44,** 271 (1957).
(5) Hartley, R. D., *Wye Coll. Ann. Rept.* **1964,** p. 14.
(6) Howard, G. A., Slater, C. A., *J. Inst. Brewing* **63,** 491 (1957).
(7) *Ibid.*, **64,** 234 (1958).
(8) Likens, S. T., Nickerson, G. B., *Am. Soc. Brewing Chemists Proc.* **1965,** p. 23.
(9) Mori, Y., *Bull. Brewing Sci.* **7,** 43 (1962).
(10) Nelson, C. E., Washington Irrigated Agricultural Research and Extension Center, Prosser, Wash., private communication, 1965.
(11) Rigby, F. L., Bethune, J. L., *J. Inst. Brewing* **63,** 154 (1957).
(12) Roberts, J. B., *Wye Coll. Ann. Rept.* **1961,** p. 21.
(13) Romanko, R. R., Skotland, C. B., *Plant Disease Reptr.* **48,** 311 (1964).
(14) Salmon, E. S., *J. Southeast. Agr. Coll., Wye, Kent,* No. **34,** 93 (1934).
(15) Schmidt, K., *Hopfen Rundschau, Sonderdrucke aus Nr.* **11,** 19 (1958).
(16) Wright, R. G., Connery, F. E., *Am. Soc. Brewing Chemists Proc.* **1951,** p. 89.
(17) Zaverin, E., Snajberk, K., *Phytochem.* **4,** 141 (1965).

Received for review October 6, 1966. Accepted January 9, 1967. A contribution of Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the Department of Agricultural Chemistry, Oregon Agricultural Experiment Station. Technical paper No. 2176, O.A.E.S., Corvallis, Ore. This work was supported in part by the U. S. Brewers Association. Mention of names of equipment or specific industrial companies does not constitute endorsement by the USDA over similar equipment or firms not named. Part I of a series entitled "Identification of Hop Varieties by Gas Chromatographic Analysis of Their Essential Oils."